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ORIGINAL RESEARCH PAPER

EVALUATION OF THE FATTY ACIDS AND AMINO ACIDS PROFILES IN SPENT GRAIN FROM BREWING AND MALT WHISKY

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Abstract: The food industry generates huge amounts of food by-products annually, most of which are considered waste. But these by-products may contain nutrients of interest and have a low-cost price. Capitalizing on these by-products is of interest to both producers and consumers, thus supporting the circular economy. In this context, the current paper aimed to evaluate the fatty acid profile and the amino acid profile of spent grain from distilleries compared to spent grain coming from the beer industry. The main fatty acids were identified linoleic, palmitic, and oleic acids. Amino acids are necessary for the production of enzymes, hormones, and even some neurotransmitters, being considered the most important nutrients of the human body. The most abundant essential amino acid is valine in both samples analyzed and the lowest level of essential amino acid is tryptophan for distilleries spent grain (DSG) and leucine for brewery spent grain (BSG).

Keywords: *alcoholic beverages, by-products, Gas chromatography-mass spectrometry (GS-MS), nutritional value, valorization*

INTRODUCTION

Whisky is one of the most popular and consumed alcoholic beverages globally, the proper distillation industry has recently grown in some countries such as Ireland and the UK [1, 2]. Whisky consumption per capita varies by country with France in the top with about 2 L per person and year, while India is the country with the highest total consumption [3]. In Romania, whisky has been produced recently by Alexandrion Group Company which is in the maturation step [4]. The whisky production process generates large amounts of by-products that contain high levels of chemical oxygen consumption (COD), biological oxygen consumption (DBO), phosphorus, ammonia, metal ions such as copper and iron, as well as complex organic materials such as lignin, yeast cells, etc. For each liter of alcohol produced it results in approximately 2.5 kg of spent grain (SG) or draff (assuming that the alcohol yield is 407 L of alcohol produced per tonne of malt) and the spent grain moisture is 74 - 80 % [5]. The management of these by-products raises significant economic and environmental problems in whisky production [6].
Beer is also one of the most consumed drinks and the production of brewer's spent grain accounts for about 39 million tonnes per year [7]. 20 kg of wet BSG are generated for every 100 L of produced beer [8, 9]. BSG is available in large quantities since the worldwide annual beer production was about 1.95 million hL [10].
Spent grain is a by-product with a complex heterogeneous structure resulting from the process of filtering the wort [11]. The process of making whisky can be divided into six main stages: grinding, malting, mashing, fermentation, distillation, and maturation [12], while in the brewing process, filtered liquor is subsequently converted into beer, and the sediment is represented by brewers spent grain (Figure 1).

