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FOREWORD

I am pleased to put into the hands of readers Volume-6; Issue-2: March-April 2021 of **"International Journal of Environment, Agriculture and Biotechnology (IJEAB) (ISSN: 2456-1878)",** an international journal which publishes peer reviewed quality research papers on a wide variety of topics related to **Environment, Agriculture and Biotechnology**. Looking to the keen interest shown by the authors and readers, the editorial board has decided to release issue with DOI (Digital Object Identifier) from CrossRef also, now using DOI paper of the author is available to the many libraries. This will motivate authors for quick publication of their research papers. Even with these changes our objective remains the same, that is, to encourage young researchers and academicians to think innovatively and share their research findings with others for the betterment of mankind.

I thank all the authors of the research papers for contributing their scholarly articles. Despite many challenges, the entire editorial board has worked tirelessly and helped me to bring out this issue of the journal well in time. They all deserve my heartfelt thanks.

Finally, I hope the readers will make good use of this valuable research material and continue to contribute their research finding for publication in this journal. Constructive comments and suggestions from our readers are welcome for further improvement of the quality and usefulness of the journal.

With warm regards.

Editor-in-Chief
Date: May, 2021

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Effect of Estrus Synchronization with Prostaglandins (PGF2A) and Gonadothropin Realising Hormone (GNRH) on the Hematological Profile of Pasundan Heifers during Pregnancy

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Abstract**—** *Twenty Pasundan cows were used in this study to know the effect of synchronization using prostaglandins and hormone gonadotropins on the picture of erythrocytes, leukocytes, and pasundan cow hemoglobin levels during pregnancy. The Pasudan heifers experimented with estrus using a combination of prostaglandin hormone (PGF2α) as much as 5 ml per head and gonadotropin realising hormone (GnRH) as much as 2.5 ml per head intramuscular to homogenize fertility conditions and improve fertility. The estrus mother cow is immediately carried out artificial insemination as much as 2 times with an interval of 6 hours. All test cows were given forage basalt food and adlibitum drinking water. Pregnancy examination is carried out on the 60th day and the 150th day of post-insemination using the rectal palpation method. Changes observed in the form of hematological concentrations include erythrocytes, leukocytes, and hemoglobin. The results showed that the concentration erythrocytes, leucocyte, and hemoglobin for Garut region respectively was 6.24±0.61 (million/µl); 11.54±0.25 (thousand/(µl); 11.54±0.61 (g/dl) higher than Bogor in a row was 5.99 ± 0.64 (million/µl); 11.46± 1.41 (thousand/(µl); 11.13 ± 0.60 (g/dl)). The results of the variance analysis showed that the synchronization of estrus with prostaglandins (PGF2α) and the gonadotropin releasing hormones (GnRH) did not differ markedly (P>0.05), between the concentrations of erythrocytes, leucocytes, and hemoglobin during the gestation period with cows that were not pregnant. It concluded that the synchronization treatment of estrus with prostaglandins (PGF2α) and gonadotropin releasing hormones (GnRH) had no effect on the profile of hematological concentrations during the gestational period (60 days and 150 days) and was no different from Pasundan heifers that are not pregnant.*

Keywords**— Pasudan heifers, synchronization estrus, haematological Pregnancy Phase.**

I. INTRODUCTION

Cultivation of Pasudan cattle as germplasm cattle is widely carried out by communities in the southern Coastal (Garut) and northern Priangan regions (Bogor), both as a primary livelihood and as an additional income. Pasudan heifers have high fertility but do not have high reproductive efficiency due to slow puberty of 22± 0.2 months, and repeated mating characterized by preconception service of 1.8±0.2 and conception rate of 60 ± 5.0% (Setiawati et al., 2018). A common reproductive problem in cows in Indonesia is low reproductive efficiency indicating reproductive disorders.

One of the symptoms of reproductive disorders is the occurrence of recurrent mating (Båge et al., 2002).

One of the efforts to obtain reproductive efficiency is through the application of synchronization of estrus in pasundan heifers that can manipulate the reproductive system to increase the potential of one head each year. Estrus synchronization is a technique of manipulation of the estrus cycle to present symptoms of estrus and ovulation in a group of cattle simultaneously. This technique proved effective for improving the efficiency of the use of artificial insemination (Patterson et al., 2005). Administration of PGF2α will cause regression of the corpus luteum followed by a decrease in plasma progesterone levels. Regression of the corpus luteum is followed by the rapid development of dominant follicles and ovulation. Additional GnRH administration before PGF2α will increase the size of the corpus luteum and maximize plasma progesterone levels during PGF2α injection, thus increasing the rate of regression of the corpus luteum and increasing the growth of dominant follicles. Synchronization of ovulation with GnRH can increase the number of follicles and corpus luteum to increase the secretion of pregnancy hormones and mammogenic hormones such as estradiol and progesterone during pregnancy that plays an important role in the maintenance of pregnancy until entering the postpartum period of cows (Rasby, 2005).

Blood is a metabolic component of living things that acts as a medium of transportation of oxygen and food juice into the tissues and transports the rest of the tissue metabolism and carbon dioxide for further excretion. On the other hand, the blood circulation system serves also as a means of channeling the secretion of endocrine glands to the target organ. Pregnancy in general causes dynamic changes in hematological parameters such as red blood cell count, hematocrit, and hemoglobin in cows (Sudjatmogo et al., 2001). The main and important thing to note in pregnant cows is their food rations and health care. Various types of diseases can interfere with the health of pregnant cows and the fetus they contain, the transmission of some types of diseases can cause infections in the placenta and fetuses. It can result in pedet being born in a weak condition or it can also result in death. Therefore it is necessary to anticipate by monitoring the hematological profile as an indicator of the level of health of the cow early before the cow shows clinical signs of pain. Hematological examinations that are often used to measure the degree of animal health are the number of red blood cells (erythrocytes), white dark cells (leucocyte), and hemoglobin (Hb) levels (Schalm, 2010). Total erythrocytes may indicate the occurrence of anemia or not, while hematocrit indicates that the cow is dehydrated or not, its total leukocytes and differentials may signal the occurrence or absence of infection (Amulic et al., 2012). There have been no reports of pasundan cow blood in pregnancy.

Based on the background description and problems in pasundan cows, this study aims to determine the effect of synchronization using prostaglandins and hormone gonadotropins on the picture of erythrocytes, leucocyte, and hemoglobin levels of Pasundan cows in the gestation period.

II. RESEARCH METHODS

This research sample uses 20 Pasundan cows, 10 each from Pasundan cattle farms in Pameungpeuk Subdistrict, Garut Regency, and 10 heads from Cariu – Jonggol Subdistrict, Bogor Regency. All test cows were adapted to the local environment and given basalt food in the form of field grass while drinking water was given adlibitum. The mother cow experimented with the hormone prostaglandins (PGF2α, dinoprost tromethamine) at a dose of 5 ml/tail intramuscular 2 (two) times with an interval of 11 days, but on the 9th day was injected with gonadotropin realising hormone (GnRH, gonadorelin) by 2.5 ml/tail intramuscular. to homogenize fertility conditions and improve fertility. The estrus mother cow is immediately carried out artificial insemination as much as 2 times with an interval of 6 hours. Pregnancy examination is carried out on the 60th day of post-insemination using the rectal palpation method. The observed changes are hematological concentrations including erythrocytes, leukocytes, and hemoglobin.

Blood sampling during pregnancy is done 3 (three) times at the time of estrus, gestational age 60 days, and 150 days. Blood is taken from the jugular vein as much as 10 ml using a disposable syringe containing anticoagulants (EDTA), then inserted into the test tube and placed in an ice-filled flask. Blood is left for 30 minutes then centrifuged at a speed of 2500 rpm for 15 minutes. The formed plasma is separated into an evendorf tube to be used for blood analysis.

Hematology examination

Eritosit. Blood samples are sucked up to the limit of 0.5 using a thinning pipette. The tip is dipped in a diluting liquid (Turk) and the liquid is sucked to the limit of 101. The pipette is lifted, then closed the tip with the thumb and the base is closed with the middle finger with a flat pipette condition. The solution with blood is flattened and mixed by making movements such as the number 8. After a partial homogeneous solution is discarded approximately 3-5 drops. The Counting Room is taken from the glass

cover, the glass cover is placed on the embankment of the counting room. The solution is filled into the counting chamber by touching the tip of the pipette on the edge between the glass plains of the cover so that the surface of the plain is filled evenly. After that, it is read under a microscope with a magnification of 40x. The cells that touch the second boundary line are calculated, the other side (right and bottom) do not enter the calculation. The five boxes that are usually counted are four corner boxes and one middlebox. The final calculation result (total number of erythrocytes), total erythrocytes = n x 10,000, where n is the sum of all cells from five squares.

Leucosit. White blood cells (leucocyte) are measured based on the number of white blood cells calculated based on the Turk method in units per mm3 of blood. According to Nugroho (2013) to calculate leukocytes, blood is diluted in leukocyte pipes and then put in the counting room. The diluent used is a solution of Turk. The test measures applied are a) Capillary blood suction, EDTA blood, or oxalate blood up to the 0.5 marks; b) Remove excess blood at the end of the pipette; c) Insert the tip into the Turk solution at a 45o angle, holding it at the 0.5 marks. suction the Turk solution until it reaches the 11 marks. Do not allow air bubbles; d) close the tip with the fingertip and remove the suction rubber; e) Shake for 15-30 seconds; f) place the counting room with the cover attached horizontally on the table; g) Shake the pipette for 3 minutes, keeping the liquid from being wasted from the pipette; h) Remove all liquid in the capillary stem (3-4 drops) and quickly touch the tip of the pipette to the counting room by offending the edge of the glass cover with a 30o angle. Let the counting room be filled with liquid with capillary power; i) Allow 2-3 minutes for leukocytes to settle; j) Using a microscope objective lens with a magnification of 10 times, the focus is placed on the divider lines; k) calculate leukocytes in four large areas from top left to right, down then left, down then left and so on. For the cells on the line, the calculated ones are on the left and top lines; l) The number of leukocytes per µL of blood is: the number of cells x 50.

Hemoglobin. The calculation of hemoglobin levels is done by the Sahli method. Sahli tubes are filled with HCl 0.1 N to the bottom line. Blood is sucked with pipette hemoglobin up to the number 20. The sucked blood is inserted in HCl 0.1 N by blowing slowly. Blood and HCl 0.1 N are mixed by blowing and sucking slowly. The formation of hematin acid is characterized by a change in color to brown or blackish brown. Aquades are dripped using a drip pipette while shaken, the addition of aquades is done until the color is the same as the comparison color. Hemoglobin levels are read by looking at the miniscus of liquid on the Sahli tube. Hemoglobin units are expressed by grams%.

Data analysis

The data obtained was analyzed using Anova's One Way analysis method with Duncan's follow-up test to see how the treatment affects variables.

III. RESULTS AND DISCUSSION

The results of the study in the form of total erythrocyte levels, total leukocytes, total hemoglobin (Hb), Pasundan heifers at the time of estrus, and period of pregnancy observed were the first quarter (gestational age 2 months), the second quarter (gestational age 5 months). The data is analyzed and presented in Table 1.

Table 1. Average Hemotological Concentration When Estrus, Pregnant 60 and 150 Days

Group (Region)	South Pesisir (Garut)		North Priangan (Bogor)	
Subgroup (Hormone)	PGF2α	PGF2α + GnRH	PGF2α	PGF2 α+ GnRH
Erythrocyte (million/µl)				
Estrus	5.75±0.25	5.72±0.86	5.15±0.43	5.42±0.29
60 days pregnant	5.63±0.35	6.61±0.19[a]	5.35±0.39	6.12±0.67[b]
150 days pregnant	5.78±0.29	6.95±0.28	5.54±0.49	6.94±1.06
Leucocyte (Thousand /(µl)				
Saat estrus	12.74±1.96	11.58±0.55	12.66±1.67	10.15±0.67
Hamil 60 hari	11.29±0.3	11.30±0.35[a]	12.09±0.88	10.25±0.61[b]
Hamil 150 hari	12.02±0.62	11.56±0.54	12.80±1.50	11.50±0.52
Hemoglobin (g/dl)				
Estrus	10.64±0.59	9.56±0.62	10.27±1.22	9.77±0.47

Hamil 60 hari	10.93±0.81	11.63±0.35[a]	11.03±0.86	10.53± 0.99[b]
Hamil 150 hari	11.66±0.61	11.95±1.15	11.57±0.57	11.39±0.51

a, b The average of each modifier on the same line with different superscripts shows a real difference (P< 0.01)

From the results of an examination of leukocytes, erythrocytes, and hemoglobin levels, Pasundan heifers in the gestation period (in Table 1) there was no difference with cows that were not pregnant (P>0.05). The data in Table 1 shows that the hematological average profile of Pasundan heifers in the South Coast and North Piangan regions is still within the normal limit of erythrocytes between 5-7 million/µl; leucocyte 10-12 thousand/(µl) and hemoglobin 9-12 g/dl. The normal range of total bovine erythrocytes is 4.9-10 µl, leucocytes 5.0 – 16 (thousand/µl), hemoglobin levels 8.4-14 g/dL (Roland et al. 2014). The concentration of hematological Pasudan heifers is quite varied, allegedly related to the management of feeding by farmers is quite varied according to the conditions of the existing grazing field location. The more adequate the nutrients in the feed will show normal total erythrocytes and are at a normal high range of cow blood (Adam et al., 2015). Furthermore, the total leukocyte plays a role in the defense of the body and its value will increase in cases of disease infection, food poisoning, anaphylactic shock, and central nervous disorders. The total decrease in leukocytes in cows can be caused when there is a decrease in leukocyte production, viral infection, shrinkage inflammation, the presence of cytotoxic substances, bone marrow disorders, and others (Roland et al., 2014). Hemoglobin functions to transport most of the oxygen and a small fraction of carbon dioxide, as well as maintain a normal blood pH (Baldy., 2003). The condition of leukocytosis is generally a physiological response to protect the body from attacks of microorganisms. In contrast, the condition of leukopenia that indicates a decreased total leukocytes can be caused by its ineffective formation process (Baldy, 2003).

Furthermore, disruption of the formation of blood cells can be caused by the administration of cytotoxic drugs, toxic substances, viral infections, starvation, normal replacement of bone marrow by malignant cells, such as in leukemia (Baldy, 2003). A significant increase in leucocyte percentage can be caused by chronic viral inflammation, adrenal cortex insufficiency disorders, and physiologically (fear, anxiety, and pain) (Vasconcelos and Galyean, 2014). Increased leucocytes can occur due to chronic diseases and increased steroids due to stress.

From the results of the examination of the concentration of leukocytes, erythrocytes, and hemoglobin, in the period of pregnancy (in Table 1) there was no difference with cows that were not pregnant (P>0.05). Similarly, after further analysis with a variety analysis in each period of pregnancy the levels of the elements studied did not show a noticeable difference with cows that were not pregnant. The concentration of erythrocytes, leucocyte, and hemoglobin in the South Coast region (Garut) is noticeably higher (P<0.01) compared to in the Northern Priangan region (Bogor). Important factors that affect the status of hematology are age, gender, status, the altitude of the region or place, feed and water balance of the body (Kadarsih, 2004). Low concentrations of erythrocytes in the North Priangan region (Bogor) are suspected to be related to inadequate feed consumed so that there are negative energy balance and environmental-climate conditions due to differences in place that can affect air oxygen pressure. One of the factors affecting the number of erythrocytes is a mineral deficiency (Njidda et al., 2014). The low concentration of erythrocytes contained in hemoglobin can be caused by the availability of feed source mineral content in a low maintenance environment (Schalm, 2010). The process of erythrocyte formation requires precursors, precursors needed i.e. supply of proteins, iron, copper, cobalt, amino acids, and hormones. The lack of precursors such as iron and amino acids that aid the process of erythrocyte formation will lead to a decrease in the number of erythrocytes (Besung et al., (2019).

Erythrocyte concentrations in pregnant Pasundan cows showed an increase as the gestational age increased from 60 days to 150 days. The increase in the number of erythrocytes occurs as compensation for changes and adaptation of the mother to the condition of pregnancy. The vascularity system and red blood cells serve to regulate the regulation of oxygen, carbon dioxide, nutrition, and circulation of important metabolites such as hormones to all tissues of the body including reproductive organs. The increased concentration of hormones is thought to trigger an increase in the number of red blood cells that are higher to supply the developmental needs of pregnancy (Kadarsih, 2004). Erythrocytes also play a role in the immune system, where when red blood cells are lysis processed by pathogens or bacteria, the hemoglobin in red blood cells will release free radicals that will destroy the walls and membranes of pathogenic cells, as well as kill them (Polizopoulou, 2010). Factors of nutritional status, blood volume, species, and altitude of the premises also affect the number of erythrocytes as well as hemoglobin. The number of erythrocytes and hemoglobin

will increase at low environmental temperatures (highlands) and will decrease at high environmental temperatures (lowlands) (Gerardo et al., 2009) Meanwhile, relatively high humidity can result in O2 levels in the air relatively lower, which can lead to hypoxia conditions. Hypoxia may result in increased erythrocyte production (Ganong, 2008).

The concentration of leucocyte and hemoglobin in the North Priangan (Bogor) region during pregnancy is relatively lower than in the South Coast(Garut). It is suspected that Pasundan cows in the North Priangan region (Bogor) experience a lack of feed balance and the unavailability of adlibitum drinking water that can have an impact on environmental stress, while in the South Coast region around (Garut) the grazing field there is a waterhole so that when the cow feels thirsty can immediately drink. Decreased leucocytes can be caused by spinal cord abnormalities and severe kaheksia due to nutritional deficiencies (Dharmawan, 2002). Deficiency of vitamin-B12, vitamin-A, vitamin-C, vitamin-E, folic acid, and riboflavin is associated with the incidence of anemia caused by nutritional factors (Jilani and Iqbal, 2011). The physiological condition of livestock is an indicator of livestock health, which has a positive implication on livestock production. Temperature and humidity are external factors that can affect comfort and productivity. Pasundan cows that experience heat insecurity due to environmental stress can result in the physiological body is not optimal. In this condition, the release of body heat energy is greater to the environment than the energy needs for optimization of production and reproduction. In addition to these conditions, cows will easily experience the stress of temperature insecurity, especially heat. Environmental heat insecurity results in the body no longer being able to expend the heat received from the environment so the body is forced to increase the metabolic rate in the heat release process. Such circumstances will increase the energy needs of metabolism and have an impact on the physiological decline of the body. Leukocyte profile may reflect increased cortisol caused by stress (Widhyari et al., 2010).

The number of leucocytes in the blood circulation can be affected by the level of production, recirculation, and the use or destruction of leukocytes. A decrease in the number of leukocytes (leucopenia) can occur due to the use of corticosteroids, thymectomy, radiation, chemotherapy, decreased production, and acute viral infections. The low value of hemoglobin (Hb) in Pasundan cattle in the northern region, is thought to be due to insufficient nutrition for basic living and reproductive needs derived from the amount of feed consumed and the difference in the height of the place. Factors of nutritional status, blood volume, species, and altitude of the premises also affect hemoglobin (Gerardo et al., 2009), hemoglobin value (Hb) is strongly influenced by the adequacy of nutrition in the body of livestock especially proteins used for the synthesis of hemoglobin (Bashar et al., 2010. Nutritional factors affect the hemoglobin concentration of cows, the more adequate nutrients in the feed will show normal total hemoglobin and is in the normal high range of cow's blood (Adam et al., 2015). The need for O2 increases in areas with low temperatures (highlands) as well as in cattle experiencing stress that has an impact on the increase in hemoglobin content (Santosa et al., 2012). Increased O2 needs at a time when livestock are stressed are necessary for the continuity of energy-intensive metabolic processes at that time. The synthesis of hemoglobin is influenced by the presence of nutrients in feed, such as protein and iron (Mohri et al., 2007). Iron deficiency causes lower levels of hemoglobin in the blood than normal, this state is called anemia, 99% of anemia is caused by iron deficiency, which will lower the body's immunity so that it is very sensitive to attacks of disease seedlings (Găvan, 2010).

IV. CONCLUSION

Synchronization of estrus with prostaglandins (PGF2α) and gonadotropin releasing hormones (GnRH) has no effect on levels of erythrocytes, leucocyte, and hemoglobin during the gestational period and is no different from that of Pasudan heifers estrus / non-pregnant cows, Thus synchronization using prostaglandins and gonadotropin hormones(GnRH), effective to be applied to Pasundan heifers.

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Prediction of High-Risk Probability Areas under Current and Future Climate Scenarios in India for the Establishment of Fall Armyworm

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Abstract**—** *The Fall Armyworm (FAW) or Spodoptera frugiperda, is an endemic and agriculturally important insect pest in tropical and subtropical regions of the Americas causing severe impact estimated at millions of dollars. FAW has been recently identified for the first time in India and is also a first record in Asia threatening the food security and livelihoods of millions of farmers. The insects are affected by climatic factors, and climate change may affect geographical distribution, abundance, growth rate, survival, mortality, number of generations per year and other characteristics. These climate change effects on insects are difficult to project due to complex interaction among insects, hosts and predators. Moreover, agricultural pest management may become more challenging under future climate change and variation. The present study aims to project the impact of climate change on future suitability for the expansion of FAW as well as highlight the high risk probability areas due to the pest using the historical and future climatic conditions. The modelling was carried out using CLIMEX model, GIS, the known distribution of the species and the CliMond meterological database. The analysis has indicated high climatic suitability for FAW occurrence in India with Eco-climatic Index (EI) values above 20. Further, the high risk probability areas for the FAW establishment up to district level were also identified for the major maize growing states. The areas where the pest is currently reported in the country are coinciding with the predicted potential areas in India validating the current analysis. The analysis using two general circulation models (GCMs), CSIRO MK3.0 and MIROC-H, for 2030 and 2050 under the A2 Special Report on Emissions Scenarios (SRES) indicated the possible reduction of climatically suitable areas for the FAW establishment in India. This kind of analysis assessing the possible impacts of FAW under future climate conditions is essential for the future economic production of crops.*

Keywords**— Fall army worm, Spodoptera frugiperda, Endangered areas, High Risk Probability, Ecoclimatic index (EI), CLIMEX.**

I. INTRODUCTION

The fall armyworm, *Spodoptera frugiperda* (J.E.Smith) is an agriculturally important insect of more than 80 plant species, causing damage to economically important cultivated cereals such as maize, rice, sorghum, and also to vegetable crops and cotton with a particular preference for maize, a main staple crop around the world threatening food security and biosecurity. This highly-destructive and invasive pest has been seen in the Americas since several decades, but its prevalence outside was noted for the first time in West Africa in early 2016 (IITA 2016; IPPC 2016), and it has subsequently been recorded in most sub-Saharan countries (ACAPS 2017). It has spread to 44 countries across the continent, barring North Africa (CIMMYT 2018). Sightings of damage to maize crops in India due to fall armyworm mark the first report of the pest in Asia in 2018. In India it was reported for the first time from Karnataka (ICAR-NBAIR, 2018a) and Andhra

Pradesh (EPPO, 2018). The pest has also been reported in Telangana, Tamil Nadu, Maharashtra and Gujarat.

Fall armyworm (FAW) causes major damage to economically important crops. In sub-Saharan Africa, where fall armyworm is devastating maize crops, estimates indicate 13.5 million tons of maize valued at $3 billion are at risk in 2017-2018, which is equivalent to over 20 percent of total production for the region (CABI, April 2017). In America, FAW is listed as one of the major pest of maize and causing severe economic losses, with infection level up to 70%. According to the International Maize and Wheat Improvement Centre (CIMMYT) at Mexico, FAW has, over the last two years, damaged more than 1.5 million hectares of Africa's maize crop. (Carolyn Cowan, Jennifer Johnson / August 13, 2018, CIMMYT). Moreover, FAW has suddenly become a major pest in Kenya, causing losses of about a third of the annual maize production, estimated at about 1 million tonnes (HugoDe Groote et al 2020).

Fall Armyworm is native to tropical and subtropical regions. However, it may also be found in temperate regions (Luginbill 1928; Sparks 1979; Clark *et al*. 2007). FAW does not possess a capability to enter diapause, due to this; FAW cannot survive extended periods of freezing temperature but must migrate northwards each spring if it is to re-infest cropping areas in temperate regions (Luginbill 1928).

The life cycle, behaviour, survival and spread of insects are affected by climate factors, especially temperature, which has a strong influence on all ectothermic organisms (Rosenzweig *et al*. 2001; Fhrer 2003; Diffen). Outbreaks of FAW are closely related to climatic conditions, and migrant adults can move northwards up to 483 km/generations with good winter and spring conditions (Sparks 1979). Climate change will have different effects on insects, directly impacting their life cycle or indirectly impacting hosts or predators (Cannon 1998; Patterson *et al*. 1999; Bale *et al*. 2002). Some of the expected effects are changes in geographic range, growth rate, migration, host preferences, abundance, synchronization, survival, mortality, number of generation per year and others (Tauber *et al*. 1986; Parry *et al*. 1990).

Climate change effects on insects are difficult to project due to complex interaction among insects, hosts and predators. Some modelling techniques have been developed to forecast the species distribution *viz*., Classification and Regression Tree Analysis (CART), Logistic Multiple Regression (LMR), Regression Tree Analysis (RTA), Maximum Entropy (MAXENT) and Bioclimatic Variable (BIOCLIM). Nevertheless, CLIMatic IndEX (CLIMEX) is especially appropriate for projecting the effects of variations in climate change on insects, since the model matches the presence of a particular organism with ranges of temperature, moisture and climatic stresses. In contrast to other models, CLIMEX uses more than one limiting factor to project current and future suitability (Sutherst *et al*. 1995; Patterson *et al*. 1999; Crozier & Dwyer 2006; Shabani & Kotey 2016). It has been widely used to project the current and future potential distribution of different pest (Kriticos *et al.* 2015b).

The main aim of the current research is to predict the high risk probability areas based on historical climatological data (centred at 1975) derived from Climond: global climatologies for bioclimatic modelling developed by The Commonwealth Scientific and Industrial Research Organisation, Australian federal government agency. An attempt has also been made to project the impact of climate change on future suitability of India for FAW, based on current known distribution using CLIMEX modelling and biological data obtained from the literature.

II. MATERIAL AND METHODS

CLIMEX (version 4.0) is a computational tool for studying the effects of climatic conditions on species distribution and relative abundance. The CLIMEX Model is based on the assumption that we can infer the suitable climatic conditions for a target species when information about its habitat is known. The compare location application was used to project the current probable distribution and spread of FAW in India.

"Compare Locations," application can predict the potential geographical distribution of target species with regard to climatic conditions (Jung *et al* 2016; Kriticos and Leriche 2010; Kriticos *et al* 2015; Stephens *et al* 2007). The species information necessary for the model is a series of parameters that describes responses to temperature, moisture and climatic stresses along with long term meteorological database for the location under study. The model assumes that the known distribution of the species infers the climatic conditions in which it can survive. The model parameters are divided among the population growth indexes, stress indexes and the constraint values, such as the length of the growing season (degree day per generation, PDD), which may exclude the organism from a particular location.

Growth index describes favorable season based on temperature, soil moisture, radiation, substrate, amount of light exposed, and diapause ability, whereas stress index is calculated from four stress indices, i.e., cold stress (CS), heat stress, wet stress, and dry stress, all of which are factors that limit the species population (Byeon *et al* 2017; Hill *et al* 2014; Kriticos *et al* 2015). The results of

CLIMEX Model are represented by the Ecoclimatic index (EI), which indicates the survival and growth of a species in many different locations based on the climate. Ecoclimatic index (EI) describes the level of favourability of a location for the particular organism to survive when the conditions are favourable. The EI, expressed as numbers between 0 (Unfavourable conditions) and 100 (ideal conditions), is calculated by multiplying growth index, stress index, and interaction stress index. EI values near 0 represents a location where the species has poor conditions for long-term survival, while EI values > 30 indicate remarkably good conditions for establishment and survival of the species, and values close to 100 represent perfect conditions for the species (Sutherst *et al.* 2007; Kriticos *et al.* 2015a). These perfect conditions are difficult to achieve in nature but can be obtained in laboratory experiments (Sutherst *et al.* 2007; Kriticos *et al.* 2007a). In this present paper/study, the EI categorization was EI = 0 = unsuitable, a region where the population does not persist; EI = 1 – 10 = marginal, where the population has limited conditions to persist; EI = 10-20 = medium, where the region can support large population; and EI > 20 = optimal, where the population has highly favourable conditions to persist. These categorizations were developed by taking into account previous studies and the information in the user manual (Sutherst & Maywald 1985; Shabani *et al.* 2014; Kriticos *et al* 2015a; NYZ RAMIREZ-CABRAL *et al* 2017; Hannalene du Plessis *et al*. 2018).

For modelling the potential spread of FAW in the country (India), the current geographical distribution of FAW is necessary. The known geographical distribution was gathered from CABI (2020) and literature resources (Ramirez Cabral *et al*. 2017 and Westbrook *et al*. 2016)

CLIMEX parameters:

CLIMEX (Sutherst and Maywald 1985; Kriticos *et al*. 2015) uses a visual, iterative process to fit the stress parameters. In the present study, stress parameters that limit the species suitability have been taken from two different hypothesis of N.Y. Z. Ramirez – Cabral *et al.*, and Hannalene Du Plessis *et al.* The stress parameters of both hypotheses have been tested to ensure the similarity of the generated potential geographic distribution with the species known geographic distribution. The hypothesis of Hannalene Du Plessis *et al*., fitting stress parameters appears to be more matching with the species known geographic distribution specified in CABI, 2020. Hence, the above hypothesis has been chosen to run the model and generate the CLIMEX simulation consistent with the current distribution of FAW. CLIMEX parameter values used for modelling the distribution of FAW is given in the table 1.

Table 1: CLIMEX parameter values for modelling the distribution of FAW

Parameter	Description	Values
Moisture		
SM0	Lower soil moisture threshold	0.15
SM1	Lower optimal soil moisture	0.8
SM2	Upper optimal soil moisture	1.5
SM3	Upper soil moisture threshold	2.5
Temperature		
DV0	Lower temperature threshold	12 ^{o}C
DV1	Lower optimal temperature	25 ^{o}C
DV2	Upper optimal temperature	30 ^{o}C
DV3	Upper temperature threshold	39 ^{o}C
Cold Stress		
TTCS	Cold stress temperature threshold	12 ^{o}C
THCS	Cold stress accumulation rate	0.001 week^{-1}
Heat Stress		
TTHS	Heat stress temperature threshold	39 ^{o}C
THHS	Heat stress accumulation rate	0.005 week^{-1}
Dry Stress		

SMDS HDS	Soil moisture dry stress threshold Dry stress accumulation rate	0.1 -0.005 week^{-1}
Wet Stress		
SMWS HWS	Soil moisture wet stress threshold Wet stress accumulation rate	2.5 0.002 week^{-1}
Threshold Annual Heat Sum		
PDD	Minimum degree day sum needed to complete a generation	600 ^{o}C

Once the parameters were included, the CLIMEX output was exported to Arch Map GIS and maps of climate suitability (EI) for establishment of FAW were generated for India and its individual states (Source of GIS maps: Indian Space Research Organisation, Dept. of Space, Govt. of India, Balanagar, Hyderabad). Validation was also done with the existing locations of occurrence in India.

An attempt was also made to project the future climatic suitability for the years 2030 and 2050. The current suitability was modelled with the Climond 10' baseline data, gridded historical climate data and the average 1961-90 baseline period in Climex format (Kriticos *et al*. 2012b). Among the different future climate scenarios (A1B, A2, B1 and B2), A2 scenario was chosen to model the future climatic suitability owing to its emission scenario with actual CO_2 emissions, increase in greenhouse gases and agricultural productivity. Two Global Climate Models (GCM's) were used to model the future climatic suitability for FAW with future data, *viz*., CSIRO MK3.0, from the Common Wealth Scientific and Industrial Research Organisation from Australia (Gordon *et al*. 2010), and MIROC-H from the Centre for Climate Research in Tokyo, Japan (Shiogama *et al*. 2010). These GCMs were chosen because of the availability of climatic variables required for CLIMEX.

OBSERVATIONS:

The parameter values from the hypothesis of Hannalene Du Plessis *et al*., relevant to species biology under "Wet Tropical" species condition were considered to project potential distribution of FAW. The parameters are explained in detail below-

- The Moisture Index is estimated from a hydrological model based on soil moisture, rainfall and evaporation, with information of the previous and current week. This index denotes the response of the organism to soil moisture and assumes it to be constant over a 24h period. A value of zero indicates no soil moisture and no growth. The parameters are SM0 = lower soil moisture threshold, SM1 = lower optimal soil moisture, SM2 = upper optimal soil moisture and SM3 = upper soil moisture threshold (Sutherst *et.al* 2007). Because FAW depends on a host survival, the insect will be unable to survive if the crop or plant is dead. Based on this assumption, the SM0 was set at 0.15, the wilting point value most frequently used (Kriticos *et al.* 2014), which is almost in agreement with the SMDS. Moisture is known to affect the pupal stage, and excessive dryness retards emergence (Vickery 1929). Soil moisture parameters were set to biologically reasonable values. The soil moisture limit for growth was set to approximate permanent wilting point (Kriticos *et al*. 2003). The upper limit for optimal growth (SM2) was set to 2.5, acknowledging that *S. frugiperda* can tolerate conditions with substantial water-logging. The lower limit for optimal growth (SM1) was adjusted to provide appropriate suitability in marginally dry areas.
- The Temperature Index is one of the main components of the growth index. It denotes suitable temperature ranges in which the organism can live, with values ranging from 0 to 1. There are four parameters of this index: the lower temperature threshold (DV0), the lower optimum temperature (DV1), the upper optimum temperature (DV2) and the upper temperature threshold (DV3) (Sutherst *et al.* 2007). The lower temperature threshold for growth is 12°C which reflect the tropical distribution of *S. frugiperda*. The lower temperature for optimal growth is 25 °C, the upper optimal temperature for growth is 30 °C (Simmons 1993), and the maximum temperature is 39 °C, near the threshold of 39.8°C reported by Valdez- Torres *et al*. (2012). Taking into account the above experimental results, the value for DV0 was set at 12^{o}C. DV1 and Dv2 were set as 25 ^{o}C and 30 ^{o}C respectively.
- FAW does not diapause and cannot survive the winters in temperate areas (Luginbill 1928;

Johnson 1987). Diapause was therefore not included in this model.

- The cold stress temperature threshold (TTCS) and the accumulation rate of this threshold (cold stress temperature rate, THCS) were used to project current suitability for FAW. A temperature threshold model of Cold Stress was used, with a 12°C threshold and a stress accumulation rate of -0.001 week^{-1}. CLIMEX can calculate the cold stress and exclude the survival of an insect when exposed to low temperatures (Sutherst *et al.* 2007). Although FAW does not tolerate prolonged and extreme cold periods, periods of mild cold and rainfall promote the abundance of the insect (Luginbill 1928; Westbrook *et al.* 2016).
- The heat stress temperature threshold (TTHS) and its accumulation rate (heat stress accumulation rate, THHS), were used to limit the survival of FAW at high temperatures. When the average maximum temperature is below TTHS, the heat stress is equal to zero (Sutherst *et al* 2007). The threshold of 39 °C is the same as the upper temperature limit for development.
- CLIMEX accumulates dry stress when soil moisture is lower than the dry stress threshold (SMDS). This stress is accumulated weekly and is multiplied the dry stress rate (HDS) (Sutherst *et al* 2007). There is evidence that during a dry season, few adult moths are trapped and the population peaks are delayed (Andrews 1988). The dry stress was set at SMDS= 0.1 to avoid the persistence of FAW. Dry Stress was given by using the lower soil moisture growth threshold and adjusting the rate to limit the distribution to tropical and subtropical regions where it has been reported.
- Wet stress accumulates when soil moisture exceeds the wet stress threshold (SMWS). Annual wet stress depends on the sum of weeks per year during which this stress occurs. Wet stress accumulates at a wet stress rate (Sutherst *et al.* 2007). A higher number of FAW moths have been recorded when rainfall is plentiful. While heavy rains reduce the population density of larvae in the early instars, this does not affect late instars or the adult stage (Luginbill 1928; Andrews 1988). The SMWS was set high at 1.5, with an accumulation rate of 0.002/week^{-1}, to allow persistence throughout the known distribution.
- This parameter describes the necessary number of growing degree days to complete a generation. It acts as a constraint, limiting the organism according to the level of suitability of a specific location. If the minimum PDD is not met, the EI equal to 0 (Sutherst *et al*. 2007). Studies have reported that the life cycle takes between 38 and 62 calendar days to complete, depending on temperature and humidity (Vickery 1929; Sparks 1979). However, this is not an exact measurement in that it varies according to climatic zones. Growing degree days allow a more accurate measurement of the growth and development of insects during the growing season. Valdez-Torres *et al*. (2012) calculated the PDD for FAW at 504, with a temperature base (T_b) of 8.7^{O}C, while another experiment used PDD = 559.1, with T_b = 10.9^{o}C (Ramirez Garcia *et al*. 1987). In the current study, PDD was set at 600, as per Ramirez Cabral *et al*. 2017, because T_b is closer to DV0 i.e., 12^{o}C. This value is the time from egg to adult.

The resulting maps show areas that are likely to be at different level of risk of FAW as determined from the climatic suitability range (Fig 1). The current climate conditions modelled for FAW showed suitability for FAW occurrence in India.

- Tropical regions were found to be more suitable for its establishment which is similar to the observations of Luginbill 1928. High risk suitability areas with EI=>20 for FAW occurrence in India are projected in Fig 2.
- Currently, infestation of FAW is reported in twelve states in India *viz*., Andhra Pradesh, Bihar, Chhattisgarh, Goa, Gujarat, Karnataka, Madhya Pradesh, Maharashtra, Odisha, Tamil Nadu, Telangana and West Bengal (CABI, 2020). The current analysis with Compare locations (Species 1) application has also indicated high climatic suitability in the above states with 20-95 EI values validating the present study (Fig 2).
- Climate is not the only factor limiting species geographical distribution (Brown *et al.*, 1996). Host availability is also important in actual determination of potential geographic areas. Though, FAW is polyphagous attacking about 80 plant species, it is reported mainly on maize in India. Therefore, 12 major Maize growing areas *viz*., Andhra Pradesh, Bihar, Gujarat, Jharkhand, Karnataka, Madhya Pradesh, Maharashtra, Rajasthan, Tamil Nadu, Telangana, Uttar Pradesh

and West Bengal are considered while predicting the climatically suitable potential areas showing EI values between 20 - 95 for FAW establishment and the potential areas were given up to Taluk level (Table 2).

- High risk suitability areas are mostly noticed in South India and North east India. High risk potential areas were noticed in eight major maize growing states *viz*., Andhra Pradesh, Bihar, Jharkhand, Karnataka, Madhya Pradesh, Maharashtra, Tamil Nadu and Telangana while risk potential was medium to low in states of Rajasthan, Gujarat and Uttar Pradesh.
- The probable areas for the establishment of FAW based on climatic suitability and presence of major host, *i.e.*, Maize are projected in the Indian map (Fig 3), while the State maps are projected in Fig 5.

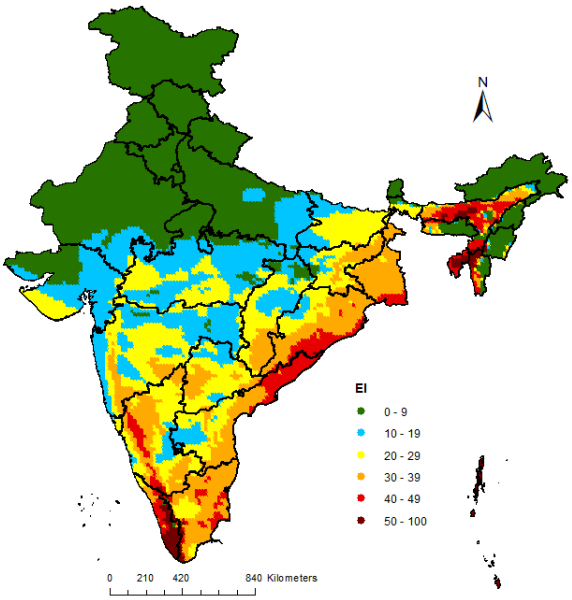

Fig 1: Different Risk probability areas in India for the Establishment of Fall army worm

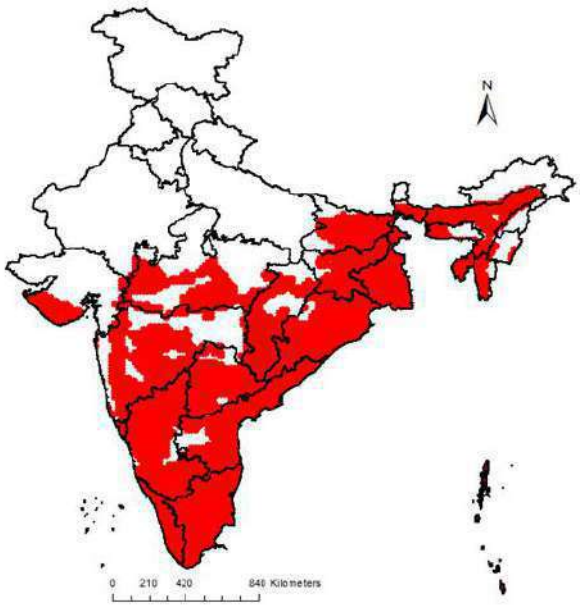

Fig 2: High Risk probability areas in India for the Establishment of Fall army worm

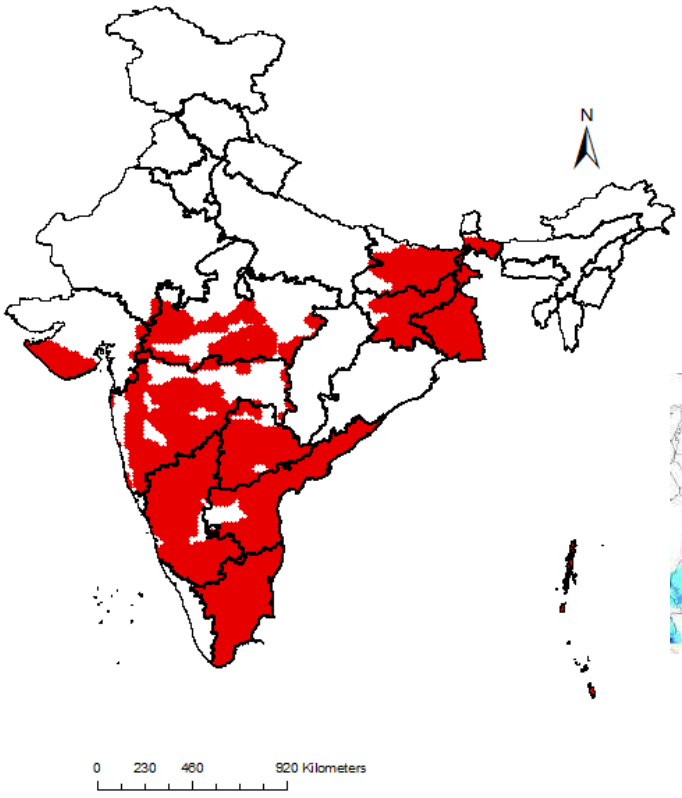

Fig 3: High Risk probability areas for the Establishment of Fall army worm in major Maize growing states in India

The CSIRO 2030, CSIRO 2050, MIROC 2030 and MIROC 2050 scenarios implemented under A2 emission scenario follow a similar pattern with different suitability with marked reduction by 2050 in the potential area for establishment of FAW (Fig 4a, 4b, 4c & 4d). The potential area will decrease its suitability significantly under MIROC 2050 in southern India eliminating few states *viz*., Madhya Pradesh, Maharashtra, Telangana and Andhra Pradesh compared with the current scenario for FAW risk.

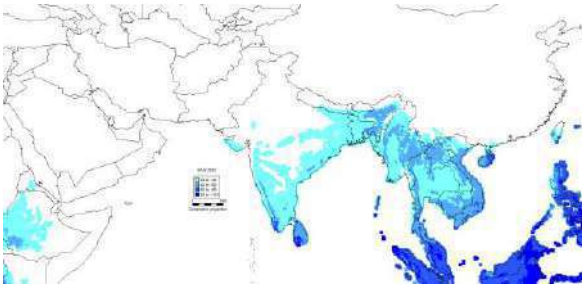

Fig 4a: CSIRO 2030

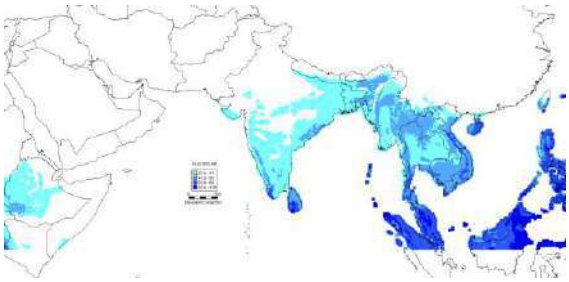

Fig 4b: MIROC 2030

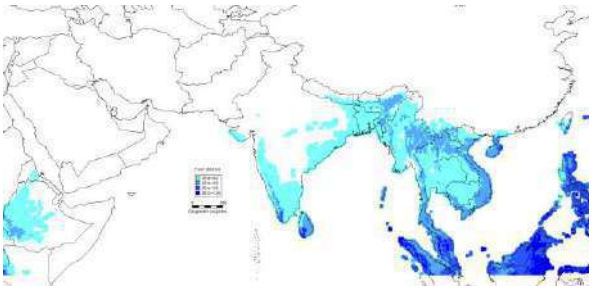

Fig 4c: CSIRO 2050

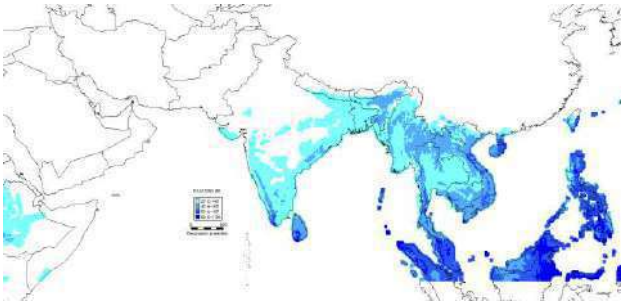

Fig 4d: MIROC 2050

III. DISCUSSION

The current FAW climate conditions projected for the model closely matches previous distributions, where FAW is listed as the major maize pest. In the present study, the reliability, accuracy and robustness of Climex model are reflected in high proportions from the validation area falling within the modelled area. The currently generated potential areas for fall army worm also include the recently infested areas in India thus validating the current prediction model. The maps generated based on Compare species 1 option appears to be more precise for the FAW species establishment. Hence, this option is recommended for better analysis subjected to the availability of the data on growth and stress parameters of the species. The data

on the potential districts and their respective taluks for the establishment of pests is also generated using Arc GIS.

High suitability was shown in tropical wet and dry regions in India *i.e.,* South Indian states as per Koppen classification (Köppen, W *etal*., 1884). The marginal suitability projected by the model in few humid subtropical regions as in the case of Bihar, Jharkhand and Madhya Pradesh could be due to the fact that FAW occurs seasonally by migration from warm climates to subtropical regions. This migration has been estimated at a rate of movement of 40km/generation in years with favourable climate conditions (Westbrooks & Sparks 1986) that allow FAW to migrate.

The modelling carried out under the A2 scenario, predicts an increase in temperature for 2030 and 2050. These small increases in temperature are likely to change the current suitability of the climate for FAW in India leading to the reduction of potential area especially in Southern India. In tropical areas, insects are close to their thermal tolerance limits and so, small increase in temperature may reduce or prevent their survival. Conversely, the increase in temperature in cold places may enhance the insect fitness and survival indicating that the impact in tropical regions may be more drastic than in temperate regions and that the benefit of warmer temperatures will depend on the temperature sensitivity of the species (Helmuth *et al*. 2002, Deutsch *et al*. 2008, Tewksbury *et al*. 2008). Further, polyphagous pests adapt better to climate change due to their phenotypic and genotypic plasticity and may even feed on the hosts of lower quality when preferential hosts are not available (Sparks, 1979, Randall 1986, Bale *et al* 2002). The above results are consistent with the studies of Svobodova *et al*. 2014 and Ramirez cabral *et al*. 2017 explaining the reduction in suitable areas due to increased temperatures and reduced moisture levels.

IV. CONCLUSION AND WAY FORWARD

The FAW is polyphagous pest with many economically important hosts majorly attacking maize in India. Predicting the climatically suitable areas in India is essential to protect the future production of both staple and non-staple crops, since FAW attacks both. With this information, farmer, policy makers and government could implement adaption measures, such as the new technology, new varieties and management practices to overcome the impacts of FAW on important economic crops.

Pest management in agriculture may be more challenging under future climate and variability. The risk maps generated has application for mainly three types of personnel: pest survey specialists, program managers, and risk analysts for implementing surveys, for allocation of resources and to assess the potential establishment for high-risk pathways, commodities, or pests, respectively. Risk maps may also be useful in delimiting surveys or for managing pest eradications.

The results can be used to help guide pest risk assessments by the National Plant Protection Organization (NPPO), effective monitoring and surveillance of the unintentional introductions of this pest *via* trade from currently infested countries, and, policy makers and trade negotiators in making science-based decisions. In addition, India which is dealing with a relatively recent introduction and spread of fall army worm, the generated maps can be used to identify areas most at risk of the expansion of this pest. Efforts can be coordinated and concentrated strategically across susceptible areas to stem the incursion. An additional potential application of these maps is for Regional Central Integrated Pest Management Centres (CIPMCs) in the identification of areas most suitable for area-wide pest suppression or eradication. Areas with established populations of pests which are on the extreme margins of climate suitability can be targeted as the most likely locations for these suppression and eradication efforts.

The current modelling approach was carried out using only the climate data, which ignore potential genetic changes in species and adaption to new climatic conditions (Bradshaw & Holzapfel 2006). Future studies at global and regional levels are essential for better understanding of the FAW risk.

Table 2: Major Maize growing states with high risk probability areas for the establishment of Fall Armyworm with EI=>20.

Major Maize Growing States	**Potential Districts**	**Potential Taluks**
Andhra Pradesh	Anantapur	Gudibanda, Hindupur, Kadiri, Penukonda, Madakasira
	Chittoor	Satyavedu, Puttur, Chandragiri, Chittoor, Srikalahasti, Palamaner, Punganur, Kuppam, Vayalpad, Madanapalle
	Cuddapah	Rajampet, Rayachoti, Sidhout, Badvel, Proddatur, Cuddapah
	East Godavari	Prathipadu, Ellavaram, Razole, Amalapuram, Mummidivaram, Kakinada (Urban), Peddapuram, Rampachodavaram, Kothapeta, Kakinada (Urban), Peddapuram, Tuni, Ramachandrapuram1, Rajahmundry (Urban).
	Guntur	Repalle, Tenali, Bapatla, Guntur, Narasaraopet , Sattenapalle, Vinukonda, Gurazala
	Krishna	Machilipatnam, Avanigadda, Kaikalur, Gudivada, Nuzvid, Tiruvuru, Vijayawada (Urban), Gannavaram, Nandigama, Jaggayyapeta
	Kurnool	Nandyal, Atmakur2, Allagadda, Emmiganuru, Pattikonda, Alur1, Nandikotkur, Dhone, Kurnool
	Nellore	Sullurpeta, Venkatagiri, Gudur1, Nellore, Kavali, Rapur, Atmakur3, Kovur, Rapur, Udayagiri
	Prakasam	Markapur, Giddalur, Addanki, Kandukur, Darsi, Chirala, Ongole, Kanigiri, Podili
	Srikakulam	Palakonda, Pathapatnam, Srikakulam, Sompeta, Ichchapuram, Tekkali, Narasannapeta
	Visakhapatnam	Chodavaram, Ananthagiri, Chintapalle, Narsipatnam, Anakapalle, Visakhapatnam (Urban), Paderu, Bheemunipatnam, Yelamanchili.
	Vizianagaram	Srungavarapukota, Salur, Parvathipuram, Vizianagaram, Chipurupalle, Pusapatirega
	West Godavari	Narsapuram, Polavaram, Bhimavaram, Tanuku, Tadepalligudem, Kovvur, Tadepalligudem, Eluru.
Telangana	Adilabad	Asifabad, Utnoor, Luxettipet, Boath, Nirmal, Khanapur, Adilabad, Mudhole.
	Hyderabad	Hyderabad
	Karimnagar	Sircilla, Huzurabad, Karimnagar, Sircilla, Karimnagar, Manthani, Medipalle, Jagtial, Sultanabad
	Khammam	Burgampahad, Kothagudem1, Madhira, Yellandu, Nugur, Khammam (Urban).
	Mahbubnagar	Achampet, Farooqnagar, Kodangal, Kollapur, Mahbubnagar, Kalwakurthy, Nagarkurnool, Makthal, Wanaparthy, Gadwal, Atmakur1, Alampur.
	Medak	Zahirabad, Narayankhed, Sangareddy, Gajwel, Andole, Siddipet, Narsapur, Medak

	Nalgonda	Bhongir, Devarakonda, Ramannapeta, Nalgonda, Suryapet, Huzurnagar
	Nizamabad	Kamareddy, Yellareddy, Madnoor, Armur, Banswada, Nizamabad, Armur, Bodhan
	Rangareddi	Pargi, Tandur, Vicarabad, Chevella, Rajendranagar, Medchal, Ibrahimpatnam, Hayathnagar
	Warangal	Narsampet, Jangaon, Hanamkonda, Mulug1, Mahabubabad, Parkal
Karnataka	Bagalkot	Badami, Bilgi, Jamkhandi, Hungund, Bagalkot, Mudhol
	Bangalore	Anekal, Bangalore South, Bangalore North
	Bangalore Rural	Ramanagaram, Kanakapura, Magadi, Nelamangala, Devanahalli, Dod Ballapur, Channapatna, Hosakote.
	Belgaum	Sampgaon, Hukeri, Belgaum, Parasgad, Gokak, Chikodi, Ramdurg, Khanapur , Raybag, Athni.
	Bellary	Hadagalli, Sandur, Hospet, Kudligi, Hagaribommanahalli, Siruguppa
	Bidar	Bidar, Homnabad, Bhalki, Aurad, Basavakalyan, Aurad
	Bijapur	Bijapur, Basavana Bagevadi, Muddebihal, Sindgi, Indi.
	Chamarajanagar	Kushtagi, Yelbarga, Koppal, Gangawati.
	Chamaraja-nagar	Gundlupet, Kollegal, Yelandur, Chamrajnagar
	Chikmagalur	Narasimharajapura, Tarikere, Chikmagalur, Kadur, Koppa, Mudigere, Sringeri
	Chitradurga	Holalkere, Chitradurga, Hosadurga
	Dakshina Kannada	Sulya, Puttur, Beltangadi, Bantval, Mangalore.
	Davanagere	Honnali, Channagiri, Harpanahalli, Harihar, Davanagere, Jagalur.
	Dharwad	Kalghatgi, Hubli, Dharwad, Navalgund
	Gadag	Shirhatti, Mundargi, Gadag, Ron, Nargund.
	Gulbarga	Sedam, Chincholi, Shorapur, Gulbarga, Yadgir, Aland, Jevargi, Chitapur, Shahpur, Afzalpur.
	Hassan	Arkalgud, Hassan, Belur, Alur, Belur, Hole Narsipur, Manjarabad, Channarayapatna, Arsikere.
	Haveri	Hangal, Shiggaon, Hirekerur, Haveri, Savanur, Ranibennur, Hirekerur
	Kodagu	Virajpet, Somvarpet, Madikeri
	Kolar	Chik Ballapur, Gauribidanur, Sidlaghatta, Kolar, Chintamani, Bagepalli, Malur, Bangarapet, Mulbagal, Gauribidanur
	Mandya	Maddur, Malavalli, Shrirangapattana, Krishnarajpet, Maddur, Pandavapura, Nagamangala
	Mysore	Heggadadevankote, Piriyapatna, Hunsur, Nanjangud, Krishnarajanagara, Tirumakudal Narsipur, Mysore
	Raichur	Lingsugur, Devadurga, Raichur, Manvi, Sindhnur

	Shimoga	Shimoga, Sorab, Bhadravati, Shikarpur, Sagar, Tirthahalli, Hosanagara
	Tumkur	Kunigal, Tumkur, Gubbi, Koratagere, Turuvekere, Tiptur, Chiknayakanhalli, Madhugiri, Pavagada.
	Udupi	Karkal, Udupi
	Uttara Kannada	Haliyal, Mundgod, Yellapur, Supa, Sirsi, Siddapur, Ankola, Karwar, Kumta
Tamil Nadu	Ariyalur	Udayarpalayam, Ariyalur
	Chennai	Chennai
	Coimbatore	Coimbatore, Pollachi, Udumalaipettai, Mettupalayam, Avanashi, Palladam, Udumalaipettai.
	Cuddalore	Kattumannarkoil, Virudhachalam, Cuddalore, Chidambaram
	Dharmapuri	Harur, Denkanikottai, Pennagaram, Palakkodu, Uthangarai, Krishnagiri
	Dindigul	Kodaikanal, Palani, Dindigul, Natham, Nilakkottai, Vedasandur
	Erode	Sathyamangalam, Bhavani, Perundurai, Dharapuram, Erode.
	Kancheepuram	Cheyyur, Sriperumbudur, Uthiramerur, Chengalpattu, Kancheepuram, Tambaram
	Kanniyakumari	Vilavancode, Thovala, Kalkulam
	Karur	Kulithalai, Krishnarayapuram, Karur
	Madurai	Usilampatti, Melur, Madurai North, Vadipatti, Madurai South, Thirumangalam
	Nagapattinam	Vedaranyam, Mayiladuthurai, Tharangambadi, Sirkali
	Namakkal	Namakkal, Rasipuram, Tiruchengode
	Perambalur	Perambalur, Thuraiyur
	Pudukkottai	Aranthangi, Pudukkottai, Alangudi, Thirumayam, Kulathur
	Ramanathapuram	Tiruvadanai, Ramanathapuram, Paramakudi, Mudukulathur, Kamuthi
	Salem	Salem, Attur, Omalur, Mettur, Sankari
	Sivaganga	Tirupathur, Karaikkudi, Sivaganga, Devakottai, Ilayangudi.
	Thanjavur	Pattukkottai, Peravurani, Orathanadu, Papanasam, Kumbakonam, Thanjavur
	The Nilgiris	Udhagamandalam, Gudalur, Kotagiri
	Theni	Uthamapalayam, Periyakulam
	Thiruvallur	Tiruttani, Uthukkottai, Ambattur, Pallipattu, Ponneri, Gummidipoondi
	Thiruvarur	Mannargudi, Nannilam, Thiruvarur
	Thoothukkudi	Kovilpatti, Sathankulam, Vilathikulam, Ottapidaram, Tiruchendur, Srivaikuntam, Thoothukkudi
	Tiruchirappalli	Manapparai, Lalgudi, Tiruchirappalli
	Tirunelveli	Tenkasi, Shenkottai, Ambasamudram, Sivagiri, Sankarankoil, Radhapuram, Nanguneri, Tirunelveli, Palayamkottai

	Tiruvannamalai	Polur, Vandavasi, Arani, Cheyyar, Chengam, Tiruvannamalai, Chengam
	Vellore	Tirupathur, Vaniyambadi, Vellore, Arcot, Arakonam, Walajapet
	Viluppuram	Kallakkurichi, Tirukkoyilur, Viluppuram, Tindivanam, Gingee
	Virudhunagar	Rajapalayam, Srivilliputhur, Sattur, Tiruchuli, Virudhunagar, Aruppukkottai.
Maharashtra	Ahmadnagar	Mahal, Sangamner, Pathardi, Parner, Shevgaon, Karjat, Nevasa, Shrigonda
	Amravati	Chikhaldara, Melghat, Morshi, Achalpur, Warud.
	Aurangabad	Khuldabad, Kannad, Sillod, Aurangabad, Aurangabad, Gangapur, Vaijapur, Soegaon, Paithan.
	Bid	Kaij, Ambejogai, Patoda, Bid, Ashti1, Georai, Manjlegaon.
	Buldana	Buldana, Chikhli, Deolgaon Raja, Mehkar, Lonar, Motala, Khamgaon.
	Chandrapur	Rajura
	Dhule	Sakri, Shirpur
	Gadchiroli	Etapalli, Dhanora, Aheri, Kurkheda.
	Gondiya	Deori
	Hingoli	Hingoli, Kalamnuri, Basmath
	Jalgaon	Jamner, Jalgaon
	Jalna	Bhokardan, Jafferabad, Jalna, Ambad.
	Kolhapur	Gadhinglaj, Kagal, Karvir, Hatkanangle, Shirol, Ajra, Panhala, Radhanagari, Shahuwadi, Chandgad, Bhudargad, Bavda.
	Latur	Nilanga, Udgir, Ausa, Nilanga, Latur, Ahmadpur.
	Nagpur	Parseoni, Katol, Narkhed, Savner, Ramtek, Hingna.
	Nanded	Mukhed, Kandhar, Bhokar, Kinwat, Deglur, Biloli, Hadgaon.
	Nandurbar	Akhrani, Akkalkuwa, Nandurbar
	Nashik	Nashik, Sinnar, Dindori, Niphad, Nandgaon, Kalwan, Chandvad, Baglan, Igatpuri, Niphad, Peint, Yevla, Surgana, Malegaon.
	Osmanabad	Umarga, Osmanabad, Kalamb, Tuljapur, Bhum, Paranda
	Parbhani	Manwath, Jintur, Pathri.
	Pune	Bhor, Haveli, Mulshi, Mawal, Purandhar, Khed, Ambegaon, Junnar, Velhe, Shirur, Mawal
	Raigarh	Mahad
	Ratnagiri	Sangameshwar
	Sangli	Walwa, Shirala, Jat, Tasgaon, Miraj, Kavathemahankal, Khanapur, Atpadi
	Satara	Karad, Satara, Wai, Khandala, Koregaon, Phaltan, Man, Patan, Khatav, Jaoli, Mahabaleshwar
	Sindhudurg	Sawantwadi, Devgad, Vengurla, Malwan
	Solapur	Sangole, Barshi, Akkalkot, Solapur South, Mangalvedhe, Mohol, Madha, Karmala, Mangalvedhe, Pandharpur
	Thane	Palghar

	Wardha	Karanja
	Washim	Risod, Malegaon, Mangrulpir, Manora
	Yavatmal	Pusad, Umarkhed, Mahagaon
Madhya Pradesh	Balaghat	Baihar, Waraseoni, Lanji, Balaghat
	Barwani	Sendhwa, Barwani, Pansemal
	Betul	Betul, Bhainsdehi, Multai
	Bhopal	Huzur, Berasia
	Chhindwara	Amarwara, Chhindwara, Parasia, Sausar.
	Damoh	Damoh
	Dewas	Bagli, Dewas, Sonkatch
	Dhar	Badnawar, Dhar, Kukshi, Manawar, Sardarpur
	Dindori	Dindori
	East Nimar	Burhanpur, Khandwa.
	Harda	Harda
	Hoshangabad	Sohagpur, Pipariya, Itarsi.
	Indore	Depalpur, Mhow, Indore, Sawer
	Jhabua	Jobat, Jhabua, Petlawad, Alirajpur, Petlawad, Thandla
	Mandsaur	Mandsaur
	Narsimhapur	Narsimhapur, Gadarwara
	Raisen	Goharganj, Baraily, Udaipura, Raisen, Gairatganj, Silwani, Begamganj
	Rajgarh	Sarangpur,Narsinghgarh,Rajgarh,Khilchipur,Biaora
	Ratlam	Ratlam, Sailana, Jaora, A lot.
	Rewa	Gurh,Mauganj,Sirmour,Teonthar
	Sagar	Rehli, Sagar, Banda1.
	Sehore	Nasrullaganj,Budni,Ashta,Ichhawar, Sehore
	Satna	Maihar,Amarpatan,Nagod, Raghurajnagar
	Seoni	Seoni, Lakhnadon.
	Shahdol	Pushparajgarh,Anuppur,Sohagpur1,Jaisinghnagar,Beohari
	Shajapur	Shajapur,Shujalpur,Agar,Susner
	Sheopur	Sheopur,Vijaypur
	Shivpuri	Kolaras,Khaniyadhana,Pohari,Shivpuri,Karera
	Sidhi	Gopadbanas,Singrauli,Deosar
	Tikamgarh	Tikamgarh,Jatara, Niwari
	Ujjain	Badnagar, Khacharod, Ujjain, Tarana, Mahidpur.
	Umaria	Bandhogarh
	Vidisha	Vidisha,Basoda,Lateri,Kurwai,Sironj
	West Nimar	Bhagwanpura,Jhiranya,Segaon,Khargone,Bhikangaon,Kasrawad,Barwaha,Ma

		heshwar
Bihar	Araria	Araria
	Banka	Banka
	Begusarai	Begusarai
	Bhagalpur	Bhagalpur, Naugachhia
	Bhojpur	Arrah
	Buxar	Buxar
	Darbhanga	Benipur, Darbhanga.
	Gaya	Gaya
	Gopalganj	Gopalganj
	Jamui	Jamui
	Jehanabad	Jehanabad
	Katihar	Katihar
	Kaimur (Bhabua)	Bhabua
	Khagaria	Khagaria
	Katihar	Katihar
	Khagaria	Khagaria
	Kishanganj	Kishanganj
	Lakhisarai	Lakhisarai
	Madhepura	Madhepura, Udakishanganj
	Madhubani	Jhanjharpur,Madhubani,Benipatti
	Munger	Munger
	Muzaffarpur	Purba muzaffarpur
	Nalanda	Bihar, Hilsa.
	Nawada	Nawada
	Pashchim Champaran	Bettiah
	Patna	Dinapur-Cum-Khagaul,Masaurhi,Patna city,Barh,Patna Rural
	Purba Champaran	Motihari,Dhaka
	Purnia	Purnia East
	Rohtas	Sasaram
	Saharsa	Sonbarsa
	Samastipur	Rosera, Samastipur, Dalsinghsarai.
	Saran	Chapra
	Sheikhpura	Sheikhpura
	Sheohar	Sheohar
	Sitamarhi	Purba sitamarhi
	Siwan	Siwan
	Supaul	Supaul, Saraigarh Bhaptiyahi.

