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Chemical Composition of the Biomass of *Saccharomyces cerevisiae* - (Meyen ex E. C. Hansen, 1883) Yeast obtained from the Beer Manufacturing Process

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Abstract**—** *Brewer's yeast was subjected to analytical studies to determine the chemical composition of its biomass. To this end, traditional methods of analysis were used to determine ribonucleic acid (RNA), mineral elements, amino acids and fatty acids. The results showed that proteins (49.63%), carbohydrates (31.55%), minerals (7.98%), RNA (8.12%) and total lipids (4.64%) predominate in the biomass composition. The amino acid profile of the protein is suitable for human nutrition, exceeding the recommendations from the FAO/WHO/UNU for essential amino acids. It is particularly rich in lysine and could be recommended as protein supplement in cereals. It was also observed that the yeast was an excellent source of some microelements, such as selenium, chromium, nickel and lithium; that it is also a good source of dietary fiber, particularly soluble fibers; and that the content of lipids was low, with a predominance of saturated and mono-unsaturated fatty acids with 10, 16 and 18 carbon atoms.*

Keywords**— Brewer's yeast, Biomass, Chemical Composition, Protein Value.**

I. INTRODUCTION

The spent yeast used in the fermentation for the production of beer is an industrial residue that represents the second largest waste product generated in this sector, second only to brewers' spent grain. This residue has a high BOD (biological oxygen demand), which may represent up to 60% of the value of this indicator in the effluent (Andreis, 2012). Since Brazil is a major producer of beverages and fuels through alcoholic fermentation (fifth largest beer producer in the world) (Cetesb, 2005), the amount of yeast produced is high.

The micro-organism used in the beer production process is *Saccharomyces cerevisiae.* During the fermentation process, the yeast uses 90% of the available fermentable sugars to produce alcohol, and only 10% for the production of biomass (Cetesb, 2005). From an industrial and national perspective, however, 10% represents a large amount of residue.

The spent yeast used in the alcoholic fermentation process has been used in various sectors, such as the production of animal feed because it is an excellent source of protein (Huige, 2006); as fish feed, potentially offsetting up to 50% of the protein in the feed without any negative effect (Ferreira et al., 2010); and also as input in the biotechnology industry, e.g. for the production of pulp, in the food industry to obtain flavors for certain foods and in pharmaceutical industry (Nasseri *et al*., 2011; Lin *et al.*, 2013).

Studies with yeasts have stood out not only because they are traditionally associated with the preparation of fermented food and beverages, but also because of their versatility and ability to grow quickly on a wide variety of substrates (Guzmán-Juarez, 1983; Sgarbieri, 1987; Giec and Skupin, 1988). Yeast as a source of proteins has been studied mainly after the 1960s, and so far there is no comprehensive and suitable technology for its extensive application (Nasseri *et al.*, 2011).

The production of protein isolates and concentrates from micro-organisms such as yeast, algae and bacteria, has received considerable attention in recent decades. Due to the high protein content (45-65%), they are considered great non-conventional sources of protein (Halàsz; Baráth and Màtri, 1988).

Brewer's yeast has been poorly studied for nutritional purposes, perhaps because of its bitter taste, which results from the beer fermentation process. Some studies performed with the excess yeast from beer production, used the intact inactive cells for functionality tests,

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without addressing the nutritional aspects (Roshkova; Dukiandjiev; Pavlov, 1986). The two main factors cited as limiting factors for the biological use of yeast nutrients are its high content of nucleic acid (RNA) and the very thick and resistant cell wall, which interferes with its digestibility (Kihlberg, 1972; Nasseri *et al.*, 2011).

Considering the nutritional potential of waste arising from the beer manufacturing process, the objective of this work was to evaluate the influence of the mechanical rupture of the cell walls for the biological use of the protein biomass from yeast cells obtained from a craft beer brewery in the southwestern region of the state of Paraná, Brazil.

II. MATERIALS AND METHODS

Yeast:

The yeast *Saccharomyces* sp., from a brewery located in the southwestern region of the state of Paraná, Brazil, was obtained in the form of fresh, already debittered cells suspended in water.

Analytical Methods:

Proximate composition - total protein, moisture and ashes were determined in accordance with the AOAC procedures (1975; 1990). Total carbohydrates were determined through the colorimetric method from Dubois *et al.*, (1958). Total lipids were extracted through the procedure of Blight and Dyer (1959) and determined gravimetrically. Soluble and insoluble fibers were quantified through the method from Asp *et al.*, (1983).

Nucleic Acid (RNA):

The nucleic acids in yeast, which consist mainly of RNA, were determined through the method from Hebert et al., (1971). The RNA was extracted with 0.5m perchloric acid at a temperature of 37° C for 2 hours. It was then hydrolyzed with 0.5m perchloric acid at a temperature of 100° C for 15 minutes.

The quantification of ribose was done with the orcinol reagent, which produces a greenish color and absorbs at 670 nm. The readings were compared with those of the standard curve made with the purified RNA of yeast (Sigma).

