Romanian Biotechnological Letters *Vol. 19, No. 5,2014*

ORIGINAL PAPER

NUTRITIONAL PROPERTIES AND VOLATILE PROFILE OF BREWER'S SPENT GRAIN SUPPLEMENTED BREAD

Received for publication, August 20, 2014
Accepted, October 10, 2014

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Abstract

The brewer's spent grain is a low-cost by-product of the brewing process but at the same time it is a valuable source of dietary fibre, protein and essential amino acids, minerals, polyphenols, vitamins and lipids. The aim of this research was to incorporate the brewer's spent grain, provided from black beer production, into a simple bread formulation and to evaluate its contribution to the nutritional composition, volatile profile as well as on the sensorial properties of the enriched bread. The substitution of wheat flour with 5%, 10%, 15% and 20% brewer's spent grain flour resulted in bread formulations with enhance nutritional value (increased fibre, protein, fat and minerals content) and flavour attributes imparted by the characteristic volatile compounds. The sensorial analysis revealed that, compare with the 100% white flour bread, the bread formulations with up to 10% added brewer's spent grain flour had good organoleptic attributes and overall acceptability, indicating the opportunity of using this by-product as a fortifying ingredient in bakery products.

Keywords: brewer's spent grain, bread, volatile compounds, nutritional value, functional ingredient

1. Introduction

Nowadays, it is shown an increasing interest in stimulating people to consume healthy foods - known as functional foods, i.e. foods containing ingredients that provide additional health benefits beyond basic nutritional requirements (MANEJU & al. [1]). Functional foods are products that contain biologically active compounds that positively affect key functions of human organism, are relevant to human health or may reduce the risk of chronic diseases (MUREȘAN & al. [2]).

The consumers' preference for white bread resulted in the removal of 70-80% of the initial nutrient content of wheat due to grinding and sieving processes. In particular, the elimination of fibres from the diet resulted in an increased incidence of cardiovascular diseases and gastrointestinal disorders (WINDER & al. [3]). In terms of nutrition, bread reflects largely the nutritional value of flour and other raw materials from which it is obtained, therefore, it is necessary to continuously improve its nutritional and organoleptic attributes. Being one of the most widely used food products in the huma's diet (FAO STAT [4]), through bread one can achieve an efficient way to supplement the diet with biologically active compounds.

Brewer's spent grain (BGS) is a by-product of the brewing process, consisting of the solid residue remaining after mashing and lautering. It primarily consists of grain husks and other residual compounds not converted into fermentable sugars in the mashing process (XIROS & CHRISTAKOPOULOS [5]). Traditionally this material is either discarded or sold as animal feed. On the other hand, the food industry is seeking to find new added-value applications that will change the traditional view on 'waste' products and re-classify them as 'co-products'. Using the brewer's spent grain by-product, which has a low monetary value, as a high-nutrient functional ingredient may enhance the economic potential of brewhouse and improve the dietary attributes of different food formulations (WATERS & al. [6]).

In the brewery, malted barley is milled, mixed with water in the mash tun and the mash temperature is then slowly increased from 37ºC to 78ºC to promote the enzymatic hydrolysis of malt constituents. The sweet liquid produced during this mashing stage is known as wort. The insoluble, un-degraded part of the malted barley grain is allowed to settle to form a bed in the mash tun and the sweet wort is filtered through it (LINKO & al. [7]). After the saccharification process is finished, the clear sweet wort is separated from the solid components - the spent grain. Then, the wort is transferred into the wort kettle, while the spent grain is removed from the lauter tun.

Beside its nutritional content, one of the main advantages of using BSG in human diet is that the brewing process uses ingredients approved for human consumption. Therefore, this particular by-product can be successfully used for the development of new food products that can meet full health regulatory approval (MANDALARI & al. [8]).

