Original Research Article

Potential Applications Of Spent Brewer's Yeast, A Residual By-Product Of Beer Production In Ivorian Breweries, Through The Identification Of Biochemical Characteristics

ABSTRACT

The recovery of by-products from food processing chains represents an area of interest. Waste from beer production such as beer lees or spent brewer's yeast constitute between 1.5 and 3 kg/100 L of beer, representing a source of pollution but a by-product available in quantity. This study aims to characterize the beer lees from breweries in Côte d'Ivoire and propose possible ways of recovery. The usual methods have made to highlight the nutritional potential of this by-product. Spent brewer's yeast is a high biological value protein source (46.77%) with a well-balanced amino acid profile. Amino acids (glutamic acid and aspartic acid) and the essential amino acids leucine, lysine and valine are the most abundant, while sulfur amino acids, such as methionine and cysteine, are the least abundant. With a high mineral content (7.3%), the spent brewer's yeast contains significant amounts of manganese 5.54.10-2 mg/k, sodium 3.92.10-2 mg/k, and potassium 3.59.10-2 mg /k. The B complex vitamins are present in large quantities followed by vitamins C, A and D. Less than 3% of the composition of the spent brewer's yeast is made up of lipids. The most abundant of fatty acids determined for saturated is palmitic acid, oleic acid represents monounsaturated fatty acid, polyunsaturated fatty acids are represented by linoleic acid and linolenic acid. Carbohydrates (32.77 %) are also main constituents of the dry matter of this by-product. This study showed that spent beer yeast is a source of polyphenol.

Spent brewer's yeast have advantages for being incorporated into animal nutrition to meet the nutritional needs. Further studies could lead to the production of activated carbon and that of hydrogen as an excellent source of alternative energy.

Keywords: Brewery waste, spent brewer's yeast, added value, applications, Côte d'Ivoire

1 INTRODUCTION

To meet the growing needs and ephemeral preferences of populations, waste from production and innovation processes is also increasing. Thus, the agricultural and food industries generate significant amounts of organic residues because of the processing of raw materials [1] (Nayak and Bhushan 2019). These authors indicate that according to [2] Baiano (2014), the largest percentage (26%) of food waste comes from the beverage industry. Beer is one of the most consumed thirst-quenchers in the world, obtained from certain cereals, including barley, corn, rice, and wheat, combined with water and hops [3] (Caporale et al., 2004). Its global production is estimated at 1.86 billion hectoliters in 2021 [4] (Statista Research Department, 2023). As a result, the production of beer involves the production of several residues and by-products, for example spent grains, hops and yeasts [5, 6] (Mussatto, 2009, Mathias et al., 2015).

These wastes must be properly treated to avoid their uncontrolled dumping which could cause environmental problems. Environmental pollution, one of the major concerns of every government, has increased in developing countries where, unlike industrialized nations, a large portion of environmental pollutants consist of agricultural by-products considered waste. This situation results from the lack of recovery of these by-products.

In Côte d'Ivoire, the rational management of waste from industrial activities is one of the environmental priorities of the Ivorian state. Among these wastes are those resulting from the production of the beer. Ivorian beer production is estimated at 3.2 million hectoliters generating waste (reference) including residual brewer's yeast which is the second largest by-product of the food industry [5] (Mussatto, 2009). These residues and by-products are usually underutilized, displaying little or no commercial value [1] (Nayak and Bhushan 2019). Moreover, this waste has a high organic matter content and requires adequate treatment for its disposal, which entails considerable costs ([7] Briggs et al., 2004; [1] Nayak and Bhushan 2019). There are various recovery methods that meet the challenges of the economy. However, any recovery imperatively requires characteristic information on these residual beer yeasts, in particular their biochemical composition.

A thorough knowledge of the macro and micronutrient composition of residual brewer's yeast is the key to discovering the possible application routes of this important by-product.

The objective of this study is therefore to characterize the beer lees from breweries in Côte d'Ivoire and to propose possible ways of recovery.