Mineral Elements:

The samples were first burned and left in the oven at 450°C for several days, until they were completely white. They were then dissolved in nitric acid at 5%. Aliquots were injected in an Argon Plasma Emission Spectrometer (ICT 2000 Baird). The operating conditions were: radio frequency, 40.68 Mhz; concentric pneumatic nebulizer; entry flow of the sample to be nebulized, 4 mL/min; cooling gas flow, 70 mL/min; position of the vertical torch, 9.8 mm; power applied, 100W.

The quantification of minerals was performed using the standard curve constructed based on a solution (100 µg/mL) of the BAIRD analytical grade in nitric acid at 5%.

Amino Acids:

Amino acids were determined in a laboratory analyzer with a cation exchange column and post-column derivatisation with ninhydrin. The samples were hydrolyzed in advance with HCl 6N at 110°C for 22h, with the exception of tryptophan, for which the hydrolysis was performed with LiOH 4N for 24 h at the same temperature.

Fatty Acids:

The fatty acid composition was determined by gas chromatography of the methyl esters of the fatty acids, obtained according to the method described by Hartman and Lago (1973). A Variam 3400/3300 gas chromatograph was used with a flame ionization detector (FID), OV 275-15% Carbowax (1/8" x 2m) column. The fatty acids were identified by comparing the retention time with the standards (Sigma) and quantified through the automatic calculation of the area with the Perkin-Elmer-100 integrator.

Statistical Analysis:

The statistical analysis was performed with the SANEST software (Statistical Analysis System), submitting the experimental results to analysis of variance and Tukey's test at a confidence interval of 95% (Gomes, 1982).

III. RESULTS AND DISCUSSION

The proximate composition of the brewer's yeast biomass of *Saccharomyces cerevisiae* is shown in table 1.

For the calculation of the crude protein (49.63%), the conversion factor of 5.8 was used, calculated after subtracting the non-protein nitrogen corresponding to the RNA of the biomass. The composition is characterized by high levels of protein, ashes, RNA and soluble fiber. The total lipid content is low and total carbohydrates represent approximately one third of the biomass.

When the data presented in Table 1 is compared with other studies, one can see that the data is similar to those obtained by Farnun and Cleland (1975); Guzmán-Juarez (1983) and Caballero-Córdoba *et al.*, (1997). The results of these studies are very similar and are very close to those found in the study presented here.

In Table 2 , the data of the amino acid composition of the yeast biomass as compared with the reference standard of FAO/WHO/UNU (1985), is presented.

One can see that all essential amino acid profiles of the yeast cells exceed the recommended amino acid quantities by the three organizations of the United Nations. It should be noted that the high concentrations of lysine (LYS) and threonine (Thr) in the yeast biomass, turn it into an exceptional material for the supplementation of cereals,

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since the protein content of cereals is commonly deficient in these two amino acids and also in tryptophan.

The fatty acids profile of the lipid fraction of the biomass of *Saccharomyces cerevisiae* is shown in Table 3.

After the performed analysis, 11 fatty acids (C8-C18) could be identified, including palmitic acids (34.33), oleic acids (11.02), stearic acids (9.56), capric acids (6.26), linoleic acids (4.37) and palmitoleic acids (2.99). Fatty and mono-unsaturated acids, therefore. The linoleic acid content (C18:3) was low, as it was only 0.63%. It should be emphasized that the data obtained in this study are in line with the data from Caballero-Córdoba *et al.*, (1997), who analyzed fatty acids levels in yeast using the same methodology, presenting very similar rates, but they are not in agreement with the results from Halász and Lástity (1991), both from a quantitative and qualitative perspective.

Accordig to Halász and Lástity (1991), the lipid content of yeast varies from 7 to 15% and the fatty acid composition is characterized by a high content of unsaturated fatty acids, with the oxygen flow during the cultivation of the cells being the parameter that most influences the fatty acid composition.

In Table 1, the total lipid content is 4.64% lower than the range of 7-15%, but very similar to the data presented by Farnun and Cleland (1977); Guzmán-Juarez (1983) and Caballero-Cordoba *et al.*, (1997) with values of 3.44; 4.91 and 4 to 7%, respectively.

In the data observed in Table 3, on the other hand, the saturated and mono-unsaturated fatty acids predominated instead of the poly-unsaturated fatty acids. It is likely that this difference reflects the different physiological and nutritional conditions of the different biomasses. In the case of the results reported in this work, the biomass was more spent after several recyclings in the fermentation process. In the example of the literature, the biomass used was grown in ideal conditions for nutrient concentration in the medium and oxygen supply.

The mineral composition of the biomass is presented in Table 4. Considering the high content of nucleic acid in the yeast (Table 1), which limits its daily intake to a maximum of 20-30 g of dry yeast per day, the yeast will not be able to contribute with very significant quantities of macro-elements. With respect to the micro-elements, it can be considered an excellent source of selenium, manganese, chromium, nickel and lithium, with observed values of 25.12; 14.98; 10.11; 9.05, 8.23 and 6.13 mg for every 100 grams of biomass evaluated.