	Vaishali	Hajipur
Gujarat	Amreli	Jafrabad, Dhari, Rajula, Savar Kundla, Kunkavav Vadia, Amreli, Lilia, Babra.
	Bhavnagar	Mahuva, Talaja, Palitana.
	Dohad	Dohad, Limkheda, Jhalod.
	Jamnagar	Kalyanpur, Bhanvad, Jamjodhpur, Khambhalia, Lalpur, Kalavad.
	Junagadh	Patan-Veraval, Kodinar, Una, Malia, Talala, Manavadar, Mangrol, Keshod, Mendarda, Visavadar, Vanthali, Junagadh, Bhesan.
	Narmada	Dediapada
	Porbandar	Kutiyana, Ranavav, Porbandar.
	Rajkot	Upleta, Dhoraji, Jetpur, Jamkandorna, Gondal.
	The Dangs	The Dangs
	Vadodara	Chhota Udaipur
West Bengal	Bankura	Vishnupur, Bankura
	Barddhaman	Burdwan – I, Kalna – I, Katoya.
	Birbhum	Bolpur Sriniketan, Rampurhat, Suri – I.
	Dakshin Dinajpur	Balurghat
	Darjiling	Darjeeling Pulbazar
	Haora	Shyampur-I, Sankrail, Uluberia-I
	Hugli	Arambag, Serampur Uttarpara, Hugli.
	Jalpaiguri	Jalpaiguri, Alipurduar
	Koch Bihar	Dinhata – I, Tufanganj-I, Mathabhanga – II, Cooch Behar – I, Mekliganj,
	Maldah	Maldah (Old)
	Medinipur	Contai – I, Tamluk, Midnapore, Jhargram, Ghatal.
	Murshidabad	Murshidabad Jiaganj, Berhampore, Jalangi, Kandi1.
	Nadia	Kalyani, Ranaghat, Krishnagar – II.
	North Twenty Four Paraganas	Basirhat – I, Bongaon
	Puruliya	Puruliya-1
	South Twenty Four Paraganas	Diamond Harbour – I, Bhangar-I.
	Uttar Dinajpur	Raiganj, Islampur.
Jharkhand	Bokaro	Bermo
	Chatra	Chatra
	Deoghar	Deoghar
	Dhanbad	Baghmara-Cum-Katras, Dhanbad-Cum-Kenduadih-Jagta.
	Dumka	Dumka, Jamtara.
	Giridih	Giridih

	Godda	Godda
	Gumla	Simdega, Gumla.
	Hazaribagh	Hazaribag
	Kodarma	Kodarma
	Pakaur	Pakaur
	Palamu	Latehar, Daltonganj.
	Pashchimi Singhbhum	Chaibasa, Kharsawan, Chakradharpur.
	Purbi Singhbhum	Dhalbhumgarh
	Ranchi	Khunti, Ranchi.
	Sahibganj	Rajmahal
Uttar Pradesh	Agra	Kheragarh,Bah,Kiraoli,Agra,Etmadpur
	Aligarh	Khair,Atrauli
	Allahabad	Meja,Karchhana,Allahabad,Phulpur1,Handia, Soraon
	Ambedkar Nagar	Akbarpur1,Tanda
	Auraiya	Auraiya,Bidhuna
	Azamgarh	Lalganj,Azamgarh,Phulpur, Sagri
	Baghpat	Baghpat
	Bahraich	Kaiserganj,Nanpara
	Ballia	Ballia,Rasra,Bansdih
	Balrampur	Utraula,Balrampur
	Banda	Naraini,Banda,Baberu
	Barabanki	Haidergarh,Nawabganj,Ramsanehighat
	Bareilly	Aonla,Bareilly,Faridpur,Baheri
	Basti	Basti,Harraiya
	Bijnor	Bijnor,Dhampur,Nagina,Najibabad
	Budaun	Dataganj,Budaun,Gunnaur,Sahaswan,Bisauli
	Bulandshahar	Khurja,Bulandshahr,Anupshahr,Sikandrabad
	Chandauli	Chakia,Chandauli,Sakaldiha
	Chitrakoot	Karwi,Mau
	Deoria	Salempur,Deoria
	Etah	Jalesar,Etah,Aliganj,Kasganj
	Etawah	Bharthana,Etawah
	Faizabad	Bikapur,Faizabad
	Farrukhabad	Kaimganj
	Fatehpur	Khaga,Fatehpur,Bindki
	Firozabad	Shikohabad,Firozabad,Jasrana
	Gautam Buddha Nagar	Dadri

	Ghaziabad	Hapur,Ghaziabad,Garhmukteshwar
	Ghazipur	Saidpur,Ghazipur,Mohammadabad
	Gonda	Tarabganj,Gonda
	Gorakhpur	Bansgaon,Gorakhpur
	Hamirpur	Maudaha,Rath,Hamirpur
	Hardoi	Sandila,Bilgram,Hardoi,Shahabad
	Hathras	Sadabad,Hathras,Sikandra Rao,Sasni **
	Jalaun	Konch,Orai,Kalpi,Jalaun
	Jaunpur	Machhlishahr,Mariahu,Kerakat,Jaunpur,Shahganj
	Jhansi	Jhansi,Mauranipur,Garautha,Moth
	Jyotiba Phule Nagar	Hasanpur,Amroha
	Kannauj	Chhibramau,Kannauj
	Kanpur Dehat	Bhognipur,Pukhrayan,Derapur,Akbarpur2
	Kanpur Nagar	Kanpur
	Kaushambi	Manjhanpur,Sirathu
	Kheri	Mohammdi,Nighasan,Lakhimpur
	Kushinagar	Hata,Padrauna
	Lalitpur	Mahroni,Lalitpur
	Lucknow	Mohanlalganj,Lucknow,Malihabad
	Mahoba	Kulpahar,Mahoba,Charkhari
	Mahrajganj	Pharenda
	Mainpuri	Karhal,Bhogaon,Mainpuri
	Mathura	Mathura,Chhata,Mat
	Mau	Maunath Bhanjan,Ghosi
	Meerut	Meerut,Mawana,Sardhana
	Mirzapur	Mirzapur,Chunar
	Moradabad	Sambhal,Bilari,Moradabad,Thakurdwara
	Muzaffarnagar	Budhana,Jansath,Kairana,Muzaffarnagar
	Pilibhit	Bisalpur,Puranpur,Pilibhit
	Pratapgarh	Kunda,Patti
	Rae Bareli	Salon,Dalmau,Rae Bareli,Maharajganj
	Rampur	Rampur,Suar
	Saharanpur	Nakur,Deoband,Saharanpur
	Sant Kabir Nagar	Khalilabad
	Sant Ravidas Nagar Bhadohi	Gyanpur
	Shahjahanpur	Farrukhabad,Shahjahanpur,Tilhar,Powayan
	Shrawasti	Bhinga
	Siddharthnagar	Domariyaganj,Bansi,Naugarh

	Sitapur	Misrikh,Sidhauli,Biswan
	Sonbhadra	Dudhi,Robertsganj
	Sultanpur	Amethi,Sultanpur,Kadipur,Gauriganj,Musafirkhana
	Unnao	Purwa,Unnao,Safipur,Hasanganj
	Varanasi	Varanasi
Rajasthan	Ajmer	Beawar,Kekri,Bhinay,Sarwar,Ajmer,Nasirabad,Kishangarh
	Alwar	Thanagazi,Rajgarh1,Lachhmangarh,Alwar,Bansur,Ramgarh,Mandawar,Kishangarh Bas,Behror,Tijara
	Banswara	Kushalgarh,Kalinjara,Banswara,Garhi
	Baran	Chhipabarod,Chhabra,Atru,Baran,Kishanganj,Shahbad
	Barmer	Chohtan,Gudha Malani,Siwana,Sheo,Barmer,Pachpadra
	Bharatpur	Bayana,Weir,Rupbas,Nadbai,Nagar,Deeg,Kaman
	Bhilwara	Sahara,Mandalgarh,Bhilwara,Kotri,Mandal,Jahazpur,Asind,BaneraShahpura, Hurda
	Bikaner	Kolayat,Nokha,Bikaner,Lunkaransar
	Bundi	Bundi,Hindoli,Keshoraipatan,Nainwa
	Chittaurgarh	Arnod,Pratapgarh,BariSadri,Chhoti,Sadri,Nimbahera,Bhadesar,Kapasan, Begun,Rashmi,Gangrar
	Churu	Sujangarh,Dungargarh,Ratangarh,Churu,Sardarshahar,Taranagar
	Dausa	Lalsot,Dausa,Sikrai,Mahwa,Baswa
	Dhaulpur	Baseri,Bari,Dhaulpur
	Dungarpur	Sagwara,Simalwara,Dungarpur,Aspur
	Ganganagar	Gharsana,Anupgarh,Vijainagar,Suratgarh,Raisinghnagar,Padampur, Karanpur,Ganganagar,Sadulshahar
	Hanumangarh	Rawatsar,Nohar,Bhadra,Pilibanga,Tibi,Hanumangarh,Sangaria
	Jaipur	Dudu (Hq. Mauzamabad),Phagi,Chaksu,Sanganer,Bassi, Phulera (Hq.Sambhar),Jaipur,Jamwa Ramgarh,Amber,Chomu,Viratnagar,Kotputli
	Jaisalmer	Jaisalmer,Pokaran
	Jalor	Sanchore,Raniwara,Bhinmal,Jalor,Ahore
	Jhalawar	Gangdhar,Pirawa,Jhalrapatan,Aklera,Pachpahar,Khanpur
	Jhunjhunun	Nawalgarh,Udaipurwati,Khetri,Jhunjhunun,Chirawa
	Jodhpur	Jodhpur,Shergarh,Bilara,Bhopalgarh,Osian,Phalodi
	Karauli	Sapotra,Karauli,Nadoti,Todabhim,Hindaun
	Kota	Ramganj Mandi,Sangod,Ladpura,Digod,Pipalda
	Nagaur	Merta,Degana,Parbatsar,Nagaur,Jayal,Nawa,Didwana,Ladnu
	Pali	Bali,Desuri,Pali,Jaitaran,Sojat
	Rajsamand	Nathdwara,Railmagra,Kumbhalgarh,Rajsamand,Amet, Deogarh,Bhim
	Sawai Madhopur	Sawai Madhopur,Khandar,Bonli,Bamanwas
	Sikar	Sikar,Sri Madhopur,Neem-Ka-Thana
	Sirohi	Abu Road,Reodar,Pindwara,Sirohi,Sheoganj

	Tonk	Uniara,Todaraisingh,Tonk,Malpura,Niwai
	Udaipur	Kherwara,Phalsiya,Sarada,Salumbar,Kotra,Girwa,Vallabhnagar, Gogunda

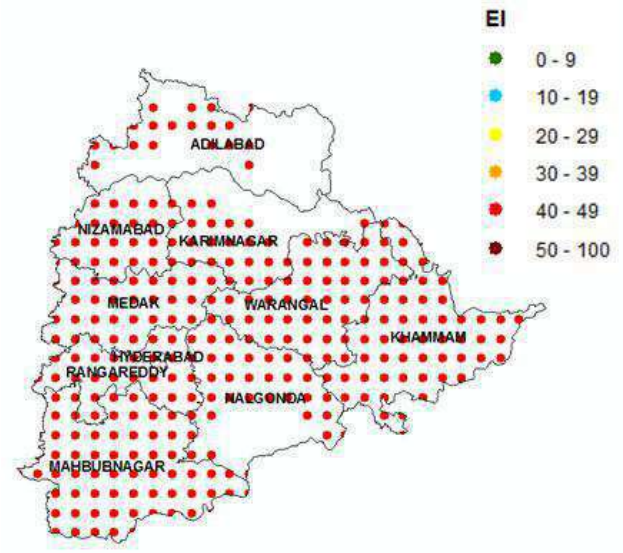

Fig 5a: Telangana

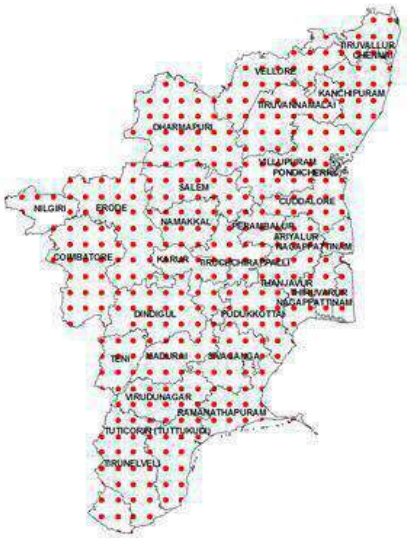

Fig 5c: Tamil Nadu

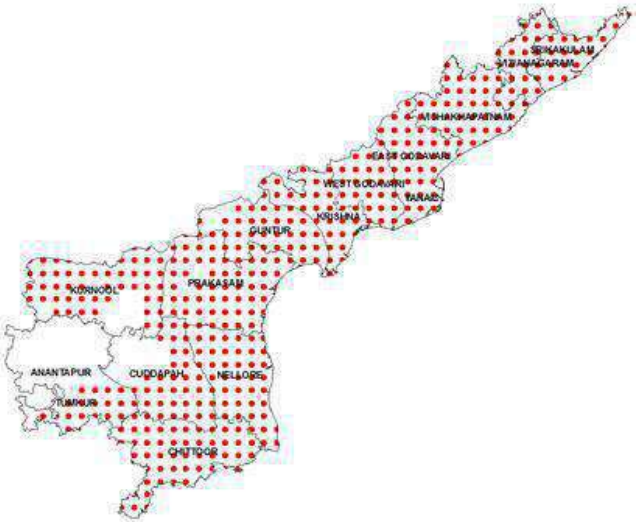

Fig 5b: Andhra Pradesh

Fig 5d: Karnataka

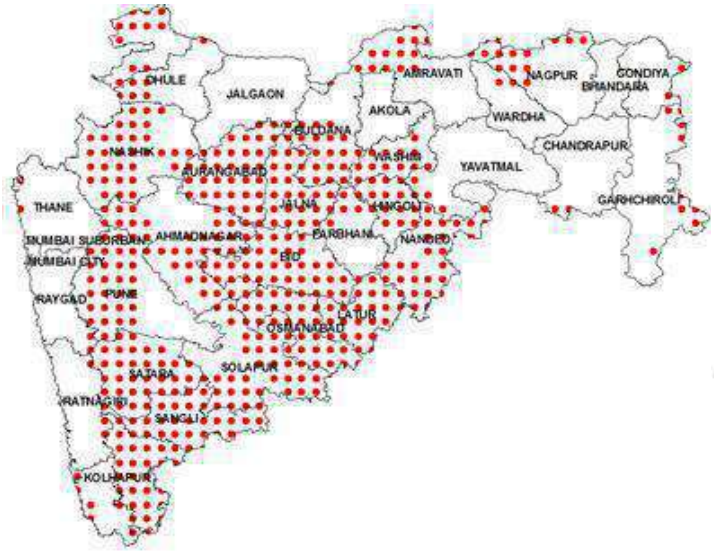

Fig 5e: Maharashtra

Fig 5h: Bihar

Fig 5f: Madhya Pradesh

Fig 5i: Rajasthan

Fig 5g: Uttar Pradesh

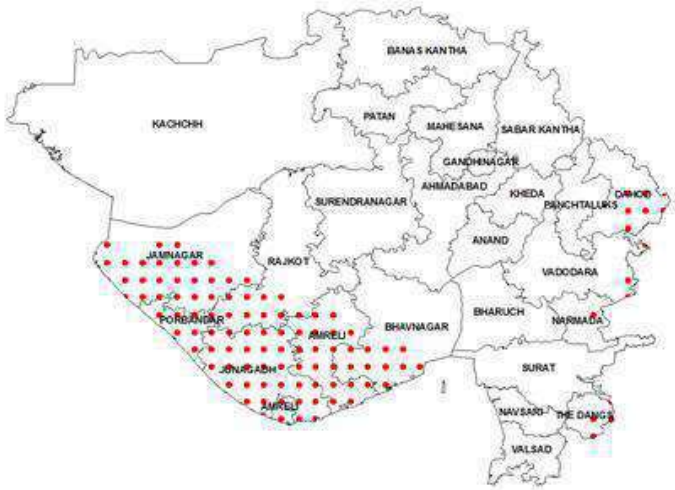

Fig 5j: Gujarat

Fig 5l: Jharkhand

Fig 5k: West Bengal

Fig 5: Risk probability areas in the Major Maize Growing States

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Induction of genetic variation and variability of Saudi Arabian cultivars of wheat

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Abstract *—This study was undertaken to explore the possibility of inducing micromutations in quantitative traits and meiotic anomalies of bread wheat (T. aestivum L.) after irradiated dry and soaked seeds with 0.0, 0.5, 5 and 10 Krad of gamma rays. Traits (number of spikes per plant, number of grains per spike, number of spikelets per spike, spike density, grain yield per plant, weight of grain yield per plant and weight of 1000-grain per plant) were analyzed quantitatively to assess the extent of the variation in M1 and M2 generations. At the same time, the number of economical traits (heading date, plant height, number of tillers per plant, average of spike length, total protein percent and wet and dry gluten percent) were also investigated.*

Results showed that all quantitative traits varied significantly in M1 and M2 at doses rather than seed condition. Specific action of dose 0.5 Krad showed significant increase for some traits for three lines in M1 and M2, and the magnitude and direction for number of spikes per plant, grain yield per plant and weight of grain per plant was significant for all three lines at treatments. There was a considerable increase in genotypic variance, heritability and genetic advance indicating the effectiveness of gamma doses in inducing polygenic mutation. The treatment with different doses caused a highly significant increase in abnormal cells, while pollen fertility percent decreased with increasing gamma ray doses. M1 and M2 irradiated generations showed presence of significant differences at doses rather than seed conditions. A 5 Krad dose showed a significant increase in some traits for dry and soaked seeds for three lines at <1 and M2 generations. There was also a considerable increase in genotypic variance, heritability and genetic advance for some traits. Radiation was shown to change the degree of association between traits.

Keywords **— Genetics, Cultivars, Wheat, Gamma Rays.**

I. INTRODUCTION

Mutation breeding or variation breeding is a new paradigm of exposing plant components as additional source for creation of variability far from the conventional breeding procedures and can also be utilized to rectify one or a few undesirable traits exhibited in commercial varieties of plants (1). The mutation are rare in nature, their frequency can be enhanced through the use of certain mutagens such as ionizing radiation (2). Chromosomal aberrations such as lagging chromosomes, premature bivalence, disjunction, tripolar cells, bridges and micronuclei occur following irradiation (3). Most of the work on mutation and its application in crops deal with induction, identification, isolation and use of changes of phenotype involving major loci (1,4,5). Atak et al (2004) induced plastid mutations in soybean plant (Glycine max L. Merill) through 200 Gy gamma radiation dose and determined the mutations with RAPD. Induced mutations increased food production by at least 70% through unmasking of novel alleles that were harnessed to breed superior crop varieties (6).

On the other hand, the effectiveness of selection of breeding programs depends mainly on the amount of genetic variability among population of plants and crops. Induced genetic variation by irradiation in many characters of a given crop were reported by many investigators(7–11).

This study aimed to investigate on induction of polygenic variability and meiotic anomalies by gamma-rays, and

determine the effects of gamma rays on the genetic variance of vegetative and chemical traits on three Saudi Arabian cultivars of bread wheat *Triticum aestivum L*.

II. MATERIALS AND METHODS

Three local cultivars of bread wheat of Saudi Arabia, L (5-130), L (17-41-90) and L (15-3-83) named L (1), L (2) and L (3) were used as genetic materials. Prior to irradiation, different seed lots from each line were soaked in water overnight and other were kept air dry. Dry and soaked seeds from each cultivar were irradiated with 0.0, 500, 5000 and 10,000 rad of gamma rays. Treated and untreated seeds were sown in pots filled with air-dried loam soil mixed with peat moss w/w 1:1 ratio. The experiment was planned in a completely randomized block design with four replications in a greenhouse in the College of Science in Riyadh,Saudi Arabia on November2018 to May 2019 to obtain M1 and M2 plants. Quantitative and cytological traits were studied.

The measurements for the induced variability were taken on a single plant basis for the following traits: heading date, plant height in cm, number of tillers, and average of spike length per plant cm, total protein percent, and dry and wet gluten percent. The statistical analyses were made for M1 and M2 irradiated generations according to Cohran and Cox (1957). The comparison test between treatments was made according to the least significant differences method (LSD). The broad sense heritability of characters was estimated according to the following formula: h^2 % = σ^2g/ σ^2ph X 100, where σ^2g is the induced genetic variance, σ^2ph is the total phenotypic variance. The genetic advance (GA) was estimated according to the formula GA=K σph x $h^{2,}$ where σph is the phenotypic deviation, K is the constant (2.64 for 1% selection differential).

III. RESULTS AND DISCUSSION

Quantitative traits

The analyses of variance and mean squares for yields and yield components of lines, radiation doses, condition and the interaction between them in M1 and M2 irradiated generations are presented in (Table1). The mean squares of treatments in M1 and M2 generations were highly significant for all traits. On the other hand, differences between radiation doses were highly significant for number of spikes per plant, number of grains per plant and weight of grains yield per plant in M1 and M2 irradiated generations. Test of significance indicated no presence of significant differences between condition for all traits except spike density in M1 generation and number of spikes per plant and number of gains per plant were highly significant in M2 generation. The interaction between lines and doses were significant for all traits in M1 and M2 generations except number of grains per spike, number of spikelets per spike and weight of 1000-grain and spike density in M1 generation. The lines of condition and doses by condition were highly significant for number of spikes per plant and number of grains per plant in M2 generation. The mean squares of the interaction between lines, doses and seed conditions were not significant for all traits in M1 and M2 irradiated generations which indicates that there was no preference between the levels of radiation and the states of the seeds being soaked or dry. It appeared that the significance of treatments is mainly due to the significance of lines and radiation of doses rather than seed condition, since the mean squares of lines were highly significant for all studied traits except the number of spikelets per spike in M1 and M2 generations respectively. This indicates that the effect of either radiation doses or seeds condition varied from one trait to the other.The mean performances of the three cultivars for all doses of radiation for both dry and soaked seeds were calculated in M1 and M2 irradiated generations. (Table 2) The shift of treatment progenies mean from the control mean was not distinguishable in dry and soaked seed for any dose of gamma rays.

Table 1. Yield and yield components in M1 and M2 irradiated generations of three lines (ANOVA and mean squares).

Sources of variance S.V.	df	No. of spikes per plant	No. of grains per spike	No. of spikelets per spike	Spike gdensity	No. of grains per plant	Weight of grain yield per plant	Weight of 1000 grain per plant
Reps	3	0.81 0.88	27.05 296.10	9.40 22.79	0.67 2.34	2051.47 3382.59	5.32 9.89	33.49 53.97
Treats.	23	12.54 ** 13.34 **	2662.77 ** 213.69 **	371.02 ** 13.85 **	7.87 ** 6.98 **	7194.89 ** 24939.84 **	3.49 ** 9.35 **	34.4 * 92.59 **
Line	2	122.20 ** 71.01 **	29922.57 ** 1571.45 **	179.53 134.07 **	72.19 ** 64.28 **	35597.52 ** 106650.59 **	9.13 ** 16.93 **	280.12 ** 888.89 **
Dose	3	4.04 ** 15.95 **	58.24 78.55	2.94 4.67	1.44 2.03	8575.48 ** 45705.71 **	5.05 * 26.84 **	6.94 10.15
Cond.	1	0.07 12.91 **	19.00 2.87	4.08 0.83	5.62 ** 0.04	815.50 28593.61**	0.20 1.57	2.10 19.56
Line x dose	6	4.81 ** 5.72 **	95.53 67.63	5.05 3.13	1.99 * 2.73	10144.20 ** 16875.30 **	5.14 ** 8.01 **	6.13 19.47
Line x cond.	2	0.05 8.54 **	10.82 59.45	2.22 2.54	1.69 2.71	317.11 13728.14 **	0.15 5.56	17.64 21.39
Dose x cond.	3	0.36 10.77 **	96.23 154.70	3.28 1.22	1.11 0.19	142.49 11997.59 **	1.19 4.59	21.56 22.30
Line x dose x cond.	6	0.29 3.40	53.58 90.80	0.78 1.35	1.36 0.58	969.80 4983.91	1.99 4.35	11.60 12.56
Error	69	0.81 1.72	146.95 78.07	117.57 2.03	0.75 1.29	798.55 2701.20	1.24 2.39	17.57 15.24

*** , ** : significant at : 5 % and 1 % level of probability respectively (* : significant. - ** : highly significant.)**

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Table 2. Responses to different doses of radiation and condition on the means yield and yield components in M1 and M2 irradiated generations for three lines of wheat.

Treatment / lines	Doses	M1 M2	No. of spikes / plant			No. of grains / spike			No. of spikelets / spike			Spike density			No. of grains / plant			Weight of grains / plant			Weight of 1000 grains / plant		
			L	M	H	L	M	H	L	M	H	L	M	H	L	M	H	L	M	H	L	M	H
1 Dry	Control	1 2	1 1	1.48 3.85	5 9	30.5 24	85.48 48.85	123 92	14.5 11	20.34 18.75	24 25.5	13.81 16.8	16.45 18.60	19.09 21.70	47 24	119.68 185.93	373 687	1.75 0.17	4.56 3.91	10.71 13.71	23.0 5.2	36.38 20.29	44.4 20.08
	0.5 Krad	1 2	1 1	2.58 4.40	6 9	33 24	84.35 95.48	140 120.3	13.3 12.3	19.53 21.45 **	25 28	14.47 15.40	17.56 18.65	19.85 22.55	50 24	177.95 ** 256.70	429 529	1.65 0.47	5.66 5.18	14.93 15.07	19.10 9.6	32.50 19.73	45.30 32.6
	5 Krad	1 2	1 1	1.53 6.35	3 10	19 33.6	86.88 49.53	158 84.3	10 16.2	20.35 19.68	25 24.6	12.50 16.53	17.38 18.48	2088 21.35	57 50	112.95 309.45 **	349 584	1.48 0.25	4.00 6.38 *	9.88 13.29	24.2 5	35.13 20.13	42.1 27.5
	10 Krad	1 2	1 1	1.78 4.70	6 10	21.3 18	87.58 59.08	142 111.7	11.7 9	20.73 21.10 *	25 27.7	13.92 15	16.94 19.15	23.07 23.72	64 18	140.68 254.00	479 569	1.78 0.25	4.84 4.98	9.10 12.42	18.2 4.6	34.33 19.83	43.2 31.6
1 Soaked	Control	1 2	1 1	1.28 3.80	6 11	44.6 30.5	96.95 49.85	140 107.3	14 12	21.58 18.98	25 28.8	13.44 13.19	17.51 19.08	20.83 22.35	45 41	114.80 192.15	388 522	0.74 0.40	4.06 4.01	11.76 12.32	1.4 8.5	34.38 21.03	40.6 28.9
	0.5 Krad	1 2	1 1	2.25 4.90	4 11	28.5 30.5	76.23 * 56.58	142 112	14.3 13.5	19.30 21.33	24 26.5	13.95 14.29	17.42 19.33	19.07 25.61	57 56	157.48 * 257.45	298 607	2.21 0.71	5.11 6.10	8.24 16.69	24.7 6.7	33.13 22.93	45.7 36.4
	5 Krad	1 2	1 1	1.83 4.90	5 17	13 16	86.48 66.43	135 107.3	12.9 14	20.73 21.83 *	26 27	13.33 11.24	16.74 18.03	20 20.56	13 16	142.45 287.65	271 548	0.05 0.02	4.99 6.43 *	9.03 15.31	3.9 0.7	36.20 21.25	47.5 38.8
	10 Krad	1 2	1 1	1.95 6.25 **	5 11	35 30.3	85.60 51.38	139 120	11 16.3	19.80 20.35	25 26.7	14.67 16.06	15.94 * 19.43	17.78 23.54	35 46	141.60 314.60 **	242 642	1.16 0.20	5.13 5.95	7.28 15.28	26.9 4.10	35.70 18.18	40.7 32.4

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The number of spikes per plant showed positive direction for dry and soaked seeds for line (1) in M1 and M2 irradiated generations. On the other hand, the mean values were of positive and of negative direction in M1 and M2 generation for line (2) and (3) at seed conditions. Dose of 5 Krad caused significant increase in the number of spikes per plant at dry seed in M1 generation for line (2) and in M2 generation at dry and soaked seeds for line (3). The mean values in treated progenies for number of grains per spike were significantly lower in the control than at 0.5 Krad, while dose of 5 Krad caused significant increase for line (1) in M1 and M2 generations. The positive or negative direction of means for lines (2) and (3) were not significant. The means of spike density significantly decreased at soaked seeds in M1 generation with 10Krad for line (1) and with 5 and 10 Krad for line (3). In M2 generation, dose of 0.5 Krad seemed the same effect for line (3) at soaked seeds. The non-significance in the directions for number of grains per spike for lines (2) and (3) were similar to the findings of Jamil and Khan (2002) where they stated that 5, 10, 15 and 25 Krad doses of gamma radiation showed minor fluctuations in their effects. The significant decrease in spike density may be due to scattering of spikelets on the axle of spike. However, studies have suggested that uniformity in low dose radiation response in wheat is essentially at physiological level than at genetic level and the role of growth hormones could be crucial(12). Mutagenic treatment could not provide appreciable amount of genetic variation for number of spikelets per spike and spike density. It appears that these traits in spring wheat varieties could be improved through hybridization with divergent lines.

The mean performance for weight of grain per yield of plant was of positive or negative direction for three lines in M1 and M2 generations. Significant action of 5 Krad caused significant increase for lines (1) and (3) at dry and soaked seed win M2 generation. Also line (2) indicated significant increase with 5 Krad at dry seeds in M1 generation and with 0.5 Krad at soaked seeds in M2 generation. The results showed no significant increase or decrease by radiation dose for weight of 1000-grains for three lines at dry and soaked seeds except for the dose 5 Krad which caused significant increase for line (2) at soaked seeds in M2 generation. The same findings were also found by Singh et al (2010)and Jamil and Khan (2002)(12,13).

The number of spikes per plant was significantly and positively correlated to grain yield per plant and weight of grain yield per plant for three lines in M1 and M2 irradiated generations at dry and soaked seeds. This is consistent with the findings of Hanafy and Mohamed (2014)where they found that irradiation increased the grain yield through subsequent generations of wheat. These results were expected since each of these traits is dependent on each other(14).

Table 3 shows the genetic, phenotypic variance, heritability, genotypic and phenotypic variances. The highest genotypic coefficient of variation was 54.8% and the genetic advance was 8.71. Heritability was 96% for weight of grain yield per plant with 0.5 Krad at soaked seeds. At the same dose, genotypic variance increased by 89.46 and genetic advance was 23.37 for the number of grains per spike. This suggests a high degree of genetic variability at this dose. The weight of grains per plant with 5 Krad at soaked seeds showed the lowest genotypic variance and genetic advance. Heritability estimates were towards the higher side. It may be due to estimation from three lines and single generation. Fairly high estimates of heritability and genetic advance for plant height, number of tillers, and grains per spike suggested that selection for these traits could be practiced more effectively (15).

Table 3. Genotypic and phenotypic variance, heritability, phenotypic and genotypic coefficient of variation and genetic advance at dry and soaked seeds in M2.

Character	Treatment	Seed type	Induced genetic variance (σ^2 g)	Total phenotypic Variance (σ^2ph)	Heritability (h^2)	Genetic coefficient of variation (G.C.V)	Phenotypic coefficient of variation (P.C.V.)	Genetic advance (G.A.)
Number of spikes per plant	Control	Dry	5.33	5.94	0.89	34.72	36.65	5.75
		Soaked	1.11	1.33	0.83	20.51	22.52	2.53
	0.5 Krad	Dry	5.15	5.37	0.96	36.29	37.08	5.85
		Soaked	2.12	2.89	0.73	21.98	25.68	3.29
	5 Krad	Dry	5.78	6.8	0.85	27.64	29.97	5.85
		Soaked	2.26	3.37	0.67	23.10	28.22	3.24
	10 Krad	Dry	0.75	1.01	0.74	15.25	17.76	15.25
		Soaked	0.98	1.25	0.79	16.3	18.39	2.33

Number of grains per spike	Control	Dry	4.16	18.59	0.22	4.52	9.54	2.55
		Soaked	8.11	12.79	0.63	6.22	7.81	5.99
	0.5 Krad	Dry	12.96	57.24	0.22	7.04	14.80	4.54
		Soaked	89.46	103.0	0.87	20.34	21.83	23.37
	5 Krad	Dry	13.08	14.94	0.87	7.92	8.48	8.95
		Soaked	89.80	141.1	0.64	17.93	22.47	19.97
	10 Krad	Dry	76.03	101.2	0.75	18.38	21.20	19.95
		Soaked	76.69	85.9	0.89	19.19	20.30	21.85
Number of spikelets per spike	Control	Dry	0.362	0.879	0.412	3.38	5.26	1.02
		Soaked	2.15	2.21	0.973	8.51	8.59	8.83
	0.5 Krad	Dry	4.95	5.83	0.849	11.88	12.80	5.40
		Soaked	6.18	6.57	0.941	13.55	13.90	6.36
	5 Krad	Dry	1.60	2.12	0.755	6.96	8.03	2.89
		Soaked	8.01	8.53	0.939	15.23	15.72	7.22
	10 Krad	Dry	4.83	5.13	0.94	12.33	12.75	5.64
		Soaked	7.55	8.50	0.989	14.57	13.40	8.76
Spike density	Control	Dry	0.035	0.698	0.05	1.051	4.72	0.110
		Soaked	1.004	1.76	0.57	5.71	7.55	1.99
	0.5 Krad	Dry	2.39	2.50	0.956	8.91	9.16	3.99
		Soaked	4.89	5.14	0.95	12.55	12.89	5.7
	5 Krad	Dry	1.55	1.72	0.90	7.29	7.67	3.11
		Soaked	1.35	1.68	0.80	6.84	7.63	2.75
	10 Krad	Dry	3.48	3.58	0.972	10.82	10.94	4.85
		Soaked	4.04	4.23	0.96	11.43	11.69	5.16
Weight of grains per plant	Control	Dry	5.21	6.41	0.812	39.28	43.58	5.43
		Soaked	1.04	1.35	0.77	23.12	26.30	3.36
	0.5 Krad	Dry	0.18	0.398	0.452	7.48	11.11	0.752
		Soaked	11.40	11.92	0.96	54.8	56.20	8.71
	5 Krad	Dry	1.53	1.98	0.77	16.18	18.49	2.87
		Soaked	0.196	0.358	0.55	6.2	8.39	0.86
	10 Krad	Dry	0.451	0.593	0.761	13.62	15.61	1.54
		Soaked	0.356	1.143	0.311	11.21	20.10	0.872
Weight of 1000 grains per plant	Control	Dry	28.02	35.73	0.78	26.70	30.13	12.35
		Soaked	22.69	24.76	0.912	25.04	26.20	11.93
	0.5 Krad	Dry	9.61	15.54	0.62	16.34	20.78	6.43
		Soaked	48.02	51.67	0.93	32.75	36.90	15.16
	5 Krad	Dry	5.08	5.93	0.86	11.23	12.14	5.52
		Soaked	27.8	29.85	0.93	23.75	24.61	13.42
	10 Krad	Dry	34.96	36.48	0.96	28.8	29.46	15.28
		Soaked	42.06	53.63	0.78	32.75	36.90	15.16

Cytological investigations

here were highly significant differences between treated and untreated plants at different meiotic stages, pollen viability and means of total percent of abnormal cells. This is due to the differences between lines and radiation doses. The significance in the interaction between lines and radiation doses may mean that there was a preference between the levels of radiation doses. The percentage of abnormal cells was found to increase with increase of radiation doses both at soaked seeds and dry seeds. There was also a significant decrease in pollen viability for dry seeds at line 3 at M1 and A1 stages. Abnormal cells also increased at 0.5 Krad for 3 lines of soaked seeds except A2. Pollen viability also significantly decreased for line 1 of soaked seeds. This suggests that pollen fertility decreased with increase gamma ray dose. (Table 4) Studies

have shown that higher gamma ray doses decreased seed emerging rate, seedling height, spike length, spikelet number and seed set, and pollen fertility is higher in untreated plants(16,17). The production of gametes with duplication and deficiencies for a certain chromosome section and to pollen sterility occurred. The role of genetic factors affecting meiosis cannot be ruled out in this polyploidy. The orientation and the unbalanced type of changes in the chromosomes is the factor influencing sterility in plants (18). Doses of 5 and 15 Gy of radiation induce chromosomal aberrations in plants (3).

Table 4. Percentage of abnormal cells in different meiotic stages and pollen viability for three lines of wheat treated by gamma rays in M1 generation (mean squares).

Sources of variance	df	Metaphase I	Anaphase I	Metaphase II	Anaphase II	Pollen viability	$\overline{X}$ of abnormal cells %
Reps.	3	93.16	196.33	46.34	20.64	115.58	19.58
Treatments	23	491.18 **	1117.36 **	198.89 **	398.30 **	701.50 **	335.00 **
Lines	2	1042.78 **	5492.35 **	150.48 **	956.60 **	668.63 **	1277.60 **
Doses	3	1909.21 **	3279.10 *	738.93 **	760.25 **	1185.71 **	1381.30 **
Conditions	1	480.40 **	1.004	664.66 **	43.87	146.20	19.17
Lines x doses	6	254.42 **	471.65 **	111.92 **	224.29 *	910.13 **	57.12 *
Lines x condition	2	123.10	348.80 *	88.76 *	316.30 *	1455.40 **	174.58 **
Doses x condition	3	200.79 **	166.25	85.57 *	185.40	20.92	28.03
Lines x doses x condition	6	104.71 *	141.68	47.75	398.30 **	443.20 **	35.09
Error	69	45.86	82.97	28.26	88.81	120.67	19.19

*** , ** : significant at : 5 % and 1 % level of probability respectively**

The more common chromosomal aberrations in the treated plants in this study include lagging chromosomes of chromosomes bridge. This was similarly reported by Han et al in 2002, who reported that the frequency of lagging chromosomes and fragments of chromosomes increased significantly by enhanced radiation (19). Ring chromosomes also appeared to increase at M1 between treatments for three lines. This ring chromosomes in wheat maybe attributed to the large size of chromosomes, presumably large segment of chromosomes were involved in interchanges or the event of translocation. Similar observation was also observed by enhanced UV-B radiation on wheat roots with polykaryocytes and ring chromosomes (20).

Induced variability

Table 5 shows the variability for vegetative and chemical traits of lines, radiation doses, conditions and interactions between M1 and M2. The mean squares of treatment were significant in M1 and M2. This is probably due to the significance of lines for all traits in M2. Furthermore the lines x doses were significant for plant height, number of tillers per plant and spike length in M1. On the other hand, lines x condition was significant for protein and dry gluten percent in M1. Also, dry and wet gluten percent showed highly significant for doses x condition at M1. These results indicate that there was no preference between level of radiation and the states of the seeds, and that the effect of either radiation doses or seed condition varied from one trait to the other.

Table 5. Vegetative and chemical traits in M1 and M2 irradiated generations for three lines (ANOVA and mean squares).

Sources of variance	df	generation	Heading date	Plant height	No. of tillers per plant	Spike length	Protein %	Wet gluten %	Dry gluten %
Rep.	3	M1	41.15	67.94	6.51	3.06	0.49	1.83	1.88
		M2	22.47	32.90	14.87	2.53	12.09	3.92	1.83
Treatment	23	M1	35.01**	1068.51**	20.25 **	12.20 **	7.67 **	42.98 *	6.27 **
		M2	162.37 **	276.81**	30.64 **	2.86 **	22.63 **	4.83 **	4.57 **
Lines	2	M1	194.49 **	11581.31**	197.71**	125.43 **	80.93 **	448.69 **	56.10 **
		M2	1545.82 **	2852.50 **	215.17 **	19.95 **	239.51 **	28.22 **	43.26 **
Doses	3	M1	50.17 *	61.96	4.94	0.99	0.63	0.898	0.297
		M2	65.41*	76.81*	14.99	3.12 *	1.31	3.740	0.230
Condition	1	M1	2.54	6.61	1.63	0.01	0.21	0.0004	0.042
		M2	0.30	4.68	19.62	0.36	1.37	1.03	0.055
Line x doses	6	M1	27.02	139.67 **	6.35 **	3.41*	0.63	3.78	1.102
		M2	34.25	47.90	6.67	1.47	4.71*	2.69	0.85
Line x condition	2	M1	17.85	41.33	1.64	0.24	1.16 *	2.71	2.88 *
		M2	0.50	16.70	16.26	0.14	1.64	2.74	0.94
Dose x condition	3	M1	3.07	14.35	1.13	0.11	0.53	11.39 **	4.71**
		M2	5.77	24.96	22.08 **	0.41	0.47	0.475	1.39
Line x dose x condition	6	M1	9.38	42.81	1.53	0.93	0.81*	4.38*	0.803
		M2	37.08	5.09	11.83	0.96	0.55	3.22	1.13
Error	69	M1	15.84	28.51	1.99	1.14	0.29	1.73	0.664
		M2	18.46	25.55	7.91	0.96	1.81	1.98	0.897

*** , ** : significant at : 5 % and 1 % level of probability respectively**

The means of all traits obtained from the effect of radiation doses, condition and the interaction were significant in M1 and M2. The direct effect of radiation doses (0.5, 5, and 10 Krad) were observed to decrease of almost all traits in M1 and M2, while spike length showed a significant increase at 5 Krad in M2. There was also a significant decrease of total protein percent at 5 Krad in M1. (Table 6) On the contrary, gamma irradiation was reported to improve plant nutrition but not improve the nutritional quality of grains (Singh and Datta, 2010), and was also reported no significantly affect the protein content of the irradiated samples (Agundez-Arvisu et al, 2006). The means of all traits showed a continuous decrease in their magnitude as the doses increased in M1 and |M2 except the number of tillers per plant, dry gluten at 0.5 Krad in M1 and plant height in M2 for line 1 and spike length for line 3 in M2. The doe of 5 Krad caused significant increase of plant height and spike length in M2 for line 1, while in M2, plant height significantly increased. The dose of 0.5 and 5 Krad was found to be a good dose for most traits in lines 1 and 2 in M1 and M2. (Table 7) This finding is similar to the findings of Ahuja et al (2014) which showed similar results, however a higher dose created some abnormalities in plant types(21).

Table 6. Vegetative and chemical traits of wheat lines in M1 and M2 irradiated generations and at different radiation doses for three lines.

Characters / Treatment		generations	Heading date	Plant height	N0. of tillers per plant	Spike length	Protein %	Wet gluten %	Dry gluten %
Line 1		M1	92.06	99.34	2.37	12.12	21.73	30.16	10.33
		M2	85.80	79.73	6.03	10.83	25.28	31.84	11.98
Line 2		M1	87.23	61.92	5.52	8.57	18.56	23.10	7.78
		M2	71.85	61.58	10.25	9.49	21.03	29.97	9.65
Line 3		M1	90.49	74.66	7.28	8.83	20.38	28.84	9.66
		M2	79.15	74.80	10.73	10.88	26.13	30.99	10.92
LSD	0.05	M1	1.98	2.66	0.70	0.53	0.27	0.654	0.405
	0.01	M2	2.64	3.54	0.93	0.71	0.36	0.764	0.473
	0.05	M1	2.14	2.59	1.40	0.49	0.67	0.700	0.471
	0.01	M2	2.84	3.34	1.86	0.65	0.89	0.820	0.552
Control		M1	90.74	78.61	5.12	10.13	20.43	27.37	9.29
		M2	81.09	70.86	8.69	10.03	23.93	31.26	10.96
0.5 Krad		M1	88.72	79.23	5.67	9.82	20.21	27.14	9.40
		M2	78.15*	72.77	9.37	10.65	24.34	30.98	10.74
5 Krad		M1	88.70	80.26	4.76	9.73	20.03	27.62	9.17
		M2	77.25	73.19	9.86	10.77	24.36	31.27	10.89
10 Krad		M1	91.55	76.46	4.67	9.68	20.24	27.36	9.18
		M2	79.20	71.54	8.10	10.16	23.96	30.48	10.82
LSD	0.05	M1	2.29	3.07	0.81	0.62	0.31	0.755	0.470
	0.01	M2	3.04	4.08	1.08	0.82	0.41	0.883	0.547
	0.05	M1	2.47	2.91	1.62	0.56	0.77	0.517	0.544
	0.01	M2	3.28	3.87	2.15	0.75	1.03	0.603	0.636

*** , ** : significant at : 5 % and 1 % level of probability respectively (* : significant. - ** : highly significant.)**

Table 7. Means of wheat lines by radiation doses for vegetative and chemical traits in M1 and M2 irradiated generations

Characters	Treatment Krad	generations	Heading date	Plant height	No. of tillers per plant	Spike length	Protein %	Wet gluten %	Dry gluten %
Line 1	Control	M1	93.98	97.85	1.64	12.59	22.00	29.36	10.20
		M2	86.05	76.29	5.18	10.02	25.27	32.01	12.43
	0.5	M1	92.33	96.03	3.14	11.25	21.69	30.50	11.02
		M2	86.15	81.76	5.75	11.32	25.25	31.48	11.71
	5	M1	91.34	100.55	2.19	12.25	21.64	30.58	9.78
		M2	85.50	82.13	6.60	11.37	25.61	31.73	11.61
	10	M1	90.59	102.94	2.51	12.40	21.60	30.19	10.32
		M2	85.28	79.24	6.53	10.67	24.99	32.15	12.17
Line 2	Control	M1	87.41	60.20	5.25	8.34	18.54	23.38	7.95
		M2	73.80	62.29	10.58	9.70	19.71	30.22	9.76
	0.5	M1	85.53	63.28	5.73	8.76	18.43	22.17	7.57
		M2	70.35	60.58	10.93	9.18	21.23	29.61	9.46

	5	M1	85.90	65.95	6.18	8.89	18.25	23.93	7.97
		M2	71.70	60.06	10.80	9.89	21.93	30.37	9.91
	10	M1	90.06	58.26	4.91	8.31	129.04	22.98	7.66
		M2	71.55	63.20	8.67	9.19	21.24	29.66	9.48
Line 3	Control	M1	90.83	77.79	8.48	9.48	20.74	29.38	9.72
		M2	83.43	74.04	10.30	10.36	26.80	31.56	10.70
	0.5	M1	88.30	78.40	8.14	9.44	20.51	28.76	9.65
		M2	77.95	75.99	11.45	11.44	26.54	31.11	11.04
	5	M1	88.85	74.29	5.91	8.08	20.20	28.32	9.72
		M2	74.55	77.37	12.20	11.05	25.54	31.71	11.15
	10	M1	94.00	68.19	6.58	8.33	20.09	28.90	9.56
		M2	80.78	71.98	9.00	10.64	25.66	29.63	10.81
LSD	0.05	M1	3.97	5.32	1.41	1.07	0.54	1.31	0.812
	0.01	M2	5.27	7.57	1.87	1.41	0.71	1.53	0.949
	0.05	M1	4.283	5.04	2.804	0.975	1.341	1.40	0.943
	0.01	M2	5.687	6.69	3.723	1.295	1.782	1.64	1.104

The vegetative and chemical traits inM2 showed a recovery from radiation effect, although all traits were not significantly deviated from the control, except that the gluten percent significantly decreased at 0.5 Krad at dry and soaked seeds for line 1. Also, the heading date had a significant decrease with 0.5 and 5 Krad and wet gluten percent at 10 Krad for dry seeds line 3. There was also a large increase in spike length at 0.5 and 5 Krad for dry and soaked seeds for line 1. At the same conditions, protein percent significantly increased with 5 Krad for line 2. Dose of 10 Krad affected the dry seeds on the same trait. (Table 8).

Table 8. Means of wheat lines by conditions (line x condition) of dry and soaked seeds for vegetative and chemical traits in M1 and M2 irradiated generations

Characters	**condition**	**generations**	**Heading date**	**Plant height**	**No. of tillers per plant**	**Spike length**	**Protein %**	**Wet gluten %**	**Dry gluten %**
Line 1	Dry	M1	91.67	98.44	2.41	12.02	21.93	30.44	10.67
		M2	85.70	76.79	6.10	10.80	25.19	31.82	11.79
	Soaked	M1	92.44	100.24	2.33	12.23	21.53	29.88	9.99
		M2	85.80	82.66	5.96	10.85	25.37	31.86	12.16
Line 2	Dry	M1	87.09	62.24	5.48	8.64	18.67	23.15	7.75
		M2	71.80	61.92	10.28	9.62	21.12	30.41	9.81
	Soaked	M1	87.36	61.60	5.56	8.51	18.46	23.08	7.81
		M2	71.90	61.23	10.21	9.37	20.94	29.53	9.49
Line 3	Dry	M1	91.51	76.03	6.89	8.83	20.22	28.53	9.41
		M2	79.40	75.70	12.00	10.94	26.48	30.89	10.86
	Soaked	M1	89.48	73.29	7.66	8.83	20.55	29.15	9.91
		M2	78.90	73.90	9.45	10.81	25.78	31.10	10.97
LSD	0.05	M1	2.81	3.76	1.00	0.75	0.38	0.925	0.573
	0.01	M2	3.73	5.00	1.32	1.00	0.50	0.083	0.673
	0.05	M1	3.029	3.56	1.98	0.691	0.948	0.990	0.668
	0.01	M2	4.022	4.74	2.63	0.916	1.260	1.160	0.781

The ranges were always wider for all traits at all doses than the control. Our results indicate the possibility of using these plants at upper limits for future improvement. Therefore, it could be concluded that the medium dose of 5 Krad increase the performance of most vegetative traits. Similar results were observed with other studies on different versions of plants that agreed on the fact that a higher dose of gamma radiations may decrease the qualitative and quantitative characteristics of plant traits(12,22,23).

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Effect of Poultry Manure Amendment on the Distribution and Mobility of Heavy Metals in Naturally Contaminated Dump Soil

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Abstract**—** *In this study, the effect of poultry manure amendment on the availability of some heavy metals, (Cu, Cr, Mn and Zn) was evaluated. The uptake of the metals by Ricinus communis (castor oil) with and without amendment was conducted in a green house. Soil sample was treated with 5%, 10% and 20% of poultry manure in a pot experiment. There was an increase in physicochemical properties of the soil such as pH, organic matter content and ECEC on treatment. Chemical speciation of the parent soil indicated that there was appreciable concentration of the metals in the extractable fraction. After three months of planting, the results showed that the extractability of the metals decreased significantly mostly with increase in percentage amendment. Residual fractionsgave the highest concentration of the metals and extractable having the least. 20% amendment has the best immobilization potential for Cu (7.07%), Cr (9.68%) and Mn (15.17%). The results also showed that amendment decreased plant metal uptake, generally decreasing as percentage amendment increased. These findings will be useful in the assessment and remediation of heavy metal-contaminated soils.*

Keywords**— poultry manure, soil amendment, heavy metal.**

I. INTRODUCTION

Soil contamination by heavy metals is a major problem that has attracted much research interest the world over. In 2005 for instance, metals were the main contaminants reported to affect over 6000 sites studied in Denmark (Jensen *et al.*, 2009) just as about 20000 sites were reported in England and wales (Environmental-Agency, 2004). Similarly, over 100000, 80000 and 50000 pollution sites were reported in USA, European Union and Australia respectively (He *et al.* 2015). China may be the worst hit with about 14000000 ha sites reportedly contaminated by heavy metals (Sun *et al.* 2009). Not only do heavy metals occur naturally in parent rocks, but they emanate from other specific sourcesand anthropogenic activities such as mining, smelting, use of pesticides, fertilizers, sludge and emission from industries (Sawidis*et al.*, 2014).

Following lessons from historic catastrophic heavy metal contamination, public awareness has grown with focus on the implications of contaminated soils environment on human and animal health (Mulligan *et al*., 2001; Bolan *et al*., 2003). Soil remediation has become expedient and has been so promoted. The remediation of metal contaminated sites is howevercost intensive and employs environmentally invasive practices. Different remediation strategies have been employed for metal contaminated soils, includingphysical and chemical treatments such as acid leaching and electro-reclamation, excavation and landfilling and thermal treatments(EPA 2006). These are however only effective enough to lower the risk (Jiang *et al*., 2009) and would be more ideal for relatively small contaminated sites (Basta and McGowen, 2004; Debela *et al*., 2012) since these techniques are expensive.

Soil remediation using low-cost organic materials and wastes has been considered as a promising solution. Numerous amendments have been proposed and tested for soil stabilization, including agricultural and industrial by-products. Lee *et al. (*2013) studied immobilization in a contaminated rice paddy soil using egg shell waste and reported that the toxicity of Pb and Cd was reduced by 93.2% and 67.9% respectively. Walkers *et al. (*2004) reported that amending a metal contaminated soil with cow manure increased the growth of *Chenopodiumalbum* and reduced shoot concentration of Cu, Mn and Zn. Sato *et al. (*2010) evaluated Cd phytoavailibilty in soils as effected by application of chemical fertilizer and three types of animal waste compost derived from cattle, swine and poultry wastes and reported that Cd concentration in spinach grown on soil amended with animal waste compost was 38% lower than in plants cultivated on chemical fertilizer-treated soil. The effectiveness of stabilization strategy depends on the nature of the contaminants, physical and chemical characteristics of the amendment, and type of the soil.

Organic soil amendment harnesses the*in situ* stabilization of soils whereby an amendment is incorporated into a contaminated soil in order to immobilize heavy metals and reduce the uptake by plants without any side effect(Hartley and Lepp, 2008; Hosseini *et al*., 2013;). This method decreases the hazard potential of the contaminants into their least soluble, mobile or toxic form (Karbassin*et al*., 2014). Amendments may bind, absorb or co-precipitate metal contaminants, reducing metal mobility and availability. Organic amendments have been reported to decrease heavy metals bioavailability, shifting them from plant available forms that is extractable with water or neutral salts such as calcium chloride, to fractions associated with organic matter, carbonates or metal oxides (Walker *et al*., 2004). Consequently, functional groups present on the surface of organic amendments would provide binding sites for heavy metals.

II. METHODOLOGY

Sample collection

Soil samples were collected into plastic bags from several dump sites at Nnewi, Nigeria at 0-15cm depth using a spade. The samples were air-dried, sieved with 2mm sieve and then thoroughly mixed together before storing for further treatment. Poultry droppings were collected from a local farm in the study area,dried and ground for further assessment.

Pot Experiment

Pots with diameter of 14.0 cm and a height of 12.0 cm were filled with 2.0kg of soil in a greenhouse. The poultry droppings were added to the soil at 5%, 10% and 20% treatment in replicates. The treated soil samples were then thoroughly mixed, watered with deionized water and kept for two days in order to get equilibration of heavy metals between the amendments and the soils. Seeds of castor oil (*Ricinus communis*) were sown in each of the amended soil with thinning carried out on germination. The plants were uprooted after three months of germination, washed thoroughly with running water and deionized water respectively.

Plant Analysis

The harvested plants were dried for 72 hours at 60^{o}C and later ground. The plant materials were digested with concentrated nitric acid (Lina *et al*., 2009) and heavy metals (Pb, Cr, Mn, and Cu) were determined using AAS (model-PG 990).

Soil Analysis

The physico-chemical properties (pH, organic matter, exchangeable cation exchange capacity, particle size) of the parent soil samples and amended soil samples were determined by standard methods. Soil samples collected from each pot after plant harvesting and sequential extraction done for the soils. The various extracts wereanalysed for heavy metals using AAS (model-PG 990).

Sequential extraction

Sequential extraction was doneas described by Tessier *et al. (*1979) with modification as described by Sebasthiar*et al.,* (2005), replacing percloric acid with aqua regia. All extracts were analyzed using AAS (model: PG-990).

Exchangeable

To 1g of amended soil sample, 8 mL of 1M $MgCl_2$ was added with pH adjusted to 7.0 with agitation for 1hr before centrifuging for 15 mins. The supernatant was filtered into a polypropylene bottle for AAS analysis, while the residue was used for further extraction.

Carbonate bound

1M NaOAc (8mL) was added to the residue obtained from the exchangeable fraction above and then adjusted to pH 5.0 with concentrated acetic acid and agitated for 5 hrs. The mixture was then centrifuged at 15 rpm for 15 mins. The supernatant was filtered into a polypropylene bottle for AAS analysis.

Fe-Mn Oxides

20 mL of 0.04M $NH_2OH.HCl$in 25% HOAc was added to the residue obtained from the carbonate bound fraction and placed in water bath for 6 hours at 96±3ºC. The mixture was then centrifuged at1500 rpm for 15 mins beforethe supernatant was filtered into a polypropylene bottle for metal analysis.

Bound to organic

3 mL of 0.02M HNO_3 and 5mL of 30%H_2O_2 adjusted to pH 2.0 was added to the residue obtained from the step above and mixture was heated to 85±2ºC for 2 hours.3mL of 30% H_2O_2 was later added and mixture heated to 85±2ºC for 3hrs before centrifuging at 1500rpm for 15 mins. The supernatant was filtered into a polypropylene bottle for metal analysis.

Residual fraction

Residue from the organic bound extraction was digested with 8mL of aqua regia for 2 hrsbefore collecting for analysis.

Determination of mobility factor

The mobility factor which is the percentage fraction of heavy metals that are mobile or available for plant absorption was calculated thus:

$$MF = (F1 + F2)/(F1+F2+F3+F4+F5) \times 100$$

Where F1 = exchangeable fraction, F2 = bound to carbonate, F3 = bound to Fe-Mn Oxide, F4 = bound to organic, F5 = residual.

DATA ANALYSIS

Results are presented as mean value ± standard deviation and analyzed by analysis of variance (ANOVA) using SPSS software package. Multivariate analysis was also used for the comparative immobilization effect of different manure amendments and forms of heavy metals in the dump soil. Statistically significant differences between means were determined by Least Significant Difference (LSD) at 95% confidence limit.

III. RESULTS AND DISCUSSION

Physicochemical properties

Table 1 shows the physicochemical properties of the amended and unamended soil samples. The pH, ECEC and organic matter of the dump soil increased with increase in manure amendment in the order, 20%>10%> 5%resulting in change inconcentrations. The variation may be due to the different concentrations of manure especially since the change in values were statistically significant ($p < 0.05$). Increased application of poultry manure resulted to the reduction in the mobility factors of the metals. The increasein percentage organic matter detectedwhich is statistically significant ($p < 0.05$) may be due to varying concentrations of poultry amendment. Mohammadi *et al*.,(2011) andHou *et al*., (2012) suggested that manure is an organic source of nutrients which increases the soil organic matter and enhances soil quality.

	Manure Amendment			
	Control	**5%**	**10%**	**20%**
PH	6.72±0.02	6.73±0.01	6.95±0.04	7.10±0.05
%Organic matter	3.77±0.03	4.21±0.00	3.88±0.12	4.15±0.01
ECEC (cmol/kg)	14.58±0.07	15.41±0.49	19.11±2.50	25.82±0.31
% Sand	93.8±0.00	93.80±0.00	93.80±0.00	93.80±0.00
% Silt	2.8±0.00	3.40±0.00	3.40±0.00	3.40±0.00
% Clay	3.4±0.07	2.80±0.00	2.80±0.00	2.80±0.00

Metal Distribution in Amended soils

Tables 2 to 5 gives the distribution of various metals in the amended and unamended soils.

Chromium

The residual fraction of the extract contained the highest Cr for all amendments giving54.00%, 56.40 %, and 61.70 % for 5%, 10% and 20% amendment respectively. The concentrations of Cr in the 5% sequential fraction followed the order; residual > bound to Fe-Mn oxide> bound to organic > exchangeable > bound to carbonate; 10% wasresidual > bound to organic >bound to Fe-Mn oxide> exchangeable > bound to carbonate and residual > bound to organic >bound to Fe-Mn oxide> bound to carbonate> exchangeable. For 20% amendment. The mobile fractions of Cr available for plant absorption in dump soil amended

with 5%, 10% and 20% of Poultry manure ranges from 9.68% - 13.02% with the 20% amendment of poultry manure having the smallest amount.

Copper

The highest fraction obtained for Cu in dump soil amended with 5%, 10% and 20% of Poultry manure was obtained in the residual fraction while the lowest fraction was obtained in exchangeable fraction. For all the percentage amendments, the concentration of sequential fractions of Cu (mg/kg) followed the order; residual > bound to organic > bound to Fe-Mn oxide > bound to carbonate > exchangeable. Residual fraction of Cu obtained in soil amended with 5% of Poultry manure is 56.50 %, lower than residual fraction obtained in the soil amended with 10% and 20 % of Poultry manure that recorded 53.70% and 53.70% respectively. The exchangeable fraction of Cu obtained in the dump soil amended with 5% of Poultry manure is 3.70%, higher than the exchangeable fraction obtained in the soil amended with 10 % and 20% of Poultry manure that recorded 1.60% and 0.90 % respectively.The mobile fractions available for plant absorption were 10%, 7.29 %, and 7.07 % for soil amended with 5%, 10% and 20% of Poultry manure respectively. It was observed that increase in % amendment resulted to decrease in concentration of mobile fractions of Cu in dump soil.

Manganese

The residual fractions contained the highest fraction of Mn in all percentage amendments at 37.0% and 32.7% for 10% and 20% amendment respectively. Based on the Mn concentration (mg/kg), the sequential fraction as observed followed the order; residual > bound to Fe-Mn oxide > bound to organic > exchangeable > bound to carbonate fraction for 5% amendment, residual > bound to Fe-Mn oxide > bound to organic > exchangeable > bound to carbonate fraction for 10% amendment and residual > bound to organic > bound to Fe-Mn oxide > exchangeable > bound to carbonate fraction for 20% amendment. The mobile fractions of Mn available for plant absorption in dump soil in order of decreasing fraction is 17.09 % > 15.70 % > 15.17 % for soil amended with 10%, 5% and 20% of poultry manure respectively.The mobile fractions of Mn was highest in the soil amended with 10% of Poultry manure but lowest in the soil amended with 20 % of poultry manure.

Zinc

The fraction with highest concentration of Zn in dump soil amended with 5%, 10% and 20% manure was obtained in the residual fraction while the lowest fractions were obtained in the exchangeable fractions. For all the percentage amendment, the concentration of sequential fractions of Zn (mg/kg) followed the order; residual > bound to organic > bound to Fe-Mn oxide > bound to carbonate > exchangeable. Residual fraction of Zn obtained in soil amended with 5% of Poultry manure is 52.80 %, higher than residual fraction obtained in the soil amended with 10% and 20 % of Poultry manure that recorded 50.20 % and 49.20 % respectively. The mobile fractions of Zn available for plant absorption are 14.42%, 15.43 %, and 15.04 % for soil amended with 5%, 10% and 20% of poultry manure respectively. The order of mobile fractions of Zn available for plant absorption with respect to amendment with poultry manure is, 10% > 20% > 5 % poultry manure amendment.

Table 2: Distribution of Cr in soil samples amended with 5%, 10% and 20% poultry manure

	Control		5%		10%		20%	
	Mean (mg/kg)	% fraction	Mean (mg/kg)	% fraction	Mean (mg/kg)	% fraction	Mean (mg/kg)	% fraction
F1	5.25±1.05	11.41	2.58±0.36	6.50	2.62±0.37	6.60	1.79±0.29	4.50
F2	9.63±1.29	20.93	2.2±0.78	5.50	2.55±0.17	6.40	2.01±0.46	5.10
F3	6.24±0.90	13.56	7.49±2.46	18.80	5.26±1.15	13.20	3.66±0.86	9.30
F4	11.67±2.07	25.35	6.1±1.24	15.30	6.87±1.27	17.30	7.6±2.21	19.40
F5	13.23±2.59	28.75	21.58±3.6	54.00	22.41±5.05	56.40	24.21±4.71	61.70
Sum	46.03		39.95		39.71		39.27	
Mf%	32.34		11.96		13.02		9.68	

Key: F1=Exchangeable, F2= Bound to Carbonate, F3= Bound to Fe-Mn oxide, F4= Bound to Organic, F5= Residual, Mf= Mobility factor

Table 3: Distribution of Cu in soil samples amended with 5%, 10% and 20% poultry manure

	Control		5%		10%		20%	
	Mean (mg/kg)	% fraction	Mean (mg/kg)	% fraction	Mean (mg/kg)	% fraction	Mean (mg/kg)	% fraction
F1	8.28±1.07	15.88	1.41±0.25	3.70	0.66±0.06	1.60	0.46±0.08	0.90
F2	11.28±2.60	21.64	2.4±0.41	6.30	2.36±0.54	5.70	2.96±0.39	6.10
F3	7.30±0.34	13.99	4.76±0.72	12.50	7.16±1.34	17.30	8.43±1.02	17.40
F4	2.81±0.60	5.40	7.99±2.91	21.00	9.02±1.79	21.80	10.55±2.72	21.80
F5	22.47±3.41	43.10	21.55±3.93	56.50	22.25±7.67	53.70	26±3.97	53.70
Sum	52.15		38.11		41.45		48.40	
Mf%	37.52		10.00		7.29		7.07	

Key: F1=Exchangeable, F2= Bound to Carbonate, F3= Bound to Fe-Mn oxide, F4= Bound to Organic, F5= Residual, Mf= Mobility factor

Table 4: Distribution of Mn in soil samples amended with 5%, 10% and 20% poultry manure

	Control		5%		10%		20%	
	Mean (mg/kg)	% fraction	Mean (mg/kg)	% fraction	Mean (mg/kg)	% fraction	Mean (mg/kg)	% fraction
F1	21.64±3.54	13.37	14.26±3.87	10.40	14.8±3.21	10.70	15.34±2.37	8.6
F2	17.38±1.73	10.74	7.33±2.69	5.30	8.83±0.52	6.40	11.66±1.11	6.5
F3	54.27±6.31	33.52	36.16±11.87	26.30	36.33±6.02	26.30	43.46±4.08	24.4
F4	37.90±4.41	23.41	30.46±11.93	22.10	27.15±3.25	19.60	49.38±5.46	27.7
F5	30.70±4.19	18.96	49.31±14.71	35.90	51.15±11.66	37.00	58.17±18.34	32.7
Sum	161.89		137.52		138.26		178.01	
Mf%	24.10		15.70		17.09		15.17	

Key: F1=Exchangeable, F2= Bound to Carbonate, F3= Bound to Fe-Mn oxide, F4= Bound to Organic, F5= Residual, Mf= Mobility factor.

Table 5: Distribution of Zn in soil samples amended with 5%, 10% and 20% poultry manure

	Control		5%		10%		20%	
	Mean (mg/kg)	% fraction	Mean (mg/kg)	% fraction	Mean (mg/g)	% fraction	Mean (mg/kg)	% fraction
F1	36.03±4.13	19.04	4.44±1.11	4.40	4.38±1.13	3.90	3.21±0.35	2.40
F2	42.81±5.19	22.62	9.94±2.95	10.00	13.15±3.25	11.60	16.56±2.31	12.60
F3	26.36±4.47	13.93	15.76±3.75	15.80	17.24±4.68	15.20	23.02±31.91	17.50
F4	18.48±3.46	9.77	16.95±3.95	17.00	21.81±6.91	19.20	23.97±1	18.20
F5	65.56±7.56	34.64	52.62±11.15	52.80	57.06±17.62	50.20	64.7±21.91	49.20
Sum	189.24		99.71		113.64		131.46	
Mf%	41.66		14.42		15.43		15.04	

Key: F1=Exchangeable, F2= Bound to Carbonate, F3= Bound to Fe-Mn oxide, F4= Bound to Organic, F5= Residual, Mf= Mobility factor

Mobility Factor and Plant Metal Uptake

The effect of poultry manure amendment on mobility factor and plant metal uptake is shown in Figures 1 and 2.The total concentrations of the heavy metals available for absorption by *Ricinus communis* after amendment with different percentages of the manures used are calculated and presented as mobility factor. An increase inpoultry manure amendment decreased the mobility factor just as metal uptake by plant decreased. Plant metal uptake was decreased in the poultry manure amended soil compared with plant metal uptake in the unamended soil. Lowest mobility was recorded in the plant with lowest plant metal uptake. Alloway and Jackson (1991) corroborated this results in a similar study where it was reported that the metal uptake from soil to plant was slow because organic matter introduces new binding sites to the soil and therefore present fewer risk for plants, as compared to unamended soils. Similar effects for biochars and biosolids of poultry manure was studied by Uchimiya*et al*. (2012) and Wuana*et al*. (2012).