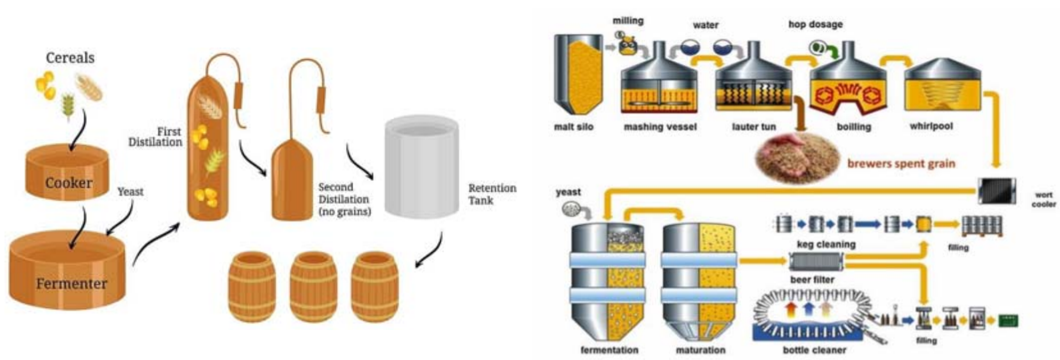

Figure 1. *The production process of malt whisky vs brewing [13]*

Spent grain is often used for animal feed due to both its nutritional content and low cost. It is used either in wet or conventionally dried form [9, 10]. The proteins in SG can be used as a substitute for fishmeal or soy flour in feed formulations [5]. Another use of SG is the generation of renewable energy to reduce the carbon footprint of alcohol production [16, 17]. The distilleries have also invested in combined plants to produce heat and electricity with spent grain pressed as fuel [18]. Spent grain contains carbohydrates up to 50 - 60 % of the dry matter consisting of carbohydrates, including glucans, starch, cellulose, and arabinoxylans. By converting these carbohydrates, various biochemical products and biofuels can be produced [19, 20]. Other products have also been obtained from spent grain through various biotechnological processes: lactic acid, xylitol, microbial enzymes, biopesticides [16, 17, 23]. Spent grain contains kernel shell, pericarp, and seed sheath, with high levels of cellulose (16.8 - 25.4 %), hemicellulose (mainly arabinoxylans) (21.8 - 28.4 %), lignin (11.9 - 27.8 %), protein, and fiber. The proteins in spent grain contain valuable amino acids of which the most abundant amino acids were glutamine/glutamate and proline [20]. In addition, spent grain were evaluated as a promising source of lipids among which triglycerides (67 % of the total extract) were predominant, followed by a series of free fatty acids (18 %) and also smaller amounts of monoglycerides (1.6 %) and diglycerides (7.7 %). Among the fatty acids linoleic (18:2), palmitic (16:0), and oleic acids (18:1) and small amounts of other fatty acids such as stearic (18:0) and linolenic (18:3) acids have been identified in spent grain [4, 21, 24].

It is necessary to add value to this by-product and to develop sustainable production methods at low cost to make it economically attractive and to ensure the continued availability of the protein and lipid component in the production of new food products [25].

The main purpose of this manuscript is to perform a comparative analysis of the profile of fatty acids and amino acids present in the spent grain samples.

MATERIALS AND METHODS

Distillery's spent grain (DSG) was kindly provided from Alexandrion Group Romania (Ploiesti, Romania) and brewers' spent grain (BSG) was purchased from a local brewery, SC Bermas SA (Suceava, Romania). Spent grain was stored at -18 °C and then dried at 50 °C for 26 hours to the moisture of 5 %. Dried spent grain was grounded and sieved through a Retsch Vibratory Sieve Shaker AS 200 basic (Haan, Germany) for 30 minutes at amplitude 70 to obtain spent grain flours. The grinded spent grain was stored in paper bags at room temperature until further use. All chemicals used in the present study were of analytical grade and were purchased from Sigma Aldrich (St. Louis, MO, USA).

Analysis of fatty acids methyl esters (FAMEs)

The fatty acids separation and quantification were made on gas chromatography–mass spectrometry (GC-MS) equipment from Shimadzu (GC-MS-QP 2010 Plus, Shimadzu, Kyoto, Japan) based on the method described by Oroian, Ursachi, & Dranca, 2020 [26]. Fatty acid derivation was made by the following procedure: 0.1 g of oil from spent grain (extracted with n-hexane) was mixed with 0.4 mL of n-hexane and 0.4 mL of 15 % BF_3 in methanol. The mix was heated at 60 °C for 15 min in a water bath, then cooled at room temperature, mixed with 2 mL of saturated NaCl solution, and centrifuged for 5 min at 3000 rpm. The supernatant was filtered through a 0.45 μm syringe filter. The initial oven temperature was 100 °C, and the injection temperature was 220 °C. The flow rate of the carrier gas (helium) was 0.83 $mL \cdot min^{-1}$ and the split ratio value was 50. A sample of 0.5 mL was injected into a 60 m × 0.25 mm i.d., 0.25 μm film thickness SUPELCOWAX 10 capillary column (Supelco Inc. Bellefonte, PA, USA), and the injector temperature were set at 220 °C. The mass scans were performed within the range of m/z: 25 - 500 at a rate of 0.14 scan·s^{-1} with an intermediate time of 0.02 s between the scans. The identification of FAMEs was accomplished by comparing their retention times with those of known standards and the resulting mass spectra to those in the database (NIST MSSearch 2.0). The composition of fatty acids was expressed as µg·mL^{-1} spent grain and as relative level (%) in fatty acids composition. Each measurement was made in triplicate.