The main goal of bread fortification with BSG is to obtain a product with highly desirable nutritional characteristic from a human dietary standpoint. The scientific research has revealed that even though the chemical composition of BSG may be influenced by some intrinsic (barley variety) or extrinsic (harvest time, malting and mashing conditions and the type and quality of secondary raw materials added in the brewing process) factors, always include considerable amounts of dietary fibre, protein and essential amino acids as well as appreciable levels of minerals, polyphenols, vitamins and lipids (SANTOS & al. [9], MUSSATTO & al. [10]).

BSG is an excellent source of dietary fibre (60-71%). Cellulose and hemicellulose together comprise almost 50% (w/w) of the BSG composition, revealing the presence of a large amount of sugars in this material, with xylose, glucose and arabinose being the most abundant (MUSSATTO & ROBERTO [11]). Nutrition studies showed that the intake of dietary fibre (i.e. lignin, cellulose and hemicelluloses) serve different functions in the human organism exerting health-promoting effects, including lowering the risk of cardiovascular diseases, constipation, irritable colon, colon cancer, obesity and diabetes (RODRIGUEZ & al. [12], MAKOWSKA & al. [13], KAMALJIT & al. [14]).

The BSG is also a source of valuable protein, the essential amino acids representing approximately 30% of the total protein content. Lysine, known to be the limiting amino acid in cereal derived foods, accounts for 14.3 % of the total BSG protein content (YOUNG & PELLETT [15], WATERS & al. [6]). Taking in consideration the aforementioned nutritional composition, the BSG is an ideal candidate for bread fortification.

The aim of the present study was to evaluate the contribution of BSG flour to the nutritional composition, volatile profile as well as on the sensorial properties of the enriched bread. Thus, by substituting different levels of wheat flour with BSG flour (5, 10, 15 and 20%), four types of breads were obtained and analyzed in order to assess the overall acceptability of BSG supplemented bread compared to the 100% wheat bread.

2. Material and Methods

Material

All the materials (malt, brewer's spent grain) were supplied by the Microbrewery of the Faculty of Food Science and Technology of UASVM Cluj-Napoca. The BSG used in this work was obtained as a by-product from a mashing process of dark lager beer with 100% all grain malted barley (Weyermann Specialty Malting Company, Bamberg - Germany). Caramunich and Carafa malts were added in small amount (5-10%) to obtain dark beer and to enhance flavour characteristics. A commercial wheat flour used for traditional bread making (type 650 according to ash content by Romanian classification) with 14.5% moisture, 10.6% protein, 0.9% fat, 0.65% ash, 73.2% total starch and 0.6% fibre was purchased locally, Pambac, Bacău - Romania.

BSG pre-treatment

The high initial moisture content of fresh BSG (75-80%) and the presence of considerable levels of polysaccharide and protein makes it particularly susceptible to microbial degradation within a few days (STOJCESKA & al. [16]). Therefore it is necessary to apply a method of preservation shortly after its production.

Fresh BSG samples were preserved by oven-drying at 78 ºC for 12 hours. The samples kilned to 6% moisture content were then grounded into flour using a laboratory milling machine, packed in sealed polyethylene bags and stored at room temperature until used.

Bread preparation

Experimental breads were obtained from wheat flour blends containing 0% (100% wheat flour), 5%, 10%, 15% and 20% of BSG (wheat flour replacement). The bread prepared from wheat flour without BSG substitution served as control. Dough samples were prepared by mixing 1000g flour, 18g salt (table salt purchased locally) and 25 g fresh yeast (Pakmaya Yeast Rompak, Romania) with water. After mixing, the dough was rested in a proofer (Zanolli Teorema Polis, Italy) at 35ºC and 85% relative humidity for 60 min, before it was divided into 400 g portions and placed into non-stick baking tins. The dough was then proofed for 50 min under the same conditions and baked immediately in a preheated deck oven (Zanolli Teorema Polis 3 PW, Italy) at 220ºC top and bottom heat for 45 min. The oven was pre-steamed before and again after putting the bread in.

