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Nutritional Potential of Spent Brewer's Yeast, A Residual By-Product of Beer Production in Breweries for Future Applications

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Authors' contributions

This work was carried out in collaboration among all authors. Author's NGYD designed the study, performed the statistical analysis, author OKA wrote the protocol, and wrote the first draft of the manuscript. Author BEM managed the analyses of the study. Authors AIC and EAC managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The recovery of by-products from food processing chains represents an area of interest in the current world. Waste from beer production, such as beer lees or spent brewer's yeast, constitutes 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 and propose possible ways of recovery. The scientific methods have been applied to highlight the nutritional potential of this by-

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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 $5.54x10^{-2}$ mg/k, sodium $3.92x10^{-2}$ mg/k, and potassium $3.59x10^{-2}$ 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 the saturated category is palmitic acid, while oleic acid represents monounsaturated fatty acids, and polyunsaturated fatty acids are represented by linoleic acid and linolenic acid. Carbohydrates (32.77 %) are also important 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 has advantages for being incorporated into animal nutrition to meet nutritional needs. Further studies could lead to the production of activated carbon and hydrogen as excellent sources of alternative energy.

Keywords: Brewery waste; spent brewer's yeast; nutritional potential; added value.

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]. The largest percentage (26%) of food waste comes from the beverage industry [2]. 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]. Its global production is estimated at 1.86 billion hectoliters in 2021 [4]. “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].

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 the whole world, the rational management of waste from industrial activities is one of the environmental priorities of each 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, including residual brewer's yeast which is the second largest by-product of the food industry” [5]. These residues and by-products are usually underutilized, displaying little or no commercial value [1]. “Moreover, this waste has a high organic matter content and requires adequate treatment for its disposal, which entails considerable costs” [7,1]. 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.

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 to characterize the beer lees from breweries in Côte d'Ivoire and to propose possible ways of recovery.

2 MATERIALS 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 the Chemical Composition of Spent Brewer's Yeast

2.1.1 Moisture

The water content of the spent brewer's yeast was determined by using the oven drying method described in the Association of Official Analytical Chemists (AOAC) procedure [8]. Spent brewer's yeast samples (10.00 g) were weighed out (W_1) in porcelain dishes 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 (W_2). The moisture contents of the samples were obtained from the weight difference ($W_1 – W_2$), and the percent weight losses were calculated, accordingly.

2.1.2 Crude proteins

The crude proteins of the spent brewer's yeast samples were calculated by the Kjeldahl method [9]. Nitrogen from nitrates and nitrites was not considered by using this dosing principle. The dried sample (0.50 g) was first digested by heating in strong sulfuric acid (15.00 ml) in the presence of a catalyst (1.00 g) which helps in the conversion of the amine nitrogen to ammonium ions. This digestion step ended in 30 min with the appearance of green coloration. After digestion, the ammonium ions were dissolved in distilled water (250.00 ml) containing 5 drops of phenolphthalein and 1 M sodium hydroxide (75.00 ml). The contents were heated and distilled. The liberated ammonia gas was led into a trapping solution, (10 g/l) Boric acid (10.00 ml) mixed with a few drops of a mixed indicator dye (methyl red + bromocresol green), where it dissolved and became an ammonium ion once again. Finally, the amount of the ammonia that had been trapped was determined by titration with a 0.1 N hydrochloric acid.

2.1.3 Carbohydrates

The beer lees carbohydrates were quantified by using the standard method of AOAC [10] the sample (3.00 ml) was added with acetonitrile (2.00 ml) and sodium chloride (0.10 g). The mixture was vortexed and centrifuged at 5,000 rpm for 10 min. The supernatant (100 µl) was withdrawn and evaporated to dryness under vacuum. Dichloromethane (DCM; 100 µl) was added to the dried sample, and the evaporation process was repeated. Bis (trimethylsilyl) trifluoroacetamide (BSTFA; 100 µl) was added, and the whole mixture was dried in an oven at 80 °C for 30 min, and the resulting solution was made up to 1.5 ml with acetonitrile, and vials were subjected for chromatographic analysis.

2.1.4 Sugars (glucose, fructose, and sucrose)

Simple sugars were quantified according to the AOAC method [10].