Free amino acids determination

For analyzed free amino acids were mixed 0.7 g of sample with 6 mL of 15 % trichloroacetic acid, then *p*H was adjusted to 2.2 and the extract was diluted to 10 mL with 15 % trichloroacetic acid. Samples were centrifuged at 3000 rpm for 5 min, an adapted method from Dabadé et al., 2021 [27]. The supernatant was filtrated through a 0.45 µm syringe filter was derivatized using faast Amino Acid Analysis Kit (Phenomenex Inc, Torrance, CA, USA) [28]. Amino acids analysis was made with a GC-MS-QP2010 Plus, Shimadzu gas-chromatograph coupled with a mass spectroscopy instrument (Shimadzu, Kyoto, Japan) using a Column Zebron TM ZB-AAA.
The initial temperature of the GC oven was 110 °C, which was increased until 320 °C and held for three minutes. The injection temperature was 250 °C, total flow was 64.2 mL·min^{-1}, and column flow was 3.83 mL·min^{-1}. The quadruple measured the abundance of ions from 35 to 500 m/z. Faast Amino Acid Analyis Kit included solutions with amino acids mixture, used for the calibration.

RESULTS AND DISCUSSION

Fatty acids

The methyl esters of the fatty acids in the oil resulted from spent grain were determined using GC-MS, being quantified 13 fatty acids from the DSG and 16 fatty acids in the BSG, respectively, presented in Table 1, compared to the study conducted by Ferreira et al., 2021, which reported 6 fatty acids in the BSG sample [29]. The most abundant saturated fatty acid was palmitic acid in both DSG and BSG, of the mono-saturated fatty acids cis-9-oleic acid was highlighted in both samples, and linoleic acid was the most found polyunsaturated acid. These data agree with previously published work by Niemi et al., 2012, Fărcaș et al., 2015, and Almeida et al., 2017 [30 – 32]. Ferreira et al., 2021 highlighted in their study that BSG contains methyl linoleate in the highest percentage: 53.9 %, which is in according with the results obtained in this study [29]. Becker, 2007 founded that spent grain has a fatty acid content similar to the barley it comes from, so minor changes occur during malting and mashing [33]. The lowest levels of fatty acids were recorded by caproic acid and lauric acid for DSG, respectively lauric acid and 11-eicosenoic acid for BSG. In Figure 2 and Figure 3 are presented gas-chromatographic profiles of fatty acids of DSG and BSG.