Extraction and analysis of volatile compounds

The extraction of volatile compounds was performed using the ITEX technique as described in a previous study (SOCACI & al. [17]). Shortly, three grams of grinded sample were placed in a 20 mL sealed-cap headspace vial. The sample was incubated at 60°C for 20 min, with continuous agitation and the volatile compounds from the gaseous phase were adsorbed repeatedly (30 strokes) into a porous polymer fibre microtrap (ITEX-2TRAPTXTA, Tenax TA 80/100 mesh). The thermal desorption of volatiles was made directly into the GC–MS injector and performed automatically by a CombiPAL AOC-5000 autosampler.

The analysis of volatile compounds was carried out on a GCMS QP-2010 (Shimadzu Scientific Instruments, Kyoto, Japan) model gas chromatograph and mass spectrometer. The volatiles compounds were separated on a Zebron ZB-5ms capillary column of 50 m×0.32mm i.d and 0.25μm film thickness. The carrier gas was helium, 1 mL/min, split ratio 20:1 and injector temperature 250°C. The temperature program used for the column oven was: 30°C (held for 5 min) rose to 110°C at 4°C/min, heated to 250°C at 20°C/min and held for 5 min. The ion-source temperature and interface temperature were set at 250°C and the MS mode was EI. The mass range scanned was 35–350 m/z. All samples were analyzed in triplicate.

The volatile compounds were identified by matching the obtained mass spectra with the spectra of reference compounds from NIST27 and NIST147 mass spectra libraries and verified by comparison with retention indices drawn from www.pherobase.com or www.flavornet.org (for columns with a similar stationary phase to the ZB-5ms column). All peaks found in at least two of the three total ion chromatograms (TIC) were taken into account when calculating the total area of peaks (100%) and the relative areas of the volatile compounds. Relative peak areas, being expressed in arbitrary units (a.u.), were considered to quantitatively compare the bread samples, one a.u. corresponding to 100000 units of peak area (PĂUCEAN & al. [18], DONG & al. [19]).

Chemical composition of bread

The nutritive value of bread samples was investigated using near infrared reflectance (NIRS) spectroscopy technology. NIRS is an instrumental technique based on measuring the intensity of reflectance or intensity of transmission of radiation from the near infrared region of the electromagnetic spectrum (800-2500 nm) by the test sample (POJIĆ & al. [20]). It is a non-destructive and rapid technique applied increasingly for food quality evaluation in recent years, reducing the costs and time of analysis by testing all the parameters simultaneously (RESTAINO & al. [21], BIROU & al. [22]).

Bread samples spectra were collected in the NIR regions in reflectance (1100-2500 nm) at 2 nm intervals using a NIR FOSS 5000 system (Denmark). The parameters investigated for each sample were: moisture, ash, crude protein, crude fat, total fibre, carbohydrate and caloric energy.

Sensory analysis - Acceptance test

Hedonic testing of the bread samples was conducted within 24 h after the bread was prepared, in the sensory evaluation laboratory of the Faculty of Food Science and Technology, Cluj-Napoca. Sensory profiling of bread samples was performed by 80 panellists. In every session, bread made only with wheat flour was included as reference and samples were coded with random three-digit numbers and presented in a randomized order under white light. Fresh water was used to cleanse the palate between samples. The panellists evaluated all five bread formulations for colour, aroma, taste, texture and overall acceptability using a 9-point hedonic scale with 0 being "dislike extremely" and 9 being "like extremely".

3. Results and Discussion

Compositional analysis

The moisture, total fibre, protein, fat, minerals and carbohydrate content as well as the caloric energy of the five tested bread formulations were determined using NIRS technique and are presented in table 1. As expected, the moisture, total fibre, protein, fat and minerals levels increased proportional to the quantity of added BSG, while carbohydrates and energy values decreased.