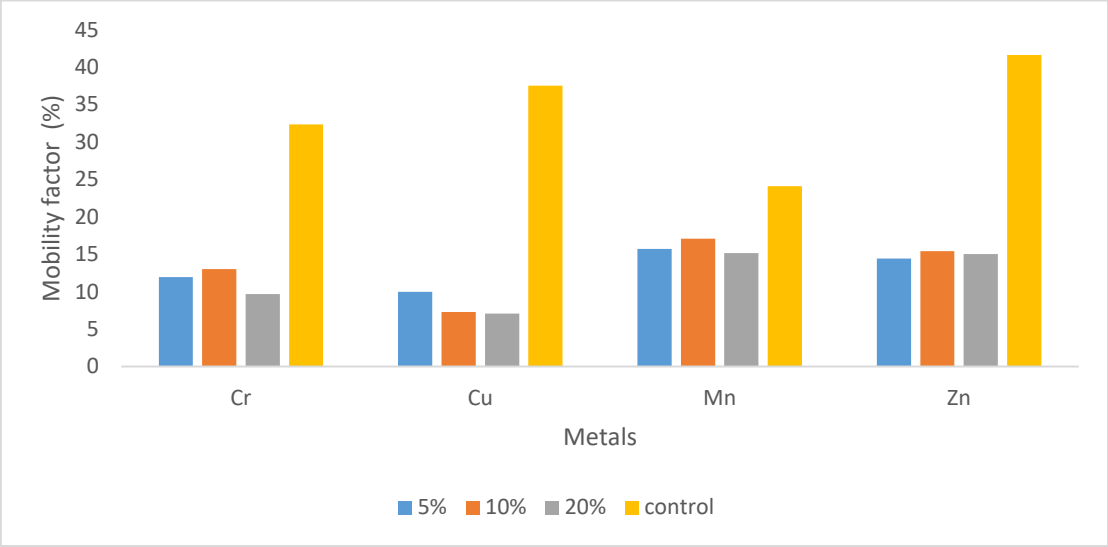

Fig.1: Effect of manure amendment on mobility factor (Mf)

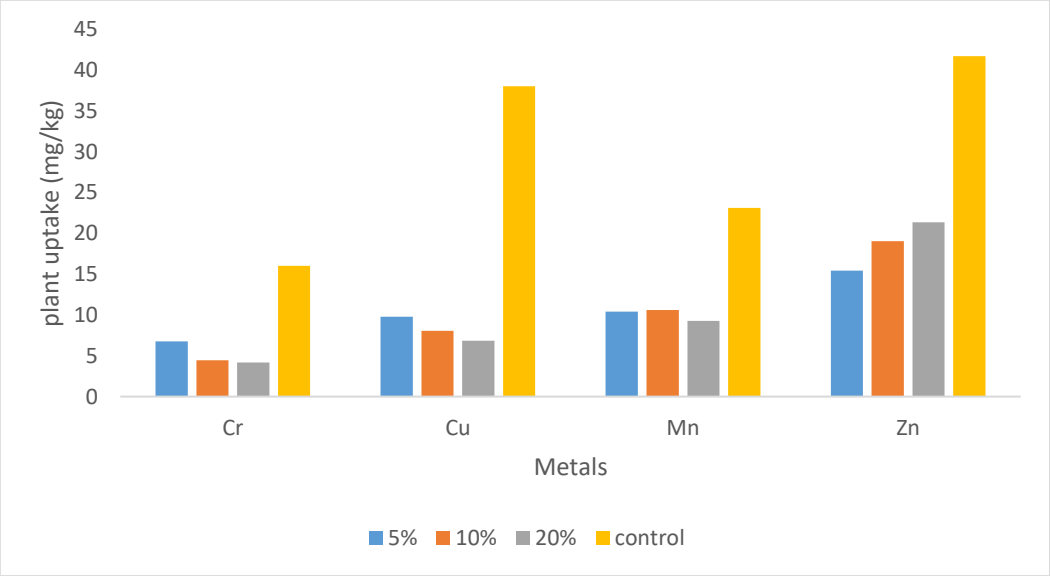

Fig.2: Effect of manure amendment on plant metal uptake

IV. DISCUSSION

Application of poultry manure increased pH, ECEC and organic matter resulting in change inconcentrations as well as reduction in the mobility factors of the metals. Generally, the concentrations of the heavy metals present in the dump soil changed in increasing order of residual >

bound organic > bound to Fe-Mn oxide >bound to carbonate > exchangeable. Results from Azeez *et al.* (2019) however disagrees with the current findings reporting anorder of abundance of the fractions in dump soil amended with poultry manure was carbonate bound >Fe-Mn oxides bound > exchangeable. Nonetheless, they reported that the application of poultry manure resulted in reduction in the available Zn, exchangeable Zn and oxide-bound Zn, Pb and Cd in line with the finding of this research. According to Azeez and his colleagues, the reason for the high carbonate-bound heavy metals in the dumpsites reported in their work could be attributed to the fact that most of the heavy metals were in association with carbonate salts. Poultry manure amendments has been reported for Cu optimization in the contaminated soil (Thomas and Dauda, 2015);but according to Walker *et al*. (2003) the release of phosphate, carbonates and other salts after the application of composted poultry manure may transform into metal insoluble compounds and decreased metal solubility.

The immobilization effect of poultry manure on various heavy metals as seen in this research is in agreement with the reports of Irshad *et al*. (2014) and Wuana*et al.* (2012). This may be due to the presence of trace metal (TM) sorbents capable of reducing TM solubility and enhancing immobility in the soil as reported by Haroon *et al.* (2019). Hanc *et al*. (2008) reported that poultry manure and compost decreased the available Cd and Cu content of contaminated soil. Lina, *et al*. (2009) also reported a decrease of soluble/exchangeable fraction of Cd and increase in organic-bound fraction when compared with the control. This also agreed with the current findings. Okieimen *et al*. (2011) while reporting stated that total uptake of Cr and Cu by maize plant decreased with increased loads of poultry amendment to the contaminated soil. These reductions were as associated with the capacity of the amendment to immobilize metals in soils. They stated that the organic matter and phosphorus content of organic amendment could account for the immobilization of metals.

Plant metal uptake shows that increase in percentage manure amendment decreased the mobility factor which also resulted in decrease in plant metal uptake this in agreement to the report of Angelove*et al.* (2010) who reported that organic amendment reduced the concentration of Pb, Zn, Cu and Cd in potato tubers . Italso showed that the immobilization potential of the manure increased as their concentration in the soil was increased implying that poultry manure is a good immobilizing agent for remediation of heavy metal contaminated soil. The manure amendment significantly ($p < 0.05$) decreased the metalconcentrations in the extractable fraction (exchangeable and bound to carbonate) when compared with un-amended soil.

V. CONCLUSION

The concentrations of all the metals in each of the soil fractions significantly ($p<0.05$) varied for amendment with poultry manure. It was observed that % amendment with poultry manure significantly affected the forms of Cd, Cu, Mn, and Zn ($P<0.05$). Multiple comparison between 5%, 10%, and 20% of poultry manure revealed that 20% of poultry manure has more effect on the forms of Mn, and Cd while 10% of poultry manure has more effect on geochemical forms of Zn and Cu.The finding also shows that higher percentage amendment immobilizes metals more than lower percentage amendment except for Cd where immobilization effect decreases with percentage amendment.

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Current biotechnological applications of L-amino acid deaminases for production of keto acids

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Abstract— *There is a growing interest in the pharmaceutical and agrochemicals industries for the use of enantiomerically-pure amino acids. α-keto acids are commonly used in feeds, food additives, pharmaceuticals, and chemical manufacturing. Commonly, most α-keto acids are manufactured by chemical synthesis, but due to the increasing concern for environment-friendly approaches, microbial fermentation and enzymatic transformation are alternative processes for the production of keto acids. Regarding this, L-amino acid deaminase (LAAD), is a major enzyme for α-keto acids production and is only found in Proteus bacteria. In this review, we discussed the recent biological applications of the enzyme LAAD in the production of keto acids, and summarized the recent advancements in the biological production of six important α-keto acids; specifically, phenylpyruvate, α-ketoglutaric acid, pyruvate, α-ketoisocaproate, α-keto-γ-methylthiobutyric acid, and α-ketoisovaleric acid.*

Keywords— ***keto acids, LAAD, enzymatic transformation, microbial fermentation.***

I. INTRODUCTION

A variety of enzymes has been used to prepare chiral pharmaceutical and agricultural compounds containing enantiomeric amine or amino acid groups. Among these are aminotransferases (EC 2.6.4.*X*), lipases (EC 3.1.1.*X*), amine oxidases (EC 1.4.3.22), amino acid dehydrogenases (EC 1.4.1.*X*), and amino acid oxidases (EC 1.4.3.*X*) (Pollegioni & Molla, 2011; Turner, 2004).The deracemization of a racemic amino acid to obtain the L-configuration was achieved by using a stereoselective D-amino acid oxidase (DAAO, EC 1.4.3.3) followed by chemical reduction. The second step iteratively converts the amino acid produced (from the D-amino acid) back into a DL-mixture to obtain the full resolution of the racemic mixture into the L-enantiomer(Turner, 2004). This approach requires stable recombinant DAAOs possessing wide substrate specificity as well as variants engineered to act on synthetic amino acids (Pollegioni et al., 2007).

Amino acid oxidases with reverse stereoselectivity are also well known flavooxidases, mainly produced by snakes or by microorganisms. In particular, L-amino acid oxidases (LAAO, EC 1.4.3.2) catalyze the stereoselective oxidative deamination of L-amino acids into the corresponding α-keto acids and ammonia; the re-oxidation of $FADH_2$ by

dioxygen then generates H_2O_2 (Pollegioni, Motta, &Molla, 2013). These flavoenzymes catalyze an irreversible reaction (differently from aminotransferases) and do not require a specific step of cofactor regeneration, as otherwise required by the NAD-dependent dehydrogenases. However, because of the problems associated with overexpression of snake venom LAAOs in recombinant hosts and the limited substrate acceptance of the microbial counterparts, no appropriate LAAOs for biocatalysis are available (Pollegioni et al., 2013). L-Amino acid deaminases (LAADs) represent a suitable alternative to LAAOs. LAAD (first identified in the genera *Proteus*, *Providencia,* and *Morganella*) catalyzes the deamination of the L-isomer of amino acids, yielding the corresponding α-keto acids and ammonia without any evidence of H_2O_2 production. LAAD from *Proteus myxofaciens* (PmaLAAD), expressed in the *Escherichia coli* K12 strain, shows a preference for L-amino acids with aliphatic, aromatic, and sulfur-containing side chains (Pantaleone, Geller, & Taylor, 2001). This review focused on the applications of LAAD for the production of α-keto acids, and summarized the recent advancements in the biological production of six important a-keto acids; specifically, phenylpyruvate,α-ketoglutaric acid, pyruvate, α-ketoisocaproate, α-keto-γ-methylthiobutyric acid, and α-ketoisovaleric acid.

II. LAAD IN BIOCATALYSIS

2.1 EXPRESSION AND PURIFICATION OF RECOMBINANT LAADS

In order to produce LAAD in bulk for biochemical characterization and biotechnological purposes, this flavoenzyme must be expressed in different microbial hosts. Recombinant type-I L-Amino acid deaminase from *Proteus myxofaciens* (PmaLAAD) was expressed as a full-length protein in *E. coli* BL21(DE3) cells (at 28 °C), obtaining an enzyme with a specific activity of 0.6 U mg^{-1} protein on the substrate L-Phe (in the crude extract) (Motta, Molla, Pollegioni, & Nardini, 2016). Chromatographic steps were ineffective for enzyme purification due to the recombinant protein and its tight association with the bacterial membrane fragments. Thus, PmaLAAD could only be separated from soluble proteins by ultracentrifuging the crude extract Resuspension of the pellet formed by the membrane fraction (and containing the active enzyme) resulted in modest increases of a2- to 3.4-fold within specific activity (Table 1) (Motta et al., 2016; Pantaleone et al., 2001). The dual objectives of producing and preparing a pure PmaLAAD aided in designing a His-tagged fusion protein. The presence of the tag located either at the protein's N- or C-terminuses have no effect on the enzyme expression yields or its specific activity in the crude extract. A His-tagged PmaLAAD variant was purified to homogeneity by immobilized metal affinity chromatography as a soluble, yet inactive enzyme. Complete activity can only be restored (and reaching the crude extract's assayed value) after adding *E. coli* membranes to the purified enzyme sample (Motta et al., 2016). Type-I PmirLAAD in its homologous form was also produced as a His-tagged recombinant protein. In this case, the recombinant protein was expressed at 20 °C to prevent the overproduced protein from accumulating in inclusion bodies. Metal-chelating chromatology followed by solubilization with the detergent n-dodecyl-β-D-maltoside was used to purify the enzyme. Unfortunately, negatively affected enzyme activity was suboptimal; this could be due to the interaction of the latter with the bacterial membranes. The specific activity of the pure protein was 0.94 U mg^{-1} on L-Phe (Table 1).

Table 1. The production level of recombinant LAADs.

Source	Variant	Specific activity (U mg^{-1} protein on L-Phe)			Reference
		Crude extract	Ultracentrifugation step	Further purification steps	
Type-I LAAD					
P. myxofaciens	Wild-type	0.6	1.2	2.9 (thawing, dilution and further centrifugation)	(Motta et al., 2016)
	C-HisTag	0.69	2.35		(Pantaleone et al., 2001)
	N-HisTag	0.8		B.D. (HiTrap)	(Motta et al.,

Source	Variant	Specific activity (U mg^{-1} protein on L-Phe)			Reference
		Crude extract	Ultracentrifugation step	Further purification steps	
					2016)
P. mirabilis	*pel*B + C-HisTag	0.02		0.94 (detergent solubilization and HiTrap)	(Hou et al., 2015)
Type-II LAAD					
P. mirabilis	N-HisTag	ND	1.54		(Baek et al., 2011)
P. mirabilis (optimized codon usage)	*pel*B + N-HisTag (deletion variant)	ND		0.74 (refolded from inclusion bodies)	(Liu et al., 2013)
P. vulgaris[a]	N-HisTag	ND	ND	ND	(Ju et al., 2016; Takahashi, Ito, & Yoshimoto, 1999)

B.D.: below detection; ND.: not determined; HiTrap: metal-chelating chromatography. [a]No information concerning the specific activity of the recombinant enzyme has been reported.

Therefore, due to partial loss of enzymatic activity and high detergent costs, the authors concluded that biocatalytic applications are better served by using whole cells to express the recombinant protein instead (Hou et al., 2015). Similar expression yields were obtained for the His-tagged Pm1LAADtoo. through preparation of a specific activity on the L-Phe of 1.54 U mg^{-1} protein (compared to 1.2 U mg^{-1} for PmaLAAD) was achieved after the ultracentrifugation purification step (Baek et al., 2011). Interestingly, by the optimized codon usage for *E. coli* expression and the gene was fused to the *pelB* leader peptide, the recombinant chimeric protein was expressed exclusively as inclusion bodies (Liu et al., 2013). This confirms that overoptimization of codon usage could induce an actual increase in protein expression but compromise of its cellular solubility. Nonetheless, the researchers were able to solubilize (to use 8 mol urea) and refold protein from the inclusion bodies (with a yield of 40 percent), obtaining an enzyme preparation with a high degree of purity but with a reduced specific activity (0.74 U mg-1 of pure Pm1LAAD) and a very high Km for L-Phe) (31.5 mM). This result indicates that the refolding approach facilitated partial recovery of the conformation/activity of the enzyme (Table 1) (Liu et al., 2013).As a general rule, the association of the enzyme to the bacterial membranes is fundamental for the catalytic competence of type-I LAADs. Consequently, when the putative N-terminal transmembrane α-helix of PmaLAAD was removed, the resulting ΔN-LAAD deletion variant is rendered nearly inactive because of its inability to accomplish membrane interaction (in the crude extract even) (Motta et al., 2016). In contrast, with type-II LAADs, the enzymatic activity is also (partially) retained in the deletion variants lacking the transmembrane α-helix. Regarding these enzymes, the insertion module is relatively sufficient for providing the necessary interaction of the protein with the bacterial membranes, even without the N-terminal transmembrane α-helix (Ju et al., 2016). Further debates have ensued since then, focusing on using whole cells to express LAAD vs. using recombinant purified enzymes. Whole cells are employed more consistently due to their production simplicity and the low cost; some authors assert that side reactions can occur, thus accumulating into small amounts of impurities and causing adverse effects on the overall yield (Ahmed, Parmeggiani, Weise, Flitsch, & Turner, 2016; Hou et al., 2016; Hou et al., 2015). In contrast, purified enzymes are more

expensive but possess a higher specific activity and lack any side reactions. (Hou et al., 2015) stressed the following. Whole-cell transformation is an attractive alternative to the laborious and costly processes of purification, stability, external FAD addition, and recycling. With that said, when taking conversion and productivity into account, the enzyme systems showed better performance overall. Therefore, both biotransformation methods are promising and need future improvement (Molla, Melis, & Pollegioni, 2017).

2.2 ELEMENTAL SOLUTIONS OF AMINO ACIDS

In 2001, Pantaleone group was the first to propose employing LAAD in biocatalysis. The recombinant enzyme from *P. myxofaciens* oxidized the 20 natural L-amino acids, thus revealing its preference for aliphatic, aromatic, and S-containing amino acids (Pantaleone et al., 2001) . This same group used a partially purified enzyme preparation to conduct specific activity on the L-Phe of 2.35 U mg^{-1} protein to determine its pH levels (which measured at 7.5). They also collaborated with Ian Fotheringham and Nick Turner the following year, using the same *E. coli* cells, which expressed the recombinant protein and 40 equivalents of ammonia-borane. The result was a deracemization of 2.5 mmol DL-Leu (in 50 mmol ammonium formate, pH 6.7), and a continuing production of the D-enantiomer, with a 90% yield and an ee> 99% (Alexandre et al., 2002). Together, these groups achieved a conversion higher than 85% obtained through racemic solutions of norleucine, methionine, O-benzyl serine, cyclopentyl-glycine (Alexandre et al., 2002), and isoleucine (Enright et al., 2003).

V_{max} (µmol min^{-1} mg^{-1} protein)	**K_m (mM)**	**Reference**	**Note**
Recombinant *P. myxofaciens* LAAD			
0.26	2.28	(Pantaleone et al., 2001)	Crude extract (PmaLAAD)

Table 2. Apparent kinetic parameters on l-Phe of various L-AAD preparations

Recombinant *P. mirabilis* LAAD:			
0.73	31.5	(Liu et al., 2013)	Refolded ΔN-L-AAD variant (Pm1LAAD)
0.34	–	(Hossain, Li, Shin, Chen, et al., 2014)	Membrane preparation from *B. subtilis* cells (Pm1LAAD)
1.64	26.2	(Hossain et al., 2016; Hou et al., 2015)	Purified *P. mirabilis* KCTC2566 L-AAD (PmirLAAD)
2.63	22.0	(Hou et al., 2016)	Purified D165K/F263M/L336M variant of *P. mirabilis* KCTC2566 L-AAD (PmirLAAD)

2.3 PHENYLPYRUVIC ACIDPRODUCTION

In recent years, phenylpyruvic acid (PPA) has been utilized as a product reference of L-AAD catalyzed L-Phe conversion (Table 2). PPA is a limiting raw material for the sweetener aspartame (production > 10,000 tons year-1) and employed primarily for patients with kidney diseases to reduce urea accumulation in their diets PPA producers were subsequently screened and selected using a variety of microorganisms such as *P. Vulgaris*, which was the most effective strain (Coban, Demirci, Patterson, & Elias, 2014). Reaching ~ 1 g L^{-1} of PPA. Pantaleone et al experimented even more with PPA production by using the *Proteus Vulgaris* B-123 strain, which reached ~ 3g L^{-1} of PPA in fed-batch fermentation (adding 4 g L-Phe at 30 hours of fermentation), and 104 and 259 mg L -1h^{-1} by conducting fed-batch and continuous fermentation, respectively (Coban, Demirci, Patterson, & Elias, 2016). *P. mirabilis* KCTC 2566 L-AAD (type-II) was overproduced in *E. coli* cells, which can be used as a whole-cell biocatalyst or as a purified enzyme preparation (Hou et al., 2015). The maximal PPA production was 2.6 and 3.3 g L^{-1} of PPA (86.7 and 82.5% conversion rate) beginning with 3 and 4 g L^{-1} L-Phe for the pure enzyme (0.2 mg mL^{-1} at pH 7.4 and 35 °C) and the whole-cell system (1.2 g cell L^{-1} at pH 7.4 and 40 °C), respectively. PPA generation through *E. coli* cells that overproduce L-AAD from *P. mirabilis* KCTC 2566 is often hampered by two critical factors: product degradation and a low substrate preference of this flavoenzyme(Hou et al., 2016). Thus, they achieved a moderate increase in PPA level (from 3.3 to 3.9 g L^{-1}) by eliminating three genes encoding aminotransferases. This led to increased PPA production on a higher scale (up to 10.0 g L^{-1} corresponding and reaching full substrate conversion) by utilizing a L-AAD variant that harbors the D165K/F263M/L336M substitutions, generated by error-prone PCR (Hou et al., 2016). The evolved L-AAD variant shows a 1.6-fold increase in maximal activity (2.63 μmol min-1 mg^{-1} protein), see (Table 2). Under optimized fed-batch conditions (i.e., feeding 4 g L^{-1} of L-Phe per hour), the maximal PPA production reached 21 g L^{-1}. However, PPA's current chemical synthesis methods have relatively low yield and result in unwanted environmental pollution. In this regard, biotransformation processes seem unsuitable as industrial alternatives.

2.4 PRODUCTION OF A-KETOGLUTARIC ACID

The Jian Chen group used L-AAD from *P. mirabilis* KCTC to transform L-glutamic acid into 2- ketoglutaric acid (α-KG), which can be utilized in various applications such as pharmaceuticals, fine chemical, and the food and animal feed industries. It is believed that the biocatalytic process bypasses the multi-step chemical process regarding succinic acid and oxalic acid diethyl esters, thereby employing toxic compounds, generates toxic waste, and showing a much lower product yield in comparison. The ΔN-L-AAD variant enzyme was used after refolding (Liu et al., 2013). This preparation of enzymes showed the optimal activity at pH 8.0 and 45 °C and an apparent Vmax = 0.73 U mg^{-1} protein and Km for L-Phe of 31.5 mmol (Table 2 at air saturation). Studies have shown that 12.6% of α-KG can be produced in 6 hours, which is considered to be optimal conditions (12 g L^{-1} L-Glu, 0.1 g L^{-1} ΔN-LAAD, 5 mmol MgCl2, pH 8.0 and 40 °C) (Liu et al., 2013). Notably, the conversion was hampered by-product inhibition (Ki = 12.6 mmol for α-KG). Notably, product inhibition (Ki = 12.6 mmol for α-KG) significantly hampered the conversion process. Later on, the wild-type L-AAD from *P. mirabilis* KCTC 2566 was overproduced in *Bacillus subtilis 168* cells (Table 2) and used as membrane fraction for the transformation of

L-Glu. Within optimal parameters (15g L^{-1} L-Glu, 20g L^{-1} cells, 5 mmol MgCl2, pH 8.0 and 40 °C) 31% of α-KG can be produced in as little as 2 hours (Hossain, Li, Shin, Chen, et al., 2014). The maximal volumetric yield reached 4.65 g α-KG L^{-1} In order to further improve α-KG production, a protein engineering study was conducted by Che's group using Pm1L-AAD (Hossain, Li, Shin, Liu, et al., 2014). By employing EP-PCR and site-saturation mutagenesis at six positions they identified the pm1-3-3 variant harboring the F110I/A255T/E349D/R228C/T249S/I352A substitutions. This variant enzyme shows a 2.4-fold higher Vmax and a 2.1-fold lower Km for L-Glu in contrast to the wild-type Pm1L-AAD does. When this variant of enzyme expression in a *B. subtilis 168* strain lacking the *sucA* gene encoding for α-ketoglutarate dehydrogenase, the α-KG titer reached 12.2 g L^{-1} (after 24 h of transformation using 15 g L^{-1} L-Glu, 20 g L^{-1} whole-cell biocatalyst, pH 8.0) (Hossain, Li, Shin, Chen, et al., 2014). After multiple rounds of error-prone PCR of *P. mirabilis* KCTC 2566 L-AAD followed by DNA shuffling with the gene coding for *pv*LAAD, they managed to isolate pm1338g4, a variant of enzyme-containing substitutions at 34 positions and further possessing maximal activity at a 4.5-fold increase and a subsequent decreased Km for L-Glu (i.e.6.6 mmol) by ~ 4-fold (Hossain, Li, Shin, Du, et al., 2014). This system produced 53.7 g α-KG L^{-1} when 100 g of monosodium glutamate was used and up to 89.1 g α-KG $L^{-1}$when the substrate was continuously fed at a constant rate of 6 g $L^{-1}h^{-1}$ with an initial concentration of 50 g L^{-1}. Regardless of the advantage of reducing environmental pollution and the significant α-KG production reached by L-AAD biocatalysis, this bioconversion system is not ready to be turned into an industrial level.

2.5 PYRUVATE PRODUCTION

Currently, the production of pyruvate by the biotechnological process has become a fast-growing trend, and fermentation processes have been extensively established in this regard. Through metabolic engineering strategies, microbes such as *E. coli* (Yihui Zhu, Eiteman, Altman, & Altman, 2008), *Torulopsisglabrata* (Yang, Chen, Xu, Liu, & Chen, 2014) *Corynebacterium glutamicum* (Wieschalka, Blombach, & Eikmanns, 2012) and *Saccharomyces cerevisiae* (Van Maris et al., 2004). have been constructed to achieve high production of pyruvate. Overall, the engineering methods target on accelerating the rate of glycolysis and on limiting cellular growth (Yihui Zhu et al., 2008). Hossain et al. attempted a production of pyruvate from DL-alanine which utilized a whole-cells system engineering of an *E. coli* cell strain (Hossain et al., 2016). The genes encoding of these two alanine uptake transporters and one pyruvate uptake transporter were eliminated to minimize their usage by the cell system. These cells also succeeded in overproduction of both the wild-type and an isolated variant (30,000 clones generated by error-prone PCR) of Pm1LAAD. Pm1ep3, in its best form, contains 9 substitutions overall (at a great distance of > 15 Å from the active site) and displaying a 1.8-fold increase in maximal activity (3.1 μmol min-1 mg^{-1} protein) with a ~ 2-fold decrease in Km for L-Ala (23.3 mmol) (Hossain et al., 2016). Interestingly, the enzyme can also be active on D-Ala (Vmax = 0.81 μmol min^{-1} mg^{-1} protein, Km= 31.5 mM), allowing further usage of the racemic amino acid to produce pyruvic acid. LAAD's ability to use the D-isomer of the amino acids as substrate require a more detailed evaluation.

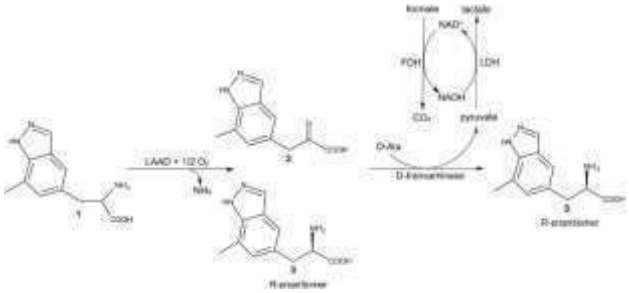

Fig.1. Enzymatic reactions by LAAD and D-transaminase used to convert a racemic amino acid (1) solution into the corresponding R-enantiomer (3), with coupling for pyruvate removal (Hanson et al., 2008). (2) S-enantiomer; LDH, lactate dehydrogenase; FDH, formate dehydrogenase.

This is due to the fact that the four-location model for substrate binding discriminates the enantiomers for their ability for hydride transferal from their αC-H to the N (5) flavin position, see above. Under ideal conditions (i.e., 50 g L^{-1} DL-Ala, 20 g L^{-1} whole-cells expressing pm1ep3, pH 8.0, at 40 °C and 220 rpm) 14.6 g L^{-1} of pyruvate was achieved, corresponding to a biotransformation ratio of ~ 29% of in just 36 hours (Hossain et al., 2016).

2.5.1 *T. GLABRATA* FOR IMPROVING PYRUVATE PRODUCTION

The earliest microbial stain used for industrial-scale pyruvate production was the multi-vitamin auxotroph *T. glabrata*. The highest pyruvate production by *T. glabrata* reached 94.3 g/L within 82 h, and the yield on glucose substrate was about 0.635 g/g by a NaCl-tolerant strain (Lütke-Eversloh, Santos, & Stephanopoulos, 2007). Further investigation was focused on controlling the NADH level to enhance the rate of glycolysis. Two different NADH re-oxidation pathways were introduced to reduce NADH in the cytoplasm and in mitochondria. The

highest pyruvate yield and productivity were increased to 38% and 21%, respectively (Qin, Johnson, Liu, & Chen, 2011). An optimal nutrient environment is also crucial to achieving a high pyruvate yield. With nitrogen optimization, the pyruvate concentration reached 85.9 g/L within 72 h with the optimal addition of thiamine, nicotinic acid, pyridoxine, biotin, and riboflavin (Yang et al., 2014).

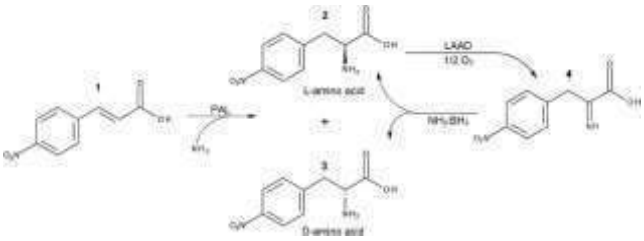

Fig.2. Amination/deracemization cascade by phenylalanine ammonia lyase (PAL) and LAAD to produce optically pure p-nitro-D-Phe (3) from p-nitrocinnamic acid (1) (Parmeggiani et al., 2015). (2) p-Nitro-L-amino acid; (4) p-nitro-amino acid.

2.5.2 CONSTRUCTING *C. GLUTAMICUM* FOR THE PRODUCTION OF PYRUVATE

C. glutamicum is well-established organic acid producer, it has been established as a microbial cell factory for pyruvate production. *C. glutamicum* strain was engineered by inactivating its pyruvate dehydrogenase complex, NAD^+-dependent l-lactate dehydrogenase, quinone oxidoreductase, and alanine transaminases AlaT and AvtA, and a reduced variant of acetohydroxyacid synthase was presented. Under low oxygen tension and in fed-batch fermentations, the strain formed 45 g/L of pyruvate, with a yield of 0.97 mol/ mol of glucose (Wieschalka et al., 2012; Zou, Hang, Chu, Zhuang, & Zhang, 2009). Even though this yield of pyruvate was minor than that of *T. glabrata*, the conversion rate from glucose was higher with *C. glutamicum*. Furthermore, *C. glutamicum* is more applicable for medicinal-grade pyruvate production owing to its generally recognized as safe (GRAS) status (Meiswinkel, Rittmann, Lindner, & Wendisch, 2013).

2.5.3 CONSTRUCTING *S. CEREVISIAE* FOR THE PRODUCTION OF PYRUVATE

S. cerevisiae does not usually produce organic acids in large scale, its tolerance to pH makes it a suitable producer of pyruvate.(Van Maris et al., 2004) reported that the highest production of pyruvate (135 g/L) was obtained by a pyruvate decarboxylase-deficient mutant of *S. cerevisiae* TAM. Yet, supplementation of a C_2 compound was required for the growth of this mutant because of the pyruvate decarboxylase deficiency. In Wang et al., (2012) study, by using rational cofactor engineering and adaptive evolution, a pyruvate concentration of 75.1 g/L with a yield of 0.63 g pyruvate/g glucose was obtained. Furthermore, by regulating the thiamine biosynthesis genes, which are related to unstable cellular growth on sole carbon sources, a pyruvate amount of 8.21 ± 0.30 g/L was achieved after 96 h of cultivation (Xu, Hua, Duan, Liu, & Chen, 2012). fermentation conditions were also optimized by focusing on the carbon and nitrogen substrates, pH, osmotic pressure, and hydrogen peroxide (H_2O_2) level for the improvement of pyruvate production by different strains (Song et al., 2016)

2.5.3 BIOCONVERSION APPROACH OF PYRUVATE PRODUCTION ENHANCEMENT

In current years, whole-cell and enzyme bioconversion methods have been developed. E.g., lactate dehydrogenase, glycolate oxidase, and lactate oxidase were used to convert lactate into pyruvate. In a previous study they used glycolate oxidase in *Pichia* pastoris to catalyze the conversion of pyruvate from L-lactate by the whole-cell transformation process (Payne et al., 1995). Acetaldehyde and carbon dioxide were converted into pyruvate by*Rhodotorulagracilis* d-amino acid oxidase (d-AAO), giving a 90% conversion rate (Miyazaki, Shibue, Ogino, Nakamura, & Maeda, 2001). In another study, a high pyruvate quantity of 111.9 g/L was obtained by expressing both glycolate oxidase and catalase together (Eisenberg et al., 1997). The catalase was needed to remove the effect of H_2O_2, which could convert the pyruvate to acetate.

2.6 PRODUCTION OF A-KETOISOCAPROATE

ketoisocaproate Essential branched-chain amino acids L-Val, L-Ile and L-Leu, alongside the corresponding keto acids, have different applications in the feed, food and pharmaceutical sector: they are produced in quantities of up to 5,000 tons year-1 (Becker & Wittmann, 2012). In a previous study, in order to establish a successful fermentation mechanism, a recombinant *Corynebacterium glutamicum* strain was designed by metabolic engineering, and the maximal α-ketoisocaproate titter was reached 9.23 g/L (Bückle-Vallant, Krause, Messerschmidt, &Eikmanns, 2014). However, with the exception of the poor yield of alpha-ketoisocaproate, an auxotroph for branch-chained amino acids is still a barrier to industrial development owing to the deletion of ilvE. Another study has fabricated a plasmid-free *C. glutamicum* to produce 6.1 g/L α-ketoisocaproate (Vogt, Haas, Polen, van Ooyen, & Bott, 2015). Yet, the production of α-ketoisocaproate of *C. glutamicum* by metabolic engineering is still narrow by the growth reliant on the L-isoleucine. The whole-cell biosynthesis mechanism offers a bright path to the low-cost development process of α-ketoisocaproate. In a study, α-ketoisocaproate was prepared using the whole-cell

transformation technique of *Rhodococcusopacus* DSM 43250, and α-ketoisocaproate titers reached 1275 mg/L (Yuhong Zhu et al., 2011). In another research, for the development of α-ketoisocaproate from leucine, an *Escherichia coli* BL21 (DE3) was constructed by whole-cell biocatalyst with membrane-bound L-amino acid deaminase (LAAD) from *Proteus vulgaris*. The highest titter was reached 69.1 gL^{-1}(Song et al., 2015). In another study conducted, an even higher α-ketoisocaproate production of 86.55 gL^{-1} and a higher L-leucine conversion rate of 94.25 percent were achieved via three engineering strategies; altering the plasmid origin with various copy numbers, modulating the mRNA composition downstream of the initiation codon, and designing the ribosome binding-site synthesis sequences (Song et al., 2017).

2.7 PRODUCTION OF A-KETO-Γ-METHYLTHIOBUTYRIC ACID

E. coli cells overproducing pvLAAD managed to convert L-Met into α-keto-γ-methylthiobutyric acid, which is an indirect inhibitor of tumor cell growth and a methionine supplement in livestock feed. By using previously optimized conditions (Hossain, Li, Shin, Chen, et al., 2014). 70 g L^{-1} L-Met was converted by 20 g L^{-1} whole-cell biocatalyst into α-keto-γ-methylthiobutyric acid with a 71.2% yield. The LAAD variant harboring the K104R/A337S substitutions, generated by error-prone PCR, possesses a lower Km for L-Met (decreased from 305 to 238 mM), and 63.6 g L^{-1} of α-keto-γ-methylthiobutyric acid could be produced in 24 hours. This single-step enzymatic process for production of α-keto-γmethylthiobutyric acid has a great potential at the industrial level compared with the traditional multi-step chemical systems.

2.8 A-KETOISOVALERIC ACID

α-ketoisovaleric acid (α-KIV) is as a precursor in leucine and valine synthesis. It also serves as an initial compound in vitamin B5 biosynthesis (Chassagnole, Diano, Létisse, & Lindley, 2003). α-KIV is mainly synthesized via a multistep chemical method (i.e. the hydrolysis of azlactones and the Grignard reagents with diethyloxamates) (Cooper, Ginos, & Meister, 1983; Waters, 1947). These chemical processes are costly and complex which restrain the high industrial production of α-KIV. i et al. (2017) studied an alternative biotechnological way by expressing the L-AAD from *Proteus myxofaciens* ATCC 19,692 in *E. coli BL21* (DE3) as a whole-cell biocatalyst system. Under the optimized conditions, the α-ketoisovaleric acid production with the wild type L-AAD was 2.014 g/L. Using the 3D structural model of L-AAD from *P. myxofaciens* and the simulation results when docking with the L-valine, key amino acid residues (N100, Q276, R316, and F318) were identified as potential target for site-saturation mutagenesis. The evolved L-AAD improved the biotransformation to 8.197 g/L after combining the mutated sites (Li et al., 2017). the rational molecular engineering of the L-AAD using site-saturation mutagenesis improved the efficiency of the biocatalysis.

2.9 2,5-DIKETO-D-GLUCONATE

In one study, 50 g of d-glucose was converted to 2,5-DKG with a 92% conversion rate in 150 h by a mixed culture of two newly isolated strains, *Flavimonasoryzihabitans* and *Pseudomonas cepacian* (Sulo, Hudecová, Properová, Bašnák, & Sedláček, 2001) . The metabolic engineering of bacteria has also been described for the production of 2,5-DKG from glucose. *Gluconobacteroxydans* was used for the oxidation of 2-ketogluconate to 2,5-DKG by engineering the overexpression of 2-ketogluconate dehydrogenase (Kataoka, Matsutani, Yakushi, & Matsushita, 2015). 2,5-DKG was also prepared in high yield and in high broth concentration by a newly isolated strain of genus *Erwinia* cultivated in an aqueous nutrient medium in the presence of d-glucose, where the conversion of d-glucose to 2,5-DKG reached 90% yield within 31 h (Sonoyama, Yagi, Kageyama, & Tanimoto, 1984).

2.9 OTHER APPLICATIONS INVOLVING LAAD

P. mirabilis LAAD's reaction (whole *E. coli* recombinant cells) and a commercially available D-transaminase aided in resolving a racemic mixture of 2-amino-3(7-methyl-1-H-indazol-5-yl) propanoic acid (Hanson et al., 2008). This resulted in an intermediate synthesis of antagonists of calcitonin gene-related peptide receptors. By making use of a 1 L batch scale and 20 g of racemic substrate, 40 g D-Ala (as amino donor for D-transaminase), 100 g *E. coli* cells that express LAAD (27 U g^{-1} cell wet weight), 200 mg D-transaminase (Biocatalytics, 4.4 U mg^{-1} protein), at pH 7.5, with 1 L min^{-1} aeration and following downstream processing, a final yield of 68% with ee> 99% was reached (Hanson et al., 2008). By coupling this two-step reaction with formate dehydrogenase and lactate dehydrogenase to remove pyruvate (Fig. 1) reaction rate and product yield can be increased. Substituted D-phenylalanines are present in natural products (such as macrolide antibiotics) and active pharmaceutical ingredients and are employed as chiral structural fragments or peptide components. Nick Turner's group paired a phenylalanine ammonia lyase (PAL, EC 4.3.1.24) with *P. mirabilis* LAAD and converted p-nitrocinnamic acid into p-nitro-D-phenylalanine. The outcome was a 71% conversion and a 96% ee rate (Fig. 2) (Parmeggiani, Lovelock, Weise, Ahmed, & Turner, 2015). By using the H359Y PAL variant containing a 3.5-fold increase in D-formation

activity, p-nitro-D-Phe'see value increased to > 99% with a rate of 78% conversion (Parmeggiani et al., 2015). Reaction conditions were: 5 mM substrate, 5 M NH4OH, 25 mg mL^{-1}*E. coli* cells expressing PAL, 35 mg mL^{-1}*E. coli* cells expressing LAAD, and 40 equivalent ammonia-borane, at pH 9.6 and 37 °C.

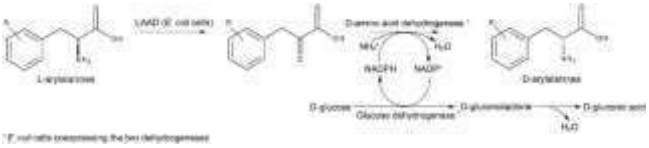

Fig.3. Enzymatic cascade by LAAD, D-amino acid dehydrogenase and glucose dehydrogenase for the synthesis of D-arylalanines by deracemization or stereo inversion (Parmeggiani et al., 2016).

This amination/deracemization cascade began at an efficient pace by utilizing numerous electron-deficient cinnamic acids that produced D-phenylglycine derivatives with sufficient optical purity. This led to increased L-isomer production when DAAO replaced LAAD. The same group paired the deamination of L-arylalanines by LAAD with the consumption of the produced keto acid by an engineered D-amino acid dehydrogenase to generate the corresponding D-amino acid (to achieve NADP+ recycling through glucose dehydrogenase, (Fig. 3) (Parmeggiani et al., 2016).

This could be a more beneficial way of generating the D-enantiomer when the corresponding L- or the DL-amino acid is commercially available in the future. Lysed *E. coli* cells expressing PmaLAAD converted natural L-amino acids into conforming α-keto acids, then asymmetrically reduced by L- or D-isocaproate reductases into (R)- or (S)-2-hydroxy acids (Fig. 4) (Busto, Richter, Grischek, & Kroutil, 2014).

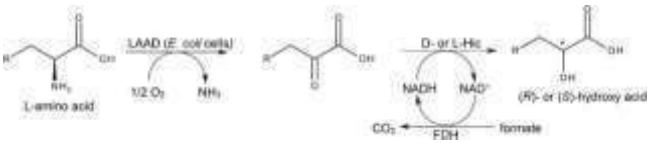

Fig.4. One-pot cascade enzymatic system based on LAAD and isocaproate reductases for the synthesis of enantiomerically pure (R)- or (S)-2-hydroxy acids starting from L-amino acids (Busto et al., 2014). D- or L-Hic, D- or L-isocaproate reductases; FDH, formate dehydrogenase.

Under 1 bar O2 pressure, 50 – 200 mM L-amino acids were fully converted into (S)- or (R)-hydroxy acids with a > 99% ee L-Tyrosine was transformed at 200 mM concentration (a value higher than its solubility) and 100 mg scale, and up to 0.5 g of L-isoleucine was converted (Busto et al., 2014). Among the production of 2-hydroxy acids, (R)- and (S)-4 hydroxy lactic acids are used to prepare various biologically active compounds such as Saroglitazar (Lypaglyn), which can treat type II diabetes.

III. CONCLUSION

Regardless, the fermentation method yielded higher amounts of pyruvate and a-KG, other a-keto acids yielded lower amounts. As a result, the genome-scale metabolic model of amino acid synthesis pathways should be investigated in order to accumulate more a-keto acids. Furthermore, because of its high conversion rate and simple and cost-effective separation process, the enzymatic bioconversion method may be a more effective approach for a-keto acid production. Enzymes with high substrate affinity and high conversion efficiency are expected to be developed in the future as new enzyme discovery tools and genetics information become available.Lastly, advances in metabolic engineering of whole-cell biocatalysts are propelling biocatalyst use to new heights. The ability to improve the overall performance of biocatalytic processes has been demonstrated by combining enzyme engineering with the modification of recombinant strain mechanism pathways. However, some work remains to be done, such as a better understanding of enzymes.

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Optimized recombinant *Bacillus Subtilis* 168 whole-cell catalyzes one-step biosynthesis of high fructose syrup

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***Abstract*—** *High fructose syrup is a sweetener that can replace sucrose, which is widely used in the food and beverage industries. In this study, codon-optimized Actinoplanes missouriensis CICIM B0118 (A) derived glucose isomerase heterologous expression was realized in the food-grade strain Bacillus Subtilis 168, and the recombinant Bacillus Subtilis 168/pMA5-xylA was successfully constructed. The whole-cell bioconversion system of D-glucose isomerization to the biosynthesis of D-fructose was optimized. The results showed that the concentration of biocatalysts was DWC 40 g/L, and the concentration of substrate D-glucose was 180 g/L, Mg2+ concentration 10 mmol/L, Co2+ concentration 1 mmol/L, continuous conversion at 70℃、220 R/min for 18 h, D-fructose concentration reached 103.32 g/L, the conversion rate was 57.4%, realizing the high fructose syrup one-step safe biosynthesis. This research provided an experimental and theoretical basis for the industrialized and safe biosynthesis of high fructose syrup and had an important reference value.*

Keywords*—** ***Glucose isomerase; isomerization; whole-cell catalysis; Bacillus Subtilis 168; high fructose syrup; biosynthesis.

I. INTRODUCTION

High Fructose Corn Syrup (HFCS), a purified, concentrated, an aqueous solution of functional saccharides currently used as a sugar substitute, is one of the most commonly used sweeteners in the production of nutritive beverages and foods, including soft drinks, ketchup, yogurts, ice-cream, chocolate milk, candies, jams, condiments, canned and packaged foods[1–3]. Besides, HFCS has been recently considered as a renewable resource for the production of 5-hydroxymethylfurfural and levulinic acid, which can be used in the synthesis of other valuable biopetrochemicals: plastics, green solvents, lubricants, and valuable biofuels[4–8]. The health benefits of HFS intake have been proven by insulin-independent metabolism, increased absorption of iron and zinc, enhanced ethanol metabolism, low-sugar and low-calorie contents, and desirable organoleptic properties [2,9]. Other advantages of HFS compared with other types of sugars include high sweetness, high solubility, and low viscosity, flavor enhancement, good humectant, does not cause any side effects in acidic foods and doesn't form crystals[3,9,10].

According to the fructose content, HFCS can be divided into three types HFS-42, HFS-55, and HFS-90. However, due to the low fructose content of HFS-42, the medical and health care value cannot be brought into full play, and it is easy to crystallize and precipitate during low-temperature storage and transportation, so HFS-55 with higher fructose concentration has become the mainstream product[1,2].The production methods of HFCS include chemical catalysis and enzymatic biocatalysis. The chemical catalytic method is that glucose is isomerized or acid hydrolyzed to fructose in an alkaline environment[2].

For more than a century, people have known that glucose can isomerize fructose through alkaline isomerization or acid hydrolysis. Therefore, this is a harsh process that leads to unacceptable sugar breakdown. Besides, the chemical synthesis of HFCS involves high calcination temperature and is not environmentally friendly[11–13]. The homogeneous Lewis acid generally has the problem of separation and recovery, and the synthesis of Sn-β zeolite is more complicated. On the other hand, Brønsted bases such as sodium hydroxide can also effectively isomerize glucose to fructose. In contrast, due to the severe degradation of fructose and glucose, they usually obtain a lower yield of glucose to fructose, and they also face the problem of separation and recovery[4,12,14].

Owing to the aforementioned drawbacks of environmentally unfriendly chemical synthesis, commercial HFCS has been mainly produced by microbial synthesis since the early 1970s after Yoshiyuki Takasaki discovered a thermo-stable glucose isomerase (GIase) enzyme from yeast [15]. GIase, also known as D-xylose isomerase, is a widely used enzyme for the production of HFCS. It catalyzes not only the conversion of D-glucose to D-fructose but also the conversion of D-xylose to D-xylulose [16]. With increasing HFCS consumption, the production level of GIase has drawn extensive attention in recent years. Many microbial strains, including *Streptomyces* sp. CH7[17], *Lactobacillus bifermentans*[18], *Bacillus coagulans* [19], *Streptomyces murinus*, *Hyperthermophilic Thermotoga* [20], and *Pseudomonas hydrophila* [21], have been reported to produce glucose isomerase. Because of the increased global demand for HFCS, the level of GIase production has gained considerable attention, especially in the food and beverages industry. Due to the low productivity and stability of enzymes produced by wild-type microorganisms under harsh conditions, a more efficient expression system is needed for the production of recombinant GIase with desired properties for large-scale production of HFCS.

To obtain an effective expression mechanism, GIase has been heterologously expressed in a variety of hosts[22,23], and a variety of fermentation techniques, including fed-batch and high-density fermentation, have been used. As a result, GIase expression has greatly improved; for example, Akdag et al.[24] announced that using a beet molasses-based feeding method, they achieved the highest recombinant GIase production, 35.3 U/mL, in E. coli. Due to its well-known genetics' history, short generation time, and suitability for low-cost high-density fermentation, *E.coli* is a common heterologous host for the expression of recombinant proteins[25]. However, improvements in GIase production, specially concerning HFCS-55 manufacturing, are still valued for industrial applications.

Although one-step biosynthesis of HFS-55 has achieved certain results, most of its host strains previously investigated with higher potential yields are *Escherichia coli* BL21, but in the catalytic process, BL21 may bring harmful toxin that do not meet the requirements of food safety into the target products[26–28]. Therefore, it is very meaningful to realize the heterologous expression of glucose isomerase in food safety strains with clear research background and to safely synthesize HFS-55 high fructose syrup in one step.

However, food safety requires a thorough investigation of food-grade microorganisms. *Bacillus subtilis* 168 and some other non-pathogenic related *Bacillus* species, which are free of exotoxins and endotoxins, and have a recognized history of safe use in foods are very useful for fermentation and large-scale cultivation[29].

Thus, an efficient expression of recombinant GIase in a generally regarded safe strain is necessary for improved economic HFCS-55 manufacturing and can contribute more to food security. In this study, a bacterial strain *Bacillus Subtilis* 168 was used as the host cell to heterologously express the glucose isomerase from *A. missouriensis* CICIM B0118 (A). The recombinant *Bacillus Subtilis* 168/pMA5-xylA was constructed. Using recombinant *Bacillus Subtilis* 168 as a whole-cell catalyst, the whole-cell catalytic conditions for isomerization of D-glucose to D-fructose were optimized. Under the optimal transformation system, HFS-55 high fructose syrup was synthesized safely in one step.

II. MATERIALS AND METHODS

2.1. STRAIN AND PLASMID

E. coli JM109, *Bacillus Subtilis* 168, and plasmid pMA5 were all preserved in our laboratory stock. The recombinant plasmid pET28a-xylA(genebank accession number: FJ858195.1) was synthesized by Suzhou Jinweizhi Biotechnology Co., Ltd.

2.2. EXPERIMENTAL REAGENTS

Restriction enzyme NdeI and MluI, high fidelity enzyme, and homologous recombination enzyme cloning kit were purchased from Takara company. Small Plasmid Extraction Kit, agarose gel DNA recovery kit, and so on are purchased from Shanghai Jarry Bioengineering Co., Ltd., chloramphenicol, isopropyl -β-D- IPTG are purchased from Shanghai Bioengineering (Shanghai) Limited by Share Ltd, D- glucose is purchased from Mclean, D- fructose is purchased from Aladdin, glycerol, imidazole, sodium chloride and so on are all commercially analytical grades.

2.3. CULTURE MEDIUM AND CONDITIONS

LB medium: yeast extract 5 g / L, tryptone 10 g / L, sodium chloride 10 g / L (solid medium with 1.5% agar powder) for *E. coli* culture. The culture temperature was 37 ° C. TB medium: (12 g tryptone, 24 g yeast extract, 4 ml glycerol, 125.5 g K_2HPO_4, 23 g KH_2PO_4) per liter used for *B.subtilis*168 culture. The culture temperature was 30 ° C.

2.4. METHODS

2.4.1. CONSTRUCTION OF THE RECOMBINANT STRAIN

2.4.1.1. CONSTRUCTION OF RECOMBINANT *E. COLI* JM109/PMA5-XYLA

The recombinant plasmids pET28a-xylA and -xylA-F: ttattcagatgaaaa**catatg** atgagtgttcaagccacccg(**NdeI**) ,and -xylA-R: atttcgacctctaga**acgcgt** ttagcgcgcacccagc(**MluI**) were used as plasmid template and primers respectively for PCR amplification. After ligation, the conjugated products were transformed into *E. coli* JM109 competent cells for cloning purpose, coated with LB plate containing 50 μ g / mL Kanamycin, and cultured overnight at 37 ° C incubator. The transformants were selected for colony PCR verification. The correct transformants were cultured in 10 ml liquid containing 50 μ g / mL Kanamycin for 8-12 h at 37 ° C and 180 R / min. Then, the strains were preserved and the recombinant plasmids were extracted. The extracted recombinant plasmids were sent to Suzhou Jinweizhi Biotechnology Co Ltd, for sequencing analysis. The sequencing results were analyzed by snap gene software, and the correct strain was named *E. coli* JM109 / pMA5- xylA, harboring the recombinant plasmid pMA5-xylA.

2.4.1.2. CONSTRUCTION OF RECOMBINANT *BACILLUS SUBTILIS* 168/PMA5-XYLA.

The recombinant plasmid pMA5-xylA was electroporated into *Bacillus Subtilis* 168 competent cells, coated on LB solid plate containing 50 μg / mL Kanamycin, and cultured upside down in a 37 ° C incubator overnight. The correct recombinant strains were named *Bacillus Subtilis* 168/pMA5-xylA

2.5. EXPRESSION OF GLUCOSE ISOMERASE (GI)

The preserved recombinant *Bacillus Subtilis* 168/pMA5-xylA was activated on LB plate containing 50 μ g / mL Kanamycin. The single colony was transferred to a 10 mL TB liquid medium (containing 50 μ g / mL kanamycin) and cultured at 30 ° C and 200 rpm for 12-24 h. Then, the 50 ml TB liquid medium (containing 50 μ g / mL kanamycin) was transferred to a 1% inoculation amount and cultured at 30 ° C and 200 rpm for 3-4 h. IPTG was added and cultured in a 30 ° C shaker for 12 h. The cells were centrifuged at 4 ° C and 10 000 rpm for 10 min. The cells were washed twice with PBS (pH 7.4, concentration 50 mmol / L) buffer and then suspended. After adding the appropriate amount of lysozyme, the cells were placed on ice for 2 ~ 3 h. The cells were broken by an ultrasonic crusher. The resultant solution was centrifuged for 20 min at 4 ° C and 12 000 R / min. The supernatant was used for SDS-PAGE analysis.

2.6. THE WHOLE-CELL CATALYTIC ACTIVITY OF RECOMBINANT *BACILLUS SUBTILIS* 168

Reaction system[30] (25 ml): 2.5 ml 8 mM $MgCl_2$, 2.5 ml 200 μ mol / L $CoCl_2$, 12.5 ml, 2 mol / L D-glucose and 1 g recombinant *Bacillus Subtilis* 168. The reaction was incubated at 70 ° C for 1 h and stopped ice bath for 5 min. The supernatant was centrifuged and analyzed by HPLC. The HPLC conditions were: RID differential detector, Hi-plex-Ca (300 mm × 7.7 mm) column, ultrapure water as mobile phase, the flow rate of 0.6 ml/min, column temperature of 80 ° C, injection volume of 10 μ L, detection wavelength of 210 nm.

2.7. OPTIMIZATION OF WHOLE-CELL CATALYSIS CONDITIONS

2.7.1. EFFECT OF CELL CONCENTRATION ON WHOLE-CELL CATALYTIC SYSTEM

Under the conditions of substrate D-glucose concentration of 1 mol / L, pH 7.0, Mg^{2+} concentration of 10 mmol / L, Co^{2+} concentration of 1 mmol / L, DCW of 20, 30, 40, 50, and 60 g / L, respectively, the reaction time was 1 h at 70 ° C, and the D-fructose content in the transformation solution was detected by HPLC to determine the optimal bacterial concentration.

2.7.2. EFFECT OF METAL IONS ON WHOLE-CELL CATALYTIC SYSTEM

Under the conditions of cell concentration 40 g / L, substrate D-glucose concentration 1 mol / L, pH 7.0, different metal ions Ba^{2+} (1 mmol / L), Cu^{2+} (1 mmol / L), Fe^{2+} (1 mmol / L), Mg^{2+} (10 mmol / L), Ca^{2+} (1 mmol / L), Mn^{2+} (1 mmol / L), Co^{2+} (1 mmol / L) and Zn^{2+} (1 mmol /

L) were added respectively, and the reaction time was 1 h at 70 ° C. The content of D-fructose in the conversion solution was determined by HPLC.

2.7.3. EFFECT OF SUBSTRATE D-GLUCOSE CONCENTRATION ON WHOLE-CELL CATALYTIC SYSTEM

Under the conditions of DCW 40 g / L, pH 7.0, Mg^{2+} 10 mmol / L and Co^{2+} 1 mmol / L, the concentration of substrate D-glucose in the whole-cell transformation system was controlled to be 0.5 mol / L, 1.0 mol / L, 1.5 mol / L and 2.0 mol / L, and the reaction time was 1 h at 70 ° C. The content of D-fructose in the transformation solution was determined by HPLC.

2.7.4. EFFECT OF PH ON WHOLE-CELL CATALYTIC SYSTEM

Under the conditions of cell concentration DCW 40 g / L, substrate D-glucose 1 mol / L, Mg^{2+} 10 mmol / L and Co^{2+} 1 mmol / L, the reaction was carried out at pH 4.0-10.0 (interval 1.0) and 70 ° C for 1 h respectively. The content of D-fructose in the conversion solution was determined by HPLC to determine the optimal reaction pH. The buffer solutions included acetic acid sodium acetate buffer with pH 4.0, 5.0, and 6.0, Tris-HCl buffer with pH 7.0 and 8.0, glycine NaOH buffer with pH 9.0 and 10.0, and the concentrations were all 50 mmol / L.

2.7.5. EFFECT OF REACTION TEMPERATURE ON WHOLE-CELL CATALYTIC SYSTEM

Under the conditions of cell concentration DCW 40 g / L, substrate D-glucose 1 mol / L, pH 7.0, Mg^{2+} 10 mmol / L and Co^{2+} 1 mmol / L, the whole-cell catalytic system was placed in a constant temperature water bath at 40, 50, 60, 70 and 80 ° C for 1 h and the D-fructose content in the conversion solution was detected by HPLC to determine the optimal reaction temperature.

2.8. WHOLE-CELL BIOCATALYTIC BIOSYNTHESIS OF HIGH FRUCTOSE SYRUP

The preserved recombinant *Bacillus Subtilis* 168/pMA5-xylA was activated on an LB plate containing 50 μ g / ml kanamycin. The single colony was transferred to 10 ml TB liquid medium (containing 50 μ g /mL kanamycin) for 12 h at 37 ° C and 200 rpm/min, and then transferred to 200 ml TB liquid medium (containing 50 μ g / mL kanamycin) at 1% inoculum for 3-4 h at 37° C and 200 rpm/min. IPTG was added and cultured in a 30 ° C shaker for 12 h. After centrifugation for 10 min at 4 ° C and 10 000 rpm/min, the cells were collected and used as the whole-cell biocatalysts.

Under the optimal conditions of whole-cell transformation (cell concentration DCW 40 g / L, substrate D-glucose concentration 1 mol / L, pH 8.0, Mg^{2+} concentration 10 mmol / L, Co^{2+} concentration 1 mmol / L), the reaction was carried out on a magnetic stirrer at 70 ° C and 220 rpm for 18 h. 1 ml sample was collected every 3h, centrifuged at 4 ° C and 12000 R / min for 15 min, and the supernatant was analyzed by HPLC to detect the content of D-fructose.

III. RESULTS AND DISCUSSION

3.1. CLONING AND EXPRESSION OF XYLA GENE ENCODING GLUCOSE ISOMERASE

The gene sequence of glucose isomerase from *A. missouriensis* CICIM B0118 (A) was retrieved from the NCBI database [31], with a length of 1185 bp, which was submitted to Suzhou Jinweizhi Biotechnology Co Ltd for artificial synthesis, and the recombinant plasmid pET28a-xylA was obtained. The plasmid was used as a template for PCR amplification. The electrophoretic results are shown in Figure 1a. The specific bands (Lane 1 and 2 in Figure 1a) are consistent with the target. The recombinant *E. coli* JM109 / pMA5 -xylA was constructed by transferring the ligation product into *E. coli* JM109 competent cells. The recombinant plasmid pMA5-xylA obtained from the culture of recombinant *E. coli* JM109 /pMA5-xylA was transferred into *Bacillus subtilis* 168 to construct recombinant *Bacillus subtilis* 168/pMA5-xylA. The expression of GI was induced by IPTG and analyzed by SDS-PAGE. The results are shown in Fig. 1b. It can be seen from Lane 2 and 3 in the figure that the recombinant strain *Bacillus subtilis* 168/pMA5-xylA has an obvious protein expression band at the molecular weight of 43kDa as reported previously[30], that is, GI was successfully expressed in *Bacillus subtilis* 168.

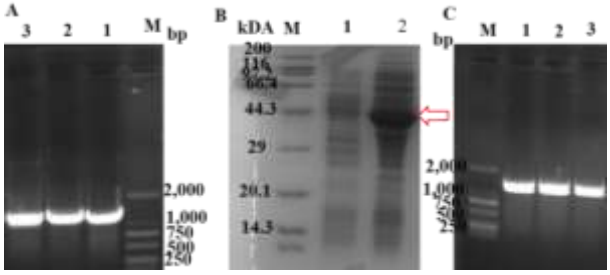

Fig. 1 Gene cloning and expression analysis of GI

Fig. A: PCR amplification of xylA gene, M: 2,000 BP nucleic acid marker; Fig. B: heterologous expression of glucose isomerase (GI), M: protein marker, lane 1: *B.subtilis* 168 wild type broken cells supernatant, lane 2: *B.subtilis* 168/pMA5-xylA broken cells supernatant; Fig. C: M:2,000 BP nucleic acid marker ,lane 1,2,& 3:*B.subtilis* 168/pMA5-xylA transformant colony PCR verification results.

3.2. WHOLE-CELL CATALYTIC PERFORMANCE TEST

Using whole-cell as a catalyst has gradually replaced crude enzyme or pure enzyme in the biosynthesis of target products because cells can protect the enzyme from the adverse environment and shear force, and batch conversion can be repeated, and no cofactor or coenzyme need to be added to the reaction process[32]. Therefore, the whole cell of *Bacillus subtilis* 168 will be used as a catalyst for D-glucose isomerization to synthesize D-fructose.

Firstly, the whole-cell catalytic performance of recombinant *Bacillus subtilis* 168 was tested to see whether it can isomerize D-glucose to D-fructose. Results as shown in Figure 2, the whole-cell biocatalyst of *Bacillus subtilis* 168 could isomerize D-glucose to produce D-fructose. In the figure, the peak time of D-glucose and D-fructose was 17.106 min and 21.380 min respectively, which indicated that *Bacillus subtilis* 168 had the potential to produce high fructose syrup. The next step is to optimize the whole-cell catalytic system of D-glucose isomerization to D-fructose, and explore the effects of cell concentration, divalent metal ions, substrate D-glucose concentration, reaction pH, and reaction temperature on D-fructose synthesis.

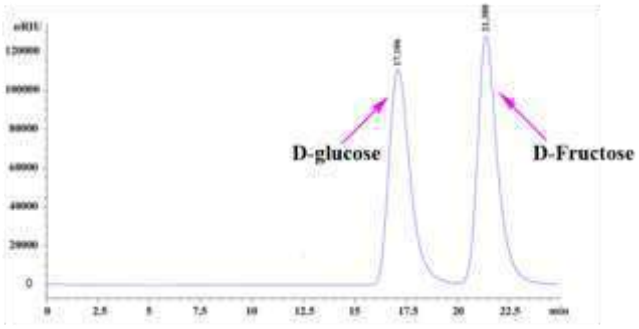

Fig. 2 HPLC detection results of the conversion solution

3.3. OPTIMIZATION OF WHOLE-CELL CATALYSIS CONDITIONS

3.3.1. EFFECT OF CELL CONCENTRATION ON BI OTRANSFORMATION OF D-GLUCOSE TO D-FRUCTOSE

The cell concentration reflects the content of glucose isomerase GI in the conversion system to a certain extent. Therefore, the different cell concentrations may affect the catalytic process of D-glucose isomerization to D-fructose, as shown in Figure 2. With the increase of cell concentration, D-fructose content increased; when cell concentration exceeded 40 g / L, although substrate concentration increased, D-fructose content decreased. When the cell concentration DCW was 40 g / L, the D-fructose content was the highest, which was 3.1 g / L. the optimal cell concentration for whole-cell catalytic synthesis of D-fructose was 40 g / L.

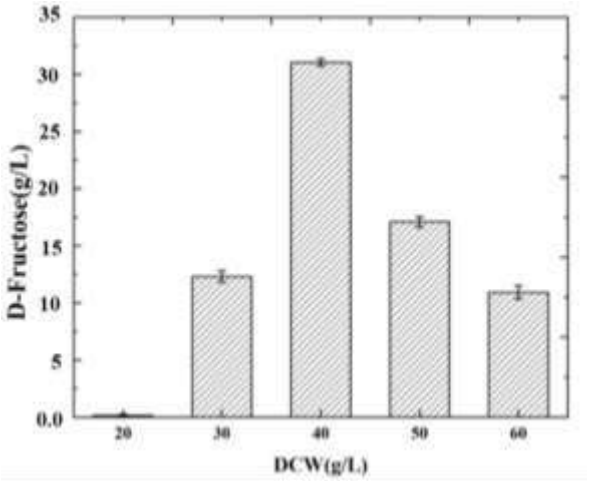

Fig. 3 The effect of cell concentration on D-glucose biotransformation to D-fructose

3.3.2. EFFECTS OF DIVALENT METAL IONS ON BIOTRANSFORMATION OF D-GLUCOSE TO D-FRUCTOSE

GI is a metal enzyme, combined with divalent metal ions, in the process of D-glucose biotransformation to D-fructose, metal ions are needed to assist the isomerization reaction, and divalent metal ions are very important for the activity and stability of GI [25]Different GI needs different divalent metal ions. Most of the reported GI mainly use Mg^{2+}, Mn^{2+}, Co^{2+} [33], or two metal ions as auxiliary catalysts [24,33].