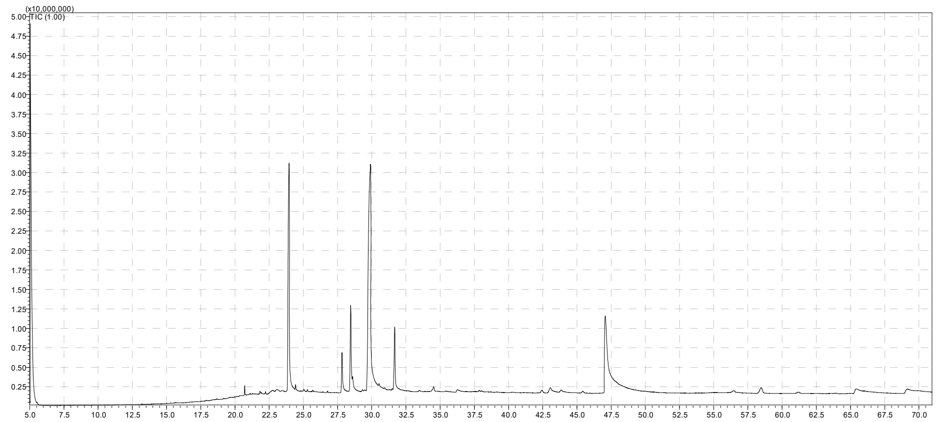

Figure 2. *Gas-chromatographic profiles of fatty acids from DSG*

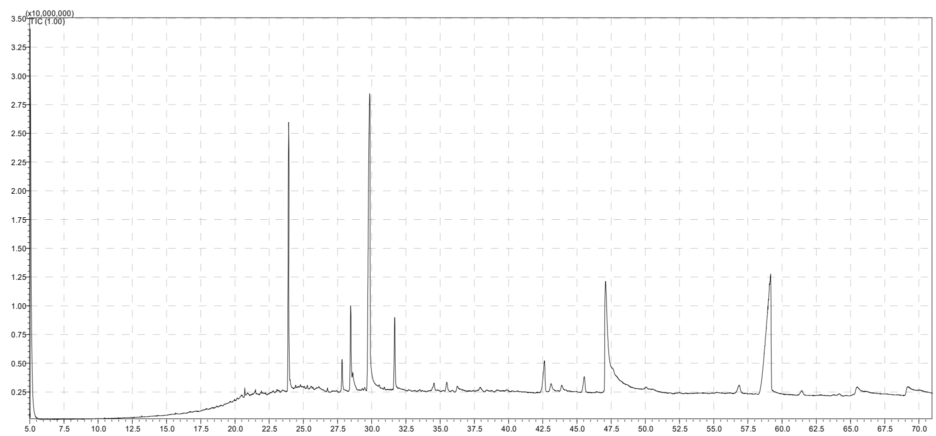

Figure 3. *Gas-chromatographic profiles of fatty acids from BSG*

The saturated fatty acids represented 16.94 % for DSG and 16.38 % for BSG, an appropriated percent for both samples, while unsaturated fatty acids represented 83.03 % for DSG, respectively 83.62 for BSG (poly-unsaturated fatty acids represented 64.22 % and 71.54 %, respectively). Eight saturated fatty acids (SFAs) were found for DSG and nine saturated fatty acids (SFAs) were found for BSG, with palmitic acid being the predominant one in both analyzed samples, but with an increased concentration for brewery spent grain, identified as double comparative with distillers spent grain. Regarding monounsaturated fatty acids (MUFAs), two compounds were found for DSG, four for BSG respectively, the most abundant being oleic acid in both samples. Concerning polyunsaturated fatty acids (PUFAs), three compounds were found, standing out linoleic acid as major compound in both samples. The role of fatty acids in the body is to facilitate communication between the cells of the body and to control inflammatory reactions. They are vital for the proper functioning of the brain and for the improvement of vision.