The data presented here are in line with the data obtained by Caballero-Cordoba *et al.*, (1997) in studies that also considered the yeast biomass of the same species and in which they observed the presence of the same chemical elements in the performed quantifications. In the two works compared here, the macro-elements phosphorus, potassium, sodium, magnesium, aluminum, calcium and iron were observed. The micro-elements obtained in this work are the same observed in the work described here.

Yeast is considered an excellent source of selenium and chromium, with its intake being recommended as a dietary supplement to prevent deficiencies of these elements, which are characterized by hair loss, growth retardation, reproductive deficiency, heart diseases, necrosis and degeneration of the liver and pancreas (Levander, 1989).

The presence of lithium should also be emphasized, because it has been used in the treatment of various problems associated with neuropsychiatric disorders in the past three decades. It is particularly beneficial for the acute treatment of mania, and usually for the prophylaxis and treatment of depression in bipolar patients. Currently, lithium is the treatment of choice for bipolar disorder, preventing relapses and suicide attempts. Its use is successful in dramatically reducing depressive and manic symptoms in 70% to 80% of patients (Muller-Oerlinghausen *et al.*, 2002).

IV. CONCLUSION

With the data obtained, the conclusion can be drawn that the biomass of the brewer's yeast of *Saccharomyces cerevisiae* - (Meyen ex E. C. Hansen, 1883) obtained from the beer production process is a rich source of protein with good nutritional value, taking into account the various levels of biological evaluation.

With a high content of lysine, this protein source proves to be of special interest as a protein supplement for cereals. The rupture of the cell wall significantly improved the digestibility and use of the net protein from the biomass.

It was also observed that the yeast proved to be an excellent source of some microelements, such as selenium, chromium, nickel and lithium; that it is also a good source of dietary fiber, particularly soluble fibers; and that the content of lipids was low, with a predominance of saturated and mono-unsaturated fatty acids with 10, 16 and 18 carbon atoms.

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Table 1: Proximate composition of the yeast biomass obtained from breweries - Saccharomyces cerevisiae.

Components	Yeast[1]	Yeast[2]	Yeast[3]	Yeast[4]
Protein	49.63±2.43	48.51	49.80	45-49
RNA	8.12±1.54	7.52	8.40	8-12
Lipids	4.64±0.52	3.44	4.91	4-7
Ashes	7.98±0.76	8.33	5.10	5-10
Total Carbohydrates	31.55±4.32	32.86	-	26-27

Soluble Fibers	9.12±1.22	9.59	-	-
Insoluble Fibers	2.87±0.87	2.60	-	-

Yeast[1] – Conversion factor to protein = at 5.8.
Yeast[2] - Farnun and Cleland (1975).
Yeast[3] - Guzmán-Juarez, (1983).
Yeast[4] - Caballero-Córdoba et al., (1997).

Table 2: Composition of amino acids (g/100g of protein) of the brewer's yeast biomass - Saccharomyces cerevisiae.

Not Essential	BI	Essential	BI	PR
Cys	1.24±0.21	Lys	6.73±1.21	5.8
Tyr	4.12±0.32	Leu	8.75±0.67	6.6
Glu	8.56±0.45	Ile	4.63±0.32	2.2
Asp	10.05±0.56	Thr	6.09±0.57	3.4
Ser	5.45±0.31	Try	0.96±0.04	1.1
Pro	5.11±0.67	Val	5.34±0.43	3.4
Ala	6.89±0.12	Met+Cys	3.55±0.64	2.5
Gly	5.23±0.87	Phe+Tyr	8.36±0.89	6.3
Arg	4.02±0.64	His	2.78±0.06	1.9
Phe	5.57±0.10	Met	3.12±0.21	-

BI = whole biomass; PR = reference standard of the FAO/WHO/UNU (1985).

Table 3: Composition of fatty acids and total lipids of the brewer's yeast biomass - Saccharomyces cerevisiae.

Fatty Acids	Structure	Total Concentration (%)
Caprylic	C8:0	0.29
Capric	C10:0	6.26
Lauric	C12:0	1.26
Myristic	C14:0	0.78
Myristoleic	C14:0	0.39
Palmitic	C16:0	34.33
Palmitoleic	C16:1	2.99
Stearic	C18:0	9.56
Oleic	C18:1	11.02
Linoleic	C18:2	4.37
Linolenic	C18:3	0.63

Table 4: Mineral composition (macro and micro-elements) of the brewer's yeast biomass - Saccharomyces cerevisiae.

Macro-elements	Mg/100g	Micro-elements	Mg/100g
Phosphorus	17.31	Selenium	25.12
Potassium	14.21	Manganese	14.98
Sodium	9.13	Lead	10.11
Magnesium	3.02	Chromium	9.05
Aluminum	1.12	Nickel	8.23
Calcium	0.87	Lithium	6.13
Iron	0.17	Zinc	4.89
		Copper	4.19
		Vanadium	0.56
		Cadmium	0.45