Different divalent metal ions may have different effects on the catalytic process of D-glucose isomerization to D-fructose. Therefore, the effects of different divalent metal ions Ba^{2+} (1 mm), Cu^{2+} (1 mm), Fe^{2+} (1 mm), Mg^{2+} (10 mm), Ca^{2+} (1 mm), Mn^{2+} (1 mm), Co^{2+} (1 mm) and Zn^{2+} (1 mm) on D-glucose isomerization to D-fructose were tested in this study. The results are shown in Fig. 3. The results showed that the presence of divalent metal ions Cu^{2+}, Ca^{2+}, and Zn^{2+} was not conducive to the isomerization of D-glucose to produce D-fructose. The divalent Cu^{2+}, Ca^{2+}, and Zn^{2+} exerted the inhibition effect on the activity of GI, so it was not conducive to the synthesis of D-fructose. The presence of bivalent metal ions Ba^{2+}, Fe^{2+}, Mg^{2+}, $Mn^{2+,}$ and Co^{2+} promoted the synthesis of D-fructose, especially the presence of Mg^{2+} and Co^{2+}, which made the D-fructose content reach 4.95 g / L and 2.96 g / L respectively, 4.85 and 2.9 times of the control (1.02 g / L).

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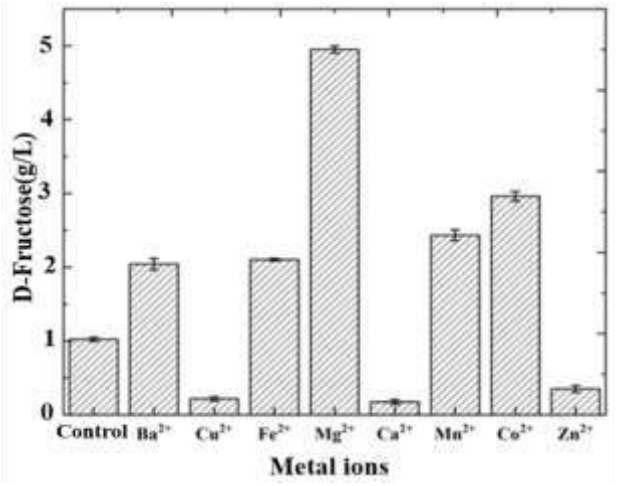

Fig. 4 The effect of divalent cations on the biotransformation of D-glucose into D-fructose

3.3.4. EFFECT OF SUBSTRATE D-GLUCOSE CONCENTRATION ON THE BIOTRANSFORMATION OF D-GLUCOSE TO D-FRUCTOSE

An appropriate increase of substrate concentration can accelerate the reaction rate, which is conducive to product synthesis. It can be seen from Fig.5 that the D-fructose content increases with the increase of D-glucose concentration when the substrate D-glucose concentration is in the range of 0 ~ 1.0 mol / L. when the substrate D-glucose concentration is 1.0 mol / L, the D-fructose content reaches the highest, 27 g / L. When the concentration of substrate was higher than 1.0 mol / L, the concentration of D-glucose increased, but the content of D-fructose decreased. It may be that if the concentration of D-glucose exceeded a certain value, the activity of the GI enzyme would be inhibited, which was not conducive to the isomerization of glucose to D-fructose. Therefore, the content of D-fructose decreased.

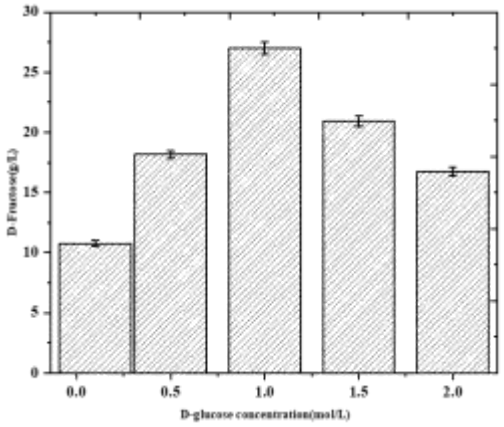

Fig. 5 The effect of substrate D-glucose concentration on D-glucose biotransformation to D-fructose

3.3.5. EFFECT OF PH ON BIOTRANSFORMATION OF D-GLUCOSE TO D-FRUCTOSE

In the process of isomerization of D-glucose to D-fructose, the optimal pH is generally 7.0 ~ 9.0, and pH has a significant effect on the biotransformation rate of D-fructose. A few GI have good catalytic activity in weak acid pH environments, while isomerization under weak acid conditions can reduce the formation of by-products [35],35]. It can be seen from Fig. 6 that the GI from codon-optimized *A. missouriensis* CICIM B0118 (a) in this study is at a high level in the range of pH 7.0 ~ 9.0, reaching 44.83 g / L, 48.10 g / L, and 46.56 g / L, respectively. However, under acidic pH 4.0 ~ 6.0 and peralkaline pH 10.0, the content of D-fructose was not high, which was not conducive to the isomerization of D-glucose to D-fructose.

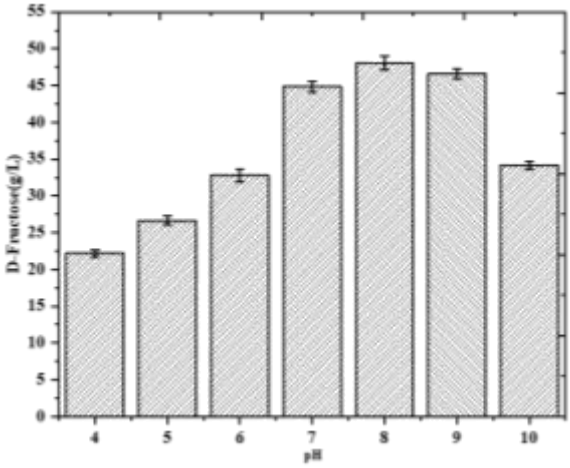

Fig. 6 The effect of pH on D-glucose biotransformation to D-fructose

3.3.5. EFFECT OF REACTION TEMPERATURE ON BIO-TRANSFORMATION OF D-GLUCOSE TO D-FRUCTOSE

Previous studies have shown that the isomerization of D-glucose catalyzed by GI is a thermodynamic equilibrium reaction. With the increase of temperature, the catalytic equilibrium moves towards the formation of D-fructose, and high temperature is conducive to the formation of D-fructose [1,10]Therefore, this study also studied the reaction temperature of the whole-cell catalytic system for D-glucose isomerization to D-fructose, and the results are shown in Fig.7. It can be seen from the figure that GI from *A. missouriensis* CICIM B0118 (a) can tolerate a wide range of temperatures 70 ℃ is the optimal temperature for biotransformation. At this time, the D-fructose content is 50.34 g / L; at 90 ℃, the D-fructose content is still at a high level, 36.23 g / L, only 16.22% lower than the

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optimal temperature. Due to the rapid decrease of enzyme activity at 80 ℃ in Sweetzyme®, a commercial enzyme, the content of D-fructose decreased critically. In contrast, GI from *A. missouriensis* CICIM B0118 (A) has higher catalytic activity at high temperatures, which is beneficial to the industrial production of high fructose syrup.

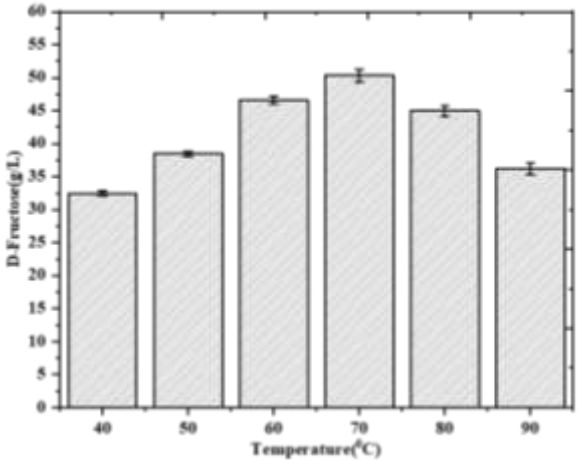

Fig. 7 The effect of reaction temperature on the biotransformation of D-glucose to D-fructose

3.3.6. WHOLE-CELL CATALYTIC SYNTHESIS OF HIGH FRUCTOSE SYRUP

Under the optimal transformation conditions, 4 g DWC were added to 100 ml Tris-HCl buffer (pH 7.0) of 180 g / L glucose, 10 mmol / L Mg^{2+} and 1 mmol / L Co^{2+}, and transformed at 70 ° C and 220 rpm. The supernatant was centrifuged every 3 h, and the content of D-fructose was determined by HPLC. Results as shown in Fig. 8, in the early stage of transformation (0 ~ 15 h), D-fructose concentration gradually increased with the consumption of substrate D-glucose. At 15h, the remaining D-glucose concentration in the transformation solution was 71.87 g / L, D-fructose concentration reached 103.23 g / L, and the conversion rate was 57.35%. After that, D-glucose consumption slowed down, the reaction basically tended to balance, and D-fructose concentration almost remained constant, 18.5% 23 g / L, D-fructose concentration reached 103. 32 g / L and the conversion rate was 57. 4%. It can be seen that the recombinant strain *B.subtilis* 168/ pMA5-xylA can safely synthesize HFCS-55 high fructose syrup in one step, which provides an important reference for the sustainable and safe industrial production of HFCS-55 high fructose syrup.

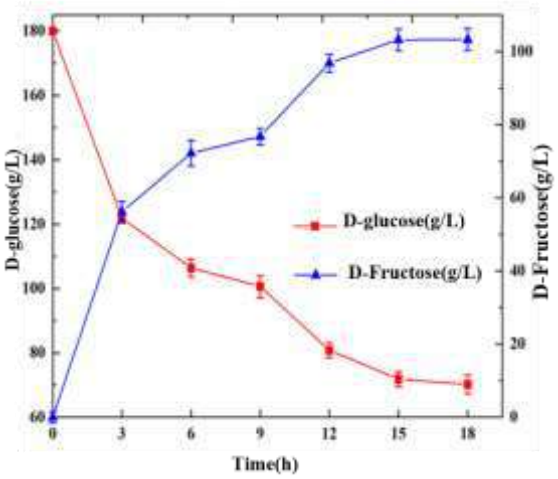

Fig. 8 Whole cells catalyze the isomerization of D-glucose to D-fructose

IV. CONCLUSION

In this paper, the heterologous expression of GI from *A. missouriensis* CICIM B0118 (A) in food safety bacterial strain *Bacillus subtilis* 168 was successfully realized for the first time, and the recombinant Bacillus subtilis 168 /pMA5-xylA was constructed to construct the recombinant *Bacillus subtilis* 168 pMA5-xylA and was used for the whole-cell biocatalysis, the whole-cell transformation conditions (cell concentration, a divalent cation, substrate concentration, pH and temperature) of D-glucose isomerization to D-fructose were optimized. Under the optimal transformation conditions, the concentration of D-fructose reached 103.32 g / L and the conversion rate was 57.4% for 18 h. One-step safe biosynthesis of HFCS-55 was realized. It is found that GI from *A. missouriensis* CICIM B0118 (A) has good activity in a wide range of temperatures and pH, respectively 60 ~ 85 ° C and pH 7.0 ~ 9.0, which are suitable for industrial production of HFCS-55 high fructose syrup. The isomerization of D-glucose to D-fructose needs the assistance of divalent metal ions. Divalent metal ions have three functions on GI: activating, stabilizing, and improving the affinity of the D-glucose- GI enzyme. The next step will focus on the effect of divalent metal ions on the biotransformation of D-fructose to realize the sustainable and safe industrial production of high fructose syrup, we should improve the enzyme activity of GI and accelerate the biotransformation rate.

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Effect of Polysaccharides (pectins) on Postprandial Glucose

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Abstract— *The cultivar Ghars is the second cultivar of economic importance for Algeria. It's a soft date. So far, the only product derived from this date is "date's syrup". This product is developed by diffusion. The date is a food rich in nutrients. It is known that the consumption of carbohydrate foods causes different elevations of blood sugar. Some studies classify dates as a high glycemic food. The objective of this study is to follow the evolution of the postprandial glycemia of these foods and to characterize them nutritionally. This analysis is carried out according to the method recommended by FAO. Fourteen healthy, non-diabetic volunteers participated in the test. The results show a peak of control hyperglycemia (glucose) is significant (1.82 g / l ± 0.25) compared to dates (1.30 g / l ± 0.20) and their syrup (1.55 g / l ± 0.1), because glucose is a simple carbohydrate, its absorption is fast. These results are explained by the composition of these foods in polysaccharides (date 4.15% ± 0.02) (syrup 3.86% ± 0.38). Since polysaccharides facilitate intestinal transit, slow gastric emptying and slow the absorption of glucose. These results likely suggest that dates of this cultivar could be non-hyperglycemic food.*

Keywords— ***Algeria, Dates, Hyperglycemia, Polysaccharides, Syrup.***

I. INTRODUCTION

Dates constitute the main food for the Saharian population, rich of nutrients (Al-hooti et al, 2002 ; Sidhu et al., 2003). They are available throughout the year. However, many date cultivars remain poorly exploited or even marginalized. And some are endangered others have disappeared. It is therefore, important to take in consideration this palm date's heritage.

In addition, carbohydrate foods can contribute to rise blood glucose levels in a different way depending on the type of carbohydrate in a food (David, 2011). So, we are talking about the food's glycemic power. Indeed, the speed of digestion of carbohydrates in a food depends on its complexity of nutrient interaction in the food bolus (fiber content, fat content, technological treatments, varietal differences in raw materials, etc.) (David, 2011).

Recently, the concepts of glycemic index (GI) and glycemic load (G l) are two tools allowing a qualitative and quantitative estimation of ingested carbohydrates and their impact on their assimilation in the body. Thus, glycemic index measurement was developed as a method of classifying carbohydrates food according to the rise of blood sugar or postprandial blood glucose (glycemic effect) following food intake. Many studies confirm the virtues of food with low glycemic index, for health in general especially during physical activity but also in the case of pathologies such as diabetes and obesity.

The date is a carbohydrate nutrient. It is classified as a hyperglycemic food (GI 95-107) (cultivar not specified). However, the indigenous consumer shows the opposite, in particular for some cultivars (Mimouni et al, 2014). Soft dates of the cultivar "Ghars" are apt to produce a locally popular co-product known as "date's syrup". This co-product is highly recommended by the population of the Saharian region (Mimouni and Siboukeur, 2011). It is exceptionally used to sweet some dishes including couscous. In this context, we proposed to study the glycemic effect of these dates and their product

in order to manage the consumption of this fruit for the different categories: healthy, diabetic, obese.

II. MATERIALS AND METHODS

2.1. Plant material

Dates: Dates, the study material, are harvested at maturity from the cultivars: "Ghars" planted in the south-eastern Algeria, Ouargla (Figure 1A).

Date's syrup: Ghars cultivar dates were used to make the syrup. Our choice is based on their ability to turn into syrup due to their soft consistency which allows them to give good results (Figure 1B).

2.2. Other Material (glucose)

An amount of glucose about 50 g, equivalent to 166.66 ml of 30% glucose solution was used as a reference food (glycemic index = 100 by reference). This solution was provided by the laboratory of Mohamed Boudiaf hospital in Ouargla

Volunteers: According to Fao (1997) recommendations, a minimum of 6 subjects is required for the determination of the GI of a food taking into account the intra-subject variability. In our case, 12 non-diabetic, healthy and non-obese volunteers (BMI <40 kg / m^2) divided into two groups according to the nature of the food tested took part in the tests.

(A) (B)

Fig.1: Plant material. A: Ghars date's cultivar, B: Date's syrup

2.3. Method of analysis

2.3.1. Sample preparation

First, date defecation is performed. It consists of boiling 100 ml of the sample in a boiling water bath for 30 minutes. After cooling, the volume is adjusted to 100 ml and then filtered. Ten milliliters of 10% lead acetate are added to the filtrate. After stirring the solution, it is filtered. The excess lead is removed by adding about 1 g of sodium carbonate to the filtrate. A second filtration is carried out to verify the definitive absence of lead (absence of precipitate) (Girard, 1962).

2.3.2. Syrup preparation

The extraction technique involves the preparation of the sample and the extraction of soluble solids by a physical process. To have a good quality product, you have to start from a good quality of raw material. This is why we started with the dates sorting**.** The extraction method adopted is inspired by the extraction of sugar from beet and whose principle is based on the passage in solution through a permeable cellulose membrane (according to the laws of diffusion by passive transport) of juice's soluble materials (Alberts et al., 2002). For the present study, sugars are extracted by diffusion using water heated to 30 ° C as a solvent. This temperature has the advantage of limiting the transfer of impurities into the date juice. The diffusion phenomenon is based on the movement of molecules from high concentration (sugars and soluble substances stored in cell tissue) towards the low concentration (water). Then the filtrate is subjected to a concentration at 60 ° C (Figure 2). The purpose of this operation is to avoid syrup's microbial deterioration and thus to obtain a saturated syrup with a degree of Brix 75 ° Bx. This temperature was used to avoid the destabilization of sugars (caramelization, formation of furfural derivatives, etc.) (Mimouni and Siboukeur, 2015 (a); Mimouni et al., 2015 (c)).

2.3.3. Biochemical analysis

Total sugars are determined according to the Dubois method (Dubois et al., 1956). The principle is to measure the neutral carbohydrates with the phenol-sulfuric reagent of 1 ml of sample in the presence of 1 ml of phenol (5%) and 5 ml of concentrated sulfuric acid, then to measure their absorbance at 485 nm. The reduction in the sugar content is determined by the Bertrand method (Audigie et al., 1995). In an alkaline and hot medium, reducing carbohydrates have reducing properties vis-à-vis the copper ion (Cu_2^+). This method is based on the reduction of cupro-alkaline liquor. The sucrose content is

determined by the following formula: Sucrose% = (total sugars% - reducing sugars %) x 0.95. The determination of glucose is carried out by an enzymatic - colorimetric method. Glucose is oxidized by dissolved oxygen to gluconic acid. The reaction is catalyzed by glucose oxidase). The optical density (OD) of the mixture is read after 10 minutes of incubation at 505 nm (Aoac, 2005). The fructose dosage is determined by a chemical method. Ketohexoses are much less resistant to the action of hot hydrochloric acid than aldohexoses. They give rise to hydroxy-methyl-furfural, which reacts with resorcinol to form a colored complex, in red the optical density is read at 420 nm (Aoac, 2005). The polysaccharides (pectins) are determined in the form of calcium pectate, after extraction with hot water, then by saponifying with NaOH, and precipitation with $CaCl_2$ in acetic medium (Aoac, 2005). The pectin content is expressed as a percentage of dry matter.

2.3.4. Postprandial response Monitoring

This step involves volunteers whose thier blood sugar is tested after ingesting the reference food (glucose) for 120 minutes (1st visit) and the test food (dates and syrup) (2nd and 3rd visit) (Figure 2).

Conduct of testing

The tests take place in the same laboratory of the Biological Sciences Department at the University of Ouargla. Before tests, recommendations are made to the volunteers. They should have a light dinner of low GI foods the night before and the timing should ensures that they have fasted for 10 hours before the first tests. Testing begins at around 8 a.m., with a basal blood test using a lancing device. During the first visit, 50 g of glucose are served and consumed by each volunteer. During the second and third visit, a quantity of dates (70g) or syrup (62g) able to provide respectively around 50g of carbohydrates is served and consumed under same conditions. The blood glucose reading is taken every 15 min. then every 30 min. The count begins when the subject is feeding (t0). In that respect, blood samples are taken at t0, at t0 + 15, at t0 + 30, at t0 + 45, at t0 + 60, at t0 + 90 and at t0 + 120 min (David, 2011). The evolution of postprandial glucose is carried out according to the Matlab program (trapezoids), using an application called “IG Dates” (Figure 2) (Fao, 1997).

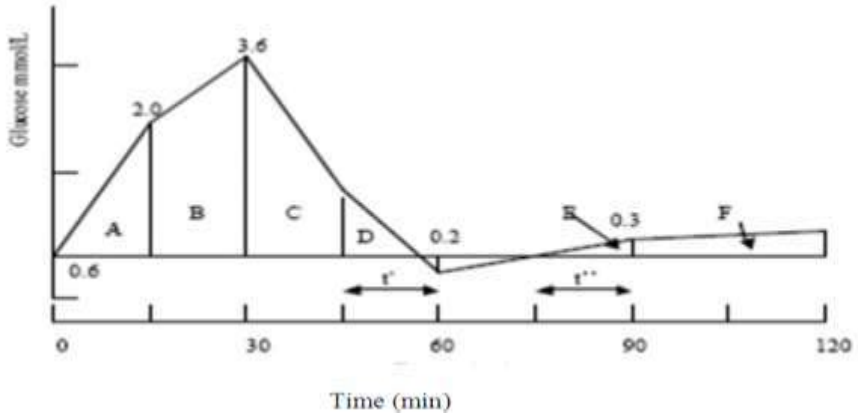

Fig.2: Illustration of the glycemic response evolution (according to Fao / Who, 1997)

III. RESULTS AND DISCUSSION

3.1. Biochemical characterization of dates and their syrup

The biochemical composition of dates and their syrup is illustrated in Table 1. These results show that the dates of cultivar Ghars contain a significant amount of total sugars (72% ± 0.02), a content of reducing sugars (70.98 % ± 0.02), sucrose (1.02% ± 0.2), glucose (32% ± 0.01), fructose (38.98% ± 0.04) and pectins (4.15% ± 0, 02). Many authors, having worked on several date cultivars, affirm that date sugars will vary according to cultivar, pollen, maturity and climate (Munier, 1973; Ahmed and Ramaswamy, 2006; Elleuch et al, 2008; Kulkarni et al, 2008; Biglari et al, 2009; Iqbal et al, 2011). The sugars nature will also vary depending on the consistency of the date (Mimouni and Siboukeur, 2011; Mimouni et al, 2014). Soft cultivars are very rich in reducing sugars, unlike sucrose (Elleuch et al, 2008; Siboukeur, 1997). Several authors report that dates contain significant amounts of total sugars. Namely: (Aleid, 2006) (81%); (Mimouni, 2009) (67.33 - 71.79%); (Al-gboori and Krepl, 2010) (86.10 - 87.91%). The values recorded in this study are within the range cited by these authors; results reported in a lot of works depend in part on the analytical method used. Glucose and fructose result from the reversal of sucrose by invertase during date’s maturity. In comparison with the total sugar content, it is

observed that reducing sugars predominate in the cultivar studied. Dosing sugar of the cultivar Ghars at the same tmar stage by an auto-analyzer (SKALAR) show that the content of reducing sugars is equal to 62.41% (Siboukeur, 1997). Values recorded by Al-gboori and Krepl. (2010) are 73.40 - 82.70%, which indicate that their study material is composed of soft cultivars. Soft dates contain a low level of sucrose. This may be due to the high water content that provides a favorable environment for invertase activity. In addition, the polysaccharide content recorded in this study is within the range reported by the authors (5 to 8%) (Gamal et al, 2009).

Regarding, the date syrup developed by the adopted method has a clear appearance (Figure 1), which allowed us to avoid resorting to clarification processes. The date-derived product is characterized by a predominance of carbohydrates (Bahramian et al, 2011; Ganbi, 2012; Queshi et al, 2012; Siboukeur et al, 2013). Date syrup contains total sugars (83.5% ± 0.28), reducing sugars (78.12% ± 0.12), sucrose (0.5% ± 0.02), glucose (27%) ± 3.23) and fructose (35% ± 0.001). Many authors claim that date extracts have appreciable contents of three major sugars, namely glucose, fructose and sucrose (Bahramian et al, 2011; Ganbi, 2012; Siboukeur et al, 2013). The total sugar content is comparable to that reported by some authors in Saudi Arabia (Gamal et al, 2009) (74%) and (Ammar, 2012) (73%). The content of reducing sugar is very high compared to the sucrose one. These results are similar to those found by some authors for syrup from the soft date cultivar (Khalas) (Aleid, 2006). The author reports that reducing sugars predominate (81% against 1% for sucrose). The pectin contents seem high, they are from 1.46% to 1.8% (Aleid, 2006; Alanazi, 2010). Values recorded in the present study could be explained by the fact that the extraction methods caried out without recourse to pectinases (Gamal et al, 2009).

Table 1: Biochemical composition of dates and its syrup Content

Characteristics	Average (n = 12)
Fasting glycemia (g / l) (before t_0)	0.95±0.05
Age (years)	22.14 ± 0.134
Weight (Kg)	55.9 ±5.49
Size (m)	1.63±0/034
BMI (Kg / m^2)	21.21±0.08

3.2. Postprandial glycemia

The postprandial evolution response is based on hyperglycemia caused by the reference food (glucose) and the food to be tested (dates or syrup) and glycemia monitoring for 120 min. as recommended by Fao / Who. The subjects selected for these tests must be in good health and not diabetic. They were selected on the basis of fasting glycemia and certain biological indices. From Table 2, the fasting glucose level is 0.97 (g / l) ± 0.05. The average age of this human cohort is equal to 22.14 years ± 0.13, the average weight is equal to 55.9 kg ± 5.49, the height is equal to 1.63 m ± 0.034 and the index body mass (BMI) is approximately 21.21 kg / m^2 ± 0.08. The results show that all the volunteers are in good health, non-diabetic and non-obese. The normal value of blood sugar varies between 0.70 g / l and 1.10 g / l. Diabetes occurs when blood sugar is above 1.26 g / l on an empty stomach. You are also diabetic if, at any time of the day, your blood sugar is greater than or equal to 2 g / l at least twice (Snow and O'dea, 1981; Grimaldi and Heurtier, 1999).

Table 2: Biological Indices of Volunteers

Analysis (%)	**Ghars Cultivar**	**Date Syrup**
Total sugar	72± 0.02	83,5±0.28
Reducing sugars	70.98± 0.02	78,12 ±0.12
Sucrose	1.02±0.2	0,5± 0.02
Glucose	32 ±0.01	40.86 ± 3.23
Fructose	38.98±0.04	39.10 ± 0.001
Pectins	4.15±0.02	3.38±0.38

BMI: Body Mass Index

Carbohydrate foods are responsible of glycemia rising and insulin secretion. Results of blood glucose evolution are illustrated in Fig. 2. The curves are plotted using a computer tool "IG DATE". Values used to draw the curves represent average results obtained with the 12 volunteers. The variation caused by the ingestion of glucose shows a significant difference compared to the foods tested (F = 3.69, p = 0.015), this could be justified by the difference in biochemical composition of both foods tested (glucose and dates / syrup).

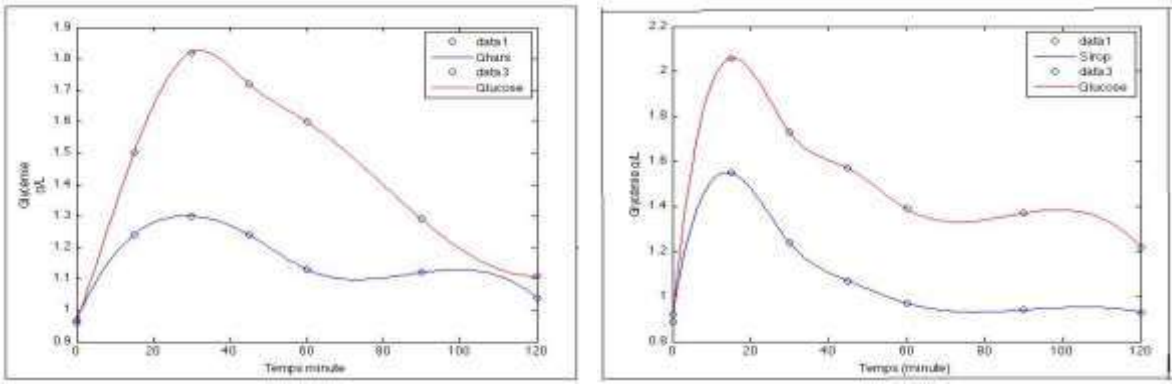

Fig. 2 Evolution of glycemia (g / l) after ingestion of the reference food (glucose) and the test food (A, Dates / B, Syrup)

3.3. Spike of hyperglycemia and postprandial

Figure 2 shows glycemia average values obtained after ingestion of each food tested, for the reference food (glucose) and the test food (dates). The hyperglycemic spike is at t_0 + 30 min. These results are comparable to those mentioned by (Hlebowicz et al, 2009), these show that the average hyperglycemia peak for ten healthy subjects after eating a meal containing whole rye bread and white bread was at after 30 min, and the research of Gunnerud et al. (2012) have shown peaks in hyperglycemia for foods prepared from (humans and cattles) milk at 30 min.

The hyper-glycemic peak with date syrup, is the same like the control food (glucose) is reached at T_0 + 15 min. Usually, this peak coincides with insulin secretion (Fao, 1997; David, 2011; Alkaabi, 2011). Not all foods containing carbohydrates induce the same glycemic and insulin response in the body. A food with an early peak hyperglycemia is a major problem for diabetics because insulin secretion occurs after the peak of hyperglycemia and will not allow coincidence between the postprandial response and insulin secretion (Garcin, 2001**).** For the tests carried out, the peaks are obtained relatively later at T0 + 30 (glycemia equal to 1.82 g / l ± 0.25 and 1.30 g / l ± 0.20) for glucose and dates, respectively. Interesting results, the insulin secretion coincides with the postprandial response. This positive aspect in the case of dates is due to the beneficial action of the polysaccharides (fibers) contained in dates. However, the value recorded in the case of syrup is quite interesting because it is lower than that of glucose (1.55 g/l against 2.3 g /l). The peak hyperglycemia reached with pure glucose at t_0 + 30 min. and at t_0 + 15 min is important. Pure glucose is a simple sugar; its absorption is easy, accompanied by an intense and short hyperglycemic peak. On the other hand, the hyperglycemic peaks of dates are less important. In general, this peak is much lower than that of the reference food. The results recorded in this study are consistent with those found by Gunnerud et al. (2011). These authors report that maximum hyperglycemia values for five varieties of dates are between 1.35 and 1.39 g / l. These results could also be explained by the date's composition of polysaccharides (4.15%). Indeed, the presence of fibers, lipids and proteins limits the peak of postprandial hyperglycemia, which is mentioned in many researches (Normand et al, 2001; Mimouni, et al, 2015 (b)).

Mimouni et al. (2015)(b) agree on the effect of dietary fiber intake on reducing the risk of developing diabetes According to their study, no increase in the level of glycemia, after the ingestion of a meal based on whole rye grains (rich in fiber) compared to white bread which reveals a peak reached at 40 minutes after the ingestion with a value of 0.48 g / l. Postprandial reduction in glycemia is achieved after the incorporation of viscous polysaccharides in cereal and protein foods (Hlebowicz et al, 2009; Mimouni, et al, 2015(a)).

The low peak of hyperglycemia recorded with date syrup may be due to the presence of soluble fiber and mineral elements, as well as the hypoglycemic effect of fructose (GI = 20) (39.10% ± 0.001). Our results are consistent with those mentioned by David (2011). The author shows that the consumption of fruit fructose (FructiLight) leads to a very weak postprandial glycemic response, an hyperglycemic peak about 0.12 g / l against glucose (3.07 g / l).

For a diabetic, the difference observed between postprandial glucose and fasting glucose called "postprandial delta" is a good marker of blood sugar. A normal postprandial delta should not exceed "0.5". In the case of this work, the postprandial delta calculated for Ghars dates and syrup is 0.07 and 0.10, respectively.

Postprandial glycemia (glycemia at T_0 + 120 min) is equal to 0.9 g/l. This value is low compared to that recorded with glucose (1.11 g / l), date (1.34 g / l) and syrup (1.37 g / l). This shows that the volunteers are

healthy. Noting that desired postprandial glucose should not exceed the value of 1.4 g / l (Hlebowicz et al, 2009; Rosen et al, 2009).

Nutrition researchers, including David, (2011) have shown that consumption of different carbohydrate foods leads to different elevations in blood sugar for an equivalent carbohydrate intake. Thus, the speed of digestion of carbohydrates in a food depends on its complexity (fiber and fat content, technological treatments, varietal differences in raw materials, etc.). In this search, the syrup shows a slight increase compared to the dates. This could be explained by the physical state of the syrup (liquid), but dates are eaten in a solid state. According to the same authors , the heat treatment and the physical state of a food have a direct influence on the speed of the digestion's and absorption's physiological process , and therefore on the glycemic index. Thus, a food in liquid form, more quickly digested and absorbed, has a higher glycemic index than the same solid food (grapes: GI = 45 and grape juice: GI = 55) (David, 2011).

IV. CONCLUSION

According to this study, the Ghars date cultivar studied allows to obtain an easily product that could be produced by the adopted method in this study at the household level. Its composition is interesting both nutritionally and dietetically. It is rich in soluble nutrients. It could also be provided to children, athletes, pregnant women, nursing mothers and convalescents. Dates and their syrup have lower glucose evolution kinetics. This justifies the effect of polysaccharides which slow down the absorption of glucose. These results obtained are likely to suggest that this cultivar of soft dates does not cause a significant increase in postprandial blood sugar. Therefore, it is required in nutrition, especially for diabetics and obeses, but with controlling the quantity ingested.

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Development and Quality Evaluation of Ragi Supplemented Cupcakes

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Abstract—*India consumes large amount of the bakery products and cupcake is one of them which is being largely consumed by children. The improvement in living standard and awareness towards health food have diverted the people mindset of food consumption that generated immense scope in value addition of bakery products so present investigation was undertaken to prepare nutritionally rich cupcakes by partial replacement of maida with ragi flour which is rich in calcium, iron and fibers. Cupcakes were prepared with different proportions (100:00, 70:30, 60:40 and 50:50) of maida and ragi and proven acceptability of ragi supplemented cupcakes (60:40 proportions of maida and ragi) with overall acceptability of 7.5 that justified mineral content as 166.34mg/100g calcium and 1.58mg/100g iron.*

Keywords— ***Bakery products, cupcakes, maida, ragi, sensory evaluation.***

I. INTRODUCTION

Bakery products are gaining much importance due to increasing demand of convenience food and so becoming popular among children as well as adults. Wheat is the most prevalent element in bakery products like bread, biscuits and cakes. Recently improvement in living standard and awareness towards health food have diverted the people mindset of food consumption that generated immense scope in value addition of bakery products like bread, biscuits, cakes etc. Cupcakes are specialty small cakes that are popular among home and commercial bakers. Initially, cupcakes were popular in western countries only and were considered as rich men's food. However with rejuvenation of society they are acquiring importance all over the world. They are desired for brunch and/or supplementary meals and are on the other hand served with tea. Several varieties with different flavors such as strawberry, chocolate, vanilla, butterscotch etc. of cupcakes are available all over the world.

Ragi (Eleusine Coracana L.), also known as finger millet is popular in India mostly consumed without de-hulling [1]. It is vibrant millet grown in several states of India and Africa and established as a principal food for a huge section of the residents in these countries [2]. Ragi is distinguished cereal rich in protein, fiber and minerals like iron, calcium and phosphorus along with essential amino acids and vitamins A and B [1, 3]. Thus ragi has proven its nutritional goodness as a functional ingredient in development of food for children, pregnant women, sick and old age people. Being a major source of calcium, dietary fibers and polyphenol, it is also acknowledged for health benefit potential, such as anti-diabetic, anti-tumerogenic, atherosclerogenic effects, antioxidant and antimicrobial properties. The deliberate assimilation rate of ragi proficiently supports to regulate blood glucose levels in diabetic patients [2, 4]. The ragi millet grains are versatile ingredient that opens many doors for health food development inclusive with appropriate processing techniques [5]. The present study was undertaken to develop the process technology for ragi millet supplemented cupcakes with recipe standardization and nutritional characterization.

II. METHODOLOGY

1. Materials

Wheat flour (Maida), ragi and various other ingredients like cocoa powder, milk powder, baking powder, baking

soda, chocolate essence, margarine, sugar, and eggs were procured from the local market for preparation of the cupcakes.

2. Packaging Material

Paper cups were used as primary packaging material, polypropylene trays for holding the cups and the HDPE bags were used as secondary packaging material.

3. Processing of ragi cupcakes

Good quality raw material were received from local market and preliminary cleaning operations were undertaken. Ragi was subjected to grinding to obtain fine texture. All dry powder ingredients were sieved properly to eliminate foreign particles and course material followed by weighing as per the formulation shown in Table 1. Beating of margarine with sugar was carried out to prepare cream and batter was prepared by mixing of all dry ingredients to it. Paper cups were placed in moulds and weighed quantity of batter was filled in cups with help of cone filler. Thereafter moulds were kept in preheated baking oven (140˚C for 15min) for baking which was followed by cooling of cupcakes at room temperature. Cooled cupcakes were packed in the polypropylene trays and covered by HDPE which were then sealed and labeled.

4. Quality analysis

Maida, ragi flour and prepared cupcakes were analyzed for nutritional and organoleptic characteristics using standard methods. Data generated was analyzed for statistical significance by ANOVA using 5% level of significance.

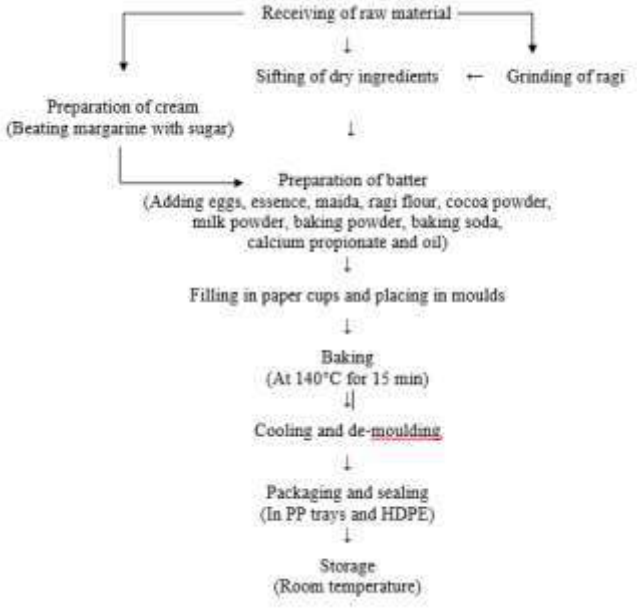

Fig.1: Process flowchart for preparation of ragi cupcakes

Table 1: Formulation of ragi cupcakes

Ingredient	S_0	S_1	S_2	S_3
Maida (g)	100	70	60	50
Ragi flour(g)	0	30	40	50
Sugar(g)	120	120	120	120
Margarine(g)	100	100	100	100
Eggs(No.)	4	4	4	4
Oil(ml)	25	25	25	25
Cocoa Powder(g)	5	5	5	5
Milk Powder(g)	5	5	5	5
Baking Powder(g)	2	2	2	2
Baking Soda(g)	2	2	2	2
Chocolate Essence(ml)	2	2	2	2
Calcium Propionate(g)	0.5	0.5	0.5	0.5

5. Methods of analyzing of nutrients

Moisture content was determined by standard oven method [6]. Values of crude ash, crude fibers, crude fat and proteins were determined by using muffle furnace, Fibrotron, Soxhlet apparatus and Micro-Kjeldahl method, respectively. Carbohydrate contents were determined by calculation method [7, 8].

6. Physical parameters

Ten different samples of cupcakes were analyzed for size (vernier caliper) and weight (weighing balance) and average values calculated.

7. Sensory Evaluation

Sensory evaluation of different organoleptic properties *viz* color and appearance, texture, taste, flavor and overall acceptability were carried out by a semi-trained panel of judges using 9 point hedonic scale [9]. The average score was calculated for individual organoleptic properties and presented graphically.

III. RESULTS AND DISCUSSIONS

1. Proximate composition of maida and ragi flour

The proximate composition of maida and ragi flour were determined and tabulated in Table 2.

Table 2 Proximate composition of raw material

Proximate Composition	Maida	Ragi flour
Moisture (%)	12.9 ± 0.63	12.57 ±0.24
Ash (%)	0.86 ±0.03	1.41 ±0.08
Crude fiber	0.35 ±0.04	3.51 ±0.06
Fat (%)	1.61 ±0.10	1.08 ±0.02
Protein (%)	9.96 ±0.29	7.45 ±0.12
Carbohydrate (%)	74.44 ±0.07	75.04 ±0.17

2. Nutritional composition of ragi supplemented cupcakes

Data on nutritional composition of ragi cupcakes depicted in Table 3 indicates the effect of ragi supplementation on nutritional composition of cupcakes. Moisture content was found maximum (24.37%) in control cupcake containing no ragi in it whereas minimum (23.31%) in cupcakes with 60:40 proportions of Maida: Ragi. Ash content in ragi cupcakes were found in the range between 1.23 to 2.31%. It was found maximum (2.31%) in the cupcakes with 60:40 proportions of maida and ragi whereas minimum (1.23%) in control sample. Crude fiber content were found more (0.63-1.11%) in ragi supplemented cupcakes as compared to cupcakes without ragi (0.32%). Fat and protein contents in the cupcakes were decreased with increase in ragi flour. Fat content was found maximum (23.13%) in control cupcakes whereas minimum (20.02%) in cupcakes with 60:40 proportions of maida and ragi. The significant effect of ragi supplementation was observed in protein content of cupcakes. It was found maximum (7.73%) in cupcakes without ragi whereas minimum (7.03%) in the cupcakes with 60:40 proportion of maida and ragi. Carbohydrate content in the ragi cupcakes was found in the range from 43.23 to 46.22%. Maximum carbohydrate content (46.22%) was found in in the cupcakes with 60:40 proportion of maida and ragi whereas minimum (43.23) in cupcakes without ragi.

Calcium content in ragi cupcakes were found in the range from 140.11 to 168.45mg/100g of cupcakes. The significant increase in calcium content was observed with increase in ragi flour supplementation in cupcakes. It was found maximum (168.45mg/100g) in the cupcakes with 60:40 proportions of maida and ragi whereas minimum (140.11mg/100g) in cupcakes without ragi. The significant effect of ragi flour supplementation was observed on iron content of cupcakes It was found maximum (1.61mg/100g) in the cupcakes with 50:50 proportions of maida and ragi whereas minimum (1.17mg/100g) in control sample. Increase in minerals content of halwa mix with increased level of ragi might be due to presence of more minerals content in ragi [10].

Table 3: Nutrient composition of ragi cupcake

Sample	Moisture (%)	Ash (%)	Crude fiber (%)	Fat (%)	Protein (%)	Carbohydrates (%)	Calcium (mg/100g)	Iron (mg/100g)
S_0	24.37	1.23	0.32	23.12	7.73	43.23	140.11	1.17
S_1	24.11	1.56	0.63	22.4	7.41	43.89	162.61	1.47
S_2	23.86	2.15	0.87	21.25	7.16	44.71	166.34	1.58
S_3	23.31	2.31	1.11	20.02	7.03	46.22	168.45	1.61
SE (±)	0.23	0.25	0.17	0.68	0.15	0.64	6.53	0.10
CD (5%)	0.55	0.61	0.41	1.64	0.37	1.56	15.83	0.24

Physical properties

Physical properties of cupcake are shown in Table 4. The size of cupcake was found between 7.26 and 7.33cm. The weight of cupcake was found in the range from 102.29 to 105.60g.

Table 4 Physical properties of cupcake

Sample	Size (cm)	Weight (g)
S_0	7.26 ± 0.01	102.29 ± 0.05
S_1	7.31 ±0.03	104.51 ±0.06
S_2	7.28 ±0.03	103.34 ±0.03
S_3	7.33 ±0.0.02	105.60 ±0.03

Organoleptic properties

The data on sensory evaluation of cupcakes tabulated in Table 5 and presented in Fig. 2 indicated effect of ragi supplementation on organoleptic properties of cupcakes. The sensory score of organoleptic properties (7.3-7.8) and overall acceptability (7.5) of cupcakes 60:40 proportions of maida and ragi was found very close to the cupcakes without ragi, hence were found acceptable.

Table 5: Organoleptic characteristics of the ragi cupcakes

Sample	Organoleptic characteristics				
	Colour and Appearance	Texture	Taste	Flavour	Overall Acceptability
S_0	7.6	7.4	7.7	7.7	7.6
S_1	6.9	7.2	6.2	6.5	6.7
S_2	7.6	7.8	7.5	7.3	7.5
S_3	6.9	7.3	7.1	7.2	7.1
SE (±)	0.20	0.13	0.33	0.25	0.21
CD (5%)	0.65	0.43	1.08	0.81	0.67

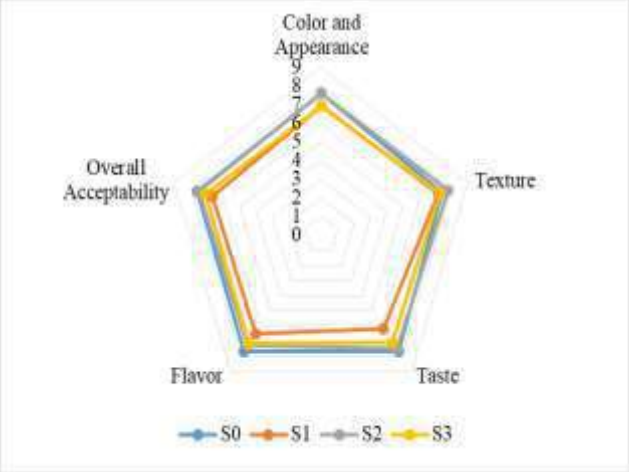

Fig.2 Organoleptic characteristics of ragi cupcakes

IV. CONCLUSION

It is evident from ongoing discussion that ragi supplemented cupcakes can be prepared by partial replacement (40%) of wheat flour with ragi. The organoleptic score of cupcakes with 60:40 proportions of maida and ragi was found very close to the control sample with overall acceptability of 7.5. Hence it can be concluded that 40% of wheat flour can replaced with ragi for making acceptable cupcakes rich in calcium and iron.

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Assessment of Food Safety and Physicochemical Properties of Sous-vide Cooked Steak in Lebanon

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Abstract**—***Sous-vide cooking was recently introduced to the meat catering and hospitality in Lebanon. It is a heat cooking process that includes either precooked, packed products that need little or no additional heat treatment prior to consumption, doesn't have low pH or low water activity, has an extended chilled shelf life, or ismarketed in sealed packages or containers. The aim of this study is to assess the microbial load and food safety of sous-vide meat produced in Lebanon while maintaining quality and palatable characteristics.*

Sous-vide process used tenderloin meat as raw material. Tenderloin meat was trimmed, cleaned, vacuum packed in plastic pouches and cooked atdifferent low heat, followed by chilled storage. After 6 hours, 24 hours, 5 days and 10 days of chilled storage samples were unpacked, portioned into steakandreheated. Samples were evaluated physicochemically by analysing pH, moister content and extract release volume and microbiologically by preparing a culture media followed by API experimental procedure. Results showed that ERV and moisture content decreased significantly but within acceptable limits and that the cooking method had no effect onpH.A significant decrease to zero microorganisms was observed after sous-vide cooking which remained constant throughout the storage period and after reheating, while pathogenic organisms were not detected. Thus, the present study indicates that sous-vide cooking is a safe technique for mass production of steak with an improved shelf life.

Keywords**—Food Safety, HACCP, Microbiology, Physicochemical assessment, Sous-vide.**

I. INTRODUCTION

The culinary world is rapidly shifting to accommodate the present lifestyle demands. New food processing technologies are applied to produce ready to eat products with improved characteristics and marginal discrepancies. Sous-vide is a fairly novel cooking method that extends shelf life and palatable qualities of food in a way that cannot be achieved through conventional cooking. It involves packing food in heat stable vacuum sealed bags followed by controlled cooking at low temperatures [1]. In meat products, low cooking temperatures help break down of collagen in the connective tissues, preserving tenderness and moisture content whilst improving sensory and nutritional quality of cooked meat [2]. More importantly, sous-vide facilitates safe reproducibility and mass production in a short and controlled timely manner, which is especially valuable for the catering industry. However, there are some limitations to sous-vide cooking. A substantial erraticism in knowledge and training of food handlers applying sous-vide escalates the potential for improper use of sous-vide cooking [3]. The survival of vegetative pathogens is another concern in low temperature long time sous-vide cooking [3]. Furthermore,

protein degradation and lipid oxidation, during storage, may produce off odours and flavours [4].

Sous-vide cooking has been recently introduced to the catering and hospitality industry in Lebanon. In the last three to four years, many catering companies and restaurants started adopting sous-vide as a primary production procedure. Appropriate local food safety standards that regulate the safe production, handling and trade of sous-vide have beenlimited as the Lebanese food safety law has been governed by legislative decrees that date back to the 1960's and 1970's [5]. A centralized and integrated approach that food businesses can consult andconform to have not been available[5]. The accessibility of systematic preventive system that provides basic advice and guidancesuch as Hazard Analysis and Critical Control Points planhas been scarce. The food industry had to rely on self-imposed internal frequent testings to ensure safe food production and avoid food borne illness. Proving that sous-vide produces food that is safe for human consumption demanded research on the safety and quality of this cooking process. The significance of this study is based on the lack of national food safety standards and the rise in public concerns about food safety [5]. The context of work focuses on microbiological assessment in conjunction with physicochemical analysis as safety is not sufficient to indicate palatable quality of food. A suggested HACCP plan offers food businesses a model for formulating a proper food safety management system.

The aim of this work is to evaluate the effect of different time, temperature and storage combinations on microbiological and physicochemical properties of sous-vide cooked steak.

Research Hypothesis

Sous-vide cooking method produces steak that is safe for human consumption while maintaining its quality.This research gave rise to the following questions to be inspected:

1. Does sous-vide produce safe to eat steak?
2. Does sous-vide preserve the physicochemical properties of steak?

1.1 History

What started as a concept to overcome recipe development issues in 1970's by George Pralus developed into a cooking method that became prominent in today's cooking industry[1]. Exceptional outcomes inspired Pralus to adopt this technique and set some rules for achieving perfection. His specifications comprise the acquisition of the highest quality raw material, coordinated with strict hygiene standards and controlled cooking procedure[1]. While others claimed that they are the pioneers of this cooking technique, Pralus' ability to apply this approach in practice and tackle challenges as they arise earned him his title as the founder. In 1971 W.R. Grace, a leading packaging company is USA, took out a patent on the basic concepts of the process [1]. Sous-videhas become widely applied in restaurants and catering industries in 2000's [6]. In Lebanon, utilization of sous-vide in the hospitality industry is fairly new and the first scientific report about it was in 2017 [7].

1.2 The Process

Sous-vide is French term for "under vacuum". It is defined by the Sous-vide Advisory Committee as "interrupted catering system in which raw or par-cooked food is sealed into a vacuumised laminated plastic pouch or container, heat treated by controlled cooking, rapidly cooled and then reheated for service after a period of chilled storage" [1, 8].

The technology involves packaging raw or precooked food under vacuum in hermetically sealed bags then cooking at low temperature. Convection steam ovens and water baths, set at specific precise temperatures, are generally used as a cooking medium. Steam ovens accommodate large quantities of food and distribute heat uniformly as long as they aren't over loaded, while circulating water baths also heat very uniformly when food pouches are completely submerged in water and loading capacity is not exceeded. Probe thermometers, inserted through a closed cell foam tape mounted on vacuumed pouches, are used to monitor and control core temperature of food [6].

Sous-vide differs from conventional cooking as it stipulates several advantages [6]. Vacuum packing inhibits production of off flavours caused by oxidation and prevents evaporative losses of moisture and flavour [10]. It eliminates the risk of contamination during storage and reduces aerobic bacterial growth, thus extending food's shelf life [1,10,11]. Moreover, it promotes efficient heat transfer from the water bath or oven steam to the product [6]. Accurate temperature control permits holding food at low temperatures long enough for fast and slow changes to take place, such as protein denaturation. Also, it allows control over doneness and perfect reproducibility [6]. Sous-vide is known to reduce material costs and enhances superior retention of texture, aroma, flavour and nutrients [12]. Sous-vide can be applied to meat, seafood, fruits and vegetables and supermarket retailed products [13].

1.3 Effect of Heat on Muscle Meat A distinctive feature of sous-vide technique is its ability to cook food at low temperature for a long time. It is particularly favourable for cooking tough meats that require application of low heat for a long time to weaken the connective tissues and

decrease myofibrillar tensile strength [6]. Upon heating, proteins denature and change. The extent at which these changes occur depends mostly on time followed by temperature. Both myofibrillar protein and connective tissues contract and shrink quickly when heated. Sarcoplasmic proteins expand, aggregate and gel also quickly. Collagen dissolves into gelatine, reducing inter-fibre adhesion. However, changes in collagen to allow tenderness of muscle meat demand more time. Moreover, sarcoplasmic protein enzyme, collagenase, remains active at temperature below 60 °C, which can considerably tenderise meat when held for more than 6 hours. Sous-vide grants the flexibility of holding food at desired temperature for enough time to achieve pleasant tenderness of different meats cuts [6].

1.4 Other Physicochemical Changes

Meat undergoes physicochemical changes upon cookingwhich affect quality parameters including colour, flavour, texture, pH, and water holding capacity. Water holding capacity is an imperative property of meat quality. It is influenced by the pH of the tissue and the amount of myofibril in which water resides. Upon heatingmuscle proteinscoagulate and shrink releasing water out of the meat. More water is released as cooking duration increases and juices are lost through drip and evaporation, resulting in dryand tough end product[14].

Previous studies that examined the effect of sous-vide cooking on physicochemical changes have shown that as cooking time and temperature increase weight loss increases [15]. Extract Release Volume (ERV) decreases upon prolonged storage and pH increases [17, 21]. Some studies have shown that sous-vide cooking enhances texture, tenderness and chewiness of meat products [16, 20] while others reported no change [1,18].

1.5 Microbiology and Food Safety

Raw untreated foods are expected to contain varying counts of bacteria, yeasts and moulds.

However, plants and animals which serve as food, have developed defence mechanisms to combat the proliferation and invasion of microorganisms. These mechanisms have become an inherent part of their tissues known as intrinsic parameters. Intrinsic parameters such as pH, moisture contents, oxidation-reduction (Eh), nutrient content, antimicrobial constituents and biological structures govern the initial microbial load of a food product [22].

Bacterial microbiota, in meat, are mostly Gram-negative with some Gram-positive such as enterococci and lactobacilli. A large number of moulds (*Penicillium* and *Mucor*) and some yeasts (*Candida* and *Rhodotorula*) may also be present [22]. *Escherichia coli* (biotype 1) is commonly found in meat products with high rates of incidence. It is regarded as an indicator organism in assessing the sanitary state of fresh food and safety of beef. *Salmonella* is another pathogen common to commercially prepared and packaged food[22]. *Listeria* is also a prevalent microbiota in red meats. Twenty-two percent of tested beef carcases in Belgium reported positive results for Listeria [23].

Meats contain a high amount of water (about 75% when raw) and abundance of nutrients encouraging the growth of microbiota. However, only a few types of spoilage microorganisms can be found in spoiled meat due to intrinsic factors of the product. The surface of meat products tends to create adequate environment for growth of potential aerobes, facultative and strict anaerobes, which may be amplified when extrinsic factors such as suitable growth temperatures are reached [22].

The microbial load in fresh meat may range from a minimum of 10^3 cfu/g to a maximum of 10^{10} cfu/g. Microbial spoilage is generally not recognised in the range of 10^3 cfu/g to 10^6 cfu/g except for milk which might develop a sour taste. Vacuum-packed meats might acquire odours and might be spoiled within a range of 10^6 cfu/g to 10^7cfu/g. Off odours associated with aerobically stored meats occur at microbial count of 10^7cfu/g to 10^8cfu/g. Obvious signs of spoilage are displayed at 10^8cfu/g to 10^9cfu/g in all most all foods. And, a definite change in structure occurs at 10^9cfu/g to 10^{10}cfu/g [2].

Bringing too much technology into sous-vide processing and cooking at lower temperatures raises the risk of growth of pathogenic bacteria [1]. Accordingly, a great deal of research on sous-vide food has focused on the safety of the procedure and on investigating its effect on shelf life extension. Most studies have shown that pathogens and spoilage microorganisms were reduced to an acceptable level [16, 25, 26] and their presence in the final sous-vide product probably results from microbes being in raw ingredients and surviving during processing [24].

1.6 Food Safety Standards

Some countries, such as Australia, Canada and the United States, have set out food safety standards and requirements for sous-vide processing method at a national level. Others have developed guidelines including legal requirements and control measures for food processing industries. Food safety authorities place requirements on food businesses to produce food that is safe and suitable for consumption. They all focus on microbial hazards of concern such as *Clostridium perfringens*, *Staphylococcus aureus*, *Listeria* and *Salmonella* and recommend cooking times and temperatures. Lebanon like many other countries doesn't

have a current standard for this particular procedure [27, 28, 29].

II. MATERIALS& METHODS

2.1 Raw and Cooked Material

Raw and sous-vide cooked steak samples were provided by Sofil Catering, Beirut Water-Front. Three batches of samples were prepared on three successive weeks starting 15th of April 2019 till 8th May 2019. They were divided into 3 groups or blocks (weeks) where each group was considered a replication. Samples were collected as soon as they were prepared and ready for testing. Three replicated identical lots of each batch were preserved in an appropriate freezer where one lot was physiochemically analysed at the Food Science Research Laboratory - Lebanese International University and two lots were microbiologically analysed at the Centre for Infectious Disease Research Laboratory - American University of Beirut.

2.2 Sous-vide Cooking Process, Sampling and Sample Preparation

Nine samples were collected from 9 different stages of sous-vide cooking. Raw vacuum-packed tenderloins were unpacked, drained, trimmed and cleaned then covered with stockinets. They were then processed in two phases. In phase one, tenderloins were cooked, where they were seared, vacuum packed then sous-vide cooked in a steam oven (temperature and time) followed by chilling then refrigeration. In phase two, after being held at chilled storage for varying durations, steak was unpacked, portioned then reheated (time and temperature).

Triplicate batches were collected for three successive weeks from Sofil's kitchen, at various stages of the cooking procedure as described in Table.1 to ensure the method's efficiency and absence of cross contamination.

Fig.1exhibits a step by step chart of sous-vide cooking procedures as well as the stages at which each sample was taken. It is based on an interview with the executive head chef followed by an on-site verification done a week later.

2.3Physicochemical Analysis

Duplicate batches of nine samples each were cooked, collected and tested on differentdates for ERV and moisture content. This was replicated over three weeks.

2.3.1 Determination of Extract Release Volume (ERV)

Sous-vide samples weighing 25g each were blended with 90ml distilled water in an electric blender for 2 minutes. The mix was then poured into a funnel fitted with Whatman filter paper No. 1 folded thrice to make 8 sections. The homogenate was left to seep between the fold into a graduate cylinder for 15 minutes [30]. The released volume was then measured by graduated cylinder to the nearest millilitre.

2.3.2 Moisture Content in Meat

The moisture content of the sous-vide cooked steak samples was measured following the *Official Methods of Analysis of AOAC International* Method 950.46[31].Two grams of sous-vide cooked samples were weighed to the nearest mg and placed in a hot oven at 100°C ±2°C for 16 ±0.5 hours. Final weight of the samples was measured and the moisture content was calculated according to the following formula:

Moisture content = (Initial weight – dry weight)/initial weight *100

2.3.3 pH Test

A calibrated pH meter (Thermo Electron Corporation) adjusted to the temperature of tissues was used to measure the pH.A sample of 15 grams of sous-vide was blended with 30ml of distilled water using a stomacher(BLSmart) at 27-30°Cfor 2 minutes. The pH was then measured by inserting a glass electrode in the prepared sample [30].

2.4Microbiological Assessment

Duplicate batches of 9 samples each were also cooked, collected at the same time as those prepared for physicochemical analysis and tested for spoilage and pathogenic bacteria. This was however replicated over two weeks period.

2.4.1 Sample preparation and culture

Steak samples were plated using standard techniques. Homogenized 1 g of steak in 20 mL of PBS buffer and the supernatant were inoculated on specific agar for bacterial growth.

Serial dilutions of the supernatant plated to MacConkey agar for detection of Gram-negative bacterial species; SS (*Salmonella- Shigella*) agar was used for easier selection of*Salmonella* spp., LB agar (Luria Bertani) used for overall count detection, and shedding samples were directly collected on Brain Heart Infusion Agar for a total viable count.

Plates were incubated at 37 °C in a humidified incubator with ambient air; all other media. For anaerobic bacteria, an anaerobic chamber wasused for incubation. Plates were read at 24 hour and 48 hours of incubation.

Each different isolate was later inoculated in 2 mL of LB broth cells were harvested after 4 hours at 37 °C[32].

2.5 Bacterial Molecular Testing

2.5.1 Analytic Profile Index (API) experimental procedure:

All isolates were grown and streaked on the same type of agar media that they were isolated from and incubated at 37 °C overnight. Fungal growth was eliminated from the study based on colony morphology, and oxidase test was performed on all remaining isolates.

API 20E test was performed on all oxidase negative isolates according to the manufacturer's instructions as follows; isolated bacterial colonies were suspended in 5ml of sterile distilled water and inoculated into the API 20E test strip microtubes. Water was added to the incubation tray to provide a humid setting and the strips were incubated at 37 37 °C for 18 hours after capping them with the provided plastic lid. TDA (Tryptophan deaminase), JAMES, and VP (Voges-Proskauer test for detection of acetoin) (1 and 2) reagents were added to the TDA, IND (Indole Test), and VP microtubes respectively after incubation, and the results were recorded as 7-digit number (excluding the oxidase test). Bacterial samples were characterized and identified with APi LAB Plus V.3.3.3 [32].

API 20NE was performed on all oxidase positive isolates according to the manufacturer's instructions as follows; isolated bacterial colonies were dispersed into 5ml of sterile saline solution (0.85% NaCl) with a turbidity of 0.5 McFarland. API 20NE microtubes NO_3 to PNPG (4-nitrophenyl-βD-galactopyranoside) were inoculated with the saline suspension. 200 µL of the remaining saline suspension was introduced to the API AUX medium and dispensed into GLU (fermentation of glucose test) to PAC (phenylacetic acid) microtubes. Water was added to the incubation tray to provide a humid setting and the strips were incubated at 37 °Cfor 18 hours after capping them with the provided plastic lid. NIT (1 and 2), JAMES reagents were added to the NO_3 (Potassium nitrate)and TRP (L-tryptophane) microtubes respectively after incubation, 2-3 mg of zinc were then added to the negative NO_3 microtubes for confirmation. The results were recorded as 7-digit number (excluding the oxidase test). Bacterial samples were characterized and identified with APiLAB plus V.3.3.3[32].

2.6 Statistical Analysis

The experimental design was a randomized complete block design. The effect of time and temperature combination on Extract Release Volume, Moisture Content, and pH were evaluated using one-way Analysis of variance- ANOVA, where ($p< 0.05$) indicated significant difference between the treatments. Duncan's multiple range test was carried out to determine homogeneous groups. And two-way ANOVA was used to determine the effect of treatments on microbiological content. All ANOVA analysis were performed using IBM SPSS V 22.

III. RESULTS AND DISCUSSIONS

3.1 Extract Release Volume

Extract release volume is a procedure used to indicate spoilage of beef based on the amount of aqueous extract released from a slurry of meat, when allowed to pass through filter paper for a given period of time.

In this study means of all tested samples are expressed in Table.2,where different subscripts denote means of significant difference. Extract release volume ranged between a minimum of 41.00ml and a maximum of 70.67 ml. Raw steak reported 70.67 ml which was the highest. Treatment 1 reported a significantly different ERV than that the rest of treatments and so did treatment 9. A decrease in ERV was noted as cooking temperature and storage duration increased and it reached a minimum for reheated, ready to serve final product at 10 days of chilled storage. The effect of the treatments(Table 3) was significant (p value <0.01) in ERV results as samples were subjected to higher temperatures during the reheating stage and as cold storage duration escalated.

Treatments 2, 3, 4 and 5 reported no significantly different ERVs. This implied that cooking temperature of 57°C in combination with different storage durations had no effect on ERV. Also, treatments 6 and 7 reported no significantly different ERVs. This explains that short durations of cold storage had no effect on extraction release volume. Treatment 5, however, reported a significantly different ERV than treatments 6, 7, 8 and 9. This explains as cooking temperature increased, from 57°C to a reheating temperature of 63°C, ERV decreased. Treatments 7,8 and 9 reported significantly different ERVs proving that prolonged chilled storage of 5 days and above resulted in a decreased extract release volume. The sinusoidal pattern displayed in ERV results may be attributed to deteriorating protein capturing water in the hydrated state or to the accumulation of water vapor on the steak sample during refrigeration and reheating or to an experimental error.

In a similar studies Anandh [17] described the gradual significant decrease of ERV in vacuum packed boiled restructured meat rolls with prolonged refrigerated storage might be attributed to the increase in microbial population. Jay *et al*., [21], explained that fresh beef releases high volumes of extract as opposed to spoiled meat. Additionally, in a study on meat processing, scientists explained that as meats undergo microbial spoilage,

proteins hydrolyse completely bringing down the extract release volume [33].

Nevertheless, all samples exhibited a volume above 25ml indicating good quality of meat as per standards of Food Safety of India which are internationally recognised and easily accessible standard[30].

3.2 Moisture Content

Moisture content values decreased gradually from a top of 77.5% for raw beef to a low of 58.8% for reheated steak held at cold storage for 10 days, as shown in Table 2. Statistical analysis indicated a significant difference in moisture content for raw (treatment 1), cooked (treatment 2, 3, 4, 5) and reheated ready to serve meat (treatment 6,7,8, 9). No significant difference was reported between samples that were only cooked and held at cold storage for 6 hour, 48 hours, 5 days and 10 days. Also, the difference between samples that were reheated after the storage time aforementioned was not significant. This indicates that reheating caused a decrease in moisture content for sous-vide cooked steak as opposed to cooking. On the other hand, different cooking temperatures and duration of chilled storage had no effect on the extract release volume. The slight insignificant rise in moisture content in treatment 5 is probably due to an experimental error.

Previous research described results comparable to this study. Moisture content of meat cooked using three different methods (pressure, microwave and atmospheric cooking) decreased regularly. This was attributed to the fact that as meat cooked progressively the water was being forced out [34]. Moisture content decreased gradually but not significantly during 30 days of storage in a study on vacuum packed restructured buffalo meat rolls [17]. Roldan *et al.*, [35] also reported a difference in moisture content in sous-vide lamb cooked at higher temperatures. Samples cooked at lower temperatures exhibited higher moisture content

3.3 pH

Changes in pH at different stages of cooking for this study are given in Table 2.The highest mean pH was 6.3, reported in sous-vide cooked beef steakchill stored for 6 hours. The lowest mean pH was 5.90, reported in sous-vide cooked samples after 5 days of chilled storage. Analysis of variance showed that different treatments had no significant effect (p value= 0.137) on the pH of meat where samples held at cold storage for 6 hours reported very similar pH readings to those held for 10 days. The pH of beef steak in this study didn't exceed 6.03, which is still within the acceptable range. Since pH plays a role in media for bacteria, the insignificant difference in results suggest a low microbiological activity throughout the samples.

Similar results were depicted in previous studies. When beef pH exceeds 6.0 within 24 hours of harvest, meat quality deteriorated, consumers' eating experience became undesirable and economic losses increased[36]. Özcan[34]studied the effect of different cooking times and treatments (atmospheric, pressure, microwave cooking) on the physical and chemical attributes of ready to eat meat. pH differed significantly between different cooking methods. However, meat of highest quality was indicated by a pH range of 5.7 to 6.0.

Other studies explained that in rested animals the conversion of glycogen to lactic acid causes a depression in pH from 7.4 to 5.6. In fatigued animals, glycogen was utilized, and less lactic acid was formed. Consequently, meat from stressed animals had a pH above 6 upon completion of rigor mortis and spoil faster[37]. This made meat more susceptible to bacterial, mould and yeast spoilage, whereas microorganisms grew best at pH value of 6.6- 7.5[22].