2 MATERIAL AND METHODS

The beer lee sample used in this study was obtained from the main beer industry of Côte d'Ivoire, Abidjan. The beer lee sample was residue of dead yeast which settles at the bottom of the container after fermentation.

2.1 Determination of chemical composition of spent brewer's yeast

2.1.1 Moisture

The water content of spent brewer's yeast was determined by oven drying using the procedure of [8] AOAC (1990). 10 g (W1) of spent brewer's yeast were weighed out in a porcelain dish and were kept for drying at 80°C for 3 to 6 hours. Then, the sample was removed from the oven, cooled in a desiccator for 30 to 45 minutes and weighed again (W2). The moisture content of the samples was obtained from the mass difference (W1 – W2).

2.1.2 Crude proteins

The crude proteins of the spent brewer's yeast samples were calculated by Kjeldahl [9] (ISO1871 2009). Nitrogen from nitrates and nitrites is not considered by using this dosing principle. 0.5 g of dried sample was first digested by heating in 15 ml of strong sulfuric acid in the presence 1 g catalyst which helps in the conversion of the amine nitrogen to ammonium ions. This digestion step ends in 30 min with the appearance of a green coloration. After digestion, the ammonium ions were dissolved in 250 ml of distilled water containing 5 drops of phenolphthalein and 75 ml of a 1 M sodium hydroxide solution. The contents were heated and distilled. The liberated ammonia gas was led into a trapping solution of 10 ml of boric acid solution (10 g/l) and few drops of a mixed indicator dye (methyl red + bromocresol green) where it dissolved and became an ammonium ion once again. Finally, the amount (150 ml) of the ammonia that has been trapped was determined by titration with a 0.1 N hydrochloric acid solution.

2.1.3 Carbohydrates

The beer lees carbohydrates were quantified by using the standard method of [10] AOAC 938.02 (1938). To 3 mL of the sample, add 2 mL of acetonitrile and 0.1 g of NaCl and the mixture was vortexed and centrifuged at 5,000 rpm for 10 min. 100 µL was withdrawn from the supernatant layer and evaporated to dryness under vacuum. Add 100 µL of dichloromethane (DCM) to the dried sample and repeat the evaporation process. Again add 100 µL of bis (trimethylsilyl) trifluoroacetamide (BSTFA). Finally, the whole mixture was dried in an oven at 80°C for 30 min and the resulted solution made up to 1.5 mL with acetonitrile then put in a vial for chromatographic analysis.

2.1.4 Sugars (glucose, fructose, and sucrose)

Simple sugars were quantified according to the AOAC 938.02 (1938) method [10].
Free sugars contents: 10 g of beer lees sample were dissolved in 100 mL of a distilled water. 10 mL of the dissolved sample was taken in a 250 mL Erlenmeyer flask and 20 mL of a 5 mM iodine solution and 5 mL of 2 M sodium hydroxide (NaOH) were added. After homogenization, the mixture was kept in

dark for 20 min and then, 7 mL of hydrochloride acid were added and titrated using 0.1 N of sodium thiosulfate.
Total sugars: 1 g of beer lees was dissolved in 50 mL of a 2 M hydrochloric acid solution and boiled for 30 minutes. 10 mL of this solution was boiled again for 30 min with 40 ml of 2 M hydrochloride acid. After cooling, mix 10 mL of the mineralized solution with 20 mL of a 5 mM iodine solution and 10 ml of 1 M NaOH. The mixture was kept in the dark for 20 min and then. 7 mL of hydrochloride acid were added and titrated using 0.1 N of sodium thiosulfate.

2.1.5 Ash and inorganics

The minerals were quantified by following the ICUMSA method GS1-10 (1998) [11]. 2 g of the previously dried sample were incinerated at 600 °C for 3 h in a graphic oven. After cooling in a desiccator for 45 min, the resulted powder was weighed, and the ash content was determined.