Free sugars content: The beer lees sample (10.00 g) was dissolved in distilled water (100.00 ml). The dissolved sample (10.00 ml) was taken into an Erlenmeyer flask (250.0 ml) and 5 mM iodine (20.00 ml) and 2 M sodium hydroxide (5.00 ml) were added. After homogenization, the mixture was kept in the dark for 20 min, and hydrochloride acid (7.00 ml) was added and titrated using 0.1 N of sodium thiosulfate.

Total sugars: The beer lees sample (1.00 g) was dissolved in 2 M hydrochloric acid (50.00 ml) and boiled for 30 min. The solution (10.00 ml) was boiled again for 30 min with 2 M hydrochloride acid (40.00 ml). After cooling, the mineralized solution (10.00 ml) was mixed with 5 mM iodine (20.00 ml) and 1 M sodium hydroxide (10.00 ml). The mixture was kept in the dark for 20 min and hydrochloride acid (7.00 ml) was added and titrated using 0.1 N of sodium thiosulfate.

2.1.5 Ash and inorganic elements

The minerals were quantified by following the International Commission for Uniform Methods of Sugar Analysis (ICUMSA) method [11]. The previously dried sample (2.00 g) was 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], phosphorus [13], magnesium [14], copper [15], calcium [16], potassium and sodium [17]. The ashes obtained from sample of dry beer lees (2.00 g) were dissolved successively in nitric acid (2.00 ml) and distilled water (98.00 ml). Most of metal ions were quantified by gas chromatography (GC) coupled with an atomic Absorption spectrophotometer (AAS). Iron: The acidic solution was first neutralized (pH 7.0) using a 2 M NaOH. To 10 ml of the neutralized solution, 100 µl of a FerroVer Iron reagent was added, mixed vigorously and then, kept for a while and spectrophotometrically quantified.

Phosphorus: 1 g of dried molasses sample in 5 mL of a Mg (NO3)2 solution was boiled at 105 °C for 30 min. The homogenized mixture was incinerated at 500 °C for 3 h and the ash obtained was dissolved in 2 ml of a hydrochloric acid solution and made up to 50 ml with distilled water. Then, 20 ml of the resulted solution was withdrawn, and the pH was adjusted to the range 3-10 by adding 2 N sodium hydroxide (NaOH). The half of this solution was used for blank while

the second part, was mixed with a prepacked packet of PhosVer3 phosphate reagent, homogenized and allow to react for 3 min the absorbance value was measured at 880 nm.

2.1.6 Fatty acid profile determination

2.1.6.1 Fat extraction

The samples were minced, homogenized, and weighed. The extraction of total fat was carried out on sample (2.00 g) using a liquid extraction based on the Folch method (chloroform/methanol 2/1 v/v) [18].

2.1.6.2 Esterification of fatty acids

The fatty acid profile was determined by analysis of fatty acid methyl esters by gas chromatography-mass spectrometry (GC-MS) according to the modified method of [19]. The fat (50.00 mg) extracted using Folch's method was mixed with hexane (5.00 ml) and resulting mixture (10.00 µl) was used for the saponification/. Nonadecanoic acid (C19:0), used as an internal standard, was 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 shaker. Then, 5% sodium chloride was added, and the fatty acid methyl esters (FAMEs) were extracted twice using hexane. The extract was then washed with 2% (w/v) potassium carbonate and sodium sulfate was added to part of the extract. The final extract was evaporated to dryness to completely remove the toluene. Hexane (400.00 µl) was added, and the tube was vortexed. Finally, the sample was transferred to an injection vial.

2.1.6.3 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 $ml \cdot min^{-1}$; 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 $10°C \cdot min^{-1}$ 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, 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 was 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 with a modification of the elution gradient and flow rate, was used for the determination of the amino acid profile [20]. The dry sample (5.00 g) was mixed in 0.2 N sodium citrate (20.00 ml; pH 2.3). The protein hydrolysis was carried out using 6 N hydrochloric acid (10.00 ml) at 110 °C for 24 hours. After evaporation of the acid, the sample was collected in 70% ethanol (10.00 ml) and filtered on a Millipore filter, before injection into High-performance liquid chromatography (HPLC).