3.4 Microbial Assessment

The mean microbiological population for sous-vide steak determined at different stages of cooking and cold storage duration is presented in Table 5.

The highest reported microbial count was seen on HBI (354,900 cfu/g) and LB plates (26,040cfu/g), particularly in raw samples that have not been subjected to a heat treatment or chilled storage. The lowest count (zero cfu/g) was detected in cooked samples subjected to 6 hours of cold holding post sous-vide cooking. This pattern was sustained along the rest of the samples and no bacterial organisms were detected throughout the rest of the process.

Hence aerobic viable count and overall count detection in raw steak that wasn't subjected to heat treatment and chilled storage conditions decreased significantly ($P< 0.01$) with increasing heat treatment and reached a zero-organism showing that sous-vide cooking decreased the microbiological load to zero. While no increase was detected as refrigerated storage period advanced.

However, total coliforms, faecal coliforms, *Staphylococcus aureus*, *Clostridium*, *Salmonella* and *Shigella* were not detected at any stage of sous-vide cooking procedure nor at short and prolonged refrigerated storage conditions.

The aforementioned results signify that the time-temperature combination in the cooking phase of this sous-vide cooking and 6 hours of chilled storage at 1-3 °Cwere suitable enough to eradicate the spoilage bacteria and

pathogens of interest. The zero microorganisms observed at the rest of the stages of sous-vide cooking and chilled storage denotes that no cross contamination has emerged throughout the process and that reheating and prolonged chilled storage did not affect the safety of the final product.

The very low microbial counts recorded in this study were in accordance or the Lebanese Standards for Food Safety [38] except for the total viable count in raw steak samples which was reduced to zero microorganisms by the first treatment. However, API experimental procedures identified other oxidase negative and oxidase positive bacteria that are worth mentioning and they included *Pasteurella pneumotropica* and *Chryseonomasluteola* in raw steak as presented in Table6.

In a similar study on the microbiological safety and quality of foods processed by sous-vide for commercial catering, results showed that non-spore forming pathogenic bacteria had very low survival rates in sous-vide cooked beef products [39]. Accordingly, the scientist justified the cooking process as limiting the risk associated with these microorganisms as long as raw materials of good microbial quality are used, and the final products are restricted to low storage temperatures.

Babur *et al.*,[40] studied the microbiological quality characteristics of sous-vide cooked meat at different time combinations (2 and 4 hours) at 70°C. Microbiological analysis was performed after 0, 3, 7 days cold storage. None of the tested microorganisms were detected in sous-vide cooked meat refrigerated for 7 days. The results were attributed to good hygienic practices accompanied by proper cooking and storing temperatures.

Anandh[17] studied the shelf life of boiled restructured buffalo meat rolls in refrigerated storage under vacuum packaging conditions. Spoilage bacteria tested in vacuum packed buffalo meat reported an increase in microbial counts of psycrotrophs, *E.-coli*, *Staphylococcus*, *lactobacillus*, yeasts and moulds with increasing storage time. However, the increase was well below the standard of cooked products.

Roldan *et al*. [35]found that very short time-temperature combinations were enough to pasteurize sous-vide cooked lamb. While very low count of LAB, psychorotrophs and Enterobacteriaceae at prolonged refrigerated storage was detected in sous-vide cooked pork loin at 70°C for 11 hours and stored for 10 weeks at 2°C[4].

1. SUGGESTED HACCP PLAN

In consideration of the deficiency of national food safety standards, and a centralized integrated approach to control food safety hazards within a food business that implement sous-vide cooking, the suggested HACCP plan presents a helpful tool to ensure safe sous-vide practices for food businesses. HACCP is an internationally recognized food safety system that is recommended by World Health Organization [41].

4.1Product description

Steak is slice of meat cut from the fleshy part of a beef carcase. It is generally cut across the muscle fibre of a large section of beef and may include a bone. Most steaks come from three prime areas of a cow; short loin, tenderloin and the ribs [42]. A detailed product description is exhibited in table 2.

4.2 Preparation of Sous-vide Steak

Raw vacuum-packed tenderloins are unpacked, drained, trimmed and cleaned then covered with stockinets. They are then processed in two phases. In phase one, tenderloins are fully cooked, where they were seared, condiments were added, and they are all vacuum packed then sous-vide cooked in a steam oven followed by chilling and refrigeration. In phase two, after being held at chilled storage for 6 hours, 48 hours,5 days and 10 days, steak was unpacked, portioned then reheated. Table 4 explains the steps at which critical control point occur including the associated hazards, control measures, critical limits, monitoring tests and frequencies as well as corrective actions to be taken. Fig. 2 exhibits a flow diagram including steps at which critical control points appear.

2. FIGURES AND TABLES

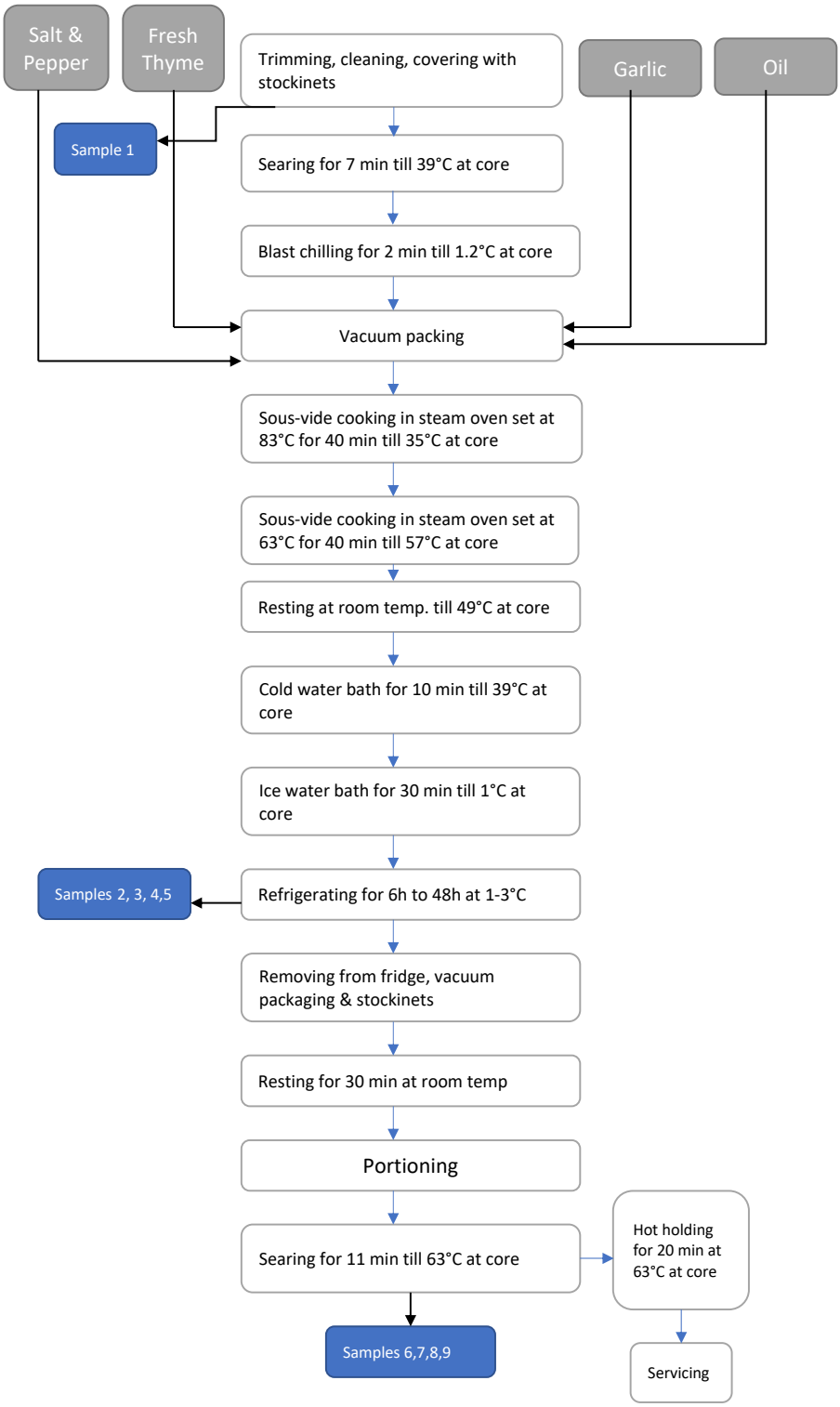

Fig. 1: Process flow diagram for the sous-vide steak showing stages where sampling took place

Fig.2: Process flow diagram for sous-vide steak

Table 1. Sample name and description.

Name	Treatments	Description
Phase 1: Cooked (57 °C at core)		
Sample 1	**1**	collected after cleaning meat and before covering with stockinets. This will be the control sample of the batch tested to identify the original state of the steak prior to handling and heat treatment
Sample 2	**2**	collected after 6 hours of cold holding post sous-vide cooking
Sample 3	**3**	collected after 48 hours of cold holding post sous-vide cooking
Sample 4	**4**	collected after 5 days of cold holding post sous-vide cooking
Sample 5	**5**	collected after 10 days of cold holding post sous-vide cooking
Phase 2: Reheated (63 °C at core)		
Sample 6	**6**	collected after portioning and reheated post 6 hours cold holding
Sample 7	**7**	collected after portioning and reheated post 48 hours cold holding
Sample 8	**8**	collected after portioning and reheated post 5 days cold holding
Sample 9	**8**	collected after portioning and reheated post 10 days cold holding

Table 2. Product Description for a HACCP Plan

Product name(s)	Sous-vide Steak
Important product characteristics	Average composition of Steakper 100 g of edible portion is 66g water, 27g protein and 8g fat per 100g [37] pH is 5.4- 5.8 [41] No preservatives are used
Intended use	*Sous-vide Steak* is prepared for either immediate consumption or long refrigerated storage It is served as a main meal and consumed by general public
Packaging	Served and dispensed on plates
Shelf life	10 days in the refrigerator (below 5 °C*)
Prepared / sold in	Restaurants, hotels, homes
Labelling instructions	Keep refrigerated (below 5 °C*)
Special distribution control	Transport, store, and display refrigerated (below 5 °C) under hygienic conditions

* As recommended by applicable Codex alimentarius standards for refrigerated foods [41, 43].

Table 3. Ingredients

Ingredients	Codex Standard
Beef Tenderloin	As per Codec STAN CXS 88-1981. AMMENDED IN 2019
Salt	As per CODEX STAN 150- 1985 [10]
Black Pepper	Freshly Ground No Codex standard available
Fresh Thyme	No Codex standard available
Garlic cloves	No Codex standard available
Olive oil	*V*egetable oils As per CODEX STAN 210- 2003 [44]

Table 4. HACCP Chart for Sous-vide Steak Production

Step	Hazard	Control Measure	CCP	Critical Limit	Monitoring		Corrective action
					Test	Frequency	
Meat	*Biological* Disease causing microorganisms	Purchase form reputable source During transport & storage temperature constant between 1-4 °C Check upon delivery. For proper shipping conditions-temperature	1	Reputable source and conformance to local specification of meat Transport and storage temperature between 1-4 °C Absence of bones	Check source certificates are consistent with specification Check fridge temperature	Each batch	Reject and change supplier
Trimming	*Physical* Foreign matter from packaging	Use well maintained equipment GMPs	1	Presence of foreign matter Adherence to GMPs	Visual examination for foreign matter	continuous	Remove for matter if possible, discard if not
Salt & Pepper	*Physical* Foreign matter from packaging *Biological* Moulds	Purchase from reputable source Sieve Visual inspection	2	Absence of impurities and foreign matter Mould growth	Ensuring purchase from reputable source Checking sieves Visual inspection	Each batch	Re-sieve salt Discard mouldy pepper
Fresh Thyme	*Biological* Disease causing microorganisms	Decontaminate using sanitizer	3	Dust and soil on produce Free available chlorine (not more or less than 0.05g/l	Measurement of chlorine in water using a certified technique	Each washing step	Re-wash with unchlorinated water in case of high doses

				to 0.1g/l) with a contact time of 30 seconds			
Cooking	*Biological* Growth of Spoilage/ pathogenic microorganisms	Avoid over cooking and charring Discard black crusts	4	Absence of charred crusts	Checking cooked meat during cooking	Each batch	Discard black crusts Readjust oven temperature and or grill
Storage	*Biological* Growth of Spoilage/ pathogenic microorganisms	Preserve meat in refrigerator at temperature between 1-4 °C GMP	5	Storage temperature between 1-4 °C	Temperature measurement	Continuous	Adjust temperature
Hot Holding	*Biological* Growth of Spoilage/ pathogenic microorganisms	Warm holding (60°C / max 1h)	6	Holding at 60°C / max 1h	Temperature and time measuring	Continuous	Adjust holding temperature to the proper level

IV. CONCLUSION

The results obtained in this study pointed out that cooking had a positive impact on quality and safety attributes of sous-vide cooked steak held at different chilled storage durations. Studied parameters were in desirable range and within the Lebanese Safety Standards, verifying the adequacy of the processing method. Further studies can be done on organoleptic and nutritional properties of sous-vide steak.

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Engineering *Bacillus Subtilis* for the production of High Fructose Syrup: Opportunities and Prospects

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Abstract— *High fructose syrup is an excellent and safe sweetener that can replace sucrose and is widely used in beverages and food products, including soft drinks, ketchup, yogurt, ice cream, chocolate milk, candies, jams, condiments, canned and packaged foods, etc. Besides, after acid dehydration, it can be used as a renewable resource to synthesize bio-petrochemicals. The advantages of high fructose syrup include high sweetness, high solubility, low viscosity, enhanced flavor, good moisture retention, no side effects in acidic foods, and no crystal formation. Due to its potential application, little has been done to satisfy the current market. It can be produced by two main approaches, (i)Chemical method of synthesis accounted several challenges and these include low yield, unacceptable products, expensive, environmentally unfriendly. This review aimed to discuss the engineering B. subtilis 168 which a food-grade microbial cell factory to solve all the challenges from chemical production processes with an improved yield, cost-effective, environmentally friendly, and quality products.GI which isomerizes a reversible reaction of D-glucose into D-fructose requires special conditions for the high-level yield of HFS biosynthesis such acid pH environment and high temperature which are harsh for wild-type enzymes. Therefore, there is a need to engineer a GRAS B. subtilis 168 for sustainable industrial production. Codon optimization and plasmid engineering have been highlighted.*

Keywords— ***Glucose isomerase, isomerization, Bacillus Subtilis, high fructose syrup, biosynthesis.***

I. INTRODUCTION

HFCS (high fructose corn syrup) is a refined and condensed functional sugar aqueous solution that is commonly used as a natural sweetener. It's among the most widely used sweeteners in soft drinks, ice cream, yogurt, tomato paste, chocolate milk, candy, condiments, jelly, and canned and processed foods [1–3]. Recently, HFCS has been regarded as a renewable resource for the manufacturing process of 5-hydroxymethylfurfural and levulinic acid, both of which can be used to synthesize other useful biopetrochemicals, such as green solvents, plastic materials, lubricants, and highly valued biofuels[4–8]. Enhanced iron and zinc absorption, insulin-independent metabolism, improved ethanol metabolism, low sugar and calorie content, and ideal sensory properties have all been demonstrated as health benefits of HFS consumption [2,9]. Furthermore, high sweetness, high solubility, low viscosity, enhanced taste, strong humectants, no side

effects in acidic foods, and no crystal formation are all advantages of HFS over other sugars. [3,9,10].

Based on the fructose content, HFCS can be classified as HFCS-42, HFCS-55, or HFCS-90. However, since HFCS-42 has a low fructose content and is easy to solidify and crystallize in low-temperature transport and storage due to its low fructose content, HFCS-55 with a higher fructose content has become the premium product [1,2]. Chemical catalysis and enzyme biocatalysis are two methods for producing HFCS. In an alkaline environment, chemical catalysis is the isomerization or acid hydrolysis of glucose to fructose [2].

For more than a century, it has been known that glucose can isomerize fructose by basic isomerization or acid hydrolysis, as shown in Figure 1. Therefore, it is a demanding process that leads to unacceptable sugar decomposition. In addition, the chemical synthesis of HFCS needs a higher calcination temperature, which is not friendly to the environment [11–13]. The problem of separation and recovery of homogeneous Lewis acid is common, and the synthesis of Sn - β zeolite is more complex. On the other hand, the isomerization of glucose to fructose can also be effectively achieved by ionic bases such as sodium hydroxide. In contrast, due to the serious degradation of fructose and glucose, the yield of fructose from glucose is low, and the separation and recovery of fructose is also a problem[4,12,14].

Since Yoshiyuki Takasaki discovered a thermostable glucose isomerase from yeast in the early 1970s, commercial HFC was mainly synthesized by microorganisms [15]. Glucose isomerase, also known as D-xylose isomerase, is widely used in the production of HFCS. It not only catalyzes the conversion of D-glucose to D-fructose but also catalyzes the conversion of D-xylose to D-xylulose [16]. In recent years, with the increasing consumption of HFCS, the production level of GIase has been widely concerned. Many microbial strains, including *Streptomyces* sp. CH7 [17], *Lactobacillus bifermentans* [18], *Bacillus coagulans* [19], *Streptomyces murinus, Hyperthermophilic Thermotoga* [20], and *Pseudomonas hydrophila* [21], it is reported that glucose isomerase can be produced. Due to the increasing global demand for hydrofluorocarbons, the production level of D-glucose isomerase has been widely concerned, especially in the food and beverage industry. Because of the increased global demand for HFCS, the level of GIase production has gained considerable attention, especially in the food and beverages industry. Due to the low productivity and stability of enzymes produced by wild-type microorganisms under harsh conditions, a more efficient expression system is needed for the production of recombinant GIase with desired properties for large-scale production of HFCS.

To obtain an effective expression mechanism, GIase has been heterologously expressed in a variety of hosts[22,23], and a variety of fermentation techniques, including fed-batch and high-density fermentation, have been used. As a result, GIase expression has greatly improved; for example, Akdag et al.[24] announced that using a beet molasses-based feeding method, they achieved the highest recombinant GIase production, 35.3 U/mL, in E. coli. Due to its well-known genetics' history, short generation time, and suitability for low-cost high-density fermentation, *Escherichia coli* is the most common heterologous host strain for the expression of recombinant proteins [25]. However, improvements in GIase production, especially concerning HFCS-55 manufacturing, are still valued for industrial applications.

Although one-step biosynthesis of HFS-55 has achieved certain results, most of its host strains previously investigated with higher potential yields are *Escherichia coli* BL21, but in the catalytic process, BL21 may bring harmful toxin that does not meet the requirements of food safety into the target products[26–28]. Therefore, it is very meaningful to realize the heterologous expression of glucose isomerase in food safety strains with clear research background and to safely synthesize HFS-55 high fructose syrup in one step.

However, food safety requires a thorough investigation of food-grade microorganisms. Bacillus subtilis 168, and some other non-pathogenic related Bacillus species, which are free of exotoxins and endotoxins, and have a recognized history of safe use in foods are very useful for fermentation and large-scale cultivation[29].

Thus, an efficient expression of recombinant GIase in a generally regarded safe strain is necessary for improved economic HFCS-55 manufacturing and can contribute more to food security.

In this review paper, HFS production approaches, Glucose Isomerase properties, plasmid vectors, hosts engineering methods strategies for efficient expression of recombinant proteins in *Bacillus subtilis* 168, are reviewed.

II. THE INDUSTRIAL PRODUCTION PROCESS FOR HFS

The chemical conversion of glucose to fructose has a history of more than a hundred years. Most methods are usually carried out at a high pH, and temperatures. The possibility of chemical conversion of D-glucose into D-fructose has been studied and reported [30]. However, this reaction is non-specific and easily produces by-products

and poor color-developing products fig.2. It is also difficult to obtain D-fructose concentrations above 40% by this method [25].

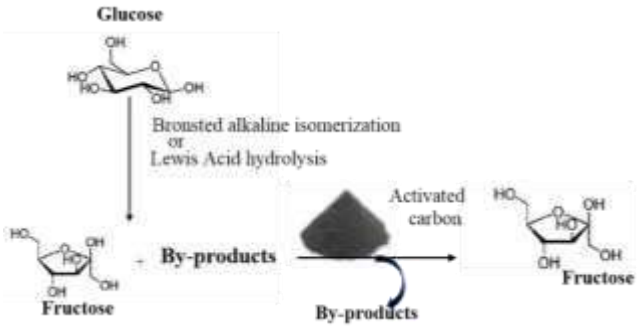

Fig. 1 Chemical process of high fructose syrup synthesis

In contrast, using glucose isomerase to convert glucose to fructose has several advantages over chemical methods, including (i) the specificity of the reaction, (ii) the mild pH and temperature conditions of the reaction, and (iii) a higher fructose ratio in the final product. Therefore, enzyme conversion is the first choice for HFS production[25].

High Fructose Corn Syrup has been produced from corn by recombinant glucose isomerase for more than 40 years. In modern industry, the production of HFC from starch includes three main processes fig.3 Glucose isomerase is used to isomerize D-glucose into D-fructose with a mild basic environment under moderate pH conditions[11].Fig.2 represents in vitro isomerization of glucose into fructose.

The production of HFS is highly industrialized, and the processing aids used are best classified in the chemical laboratory. Both of these sweeteners are extracted from complex plant sources, which contain a large number of potential colors, odor, and flavor compounds that must be removed. Since foods and beverages manufacturers require high-purity sweeteners, there are no undesirable contaminants, and because process engineers can only obtain a few refining techniques, the isomerization process is illustrated below[11,31]:

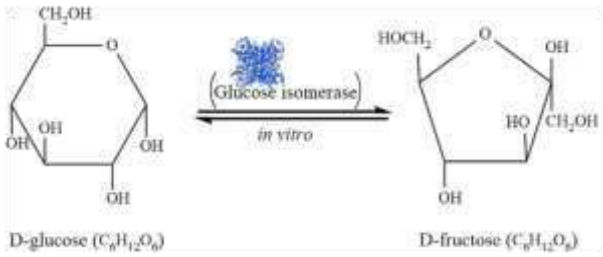

Fig. 2 Isomerization of D-glucose into D-fructose by recombinant GI

In general, the bioconversion process is referred to as **fermentation** which is a biochemical process in which carbohydrate molecules (such as glucose) are converted into energy, lactic acid, and other by-products according to the type of microorganisms involved in the fermentation processes[32]. There are two types i.e. **Solid-state Fermentation** which is related to solid-state fermentation, microbial growth and fermentation occur on the surface of a solid substrate and **submerged Fermentation** which in this fermentation process, the substrate for the growth of microorganisms is placed in a liquid solution in a big tank called a fermenter or bioreactor[33]. Submerged fermentation of fermented liquid can be subdivided into three categories:

• Batch fermentation: Put the substrates and raw materials required for fermentation and the required microbial growth into the bioreactor; when operating parameters such as pH value and thermal value are determined, culture is allowed. In the fermentation process, there is nothing except the addition of oxygen in the case of aerobic microorganisms. After each process is completed, the products are collected and the fermenter is cleaned; then, another batch of products can be prepared and the process can be restarted.

•Fed-batch fermentation: A small number of substrates and raw materials are added during the fermentation process. Both batch and fed-batch procedures are considered "closed" fermentation systems, different from "open" systems such as continuous fermentation [34]

• Continuous fermentation: In this process, the addition of substrates and raw materials is carried out continuously. Therefore, continuous fermentation is considered an open system: allowing the introduction of new raw materials, unlike a "closed" system [34].

Although batch isomerization is feasible in some modifications of stirred tank reactor systems[35].

Technically feasible, a continuous process using a fixed bed reactor has advantages.

Advantages of continuous isomerization process

Compared with batch isomerization in a stirred tank.

1. The amount and cost of enzymes for converting glucose into fructose are low.

2. Reduce capital equipment and labor costs.

3. Better and easier process control.

4. Reduce refining costs and improve product quality.

III. MICROBIAL SOURCES OF GLUCOSE ISOMERASE

The Most commercially available glucose isomerase genes are mined from some mesophilic microorganisms. The glucose isomerase is widely distributed in prokaryotes, many bacteria and actinomycetes have been found to produce Glucose Isomerase and most of them produce intracellular GI enzymes. Previous studies reported that only a few extracellular Glucose Isomerases are produced by Chainia spp, *Staphylococcus aureus*, and *Streptomyces glaucescens*. In addition to Streptomyces, some bacilli are also good producers of GI[36,37].

IV. CRYSTAL STRUCTURE OF GLUCOSE ISOMERASE

Glucose or xylose isomerase catalyzes the reversible isomerization of D-glucose and D-xylose into D-fructose and D-xylulose, respectively, and is not only involved in sugar metabolism but also has other valuable industrial application, such as the production of high fructose syrup and biofuels[38,39]. Various structural crystals of glucose isomerase demonstrate the binding configuration of the substrate and its molecular mechanism. However, the metal-binding mechanism required for the isomerization reaction has not been described[39]. Based on primary amino acid sequence homology, Glucose isomerases are divided into two categories, namely class I and class II. The main difference between them is that the second type of enzyme contains an extra insertion of approximately 40-50 amino acid residues in the N-terminal domain and although the homology of the primary sequences of the two classes is low (25–30 %), their three-dimensional structures are similar fig.3 [16,38]. The crystal structures of some GIs derived from microorganisms, such as *Streptomyces rubiginosus*, *Streptomyces olivochromogenes*, and *Actinoplanes missouriensis*, have been identified and show a high degree of structural homology[40]. The enzyme catalytic capsule at the N-terminus of the GI monomer and two metal-binding sites are folded into an eight-stranded (β/α) catalytic pocket, and the C-terminus is folded into a large ring, which can overlap with the N-terminus of another subunit to form a tightly bound dimer body which are associated with non-covalent bonds[38,41]. Currently, commercial GIs as HFS producers mainly come from *Bacillus coagulans*, *Streptomyces murinus*, and *Streptomyces rubiginosis*[21]. However, the disadvantage of using these enzymes is that when the isomerization temperature rises above 60°C, their catalytic performance is poor, and subsequent chromatographic separation is expensive to obtain the desired D-fructose concentration of HFS. Thus, looking for heat-resistant GIs that produce HFS at high temperatures and increase the yield of D-fructose is considered to be an effective way to reduce the cost of improvement[42,43].Glucose Isomerases are homo-tetramer with the monomer molecular weights ranging in between 43-53kDa.The tetramer appears to be a living unit, but the monomer is inactive. The Monomers are combined with non-covalent bonds and lack interchain disulfides. Each monomer has two metal binding sites, which are combined with $Mg^{2+,}$ Co^{2+} or Mn^{2+}, and are necessary for catalytic activity and thermal stability. The monomer has 8 α-helices and 8β sheets an alpha-beta barrel motif[36].

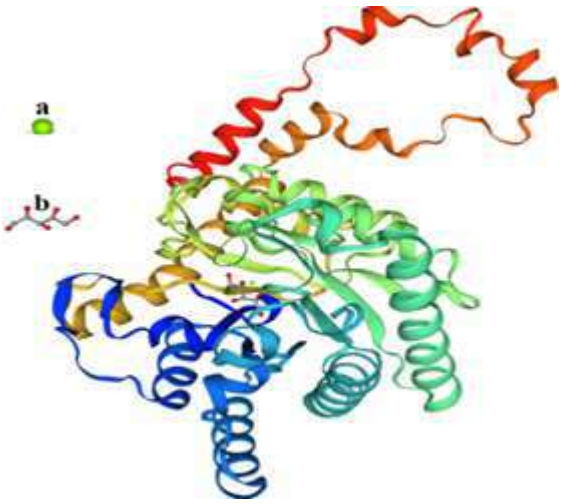

Fig. 3 3D carton Homo-tetramer structure model of GI: an overview of GI-ligands interaction in 3D dimension, green ball (a) represents Magnesium and (b) represents ligand.

(https://swissmodel.expasy.org/interactive/8S3uUT/models/)

V. PHYSICAL-CHEMICAL PROPERTIES OF GLUCOSE ISOMERASE

Various substrates. pentose, hexose, both sugar alcohol, and Phosphate can be bio-converted by enzymes. This protein has different affinities and can utilize D-ribose, L-rhamnose, L-arabinose, 2-deoxyglucose, and D-allose like the most very common substrates D-xylose and D-glucose [25]. The glucose isomerase is significantly stabilized and activated divalent cations, especially Mg^{2+}, $Co^{2+,}$ and Mn^{2+} ions. It is generally believed that Mg^{2+} is the main activator of Glucose Isomerase. The divalent Co^{2+} plays a vital role in the activity of the enzyme, but it is best to maintain the stability of the GI enzymes by maintaining the molecular structures of the proteins, especially the quaternary structures [44,45]. Other metal ions such as $Hg^{2+,}$ $Ag^{+,}$ Zn^{2+}, Cu^{2+}, Ni^{2+} can inhibit the catalytic activity of GI enzymes, especially Ca^{2+} in the initial stage of industrial biosynthesis. In the early stage of producing

HFCs, Ca^{2+} can be used as a cofactor for the glucan 1,4-a-glucosidases the next step to produce glucose syrup as a substrate for glucose isomerase. Excessive residual Ca^{2+} inevitably inhibits the glucose isomerase enzyme activity and which reduces the conversion rate, yield, and productivity of High Fructose Syrup. Therefore, the process to remove calcium ions before the isomerization of glucose syrup is an essential process in industrial production. Other known GI activity inhibitors are substrate analogs such as arabitol, xylitol, mannitol , and sorbitol[46]. The optimum temperature range for GI for most commercial applications is 55 to 65°C which increases in the presence of cobalt ions. The optimal pH ranges for GI activity generally range in between pH 7.0 ~ 9.0. According to recent reported studies, it has been found that many thermostable glucose isomerases have higher optimal reaction temperatures[47].

VI. FUNDAMENTAL ELEMENTS FOR HIGH LEVEL EXPRESSION OF RECOMBINANT PROTEINS

The expression of recombinant protein is realized by several steps, and the overall steps are shown in Figure 5. The two main components of recombinant protein expression are expression vector and expression host / compatible host.

6.1. Gene design

Direct synthesis of genes is rapidly becoming the most effective way to produce genes Construction and application of functional genes, such as codon optimization[48].

6.1.1. Codon Optimization

To select a gene for optimal expression needs to select from a large number of sequences. For example, in theory, a protein with an average size of 30 kDa could be encoded by 10^{100} possible DNA sequences. Historically, there have been two approaches to codon optimization. The first, named "one amino acid, one codon", use the most abundant codon of the host to encode all occurrences of a given amino acid in the optimized sequence. The second method, called "codon randomization", uses translation tables based on the frequency distribution of codons across the genome or in a subset of highly expressed genes[49]. Codon optimization consists of the replacement of codons to meet the host codon bias, and this aims to increase gene expression[50]. Recombinant protein expression using bacterial and other host organisms is a fundamental technology for protein production. A key step in recombinant protein expression is codon optimization where a coding sequence for a protein of interest is designed by synonymous substitution directing to increase its expression level[51]. The substitution of rare codons in the introduced gene may increase the yield dramatically. Besides, the replacement of rare codons might decrease the chance of misincorporation and protect the protein from premature turnover[52]. Current approaches to codon optimization are based on sequence features considered to influence protein expression levels. As an example, a conventional approach is to substitute rare codons with frequent codons according to the genomic codon usage in a host organism. The basis of this approach is that endogenous genes whose coding sequences consist of frequent codons have high protein expression levels, and therefore, recombinant protein expression is also considered to be improved by increasing the codon frequency. Besides, aiming to introduce synonymous substitution computationally predicted to destabilize mRNA secondary structures since the stable messenger RNA secondary structures may inhibit translation, this approach is taken into consideration to improve recombinant protein expression by enhancing translational efficiency[51,53,54].

The genetic information carried by mRNA and then translated into proteins is encoded into nucleotide triplets called codons. Four alternate nucleotidic bases (A, U, C, G) compose mRNA so that $4^3 = 64$ possibilities of codons which code for only 20 naturally occurring amino acids. The genetic code is therefore redundant: while a few amino acids correspond to a single codon, most amino acids can be encoded by different codons. Different codons coding for the same amino acid are known as synonymous codons, and in a wide variety of organisms synonymous codons are used with different frequencies “a phenomenon is known as codon bias’’[55,56]. Various factors such as expression level, GC content, recombination rates, RNA stability, codon position, gene length, environmental stress, and population size, can influence codon usage bias within and among species. These observations stress the potential of the new index to both measures and explain codon usage bias, particularly as related to speed and accuracy of gene translation and protein synthesis and this approach has been widely used for designing synthetic genes to improve their expression in the heterologous host organisms[56,57].

6.1.2. Expression Vector

The design of the vector expression vector enables the cloned gene to be transcribed and/or translated. It contains all the components that express foreign genes. Plasmid vector was used for cloning and expression of the foreign gene in the prokaryotic system[58]. Therefore, the expression vector used has elements specific to those used

in a particular system. The basic components of the expression vector are shown in Fig. 4.

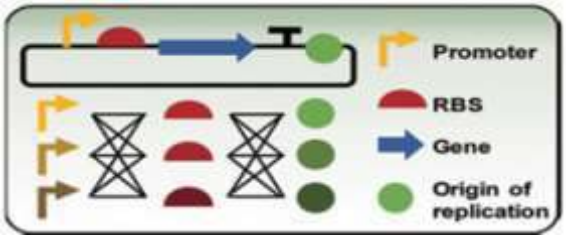

Fig. 4 Plasmid-based engineering

6.1.2.1. Promoter

The promoter is a key component of the expression system because it controls the transcription initiation of related genes. An ideal promoter should exhibit several desirable characteristics: (i) it should be strong enough to allow the product to accumulate up to 50% of the total cell protein; (ii) it should be strictly regulated to prevent product toxicity. Constitutive promoters do not allow efficient production of toxic proteins / some natural proteins that are harmful to cells when overexpressed. One example is a membrane protein, which can cause cell death when overproduced in the host, possibly by jamming the inner membrane[58,59].

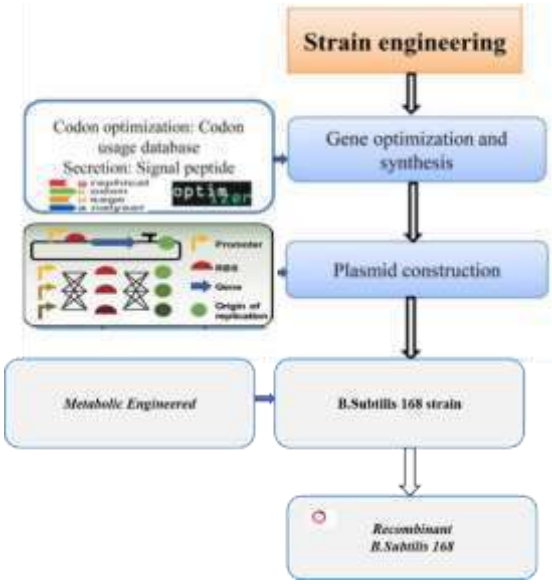

Fig. 5 Engineered recombinant B. Subtilis 168 for high-level protein expression

VII. INDUSTRIAL APPLICATIONS OF HIGH FRUCTOSE SYRUP

Currently, HFCS-55 is highly more suitable for industrial applications fig.7 [60].]. Because of its better function and Technical advantages compared with sucrose, such as better solubility, higher sweetness, stability, and lower prices[61], use of HFCS in beverages, baking, canning, and confectionery the industry as an alternative sweetener and food additive is increasingly[11,62]. 5-hydroxymethylfurfural (HMF) can be produced from a variety of raw materials, including hexose and polysaccharides [63]. Fructose is usually an effective and selective starting material for HMF production, which can achieve faster conversion at a higher yield than glucose [61]. However, due to its cost-effectiveness, glucose is often used in the production of HMF in industrial production Costs [63]. HMF is produced by dehydration of fructose and induced by acid or metal catalyst[61]. Besides, HFS can be used as raw material for valuable biobased fine chemicals such as biofuels, green solvents, and lubricants, plastics[64].

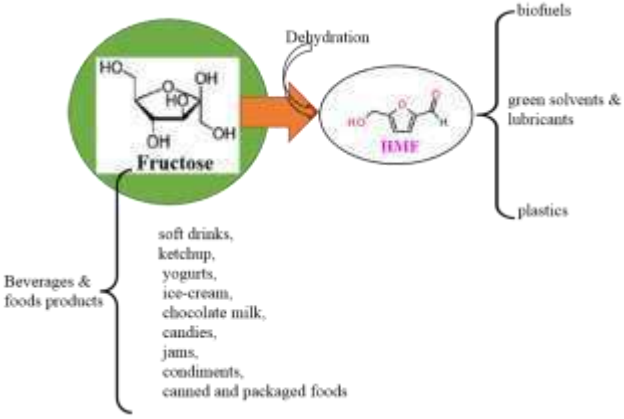

Fig. 6 Industrial applications of Fructose

VIII. CONCLUSION

Due chemical hydrolysis of D-glucose into D-fructose which has several challenges and disadvantages, in recent decades' researchers have attached their attention to Enzymatic isomerization which revealed excellent economical potentials over the classical method.

Benefits of codon optimization in Industrial Biotechnology in the past ten years, nothing has been strongly proved and this technology is rapidly being adopted to remain competitive in the current market. This example shows that there is only one or several synthetic genes a host needs to be introduced to generate new products or directly reduce the existing production costs. The cost of gene synthesis is decreasing in the past decade; and the technology of assembling large pieces and multiple worlds changing at the same time type genome becoming available. Thus, we can envision a future where custom-made microorganisms can be designed for a particular application. One of the areas in which these new technologies can play a role Dramatic contribution toward the production of chemicals by microbial cell factories.

Start moving towards that goal It is adopted in the industry.

Furthermore, engineering promoters can accelerate the level of recombinant protein expression which contributes more to large-scale protein production. Thus, strong promoters are highly recommended for microbial strain engineering.

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Observation of El Niño and La Niña Phenomena, on the island of Sulawesi - Indonesia, based on the Global Positioning System (GPS)

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Abstract— *This paper studies precipitable water vapor (PWV) in 2015-2016, an El Niño-Southern Oscillation episode (ENSO), and causes drought in Indonesia. Using the Global Positioning System (GPS), we obtained the PWV value. Expected can detect ENSO phenomenon through PWV value. We processed the GPS observation data by Precise Point Positioning (PPP) method using the goGPS software. The obtained values of Zenith Troposphere Delay (ZTD) and PWV were validated by comparing with the ZTD values from four IGS stations (BAKO, PIMO, NTUS, ALIC), i.e., there was a mean difference of -3.48, the average standard deviation is 13.36 mm, and the correlation coefficient was 0.94. The variation between the IGS ZTD and PPP ZTD estimates is within the measurement error. Our results can be used to study PWV behaviors for meteorological and climatological purposes in Indonesia. The values of PWV obtained from station GPS CMAK and CBIT were compared with PWV by radiosonde observations. The results showed a good agreement with the coefficient correlation is 0.86 at CMAK Station and 0.85 at CBIT station. The PWV values at CBIT stations varied between 37.08 and 76.01 mm with an average of 57.05 mm. For CTOL, CMAK, and CKEN stations, their average PWV is 61.17, 50.84, and 51.75 mm. The standard deviation (STD) value of the PWV at CMAK stations was 11.79 mm, higher than the other stations. During the El Niño event, the correlation between SSTa and PWV became weak at the four stations.*

Keywords— ***PWV, ENSO, GPS, PPP, ZTD, SULAWESI.***

I. INTRODUCTION

The Global Positioning System is the oldest Global Navigation Satellite Systems (GNSS). Indonesia's GPS network has been used only for positioning, surveying, and mappings, such as inland administration, mining, and transportation sectors. It has not been utilized to observe the Earth's atmosphere, including meteorological and climatological studies. In the early 90s, (Bevis et al. 1992) first proposed GPS meteorology, i.e., we can use ground GPS stations to study water vapor behaviors in the lower atmosphere (the troposphere) called precipitable water vapor (PWV).

PWV is an important parameter that has an essential role in weather and global climate. One of the factors influencing climate change is the ENSO phenomenon, El Niño, Southern Oscillation, and the phenomenon opposite ENSO (LaNiña). We used the four indicators to detect the ENSO phenomenon, the sea surface temperature anomaly (SSTa), sea surface pressure anomaly (SLPa), and multivariate ENSO index (MEI), and rainfall gradient (Curtis and Adler 2000).

This study investigates the behavior of PWV in 2015–2016. Based on data from the National Oceanic and Atmospheric Administration (NOAA), El Niño in 2015–2016 was more robust than El Niño in 1997-1998. During El Niño, many parts of Indonesia experience drought. We address the question of whether GPS-PWV can detect ElNiño, especially when El Niño grows and decays. These techniques are cost-effective because we use pre-existing infrastructure, the GNSS network in Indonesia. In this

research, we will focus on ENSO analysis, especially El Niño, by GPS PWV monitoring on the island of Sulawesi.

II. DATA DAN METHOD

PWV Retrieved from GPS

The measurement concept of PWV with GPS is illustrated in figure 1. In the model, a microwave signal propagates from the GPS satellite to the receiver on the ground, which deforms with the seawater's loading mechanisms. GPS signals that propagate through the Earth's atmosphere suffer from the variability of the ionosphere's refractive index and troposphere. Delays are caused by two factors, i.e., the excess path caused by the bending of the microwave path and the delay along the way caused by the refractive index (Suparta 2012). The tropospheric zenith total delay (ZTD) consists of zenith hydrostatic delay (ZHD) and zenith wet delay (ZWD). ZWD can be calculated based on the Hopfield model [H. Hopfield, 1969]. ZHD can be calculated using the Saastamoinen model (Saastamoinen, 1972).We used the Vienna mapping functions (VMF1) to convert zenith delays to those along the line-of-sight (Suparta et al., 2011).ZWD is calculated by subtracting ZHD from ZTD. ZWD was later transformed into approximate PWV (in mm) by employing the surface temperature measured at a particular site. To calculate the PWV, we need meteorological data, such as pressure (P), temperature (T), and relative humidity (H). Meteorological data are obtained by meteorological sensors installed on the GPS Stations to get the accurate PWV.

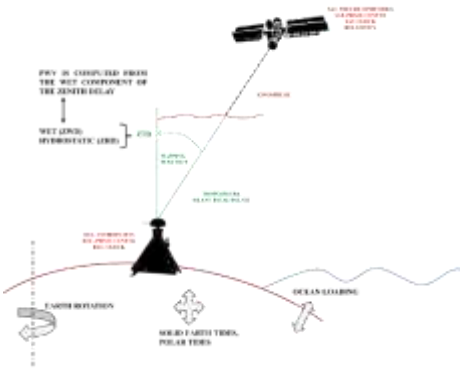

Fig. 1 Propagation of GPS signals to a receiver on the ground that covers the sea surface temperature influences

The conversion from ZWD to PWV is done using the formula by (Bevis et al. 1992),

$$PWV = \pi(Tm)\, ZWD \qquad (1)$$

Where $\pi(Tm)$ is the conversion factor that varies with the local climate and depends on weighted mean temperature (Tm, in Kelvin).

$$Tm = 0.83663Ts + 48.103 \qquad (2)$$

Where Ts is the surface temperature (in Kelvin), note that the Tm equation (2) was obtained empirically by linear regression for 15 selected radiosonde stations over the Western Pacific (Bevis et al. 1992)

Dataset

GPS data for the meteorological study were collected from the four stations in Sulawesi Island, Indonesia. We selected the El Niño episode that occurred in 2015/2016. We processed the data to study the response of PWV to ENSO. The four GPS stations are Makasar (CMAK), Kendari (CKEN), Toli-Toli (CTOL), and Bitung (CBIT) observatories. Data from the meteorological sensors were used for the individual GPS stations. All GPS stations recorded the carrier phase with an interval of 30 seconds. The stations are shown in Fig. 2. To link the activities of ENSO with PWV response, SSTa Oceanic

Niño Index (ONI) on the Niño 3.4 area was obtained daily from the National Oceanic and Atmospheric Administration (NOAA).

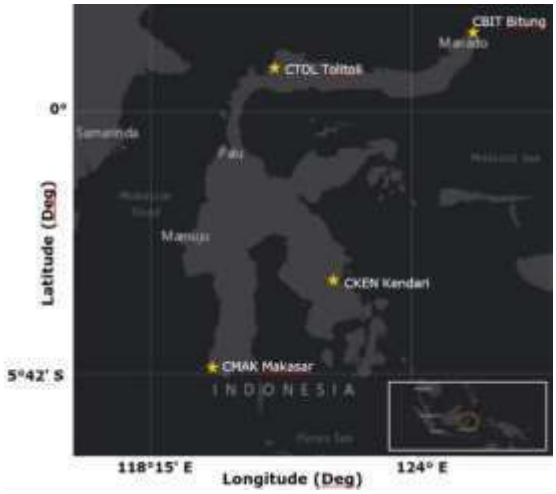

Fig. 2 Location 4 station for study

Data Processing

Ground-based GPS data can be processed to determine ZTD in two different ways : (1) Baseline network solution using double-difference observable (Bevis et al. 1992) and (2) Precise Point Positioning (PPP) using indifference observable (Byun and Bar-Sever 2009).

The baseline approach is the most commonly used in GPS processing. It is done with two or more receivers

simultaneously observing GPS satellites. This technique provides precise ZTD estimates, but the separation distance between adjacent receivers limits its effectiveness. On the other hand, PPP can be performed for a set of single GPS receivers to estimate the precise values of ZTD. It should be emphasized that in PPP, precise satellite orbit and clock correction data are needed.

In this research, the ZTD results derived using PPP processing methods were compared and evaluated with the ZTD from the International GNSS Service (IGS) final troposphere. One week of GPS observations was collected from 4 IGS network stations, i.e., BAKO, NTUS, PIMO, ALIC. This validates that the methods we used to calculate the PWV in subsequent studies. For more information on the IGS final troposphere product, refer to http://igscb.jpl.nasa.gov/.

To process and analyze the data signal and GPS meteorology in RINEX format, we used the software package goGPS in the PPP model. goGPS is an open-source software application developed by (Realini et al. 2012) since 2007 at Geomatics Laboratory of the Politecnico di Milano, Como Campus. Initially, it was created in MATLAB but was recently converted to Java to expand its users. They started to provide it as a service through the web.

To validate the PWV values from GPS, we compared them with those from radiosonde observations around the GPS stations we used. Radiosondes provide various quantities concerning the atmosphere, including temperature, pressure, and humidity. They are often used in validating GPS-PWV data as a reliable source of independent data. Although radiosondes provide meteorological observations with a high vertical resolution, their temporal and spatial resolutions are not high. Therefore, we compare GPS-PWV values observed at times when the radiosondes are released. The PWV from radiosondes is considered to be accurate to ~1.2 mm (Feng, Bai, and Williams 2001)

In this study, we used two radiosonde sites on Sulawesi Island operated by the Indonesia Agency of Meteorology, Climatology, and Geophysics. They were found to be close to two of the GNSS stations used in this study. These stations located at the Sultan Hasanudin Airport in Makasar (WAAA) and the Sam Ratulangi Airport near Bitung (WAAM). The nearest GNSS stations to these radiosonde sites are Makasar Observatory (CMAK) and Bitung (CBIT).

III. RESULTS AND DISCUSSION

Troposphere Zenith Delay

The mean, standard deviation, and the correlation between the ZTD estimate obtained from goGPS software with PPP processing and ZTD from IGS for the stations over one week are listed in Table 1. The mean difference between the two processing methods is more petite than -3.48 mm. The mean, standard deviation is 13.36 mm, and the mean correlation is 0.89. This shows good agreement results in ZTD goGPS with ZTD from IGS.

Table 1 Statistical compilation ZTD processing by goGPS software and ZTD from IGS

Station ID	Diff (mm)	STD (mm)	Correlation
BAKO	-3.77	13.58	0.87
PIMO	-6.47	13.60	0.95
NTUS	-3.28	13.06	0.76
ALIC	-0.40	13.20	0.99
Average	-3.48	13.36	0.89

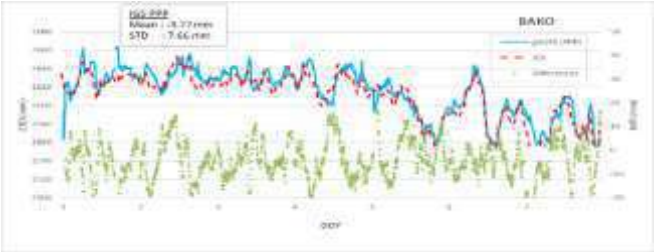

Fig. 3 One-week comparison between the IGS final tropospheric product with PPP processing solutions at BAKO GPS station

The ZTD estimates for the BAKO station from the IGS and our solution are compared in Fig. 3. The results from both are consistent, and the deviations between the two solutions are well within the level of 1–2 cm. These results conform to the findings in (Bevis et al. 1992),(Gao and Chen 2004), (Gendt, Reigber, and Dick 2001), and (Rocken et al. 1993). An error of 1–2 cm in ZTD equals an error of 1–3 mm in PWV, which is considered insignificant for this study. Therefore, we use this PPP solution in the following analysis, unless stated otherwise.

Validation of ground-based GPS-PWV with radiosondes.

One-month of GPS-PWV estimates at these stations were calculated using measurements from the nearest weather stations. The GPS-PWV and radiosonde-PWV time series at these stations are illustrated in Fig. 4

The correlation coefficient shows good agreement between the two data sets, particularly at the CMAK-WAAA station pair. The GPS receiver and radiosonde station are collocated. We use these PWV values to study the link

between PWV and climate changes, e.g., the El Niño episodes.

PWV and SSTa Variability

Figure 5 shows the variation of daily averages of the PWV from GPS measurements in 2015/2016 at four selected CBIT, CTOL, CMAK, and CKEN. This figure shows that the PWV at the four stations shows different patterns. At the CBIT station, PWV varies between 37.08 and 76.01 mm, with an average of 57.05 mm. For stations CTOL, CMAK, and CKEN, their average PWV is 61.17, 50.84, and 51.75 mm, respectively. The standard deviation (STD) of the PWV at the CMAK station, 11.79 mm, was higher than other stations.

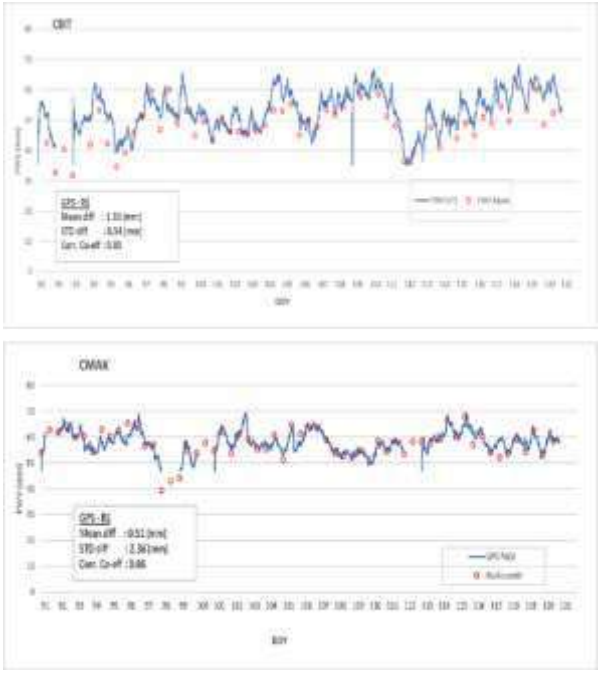

Fig. 4 Comparison between the GPS-PWV (solid line) and radiosonde-PWV for CBIT (upper) and CMAK (lower) stations, respectively. The red circles show radiosonde estimates of water vapor.

From Figure 5, PWV at CMAK and CKEN stations show a clear pattern that PWV values are higher during the rainy season (Oct-Mar) and lower during the dry season (Apr-Sept). This tendency was also seen at the CBIT and CTOL stations, but there was not so strong. The PWV at CBIT and CTOL increase during April-May 2016. Significant PWV fluctuation occurred during 2015 at the CMAK and CKEN stations in July when ENSO activity disturbed the two stations' atmosphere.

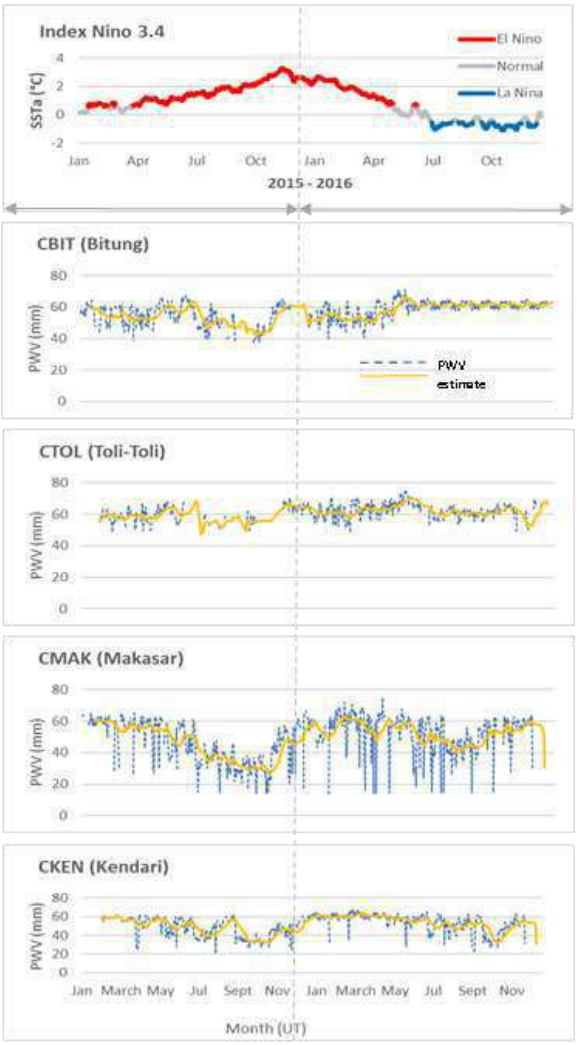

Fig. 5 The daily average of PWV variation from GPS measurements during 2015 and 2016

Intense ENSO episodes were recorded in 1982/83, 1997/1998, and 2015/2016. Such ENSO episodes play an essential role in generating extreme conditions in the Pacific Ocean and the region around Sulawesi. There are two approaches to identify the occurrence of ENSO. In the first definition, an ENSO event is considered to occur when the 5-month running average of the SST anomaly (SSTa) in the Niño 3.4 region is above 0.4 degrees for six months (Trenberth 1997). As the second definition, the Japan Meteorological Agency (JMA) uses the oceanic of Niño 3 (4N-4S and 150-90W). They consider it an ENSO event when the 5-month running average of the SST in this area (SSTa) exceeds the thresholds of 0.5 degrees or more for six consecutive months (Bove et al. 1998).

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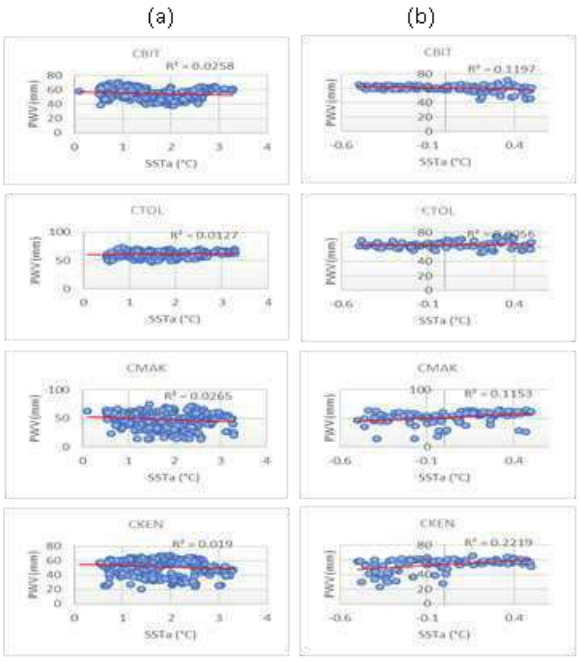

Fig. 6 Scatter plot between GPS PWV and SSTa for Index Niño 3.4 region for the year of 2015/2016

Figure 5 shows the identification of ENSO, according to NOAA, based on the first definition. The period of more than~12 months shown in red Five to an El Niño episode (SSTa> 0,5), while the part is shown in blue (SSTa <-0.5), is a La Niña event. The gray color part is neutral. From this figure, the El Niño event was from the middle of January from 2015 to May 2016. Its peak is around November 2015, where the SSTa anomaly in the Niño 3.4 region is 3.28 degrees.

Monitoring of PWV Variability from GPS During El Niño Event

The correlation analysis was done to show PWV responses to El Niño events, i.e., we compare the PWV values among the periods of El Niño, neutral, and La Niña. During the El Niño episode, its intensity (ENSO index) varies in time. We also analyze the PWV changes associated with the difference in the index. The responses seem to go among the four stations.

Table 2 The correlation coefficient between GPS PWV and SSTa during the El Niño event for the case of 2015/2016

Station (City)	Niño 3.4	Normal	LaNiña 3.4
CBIT (Bitung)	-0.1606	-0.3460	-0.1469
CTOL (Toli-toli)	0.1126	0.0748	0.0882
CMAK (Makasar)	-0.1627	0.3395	-0.2384
CKEN (Kendari)	-0.1378	0.4710	-0.0579
Average	-0.0871	0.1348	-0.0887

Below is the classification of correlation coefficients,

1. 0. “no correlation.”

2. 0.20 -0.39 "weak."

3. 0.40 -0.59 "moderate"

4. 0.60 -0.79 "strong."

5. 0.80 -1.0 "very strong"

During the El Niño episode shown in Figure .6a., the correlation between SSTa and PWV is very weak at four stations, i.e., below 0.20. For neutral conditions (Figure 6b). The correlation between SSTa and PWV is weak at CBIT, very weak at CTOL, soft at CMAK, and moderate at CKEN (0.4710). During LaNiña, a weak correlation is found only at Makasar station (CMAK). It is very weak at the other three stations.

The weak response to El Niño at these sites may be due to seasonal changes from the rainy season to the dry season. The relationship between PWV from GPS and SSTa for Niño 3.4 did not show any significant correlation, either.

During the El Niño intensity increase in April–November 2015 (Figure 7a). Of the four existing stations, a correlation between GPS PWV and SSTa is very weak at CBIT, weak at CTOL, moderate at CMAK, and soft at CKEN. The overall average correlation is very weak.

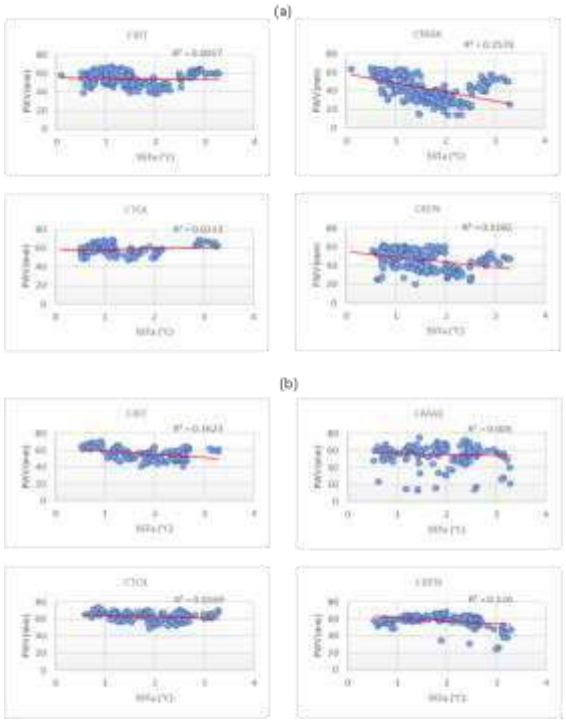

Fig. 7 Scatterplot between GPS PWV and SSTa from Niño 3.4region during El Niño event for 2015/2016 (a) the decreased intensity and (b) the increasing intensity.

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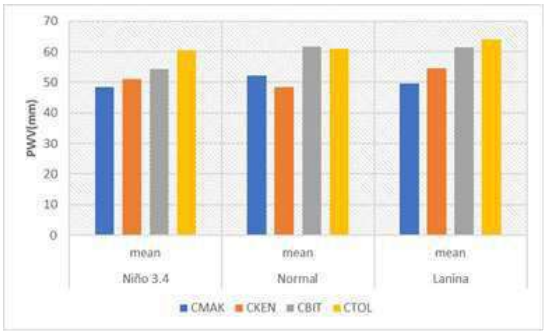

Fig. 8 Graph of average PWV value under El Niño condition, neutral condition, and LaNiña, at four stations in 2015/2016

On the other hand, decreasing the ENSO index in November 2015–June 2016 (Figure 7b) shows a moderate correlation with GPS PWV at CBIT, the weak correlation at CTOL, the very weak correlation at CMAK, and CKEN. The overall average correction between the declining El Niño intensity and PWV is soft.

Figure 8 shows that the average PWV during LaNiña is generally higher than in El Niño and Neutral conditions at all four stations. More statistics details are given in Table 2. However, further and more in-depth research is needed to address the response of PWV to ENSO.

IV. CONCLUSIONS

We studied the variability of GPS-PWV and SSTa during the 2015/2016 period covering El Niño, neutral. La Niña episodes using data from four stations in Sulawesi, Indonesia, i.e., CBIT, CTOL, CMAK, and CKEN. The GPS-PWV and SSTa showed a negative correlation. The decrease in PWV also correlates with the dry season lasting from April to September, when water vapor in the atmosphere offers a minimum. The selected GPS receivers in the Sulawesi region may not be optimal for studying PWV changes by ENSO since the correlation between PWV and SSTa was weak throughout the period. Nevertheless, by comparing the average PWV values in the El Niño, neutral, and La Niña conditions, We could show that average PWV offers smaller deals in the El Niño period than in the La Niña period. Further research is needed to find out more details.

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The impact of China's fertilizer industry de-capacity on agricultural production costs

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Abstract—*In response to the re-collection of value-added tax and the abolition of fiscal and tax preferential policies for the fertilizer industry in 2015, using provincial panel data from 2011 to 2018, a model was established to analyze the impact of policy changes on agricultural production costs. Research shows that after the implementation of the policy, agricultural intermediate consumption has increased significantly by 11%, and the proportion of fertilizer fees in total costs has increased significantly by 0.5%. It can be inferred that it has increased agricultural production costs, while the profitability of fertilizer companies has not changed significantly , Which proves that after the abolition of preferential fiscal and taxation policies for chemical fertilizers, at least part of the cost of enterprises has been transferred to farmers, which has increased the burden on farmers. Suggestions on the need to rationally guide the transformation and upgrading of fertilizer companies, formulate fertilizer subsidies for farmers, and actively promote reasonable fertilization.*

Keywords—***Fiscal and tax policy, fertilizer, agricultural production cost, VAT***

I. INTRODUCTION

Fertilizers play an irreplaceable role in modern agricultural production. Under the impact of the 2020 locust plague and the COVID-19 epidemic, world food production has generally decreased and many countries have announced restrictions on food exports, posing new challenges to China's food security. Fertilizers have a significant role in increasing food production, but the continuous expansion of fertilizer production capacity has led to greater pressure on enterprises to de-stock, and according to statistics, the problem of overcapacity in China's fertilizer industry was serious in 2015.In 2015, the Ministry of Finance, the Development and Reform Commission and other ministries issued successive notices on the reimposition of VAT on fertilizers and the cancellation of some preferential fiscal policies, and the former Ministry of Agriculture formulated the Action Plan for Zero Growth in Fertilizer Use by 2020 The Programme pointed out the problems of blind fertiliser application, low utilisation of organic fertiliser resources and unbalanced fertiliser application structure in China, requiring zero growth in fertiliser use by 2020. While these policies are guiding the fertiliser industry to remove production capacity, reduce fertiliser waste and reduce environmental pollution, it is worth exploring whether enterprises are transferring the new costs brought about by the removal of preferential policies to farmers who are in a disadvantaged position, thus leading to an increased burden on farmers.

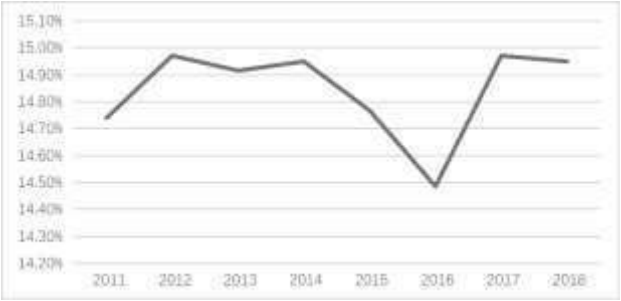

Fig. 1: Fertilizer consumption in China as a proportion of total agricultural output (Data source: China Rural Statistical Yearbook)

II. REVIEW OF THE LITERATURE

In the field of agriculture, fiscal and taxation policies have an important regulatory role. Bo Li et al (2019) summarize the content of agricultural fiscal and taxation policies into three main categories: fiscal agricultural subsidy policies, fiscal agricultural investment policies, and agricultural-related taxation policies. Among them, agricultural subsidy policy is the most commonly used policy in agricultural policy, which is more flexible and can target specific groups or be oriented to the general farmers. Xiangdong Yang (2016) found that China's agriculture-related tax policies are not perfect, and the preferential policies for agriculture-related taxes are convenient and single, mainly focusing on income, however, there are fewer preferences in terms of transfer. At the same time, tax concessions are mainly for high-quality agricultural products assessed as provincial and national level, and the concessions for basic agricultural construction are low. Although VAT is a more excellent tax, there are limitations in the operation of VAT in agriculture, and the current VAT is not strong enough to protect agricultural products and industrialized operations, and the VAT rate for processing industries is obviously high, which is not conducive to the development of agricultural industrialization. China has previously implemented preferential fiscal policies for the fertilizer industry for a long time, and Ke Feng (2015) argues that China's fertilizer industry was not yet mature enough to meet the needs of agricultural modernization in the 1990s, and that the actual proof of preferential fiscal policies for the fertilizer industry has promoted the rapid expansion of the fertilizer industry, which is conducive to safeguarding China's fertilizer supply and stabilizing food prices. After years of development, China's fertilizer industry is facing backward technology and overcapacity. It is imperative to abolish preferential fiscal policies and promote the market to play a decisive role in the allocation of resources in the fertilizer industry. Keqing Zhou et al (2019) summarised and reviewed the changes in China's preferential fiscal policies for fertilisers. With the development of economy and society, some traditional unreasonable fiscal and tax policies have not adapted to the upgrading of China's industrial structure and changes in the market environment. Faced with factors such as overcapacity, the reintroduction of VAT on fertilizers did not necessarily bring about an increase in the final price of fertilizers. Yong Fan et al (2020) found that the direction of the impact of VAT on final product prices depends on the direction of tax burden shifting. Tao Wen (2011) explored the impact of fiscal policy on agriculture and found that the effect of active fiscal policy in agriculture on food price volatility is significant. Su Chen (2017) explored and found that agricultural policy adjustments since 1994 can affect the incentive of farmers to grow food.