For elemental analyses, procedures of ICUMSA were followed: iron [12] (Method GS2/3/7/8-31 1994), phosphorus [13] (Method GS7-15 1994), magnesium [14] (Method GS7-19 1994), copper [15] (Method GS2/3- 29 1994), calcium (ICUMSA method GS8/2/3/4- 9 2000), potassium and sodium (Method GS6-7 2007). The ashes obtained from 2 g sample of dry beer lees were dissolved successively in 2 mL of nitric acid and 98 mL of distilled water. Most of metal ions were quantified by gas chromatography (GC) coupled with an atomic Absorption spectrophotometer (AAS).

2.1.6 Fatty acid profile determination

2.1.1.6.1 Fat extraction
The samples were minced, homogenized, and weighed. Then, the extraction of total fat was carried out on 2 g of sample using a liquid extraction based on the Folch method (chloroform/methanol 2/1 v/v) [12] (Folch, 1957).

2.1.1.6.2 Esterification of fatty acids
The fatty acid profile was determined by analysis of fatty acid methyl esters by GC-MS according to the method of [13] Douny et al. (2015) modified. Fifty milligrams of fat extracted using Folch's method was mixed with 5 mL of hexane and 10 µL was used for the saponification/fatty acid methylation reaction. Nonadecanoic acid (C19:0), used as internal standard, was then added and the hexane was evaporated to dryness under nitrogen flow. Toluene and sulfuric acid 2% (v/v, in methanol) were added and the closed tube was then heated in a water bath at 100°C for 1 h, with vigorous shaking carried out at using a magnetic bar. Then 5% NaCl was added, and the fatty acid methyl esters (FAMEs) were extracted twice using hexane. The extract was then washed with 2% (w/v) K_2CO_3 and finally Na_2SO_4 was added to part of the extract. The final extract was evaporated to dryness to completely remove the toluene. Finally, four hundred microliters of hexane were added, and the tube was vortexed. Finally, the sample was transferred to an injection vial.

2.1.1.6.3 Separation, detection, and quantification of fatty acids
Fatty acid methyl esters were separated on a Focus GC gas chromatograph (Themo Fisher Scientific) using a CP-Sil88 column (100 m × 0.25 mm, 0.2 µm) (Varian, Agilent Technologies, Santa Clara, California, USA) and analyzed using a Polaris Q ion trap mass spectrometer (Themo Fisher Scientific). The GC conditions were: injector: 250°C; split less type injection; helium as carrier gas at 1.5 ml·min-1; temperature gradient: 55 °C for 1 min, followed by an increase of 5 °C min–1 to 180 °C, then 10 °C min–1 to 200 °C for 15 min, then an increase of 10°C·min–1 to 225 °C for 14 min; the total analysis time was 59.50 minutes. The injection volume was 1 µl. Peaks were identified by comparing their mass spectra and retention times with those of the corresponding standards. The mass spectrometer conditions were: transfer line: 250°C; source ion: 220°C; collision energy: 35 eV; ionization in positive mode. The FAMEs were detected using the “selected ion monitoring” (SIM) mode with 5 times windows. In each analysis, different ions were monitored for each fatty acid analyzed, which made it possible to carry out the detection and quantitative analysis of these: m/z 74+143 for saturated fatty acids, 79 + 91 for mono and polyunsaturated fatty acids. For quantification, a 6-point calibration curve produced using standard solutions and an internal standard was produced for each of the 23 fatty acids analyzed. The response (ratio between the area of the chromatographic peak of each fatty acid methyl ester and that of the internal standard) was plotted as well as the concentration of the standard solutions. Linear regression was used. After determining the fatty acid

content in each sample, the sum of saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, the n-6/n-3 ratio, the PUFA/AGS ratio, were calculated.