Waters Alliance HPLC device (model e2695), equipped with an automatic 48-sample collection system and a p2895 pump system was used in the current study. The separation of amino acids was carried out using two columns in a series, of the Lichrocart (125-4 cartridge type containing a Lichrospher 100 RP18 column). The length of each of these columns was 12.5 cm, and the diameter of the particles was 5 µm. A pre-column of the same type had been placed at the beginning of each column. The detection was made using a Waters 2475 spectrofluorometer. The excitation was made at the wavelength of 340 nm and the emission at 450 nm.

The Reference solution consisted of 20 amino acids prepared at different concentrations by making dilutions with a hydro-alcoholic solution at 10% vol. Each series contained 6 concentrations. The samples and the reference solutions were introduced into glass vials (2.0 ml) and vials were placed in the autosampler/.

2.1.8 Vitamin dosage

2.1.8.1 Water-soluble vitam ins

The vitamin content of the samples was determined by high performance liquid chromatography technique using the method described by European pharmacopoeia book [21].

The sample (2.00 g) was placed in 0.1 N acid sulfuric (25.00 ml). 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 filter paper, and the filtrate was diluted with distilled water (50.00 ml) and filtered again through a Micropore filter (0.45 µm). The filtrate (20.00 µl) was 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 [22].
The sample (1.00 g) was added 10% KOH (10.00 ml) in methanol: water (1:1, v/v) mixture. To avoid the oxidation process during saponification, ascorbic acid (0.025 g) was added. The mixture was brought to reflux in a water bath at 70 °C. for 30 min. The mixture was cooled and extracted with 3 x 5 ml of hexane. The hexane phases were combined and dried over anhydrous sodium sulphate and evaporated to dryness. The residue obtained (approximately 0.30 g) was taken up in methanol (10.00 mL) for further analyses.

The evaluation of fat-soluble vitamin content was made by HPLC coupled to a fluorometric detector. The analysis was 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 was 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. Fluorometric detection was done at: 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 [23].

The dried yeast sample (1.00 g) was homogenized in 70% (v/v) methanol (10.00 ml). The mixture obtained was centrifuged at 1000 rpm for 10 min. The pellet was recovered in f 70% (v/v) methanol (10.00 ml) and centrifuged again. The supernatants were pooled into a Falcon tube.

The methanolic extract (200.00 µl) was introduced into a test tube and added with Folin-Ciocalteu reagent (200.00 µl). The tube was left to stand for 3 min, 20% (w/v) sodium carbonate (200.00 µl) was added. The contents of the tube were added with distilled water (1.40 ml) and the mixture was placed in dark for 30 min. The absorbance reading was taken with a spectrophotometer, at 760 nm, against a blank. A standard range was established from a stock solution of 0.1 mg/ml gallic acid under the same conditions as the test, making it possible to determine the quantity of polyphenols in the sample.

3. 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” [24].

The main organic compounds of the dehydrated residual brewer's yeast studied are summarized in Table 1. The results show that proteins (44.77%) were 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 and it tallies with the previous findings [5]. “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 content was not greater than 41.928% [25] while [6] obtained 52.7%. According to previous published data [26,27,28] protein content was ranged from 35.2 to 47.2%, on dry matter basis. [29] 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 [30]. "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].

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 [31].

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. 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%) is 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" [5,27]. The richness of this by-product has been proven by other researchers as well [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 corroborated 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 beer production.

In addition, this waste contains other elemental 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.

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).

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 [32]. This by-product can be used as vitamin supplements in natural foods [33].

"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)" [7,34]. "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" [33].

Table 1. Descriptive statistics on the proximate composition of beer lees

Item	Average	Standard deviation
Moisture content (%)	11.83	0.70
Crude proteins (%)	44.77	0.06
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

Table 2. Descriptive statistics on amino acid profile 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

Table 3. Descriptive statistics on inorganic compounds 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

Table 4. Descriptive statistics on fatty acids profile 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

Table 5. Descriptive statistics on vitamin 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

Authors [35] 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 [36] 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". [37] 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, [38] 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".

[33] report that yeast extract is used in microbiological media as a source of nutrients. Thus, the work of [39] 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".

5. CONCLUSION

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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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