Objectively, the use of chemical fertilizers has promoted increased food production, and in modern society chemical fertilizers are widely used in the growth stages of all types of food crops. Jikun Huang et al (1994) studied the effect of chemical fertilizers on rice crops and found that chemical fertilizers could significantly increase the yield per acre of rice, while labour inputs were found to have a limited effect on increasing rice yields. Lingling Yang (2018) noted that there were many wasted resources in agricultural production, especially the excessive use of chemical fertilizers. By constructing a model between fertilizer use and total agricultural output, the study found a "negative relationship" between fertilizer use and total agricultural output. Jun Yin et al (2020) analysed fertiliser and other factor input and price data from 2004 to 2016 and found that various price variables, such as fertiliser prices, labour prices and machinery prices, had a significant impact on the amount of fertiliser input in maize production. Yijie Deng (2019) conducted an empirical study on fertiliser application in Sichuan between 2001 and 2015 and concluded that changes in fertiliser use brought about positive changes in grain yield, while the elasticity of fertiliser application and grain yield increase showed an upward and then downward trend. Denglin Shi (2020) significantly increased soil organic carbon (SOC) content, soil microbial biomass carbon (MBC) and carbon readily available (ROC) content by reducing the amount of N fertilizer by 20% with a moderate amount of biochar, which could also increase rice yield.

In 1998, the State Council issued the "Notice of the State Council on Deepening the Reform of the Fertilizer Distribution System", which stipulated that the ex-factory price of fertilizer was changed from the previous government pricing to the government guiding price, but at the same time the fluctuation range of fertilizer prices was limited. 2009, the National Development and Reform Commission and the Ministry of Finance jointly issued the "Notice on Reforming the Fertilizer Price Formation Mechanism Notice", which adjusted the previous government-guided price to a market-regulated price. Zuli Wang et al (2011) found that fertiliser prices were mainly positively correlated with food prices, raw material prices and the supply-demand gap, and that the effect of national policies to limit fertiliser prices was not significant. Wenxiong Zhang et al (2014) showed that fertilizer price fluctuations have obvious clustering and long-term memory, and that fertilizer market price fluctuations are asymmetric, with fluctuations triggered by information on rising prices being greater than those triggered by

information on falling prices. Ming Qin (2016) found that there is a long-run cointegration relationship between energy prices and fertilizer prices.

The cost of fertilizer is positively correlated with unit price and quantity. Jingxian Ru (2008) found that fertiliser application was mainly positively related to the education level of the household head, time spent in agricultural activities, attitude towards risk, and total household income of the farming household; and negatively related to trust channels, the farming household's perception of fertiliser utilisation, and the farming household's perception of pollution. Yinglu Bu et al (2020) concluded that fertiliser application was also correlated with factors such as whether farmers participated in technical guidance on fertiliser use, whether they frequently enquired about agricultural technology knowledge, acceptance of external fertiliser promotion and arable land area and household income.

At present, the academic community has rarely explored whether policy changes affect the cost of agricultural production from a fiscal perspective. As fertiliser is an important component of agricultural intermediate consumption, a change in tax incentives for fertiliser may affect the price of fertiliser and hence the cost of agricultural production. This paper therefore proposes the hypothesis that the removal of a series of fiscal support policies in the fertiliser industry will lead to an increase in the price of fertiliser and hence in the cost of agricultural production.

III. DATA DESCRIPTION AND MODEL DESIGN

In this paper, we first collated the fertilizer inputs of seven grain crops, including early indica rice, middle indica rice, late indica rice and japonica rice, and nine non-grain crops, including peanut, rapeseed, cotton and vegetables, from 2011 to 2018 in the National Compilation of Information on Costs and Benefits of Agricultural Products, and intuitively, the mean, median and extreme values of the average fertilizer per mu for grain crops and non-grain crops were different (Table 1). The t-test shows that the difference in mean fertiliser use per acre between food and non-food crops is statistically significant, with the mean fertiliser use per acre for non-food crops being significantly higher than that for food crops (Table 2). Therefore, theoretically, there is a difference between the policy change for food crops and non-food crops.

Table 1. Descriptive statistics of fertilizer use per mu in different crop categories

Variables	Category	Total count	Average value	Standard deviation	Minimum value	Median	Maximum value
Consumption	Non-food	72	3.5685	0.4519	2.6810	3.5931	4.2099
	Food	56	2.9946	0.3594	2.1211	3.1219	3.3203

Table 2. T-test for differences in mean discounted net fertilizer use per acre for different crop categories

μ_1: Mean value of Consumption (non-food)			
μ_2: Mean value of Consumption (food)			
Difference: $\mu_1 - \mu_2$	**Difference**	**95% confidence interval of the difference**	
	0.5739	(0.4319, 0.7158)	
Original assumption H_0: $\mu_1 - \mu_2 = 0$	**T-value**	**Degree of freedom**	**P-value**
Alternative hypothesis H_1: $\mu_1 - \mu_2 \neq 0$	8.00	125	0.000

This paper selects provincial administrative units in China from 2011 to 2018 as the sample for the study, excluding Hong Kong SAR, Macau SAR, Taiwan Province and four municipalities directly under the Central Government which lack statistical data, and the data are obtained from the China Labour Statistical Yearbook, the China Statistical Yearbook, the China Rural Statistical Yearbook and the National Compilation of Information on Costs and Benefits of Agricultural Products. All data on financial resources were adjusted to comparable prices in 2011 using the Consumer Price Index for Rural Residents.

The regression model is as follows:

$$Intermediate\_consumption_{i,t} = \alpha + \beta_1 Policy_{i,t} + \beta_2 Chemical\_fertilizer_{i,t} + \beta_3 Sown\_area_{i,t} + \beta_4 Fuel_{i,t} + \beta_5 Electricity_{i,t} + \beta_6 Film_{i,t} + \beta_7 Pesticide_{i,t} + \beta_8 Mechanization_{i,t} + \beta_9 Wage_{i,t} + \beta_{10} gap_{i,t} + \beta_{11} Proportion\_of\_operating\_income_{i,t} + \beta_{12} Population\_quality_{i,t} + \mu_t + \gamma_i + \varepsilon_{i,t}$$ Equation 1

where i and t denote province and time respectively, μt is the year fixed effect, γi is the province fixed effect, and εi,t is the regression residual term. In this paper, the standard errors are clustered to the province level.

The explanatory variable Intermediate_consumptioni,t denotes the agricultural intermediate consumption in province i in year t. Intermediate consumption is a component of total agricultural output and consists of material consumption and production service expenditures, and in 2018, fertilizer expenditures accounted for about 15% of the intermediate consumption in agriculture, forestry, animal husbandry and fisheries, and about 20% to 30% of the agricultural intermediate expenditures. In addition to fertiliser, intermediate consumption related to agricultural production includes the amount of seed used, fuel, pesticides, agricultural film, electricity and small farm machinery. The amount of seed used is controlled by the total sown area of the crop (Sown_area); since more than 70% of the power in agriculture is diesel, the amount of diesel used in agriculture is used to control fuel (Fuel). In addition, other influencing factors are controlled for, such as the gap between urban and rural incomes (gap), the proportion of operating income in the disposable income of rural residents (Proportion_of_operating_income), and the education level of the regional population (Population_quality). Since the policy has different effects on food cultivation and non-food cultivation, a total of thirteen major food-producing regions in China, namely Liaoning, Hebei, Shandong, Jilin, Inner Mongolia, Jiangxi, Hunan, Sichuan, Henan, Hubei, Jiangsu, Anhui and Heilongjiang, were set as the control group (Treat=0), and the remaining fourteen provincial units were set as the experimental group (Treat=1). The policy time was 2015, so non-major grain-producing provinces in 2015 and beyond = 1 and others = 0. Table 3 shows the variables and their definitions, and Table 4 reports descriptive statistics for the core variables.

Table 3. Model variables and variable definitions

Variables	**Variable definitions**
Policy	Non-food producing provinces in 2015 and beyond = 1, others = 0
Intermediate_consumption	Ln (agricultural intermediate consumption (adjusted to 2011))
Chemical_fertilizer	Ln (amount of fertilizer applied)
Sown_area	Ln (total area sown to crops)
Fuel	Ln (agricultural diesel use)
Electricity	Ln (rural electricity consumption)
Film	Ln (amount of agricultural plastic film used)
Pesticide	Ln (pesticide use)
Mechanization	Ln (total power of agricultural machinery)
Wage	Ln (average wage of persons employed in agriculture in urban units (adjusted to 2011))
gap	Comparison of income levels of disposable income between urban and rural residents (rural residents = 1)
Proportion_of_operating_income	Net operating income as a percentage of total revenue
Population_quality	District non-attendance rate for people over 6 years old

Table 4. Model variables and variable definitions

Variables	Treat	Total count	Average value	Standard deviation	Minimum value	Median	Maximum value
Intermediate_consumption	0	104	6.6734	0.4740	5.7239	6.7192	7.5474
	1	112	5.676	1.060	2.833	6.016	6.993
Chemical_fertilizer	0	104	5.6198	0.4211	4.8138	5.5278	6.5738
	1	112	4.421	1.169	1.567	4.669	5.575
Sown_area	0	104	9.0158	0.3323	8.3099	9.0573	9.6013
	1	112	7.7931	0.9859	5.4866	8.2402	8.8811
Fuel	0	104	4.3983	0.5836	3.2696	4.2959	5.6839
	1	112	3.597	1.065	1.099	3.803	5.314
Electricity	0	104	5.2761	1.0006	3.7495	5.0518	7.5669
	1	112	4.126	1.856	-0.105	4.438	7.275
Film	0	104	11.535	0.461	10.773	11.478	12.672
	1	112	10.545	1.251	6.939	10.744	12.506
Pesticide	0	104	11.320	0.433	10.105	11.344	12.013
	1	112	9.918	1.439	6.828	10.343	11.645
Mechanization	0	104	8.5106	0.5142	7.6079	8.4342	9.4995
	1	112	7.3449	0.7150	6.0176	7.6820	8.2436
Wage	0	104	10.174	0.365	9.348	10.198	10.971
	1	112	10.333	0.327	9.450	10.351	11.004
gap	0	104	2.4953	0.2360	2.0300	2.4700	3.0700
	1	112	2.9694	0.4499	2.0400	2.9600	3.9800
Proportion_of_operating_income	0	104	0.4551	0.1030	0.2890	0.4335	0.7250
	1	112	0.4318	0.1146	0.2430	0.4320	0.7140
Population_quality	0	104	0.04953	0.01637	0.02083	0.05029	0.08440
	1	112	0.08842	0.08351	0.02379	0.06160	0.44388

IV. EMPIRICAL RESULTS AND ANALYSIS OF RESULTS

4.1 Regression results

Table 5 shows the results of the benchmark regressions, which indicate that the policy significantly increased the amount of intermediate consumption in agriculture, i.e. raised the cost of agricultural production.

Table 5. Impact of policies on intermediate consumption in agriculture

	(Equation 1)
VARIABLES	**Intermediate_consumption**
Policy	0.110**
	(2.39)
Chemical_fertilizer	-0.050
	(-0.18)
Sown_area	0.443
	(1.52)
Fuel	0.015
	(0.18)
Electricity	0.540**
	(2.41)
Film	-0.230*
	(-1.96)

Pesticide	0.064 (0.43)
Mechanization	0.086 (0.95)
Wage	0.023 (0.22)
gap	-0.073 (-0.62)
Proportion_of_operating_income	0.226 (0.82)
Population_quality	-0.262 (-0.40)
Constant	1.070 (0.50)
Observations	216
Number of Province	27
R-squared	0.666
Province FE	YES
Year FE	YES

Robust t-statistics in parentheses

*** p<0.01, ** p<0.05, * p<0.1

In order to further test the impact mechanism of the policy, a model was designed using the total cost per mu, fertiliser cost per mu and discounted fertiliser dosage per mu for various crops in each region from the National Compilation of Agricultural Cost and Benefit Information (Equation 2).

$$Proportion\_of\_fertilizer\_cost_{i,s,t} = \alpha + \beta_1 Policy_{s,t} + \beta_2 Consumption_{s,t} + \mu_t + \gamma_i + \delta_s + \varepsilon_{i,s,t} \quad \text{Equation 2}$$

where i, s and t denote province, crop species and time respectively, μ_t is the year fixed effect, γ_i is the province fixed effect, δ_s is the species fixed effect, and $\varepsilon_{i,s,t}$ are the regression residual terms. proportion_of_fertilizer_cost is the proportion of fertilizer cost per acre to total cost per acre, Consumption is the discounted pure fertilizer per acre Table 6 shows the regression results, which show that the policy has a significant positive relationship with fertilizer cost per acre to total cost per acre. Combining the results of the Equation 1 test with the fact that intermediate consumption is an important component of total cost, it can be concluded that the policy has increased the intermediate consumption of agriculture by raising the fertilizer fee.

Table 6. Impact of policy on fertiliser charges as a proportion of costs

VARIABLES	(Equation 2) Proportion_of_fertilizer_cost
Policy	0.005*** (3.44)
Consumption	0.051*** (28.88)
Constant	-0.030*** (-5.26)
Observations	3,636
R-squared	0.720
Province FE	YES
Year FE	YES
Species FE	YES

Robust t-statistics in parentheses

*** p<0.01, ** p<0.05, * p<0.1

4.2 Parallel trend test

In order to verify the validity of the baseline model in this paper, a parallel trend test was conducted on the agricultural intermediate consumption of the experimental and control groups, and the following test model was constructed(Equation 3).

$$Intermediate\_consumption_{i,t} = \alpha + \beta_1 post + \beta_2 time_{t-3}*Treat_i + \beta_3 time_{t-2}*Treat_i + \beta_4 time_{t-1}*Treat_i + \beta_5 current_t*Treat_i + \beta_6 time_{t+1}*Treat_i + \beta_7 time_{t+2}*Treat_i + \beta_8 time_{t+3}*Treat_i + \beta_9 Chemical\_fertilizer_{i,t} + \beta_{10} Sown\_area_{i,t} + \beta_{11} Fuel_{i,t} + \beta_{12} Electricity_{i,t} + \beta_{13} Film_{i,t} + \beta_{14} Pesticide_{i,t} + \beta_{15} Mechanization_{i,t} + \beta_{16} Wage_{i,t} + \beta_{17} gap_{i,t} + \beta_{18} Proportion\_of\_operating\_income_{i,t} + \beta_{19} Population\_quality_{i,t} + \mu_t + \gamma_i + \varepsilon_{i,t} \quad \text{Equation 3}$$

where time is an annual dummy variable. The test results are displayed in Table 7 and Fig 2. From the test results, it can be seen that the difference between the experimental and control groups was small and insignificant before the policy was implemented, and after the policy was implemented, the difference between the two groups widened and became significant. Therefore

the paper uses a double difference model to test that the prerequisites for the common trend hypothesis are met.

Table 7 Parallel trend test results

VARIABLES	(Equation 3) Parallel_trend_test
post	0.045 (0.37)
pre_3	-0.013 (-0.66)
pre_2	0.001 (0.04)
pre_1	0.037 (1.10)
current	0.048 (1.01)
post_1	0.104* (1.97)
post_2	0.228*** (2.85)
post_3	0.222*** (2.87)
Constant	0.523 (0.23)
Observations	216
Number of Province	27
R-squared	0.697
Control	YES
Province FE	YES
Year FE	YES

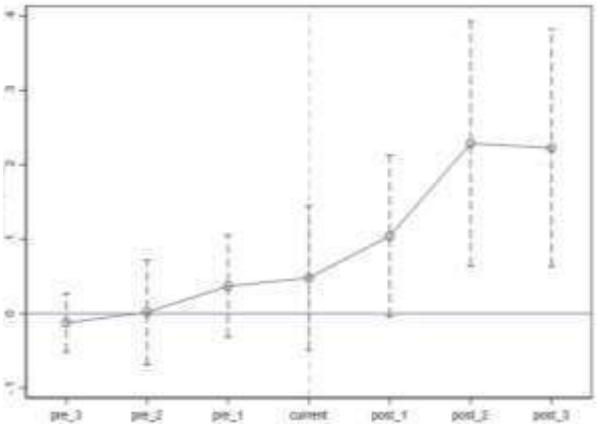

Fig. 2: Policy dynamic effects

4.3 Robustness tests

To test the veracity of the effect of the Policy variable on agricultural intermediate consumption, this paper lets the policy shocks to a particular region become random and then makes this random process repeat 1000 times, such a random treatment ensures that the policy reform does not have an effect on intermediate consumption in the responding region, extracts the regression coefficients with standard errors obtained each time, and calculates the corresponding t-values. Figure 3 illustrates the distribution of the estimated 1000 t-values, which are indeed concentrated around 0, consistent with a normal distribution, so that unobserved regional characteristics have essentially no effect on the estimation results.

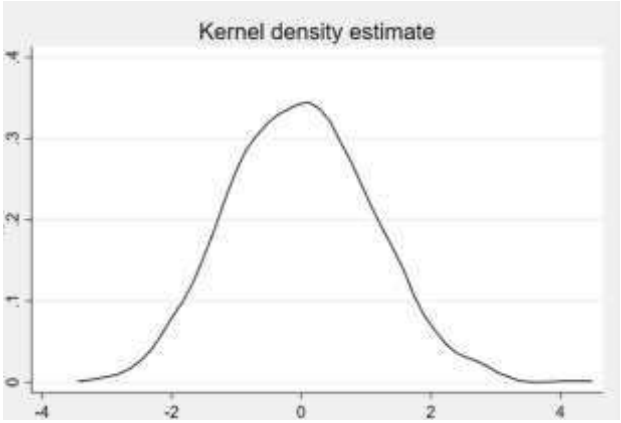

Fig. 3: Placebo test

V. CONCLUSION

5.1 Analysis of mechanisms

First, the supply and demand situation of China's fertilizer industry has changed. 2015, after the implementation of a series of policies in the fertilizer industry, fertilizer production capacity fell rapidly, and in 2017 basically reached a balance between supply and demand, but since 2018 production capacity has been lower than demand (Fig. 4), but from the total demand, farmers' fertilizer habits have not changed too much, so farmers do not use fertilizer scientifically, the fertilizer industry demand exceeds supply situation is also the cost of fertilizer This is why the cost of fertiliser has increased.

Fig.4 Supply and demand in China's fertilizer industry(Data source: China Statistical Yearbook, China Rural Statistical Yearbook)

Secondly, there is a shift of cost burden. Firstly, VAT is ring-fenced and accumulated at every level. As farmers are at the end of the industrial chain, most individual farmers are unable to offset their taxes through invoices, and the bargaining power of agriculture is weak and the added value of agricultural products is low, so at least part of the increased tax burden was passed on to farmers; secondly, after 2015, the increased costs due to the cancellation of various subsidies were at least partially transferred to farmers, as can be seen from Fig. 5 , Fig. 6 shows that the main business profit margin and asset-liability ratio of enterprises in the fertiliser wholesale industry above the limit have remained relatively stable before and after the reform, without any significant fluctuations, reflecting from the side that enterprises have transferred the rising costs to the downstream.

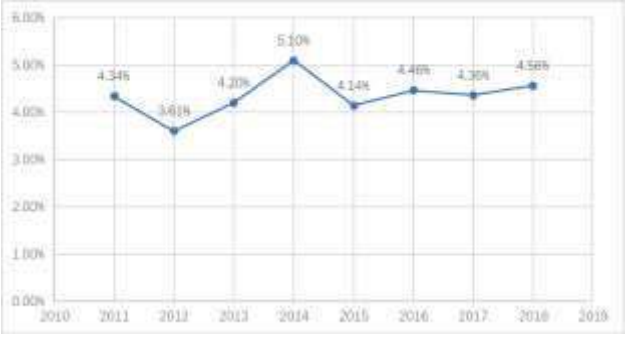

Fig. 5: Profit margin from main business of enterprises in the wholesale fertiliser industry above the limit (Data source: China Trade and Foreign Economic Statistics Yearbook)

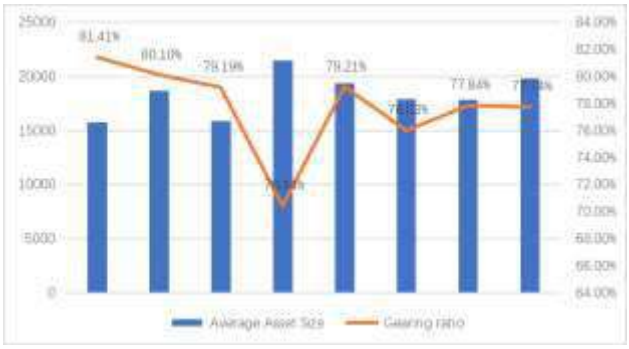

Fig. 6: Average asset size and gearing of enterprises in the fertiliser wholesale industry above the limit (Data source: China Trade and Foreign Economic Statistics Yearbook)

5.2 Policy Insights

First, China has long implemented preferential fiscal policies for the production, distribution and application of fertilisers. After years of development, China's fertiliser industry as a whole has shifted from under-capacity to over-capacity. The rise and fall of the fertiliser industry is a matter of food security. In recent years, while the fertiliser industry as a whole has continued to remove the relevant fiscal concessions, it should also be noted that after years of de-capacity in the fertiliser industry, the supply and demand for fertilisers has changed again, and therefore, while eliminating backward production capacity, quality production capacity should also be encouraged, i.e. the government is required to formulate corresponding policies to encourage and guide enterprises to innovate and thus achieve the transformation and upgrading of fertiliser enterprises. The reintroduction of VAT will inevitably lead to enterprises giving up some of their benefits and bearing part of the VAT burden themselves, which can then be considered in other taxes to compensate for the transition. For example, the corporate income tax will provide tax credits for innovative expenditures such as research and development of new types of fertilisers and innovative fertiliser production processes by fertiliser enterprises, so as to stimulate them to break away from the path dependence of the traditional fertiliser industry and innovate excellent fertilisers suitable for the actual soil in China.

Secondly, in general, the government hopes to achieve a gradual reduction in the application of chemical fertilizers while ensuring food security. This requires the active guidance of long-term and short-term fertiliser subsidy policies. In the short term, to achieve zero growth in fertiliser application and to encourage the use of organic fertilisers, a combination of encouragement and

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guidance from the central government, and specific operations by local governments in accordance with local conditions, is needed. Farmers' demand for fertilisers has not changed much due to habits and other reasons, but fertiliser production has begun to fall with the reintroduction of VAT, and changes in supply and demand, coupled with farmers bearing part of the tax burden, have necessitated appropriate government subsidies. As there are local differences in the types and demand for fertilisers, this requires the central government to co-ordinate the planning of fertiliser-related funds and local subsidies for affected farmers as needed to protect farmers' production incentives and reduce the impact of the structural transformation of fertilisers on farmers. In the long term, in order to achieve ecological civilisation and promote the development of green agriculture, fertiliser application and management needs to be combined with other policy tools. The externalities of agricultural pollution are strong and easily affect soil and water bodies, so it is also necessary for the central government to co-ordinate management and establish long-term special funds to resolutely win the "battle against pollution". This is why the central government should also coordinate and manage the establishment of long-term special funds to win the "battle against pollution". It should also explore the establishment of a dynamic monitoring mechanism for fertilizer-related funds, using modern information management technology and integrating system resources. Comprehensive coverage of all fertilizer-related funds for comprehensive management.

Third, at present, the input-output ratio of fertiliser use in China is seriously out of balance, the input utilisation rate is low, farmers lack knowledge of fertiliser, resulting in a lack of knowledge of reasonable fertiliser application, the fertiliser industry as a whole is going to capacity and inventory, but farmers apply fertiliser instead of reducing due to factors such as habits, and the relationship between supply and demand is changing again. In order to reduce fertiliser pollution and reduce the impact of the transition period of the fertiliser industry, it is necessary for the state to provide relevant training to farmers to supplement their knowledge of spending and guide them to apply fertiliser in a reasonable manner; to support and set up new agricultural business entities in the form of large planters, large farms or professional co-operatives, etc. to strengthen the degree of specialisation and control the amount of fertiliser applied in a scientific and reasonable manner according to the situation on the ground, so as to reduce the excessive amount produced by the excessive use of fertiliser burden.

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Gender Involvement in Crop Production and Livestock Related Activities in Chitwan and Lamjung District of Nepal

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Abstract— *A study was carried out during 2019 with the aim to access gender involvement in crop production and livestock sector. Two hundred and forty (240) smallholder farmers were selected randomly, 120 smallholder farmers each from Chitwan and Lamjung district of Nepal. Findings revealed that the gender involvement in most of the activities on crop production and livestock is dominated by joint participation of both male and female. However, in terms of technology adoption and control over land resources, status of female participation was quite low compared to male. Thus it is recommended to bring equal involvement of both male and female in all aspects of production and resources control.*

Keywords— ***Gender involvement, smallholder farmers, crop production, livestock.***

I. INTRODUCTION

Nepal being an Agrarian country, agriculture production accounts for around 24.26% of total GDP and about three-quarters of the population work in agriculture sector of Nepal (MoAD, 2019). Socially constructed relationship between men and women in a society is referred as 'Gender' (Eagly , 1987). Gender role refers to how men and women should think, act and feel according to the existing norms and traditions in our society (Groverman and Gurung , 2001). Gender roles are highly influenced by the interactions between individuals and their social, historical and economic environments (West and Zimmerman , 1987). Strong relationship exists between gender and agricultural activities in the Nepalese household (Bajracharya, 1994; Devkota and Pyakuryal, 2006). Gender roles are dynamic and changing as per the societal change (Devkota, 2010). This study aims to access gender involvement in crop production and livestock sector at the household of Chitwan and Lamjung district.

II. METHODOLOGY

The study was carried out in Chitwan and Lamjung district of Nepal during 2019. Smallholders constitute more than 50% of Nepalese farmers, cultivating less than 0.5 ha per household (CBS, 2011). Hence, to get the knowledge of the dominant workforce, all smallholder farmers constituted the population of the study. One hundred and twenty (120) farmers were selected randomly from each district. Information was largely collected through FGD, KII and face to face interview schedule conducted using pre-tested questionnaire with household head. Frequency was used to interpret the findings of the study.

III. RESULTS

3.1 Basic information of the respondents

Gender, age, education, ethnicity, occupation, farming experience, number of male and female per household of the respondents were measured and categorized (Table 1). Out of 240 households surveyed, findings revealed that majority of the respondents were male. Similarly, majority of the respondents were of economically active age group. Half of the respondents had intermediate and higher degree of education. Majority of the respondents were Brahmin/Chhetri. More than half of the respondents were dependent only on agriculture as source of income. Majority of the respondents had greater than 30 years of

farming experience. Number of males per household was higher compared to number of number of female in range of 5-10 size.

Table 1. Basic information of the respondents across the study districts

Basic information	Frequency
Gender of household head	
Male	164 (68.33)
Female	76 (31.67)
Age of household head (years)	
Economically active(15-59)	164 (68.33)
Dependent(<14 and >60)	76 (31.67)
Education	
Illiterate	30 (12.5)
Lower level	16 (6.67)
Secondary level	70 (29.17)
Intermediate	84 (35)
Bachelor	40 (16.66)
Ethnicity	
Brahmin/Chhetri	176 (73.33)
Janajati	60 (25)
Dalit	4 (1.67)
Occupation	
Agriculture	136 (56.67)
Agriculture + off farm	104 (43.33)
Farming experience (years)	
1 - 15	2 (0.83)
16- 30	74 (30.83)
>30	164 (68.34)
Number of males per household	
1-4	108 (45)
5-10	132 (55)
Number of female per household	
1-4	125 (52.08)
5-10	115 (47.92)

Source: Field Survey, 2019.

Note: Figures in parentheses indicate percentage

3.2 Gender involvement in crop production related activities

Both men and women were involved in the activities related to crop production (Table 2). However, the degree, level, and stage of gender participation in various activities related to the crop productionvaried from one to other. Some of the activities were predominantly done by male, some by female and some of them were done by both male and female. In agricultural activities such as buying of seeds, land preparation, planting and sowing, there was major involvement of both the gender, which was 55.83%, 52.5%, 50% and 50% respectively. In contrast to that, activities such as tilling and weeding were dominantly handled by female member. There was involvement of only 15% male in postharvest operation which was very low as compared to female involvement (42.5%). However, financial and decision making activities such as selling of land, leasing of land and adoption of technology were mainly handled by male member of the household which includes 68.33%, 68.33% and 65.83% male involvement respectively.

Table 2. Gender involvement in crop production related activities across the study districts

Activities	Gender involvement in crop production related activities (n=240)		
	Male	Female	Both
Buying of seeds	36 (15)	70 (29.17)	134 (55.83)
Land preparation	74 (30.83)	40 (16.67)	126 (52.5)
Planting	54 (22.5)	66 (27.5)	120 (50)
Sowing	54 (22.5)	66 (27.5)	120 (50)
Tilling	52 (21.67)	102 (42.5)	86 (35.83)
Weeding	52 (21.67)	102 (42.5)	86 (35.83)
Harvesting	38 (15.83)	60 (25)	142 (59.17)
Postharvest operations	36 (15)	102 (42.5)	102 (42.5)

Selling of produce	80 (33.33)	70 (29.17)	90 (37.5)
Selling of land	164 (68.33)	30 (12.5)	46 (19.17)
Leasing of land	164 (68.33)	30 (12.5)	46 (19.17)
Adoption of technology	158 (65.83)	42 (17.5)	40 (16.67)

Source: Field Survey, 2019

Note: Figures in parentheses indicate percentage respondents' responses

3.3 Gender involvement in livestock related activities

In contrast to the crop production, there was found to be higher involvement of both the gender in most of the livestock related activities (Table 3). Activities such as fodder collection, watering to the animals and overall management were carried by women in greater extent compared to the male, however, there was no such significance difference in frequencies. Male participation was found to be higher in decision making activities such as adoption of technology. Only 16.67% female own full decision making power for technology adoption whereas 63.33% male hold full power in decision making. Activities such as milking of animals and grazing were also dominated equally by both the genders which accounts for 45.84% and 44.17% frequency respectively.

Table 3. Gender involvement in livestock related activities across the study districts

Activities	Gender involvement in livestock related activities (n=240)		
	Male	Female	Both
Feed preparation to the animals	48 (20)	74 (30.83)	118 (49.17)
Fodder collection	46 (19.17)	70 (29.17)	124 (51.66)
Watering to the animals	34 (14.17)	70 (29.17)	136 (56.66)
Overall care and management of livestock	44 (18.33)	50 (20.83)	146 (60.84)
Milking animals	74 (30.83)	56 (23.33)	110 (45.84)
Grazing animals	74 (30.83)	60 (25)	106 (44.17)
Decision to sell animals	70 (29.16)	40 (16.67)	130 (54.17)
Adoption of technology	152 (63.33)	40 (16.67)	48 (20)

Source: Field Survey, 2019

Note: Figures in parentheses indicate percentage respondents' responses

IV. DISCUSSION

There was found to be varied involvement of male and female in different activities related to crop production. Greater time and effort demanding task such as land preparation, sowing and harvesting were done by involvement of both the gender. Zewdu et al. (2016) reported that males were found to be more involved in ploughing and harvesting of horticultural crop. But this was found quiet different in our study. Although tillage is considered to be extremely laborious job, it was performed by involvement of women to greater extent. Olowa and Olowa (2015) found that women are more involved in weeding, watering, transplanting and harvesting. Similarly, there was greater involvement of women in activities such as weeding, harvesting and post-harvest operation. There was greater involvement of male in adoption and decision making task such as selling of land, leasing of land and technology adoption. Result is in line with Agarwal (2015) who reported lack of ownership and access to land for women. FAO (2010) reported female have no access to and decision-making role on technology use. Ogato et al. (2009) reported man as the principal decision maker in

family being household head. Zewdu et al. (2016) also revealed that men are the heads of households and are the principal decision- makers in most of the household however it might involve some consultation with women. The patriarchal system is seen to be accelerating factor for domination of male over female. In case of livestock related activities, there was greater involvement of both the gender. Feed preparation, fodder collection, watering and other management related activities of livestock was performed jointly regardless of gender whereas male involvement was found to be higher in technology adoption related decision. But in case of selling of produce and animals, involvements were made jointly.

V. CONCLUSION

Findings of this study revealed that gender involvement in most of the activities on crop production and livestock is not single domination of either male or female, but towards joint participation of both. However, access of female in terms of technology adoption and control over land resources were quite low compared to male. Thus, it is suggested that, implementing program and policies which would bring equal involvement of both male and female in all aspects of production and resources control will be helpful.

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Effect of the Marination Time & Marinade Ingredients on Sensory Evaluation of Tawouk

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Abstract— *Tawouk is the chicken's breast meat. Traditionally, meat has been marinated to improve flavor, improve tenderness, and increase product shelf life. The purpose of the marinating time is to permit the marinade to soak as deeply into the food as potential. Here, we studied the effect of marinating time and marinade ingredients on color, flavor, juiciness, chewiness and overall acceptability of chicken breast meat. Our results showed a difference in color, flavor and juiciness between different treatments according to the panelists. Panelists preferred red-colored chicken meat with a strong flavor. We also showed a correlation between color and the overall acceptability of breast chicken meat. This work is laying the ground for better customer service for poultry businesses and restaurant chains. Our work shows that focusing on color is essential to increase consumers' acceptability.*

Keywords— ***Lebanon, Tawouk, marinade, marinating time, sensory evaluation.***

I. INTRODUCTION

Lebanon is characterized by a high level of meat consumption per capita compared to Mediterranean countries [1]. The increase in poultry demand leads to various poultry products that became one of the most popular traditional dishes in Lebanon, such as Tawouk, which is marinated chicken breast meat [1]. Traditionally, meat has been marinated to improve flavor, improve tenderness, and increase product shelf life [2]. There are three strategies for manufacturing marinated products, which are immersion, injection, and vacuum tumbling [2].

The purpose of the marinating time is to permit the marinade to soak deeply into the meat [3]. Allowing the meat to stay within the marinade for a long time might increase toughness, which is the opposite of what is desired. Marinating times vary depending on the sort, cut, and size of the meat. Thinly cut meat can infuse marinade more than thick cuts and need less marinating duration [4].

A good marinade can have the right balance of flavorings components, organic acids, and oil. Vinegar, tomato sauce, or citrus juice are commonly used acids that soften the meat by denaturing the meat proteins. Oils are used to moisten and flavor the meat. Wide varieties of ingredients are used concerning flavorings, such as fresh or dried herbs and spices [4].

Flavors and spices included in marinades upgrade meat's quality and control or limit lipid oxidation [5]. Oxidation in meat products causes muscle protein and fat changes, negatively affecting the product's consumer acceptability after few days [6].

The standard marinade components are water, salt, and phosphates [7, 8]. The salt and phosphate particles in the cells ensure the water maintenance limit of muscles by separating muscle fibers' protein structure and causing expansion of myofibrils [9]. Salt improves the growth of protein structures; however, it doesn't solubilize the greater part of the fiber proteins, autonomously [10]. Offer and Trinic proposed that the chloride particles stick to the fibers and increase electrostatic powers [11]. This permits the fiber network to grow and shape a bigger hole between the actin and myosin in myofibrils. The salt concentration in marinade influences the chemical gradients, the water-holding capacity, and the mass transfer level. Phosphates eliminate the cross-joints among actin and myosin fibrils

[11]. Hence, a mix of sodium chloride and phosphates is fundamental to improve poultry meat's general delicacy and succulence. The allowed upper level of phosphates (separately or in the blend) in the worldwide food industry is 0.5% [12].

The main reason of this study is to examine the effect of marination duration and marinade ingredients on the color, flavor, chewiness, and juiciness of Tawouk.

II. METHODS

2.1 Experimental Design

The experiment design was a 3 x 3 factorial arrangement of treatments evaluating three marination solutions and three holding times. Nine chicken breast meat samples from a local restaurant in Anjar (average weight ± SD, 1000 ± 5 g each) were randomly allocated to three marination solutions: mix one, mix two, and mix three. After marination, the meat samples from each mix were subdivided (n=20) according to holding times of 3, 6, and 12 h at 4°C.

2.2 Preparation of Marinade

The three marinade solutions were prepared, as mentioned in Table 1. These quantities were used for every 500 g of breast meat. These mixes were chosen based on a questionnaire done on 35 producers to find out the most used ingredients by Lebanese people to marinate breast meat. All spices used were from the brand Gardenia®. The used brand for mayonnaise, mustard and sunflower oil was Plein Soleil®. The brand used for grenadine molasses and vinegar was Yamama®.

2.3 Preparation Procedure

Fresh boneless breast meat of broiler chickens obtained from a local processing plant was used in this study. External fat, skin and connective tissues associated with breast meat were manually removed. The boneless breast meat was cut into 1000 g pieces. The marinating process consisted of immersion of breast meat in the prepared marinade— mix 1, mix 2, and mix 3 —. Samples from the groups were immersed in the marinade inside plastic containers. The breast meat was stored in a refrigerator at 4°C ± 1°C in atmospheric conditions. The components of each container were mixed every 1 hour.

2.4 Sensory Evaluation

Descriptive testing was used to quantify the perceived intensities of color and flavor of a certain marinated Tawouk using a 5-point anchored just about right (JAR) scale. Hedonic or effective testing was used to quantify the degree of overall liking or disliking of a certain marinated Tawouk. Juiciness and chewiness were tested by using a 9-point hedonic scale.

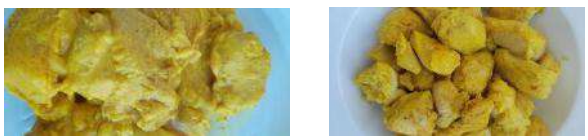
Table 1. Ingredients of the three marinade solutions per 500 g of breast meat

Ingredients	Mix 1	Mix 2	Mix 3
Salt	7.5 g	7.5 g	7.5 g
Vinegar	15 ml	15 ml	15 ml
Sunflower Oil	30 ml	30 ml	30 ml
Lemon Juice	8 ml	8 ml	8 ml
Garlic	6 g	6 g	6 g
Yogurt	15 ml	15 ml	15 ml
Tawouk Spice	3.75 ml	3.75 ml	3.75 ml
Peppercorn	3.75 m	3.75 ml	-
White Pepper	-	-	3.75 ml
Paprika	7.5 ml	-	-
Grenadine Molasses	15 ml	-	-
Mustard	-	30 ml	-
Curry	-	7.5 ml	-
Mayonnaise	-	-	15 ml

Twenty trained panelists were chosen to conduct the sensory evaluation test. The chewiness and overall acceptability were tested by 9-point hedonic scale. Also, panelists trained to use a 5-point anchored just about right (JAR) scale to evaluate flavor (1- too mild, 2-mild, 3- just about right, 4- strong, and 5-too strong) and color (1- Too light, 2-light,3- Just about right, 4-dark, and 5-too dark) attributes.

(a) (b)

Fig.1: Pictures of chicken breast samples treated with mix one marinade before cooking (a) and after cooking (b) prepared for sensory evaluation after 6 h marination



(a) (b)

Fig.2: Pictures of chicken breast samples treated with mix two marinade before cooking (a) and after cooking (b) prepared for sensory evaluation after 6 h marination.

(a) (b)

Fig.1: Pictures of chicken breast samples treated with mix three marinade before cooking (a) and after cooking (b) prepared for sensory evaluation after 6 h marination.

Raw chicken breast samples were placed on labelled cooking sheets, and the samples were then covered with a single aluminum foil layer. Covered samples were placed in the ovens' center rack and cooked in a preheated 180ºC oven for 15 min. For testing, chicken breast samples were prepared for presentation by cutting into 2 cm cubes then dispensed into lidded souffle cups with three-digit codes.
All candidates were between the age of 20 and 48 years and lives in the Bekaa area (east of Lebanon). These 20 panelists were 12 females and 8 males. We modified the sensory tests conditions due to Covid-19 circumstances so instead of indoor testing, we used an outdoor set up to ensure social distancing and safety measures. The panelists rinsed their mouth with water and crackers between samples.

2.5 Data Analysis

Differences in color, flavor, juiciness and chewiness for each treatment were assessed using one-way ANOVA, Duncan mean separation, cluster analysis and correlation.

III. RESULTS AND DISCUSSION

Using one-way ANOVA, we studied the variability between distinct treatments with respect to chicken color, flavor, juiciness, chewiness and overall acceptability (Table 2). Our results showed a highly significant difference in color (p-value <0.01), flavor (p-value < 0.01), and juiciness (< 0.05) between different treatments. In contrast, there was no significant difference among all treatments in term of chewiness and overall acceptability.

Table 2. One-way ANOVA for the difference between

Dependent Variable	Significance
Color	.000
Flavor	.000
Juiciness	.039
Chewiness	.625
Overall Acceptability	.092

Using Duncan mean separation for the treatments concerning color, treatments were grouped into four categories (Table 3). Results showed that the color of chicken breast samples treated with mix one for 12 hours were the most preferred color among the panelists. Chicken breast samples treated with mix one for 6 hours and mix three for 12 hours were not significantly different in color, but they were distinct from other treatments. The least preferred treatments concerning color were the chicken breast samples treated with mix three for 3 hours and mix two for 3 hours (*p*-value > 0.05).

To study the effect of different treatments on flavor (Table 1), treatments were grouped into four categories, and results showed that chicken breast samples treated with mix one for 12 hours and mix one for 6 hours were the *most* preferred in terms of flavor (p-value <0.05), and were significantly different from other treatments. Chicken breast samples treated with mix three for 12 hours, mix two for 12 hours, mix three for 6 hours and mix two for 6 hours were not significantly different from each other, but they were significantly different from other treatments. Chicken breast samples treated with mix three for 3 hours were not significantly different and had the least preferable flavor for the panelists (Table 3).

In terms of juiciness, treatments were grouped into three categories. Results showed that chicken breast samples treated with mix two for 6 hours were the most significant and thus preferred treatment (6.3). Chicken breast samples treated with mix two for 6 hours were significantly different in their juiciness from those treated with mix one for 3 hours (5.4), mix one for 6 hours and mix one for 12 hours. Chicken breast samples treated with mix three for 6 hours were significantly different in their juiciness from those treated with mix one for 3 hours (Table 3).

Table 3. Estimated marginal means of statistically significant (α = 0.05) sensory attributes (n =20)

Treatment	Color	Flavor	Juiciness
Mix3-3hrs	1.5^{d}	1.85^{d}	$5.9^{a,b,c}$
Mix2-3hrs	1.55^{d}	$2.3^{c,d}$	$5.9^{a,b,c}$
Mix1-3hr	$1.8^{c,d}$	$2.35^{c,d}$	5.4^{c}
Mix2-6hrs	$1.8^{c,d}$	3.15^{b}	6.3^{a}
Mix3-6hrs	2.05^{c}	$2.8^{b,c}$	$6.05^{a,b}$
Mix2-12hrs	2.1^{c}	3.8^{a}	$5.95^{a,b,c}$
Mix3-12hrs	2.65^{b}	2.9^{b}	$5.9^{a,b,c}$
Mix1-6hrs	2.95^{b}	3.15^{b}	$5.65^{b,c}$
Mix1-12hrs	3.9^{a}	3.9^{a}	$5.7^{b,c}$

^{a,b,c,d} *Values within an sensory attribute with differing superscript letters are significantly different (α = 0.05)*

Table 4. Pearson correlation between the sensory attributes based on their means

	Color	Flavor	Juiciness	Chewiness	Overall
Color	1	.716*	-.331	.212	.761*
Flavor			.067	.537	.180
Juiciness				-.030	-.570
Chewiness					-.047
Overall					1

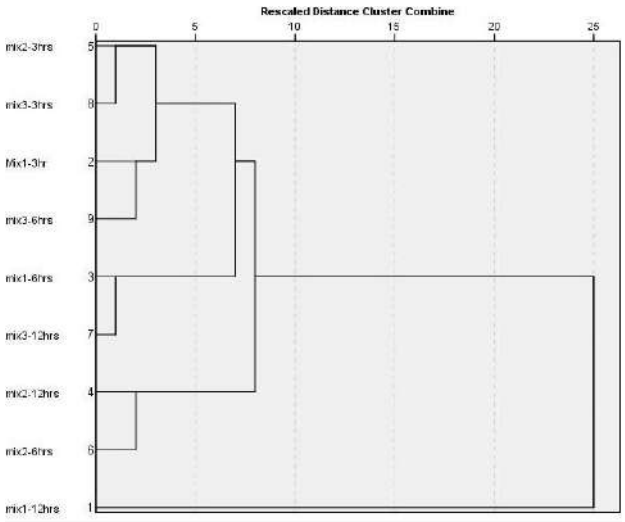


Fig.4: Dendrogram Using Average Linkage based on color, flavor, juiciness, chewiness and overall acceptability of treatments.

Next, we investigated the correlation between the five studied variables based on their means (Table 4). Our results showed a significant correlation between color and flavor, and between color and overall acceptability.

Cluster analysis based on color, flavor, juiciness, chewiness and overall acceptability, demonstrated that all treatments were similar, except chicken breast samples treated with mix one for 12 hours, which were highly favored by the panelists (Fig. 4).

IV. CONCLUSION

We studied the effect of various combinations of marination ingredients and duration on flavor, color, juiciness, chewiness, and overall acceptability of Tawouk. Our work showed a significant difference in consumer preference based on color and flavor between different treatments and a significant difference in juiciness between other treatments. Chicken breast samples treated with mix one for 12 hours were significantly preferred over all other treatments in terms of flavor and color; however, they were equally favored as samples treated with mix one for 6 hours in terms of color alone. Chicken breast sample treated with mix two for 6 hours was the most preferred treatment concerning juiciness. Our results showed a significant correlation between color and flavor and a significant correlation between color and overall acceptability.

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Nexus between Climate Change and Agricultural Production in Odisha, India: An ARDL Approach

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Abstract**—** *Climate change is an emerging issue particularly in agricultural research as it is observed that the climate change has unfavorably distressed the agricultural production in different regions in India. Therefore, the present study has empirically examined the relationship between climate change and agricultural production in the selected districts of Odisha, India using a Panel Autoregressive Distributed Lag (PARDL) model over the period 1993 to 2019. The study found that the climate variables have adversely affected the crops production in the districts of Odisha. In order to minimize the impact of climate change on crops production in the state, there must have implementation of various policies and adaptive strategies by the government and farmers.*

Keywords**— Climate change, crops production, economic impact, central revenue zone Odisha.**

I. INTRODUCTION

Climate change is an emerging issue for policy makers, scientists and academic researchers across the regions, since the impact of climate change varies from regions to regions, and have positive impact in some regions and negative in some other regions (Ninan and Bedamatta, 2012; Al-Amin et al., 2013). The existing studies on climate change have found a modest impact of climate change on developed countries, whereas the developing countries have a negative impact of such change (Mendelsohn and Dinar, 2003; Ciscar et al., 2012). The regions, which are located in tropical and sub-tropical climate, have realized more negative impact of climate change than others as it affects more to drier regions. ((Parry et al., 2007; Kurukulasuriya and Mendelson, 2006).

Empirical studies on the climate change and agricultural production have found a negative impact on developing countries, because the climate variables act directly along with input variables such as land, water, fertilizers & pesticides, etc. in agricultural production (Cline, 2007; Xie et al., 2019). Though, the agriculture production is one the major sources of livelihood in rural areas of developing regions, the policy makers and researchers have given more importance to the impact of climate change on agricultural production in rural areas. The present study has intended to empirically examine the nexus between climate change and agricultural production in Odisha, India. Odisha is one of the poor and backward states situated in the East-Cost of India and in a climatic tropical zone, where we observe high temperature along with high humidity. The state economy and people's livelihood have extensively depend on agricultural & its allied activities (Hoda et al., 2017). Since three/four decades, the agricultural production of the state has been highly affected by various climatic issues like; floods, droughts, storm, cyclones etc. (Rao et al., 2016). The agricultural production and yield have been declined over the years and stand as a major problem to food security in the state (Reddy, 2012). The state Odisha has been continuously influenced by different natural calamities such as cyclones, floods, or shortage of rainfall, etc, which badly hit the agricultural growth rate and livelihood of farmers. Therefore, the present study has made an attempt to examine the nexus between climate change and agricultural production in Odisha using an autoregressive Distributed Lag (ARDL) model. The rest of the paper is organized as follows; section-2 provides the review of literature. Section-3 describes study variables and

methodology. Section-4 analyses the empirical results and the last section-5 provides the study conclusion.

II. REVIEW OF LITERATURE

A number of studies both at international and regional level have examined the impact of climate change on agriculture production or yield, that are broadly classified into two types; general equilibrium approach and partial equilibrium approach (Mishra et al. 2015). The general equilibrium approach or models have been used by many researchers, but the application of such models in developing and under developed countries are too less due to problems like data inconsistency and reliability, parameters specification, identification of model, etc. (Gillig and McCarl 2002; Zhai et al. 2009; Ciscar et al. 2002). As a result, the researchers have more relied on partial equilibrium approach in developing or under developed countries. The partial equilibrium approach is categorized into crop growth simulation model, and the econometric or Ricardian model (Deressa 2007). The economists and agricultural researchers prefer to use Ricardian model or econometric models to examine the impact of climate change on agriculture by taking into account of land revenues and values respectively. Ricardo uses the land value to measure net productivity, but for the first time Mendelsohn et al (1994) use the value of land to demonstrate the impact of climate change on agriculture of USA. Moreover, developing countries are lack of market information and difficult to get accurate land values, therefore, the annual net revenues per hectare has been used as a proxy by the researchers as it reflects the variation due to climate change (Dinar et al. 1998). The effect of climate change on the net crop yields per hectare is shown negative at national level (Chen et al. 2013). The high temperature affects negatively the net revenue from agriculture (Kaimakamis et al. 2013).

The climate change is measured by the climatic variables such as rainfall and temperature, and their impact depends on level of change in such variables. The extreme temperature and rainfall negatively affect crops production, while the minimum temperature and rainfall positively affect the crops production (Chowdhury and Khan 2015). Moreover, the low rainfall and high temperature could lead to raise of global food prices in future, and to avoid that problem, farmers should adopt coping mechanisms to resist from climate change (Rahman et al. 2018). In recent years, the global warming is one of the most harmful factors than precipitation (Mariara and Karanja, 2007).

In India, a continuous increase of the concentration of CO2 and other GHGs in the atmospheres lead to increase in the temperature and inconsistent rainfall which put a serious concern for agriculture & allied activities (Bhattacharya and Panda, 2013). It is estimated that a 2^0C increase in temperature and 7% increase in precipitation could lead to 8.4% decrease in the total net revenue from crops production (Kumar and Parikh, 1998). A common tendency of climate change is observed through the increase in average temperature, changes in rainfall timing and intensity (Putriawanti and ASAI, 2016). Studies suggested that the effects of climate change on agriculture in India can be tackled through proper irrigation, adaptation, diversifying the crops, and mitigation process, etc. (Birthal et al., 2014; Manoj et al., 2019). The climatic effects can also be cut off through increasing the marketing facilities, diversifying the crops, increasing various social security measures etc. (Mishra et al., 2015).

Climate studies on Odisha show that the climate change has a greater and severe impact on both the costal and western zone of Odisha. The deficits of rainfall and high temperature have negatively affected the agricultural production in the state (Panda et al. 2019). The relative magnitude of rainfall and temperature changes can be tackled by using appropriate mitigation, adaptation strategies, education to farmers on climate change and climate information (Mishra, 2017).

The above literature review indicates that the study on the impact of climate change on agricultural production has been growing, but still there is lack of regional studies particularly for Odisha. There is hardly any study, which analyzed the relationship between of climate change and agricultural production in the Central Revenue districts such as Nayagarh, Khorda, Puri, Jagatsingpur, Kendrapada, Cuttack, Jajpur, Bhadrak, Balasore, and Mayurbhanj of Odisha India. Therefore, the present study attempts to examine the nexus between climate change and the production of paddy and sugarcane in the selected districts of Odisha. In Odisha, paddy is the principal crops, which is produced at a larger quantities thn any crops by the farmers. Except that, sugarcane production has grown up in the study area as it gives good market value than other crops. Therefore, the resent study examine the nexus between climate change and crops production (paddy and sugarcane) in the selected districts of odisha.

III. DATA AND METHODOLOGY

The study has collected secondary data from ten selected districts of Odisha, which are from different agro-climate zones. The agro-climate zones of the state are categorized based on various components like soils,

climate, topography, vegetation, crops, etc. The state Odisha has 10 major agro-climate zones, out of which the present study has collected data from North Central Plateau Zone (Mayurbhanj), North Eastern Coastal Zone (Balasore), East and South Eastern Coastal Zone (Bhadrak, Kendrapara, Jagtsingpur Cuttack, Puri, Khorda and Nayagarh), and Mid Central Table Land Zone (Jajpur). The details of the district agro-climate zones, climate, soils and suitable cropping system is reported in Appendix Table-1. The climatic variables such as rainfall and temperature data have been collected from the Indian Meteorological Department (IMD), Bhubaneswar for selected districts of Odisha from 1993 to 2019. The agricultural variables such as paddy and sugarcane productions, net shown area under crops, fertilizer consumptions data have been collected from the District Statistical Handbooks, Directorate of Agriculture and Statistics, Government of Odisha, India. The study selected crops i.e. paddy and sugarcane are largely produced by the selected districts of Odisha (Appendix Figure-1 &2).

This paper has examined the nexus between climatic variables and agricultural production in Odisha using the Autoregressive Distributive Lag Model (ARDL) developed by Pesaran and Shin (1998) and Pesaran et al (2001). The ARDL model of co-integration test is more superior to other cointegration tests (i.e. Engle and Granger cointegration test (1987) and Johansen and Juselius cointegration test (1990)) for various reasons such as; the ARDL model consider the small sample size, simultaneity biases, and consider both I(0) and I(1) variable or both of mixed order of integration. The relationship between climatic variables rainfall and temperature and crops production in selected districts of Odisha, India is examined in the following specific model:

$$AGC_{it} = f(LA_{it}, FC_{it} RF_{it} TEMP_{it}) \quad (1)$$

In the equation(1) AGC_{it} represents the agricultural crops production, i represents both crops Paddy and Sugarcane , LA_t represents the land area or area under selected crops, FC_t represents fertilizer consumption, RF_t represents the rainfall, $TEMP_t$ represents the average temperature in the study selected area respectively. The equation (1) can be written in the econometric form as follows:

$$AGC_{it} = \beta_{i0} + \beta_{i1} LA_{it} + \beta_{i2} FC_{it} + \beta_{i3} RF_{it} + \beta_{i4} TEMP_{it} + \mu_{it}$$

Since the study variables have different units of measurement, they may have multicollinearity and volatility, hence, for the better analysis of results, the study variables are transferred to their natural logarithm form, then the equation (2) become a log-linear model as follows:

$$LnAGC_{it} = \beta_{i0} + \beta_{i1} LnLA_{it} + \beta_{i2} LnFC_{it} + \beta_{i3} LnRF_{it} + \beta_{i4} LnTEMP_{it} + \mu_{it}$$

The ARDL model has two steps for estimation; first step, we examine the presence of a long-run relationship between the agricultural crops and study input variables are as follows:

$$\Delta lnAGC_{it} = \alpha_0 + \sum_{j-1}^{p} \alpha_{1ij} \Delta lnAGC_{it-k} + \sum_{j=1}^{p} \alpha_{2ij} \Delta lnLA_{it-k} + \sum_{j=1}^{p} \alpha_{3ij} \Delta lnFC_{it-k} + \sum_{j=1}^{p} \alpha_{4ij} \Delta lnRF_{it-k} + \sum_{j=1}^{p} \alpha_{5ij} \Delta lnTEMP_{it-k} + \beta_{1i} lnAGC_{it-1} + \beta_{2i} lnLA_{it-1} + \beta_{3i} lnFC_{it-1} + \beta_{4i} lnRF_{it-1} + \beta_{5i} lnTEMP_{it-1} + \varepsilon_{it} \quad (1)$$

Where, α_0 represents as intercept tem, p represents the lag order, Δ stands for first difference operator of variables and ε denotes the error term in the equation. The long-run equilibrium relationship between $lnAGC$, $lnLA$, $lnFC$, $lnRF$ and $lnTEMP$ is examined using the Pesaran et al. (2001) given F-test. If the estimated F-test statistic lies above the upper level of band, there exists long-run relationship between the study variables and otherwise if the estimated F-test statistic lies below the upper level band. The relations are inconclusive if the computed F-test statistic lies between the lower and upper band. In the second step, we estimate the short-run relation between the study variables using the error correction model (ECM) in ARDL model as follows:

$$\Delta lnAGC_{it} = \alpha_0 + \sum_{j-1}^{p} \alpha_{1ij} \Delta lnAGC_{it-k} + \sum_{j=1}^{p} \alpha_{2ij} \Delta lnLA_{it-k} + \sum_{j=1}^{p} \alpha_{3ij} \Delta lnFC_{it-k} + \sum_{j=1}^{p} \alpha_{4ij} \Delta lnRF_{it-k} + \sum_{j=1}^{p} \alpha_{5ij} \Delta lnTEMP_{it-k} + \alpha ECM_{it-1} + \varepsilon_{it} \quad (2)$$

The stationary condition of each variable is checked by the Levin, Lin and Chu's t-statistic.

IV. RESULTS AND DISCUSSIONS

The descriptive statistics of the study variables are reported in Table-1, which indicates that, the study variables are normally distributed, whereas the variables such as production, net shown area, fertilizer consumption

and rainfall are more volatile or fluctuate than the temperature of both paddy and sugarcane.

Table 1: Descriptive Statistics of Crops Production in the Study Area

	Mean	Median	Maximum	Minimum	Std. Dev.	Skewness	Kurtosis	J-B	Probability
PADDY									
LNAGP	12.05	12.13	13.44	9.53	0.72	-1.34	5.37	144.55	0.00
LNAL	12.18	12.06	15.02	11.29	0.59	1.27	5.49	142.36	0.00
LNFC	8.12	8.01	10.73	4.96	1.35	0.05	1.75	17.66	0.00
LNRF	4.68	4.85	6.17	2.35	0.56	-1.90	6.47	297.67	0.00
LNTEMI	3.25	3.29	3.58	2.86	0.13	-0.52	2.65	13.67	0.00
SUGARCANE									
LNSP	10.21	10.40	13.24	5.70	1.58	-0.58	3.20	15.36	0.00
LNSAL	7.32	7.32	9.98	2.51	1.47	-0.69	3.60	25.44	0.00
LNSFER	8.12	8.01	10.73	4.96	1.35	0.03	1.73	18.23	0.00
LNRAIN	4.68	4.85	6.17	2.35	0.56	-1.90	6.47	297.63	0.00
LNTEMI	3.25	3.29	3.58	2.86	0.13	-0.52	2.65	13.66	0.00

Source: Author estimated

The correlation coefficient matrix are reported in Table-2, which indicates that both paddy and sugarcane productions are positively correlated to area, fertilizer consumption and temperature, whereas there is negative relations between crops production and rainfall, which is the preliminary indication of estimating cointegration between these variables.

Table 2: Correlation Results

	LNAGP	LNAL	LNFC	LNRF	LNTEMP
PADDY					
LNAGP	1.00	0.17	0.16	-0.09	0.05
LNAL	0.17	1.00	0.12	0.19	0.01
LNFC	0.16	0.12	1.00	0.00	0.05
LNRF	-0.09	0.19	0.00	1.00	0.13
LNTEMP	0.05	0.01	0.05	0.13	1.00
SUGARCANE					
	LNSP	LNSAL	LNSFERT	LNRAIN	LNTEMP
LNSP	1.00	0.59	0.06	-0.14	0.02
LNSAL	0.59	1.00	-0.22	-0.04	0.05
LNSFERT	0.06	-0.22	1.00	-0.08	0.07
LNRAIN	-0.14	-0.04	-0.08	1.00	0.13
LNTEMP	0.02	0.05	0.07	0.13	1.00

Source: Author estimated

The cointegration between the crops productions and input variables such as net shown area, fertilizer consumption, rainfall and temperature are examined using the ARDL model, for which the stationary test is compulsory for each variable, and the stationary is checked using the Levin, Lin and Chu's t-statistics (Table-3). The estimated Levin, Lin and Chu's t-statistics indicates that all the study variables are stationary at I(0) and I(1), which indicates to use the ARDL model for the analysis of long-run equilibrium relationship between crops production and input variables.

Table 3: Results of Unit Root Test

Crops	Variables	Level Value		First Difference)	
		Statistic	Prob.**	Statistic	Prob.**
Paddy	LNPP	-3.81*	0.00		
	LNAP	4.21*	0.00		

	LNFC	-0.83	0.20	-15.20*	0.00
	LNRF	-2.47**	0.01		
	LNTEMP	-0.79	0.22	-13.48*	0.00
Sugarcane	LNSP	-1.75**	0.04		
	LNAS	3.75	1.00	-4.40*	0.00
	LNFC	-1.59	0.06	-5.72*	0.00
	LNRF	-2.49**	0.01		
	LNTEMP	-22.02*	0.00		

Source: Author estimated

The estimation of ARDL model needs to use appropriate lag-length, which is determined by the optimum lag-length criteria and the results are reported in Table-4.

Table 4: Lag Order Selection for Study Variables

Crops	Lag	FPE	AIC	SC	HQ
Paddy	0	0.00	7.73	7.80	7.76
	1	0.00	4.57	4.00*	4.74
	2	0.00*	4.38*	5.15	4.69*
Sugar	0	0.02	10.31	10.39	10.34
	1	0.00	6.42	6.93*	6.62
	2	0.00*	6.20*	7.14	6.59*

Source: Author estimated

Note: * indicates lag order selected by the criterion

FPE: Final prediction error

AIC: Akaike information criterion

SC: Schwarz information criterion

HQ: Hannan-Quinn information criterion

The Schwarz information criteria (SCI) suggests one is the optimum lag, whereas other criterions such as Final Prediction Error (FPE), Akaike Information Criteria (AIC), and Hanann-Quinn Information Criteria (HQ) suggest to use the optimum lag two in the model estimation. Since, majority criterions suggest the optimum lag two, hence the study uses two optimum lag in the ARDL model estimation. The ARDL model estimated results are shown in Table-5, which has two parts; the first part reports the long-term equilibrium relationship between the selected crops production and the study inputs, whereas the second part reports the error correction results for the short-term equilibrium relations between the study variables. The results indicate that both the paddy and sugarcane production have long-term cointegration with the study input variables. The negative coefficients of rainfall and temperature indicate the negative relationship between crops production and climatic variables. A high rainfall and temperature lead to fall of paddy and sugarcane production. The pesticide consumptions for both the crops are positive and significant, which indicates that a direct relationship exist between crops production and fertilizer consumption in the study area.

Table 5: Results of ARDL Model for Study Variables

	Paddy		Sugarcane	
Variable	Coefficient	t-Statistic	Coefficient	t-Statistic
Long Run Equation				
Area	-0.0273	-5.34	0.91* (0.07)	13.78
Fertilizer	0.08* (0.01)	7.78	0.28* (0.07)	3.93
Rainfall	-0.06** (0.03)	2.13	-0.27* (0.38)	-5.11
Temperature	-0.26*** (0.15)	-1.97	-0.85*** (0.46)	1.86
Short Run Equation				
COINTEQ01	-0.186	-4.71	-0.078	-3.47
D(Production(-1))	0.25 (0.19	1.35	-0.03 (0.08)	-0.37
D(Area)	0.19** (0.11)	1.76	0.36** (0.18)	1.99
D(Area(-1))	-0.25** (0.11)	-2.29	-0.12*** (0.06)	-1.91
D(Fertilizer)	0.02** (0.03)	-2.05	0.44* (0.13)	3.53
D(Fertilizer(-1))	-0.04 (0.04)	-0.92	0.09 (0.07)	1.36
D(Rainfall)	-0.214** (0.24)	-0.84	-0.68** (2.01)	1.93
D(Rainfall(-1))	0.19*** (1.62)	1.72	-0.47** (1.99)	2.35
D(Temperature)	-0.66** (0.65)	1.96	-0.27 (0.35)	-1.00
D(Temperature(-1))	-0.58 (0.67)	-1.00	0.25 (0.53)	1.00

Source: Author estimated

The second part indicates the short-term relationship between the crops production and selected inputs in the study area. The error correction coefficients of both the crops are negative and significant at 1% significance level, which indicates the speed of adjustment for the long-term equilibrium relationship between the study variables. In the short-run both the net shown area and fertilizer consumption have positive relations with the crops production, but there is negative relation between rainfall and temperature and crops production in the study area. The ARDL model bound test results are reported in Table-6, which indicates that the F-statistics of both the crops are significant and above the upper bound, suggest the long-term equilibrium relationship between the crops production and the study selected inputs in the study area.

Table 6: ARDL Cointegration Bound test Results

Test Statistics	Estimated Values	Crops
F- Statistics	6.36	Paddy
	4.72	Sugarcane
Critical Value Bounds (Pesaran et al., 2001)		
Significance Level	Lower Bound (I0)	Upper Bound (I1)
1%	4.99	5.85
5%	3.88	4.61
10%	3.18	4.02

Source: Author Estimated

V. CONCLUSION

Climate change is an emerging issue particularly in agricultural research, as it is projected that the climate change unfavorably distresses the agricultural production across the regions. Therefore, the present study aimed to empirically examines the relationship between climate change and agricultural production in the selected districts of Odisha, India using the Panel Autoregressive Distributed Lag (PARDL) model over the period 1993 to 2019. The study has used production inputs such as net shown area, fertilizer consumption, and two climatic variables such as rainfall and average temperature to examine their effect on the selected crops (Paddy and Sugarcane). The study found that the increase of rainfall and temperature affect the crops production negatively in the study are, which is similar to the finding of Chandio et al. (2019), and Guntukula and Phanindra (2020). In order to minimize the impact of climate variables on agricultural productions in the study area, there must have implementation of various policies and adaptive strategies. Since, the timing and adequate quantities of rainfall and temperature has been changed in the study are, the farmers must adopt new crops and diversification strategies to combat the climatic risk.