2.1.7 Determination of the amino acid profile

The method of Soufleros and Bertrand (1998) [14] with a modification of the elution gradient and flow rate, was used for the determination of the amino acid profile. Thus, 5 g of dry matter in 20 mL of 0.2N sodium citrate at a pH of 2.3. Then protein hydrolysis with 10 mL of 6N hydrochloric acid at 110°C for 24 hours. After evaporation of the acid, the sample is collected in 10 mL of 70% ethanol and filtered on a millipore filter before injection into HPLC.
The Reference solution consists of 20 amino acids prepared at different concentrations by making dilutions with a hydro-alcoholic solution at 10% vol. Each series contained 6 concentrations.
We used a Waters Alliance HPLC device, model e2695, equipped with an automatic 48-sample collection system and a p2895 pump system.
Separation of amino acids was carried out using two columns in series, of the lichrocart 125-4 cartridge type containing a Lichrospher 100 RP18 column. The length of each of these columns is 12.5 cm and the diameter of the particles is 5 µm. A pre-column of the same type has been placed at the beginning of each column. The detection is made using a Waters 2475 spectrofluorimeter. The excitation is made at the wavelength of 340 nm and the emission at 450 nm.
The samples and the reference solutions are then introduced into 2 mL glass vials. These vials are then placed in the autosampler/sampler carousel.

2.1.8 Vitamin dosage

2.1.8.1 Water-soluble vitamins

The vitamin content of the samples was determined by high performance liquid chromatography technique using the method described by European pharmacopoeia book [15].
Two (2) grams of sample were placed in 25 mL of acid sulfuric (0.1 N) solution. Then, the contents were adjusted to pH 4.5 with 2.5 M sodium acetate. The preparation was stored at 35°C overnight. The mixture was then filtered through a Whatman paper, and the filtrate was diluted with 50 mL of pure water and filtered again through a micropore filter (0.45 µm). Twenty microliters (20 µl) of the filtrate were injected into the HPLC system. Quantification of vitamins B5, B6, B9 and B12 content was accomplished by comparison to standards. Chromatographic separation was achieved on a reversed phase (RP) HPLC column through the isocratic delivery mobile phase at a flow rate of 1.5 mL/min. Ultraviolet (UV) absorbance was recorded at 270 nm at room temperature.

2.1.8.2 Fat-soluble vitamins

The extraction of fat-soluble vitamins was carried out according to the method described by [16].
To 1.0 g of the sample was added 10 mL of a 10% KOH solution in methanol-water (1:1, v/v). To avoid the oxidation process during saponification, 0.025 g of ascorbic acid was added. The mixture is then brought to reflux in a water bath at 70° C. for 30 min. The mixture is then cooled and extracted with 3 x 5mL of hexane. The hexane phases were combined and dried over anhydrous sodium sulphate then evaporated to dryness. The residue obtained (approximately 0.3 g) is taken up in methanol (10 mL) for analysis.
The evaluation of fat-soluble vitamin content was made by HPLC coupled to a fluorimetric detector. The analysis is carried out in isocratic mode on a Hypersil ODS RP18 column (stationary phase), 5 µm particle diameter and 4.6 mm internal diameter. The mobile phase is an Acetonitrile/methanol mixture (80:20, v/v) with a flow rate of 1 mL/min. The standards were prepared by dilution series (1/10th then 1/2): α-tocopherol (E): 3.4µg/100 mL; Retinol (A): 11.3 µg/100 mL; ergocalciferol (D2): 8.6µg/100mL. All calculations are made from the 100% witness. Fluorimetric detection: vit A (455 nm), vit D (245 nm) vit E (295 nm)

2.1.9 Assay of total polyphenols

The method used to assay the total polyphenols was that proposed by the reagent of [17].
One (01) gram of dried yeast sample is homogenized in 10 mL of 70% (v/v) methanol. The mixture obtained was centrifuged at 1000 rpm for 10 min. The pellet was recovered in 10 mL of 70% (v/v) methanol and centrifuged again. The supernatants were pooled in a Falcon tube.
Two hundred (200) µL of methanolic extract was introduced into a test tube and added with two hundred (200) µL of Folin-Ciocalteu reagent. The tube was left to stand for 3 min, then 200 µL of 20% (w/v) sodium carbonate solution was added. To the contents of the tube was added 1.4 mL with

distilled water and the whole was placed in the dark for 30 min. The absorbance reading was taken with a spectrophotometer at 760 nm against a blank. A standard range established from a stock solution of gallic acid (0.1mg/mL) under the same conditions as the test makes it possible to determine the quantity of polyphenols in the sample.