Table 1. *Fatty acid content from DSG and BSG*

Fatty Acid	Fatty Acids Trivial Name	Type	Distillery spent grain [µg·mL^{-1}]	Relative level [%]	Brewers spent grain [µg·mL^{-1}]	Relative level [%]
Hexanoic acid C6:0	Caproic acid	SFA	1.69 ± 0.02	0.03	-	-
Dodecanoic acid C12:0	Lauric acid	SFA	1.84 ± 0.01	0.03	1.15 ± 0.01	0.01
Tetradecanoic acid C14:0	Myristic acid	SFA	107.15 ± 0.03	1.61	49.48 ± 0.02	0.58
z-11-Tetradecenoic acid C14:1ωc	Myristoleic acid	MUFA	113.23 ± 0.05	1.71	-	-
Pentadecanoic acid C15:0	Pentadecylic acid	SFA	94.80 ± 0.02	1.43	10.13 ± 0.01	0.12
Hexadecanoic acid C16:0	Palmitic acid	SFA	1792.44 ± 0.07	8.99	3173.69 ± 0.03	12.42
Octadecanoic acid C18:0	Stearic acid	SFA	413.33 ± 0.03	3.11	386.01 ± 0.01	2.27
cis-9-Octadecenoic acid C18:1ωc	Oleic acid	MUFA	758.50 ± 0.05	17.13	663.60 ± 0.02	11.69
9,12-Octadecadienoic acid C18:2ω6c	Linoleic acid	PUFA	2726.98 ± 0.08	41.43	4269.05 ± 0.06	50.61
Eicosanoic acid C20:0	Arachidic acid	SFA	18.04 ± 0.01	0.27	23.17 ± 0.01	0.27
9,12,15 Octadecatrienoic acid C18:3	Linolenic acid	PUFA	488.72 ± 0.02	7.44	688.51 ± 0.03	8.18
Henoicosanoic acid C21:0	Heneicosylic Acid	SFA	96.87 ± 0.01	1.47	23.38 ± 0.01	0.28
Decosa-13,16-dienoic C22:2	Decosadienoic Omega 6 acid	PUFA	1019.40 ± 0.03	15.35	1085.57 ± 0.03	12.75
Hexadec-9-enoic acid C16:1cis9	Palmitoleic acid	MUFA	-	-	20.15 ± 0.02	0.24
Heptadecanoic acid C17:0	Margaric acid	SFA	-	-	11.36 ± 0.01	0.13
Heptadecenoic acid C17:1cis10	cis-10-Heptadecenoic acid	MUFA	-	-	3.31 ± 0.01	0.08
11-Eicosenoic acid C20:1	Methyl cis-11-eicosenoate	MUFA	-	-	5.98 ± 0.01	0.07
Tricosanoic acid C23:0	Tricosylic Acid	SFA	-	-	25.73 ± 0.01	0.30
Total fatty acid content			7633.005 ± 0.03	100.00	10440.28 ± 0.04	100.00
Saturated fatty acids SFA (%)			2526.16 ± 0.04	16.94	3704.1 ± 0.02	16.38
Unsaturated fatty acids UFA (%)			5106.845 ± 0.02	83.06	6736.18 ± 0.03	83.62
Mono-unsaturated fatty acids MUFA (%)			3598.725 ± 0.03	18.84	4962.1 ± 0.04	12.08
Poli-unsaturated fatty acids PUFA (%)			1508.12 ± 0.02	64.22	1774.08 ± 0.02	71.54
UFA/SFA ratio			2.02 ± 0.04	4.90	1.82 ± 0.03	5.11

Amino acids

Free amino acids contribute to nutritional value, but also the aromatization and / or interaction with sugars in the Maillard reaction [34]. The sweet taste is given by alanine, glycine, methionine, proline, serine, and threonine. The bitter taste is given by histidine, isoleucine, leucine, lysine, phenylalanine, and valine, while aspartic acid and glutamic acid are responsible for the taste of umami. Tyrosine gives a sour taste.