FUNDING

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Appendix

Table 1: Agro-Climate Zones of the Study Area in Odisha

Sl No.	Zones nd Districts	Climate	Soils	Suitable croping system
1	North Central Plateau (Mayurbhanj)	Hot and Moisture, humid to sub-humid	Red loam type, acidic in nature, light textured	Paddy, mustard, groundnut, arhar, ragi, and horsgram
2	Eastern Coastal Plain (Balasore, Bhadrak and Kendrapara)	Hot and Moisture, humid to sub-humid	Red ;aterite, alluvial and saline coastal sandy	paddy, jute, mung, mustard, groundnut, sugarcane, etc.
3	Eastern and South Eastern Coastal (Cuttack, Jagatsingpur, Khorda and Puri)	sub-topical, hot and humid, temperature lies between 11.5°C to 41°C, the average annual rainfall 1340mm	Saline and sandy soils, alluvial, lateric, black, and red lateric soils	paddy, groundnut, sugarcane, vegetables and greengram
4	Mid Central Table Land Zone (Jajpur and Nayagarh)	Hot and Dry-sub humid, temperature lies between 14.0°C to 38.7°C, the average annual rainfall is 1421mm.	light textured lateric-Rhodustalfs, mixed of red and black soils	paddy, pulses, sugarcane, cotton and vegetables

Source: Research Bulletin 22, WTCER, Bhubaneswar, Odisha

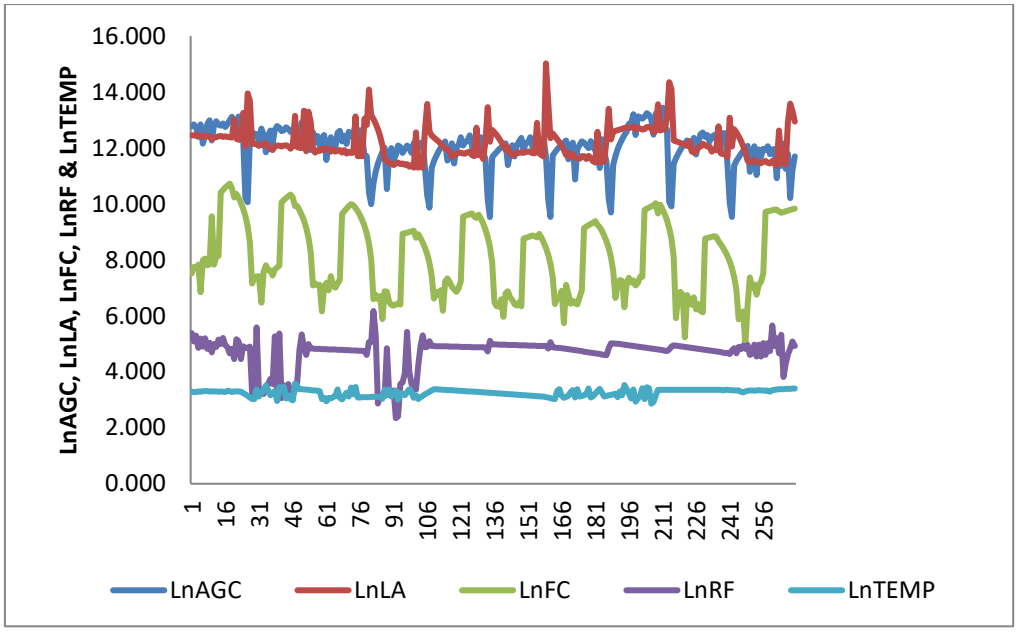

Fig.1: Trends of AGC, LA, FC, RF and TEMP of both Paddy

Source: Author estimated

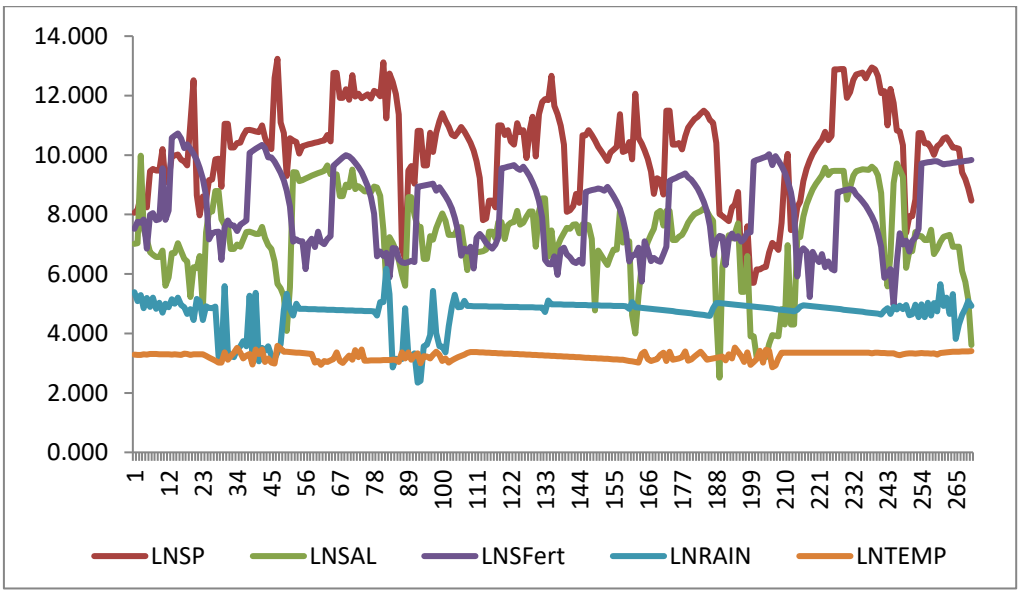

Fig.2: Trend of crops production, net shown area, fertilizer consumption, Rainfall and Average Temperature in Study Area, Odisha

Source: Author estimated

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Environmental factors and reactor configurations in biogas production from anaerobic co-digestion of fruit wastes

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Abstract— *A search for alternatives economically viable and environmentally sound to the world energy demand, stimulated the research in the field of anaerobic digestion, as a form of renewable energy and the anaerobic co-digestion is an alternative to use different types of residues, including food wastes. Therefore, this article presents an analysis of the scientific advances realized of the period of 2015 to 2018 in terms of anaerobic co-digestion, with emphasis on the use of different food residues, especially fruit and vegetable wastes, a different configuration of reactors, and kind of operational conditions used. A description of environmental factors affecting the process efficiency and the biogas generation based on substrate characteristics is presented in this review since these factors play an important role in the biogas yield and determine the metabolic conditions of the microorganism growth. Therefore, research should focus on the anaerobic digestion process balance, to identify optimal operating conditions through the use and valorization of wastes.*

Keywords— ***Anaerobic co-digestion, Biogas, Food wastes, Fruit waste, Methane.***

I. INTRODUCTION

One of the biggest environmental global problems is food waste (FW) production, which can be defined as the mass of food lost or wasted during the part of the food supply chains. According to estimates by FAO (2012), 28% of the world's agricultural area is used annually to produce food. Of this amount, 1.3 billion tons of fresh vegetables, fruits, meat, bakery, and dairy products are lost per year. This waste of food has unfavorable economic and environmental implications because represents around USD 990 billion per year, consumes 1/4 of all water used for agricultural purposes, and contributes with 8% in the emission of greenhouse gases [1, 2, 3, 4, 5].

Experts estimate that the production of FW is expected to increase by 44% until the year 2025. India ranks seventh in overall food wastage, while the Russian Federation tops the list. In China, the production of FW reached 97.7 million tons in 2017. In Europe, this raise is expected to be about 42% from 2006 to 2020, with the production of 126 million tons. The generation of FW has been noted in England with an amount of 14.257 million tons from 2009 to 2013. Germany generated about 12.258 million tons in the same period [1, 4, 6, 7, 8, 9, 10].

Food waste is organic materials constituted mainly by carbohydrates, proteins, lipids, and others traces of inorganic compounds, which can be degraded by microorganisms in an oxygen-free environment. This complex biologic treatment process performed in the

absence of oxygen is called Anaerobic Digestion (AD), which is produced biogas while stabilizing the organic matter according to [2], [5], [7], [11], [12] and [13]. According to [14] each kilogram of food waste can generate approximately 0.1 m^3 of methane gas. Therefore, methane has a high calorific value of 17 to 25 MJ/m^3, which can be converted into energy. Estimates presented by [15] pointed out that just 15% of this gas is captured for beneficial use or flaring and the remainder converts into fugitive greenhouse gas emission from landfilling of food waste, that could amount to 3.1 gigatons CO_2-eq $year^{-1}$ considering the total global of 1.6 gigatons of food waste each year.

The use of AD for treating food waste is attractive for some economic and environmental reasons, among them: (i) reduces the volume of material to be disposed of; (ii) avoids soil and water pollution; (iii) provides renewable and inexpensive energy. Therefore, the anaerobic digestion of solid wastes, such as fruits and vegetable wastes (FVW) present two important advantages, as treats the residues and simultaneously produces biogas [16]; [17]. However, this process is strongly dependent on environmental conditions such as pH, temperature, substrate typology, carbon/nitrogen/phosphorous ratio (C:N:P), particles size, presence of inhibitors, among others, that in certain unfavorable situations could cause instabilities in the process and consequently, impairing their performance [16, 18].

Co-digestion has been used to promote instantaneous digestion of two or more substrate and co-substrate mixtures, minimizing some imbalances in the process. Many researchers have been investigated co-digestion using various mixtures of industrial, farming, agricultural, and municipal waste materials according to [19] and [20]. So, this review, which cannot be exhaustive given the number of published papers about this theme, collected some papers published in the period of 2015-2018, to describe the trends for biogas from anaerobic co-digestion research, emphasizing different feedstocks, reactors, and operational conditions, that could significantly improve the biogas conversion.

II. METHODS

A summary of bioconversion of food waste into energy is presented including a brief description about energy demand with an emphasis on biogas, the anaerobic digestion process and the anaerobic co-digestion process, pointing the most used substrates, reactors and operating conditions nowadays. The literature used to compose the state of the art, the object of study of this review article, includes papers and scientific reports that have been obtained from scientific journals and online resources. To refine the search, the following keywords were used: anaerobic co-digestion, fruit residues, and methane. Due to the high number of articles published, only articles published in the period from 2015 to 2018 were considered.

III. FROM FOOD WASTE GENERATION TO ENERGY RECOVERY

According to the data presented by [21], approximately 1.3 billion tons of food is lost or wasted in the food supply chain. According to the researchers [2], [3], [5], [9], [22], [23], and [24], this food waste must increase in the coming decades, causing socio-economic and environmental problems. Fig. 1 shows an estimate of food waste in several countries as reported by these authors. The loss or waste of 1.3 billion tons of food also results in the waste of natural resources such as soil, water, and energy.

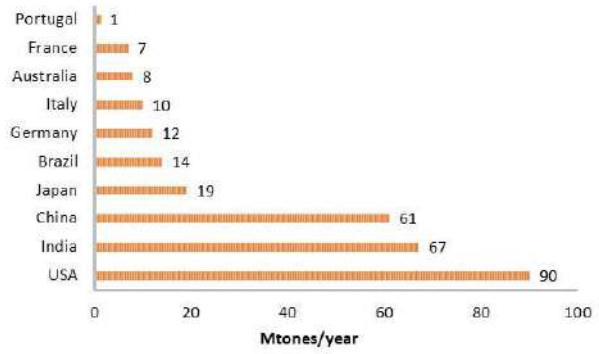

Fig.1: Food waste generation in some countries in 2017.

Estimates predict that 2.2 billion tons of food waste will be generated, as mentioned by according Food and Agriculture Organization (FAO), this would imply a total cost of approximately US$ 310 billion for low-income countries and US$ 680 billion for developed countries [7, 23, 25]. [2], [3], [5], [23] and [26] evinced that food waste cost around US$ 990 billion annually besides consuming a quarter of all the water used for agriculture purposes and contributed around 8% of total anthropogenic global greenhouse gas emission, accumulating annually 3.3 billion tons of CO_2 into the atmosphere.

The amount of fruit and vegetable represents 44% of the food waste contributor, and of this total, 25-30% are in the form of pomace, peels, and seeds [27]. According to [26] and [28], the number of food wastes in developed and low-income countries is in the range of 670 and 630 million tons, respectively. However, developed countries are produced 257 kg $year^{-1}$ compared to 157 kg $year^{-1}$ in low-income countries, on a per capita basis.

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The bioconversion process is a method to minimize easily biodegradable biomass, due to its high moisture content characteristics, and realize bioenergy recovery simultaneously [22, 24]. From this precept, the use of an anaerobic process will promote effective and environmental-friendly treatment of this type of waste and its valorization in the form of others products, such as methane and hydrogen [1, 29]. Considering the importance of the AD process in the decomposition of organic materials, such as FVW, the next section presents a review of the principles that involve anaerobic digestion.

IV. ANAEROBIC DIGESTION: THE PROCESS AND RELEVANT FACTORS AFFECTING

Anaerobic digestion (AD) is the biological degradation process of organic substrates in the absence of oxygen, applied for stabilizing the organic matter. The application of this process is attractive for economic and environmental reasons because this consolidated technology reduces the material volume to be disposed of, prevents soil and groundwater pollution, besides provides renewable energy, e.g. biogas. However, is a complex process that involves a consortium of bacteria and methanogenic archaea, which needs control of some environmental factors [16, 30, 31]. Around 1870, Jean-Louis Mouras developed the first septic tank, introducing the concept of anaerobic digestion [32]. Biogas was reported for the first time by Louis Pasteur who stated that this could be used as energy. During the petroleum crisis, in 1970, biogas had its development peak. Since then, the application of this technology has been exploited for waste treatment and energy production [32].

Several authors have reported that AD is a biodegradation process of three or four steps including phases of hydrolysis, acidogenesis, acetogenesis, and methanogenesis, and for others, the acetogenesis is suppressed. The schematic of the four phases of the anaerobic digestion process is shown in Fig 2. However, irrespective of how many steps are involved in AD and defined by the researchers, it is known that the biodegradation process principles are similar and these steps are performed synergistically by several bacteria hydrolytic, acetogenic, hydrogen-producing, acetate-forming microbes, homoacetogens, methanogenic acetoclastic arches and hydrogenoclastic methanogens arches [7, 30, 33, 34].

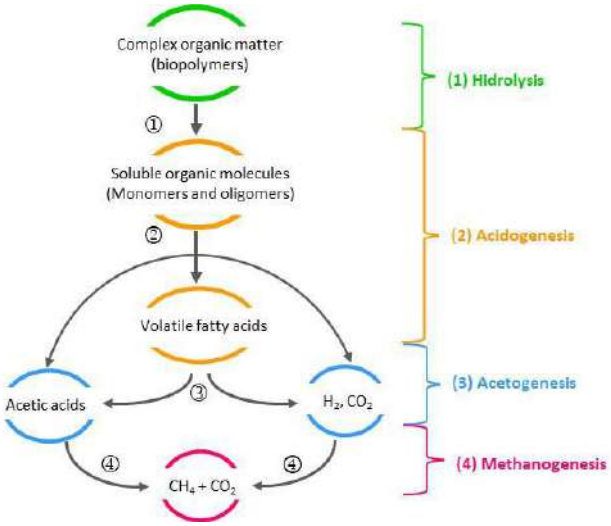

Fig.2: Schematic drawing of anaerobic digestion stages.

Environmental pollution and increase in energy demand are the greatest challenges to be faced by human beings in the coming years. One of the options currently studied is the use of biomass, which has shown to be a vast and promising source of energy [13]. The energetic usage of biomass can be enhanced by the AD, whose final product is biogas, rich in methane. The methane has a high calorific value of 17-25 MJ m^{-3}, which can be converted to energy (heat or electricity). So, the AD of organic wastes presents a double advantage, as it produces biogas and simultaneously treats the residues, reducing their disposal in sanitary landfills. Therefore, the extent to which this methane production efficiency becomes more developed and tested, allows this process to be commercially viable [14, 17]. However, to achieve this efficiency, the AD requires control of environmental conditions such as temperature, pH, alkalinity, carbon/nitrogen ratio (C/N), substrate typology, particles size, and organic loading rates (OLR) [16, 35]. Fig. 3 shows a summary of the operating conditions that allow biogas production in the anaerobic process.

4.1 Temperature

[11], [20], [36] and [37] point out that temperature is one of the most significant parameters affecting AD, especially in the enzymatic activity, thus influencing the biogas yield. Generally, anaerobic bacteria can grow at psychrophilic (10-30ºC), mesophilic (30-40ºC) and thermophilic (50-60ºC) conditions, however the mesophilic process is more stable if compared to the others and shows higher performance during the digestion process, that there is a greater microbial diversity in mesophilic conditions.

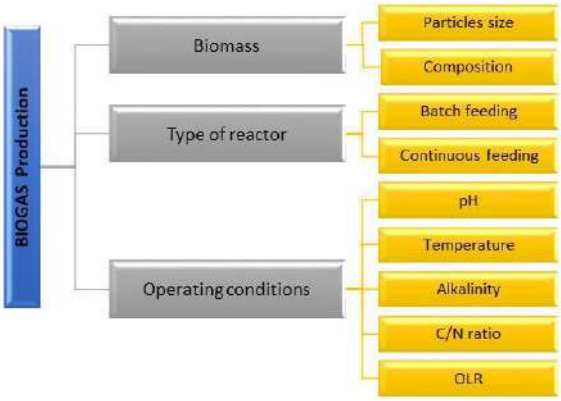

Fig.3: Operational control variables of the AD process.

These researches mentioned that in thermophilic conditions, there is an increase in AD performance and consequently of biogas production, due to the higher specific growth rates, higher metabolic rates, and higher destruction of pathogens. Despite this, there is a reduction in the methane content compromising the biogas quality by the decrease of the CO_2 solubility due to the increase in temperature. Therefore, it can be concluded that in mesophilic conditions (mainly in 32-35ºC) there is an improvement in the stability of the anaerobic digestion process, and can achieve better methane conversion.

4.2 pH value and alkalinity

When it comes to biological processes, pH is a limiting factor because influencing the ionized and non-ionized compounds forms, such as hydrogen sulfate, ammonium and other fatty acids, which are toxic to some microorganisms. On this control parameter, for AD process, the ideal range of pH is 6.8 to 7.3 [13, 20], that varies according to the microorganism's groups involved in each of the anaerobic digestion stages. For example, the optimum pH for fermentative bacteria that act on the hydrolysis and acidogenesis is between 5.5 and 6.5, while methanogens prefer a pH close to neutrality, since they are more sensitive to variations outside this range [13]. So, the anaerobic digestion, with focus in biogas production need to maintain a suitable pH range between 6.5 and 7.5 during the whole process, avoiding any sudden variation that may cause imbalance of the microorganism's metabolic functions involved [2, 30].

[14], [23] and [38] stated that alkalinity is another factor that can guarantee the stability of the pH, especially the variations that occur in the hydrolysis phase. According to [39], to ensure such stability, it is necessary to maintain the range of total alkalinity between 13,000 – 15,000 mg L^{-1} and volatile acids concentration below 1500 mg L^{-1} and the ratio intermediate alkalinity/partial alkalinity (IA/PA) equal or less than 0.3.

4.3 Carbon to Nitrogen (C/N) ratio

The performance of AD is significantly affected by C/N ratio and an optimum value is needed because anaerobic microorganisms require carbon as energy and nitrogen to form their cell proteins, i.e., an appropriate nutrient balance for their growth. This is the reason to maintain minimum levels of these nutrients in the medium and then guarantee microbial growth and performance because improper C/N ratios could result in high volatile fatty acids accumulation and/or high ammonia released, both of which are potential inhibitors and could cause the possible failure of the AD process [40].

Several researchers reported a wide range of C/N ratio, which is essential to reach an optimal biogas yield in the balance of sufficient nutrient supply [11, 30, 41]. The most recommended C/N ratio in the literature range from 20/1 to 30/1 for anaerobic bacterial growth; however, some authors point to an even lower range, such as 15-20/1, indicating that optimum C/N ratio depends on the characteristics of the substrates [11, 13, 16, 30, 42, 43].

4.4 Substrate characteristics

Another environmental factor that interferes in the anaerobic digestion process is the characteristic/ composition and size particles of the substrates [16, 44]. In the literature, this aspect has not yet been extensively treated and [45] mentioned that there are only a few studies that relate the impacts of the substrate on the anaerobic digestion process. However, the characteristics related to substrate composition in terms of carbohydrates, lipids, and proteins are key factors that influence the biological metabolism and consequently, in the performance of anaerobic digestion, because several substrates are more biodegradable while others more complex [39].

[36] affirm that a high concentration of substrate may become toxic to the agents responsible for anaerobic degradation, causing the accumulation of total ammonia (TAN), free ammonia (FAN), and volatile fatty acids (VFA). [46] identified an appropriate ratio of food waste/inoculum to maximize the methane production, with a buffered environment, avoiding low pH. [18] and [20] mentioned that particle size also influences the process since they observed that the smaller the surface area, the better the hydrolysis process because it facilitates the performance of the microorganisms.

4.5 Organic loading rate

The number of organic materials subjected to biological reaction in a certain time period, and per unit of reactor volume is called Organic loading rate (OLR). For many

authors, this is the key that defines the balance between the acidogenesis and methanogenesis phases. Therefore, to provide a maximum biogas generation, the OLR is maintained at high loads in many experiments [36].

The principle governing the best OLR values are related to the reactor configuration and the composition of substrates used. [47] reported a decrease of the volatile acids when the OLR was increased, and consequently the hydrolysis rate was reduced in the anaerobic digestion of food waste. According to [20] and [47], the OLR must be controlled as an environmental condition that interferes in the anaerobic digestion process and the range of optimum values of OLR must be calculated for each bioreactor project, associated with the type of substrate. Therefore, a general optimum range cannot be mentioned for all bioreactors because it differs according to the substrate and inoculum.

V. ANAEROBIC CO-DIGESTION OF FRUIT AND VEGETABLE WASTES

At the end of the 1970s, anaerobic digestion underwent an adaptation, known as anaerobic co-digestion (AcoD) or co-fermentation, a process in which different mixed residues can be simultaneously digested, achieving yields equal to or even higher than the anaerobic digestion process. AcoD has shown advantages over AD because it provides greater balance in terms of nutrient availability, offers better buffering to the system, depending on the blend, and also dilutes certain inhibitory compounds [48].

The synergistic effects of AcoD were pointed by [1], [16], [17], [23], [38] and [49], when they mentioned the increase in the biodegradability, increase in the active biomass concentration as a function of the increase of the microbial community involved in the process, production of a digestate with improved characteristics to use in agriculture, suggesting that co-digestion process is a feasible option to overcome the mono-digestion limitations. There is an extensive variety of organic materials that can be used as feedstock to the anaerobic co-digestion and the scientific literature presents several results that indicated correlations between substrates used and biogas yield [44].

A series of researches and their respective data will be mentioned in sequence to present the main scientific results about anaerobic co-digestion, specifically of fruit and vegetable wastes, reported in the 2015 to 2018 period. A summary of several types of research, addressing different types of reactors, substrates, and inoculum is presented in Table 1.

[50] evaluated the effect of the addition of cow manure with straw in the single-phase and two-phase digestion of fruit and vegetable wastes (FVW) and concluded that the substitution of vegetable wastes with cow manure (CM) from 20 to 40% resulted in a methane yield decrease and reduction of both mono-digestion and co-digestion. However, when comparing the equivalent waste combinations, the authors verified that the yield was higher in single-phase process, with 33% (100% FVW), 40% (80% FVW / 20% CM) and 58% (60% FVW / 40% CM).

[51] studied the effect of waste-mixed sludge (WMS) co-digested with fruit and vegetable wastes (FVW) at different organic loading rates ranging from 1.46 kgVS m^3 day^{-1} to 2.8 kgVS m^3 day^{-1} during 280 days. The results indicated that the increase in OLR showed major benefits in comparison with the other conditions analyzed and also that co-digestion with FVW led to an increase in the amount of the biodegradable organic carbon in the digester, equalizing the typical high nitrogen concentration of WMS. Therefore, the net electrical energy available achieved a maximum value of about 3,500 MWh $year^{-1}$ when operated with to an OLR of 2.1 kgVS m^3 day^{-1} (i.e. 22 tonnes day^{-1} of FVW).

Anaerobic co-digestion of cow manure, with carbon slowly released from corn straw, as well as the effect of adding available carbon quickly released, with fruit and vegetable waste was explored by [52]. Two experiments were conducted consisting of group A (FVW dosage was 5% of cow manure) and group B (FVW dosage was 1%), and Group C used as the control. The authors verified that the hydrolysis process of the anaerobic co-digestion of the cow manure and corn straw was improved by adding the FVW. The specific methane yield increased from 202.06 to 522.92 mL gVS^{-1} in group A and 174.98 to 743.24 mL gVS^{-1} in group B.

Table 1: Some scientific works about anaerobic co-digestion of fruit and vegetable waste

Reference	Reactor	Substrates	Inoculum	Remark	Methane production/yield
[17]	Batches glass reactors	Organic fraction of municipal solid waste + FVW	Anaerobic sludge	This study investigated the digestion of four different organic fraction of municipal solid waste and fruit and vegetable waste ratios that was evaluated in terms of biogas and methane yield, TVS removal rate, and stability of the anaerobic process.	396.6 N mL g VS^{-1}
[49]	Continuous anaerobic digesters	Sewage sludge + three different fruit waste	Sludge from a stable lab-scale mesophilic digester	The transitory state was evaluated with two different conditions: co-substrate changing and co-substrate stopped.	1.1 L CH_4 L_R day^{-1}
[50]	Anaerobic reactor	Cow manure with straw + FVW	Granular sludge	The influence of different proportions of lignocellulosic substrate on the single-phase and two-phase digestion of a readily biodegradable substrate was investigated to determine the optimum co-substrate ratio and the process best suited for co-digestion.	82.3 L $week^{-1}$ (single-phase) 7 L $week^{-1}$ (two-phase)
[51]	Gas-tight anaerobic reactor	waste-mixed sludge + FVW	Not mentioned	The effect of waste-mixed sludge co-digested with fruit and vegetable waste was investigated at different organic loading rates.	900 NL m^3 day^{-1}
[52]	Continuous stirred tank reactor (CSTR)	Cow manure and corn straw + FVW	Not mentioned	In this study, the anaerobic co-digestion of cow manure with available carbon was investigated to measure the effect of adding available carbon quickly released, so the fruit and vegetable waste could be exploited as substrate.	202.06 mL g·VS^{-1} (group A) 174.98 mL g·VS^{-1} (group B) 165.08 mL g·VS^{-1} (group C)
[53]	Anaerobic batch reactors	FVW	Sewage sludge	The feasibility of fruit and vegetable wastes to yield methane gas has been evaluated by adopting the automatic methane potential test system and substrate/Inoculum ratio has also been optimized to get maximum methane gas.	265-444 N mL g VS^{-1}
[54]	Anaerobic batch reactor	FVW	Swine manure effluent + cattle manure and raw cattle manure	This research investigated the chemical composition influence of twelve different batches of fruit and vegetable waste with different compositions collected over one year, on the biochemical methane potential (BMP).	288 to 516 NL CH_4 kg VS^{-1}
[66]	Gas-tight	Waste-mixed	Not	The effects of anaerobic co-	435 NL CH_4 kg

	anaerobic reactor	sludge + FVW	mentioned	digestion of waste-mixed sludge with fruit and vegetable waste on the methane generation of a mesophilic digester was investigated.	VS^{-1}
[67]	Continuously stirred-tank anaerobic bioreactor	Swine manure + a mixture of FVW	Not mentioned	Various co-substrate ratios were investigated under mesophilic conditions in a pilot-scale continuously stirred-tank bioreactor of obtaining an optimal ratio for maximizing the methane production.	0.65 m^3 kg VS^{-1} day^{-1}
[68]	Gas-tight anaerobic reactor	Waste-mixed sludge + FVW	Not mentioned	The effect of WMS co-digested with fruit and vegetable waste was investigated at different organic loading rates.	900 NL m^3 day^{-1}
[69]	Anaerobic batch reactors	Mixture of cooked vegetables, rice, bread, cereals + fruits in semi-solid forms	Seed sludge	The optimum F/M ratio was evaluated and determined the optimum temperature for anaerobic digestion of food waste charged with total solids content of 25–50%.	0.88 L CH_4 g COD^{-1} (mesophilic) 0.62 L CH_4 g COD^{-1} (thermophilic) 0.73 L CH_4 g COD^{-1} (psychrophilic)
[70]	CSTR and UASB	Mixture of waste matter consisting of watermelon, apple + potato	Anaerobic sludge of wastewater treatment plant	Two-phase anaerobic digestion in acid reactor was investigated, with a completely stirred tank (CSTR) acid reactor and an up-flow anaerobic sludge bed (UASB) methane reactor to examine the lactate degradation.	261.4 mL g COD^{-1} removed
[71]	Discontinuous anaerobic digestion reactors	Seventeen types of fruit waste, including peels, seeds, and shells	Anaerobic sludge from Biogas plant fed with pig manure	Batch tests were realized to compare the AD performance of 17 types of fruit residues as a single substrate, as well investigated the characteristics of these fruit waste in methane yield, comparing different kinetic models.	383.4 mL g SV^{-1} (Rambutan seeds)
[72]	Batches glass reactors	Ripe banana + ripe longan + rambutan	Mixed sludge from two full scale anaerobic digesters	This study was divided into two main parts: Firstly, identify the bio methane potential of key tropical fruits waste. The second part was to evaluate an appropriate digestion strategy of the selected substrate (banana peel) in continuous anaerobic digestion systems.	330.6 mL CH_4 g VS^{-1} (ground banana peel) 268.3 6 mL CH_4 g VS^{-1} (chopped banana peel) 234.66 mL CH_4 g VS^{-1} (chopped longan waste)

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[73]	Anaerobic co-digestion batch reactors	Empty fruit bunches + palm oil mill effluent + sewage sludge	Mesophilic methane production sludge	Empty fruit bunches, palm oil mill effluent, sewage chemical sludge and sewage biological sludge were evaluated for methane production under liquid-state anaerobic digestion and solid-state anaerobic digestion.	18 mL CH_4 g VS^{-1}
[74]	Semi-continuous bench scale stirred tank reactors	Poultry manure + FVW	Sludge from dairy effluents treatment anaerobic lagoon	The authors evaluated the performance of anaerobic digestion of poultry manure co-digested with fruit and vegetable waste, in terms of biogas production, organic matter reduction and release of nitrogen compounds.	0.21 NL CH_4 g VS^{-1}
[75]	Continuous stirred tank reactor (CSTR)	Peach waste + apple pulp waste	Sludge and granular sludge	This work had as objective to evaluated the performance of a two-stage anaerobic process and the optimal operational conditions, taking into account the degree of acidification and biomethane production under different operational conditions.	4.33 L CH_4 L day^{-1}
[76]	Glass bottles reactors	Durian shell + chicken manure + dairy manure + pig manure	Anaerobic sludge	Anaerobic co-digestion of Durian shell with chicken manure, dairy manure and pig manure at different ratios was performed to investigated the methane production and determined the principal synergistic effects of co-digestion.	224.8 mL g VS^{-1}
[77]	Anaerobic batch reactors	Sugarcane bagasse + FVW	Waste activated sludge	This research investigated the influence of mixture of waste activated sludge as inoculum to the ratio of sugarcane bagasse and fruit-vegetable waste as substrate, to evaluated the biogas yield during anaerobic co-digestion.	2600 mL day^{-1} (biogas yield)

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One research that sought to assess the feasibility of fruit and vegetable wastes to yield methane gas and substrate/inoculum (S/I) ratio was conducted by [53]. Methane potential obtained from the fruit waste was 0.444, 415.12, 358.27, 337.31 and 265.03 N mL gVS^{-1} to S/I ratio of 0.43, 0.67, 1.0, 1.5 and 2.3, respectively, whereas, this potential from vegetable waste was 470.91, 435.47, 403.46, 351.42 and 247.97 N mL gVS^{-1} to S/I ratio of 0.43, 0.67, 1.0, 1.5 and 2.3, respectively. According to these authors, the S-shaped cumulative methane curve indicated the lower production of methane at the highest S/I ratio, that is, as lower inoculation lower is the microbial activity and more risk of inhibition of the anaerobic digestion process.

Twelve different batches of fruit and vegetable wastes were used by [54] to investigate the influence of chemical composition on the biochemical methane potential (BMP). The authors verified that BMP ranged from 288 to 516 LN CH_4 $kgVS^{-1}$, with an average of 377 (67) LN CH_4 $kgVS^{-1}$ and 79% of biodegradability, and attributed this variation to the chemical composition over time. Moreover, they also developed statistical models by multiple linear regression to predict methane potential and affirmed that the best BMP prediction was obtained using the model including lipid, protein, cellulose, lignin, and high calorific value, with an R2 of 92.5%. The authors concluded that the high calorific value might be useful for predicting the BMP.

When analyzing the data presented in Table 1, it is understood that for each combination of substrate used, the AcoD process must be differently designed to obtain the optimal treatment efficiency. However, scientific research has indicated the feasibility of the AcoD process has been increasing, and that there is an even greater potential for biogas production using co-digestion of different feedstock wastes. Among the challenges of maintaining the AcoD process are the biogas yield rate, the substrates ratio, process stability, and nutritional balance, which require more investigation.

Various strategies have been applied to improve the biogas production from anaerobic co-digestion of food wastes, especially of fruits and vegetables, and different improving effectiveness has been reported in the scientific literature. However, these strategies cannot be compared directly with each other about their effectiveness, because operational conditions are different from each other. Thus, because it is a complex process, the efficient results of anaerobic digestion are directly related to the interaction of some factors, among them: applied organic load, operational conditions, and configuration of the reactors.

VI. REACTORS CONFIGURATION AND FLOW MODE

The anaerobic digestion system can be classified according to the substrate type, temperature, or power supply. [55] suggested three types of classification: (A) total solids: wet type (with < 10% total solids TS) or dry type (> 20% TS); (B) temperature: mesophilic (35-40°C) or thermophilic (> 55°C) and (C) reactor feeding mode: batch fed; semi-continuously fed or continuously fed. The anaerobic digestion process can occur in different types of reactors, regardless of their classification, so in this section are described some types of reactors most commonly used in scientific experiments and their results in the anaerobic co-digestion process for biogas production. Thus, this section was divided into two topics: batch and continuous reactors.

6.1 Flow mode of reactors in anaerobic co-digestion

6.1.1 Batch reactors

For [56] the most common anaerobic reactor is the anaerobic sequential batch reactor (ASBR), characterized by a single-tank unit in which occur all of the treatment steps and processes. This reactor presents some advantages such as operational simplicity, effluent quality control, fewer maintenance requirements, low cost, and high biogas yield.

[57] operated a batch reactor to investigate the synergistic effects of mono and co-digestion of six different ratios of food wastes (FW) and pig manure (PM) on the specific methane yield (SMY) and reaction kinetics. Lower average daily methane yield was observed in the PM mono-digestion (260 ± 13 mLCH_4 gVS^{-1}) than in the food waste mono-digestion (516 ± 33 mL CH_4 gVS^{-1}), with highest value of 521 ± 29 mL CH_4 gVS^{-1} to PM/FW mixing ratio of 1/4. The authors affirmed that the methane generation increased when combining PM and FW due to the synergistic effects of using two substrates.

[58] developed a compact three-stage anaerobic digester (TSAD) for food waste substrate composed of three separate chambers in a single-stage digester. TSAD achieved a higher methane yield of 24–54% with the production of 0.307 LCH_4 gVS^{-1} when compared to traditional reactors of one-stage (0.199 LCH_4 gVS^{-1}) and two-stage anaerobic digesters (0.249 LCH_4 gVS^{-1}).

To treat food waste at mesophilic conditions, [59] used a digester system consisting of three reactors operated in single and two-phase mode. Two-phase mesophilic digestion presented higher methane production (446 LCH_4 $kgVS^{-1}$) when compared to the single-stage operation (380 LCH_4 $kgVS^{-1}$). The authors concluded that although it is more complex, the reactor operated in two-phase mode has the potential to maintain the process in periods of low rate.

[23] also affirmed that two-stage AcoD is more effective if compared to the conventional single-stage, because it can improve the degradation rates, methane yield, i.e., the overall process efficiency.

6.1.2 Continuous reactors

[60] investigated the performance of a continuous flow reactor, operated at 37ºC, in the anaerobic co-digestion of food waste with high solids content. The reactor was submitted to different organic loading rates of 5, 6, and 9 kgVS m^3 d^{-1}, corresponding to the hydraulic retention time of 26, 25, and 14 days, respectively. The authors reported that the daily biogas production drastically decreased from 196 to 136 L d^{-1} when the organic loading rate was increased from 6 to 9 kgVS m^3 d^{-1}. They concluded that the increase in the organic loading rate, and consequently decrease of hydraulic retention time, contributed to the reduction in the efficiency and instability of the process.

To evaluate the effectiveness of the anaerobic co-digestion of food wastes (FW) and de-oiled grease trap wastes (GTW) in the biogas production, [61] operated three different systems - a lab-scale mesophilic digester (MD), a temperature-phased anaerobic digester (TPAD) and a TPAD with recycling (TPAD-R). Each reactor consisted of a continuous stirred tank reactor with temperature control and biogas collection and was operated under mono-digestion (FW) and co-digestion (FW + de-oiled GTW), synchronously. The authors reported greater biogas yields to mono-digestion in MD (19%) and TPAD-R (19%) than to TPAD (8%), with the maximum value of 0.62 L gVS^{-1} in the lab-scale mesophilic digester (MD) of the co-digestion system.

A two-stage anaerobic system, coupled in continuously stirred tank reactor (CSTR) and sequential batch anaerobic reactor (ASBR) was assembled by [62] to investigate the anaerobic co-digestion of fruit and vegetable wastes (FVW), waste-activated sludge (WAS), olive mill wastewater (OMW) and cattle manure (CM). The results showed that the single-stage digester was characterized by higher electric and thermic energy productions, with methane yield around 340 L $kgVS^{-1}$. However, there was an increase in the energy production associated with the two-stage system when increasing gradually the OLR and biogas recirculation. The authors verified 1765.2 kWh $tonVS^{-1}$ of electrical energy and 2942.1 kWh $tonVS^{-1}$ of thermal energy when applied OLR of 3.44 kgVS m^3 d^{-1}, and assumed that two-stage anaerobic co-digestion may be a mechanism used in pollution control and bioenergy recovery from organic wastes, including fruit and vegetable wastes as substrate.

In another study, [63] compared the performance of single and two-stage anaerobic digestion processes using food waste as substrate. CSTRs reactors were used for a one-stage process (230 L) and a two-stage system with two reactors (200 and 760 L), operated in thermophilic conditions. The results proved that the systems showed high biogas yields. The specific gas production was higher in the two-phase system with 0.88 m3 biogas $kgVS^{-1}$ than in the one-phase system with 0.75 m3 biogas $kgVS^{-1}$. The authors concluded that the methanogenic process was positively affected by the two-phase process.

6.1.3 Semi-continuous reactors

[64] studied the methane production capacity in mesophilic conditions (35ºC) treating food waste in a semi-pilot batch reactor (6 L) and pilot-scale semi-continuous reactor (300 L). The substrates used were composed of vegetable waste and pesto sauce and other kinds of sauces wastes. Already pilot-scale test (semi-continuous mode) involved only the vegetable mix waste (VMW) because of its higher heterogeneity. The results of the semi-pilot scale (five replicates) indicated biogas and methane specific production of 0.554 Nm^3 $kgVS^{-1}$ and 0.294 Nm^3CH_4 $kgVS^{-1}$, respectively. The results of biogas daily production ranged from 50 NL d^{-1} to 100 NL d^{-1} in the pilot-scale test. The higher methane specific production was obtained in the semi-continuous test with values 76% higher than those obtained in the batch test. Another important result indicated that the average methane content was 20% greater on the pilot scale than on the semi-pilot scale.

[65] evaluated the impact of digesting fruit and vegetable wastes (single substrate) at different ratios to achieve the optimal mix in a two-stage semi-continuous digester composed of a hydrolysis unit (6 L, first stage) and a vertical continuously stirred digester (35 L, second stage), both operated at 35º C. The results revealed that the biogas yield increased proportionally with the OLR. However, when the OLR exceed 3.4 kg VS m^{-3} d^{-1}, a decline in biogas yield was observed. Optimal conditions were found to the OLR range of 2.68-2.97 kg VS m^{-3} d^{-1}, resulting in a biogas yield between 2.4 and 2.8 Nm^3biogas m^{-3}reactor d^{-1}. The average specific biogas and methane were 0.87 Nm^3 $kgVS^{-1}$ and 0.49 Nm^3 $kgVS^{-1}$ at the optimal conditions, respectively.

VII. CONCLUSION

This paper described researches about the development on the anaerobic co-digestion (AcoD) process of fruit and vegetable wastes as substrates, published in the period from 2015 to 2018. From the data reported in several studies, it can be concluded that the AcoD process from different biodegradable organic materials is a technology economically and environmentally feasible to biogas

generation at both the laboratory scale and the industrial scale. However, some challenges still have to be overcome, among them the characterization of the different organic materials and the process parameters, and the behavior of microorganisms to ensure maximum process efficiency. A comprehensive analysis to combine strategies to improve the co-digestion process is still a task for the scientific field, especially to optimize the operation of anaerobic reactors for the sustainable conversion of organic waste to sustainable bioenergy.

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Flood Modeling and Vulnerability Analysis of Abia State using Remote Sensing and Flood Modeler

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***Abstract*— *This study aimed at flood modeling and vulnerability analysis of Abia State using Remote Sensing and Flood modeler. The methodology involved acquisition of Sentinel-2 imagery covering Abia State, Rainfall data and ALOS PALSAR. Image subsetting was done to extract the area of study from the acquired dataset, this was followed by analysis of DEM accuracy using root mean square error, image classification to extract the landuse/ landcover of the study area, surface runoff modelling to determine surface runoff potential in the study area and flood modelling. The flood frequency return as modeled by Flood Modeler indicated a 25.04km^2 inundation extent for 2-year return period, 28.10km^2 inundation extent for a 5-year period and 26.04km^2 inundation extent for a 10-year return period. Increasing to its peak extent by 3.67% by the 5-year return period, and then decreased by 2.24% by the 10-year return period. The surface runoff potential revealed that 35.99% of the study area with an area coverage of 1630.19 km^2 had low infiltration potential, 32.51% with an area of 1472.56 km^2 had moderate infiltration while 31.50% with an area of 1426.82 km^2 had high infiltration. This indicated that the study area had a high extent of low surface infiltration which will lead to flooding during heavy or frequent rainfalls. This study recommends flood modeler as it is reliable for flood modeling, having been proven with correlation results of 0.8196that it fits to the ground flood points gotten during field validation.*

Keywords*— *Flood Vulnerability, Flood Modeler, Remote Sensing, Sentinel-2, Surface Runoff.

I. INTRODUCTION

Floods are one of the most hazardous threats to several communities affecting mainly the economy and wellbeing of the people. Floods are usually caused by excessive runoff from precipitation or snow melt, on by coastal storm surges or other tidal phenomena (Nwilo, 2012). In 2007, the frequency distribution and causes of floods over the last thirty years has been analyzed and reported by the Dartmouth flood observatory, and a five-fold increase in the number of floods per year has been observed since the 1980s. These countermeasures rely on flood prediction capabilities, and especially, the ability to delineate potential flood inundation areas is one of the most important requirements (Hoey and Ferguson1994). Flooding in Nigeria has taken a new dimension in recent times. The latest occurred on September, 2018 where communities in about 20 states were inundated and millions of people displaced from their homes Abia state was one of the states affected by the flood event. The impact of the flood was felt by the communities, human lives were lost, properties, houses, and farmlands destroyed.

The studies of Bariweni et *al*, (2012), Dupe et *al*, (2013), Chidi et *al*, (2015), Moses and Ikechukwu, (2015) have modelled flooding in Abia State but the situation remains the same. One would argue if the problem is with the accuracy of the flood models applied or just a case of not applying the right methodology to the situation.

Flooding can be exacerbated by increased amounts of impervious surface which reduce the supply of vegetation that can absorb rainfall (Förster *et al*., 2020), runoff from sustained rainfall, flash floods resulting from convective

precipitation (intense thunderstorms) or sudden release from an upstream impoundment created behind a dam (Haghizadeh *et al.* 2012)or floods caused by blocked drainages when water accumulates across an impermeable surface (e.g., from rainfall) and cannot rapidly dissipate.

So, in order to properly solve the problem of flooding in Abia State, a deeper understanding of the factors and mechanisms that cause and contribute to the flooding event unique to Abia State can assist towards formulating an effective methodology using flood models for flood modelling and management, thus lessening the impacts of flood in Abia State.

It is to this effect that this study aimed at flood modeling and vulnerability analysis of Abia State using Remote Sensing and Flood modeler, so that planners can have an accurate data necessary for planning and managing flooding in Abia State Nigeria.

II. MATERIALS AND METHODS

2.1 Study area

Abia is a state in the south eastern part of Nigeria located between latitude 5^0 00'N and 6^0 00'N and longitude 7^0 00'E and 8^0 00'E of the equator see figure 1.0. It occupies about 6,320 square kilometers and bounded on the north by Enugu, west by Imo State, east by Akwa Ibom and to the south by Rivers State. The southern part of the State lies within the riverine part of Nigeria, which is a low-lying rainforest. The southern portion gets heavy rainfall of about 2,400 millimeters (94 in) per year especially intense between the months of April through October. The rest of the State is moderately high plain and wooded savannaand the most important rivers are the Imo and Aba Rivers which flow into the Atlantic Ocean through Akwa Ibom State.

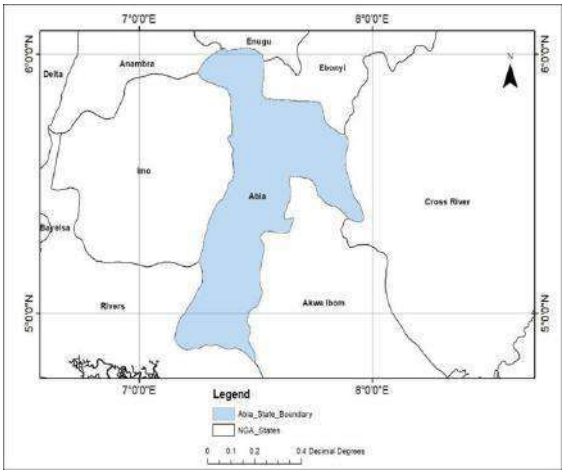

Fig.1.0: Location Map of the study area.

2.2 Methodology

This study utilized Sentinel-2 and ALOS PALSAR imageries of the study which were downloaded from the USGS website using the Earth explorer as the primary data. Digital administrative maps of Nigeria, Abia State which was sourced from the Department of Surveying and Geoinformatics, Nnamdi Azikiwe University Awka. Rainfall and soil data were gotten from NIMET Agency as the secondary data. Software used includes ArcGIS 10.6, Erdas Imagine 2014.

III. RESULTS

3.1 Analysis of DEM Accuracy

Before theALOS PALSAR DEM could be used in flood modelling, it had to be validated first using ground control points. The elevation points obtained from the ALOS PALSAR DEM were compared with the elevation points picked from ground to obtain the horizontal and vertical accuracy of ALOS PALSAR using root mean square error. This returned vertical and horizontal accuracies of 5.64m and 14.39m respectively indicating thatALOS PALSAR was a good fit and represents the elevation values on ground.

3.2 Land Use Land Cover and Surface Runoff Modelling.

Abia State shape file was used to subset the area of study from Sentinel 2 imagery and the resulting image was classified using supervised classification in order to obtain its LULC. The landcover/landuse distribution (figure 3.1) indicated that grassland, accounted for the largest land cover/use of about 52.06% and an area of about 2459.28 km^2. Urban area had 23.21% and a coverage area of 1096.41 km^2, forest had 18.36% with an area of 867.44 km^2 and water body had the lowest turnout with 6.35% with an area of 300.32 km^2. The precision of the classified images was ascertained and accuracy assessment was carried out by comparing the classified Landsat image with known reference pixels. The overall classification accuracy gotten was 89.23% and the overall kappa was 0.8935.

The landcover/landuse map, rainfall data and soil map were used to model the surface runoff potential in Abia state. The landcover/landuse map, soil map and rainfall data were then used to derive the curve number map, the curve number being the one of the major governing factors that predominantly affect the runoff amount that flows over the land after satisfying all losses plays an important role in defining hydrological response of catchment. The surface runoff potential (figure 3.2) revealed that 35.99% of the study area with an area coverage of 1630.19 km^2 had low infiltration potential, 32.51% with an area of

1472.56 km^2 had moderate infiltration while 31.50% with an area of 1426.82 km^2 had high infiltration. This indicated that the study area had a large extent of low surface infiltration which will lead to flooding during rainfalls.

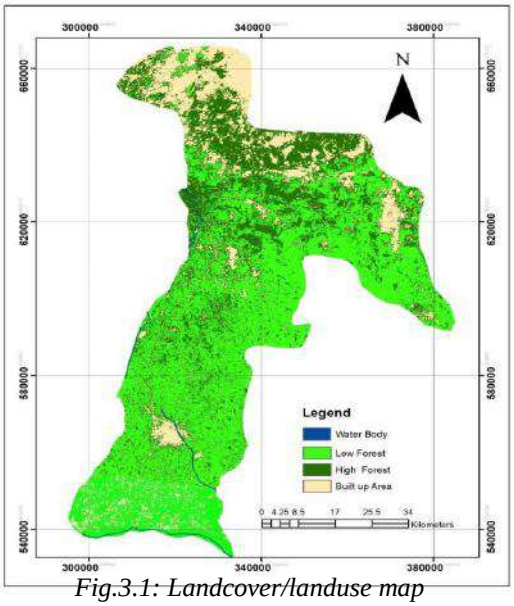

Fig.3.1: Landcover/landuse map

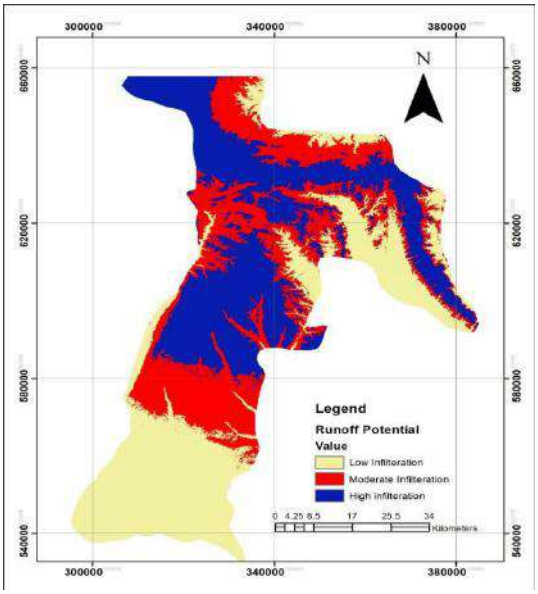

Fig.3.2: Surface runoff potential

3.3 Flood Frequency Analysis

The flood frequency return by flood modeler revealed a 25.04km^2 inundation extent for 2-year return period, 28.10km^2 inundation extent for a 5-year period and 26.04km^2 inundation extent for a 10-year return period. Increasing to its peak extent by 3.67% by the 5-year return period, then decreased by 2.24% by ten-year return period see figure 3.3.

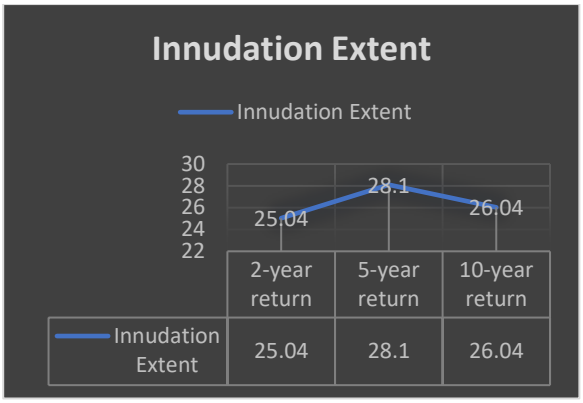

Fig.3.3: Flood frequency as modeled by Flood modeler

3.4 Flood Vulnerability Mapping

The results of the vulnerability modelling with flood modeler, produced a layer showing four vulnerable zones; namely very high risk, high risk, moderate risk and low risk flood zones in the study area. The results obtained from the flood modeler modelled vulnerability revealed that very high-risk zone occupied 9.69% of the entire study area, covering an area of 439.10km^2, while high risk zone occupied 27.87%, covered an area of 1262.53km^2. Moderate risk zone occupied 32.87% covering 1489.25km^2 while low risk zone occupied 29.55% covering an area of 1338.71km^2 See table 3.1.

Table 3.1 Flood vulnerability distribution by flood modeler.

	Flood Modeler		
		Area (Hectare)	%
1	Very High Risk	439.10	9.69
2	High Risk	1262.53	27.87
3	Moderate Risk	1489.25	32.87
4	Low Risk	1338.71	29.55
	Total	4529.59	100

An overlay analysis was done, overlaying the flood risk layer with the landcover/landuse layer to determine areas at crucial very high risk. The results as modeled by flood modeler showed that built up area had 34.64% with an area of 152.12km^2, high forest had 30.94% and an area of 135.89km^2, low forest had 23.55% with an area of 103.45km^2 and waterbody had 10.96% with an area of 48.13km^2. see figures 3.4.

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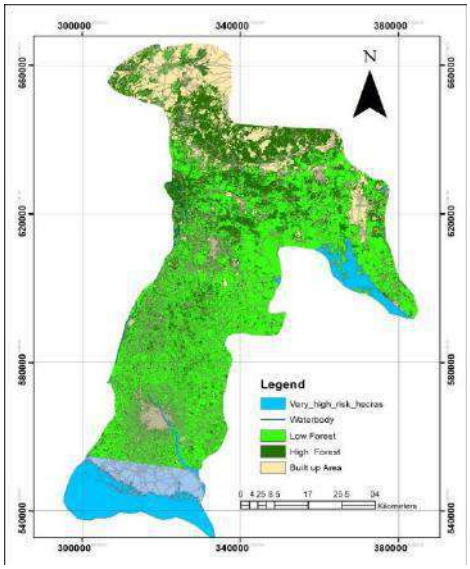

Fig.3.4: Feature at very high risk

3.5 Ground Validation of modeling results.

In order to determine if the results obtained fromthe modeling are reliable for flood modelling in the study area, ground validation was needed to determine the reliability and accuracy of the results.The modeled zones points were compared to flood points obtained from ground. These sample points were coded and compared using correlation coefficient.

The results of the correlation analysis revealed that the modelled result obtained a coefficient of 0.8196 and standard error of 0.48 against the ground flood points. This result indicated that flood modeler is reliable as it has a close fit to the ground flood points, see figure 3.7.

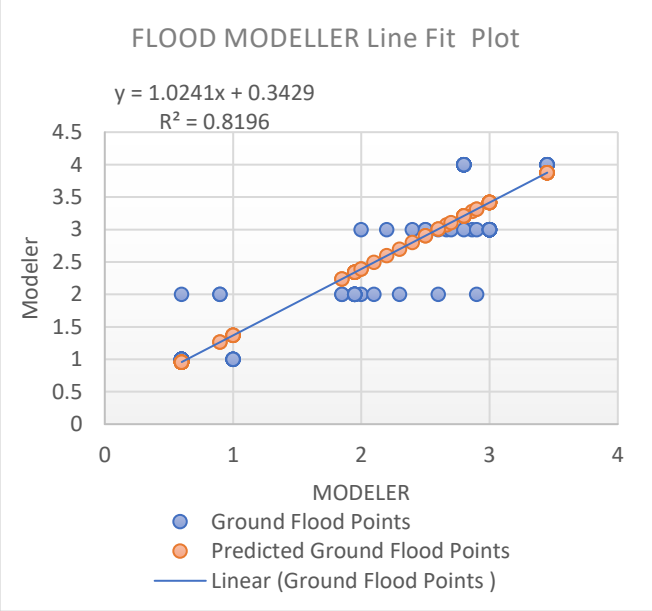

Fig.3.7: Flood modeler line fit plot against ground flood points

IV. CONCLUSION

Flooding is considered as one of the most devastating events in many parts of the world. In terms of its frequency and distribution river flooding remains as a frequent disaster that has to be faced by civilization in flood plain. Over the years now a lot of areas in Abia State have been vulnerable to flooding. Landuse, topography, and heavy rainfalls have been the major causes of flooding in Abia State, thereby resulting in mass causalities after a flooding event.

The study was able to determine the fit and accuracy of ALOS PALAR for flood modelling in Abia State as backed by the vertical and horizontal accuracies of 5.64m and 14.39m respectively.

The study was able to determine the flood frequency return using Flood Modeler, which revealed a 25.04km^2 inundation extent for 2-year return period, 28.10km^2 inundation extent for a 5-year period and 26.04km^2 inundation extent for a 10-year return period. increasing to its peak extent by 3.67% by the 5-year return period, then decreased by 2.24% by the 10-year return period. The study was also able to determine the surface runoff potential in Abia State. It revealed that 35.99% with an area of 1630.19 km^2 had low infiltration potential, 32.51% with an area of 1472.56 km^2 had moderate infiltration while 31.50% with an area of 1426.82 km^2.

The approach used in this study is recommended as it is a robust and efficient tool for flood modelling in Abia State as it represents closely the flood situation on ground, also the study results is recommended to be used as tool for planning and decision making in flood control and management in the study area.

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Growth, Yield and Yield components of Sesame (*Sesamumindicum* L.) as Influenced by Crop Variety and Different Rates of Herbicides in Mubi, Adamawa State, Nigeria.

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Abstract— *The experiment was conducted to evaluate the growth and yield performance of sesame (SesamumindicumL.) using four levels of herbicides (butachlor and diuron) to control weeds. Field trials were conducted in 2011 and 2012 cropping seasons at the Food and Agricultural Organization (FAO)/Tree Crop Programme (TCP) Farm, Adamawa State University Mubi, Adamawa State, Nigeria. The experiment was laid out in a Complete Randomized Block Design with sesame varieties (Kenana and Muva Local) assigned to the main plot and herbicide levels: Butachlor and diuron (0.5, 1.0, 1.5, 2.0 kg a.i.ha^{-1}) assigned to the sub plot treatment which was replicated three times. Growth and yield performance parameters measured were plant height, number of leaves per plant, stem girth, number of capsules per plant and total yield (kg/ha). Result showed that both kenana and muva local were significantly affected by herbicide levels in terms of plant height, number of leaves per plant, stem girth, number of capsules per plant and the total yield.Kenana variety showed superior performance in terms of yield over Muva Local. Application of butachlor at the doses of 0.5, 1.0, 1.5, 2.0 kg a.i.ha^{-1} decreased weeds infestation up to 6WAS compared to the unweeded check and was at par with the hoe – weeded check, but inferior performance to the hoe-weeded check in 2011and combined analysis. Diurons at 1.0-2.0 kg a.i.ha^{-1} suppressed weed infestation up to 9WAS compared to the unweeded and were at par with the hoe-weeded treatment. Among the herbicides butachlor at 1.0 kg a.i.ha^{-1} and diuron 0.5 kg a.i.ha^{-1} produced comparable grain yield to the hoe-weeded treatment in the two seasons and the combined analysis. It is recommended that kenana variety and butachlor at 1.0 kg a.i.ha^{-1} and diuron 0.5 kg a.i.ha^{-1}should be used by farmers for effective weed control and increased yield.*

Keywords— ***Herbicides, variety, growth, sesame, yield***

I. INTRODUCTION

Sesame (*Sesamumindicum*. L.) also known as beniseed is one of the oil seed crops grown in Nigeria. The crop originated in southern parts of Africa andsesame seeds are rich in edible oil (46-52%). After extraction of oil the sesame cake makes very good feed as it is a rich source of protein, carbohydrate and minerals such as calcium and phosphorus (Singh, 2005). The oil is used as cooking oil, anointing oil, manufacturing of perfumed hair oil and medicinal purposes. In Nigeria it is common to find fried sesame seed sold sole or mixed with groundnuts and taken as snacks. The young leaves of the crops are used in soup making in some areas, while the dried stems may be burnt as fuels with the ash used for local soap making (Singh *et. al., 2007*).

In response to the growing export market demand, Nigeria's production of the crop has increased consistently from about 15,000 metric tonnes in 1980 to about100,000 metric tonnes in 2006. Annual exports of sesame from Nigeria have been valued at about US $200 million (USAID, 2010). The country is the main supplier of sesame seed to Japan which is the world's largest importer. Although the country has an estimated 3.5 million hectares of land suitable for the crop, only about 335,000 ha are currently used for sesame production (USAID, 2010). Therefore, there is a vast potential for increased production. In view of the potentials of sesame in contributing significantly to livelihood of rural dwellers and the growth of national economy the Raw Materials Research and Development Council (RMRDC ABUJA), commenced the promotion of the crop through the establishment of 500 ha foundation seed farms in Nasarawa and Benue states in 2006 (USAID, 2010).

Sesame is basically a crop of the warm regions of the tropics and sub-tropics. It grows in plains as well as up to an elevation of 1250 meters. It requires fairly hot conditions during growth to produce maximum yield. A temperature of 25-27^{0}C encourages rapid germination and initial flower formation. Low temperature during flowering can result in the production of sterile pollen or pre - flower drop (Singh, *et. al., 2007*). Sesame can be grown on a wide variety of Soil types ranging from sandy-loam to heavy black soil, with a pH ranging from 5.5 to 8.2 (Singh, 2005).

Weed infestation has been one of the major threats to sesame production. Poor weed management could cause significant reduction in yield. The traditional methods of weeds control, hoe weeding and hand pulling are the commonest methods used by farmers in Nigeria (Imoloami, *et. al,* 2011). This method is not only labor intensive, expensive and strenuous, but can also cause mechanical damage to growing branches and roots of plants. In addition to high cost, labor availability is uncertain thus making timeliness of weeding difficult to attain, which could lead to greater yield losses (Adigun *et al.,* 1993).

Therefore, it is important to employ various weed control practices which is economically viable and does not tamper with plant growth and yield.

II. MATERIALS AND METHODS

Experimental site

The experiment was conducted during 2011 and 2012 cropping seasons at Food and Agriculture Organisation (FAO)/ Tree Crop Programme (TCP), Adamawa State University, Mubi, (latitudes 9^{o}30' to 11 ^{o} 00'N and longitudes 12^{o}00' to 13^{o}45'E), at an altitude of 696 metres above sea. In the area rainfall starts in the month of April and terminates in the month of October with a unimodal peak in the month of August (ADSU Metrological Unit, 2011).

Treatments and Experimental Design

The experiment was laid out in split – plot design with three replications. Kenana and Muva local varieties comprised the main treatment. The sub- treatments consisted of butachlor at four rates (0.5, 1.0, 1.5, 2.0 kg a.i. /ha) and diuron at four rates (0.5, 1.0, 1.5, 2.0 kg a.i. /ha) along with hoe weeded and unweeded checks.

Land preparation

The experimental site was ploughed and harrowed with tractor. Suitable seedbed was then prepared before sowing.

Crop establishment

Sowing was done on July 26 and August 1,in 2011 and 2012 respectively.Seeds were sown on the flat at the spacing of 45cm ×15cm at 5-6 seeds/hill and thinned to 2 plants / hill at 2 Weeks After Sowing(WAS).

Herbicides application

Herbicides were applied using knapsack sprayer (CP15) with red nozzle. After calibration using area – volume method, the herbicides were applied at the rate for each treatment.

Fertilizer application

NPK(15:15:15) and Urea(46%) fertilizers were applied at the rate of 30 kg N, 60 kg P_20_5 and 30 kg K_20 per hectare (Singh,*et.al*.,2007). The whole doses of P_20_5 and K_20 along with half the rate of N was applied at sowing. The remaining half dose of N was applied at 6 weeks after sowing (WAS).

Harvesting

Harvesting was done manually by cutting at the base of the crop using sickle and the harvested plants were assembled on a polythene bag for sun drying after which it was threshed by gentle shaking and winnowed.

Data collection

Data on the following parameters on sesame growth and development were collected by observation and measurement.

Plant height (cm)

Plant height was taken at 4, 8, and 12 WAS. This was done by selecting and tagged three plants randomly from each net plot and taking their height from

ground level to the tip of its highest point and the mean determined.

Number of leaves per plant

Number of leaves per plant was taken at 4,8, and 12 WAS. Leaves were counted from 3 randomly selected and tagged plants per plot and the mean determined.

Stem girth

Stem girth was taken at 6, 9 and 12 WAS from three randomly selected plants using Vernier calipers and the mean determined.

Number of capsules per plant

The number of pods from each net plot was counted at harvest and divided by number of plants in the respective plot.

Grain yield (kg/ha)

Grain yield was determined by weighing the grain from each net plot after threshing and winnowing. The weight was converted to yield per hectare using the formula below:

$$\text{Grain yield} = \frac{\text{(Grain yield/plot (kg) x } 10{,}000\text{m}^2)}{\text{Net plot size } (20\text{m}^2)}$$

Statistical Analysis

The data collected was subjected to statistical analysis of variance (ANOVA) using Statistical Analysis Software (SAS:R-Version,2005) package.

and the treatment means was separated using Duncan Mutiple Range Test (DMRT) at 5 % (0.05) level of probability.

III. RESULTS

Influence of crop variety and herbicide on plant height.

The data on plant height at 4, 8 and 12 WAS are presented in Table 1. At 4 WAS Kenanavariety produced significantly taller plants than Muva in 2011 and the combined analysis; but was at par in 2012. At this stage in the two years and the combined analysis, the unweeded check produced the tallest plants that were only comparable to the hoe- weeded and to butachlor at 0.5 kg a.i. in 2011 only. The hoe- weeded treatment in turn exhibited plants that were appreciably taller than that of the remaining treatments except butachlor at 1.0kg a.i.in 2012, butachlor at 0.5 kg a.i. in both years and combined analysis. Butachlor at 0.5 kg a.i. dose produced plants that were markedly taller than those of butachlor at 2.0 kg a.i. diuron at 1.5 and 2.0 kg a.i rates in the two years and combined analysis. Throughout the study at this stage, diuron at 2.0 kg a.i. showed the shortest plants.

At 8WAS, the two varieties exhibited plants of similar height in the two years and combined analysis. At this growth stage also in the two years and the combined analysis, the hoe- weeded treatment produced the tallest plants which were at par with plants of unweeded- check and diuron at 0.5kg a.i. rate. In 2011 and 2012 plant heights of hoe- weeded treatment was also comparable to those from butachlor at 0.5 and 1.0 kg a.i. rate. It was followed by the unweeded- check, which exhibited appreciably taller plants than butachlor at 2.0 kg a.i.,diuron at 1.5 and 2.0 kg a.i. rates in the both years and the combined analysis. The shortest plants were produced by diuron at 2.0kg a.i./ha in the investigation at this stage.

At 12 WAS the two varieties did not differ significantly in height in the two years and combined analysis. At this growth stage in 2011 and 2012 and the combined analysis, hoe-weeded treatment had the tallest plants. In 2011 plants of the treatment were comparable in height to those of butachlor and diuron at 0.5 kg a.i./ha; and only surpassed plants treated with 2.0 kg a.idiuron rate in 2012. In the combined analysis, plants of the treatment were considerably taller than those of butachlor at 2.0 kg a.i and diuron at 1.5 and 2.0 kg a.i/ha. The 2.0 kg a.idiuron rate exhibited the shortest plants throughout the study. There was no significant interactive effect of variety and herbicide on plant height at 4, 8 and 12WAS.

Table 1: Influence of crop variety and herbicides on plant height of sesame at 4,8,12 WAS grown atMubi, in 2011 and 2012 rainy seasons.