RESULTS AND DISCUSSION

Residual brewer's yeast is a brewing by-product that deserves considerable attention, due to the large amount produced. This by-product, once collected, can be marketed in the form of paste, powder, or even in liquid form [18].

The main organic compounds of the dehydrated residual brewer's yeast studied are summarized in Table 1. The results show that proteins (46.77%) are the main constituents of the dry matter of this by-product followed by carbohydrates (34.77%). Thus, residual beer yeasts are mainly composed of proteins and carbohydrates [5]. Also, the most abundant element in yeast cells is carbon, which represents just under 50% of the dry weight. The other major elemental components are oxygen (30-35%), nitrogen (5%), hydrogen (5%) and phosphorus (1%) [5]. The total crude protein obtained was net greater than 41.928% obtained by [19] while [6] obtained 52.7%. These authors indicate [20,21,22] found protein content to range from 35.2 to 47.2% on a dry matter basis. [23] states that brewer's yeast powder contains 46.1% protein in its percentage composition and the idea that protein makes up 35% to 69% of dry yeast has been supported by [24]. In general, the protein content of yeast is 45%, and can reach 70%, depending on the physiological state and phase of the growth cycle [7].

Table 1. Descriptive statistics of moisture and main organic compounds content of beer lees

Item	Average	Standard deviation
Water content (%)	11.83	0.70
Crude proteins (%)	44.77	0.06
Crude fat (%)	1.33	0.10
Carbohydrates (%)	34.77	1.56
Total polyphenol (mg/g)	4.18	0.16
Sucrose (%)	1.49	0.20
Glucose (%)	1.49	0.03
Fructose (%)	2.98	0.70

The high protein content of yeast thus offers a wide range of uses. Residual brewer's yeast is also a good source of essential amino acids because neither human nor other mammal can synthesize them and therefore must ingest them from food [25].
These high values of the protein content of residual brewer's yeast indicate that the latter can easily replace local soybean meal (45% protein) in animal nutrition to meet the nutritional needs of animals. Also, the low lipid content (1.33%) of beer lees would be an advantage in feed to produce animals with the less fatty flesh sought by consumers. Indeed, some studies indicate that the main current destination of residual brewer's yeast is to formulate animal feeds and to mix it with spent grains generated in the process to increase their nutritional value. The low content of reducing sugars (glucose 1.49% and fructose 2.98%) are close to that (1.3%) obtained by [6] determined in the residual yeast is in line with expectations. They justify this low by the fact that the residual brewer's yeast mainly presents polysaccharides (and not reducing saccharides) as constituents of the cell wall.
Additionally, this study showed that spent beer yeast is a source of polyphenol as some studies have reported the ability of yeast to absorb polyphenols during fermentation processes. This fact suggests

the potential value of residual brewer's yeast as a valuable source of value-added bioactive polyphenols.

Residual brewer's yeast is a source of protein which has a high biological value with a quantity of essential amino acids in its structure (Table 2). The amino acids present in greater quantities are lysine, leucine, isoleucine, valine, threonine and phenylalanine, and there may be a slight deficiency of sulfur amino acids [5,21]. This richness of this by-product has been proven by other researchers [6]. According to them, among the waste products from the production of beer, brewer's yeast is the by-product richest in amino acids and corroborates with its high composition in soluble proteins. These authors argue that this result can be attributed to cellular constituents that are dispersed in the medium, possibly due to cell disaggregation, fragile and susceptible to autolysis at the end of fermentation, as well as during atomization during of beer production.

Table2. Descriptive statistics of amino acid profile compounds content of beer lees

Item	**Average (g/16 g N Protein)**
Histidin	1.35
Isoleucin	4.30
Leucin	6.69
Lysin	8.41
Methionin	2.44
Phenylalalin	4.10
Threonin	3.58
Cystein	ND
Glutamic acid	11.67
Valin	5.57
Arginin	4.44
Prolin	5.15
Alanin	6.24
Serine	3.37
Tyrosin	4.31
Aspartic acid	7.21

In addition, this waste contains other substances, such as minerals (Table 3). The total mineral content of the yeast is 7.3% corresponding to the values (5 to 10% of the dry weight of the cell) obtained from certain works [5,6]. This fraction includes a multitude of elements, including manganese (54.5 10-3 mg/kg), potassium (35.9 10-3 mg/kg), sodium (39.2 10-3 mg/kg), iron (31.5 10-3 mg/kg) and calcium (24.5 10-3 mg/kg) and zinc (23.4 10-3 mg/kg). Besides the mentioned minerals, phosphorus, and copper are also determined in lower proportions.