Table 2. *Amino acid content of DSG and BSG*

Amino Acid	Distillery spent grain [nmol·g^{-1}]	Brewers spent grain [nmol·g^{-1}]
Hystidine (Hys)*	1203.78 ± 0.02	-
Methionine (Met)*	730.09 ± 0.03	-
Phenylalanine (Phe)*	4355.32 ± 0.02	455.72 ± 0.03
Tryptophan (Trp)*	663.79 ± 0.06	-
Alanine (Ala)	4428.57 ± 0.02	555.89 ± 0.02
Glycine (Gly)	1136.67 ± 0.04	10.04 ± 0.03
Proline (Pro)	4191.95 ± 0.03	697.96 ± 0.03
Serine (Ser)	2397.79 ± 0.04	774.86 ± 0.05
Leucine (Leu)*	4197.84 ± 0.01	290.64 ± 0.04
Glutamine (Glu)	824.42 ± 0.02	4172.62 ± 0.03
Asparagine (Asp)	627.80 ± 0.03	467.58 ± 0.04
α-aminobutyric acid	503.95 ± 0.02	-
Valine (Val)*	4469.72 ± 0.03	1236.46 ± 0.02
Isoleucine (Ile)*	3747.83 ± 0.03	587.41 ± 0.01
Threonine (Thr)*	1348.23 ± 0.02	-
Lysine (Lys)*	825.06 ± 0.01	276.52 ± 0.02
Aspartic acid (AAsp)	8704.22 ± 0.06	8802.8 ± 0.06
Glutamic acid (AGlu)	13255.07 ± 0.02	11307.93 ± 0.04
α-aminopimelic acid (AAminop)	29292.78 ± 0.04	3615.95 ± 0.02
Hidroxylysine	2456.1 ± 0.02	-
Proline-hydroxyproline	6088.59 ± 0.01	700.19 ± 0.03
Tyrosine (Tyr)	1616.22 ± 0.03	611.85 ± 0.04
Total amino acid content	97065.79 ± 0.06	34564.42 ± 0.09
Essential amino acids	21541.6 ± 0.03	2846.75 ± 0.04
Conditional amino acids	68070.61 ± 0.04	26023.24 ± 0.05
Nonessential amino acids	8278.58 ± 0.07	5970.95 ± 0.02

*Essential amino acids

From Table 2 it can be seen that spent grain from distilleries has a more pronounced sweet taste than spent grain from the beer industry, methionine and threonine being present only in DSG, but all amino acids responsible for the sweet taste were present in higher quantities than BSG. Amino acid responsible for the bitter taste is present in both samples, histidine being present only in the DSG. The values of aspartic acid and glutamic acid are similar for the two samples. Tyrosine is found in greater quantity in DSG compared to BSG. In Figure 4 and Figure 5 are presented gas-chromatographic profiles of amino acids of DSG and BSG.