	Plant height (cm)								
	4 WAS			8 WAS			12 WAS		
Treatment	2011	2012	Combined	2011	2012	Combined	2011	2012	Combined

Variety									
Kenana	16.88a	19.48	18.18a	82.07	93.81	87.94	93.65	166.16	127.47
Muva	12.04b	16.04	14.04b	72.82	85.51	79.17	88.78	147.58	120.61
SE ±	0.78	0.66	0.46	8.29	5.02	4.09	11.55	5.81	5.33
Level of signif.	*	Ns	*	Ns	Ns	Ns	Ns	Ns	Ns
Herbicide									
Butachlor (kg a.i.ha^{-1})									
0.5	16.98ab	23.41bc	20.20bc	87.30ab	104.79abc	96.04b	103.62ab	163.70a	133.66ab
1.0	15.28bc	20.26bcd	17.77cd	82.87ab	102.26abc	92.56b	96.12b	166.97a	131.55ab
1.5	14.72bc	17.64de	16.18de	82.03abc	96.97bcd	89.50bc	95.55b	163.52a	129.53ab
2.0	10.95de	15.78def	13.36ef	64.66c	90.36cd	77.51d	77.47c	163.97a	120.72b
Diuron (kg a.i.ha^{-1})									
0.5	14.96bc	19.82cd	17.39cd	92.53a	103.47abc	97.99ab	107.13ab	150.53a	128.83ab
1.0	15.10bc	12.63ef	13.86 ef	80.54b	75.89de	78.21cd	96.80b	165.77a	131.28ab
1.5	12.53dc	12.20f	12.37f	64.32c	59.44ef	61.88e	76.55c	152.74a	114.64b
2.0	8.48e	5.81g	7.15g	47.99d	43.06f	45.52f	60.57d	103.94b	82.25c
Hoe-Weeded	19.07a	25.22ab	22.14ab	94.80a	123.68a	109.23a	113.07a	183.37a	148.22a
UnweededCheck	19.23a	28.86a	24.05a	86.87ab	114.39ab	100.63ab	96.60b	165.87a	131.23ab
SE ±	1.05	1.64	1.03	3.83	6.65	3.83	4.47	12.02	11.93
Level of sign	*	*	*	*	*	*	*	*	*
Int. var x trt.	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns

Mean values followed by the same letter(s) in each treatment group are not significantly different at P=0.05 (DMRT)

WAS= Weeks after Sowing

*=Significantly different at 5% level of probability.

Ns= Not significant at 5% level of probability

Influence of crop variety and herbicide on number of leaves per plant.

Numbers of leaves per plant at 4, 8 and 12 weeks after sowing are shown in Table 2. In both years and the combined data, the two varieties recorded similar number of leaves per plant at 4 and 8 WAS. However at 12 WAS Muva local exhibited higher number of leaves per plant than Kenana variety.

At 4WAS in the two years and the combined analysis, the hoe- weeded treatment produced the highest number of leaves per plant, which was comparable to those of unweeded- check, butachlor and diuron at 0.5kg a .i .rates. Also it was at par with butachlor at 1.0 kg a.i. rate in the two years and diuron at 1.0 kg a.i.in 2011 only. Increasing butachlor rate to 2.0 kg a.i. only reduced number of leaves per plant below that of the lower doses of butachlor and diuron in 2011; while diuron at 2.0 kg a.i.

reduced the number of leaves significantly below all other treatments throughout the study at this stage.

At 8WASand in 2011 and the combined analysis, all rates of butachlor, the hoe-weeded treatment, the unweeded check,0.5 and 1.0 kg a.i. diuron rates exhibited comparable number of leaves. Application of 2.0 kg a.i. diuron produced the lowest number of leaves per plant at this stage but was comparable only to diuron at 1.5 kg a.i. in 2012 and the combined data. Neither herbicide treatment nor hoe- weeding had any significant effect on number of leaves per plant in 2012.

At 12WAS in the two years and the combined data, application of 1.0 kg a.i. diuron produced the highest number of per plant but comparable with diuron at 0.5 kg a.i. rate. It was also at par with butachlor at 0.5, 1.0, 2.0 kg a.i.anddiuron 1.5 kg a.i. dose in 2011. Similarly it was comparable to butachlor at 1.0, 1.5 kg a.i. and diuron at 1.0 kg a.i. rate in 2012. Varying rates of butachlor had no significant effect on number of leaves per plant at this stage in the study. In the two years and the combined data at this stage the unweeded check exhibited the lowest number of leaves per plant but at par with hoe –weeded and diuron at 2.0 kg a.i. rates.

Table 2: Influence of crop variety and herbicides on number of leaves per plant of sesame at 4, 8, 12 WAS in 2011 and 2012 cropping seasons.

	Number of leaves per plant								
Treatment	4 WAS			8 WAS			12 WAS		
	2011	2012	Combined	2011	2012	Combined	2011	2012	Combined
Variety									
Kenana	7.03	14.34	10.68	38.86	38.00	35.69	79.10b	91.66b	85.38b
Muva	6.07	14.46	10.28	33.38	45.25	42.05	118.18a	139.50a	128.84a
SE ±	0.35	0.13	0.47	7.78	5.49	4.30	6.06	4.75	3.16
Level of sign.	Ns	Ns	Ns	Ns	Ns	Ns	*	*	*
Herbicide									
Butachlor									
(kg a.i ha^{-1})									
0.5	7.33ab	16.68ab	12.00ab	43.00a	45.68	44.33a	101.17abc	113.00bc	107..08bc
1.0	7.0 0ab	15.33abc	11.17bc	38.17ab	45.68	41.92a	109.17abc	123.33ab	116.25bc
1.5	6.67bc	15.00bc	10.83bc	38.33ab	42.18	40.25a	86.67bcd	120.67abc	103.67bc
2.0	5.00d	15.68abc	10.33bc	36.17ab	44.00	40.08a	99.33abcd	115.00bc	107.17bc
Diuron (kg a.iha^{-1})									
0.5	7.00ab	16.50ab	11.75abc	43.00a	45.33	44.17a	118.83ab	135.67ab	127.25ab
1.0	7.33ab	13.00cd	10.17c	35.68ab	42.67	39.17ab	135.17a	157.00a	146.08a
1.5	6.00c	10.83d	8.42d	29.33b	32.68	31.00bc	107.33abc	123.67ab	115.25bc
2.0	4.00e	6.50e	5.25e	19.00c	26.00	22.50c	69.83cd	83.33cd	76.58de
Hoe-Weeded	8.00a	18.68a	13.33a	38.33ab	48.33	43.33a	90.67bcd	100.00bcd	95.33cd
Unweeded Check	8.00a	18.00ab	13.00a	40.83a	46.50	43.67a	61.00d	72.33d	66.67e
SE ±	0.30	0.99	0.52	3.24	4.74	3.46	11.73	11.60	8.25

Level of sign	*	*	*	*	Ns	*	*	*	*
Int. var x trt	Ns	Ns	NsNsNs	Ns	NsNs*				

Mean values followed by the same letter(s) in each treatment group are not significantly different at P=0.05 (DMRT)

WAS= Weeks after Sowing

*= Significantly different at 5% level of probability.

Ns= Not significant at 5% level of probability

Influence of crop variety and herbicides onstem girth.

Data on stem girth at 6, 9 and 12 WAS are shown in Table 3. At 6 WAS, in the two years and the combined analysis, the two varieties exhibited stems of similar girth. It was only in 2012 that herbicides affected stem girth significantly at 6 WAS. In 2012, butachlor at 0.5 kg a.i. produced stems with the thickest girth which were only considerably thicker than those of diuron at 1.0, 1.5 and 2.0 kg a.i./ ha and unweeded check. Diuron at 2.0 kg a.i dose exhibited stems with thinnest girth at his stage.

At 9 WAS, in the two years and the combined data Muva local produced stems with significantly thicker girth than Kenana variety. At this stage herbicide showed significant effect on stem girth only in 2012 and combined analysis, whereby only unweeded check recorded significantly thinner stem girth than any of the other treatments which were all invariably at par.

At 12WAS, varieties only differed in plant height in the combined analysis with Muva local producing stems of thicker diameter than Kenana. At this stage herbicide affected stem girth significantly. In 2011and combined analysis, whereby in both cases, the hoe- weeded treatment produced stems with the thickest girth. However in 2011 the hoe-weeded treatment produced stems that were only significantly thicker than unweeded check and diuron at 2.0 kg a.i/ha. However, in the combined analysis, the hoe-weeded treatment exhibited stems with similar stem girth only with butachlor at 1.0 kg a.i./ha, diuron at 0.5 a.i./ha and 1.0 kg a.i./ha. Application of iuron at 2.0 kg a.i. produced stems with the thinnest diameter in 2011 and combined analysis.

There was no interaction between variety and herbicide treatments at 6 and 12 WAS, while at 9 WAS in 2012 and combined analysis showed significant interaction was observed between variety and herbicide.

Table 3: Influence of crop variety and herbicides on stem girth of sesame at 6, 9 and 12 WAS in 2011 and 2012 cropping seasons.

	Stem Girth (mm)								
	6 WAS			9 WAS			12 WAS		
Treatment	2011	2012	Combined	2011	2012	Combined	2011	2012	Combined
Variety									
Kenana	10.19	7.90	9.04	9.77b	10.43b	10.10b	19.99	20.84	20.41b
Muva	8.04	8.67	8.35	11.77a	12.51a	12.14a	21.08	22.48	21.78a
SE ±	2.12	0.39	1.06	0.15	0.07	0.13	0.29	0.54	0.26
Level.ofsigf.	Ns	Ns	Ns	*	*	*	Ns	Ns	*
Herbicide									
Butachlor (kg a.i.ha^{-1})									
0.5	22.64	10.50a	16.57	10.58	11.64a	11.11a	20.30abc	21.75	21.03c
1.0	8.36	9.04abc	8.70	11.30	11.96a	11.63a	21.65ab	22.75	22.20ab

1.5	8.21	8.75abcd	8.48	10.89	11.55a	11.23a	20.23abc	21.00	20.62bcd
2.0	8.10	8.67abcd	8.38	10.98	11.83a	11.41a	20.65abc	21.68	21.16bcd
Diuron(kg a.i.ha^{-1})									
0.5	8.93	9.56ab	9.24	11.30	11.83a	11.57a	21.45ab	22.92	22.18abc
1.0	6.98	7.33cd	7.16	11.39	11.92a	11.65a	21.47ab	22.18	21.82ab
1.5	6.78	6.71de	6.75	10.51	11.09a	11.80a	20.18abc	20.33	20.26dc
2.0	4.67	5.30e	4.99	10.62	11.50a	11..06a	18.55c	20.25	19.40d
Hoe- Weeded	9.87	10.28a	10.07	10.70	11.35a	11.03a	22.17a	24.33	23.25a
Unweeded Check	7.14	7.62bcd	7.38	9.21	9.79b	9.50b	19.30bc	20.68	19.98d
SE±	4.42	0.61	2.23	0.51	0.43	0.33	0.70	0.80	0.53
Level of Sign	Ns	*	Ns	Ns	*	*	*	Ns	*
Int. var x trt.	Ns	Ns	Ns	Ns	*	*	Ns	Ns	Ns

Mean values followed by the same letter(s) in each treatment group are not significantly different at P=0.05 (DMRT)

WAS= Weeks after Sowing

*= Significantly different at 5% level of probability

Ns= Not significant at 5% level of probability

Influence of crop variety and herbicide on number of capsulesper plant.

Influence of crop variety and herbicide on number of capsules per plant in 2011 and 2012 cropping seasons are presented in Table 4. In the two years and the combined analysis Kenana variety produced appreciably higher number of pods than the Muva variety. In 2011 and 2012, the hoe-weeded treatment and all herbicide treated plots produced similar number of pods per plant that were appreciably higher than that of the unweeded check, except that it was at par with the 2.0kg a.i. rate of diuron in 2011. In the combined analysis the hoe-weeded treatment and all rates of Butachlor, 0.5 and 1.0 kg a.i. rates of diuron exhibited comparable number of pods per plant which were significantly higher than that of unweeded treatment. Also diuron at the rates of 1.5 and 2.0 kg a.i.ha^{-1} recorded similar number of pods per plant that were remarkably higher than that of unweeded check. It was only in the combined analysis that there was significant interaction between variety and herbicide.

Table 4.Effect of variety and herbicides on number of capsules per plant in 2011 and 2012 cropping seasons.

		Number of capsules /plant	
Treatment	2011	2012	Combined
Variety			
Kenana	90.80a	103.11a	96.96a
Muva	61.36b	74.40b	67.88b
SE ±	4.61	1.58	2.09
Level of signf.	*	*	*
Herbicides			
Butachlor**(**kga.i.ha^{-1})			

0.5	100.67a	117.17a	105.92ab
1.0	84.33a	95.17a	89.75abc
1.5	72.33a	83.33a	77.83abc
2.0	70.17a	84.67a	77.83abc
Diuron(kga.i.ha^{-1})			
0.5	93.67a	121.33a	106.42a
1.0	69.67a	79.83a	74.75abc
1.5	66.00a	77.67a	71.83bc
2.0	57.83ab	70.00a	63.92c
Hoe- Weeded	102.00a	110.83a	106.42a
Unweeded Check	17.17b	23.10b	20.13d
SE ±	14.08	15.23	10.37
Level of sign	*	*	*
Int. var x trt.	Ns	Ns	*

Mean values followed by the same letter(s) in each treatment group are not significantly different at P=0.05 (DMRT)

WAS= Weeks after Sowing

*= Significantly different at 5% level of probability

Ns= Not significant at 5% level of probability

Influence of crop variety and herbicide on grain yield.

The data on sesame grain yield is presented in the Table 5. The two varieties did not differ in grain yield in 2011 and 2012; but in the combined analysis Kenana variety gave significantly higher yield than Muva variety. In the two years and combined analysis, the hoe - weeded treatment out yielded all the herbicide treatments and the unweeded check. This was followed by Diuron at 0.5 kg a.i. ha^{-1} which produced comparable grain yield to butachlor at 1.5kg rate in the two years and the combined analysis.

However it out - yielded the remaining treatments in 2011 and the combined analysis and butachlor at 1.0 kg rate in 2011, diuron at 2.0 kg rate and unweeded check in 2012 only. All the other treatments exhibited comparable grain yield to the unweeded check in both years and the combined analysis, while diuron at the rate of 2.0 kg a.i. ha^{-1} gave the least grain yield throughout the period of the trial. There was significant interaction between variety and herbicide treatments in the two years and combined analysis.

Table 5: Effect of variety and herbicide on the grain yieldin 2011 and 2012 cropping seasons.

	Yield (kg/ha)		
Treatment	2011	2012	Combined
Variety			
Kenana	333.14	256.53	294.84a
Muva	176.24	107.77	142.00b
SE ±	51.76	51.81	36.62
Level of signif.	Ns	Ns	*
Herbicide			
Butachlor (kg a.i./ha)			

0.5	216.39cd	166.74bcd	191.56cd
1.0	192.20cd	61.01cd	126.60cde
1.5	303.62bc	163.91bcd	233.76bc
2.0	192.31cd	135.79bcd	164.05cd
Diuron (kg a.i./ha)			
0.5	430.08b	264.18b	347.13b
1.0	190.47cd	190.75bc	190.61cd
1.5	175.92cd	147.67bcd	161.79cd
2.0	39.74d	33.29d	36.51e
Hoe-Weeded	717.43a	548.59a	630.51a
Unweeded Check	88.72d	114.58cd	101.65de
SE ±	59.91	41.90	36.56
Level of sign	*	*	*
Int.var ×trt.	*	*	*

Mean values followed by the same letter(s) in each treatment group are not significantly different at P=0.05 (DMRT)

WAS= Weeks after Sowing

*= Significantly different at 5% level of probability

Ns= Not significant at 5% level of probability

IV. DISCUSSION

Kenana variety exhibited superior performance over Muva local in number of pods per plant. This could be attributed to the better genetic composition of the improved variety (Kenana) which is probably more efficient in utilizing growth factors than the Muva local variety. This agrees with the findings of Azeez*et. al*., (2003) who noted that improved varieties of crops exhibit superior performance of growth and yield characters over local varieties.

Plant height of sesame was significantly affected by herbicide treatment at 4, 8 and 12 WAS. Generally, plant height decreased with increasing rate in both herbicides. This could be due to the phytotoxic effect of the herbicides at higher rates, which might have caused a depressive effect on crop growth. At higher dose of butachlor and diuron there was suppressed crop growth resulting in significantly short plants. The height of plants under 0.5-1.5 kg a.i. dose of butachlor and 0.5-1.0 kg a.i. diuron were comparable to that of hoe - weeded treatment at 12 WAS. This showed that sesame plants are able to overcome the phytotoxicity effect at these rates possibly as the herbicide became degraded or leached out as observed by Imoloame*et al., (*2011).

The number of leaves per plant varied with herbicide treatments at 4 WAS. This could be as a result of the phytotoxic effect of the herbicide on the crop growth. The depressive effect of the herbicides at higher rates resulted in lower number of leaves. However at 8 WAS, with the exception of diuron at 1.5and 2.0 kg a.i. a rate in the combined analysis, the numbers of leaves from herbicide treatments were comparable to that of hoe-weeded treatment. The overcoming of the depressive effect of the herbicides agrees with the findings of Ishaya*et. al.*, (2009).

The mean performance at various growth stages showed that all herbicide treatments and hoe- weeded treatment suppressed weed infestation compared to unweeded-check up to 9 WAS. This can be attributed to efficient residual effect of these herbicides on weed control as noted by (Zimdahl and Gwynm, 1977; Rao, 2000). The ability of diuron to provide season-long effective weed control, could be due to the persistence of this herbicide in the soil (Akobundu , 1987; Beyer,1988; Imoloame, 2004).

Butachlor at 2.0 kg a.i. dose and diuron at 1.0-2.0 kg a.i / ha reduced the weed dry weight significantly in the combined analysis compared to the unweeded and gave comparable weed dry weight to the hoe- weeded check at 6 WAS. The effectiveness of these rates of butachlor and diuron in weed suppression up to 6 WAS

indicates that , these herbicides at these rates can be used as an alternative weed control weed control methods to replace to hoe -weedings at 3 and 6 WAS. This agrees with findings of Ibrahim *et al*., (2009) that application of diuron at 0.960 kg a.i./ha gave excellent broad- leaved weed control in sesame in Egypt. It also agrees with Gritcher*et al.*,(2001) who reported that butachlor among all the herbicides evaluated, provided best control of weeds and least sesame injury.

The combined analysis has shown that all rates of butachlor (0.5- 2.0 kg a.i.) and 0.5- 1.0 kg a.i. rates of diuron produced appreciably higher number of pods/plant than unweeded check but comparable to hoe-weeded treatments. Since these treatments had less weeds infestation, they had less competition from weeds for growth factors such as nutrients and water. Therefore they possibly had more available assimilates for production of higher number of pods.

Throughout the two years and the combined data, it was only butachlor at 1.0 kg a.i. that produced comparable yield to the hoe-weeded treatment which gave the highest yield. This shows that butachlor at 1.0 kg a.i./ha is a promising herbicide for use in sesame. From this research, it can be recommended that butachlor at 1.0 kg a.i./ha and diuron 0.5 kg a.i./ha are possible rates for use in sesame production as alternative to first two hoe weeding for effective weed control and high yield in sesame in Mubi, Northern Guinea savanna of Nigeria.

V. SUMMARY

The experiment was conducted during 2011 and 2012 rainy seasons, to evaluate the effect of herbicides for weed control in sesame (*Sesamumindicum* L), at Food and Agriculture Organisation (FAO)/ Tree Crop Programme (TCP), Adamawa State University, Mubi, latitudes 9º30', to 11 º 00'N and longitudes 12º00' to 13º45'E) in the northern Guinea savanna ecological zone, Nigeria.

The experiment was laid out in a split-plot design replicated three times. Two varieties of sesame (Kenana and Muva Local) were allocated to the main plots, while butachlor and diuron at each at four rates (0.5,1.0,1.5, and 2.0 kg a.i. ha^{-1}), a hoe-weeded and un-weeded checks were assigned to the sub-plots.

Kenana variety showed superior performance compared to Muva local variety in number of pods per plant, but higher fresh weed infestation in the combined analysis at 6 and 9WAS.Muva local exhibited significantly higher number of leaves per plant in both seasons and combined analysis at 12WAS and gave appreciably higher straw yield in the combined analysis.

Generally plant height decreased with increasing rates in both herbicides up to 8 WAS. The combined analysis at various growth stages showed that all herbicide treatments and hoe-weeded check suppressed weed infestation up to 9 WAS.

Throughout the two years and the combined data, it was only butachlor at 1.0 kg a.i/ha that produced comparable yield to the hoe- weeded treatment which gave the highest yield. This shows that butachlor at 1.0 kg a.i/ha is a promising herbicide for use in sesame.

VI. CONCLUSION

This research revealed that butachlor at 1.0 kg a.i./ha^{-1} rate and can be used as alternative to hoe weeding for effective weed control and higher yield of sesame.

VII. RECOMMENDATION

Potentials exist for the production of sesame using herbicides as an alternative to hoe weeding. The information thus generated in this study should further be studied for sesame production by using other herbicide rates.

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The Value Connotation of Evidence Evaluation on Forensic Conclusions

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Abstract—*Forensic appraisal is inherently uncertain, and the evidence used to determine the facts of a case must be certain. This creates a tension between the uncertainty contained in forensic appraisal itself and the certainty as evidence, and makes technical authority and legal authority confused in confirming the facts of the case. The forensic conclusions can only be used as evidence after it has been evaluated .Evidence evaluation on forensic conclusions should adhere to the goals of science and truth, and comply with the value of justice and efficiency, to ensure the scientific nature of forensic conclusions, and realize judicial justice.*

Keywords— ***forensic conclusions, evidence evaluation, value, science, justice.***

I. INTRODUCTION

With the continuous improvement of the level of rule of law, the way the public resolve disputes through litigation in accordance with the law is more and more popular. The main basis for the court to confirm the facts of the case and resolve disputes is evidence. Forensic conclusions, a form of evidence that introduces science into litigation procedures to resolve disputes, make judgments which have no definite and unified standards can be tested and repeatedly practiced by using natural scientific methods [1]. This makes the role of forensic conclusions in litigation more and more critical. In judicial practice, unjust, false, and wrong cases caused by forensic conclusions without evidential ability have occurred frequently. The applicability of forensic conclusions in litigation is increasingly being questioned. This will not only affect judicial justice and the maintenance of the legitimate rights and interests of the masses, but also seriously damage judicial authority and is not conducive to promoting social harmony and stability. Accordingly, in order to ensure that the forensic conclusions entered into the litigation process have evidence capability and probative force , the identification of specialized issues in the case is based on objective and reliable scientific evidence to ensure that the people can feel fairness and justice in every judicial case. It is necessary to conduct a scientific, objective, and fair evidence evaluation of the forensic conclusions, to ensure the correctness of the forensic conclusions and prevent pseudo-science from entering the litigation.

II. THE VALUE GOAL OF SCIENCE AND TRUTH

The purpose of establishing the forensic appraisal system is to improve the judge's ability to ascertain the facts of the case, and to make up for the lack of professional knowledge through the activities of the appraiser, so as to obtain a more objective understanding of the facts of the case and make

judgments on the basis of this. The forensic conclusion is inferred by the appraiser based on scientific principles and technical methods. Whether the scientific principles and methods of appraisal activities are scientific, this will affect the reliability and credibility of the forensic conclusion from the source.

From the point of view of the legality of evidence, the forensic conclusions as evidence should meet the requirements of legality. Judges' understanding and review of forensic conclusions are primarily to examine the legality of the forensic conclusions, that is, to verify whether the production and formation of the forensic conclusions comply with legal procedures. If the forensic conclusion is not legal, the judge will deny the evidence qualification of the forensic conclusion and no longer consider reviewing its scientific. Legitimacy is the prerequisite for the development of forensic appraisal activities, and science is the basis of forensic appraisal. The forensic appraisal system must be able to guarantee its scientific of realization [2].Therefore forensic conclusions can be used as evidence to be legal and scientific.

Forensic appraisal usually has three possible results: one is a definitive conclusion; the other is an uncertain conclusion or multiple possible conclusions; and the third is the inability to draw a conclusive conclusion [3]. In litigation, all materials that can be used to prove the facts of the case, but the law requires that the proof materials should have legality, relevance, authenticity and certainty, and "ambiguity", "indeterminate" or "paradoxical" proof materials should not be used as evidence, and forensic conclusions is no exception. For the "deterministic conclusion" of forensic appraisal, because it meets the evidence standard stipulated by law, the referee can directly choose it as the evidence, and the "unable to draw a conclusive conclusion" does not include the scope of evidence. The "uncertainty conclusions" of forensic appraisal are manifested as tendencies. From the outside, they do not meet the evidence standards stipulated by the law. There is considerable controversy about using them as the basis for verdicts, but it also has a certain degree of scientific.

Science and technology have errors, and the results are not absolutely accurate. In the practice of forensic appraisal, although the appraiser has issued a "certainty" conclusion, it is only a formal "certainty", not "absolute certainty" still has probabilistic conclusions, which is determined by the nature of science itself. The "uncertainty" of forensic conclusion comes from science itself, is a scientific understanding that tends towards correctness with a high probability.

Whether the "uncertainty conclusion" of forensic appraisal can be used as evidence not only depends on the scientific and legitimacy of the forensic appraisal itself, but also affected by the scientific degree of the evidence standard setting which related to the proof of objective facts. The objective reality is the fact that things originally exist. Since the facts of the case dispute itself are not permanent and existing, but are lost due to the passage of time and never return, the objective facts cannot be reproduced truthfully, even if the audio and video are played again, they are not the original truth. If "objective truth" is used as the proof standard in litigation, it appears that the proof requirements are too harsh, and it is even suspected of violating the laws of nature. In litigation, the referee cannot reproduce the "objective facts" as they are in the court, but they can restore the authenticity of the facts of the case through investigation and collection of evidence, which conforms to the requirements of modern science and reaches the level of scientific, reasonable and reliable. Therefore, the "standard of evidence" stipulated by the law needs to be based on the requirements of the essence of science, reflect the nature of science, and conform to the spirit of science.

III. THE PRACTICAL VALUE OF JUSTICE AND EFFICIENCY

Facing a competitive environment that tends to be market-oriented, the development of appraisal agencies presents a situation of uneven levels. At the same time, because forensic appraisal is different from ordinary scientific inquiry activities, the purpose of forensic appraisal activities is clearer, the restrictions on experimental materials are stricter, and the appraisal process is more susceptible to human factors. These characteristics also lead to the possibility of errors in forensic conclusions. Wrong forensic conclusion is a

long-standing and inconclusive problem in forensic appraisal. Forensic appraisal is reproducible. In litigation activities, flaws or faults in the forensic conclusions occur from time to time. Different appraisal institutions and appraisers use the same method for appraisal under the same conditions at different times and places, and they will get the same or extremely similar conclusions, means that the conclusions of the forensic appraisal are accurate. It is correct, reflecting the scientific and reliability of forensic appraisal. So re-appraisal is a frequently used evaluation method to correct defects or errors in the original forensic appraisal. However, all technologies, all plans, all practices and choices should aim for a certain kind of good [4]. Therefore, re-appraisal should avoid abuse. Unnecessary or unreasonable re-appraisal will have negative effects such as chaos in the appraisal order, reduced litigation efficiency, and increased litigation costs.

In practice, there is indeed a phenomenon in which judges rely on forensic conclusion to cause judgment errors. Some scholars have proposed that "authentication is the process of the judge's heart testimony". The effective conduct of court cross-examination relies on judges' command and review functions. This is also the basic requirement for protecting the legitimate rights and interests of the litigants and achieving procedural justice. In order to improve the error correction and error prevention mechanism so that the wrong forensic conclusions can be corrected in time, it is necessary to play the role of evidence evaluation in restricting judge's discretional evaluation of evidence. To a certain extent, it shows the attitude of correcting the defects of forensic conclusions in litigation, and reflects the open, fair and just principles of forensic appraisal.

In the whole process of the formation of evidence from evidence to fact, there are two links: one is the determination of evidence, and the other is the process of proof from evidence to fact. Control of the subjective assumptions or abuse of liberty in these two links usually guarantees that the results of the forensic conclusions can be relied upon out of reason of legitimacy and reasonableness. Control is carried out mainly through four aspects: the law by setting the capacity for evidence to ensure the basic security of the evidence database; the evidentiary power of the evidence is pre-defined; the basis of the judge's testimony can only be the law of experience, not subjective speculation, speculation or inference, the law of experience has the commonality in the common sense, easy to judge, but also the objective performance of the evidence; It is required to adopt a comprehensive judgment method for the final determination of the magnitude of evidence force, and must be combined with the whole case rather than determine separately. In this case, the judge's discretional evaluation of evidence of the forensic conclusions should be reasonable and scientific.

IV. THE INSTITUTIONAL SIGNIFICANCE OF RATIONALITY AND NORMS

The ordinary way of cross-examination by judges to review and cross-examine the forensic conclusions cannot achieve a scientific and objective evaluation of its proving power. In addition, the attendance rate of appraisers is low, which puts the forensic conclusions in an embarrassing situation. Forensic conclusions not only failed to become an effective means for judges to ascertain facts, but instead became a fuse that exacerbated the conflicts between the litigants. From a normative point of view, since the forensic appraisal standards and other related systems are formulated by functional departments, and there is a phenomenon of cross-management by multiple departments, the forensic appraisal standards, technical specifications, and procedures cannot be unified, and the quality of the forensic conclusion is questioned. This is also one of the important reasons why people entrust different appraisal agencies to repeatedly appraise the same appraisal item. In the final analysis, they are all due to the lack of a unified forensic conclusion evaluation system [5]. The evaluation effect of the forensic conclusion has been criticized, and it cannot be able to improve and maintain its credibility inevitably. In order to ensure that judges make a fair judgment on the forensic conclusions, the establishment of a unified forensic conclusion evaluation system should be put on the agenda as soon as possible.

The evaluation of forensic conclusions is mainly reflected in the system of appraisers' appearance in court and the cross-examination process [6]. The system of appraisers appearing in court can fully guarantee the realization of the cross-examination process. Regarding the

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forensic conclusions, the appraiser can make detailed explanations when appearing in court, and accept the cross-examination of both litigants and the court questioning. This process is the basis for the review and judgment of the forensic conclusion and the evaluation of the evidence. The appearance of the appraiser in court to testify is of great significance both in terms of procedure and substance.

The forensic conclusion is verbal evidence, and its own scientific nature needs to rely on the appraiser to appear in court to explain on the spot, and through cross-examination to improve the objective and just of the forensic conclusion. At the same time, the forensic conclusion is only the judgmental opinion of the appraiser on specialized issues. The court exercises jurisdiction and has the responsibility to ascertain the facts of the case, while the appraiser answers the judge's questions, which can improve the accuracy of the judge's trial and help the judge accurately determine the facts. According to the basic requirements of procedural justice, the appraiser needs to testify in court. The presence of an appraiser in court to testify allows the judge to fully contact and comprehensive review the forensic conclusion, and finally make a correct judgment, thereby guaranteeing the litigation rights of litigants. The essential feature of court cross-examination lies in cross-examination, and perfecting the system of appraiser appearance in court is conducive to improving the legitimacy of the cross-examination procedure. The essential feature of court cross-examination lies in examination. Improving the system of appraisers' appearance in court will help improve the legitimacy of the cross-examination procedure [7]. Therefore, by improving the system of appraisers' appearance in court, giving full play to the commanding role of judges in court trials, ensuring the smooth progress of court cross-examination, so as to conduct objective evidence evaluation of forensic appraisal.

V. CONCLUSION

The forensic conclusions must be cross-examined and evaluated in order to be used as evidence in litigation. Scientific is the essential attribute of forensic, not only the basis of forensic conclusions as evidence, but also the prerequisite for the litigation to use the forensic conclusions for assisting the judicial determination of the facts of specific issues. Evidence evaluation of forensic conclusions, on the one hand, excludes wrong forensic conclusions from litigation, on the other hand, it forces appraisal institutions and appraisers to improve their appraisal level, thus forming a virtuous circle and improving the authority and credibility of forensic appraisal. Based on this, the evaluation of the evidence of the forensic conclusions should reflect the value goals of science and truth, the practical value of justice and efficiency, and the rational and standardized institutional significance, ensure the objectivity and science of the forensic, and ensure the scientific nature of the forensic conclusion as the basis for the verdict, so that the confirmed case facts are reasonably acceptable, so as to achieve the goal of true discovery and judicial justice.

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Supply Chain of Fish Performance and Analysis Fisherman Share in Paotere Landing Fish, Makassar City

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Abstract— *Fishing port is a center for marketing of catches, where the role and facilities are very important in the economy of a port, starting from landing to marketing the catch. Several studies can be carried out to overcome this problem, one of which is the supply pattern which can be described in study of the supply chain at the Paotere Fish Landing Base which is located in the middle of Makassar City. The purpose of this study is to explain how the supply chain system, supply chain flow with the problems and chain models that exist in PPI Paotere. The author also relates using the Fiherman Share analysis. The method used in this research is purposive sampling with descriptive analysis approach. The results showed that business actors and the supply chain system at the Paotere Fish Landing Base consisted of 2 supply chain systems consisting of 1 channel, namely, Fishermen-Collectors-Retailers-Consumers, the highest occurred in flow chain 1 by 68%, - where The percentage is greater than flow 2, namely the final 62%, while for channel 2 it consists of fishermen-collectors-exporters (UPI)-final consumers. fisherman's share*

Keywords— ***Supply Chain, Marketing, Fishing Port, Paotere, Fisherman Share.***

I. INTRODUCTION

The marine and fisheries sector is one of the important sources of economic growth due to its large supply capacity and increasing demand. The high demand mainly comes from developing countries with an increasing population (Choir, 2018). About 70 percent of the fish demand for world consumption is supplied by developing countries.

South Sulawesi capture fisheries production in 2017 amounted to 362,038 tons. This shows that marine fishery commodities in South Sulawesi can be used as a superior commodity with an important economic value. Judging from the length of the coastline covering an area of 2,500 km with an area of 62,482.54 km2, part of the area is directly adjacent to 3 (three) coastal areas, namely the southern coast of the Flores Sea with marine fishery potential of 168,780 tons / year. the eastern part is Bone Bay with 144,320 tons / year, and the western part of the Makassar Strait with 307,300 tons / year (Central Statistics Agency, 2020)

A fishing port is a center for marketing of catches, where the role and facilities are very important in the economy of a port, from landing to the marketing of catches (Lubis, 2016). The function of fishing ports in marketing the catch includes the availability of facilities, services and the availability of information systems . These three indicators are very important in a fishing port. Fishery commodities fluctuate according to the amount of production and prices. Production fluctuations are caused by catches that depend on the season, while price fluctuations are caused by shifts in demand and supply of fresh fish. Several studies can be carried out to overcome this problem, one of which is the supply pattern which can be described in a study of the supply chain at the Paotere Fish Landing Base (PPI) which is located in the middle of the city of Makassar (Danial, 2018).

One of the PPIs in South Sulawesi, which is located in Makassar City, is PPI Paotere, which is a type D landing point for fish, where capture fisheries in Paotere are a parameter to improve the economy in the South Sulawesi region.

The Paotere Fish Landing Base (PPI) is one of the Regional Technical Implementing Units (UPTD) which is directly responsible for the Makassar City Office of Marine, Fisheries, Agriculture and Livestock. PPI Paotere functions as a place for boats / fishing boats to be anchored or moored. In addition, it functions for marketing and processing of fishery products as well as a place for fostering fishing communities. PPI Paotere managers collaborate with the Fisheries Insan Cooperative in order to provide convenience and benefits for fishermen's businesses in utilizing existing facilities at PPI. This collaboration can support the income of the Makassar City fishery community and increase local revenue (PAD) (Arbi, 2016)

The function of PPI Paotere it is not only as a place to land, marine fish, but fish originating from inland fisheries (ponds) are also landed and auctioned off at PPI Paotere . The fish caught are then distributed to the final consumer in a number of ways. According toto (Indrajit, 2010) in Anatan and Ellitan (2015), supply chain is a new concept in implementing an integrated and structured logistics system, which is the process of supplying raw materials to the final buyers.The fish supply chain process needs to be distributed quickly and use cold chain.Fish has a fast-rotting nature, so poor handling and supply chain processes will reduce the quality of the fish. Apart from being a fish distributor, the supply chain system also acts as a price differentiator in each fishery chain. The supply chain system also functions to determine traceability.

Currently, PPI Paotere is the center where fishermen, traders and fish buyers meet. PPI Paotere is an inter-island market that connects fishermen from islands around Makassar including Lae-Lae Island, Kodingareng Lompo Island, Kodingareng Keke Island, Barrang Caddi Island, Barrang Lompo Island and other islands. Seeing the current strategic conditions in PPI Paotere as well as the demand for fresh fish quality must be met, it is necessary to study how fish supply in PPI Paotere is, what are the characteristics of the actors involved in fish procurement, how to manage fish from the supplier to the final distributor, diversity Fresh fishery products and fish supply chain management problems at the Paotere Fish Landing Base (PPI), from the above problems, the authors examine the performance of the fish supply chain and Fisherman Share analysis at the Paotere fish landing base (PPI) Makassar.

➢ *Time and Place of Research*

This research was conducted at the Paotere Fish Landing Base (PPI), which is located on the northern coast of Makassar City, at Sabutung, Pattingan Loang, Wajo Subdistrict, Makassar City in September-November 2020. Starting with preparation, data collection, data analysis and finally a discussion of the research results as outlined in the Thesis.

Picture 1. Research Location

➢ *Sample and Population*

The population chosen is the stakeholder who knows internally and externally the condition and is able to provide answers and confirmation of the questions asked by means of interviews (depth interview). The samples in this study were 27 fishermen with ship's engine power <15 GT, 32 collectors, 8 big traders and 39 retailers. While the sample which is representative of the population, was taken by using purposive sampling method.

➢ *Method of CollectingData*

As a complement to fulfilling the required data in this study, it is very important to use the interview method (deep interview) in data collection used to answer the problems in the study to get the correct conclusions. Therefore, researchers use data collected through observation, interviews with stakeholders, and Gathering information or documentation

- *DataAnalysis*

The data analysis carried out in this research is qualitative analysis and quantitative analysis, based on the formulation of problem number 1 and 2 used a qualitative analysis using a descriptive analysis approach, the researcher will describe who the actors are involved and will be presented through a flowchart, agreement between actors, stocks. available, information, problems, and financial systems. The solution to problem number 3 can be used quantitative analysis with the calculation of Fisherman Share. the results obtained are in accordance with the facts.

II. RESULTS AND DISCUSSION

After conducting research and conducting some analysis of the data obtained in the field, we will then conclude the results and discussion, as follows:

1. *Main Actors in the PPI Paotere Supply Chain*

The fishermen in PPI Paotere consist of catch fishermen, collector fishermen and fishermen outside the region. Catch fishermen are fishermen who carry out fishing activities directly on the sea. Collectors in the supply chain process at PPI Paotere are tasked with buying fish caught directly from fishermen. The price offered by collectors is low. Exporters in the supply chain structure are responsible for distributing or distributing fish to corporate / consumer partners. Before distributing this company, sorting fish size and fish quality for export, retailers (Bakul / Pagandeng) in the supply chain process are tasked with buying small-scale catches. Bakul at PPI Paotere only buys fish from collectors, not directly from fishermen. Bakul sells fish on a port scale, and around auctions in Makassar.

The supply chain system in PPI Paotere consists of 2 chains. In channel 1, fishermen sell 100% of the fish they catch every day to collector traders, whether they are partnered or not. The relationship that is bound between fishermen, boat owners and collectors makes fishermen have to give all their catch to the collecting traders. Giving and investment is given by collecting traders to fishermen and boat owners, so that fishermen are greatly assisted in carrying out fishing operations. the collectors docked at the PPI jetty to pick up fish from fishermen who mostly come from the Pangkep Islands and Makassar City then the collectors sell fish to the retailers who come. The payment system from collectors to retailers is debt or paid in full according to the belief and subscription concept that is carried out. In this second channel chain is slightly different, fishermen who have the fish catch will distribute their catch to collectors as fishing business financiers then distributed again to the UPI (fish processing unit) to then be processed according to the contract or the target market, whether it is local and international market until it reaches the end consumer.

2. *Information Flow System, Goods, Finance*

The flow of material feeds the production of fish caught from the PPI Paotere fishing fleet. Domersal and pelagic fish that are unloaded from ships are landed without going through an auction or what is commonly called the opow system. Fishermen land the fish in the port dramaga, then collectors or ship owners accompany the calculations carried out by the weigher. In supply chain flows 1 and 2, all catch is distributed to collector traders, then the fish is distributed to retailers or stored in cold storage for sorting and storage.

Information management is related to how the actors involved manage the existing information, while the ordering process is how the retailer and exporter order fish from the supplier (fisherman). The agreement between the actors is related to how the fishermen, collectors, exporters and retailers make agreements with related parties. The source of information for each fish collector and retailer is different. Fishermen play an important role because they know the price of fish based on the type, condition and stock of fish. Fish traders at PPI Paotere order fish from suppliers by using short messages (SMS), telephone or Whatsapp (WA).

The system for determining the price of fish at PPI Paotere starts from sales made at PPI. After a purchase has been made by the trader, each trader determines the price according to their respective market conditions. Fish market retailers determine their own prices, but the price set by these traders is sometimes based on observations on prices in the market or prices set by other retailers.

3. *Fisherman Share*

Fisherman's share serves to see how much percentage of the share is received by fishermen. The value of fisherman's share is obtained by comparing the price at the fishermen level with the price at the end consumer level.

Table I. Fisherman Share Analysis of Fish Supply Chain in PPI Paotere

Description Supply Chain	Average Initial Price (Rp / Kg)	Average Final Price (Rp / Kg)	Percentage (%)
Chain I	Rp.41.667.00,-	Rp 61.026.00,-	68%
Chain II	Rp.41.667.00,-	Rp66.625,00	62%

Data source, primary data processing 2020

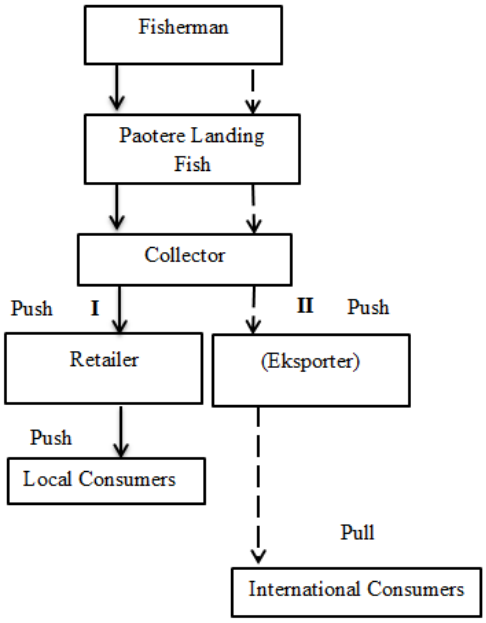
Based on the data in table 1 above, the highest fisherman's share occurred in flow chain 1 at 68%, with an average price from the initial producer of IDR 41,667.00 and an average final price of IDR 61.026.00 where the percentage is greater from flow 2 that is 62%. Supply chain flow I is a flow that involves fishermen, collectors, retailers and consumers. Thus, this flow is a flow that has the most efficient marketing efficiency compared to other streams. This flow is included in indirect distribution, indirect distribution is a flow that uses two or more intermediaries before going to consumers (Mursid, 2016).

Meanwhile, stream 2 becomes a stream that has a smaller fisherman's share value with a value of 62%, this indicates that the price received by fishermen tends to be less. This is in accordance with the opinion (Sudiyono, 2001) that commodities that are produced at a high unit cost must be sold at a high price, so that the share received by fishermen (fisherman's share) is smaller. This shows that flow 1 and 2 are the same flow which is quite beneficial for fishermen, because according to (Erzal et al. 2016), if the fisherman's share value is above 50%, it can be ascertained that the marketing that occurs is efficient, while below 50% marketing can be ascertained. what happens is inefficient.

In the second chain flow, the percentage value is smaller, because there is a high margin difference between the buying price of collectors and the selling price to export. This is due to the demand for fish by exporters to collectors who choose fish of good quality and size so that the selling price is high, so the fisherman share analysis at PPI Paotere is efficient because every marketing channel from the beginning to the end already has a percentage of fisherman's share. range above 50%.

4. Supplay Chain Model

The performance of the fish supply chain at PPI Paotere which is carried out by institutions / stakeholders that play a role in the supply chain process results in 2 supply chain flow schemes. The Supply Chain scheme at PPI Paotere can be seen from the picture below:



Picture 2. Scheme Of Supplay Chain In Paotere Fish Landing Station

The results of descriptive analysis, which can be seen in the supply chain flow diagram I note that most of the processes in the supply chain process are push processes. This occurs because the demand for fresh fish has to adapt to the catch of fishermen's fish which is not always the same and the berthing time is uncertain, so there is no dependence on fish demand from collectors and is also not dependent on time.

The relationship between supply chain actors I and II in business activities is a relationship that brings together suppliers, distributors, retailers, companies, retailers to the final consumer, in the flow of a supply chain to be able to produce a fish product and distribute the product in the right amount, at the right time. precise, good quality, and cost efficient to meet needs. This good relationship occurs in supply chain II where demand for fish species by UPI (fish processing units) which aims to be exported must match the right amount, right time and good quality, therefore, supply chain actors are already familiar with and trust each other.

III. CONCLUSION AND SUGGESTION

Based on the results of research conducted at the Paotere Fish Landing Base (PPI), the following conclusions can be drawn:

- Supply chain business actors at the Paotere Fish Landing Base (PPI) consist of fishermen, collectors, retailers, exporters, and final consumers.
- The flow of information, goods, finance and problems in the fish supply chain at the Paotere Fish Landing Base (PPI) includes, distribution systems, payment systems and information related to fish availability. The problem in the supply chain system at PPI Paotere is that the loading and unloading process of fish at PPI Paotere is mixed with other activities. The mixing of activities in one dock causes fish landing activities to be disrupted.
- Based on research data, the highest Fisherman's share occurred in flow chain 1 by 68%, and the smallest was in flow 2, namely 62%
- The supply chain scheme in PPI Paotere for Flow 1 is Fishermen-Collectors-Retailers-End Consumers. Channel 2 consists of fishermen-collectors-exporters (UPI) -the final consumers

Based on the results of research carried out at PPI Paotere, several suggestions are needed, namely the need for efforts from the port, fishermen and all facility users at PPI Paotere to optimize PPI facilities, for example by managing existing facilities, Need to improve service quality, improve human resource quality , as well as the development of environmentally friendly fishing gears. It is necessary to do counseling related to high and low economical fish processing and the need to develop fish supply chain problems to identify market segmentation.

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A Review on Feed Additives used in Fish Diet

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Abstract**—** *The feed additives are the essential compound of the fish diet. The use of feed additives in the animal's diet is a vital role to rising growth and immunity of the aquatic animals. Feed additives are substances which are added in trace amounts in fish diet it is also act as ingredients improver or preserve it. The major feed additives are act as preservatives, binders, feeding stimulants, and food colorants etc. In favor of growing aquaculture in a supportable fashion, the use of additives must be in correct amount. The requirement of additives are depends on species and their availability. Some authors exposed that the feed additive to have antimicrobial, anti-oxidative, growth promoting ability as well as it is improve the fish immune system.*

Keywords**— Feed additives, ingredient, oil, sustainability, digestibility, attractants, flavors etc.**

I. INTRODUCTION

Feed additives are substances which are added in trace amounts to a diet or feed ingredient for improve or preserve it. Major feed additives are also act as preservatives, binders, feeding stimulants, and food colorants. An ingredient or combinations are added to the basic feed mix there to fulfill a specific need and usually used in micro quantities. For making the feed more attractive, palatable and digestible- attractants, flavors and digestive aids are added. Binding materials are used to prevent disintegration of feed under water column. Feed devoid of nutrient ingredients are additives that are incorporated in diets for reasons other than to offer nutrients [1]. For the most part, these compounds have little or no nutritional value, yet they are important constituents of fish feeds, increasing pellet stability, diet safety, diet flavour, and animal and fish performance and health status and influencing the quality of the final product. Non-nutritive feed ingredients include feed binders, carotenoid supplements, drugs and antibiotics, hormones, antifungal, antioxidants, fibre, flavourings, and water [2].

II. CLASSIFICATION OF ADDITIVES:

According to [3] the main objective of additives in feed is to achieve healthy and faster growth leading to higher production.

The various additives used for these purpose may be classified in to –

1. Essential additives.
2. Growth promoting but non-essential additives.
3. Auxiliary additives.

2.1 Essential feed additives:

Essential additives are supplemented in small quantities to make the formula nutritionally better balanced to promote healthy growth. Their prolonged absence in feed may cause deficiency disease [4]. Ex. Vitamins, minerals etc

2.1.1 Vitamin

According to [5] studying the feed additives vitamin premixes is concentrate in which constant forms of necessary vitamins are mixed with basal feed. In the vitamin premixes choline chloride is not included because it shown to decrease the stability of other vitamins. Added at levels ranging from 0.5- 4% of the diet. Vitamins are important food factors and are involved in many metabolic bio-chemical reactions of animal body.

2.1.2 Minerals:

Inorganic elements, calcium, phosphorus, sodium, potassium, iron, manganese, magnesium, copper, chloride, iodine, cobalt and zinc are essential minerals in the diet. Their insufficiency causes deficiency disease. Trace elements such as copper, zinc, cobalt, iodine and manganese when supplemented in the diet improve growth. Calcium and phosphorus are found in bone and exoskeleton, sodium, potassium, magnesium and chloride are associated with osmoregulation. Magnesium, Manganese and zinc are found as co-factor of metabolic enzymes. [6]

2.1.3 Fish oils:

Authors [7] suggested that fish oils such as cod liver oil, sardine oil, squid oil and clam oil are rich in PUFA. Fish oil is added at 2-3 per cent level in feeds to improve growth and food conversion ratio. Oils provide dietary energy as well as fatty acids essential for aquatic animals.

2.1.3 Fatty Acids:

Highly unsaturated fatty acids (HUFA) such as eicosatetraenoic acid (20:5 W3) and docosahexaenoic acid (22:6 W3) may be added at 1% level to improve growth. [8]

2.1.4 Phospholipid:

Phospholipids such as phosphatidylcholine are essential in the diet of fish for growth and survival. Addition of 1-2 per cent of soybean lecithin in the diet promotes faster growth and improves feed conversion ratio. Phospholipids are physiologically important in transportation of lipids in the body according to [9].

2.1.5 Cholesterol:

Among the steroids, cholesterol is nutritionally essential in the diet. Addition of 0.1- 0.5 % of cholesterol in feed enhances growth and survival. Cholesterol is present in the shrimp head waste meal [10]

2.2 Growth promoting but non-essential additives:

Materials derived from plant and animals, single cell proteins and some synthetic substances are used as additives in feeds for achieving faster growth and higher production. If they are not included in the feed they will not cause any deficiency disease [11]. On the other hand they are beneficial when added to feed. These are growth promoters and attractants. Ex. Plant materials, Animal materials, Single cell proteins, Antibiotics, Drug etc.

2.2.1 Antibiotics:

Authors [12] suggested that antibiotics are generally found to stimulate growth of young animals rather than adults. Antibiotics show positive response in feeds formulated with vegetable proteins. Reduce or eliminate the activity of pathogens, Eliminate bacteria which produce toxins that reduce growth, Stimulate growth of beneficial micro-organisms which synthesize nutrients, Reduce micro-organisms which compete with host for nutrients Increase the absorptive capacity of intestine.

2.2.2 Drugs:

Drugs like arsenicals and sulpha drugs are added to feed for improving growth. 3-nitro-4-hydroxy phenyl arsenic acid, para-amino-phenyl arsenic acid and its sodium salt are the arsenicals used for this purpose. Among the sulpha drugs, sulphanamides are employed. Drugs are act as tonics for animals which contribute to general wellbeing and appearance. The exact mode of action of these drugs is not known, their functions seems to be similar to that of antibiotics [13].

2.2.3 Hormones:

Various natural and synthetic hormones have been used in aquaculture for-

1. Inducement of spawning,
2. Sex reversal,
3. Production of mono-sex population,
4. Growth enhancement.

Hormones responsible for fish growth are growth hormone, thyroid hormone, gonadotropin, prolactin, insulin and various steroids. Steroid hormones- androgen, estrogens and progestogens and non-steroidal hormones-thyroxin are used as growth promoters [14].

2.2.4 Enzyme:

Researchers [15] concluded that enzymes are improving the digestion of feed to the fish also can't digest and can't digest competently. Enzyme also used in the digestion of complex carbohydrates, collagen in skin and bones, and other feed constituents. Enzymes are typically denatured at temperatures above 65º C. Thus enzyme supplements are typically spread on feeds after pelleting.

2.2.5 Probiotics:

Probiotics are live microbial feed supplements that stimulate fish growth by affecting the microbial flora population in the gut. Probiotic may be a single species of microorganisms or a mixture of species. The species of microorganisms present in the supplement colonizes the gut and detrimental species of microorganisms. Allowing the fish are to avoid wasting metabolic energy fighting the effects of detrimental organisms. Probiotics must be added to the feeds after pelleting [16].

2.2.5 Prebiotics

Prebiotics are mostly fibers that are non-digestible food ingredients and beneficially affect the host's health by selectively stimulating the growth and activity of some genera of microorganisms in the colon, generally lactobacilli and bacteria [17].

2.2.6 Synbiotics

When Gibson introduced the concept of prebiotics he speculated on the extra benefits if prebiotics were combined with probiotics to make what he termed as Synbiotics [17]. A symbiotic product beneficially affects the host in improving the survival and implantation of live microbial dietary supplements within the alimentary canal by selectively stimulating the expansion.

2.2.7 Phytobiotic

The phytobiotic canister is regarded as plant-derived foodstuffs inoculation to feed in arranges to improve performance in aquatic species. Generally the plants leaves, roots, tubers, and fruits of herbs, spices and other plants also used as pytobiotics. The pytobiotics menially used to enhance the growth performance in fish and shrimp culture [18]**.**

2.3 Auxiliary additives:

According to [19] the certain components added to feed formula act as components to the physical properties of feed. This in turn helps in better utilization of feed resulting in feed efficiency. Such additives may be considered as auxiliary additives. These are feed colour, binders, molasses and fats. Ex. Attractants, Colour, Binders, Molasses, etc

2.3.1 Feed colorants:

Over 300 pigments are found in various plant and animals. Carotenoids and astaxanthins are either present in some natural or as synthetic substances. Xanthophyll and carotenoids are being the most important classes of pigments. Most part xanthophyll are found in plants and carotenoid pigments are in crustaceans and in fish [20]. They are also found to improve growth and survival of fish. The function of these colors is mainly for proper pigmentation of cultured species.

2.3.2 Binders:

Fish feed must be strong enough to withstand normal handling and shipping without disintegration. Moreover, fish feed must be somewhat water-stable. Starch present in basic feed ingredients is gelatinize during processing and act as binder in feed. Materials such as agar-agar, carboxymethylcellulose (CMC), bentonite, guar gum, lignin sulfate, plaster of Paris, polyvinyl alcohol, sodium alginate and wheat gluten are used as binder. Used at 2 to 8 % level for higher stability of pellets [21].

2.3.3 Molasses:

Addition of molasses to feed often helps in smooth pelleting of feed. Also improves its palatability. It is also a good source of energy in feed [22].

III. IMPORTANCE OF FEED ADDITIVES

1. The feed additives are used for their importance in fish feed and their outcome on the stability and texture of the fish diet.
2. The feed additives for example the pigments increase the economic otherwise nutritional value of fish.
3. Additives are increases fish growth, feed conversion ratio, protein efficiency ratio, and specific growth**.**
4. Feed additives are also act as antimicrobial activity, ant-oxidative effects, it enhances the feed palatability, improves the gut functions.

IV. AN OVERVIEW ON THE RECENT STUDY RELATING THE INCORPORATION OF FEED ADDITIVES IN FISH DIET

Authors [23] suggested that the new aspects of using some safe feed additives on alleviated imidacloprid toxicity in farmed fish: a review. Investigators was [24] reviewed that the fish and shellfish are precious and cheap sources of Omega fatty acids and several other important nutrients for human consumption. There is critical need to study further on novel feed additives similar to inclusion of herbs on fish feeds which decrease feed expenditure, highest digestibility and prevention of remaining effects of hormones and antibiotics on fish muscles which in returns have effects on human that consumes them. The fishing have also been connected with a fast increase in fish consumption, as well as the presence of omega-3 and omega-6 fatty acids, which are essential for humans other than high quality and feed additives. According to [25] the feed contain useful feed additives encourage the growth and physical condition of tilapia, get better their immune systems, and encourage physiological benefits away from traditional feeds. The feed additives such as probiotics, prebiotics, phytogenic substances, immune-stimulants, enzymes, hormones, mycotoxin binders, organic acids etc. are best functional feed additives.

Authors [26] reviewed the feed additives in aqua feeds. Aquaculture trade show swift growth in our country. The reason of the use of feed additives in aquaculture is to raise efficiency, superiority and income. On the other hand, due to ecosystem dilapidation, environmental pollution and leaving residues of the feed additive to the environment, a judgment alternative feed additive has been mandatory. Performing additional researches intended at raising the

productivity of fish through by additives will allow the appropriate fish feed. The according to [27], [28], [29] has been analyzed some researches about enzymes, herbal feed additives and probiotics used as an additive in fish feed. Those feed additives are increase the feed palatability, feed colour, and feed nutrient quality as well as improve the feed digestibility and ultimately enhance the fish production.

Researchers [30] have evaluated the feed additives in animal diets be able to work as chemo preventive agents, which have the possible to reduce the toxicity risk of several pollutants and detoxification of activated metabolites. Understanding the interior act mechanisms of different protected feed additives could lead to the novel therapeutic approaches for acute and chronic pesticide toxicity. According to authors [31], [32], [33], [34] studding the fish nutrition should be cautiously analyzed and there is a requirement to investigate for the novel feed additives and supplements which make sure low feed price, maximum digestibility with least side effects and high feed conversion ratio. Authors are [35] studied on the high-quality feed is arranged from appropriate and essential feed additives. Useful feed additives not only pick up the growth performance of the fishes other than also get better the health performance of the fishes. These useful feed additives include prebiotics, probiotics, seaweeds, mushrooms, microalgae, enzymes, organic acids, mycotoxin binders, photogenic or phytobiotic compounds and yeasts. Researchers [36] has reviewed on utilize of antibiotics in the aqua feeds to moderate infectious diseases and to improve growth performance is commonly practiced. A moment ago, the prophylactic use of antibiotics and chemotherapies have been criticized which ultimately led to their ban in aquaculture by law on several countries. One possible replacement for antibiotics in aqua feeds is the use of useful feed additives. The present review is a whole and an efficient collection of the available works on different feed additives, their examples including probiotics, prebiotics, synbiotics, immunostimulants, organic acids, nucleotides and medicinal herbs.

According to reviewers [37] has reviewed on application of probiotic, prebiotic and synbiotic for sustainable development of aquaculture. The review summarizes and discusses the effects of probiotic, prebiotic or synbiotic administration on growth performance, stress tolerance, intestinal microbiota, immune response and health of aquatic organisms, [38] has evaluated the growth and metabolism of *Labeo rohita* (Hamilton, 1822) fingerlings feed with *Aloe vera* supplementation diet. They found a significant role of *Aloe vera* as an herbal growth advertiser when varied in the basal diet of groundnut oil cake, rice bran and wheat flour for *L. rohita* fingerling. The incorporation of *Aloe vera* in fish diet does not show adverse impact on health of *L. rohita* and it is environment friendly. On the basis of the results obtained in the study, it was concluded that *Aloe vera* supplementation @ 400g/kg diet has paramount importance in enhancing the growth performance and metabolism. The researches [38] studied on the role of *Pedalium murex* in the enhancement of growth, metabolism and immunity of *Labeo rohita* (Hamilton, 1822) fingerlings. They concluded that a *Pedalium murex* extract supplemented diet has the significant role in improving the growth of *L. rohita* besides its ability to enhance metabolism and immunity of the fish. The optimum dose (0.08 gm/100 gm diet) in the feed of *L. rohita* need to be further tested under field condition so that the *Pedalium murex* may be recommended for the commercial aquaculture.

Authors were [39] studied on the effect of ethanolic extract of *Mucuna pruriens* on growth, metabolism and immunity of *Labeo rohita* (Hamilton, 1822) fingerlings. They reported that the *Mucuna pruriens* extract supplemented diet has significant role in improving growth of *L. rohita* besides its ability to enhance metabolism and immunity, [38] studied on the growth, metabolism and haematological parameters of *Labeo rohita* (Hamilton, 1822) fingerlings fed with herbal supplemented diet. They found that *Pedalium murex* and *Mucuna pruriens* combination (1:1) extract supplemented diet has significant role in improving growth of *L. rohita*. Besides, it has ability to enhance metabolism and immunity of the fish. Authors [40] studied on the effects of varying levels of *Moringa oleifera* leaf meal diet on growth performance, hematological indices and biochemical enzymes of African catfish *Clarias gariepinus* (Burchell 1822). The authors are recommended that the fish is a essential source of high-quality protein, providing approximately16 per cent of the animal protein consumed by the world's population (FAO 1997). It is a mainly vital protein source in regions where livestock is relatively scarce. The investigators [41] have studied the nutritive importance of water hyacinth (*Eichhornia crassipes*) leaf meal in mix diets for rohu, *Labeo rohita* (Hamilton, 1822) fingerlings after fermentation through two bacterial strains isolated from fish gut. They suggested that 40 per cent of fish meal can be replaced with water hyacinth leaf meal fermented with fish gut bacteria devoid of any undesirable effect on growth of the fish to produce cost effective formulated fish feed. The nutritional properties of *Mucuna utilis* seed meal and its consumption in the diet of *Clarias gariepinus* (Burchell, 1822), [42] concluded on that toasting significantly enhanced the nutritional quality of this original feedstuff, allowing better consumption at up to 200 g/kg inclusion level in the diet of

African catfish *C. gariepinus*, [44] studied on the effect of natural β-carotene from-carrot (*Daucus carota*) and Spinach (*Spinacia oleracea*) on colouration of an ornamental fish - swordtail (*Xiphophorus hellerii*). They concluded that dietetic supplementation of carrot at the rate 1.82 g/100 g of diet and spinach at the rate of 1.33 g/100 g of diet may be used for pretty the orange coloration in swordtail (*Xiphophorus hellerii*). Because synthetic carotenoid are expensive, cheap and readily available natural carotenoid sources such as carrot and spinach can be incorporated into the diet to enhance better coloration in swordtail, [43] studied on the valuable effects of probiotics in aquaculture. The uses of probiotics in aquaculture have exposed many valuable impacts on fish health by decreeing the risk of diseases, which also measured as a vital step in maintaining sustainable aquaculture. While the favorable effect of probiotics is well recognized, the huge scale use of probiotics in the development of commercial aquaculture.

The effects of fish oil substitution with sunflower oil in diet of juvenile *Catla catla* (Ham) on growth performance and feed utilization. Authors [45] results clearly revealed that sunflower oil could be partially 50 per cent replaced without any adverse effect on the growth performance. The significance of oils in fish diet has been reviewed by [2] they suggested that the oils are cheap sources of essential fatty acid. The uses of dietary oils in accurate quantities as per the required of indivisible animal best the growth and body composition are must. Further they suggested that the oils are beneficial for fish because there antimicrobial, anti-oxidative; growth promoting ability and also as enhances of feed palatability, digestibility & binding capacity.

V. CONCLUSION

The various additives used for these purpose may be classified in to essential additives, growth promoting but non-essential additives, and auxiliary additives. Major feed additives; preservatives, binders, feeding stimulants and food colorants and they are incorporated in feed to achieve healthy and faster growth leading to higher production. Finally it can be concluded that feed additive are added in feed to enhance growth and production, to make diet more attractive, more palatable and digestible- attractants, flavors and increase digestibility.

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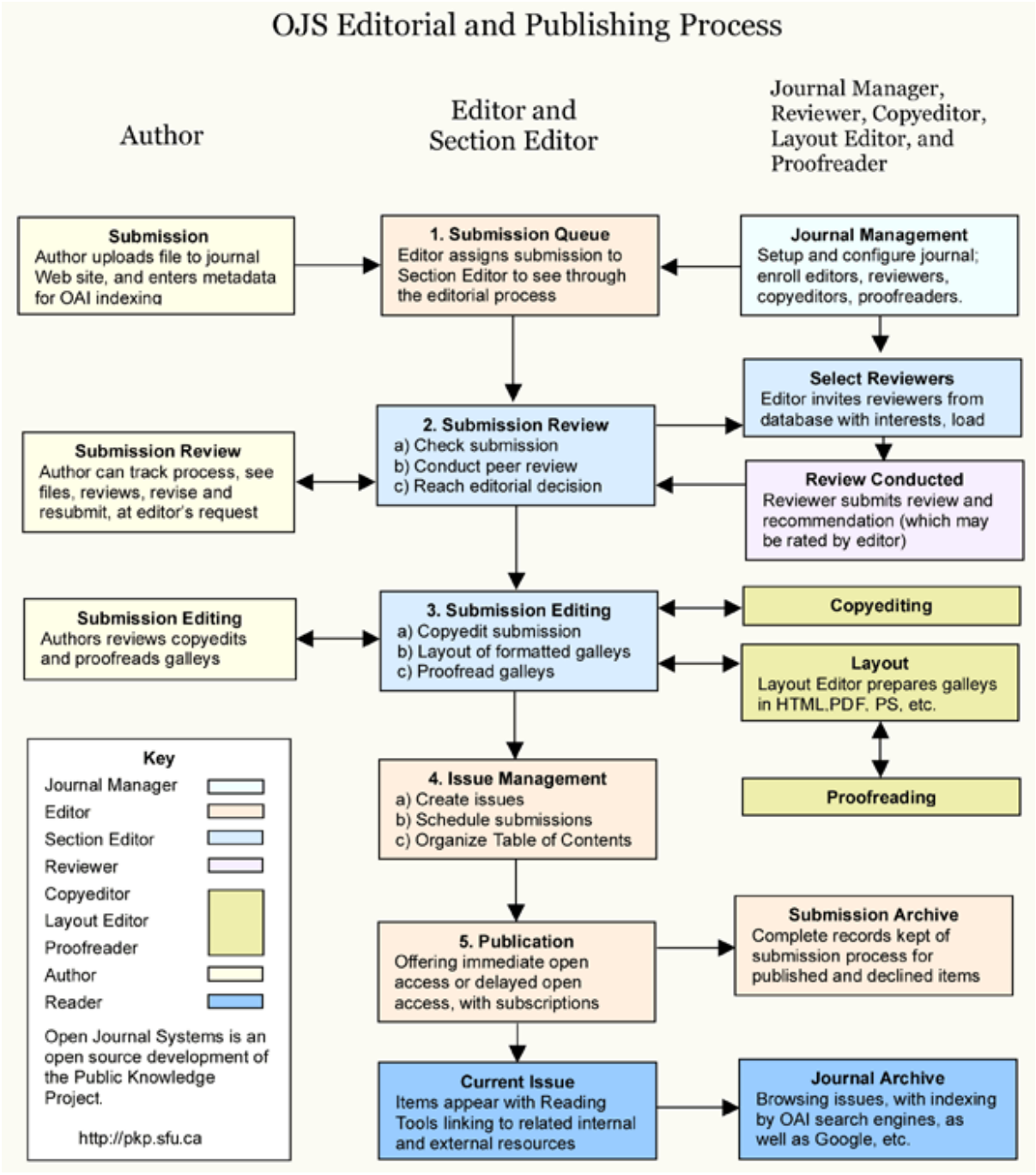

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