Table 3. Descriptive statistics of inorganic compounds content of beer lees

Item	Average	Standard deviation
Ash (%)	7.3	0.84
Calcium (Ca; mg/kg)	0.0245	0.0003
Phosphorus (P; mg/kg)	0.0177	0.0004
Magnesium (Mg; mg/kg)	0.0145	0.0005
Potassium (K; mg/kg)	0.0359	0.0005
Sodium (Na; mg/kg)	0.0392	0.0500
Manganese (Mn; mg/kg)	0.0545	0.0005
Zinc (Zn; mg/kg)	0.0234	0.0003
Copper (Cu; mg/kg)	0.0027	0.0003
Iron (Fe; mg/kg)	0.0315	0.0013

Residual brewer's yeast with a lipid content of less than 2% is rich in unsaturated fatty acids including oleic acid (2.1%), linoleic acid (0.38%) and linolenic acid (0 ,35%). The saturated acids determined are mainly palmitic acid (1.46%), stearic acid (0.55%), myristic acid (0.46%) and a small amount of lauric acid (0.02 %) (Table 4).

Table 4. Descriptive statistics of fatty acids profile compounds content of beer lees

Item	Content (g/100g MS)
Palmitic acid	1.46
Arachidonic acid	0.11
Oleic acid	2.1
Stearic acid	0.55
Lauric acid	0.02
Linoleic acid	0.35
Linolenic acid	0.38
Myristic acid	0.46

It is also rich in vitamins (vitamin A (35µg/100 g), vitamin E, vitamin D, vitamin B3, vitamin B6, vitamin C), mainly in niacin or vitamin B3 (Table 5) as mentioned by [26]. This by-product can be used as vitamin supplements in natural foods [27].

Table 5. Descriptive statistics of vitamins compounds content of beer lees

Item	Content

Vitamin A (µg/100g)	35.1
Vitamin E (mg/100g)	0.58
Vitamin D (µg/100g)	6.69
Vitamin B3 (mg/100g)	5.45
Vitamin B6 (mg/100g)	0.71
Vitamin C (mg/100g)	0.27

Due to the composition rich in proteins, amino acids, minerals and other compounds of interest, several attempts have been made to reuse the yeast surplus in biotechnological processes such as obtaining products with high nutritional value in application in the pharmaceutical industry and in human food as food supplements due to their rich composition and to be generally recognized as safe (GRAS) [7,28]. Such a compound is of particular interest for use in the food industry as a flavoring agent in soups, sauces, gravies, stews, snacks, and canned foods [27].
Authors [29] point out that residual brewer's yeast has the potential to increase ham quality due to its high protein content which can improve texture. Moreover, Others [30] showed that it contains nucleotides that can act as flavor enhancers and increase the sensory characteristics of ham. It is also a good source of minerals and vitamins which will improve the nutritional composition of the ham. [31] showed that cooked ham supplemented with yeast extract had higher hardness, chewiness, ash, protein, and free amino acids than control hams. These authors concluded that spent yeast extract can be used as a gel stabilizer in cooked ham formulations.
However, [32] report that some factors limiting their application for human consumption are presence of bitter compounds, difficulty in digesting thick cell wall and high RNA content, which may lead to increased uric acid levels in blood and tissues.
[27] report that yeast extract is used in microbiological media as a source of nutrients. Thus, the work of [33] demonstrated that the use of brewer's yeast autolysate during the fermentation of vegetable juices by Lactobacillus acidophilus, favorably affects the increase in the number of lactic bacteria, the reducing fermentation time and enriching vegetable juices with amino acids, vitamins, minerals, and antioxidants.