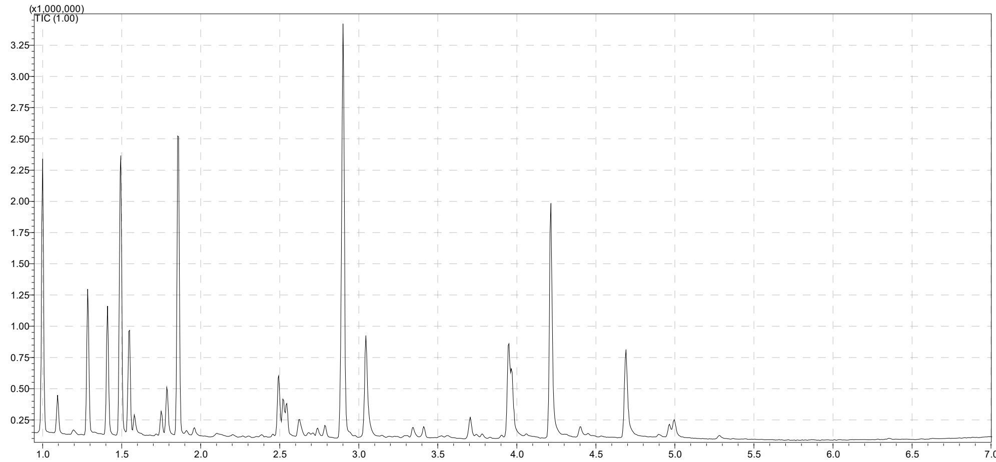

Figure 4. *Gas-chromatographic profiles of DSG*

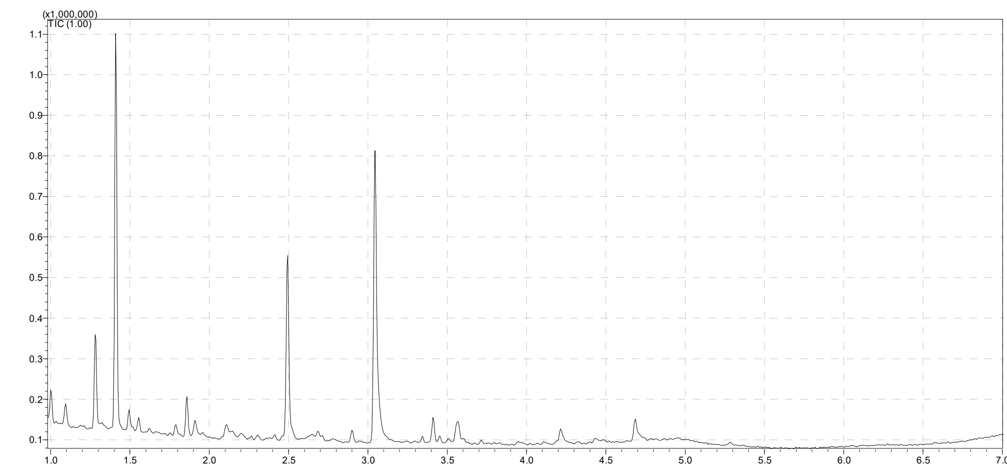

Figure 5. *Gas-chromatographic profiles of BSG*

Essential amino acids are represented in the ratio of 19.95 % in spent grain from distilleries, while in spent grain from the beer the percentage is lower, at 7.44 %. At the same time, nonessential amino acids are represented in a percentage of 8.53 % in spent grain from distilleries and 17.27 % in that from breweries. The most abundant essential amino acid is valine in both samples analyzed, followed by phenylalanine, leucine, and isoleucine in different ratios (valine > phenylalanine > leucine for DSG, and valine > isoleucine > phenylalanine for BSG). The lowest level of essential amino acid is tryptophan for DSG and leucine for BSG. Essential amino acids are those amino acids that cannot be synthesized by the human body, so their intake must take place in a balanced way. Valine plays an important role in maintaining emotional balance and the ability to concentrate, isoleucine helps heal wounds and supports the production of hormones in the body, leucine has an essential role in the production of growth hormones, and phenylalanine stimulates the body to use other amino acids, proteins, and enzymes. Amino acids contribute to health, especially through their content in essential amino acids, which makes spent grain ideal for improving the protein quality of food.

Jin et al., 2022 showed in their study of brewers' spent grains obtained from craft brewing operations that tyrosine is the most abundant amino acids in craft beers, and the most abundant amino acids essential were leucine, phenylalanine, and methionine, accounting for 56.5 % of the total essential amino acids [34]. Stein et al., 2006 were analyzed in their study 10 samples of distillers spent grain according to their amino acid profile. 5 samples had the most abundant amino acid leucine, 3 samples methionine, a sample tyrosine, and a sample tryptophan [35]. Batal & Dale, 2006 obtained an average of 8 samples of distillery spent grain where glutamine had the highest share, followed by leucine and proline [36].

CONCLUSION

For an industrial use as efficient as possible it is necessary to know the chemical composition of the spent grain, in particular the profile of fatty acids and amino acids. The two spent grain samples contain in the highest amount palmitic, oleic and linoleic acid, both in the case of samples from distilleries and those from the beer industry. High protein content and a good amino acid profile can provide an economical alternative with good nutritional properties of spent grain. In the present work, we have successfully applied the Phenomenex EZ:faast^{TM} amino acid analysis kit for gas-chromatographic (GC) determination of spent grain amino acid concentrations. Among the advantages of this method are: simple, fast, sensitive method, without prior removal of proteins, urea, or other interfering substances. Research conducted lately has suggested a wide range of possible applications of spent grain, including in value-added food products and pharmaceuticals, highlighting the importance and opportunities of reusing this agro-industrial by-product.

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