It appears from this study that the residual brewer's yeast, a by-product of the brewing industry and contains a large amount of proteins, carbohydrates, vitamins and minerals, which is why this residue can be used as an ingredient in the animal feed and as food supplements for an enhancement of this by-product from Ivorian breweries as it is in other countries.

COMPETING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

AUTHORS' CONTRIBUTIONS

N'Guessan Yevi Delphine. designed the study, performed the statistical analysis, Otchoumou Kraidy Athanase wrote the protocol, and wrote the first draft of the manuscript. Bedikou Ehuie Micaël managed the analyses of the study. Assemian Ines Christelle and Ehon Ayawovi Charlotte managed the literature searches. All authors read and approved the final manuscript.

REFERENCES

[1] Nayak A., Bhushan B. (2019), An overview of the recent trends on the waste valorization techniques for food wastes, J. Environ. Manage. 233, 352–370.

[2] Baiano A., (2014) Recovery of biomolecules from food wastes – a review, Molecules 19, 14821–14842.

[3] Caporale, G., Monteleone, E. (2004). Influence of information about manufacturing process on beer acceptability. Food Quality and Preference, 15 (3) 271-278

[4] Statista Research Department, (2023). Production mondiale de bière 1998-2021

[5] Mussatto SI. Biotechnological potential of brewing industry by-products. In: Singh nee' Nigam P, Pandey A, editors. Biotechnology for Agro-industrial Residues Utilization. Berlin, Germany: Springer; 2009. pp. 313-326

[6] Mathias T. R. S., Alexandre V. M. F., Cammarota M. C., Mello P.P. M. and Sérvulo E. F. C. (2015) Characterization and determination of brewer's solid wastes composition. Institute of Brewing & Distilling 121: 400–404

([7] Briggs DE, Boulton CA, Brookes PA, Stevens R (2004) Brewing: Science and Practice, Woodhead Publishing, Cambridge

[8] AOAC (1990). Official methods of analysis. Association of Official Analytical Chemists Ed., Washington DC, 684 p.

[9] (ISO1871 2009) International Organization for Standardization 1871. 2009. Food and feed products – general guidelines for the determination of nitrogen by Kjeldahl method. Available from: https://www.iso.org/fr/standard/41320.htm

[10] AOAC 938.02 (1938). Association of Analytical Communities 938.02. 1938. Glucose in cacao products.Virginia: Association of Official Analytical Chemists, Inc.

[11]. [ICUMSA] International Commission for Uniform Methods of Sugar Analysis. Method GS1-10. 1998. The determination of ash in raw sugar by single sulphation. Available from: https://www.icumsa.or g/methods/icumsa-method-gs1-10-1998/

[12] Folch J, Lees M, Sloane Stanley G H (1957). A simple method for the isolation and purification of total lipides from animal tissues. Journal of Biology and Chemistry, 226(1):497-509.

[13] Douny, C ; Tihon, A ; Bayonnet, P ; Brose, F ; Degand, G ; Milet, Jérôme Ribonnet, Laurence Larondelle, Yvan Scippo, M-l . (juni 2015) Validation of the Analytical Procedure for the Determination of Malondialdehyde and Three Other Aldehydes in Vegetable Oil Using Liquid Chromatography Coupled to Tandem Mass Spectrometry (LC-MS/MS) and Application to Linseed Oil. In: Food Analytical Methods, Vol. 8, no. 6, p. 1425-1435

[14] Soufleros E., Bertrand A. (1998) Evaluation d'une méthode CLHP adaptée au dosage des acides aminés du vin. Vitis 37 (1), 43-53

[15] European pharmacopoeia book (2014). European pharmacopoeia, 8th Edition, tome 1, p: 96-97

[16] Jedlicka A., Klimes J. 2005. Determination of Water- and Fat-Soluble Vitamins in Different Matrices Using High-Performance Liquid Chromatography. Chemistry Paper. 59 (3) 202-222

[17] Singleton, V. L., Orthofer, R., Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods in enzymology 299, 152-178

[18] Tanguler H, Erten H (2008). Utilisation of spent brewer's yeast for yeast extract production by autolysis: the effect of temperature. Food Bioproducts Processing. 86:317-321.

[19] Zhu, L.; Wang, J.; Feng, Y.; Yin, H.; Lai, H.; Xiao, R.; He, S.; Yang, Z.; He, Y. (2022) Process Optimization, Amino Acid Composition, and Antioxidant Activities of Protein and Polypeptide Extracted from Waste Beer Yeast. Molecules 27, 6825. https://doi.org/10.3390/ molecules27206825

[20] Vilela, E. S. D., Sgarbieri, V. C., Alvim, I. D. (2000a) Determination of the protein value of intact cells, total autolysate and yeast extract (Saccharomyces sp.). Annual Review of. Nutrition. 13, 185–192.

[21] Yamada EA, Alvim ID, Santucci MCC, Sgarbiere VC (2003). Proximate composition and protein value of residual yeast from ethanolic fermentation and its derivatives. Revista de Nutrição.16(4):423-432.

[22] Pinto, L.; Lopes, M.; Carvalho Filho, C.; Alves, L.; Benevides, C. (2013) Determination of the nutritional value of brewer's yeast (Saccharomyces spp.) derivatives. Brazilian Journal of Agroindustrial Products, 15, 7–17.

[23] Franco, G. (1989) Table of chemical composition of foods, 8th ed., pp. 92, 135, 164, 196, 197, Atheneu Editora, Rio de Janeiro.

[24] Amorim, M.; Pereira, J.O.; Gomes, D.; Pereira, C.D.; Pinheiro, H.; Pintado, M. (2016) Nutritional ingredients from spent brewer's yeast obtained by hydrolysis and selective membrane filtration integrated in a pilot process. Journal of Food Engineering, 185, 42–47.

[25] Berlowska J, Dudkiewicz-Kołodziejska M, Pawlikowska E, Piel- ech-Przybylska K, Balcerek M, Czysowska A, Kregiel D (2017) Utilization of post-fermentation yeasts for yeast extract production by autolysis: the effect of yeast strain and saponin from Quillaja saponaria. Journal of the Institute of Brewing 123:396–401

[26] Huige NJ (2006) Brewery by-products and effluents. *In*: Priest FG, Stewart GG (eds) Handbook of Brewing, 2nd edn. CRC Press, Boca Raton

[27] Chae HJ, Joo H, In M-J (2001) Utilization of brewer's yeast cells for the production of food-grade yeast extract. Part 1: Effects of different enzymatic treatments on solid and protein recovery and flavor characteristics. Bioresource Technology 76:253–258

[28] Man-Jin I, Dong CK, Chae HJ (2005). Downstream process for the production of yeast extract using brewer's yeast cells. Biotechnology and Bioprocess Engineering. 10:85-90.

[29] Caballero-Córdoba, G. M., & Sgarbieri, V. C. (2000). Nutritional and toxicological evalua- tion of yeast (Saccharomyces cerevisiae) biomass and a yeast protein concentrate. Journal of Science Food and Agriculture, 80(3), 341–351.

[30] Vieira, E., Brandao, T., & Ferreira, I. M. (2013). Evaluation of Brewer's spent yeast to pro- duce flavor enhancer nucleotides: Influence of serial repitching. Journal of Agricultural and Food Chemistry, 61(37), 8724–8729.

[31] Pancrazio G., Cunha C.S., Pinho G.P., Loureiro M., Meireles S., Ferreira O.V.L.P.M.I., Olívia Pinho O. (2016) Spent brewer's yeast extract as an ingredient in cooked hams. Meat Science 121, 382–389

[32] Sgarbieri VC, Alvim ID, Vilela ESD, Baldini VLS, Braganolo (1999). Produção piloto de derivados de levedura (*Saccharomyces* sp.) para uso como ingredients na formulação de alimentos. Brazilian Journal of Food Technology 2:119-125.

[33] Rakin M, Vukasinovic M, Siler-Marinkovic S. (2007) Contribution of lactic acid fermentation to improved nutritive quality vegetable juices enriched with brewer's yeast autolysate. Food Chemistry 100:599–602