Table 1 Compositional analysis of bread containing different levels of BSG

BSG, (%)	Moisture (%)	Total fibre (%)	Protein (%)	Fat (%)	Minerals (%)	Carbohydrates (%)	Energy (kcal/100g)
0	37.43±2.12	0.81±0.06	6.64±0.3	0.39±0.02	0.44±0.02	53.69±3.75	244.83±6.12
5	38.51±1.23	1.91±0.08	7.49±0.86	0.48±0.09	0.68±0.01	49.16±1.96	230.92±4.27
10	40.02±0.91	2.8±0.21	8.28±0.44	0.61±0.01	0.92±0.06	46.22±2.14	223.49±5.09
15	41.14±1.02	3.76±0.34	9.13±0.67	0.72±0.01	1.17±0.21	43.82±1.82	218.28±2.98
20	42.09±0.83	4.52±0.18	10.03±1.12	0.97±0.00	1.29±0.07	40.46±2.03	210.69±3.56

The incorporated BSG had greatly influenced the total fibre content of the fortified bread samples. Thus, a 5% BSG flour addition was shown to double the total fibre amount of the bread while a 20% BSG bread formulation had a total fibre level five times higher than the reference bread sample (100% wheat flour). STOJCESKA & AINSWORTH [23] reported also high levels for fibre content of BSG supplemented bread (6.3-11.5%) in comparison with the control sample (2.3%). Although the consumption of dietary fibre has important implications in human health (TUNGLAND & MEYER [24]), the fibre intake is commonly lower than recommended. Thus, the BSG enriched breads may be considered a good source of dietary fibre in order to attain the necessary daily fibre intake (28-36g/day) required for a healthy nutrition (ANDERSON & al. [25]).

The increase in moisture content from 37.43% for the control sample to 42.09% for 20% BSG containing sample can be explained by the increasing fibre content which leads to higher water absorption during dough preparation. Contrariwise, total carbohydrates amount decreased (from 53.69% to 40.46%) as the content of BSG in the samples increased. The main carbohydrate in the wheat flour is represented by starch, while the BSG contains only residual amounts of starch, this compound being consumed by the extensive amylolysis during the mashing process (WATERS & al. [6], MAKOWSKA & al. [13]).

According to recent studies, the predominant lipids identified in BSG were triglyceride (55% to 67% of all identified lipid compounds), followed by a notable amount of free fatty acids (from 18% up to 30%) with beneficial properties for health (NIEMI & al. [26], DEL RÍO & al. [27]). An addition of BSG up to 20% increases the amount of total lipids by almost 3 times compared to control sample. The obtained data agree with previously published work that also found a 2.5 to 4 times increase of the fat content of baked snacks containing 25% BSG (KTENIOUDAKI et al. [28], KTENIOUDAKI et al. [29]).

Micronutrients as minerals are also important components when considering the nutritional characteristic of a potential food ingredient. The minerals content was 0.44% for the control sample and increased to 1.29% for the bread with 20% BSG supplementation. The level of minerals reported in BSG by WATERS & al. [6] is of 1.13% (w/w) including relatively high amounts of calcium, magnesium and phosphorus. Also, AJANAKU & al. [30] reported an increase in the iron level from 0.09% in the control sample to 0.18% in cookies supplemented with 15% BSG.

Following the results obtained for the chemical composition of the analyzed bread samples, it can be stated that an increase of BSG in bread formulation led to a significant increase in the nutritive value of bread. These results are also sustained by other studies in which the addition of BSG into dough formulation has shown to significantly improve the dietary fibre, protein, fat and minerals content of baked products (STOJCESKA & AINSWORLT [23], MANEJU & al. [1], KTENIOUDAKI & al. [28], KTENIOUDAKI & al. [29]).

Volatile analysis

One of the main issues associated with the incorporation of BSG in food products is the effect on flavour. The compounds identified from the volatile profile of the bread samples and their concentrations expressed as relative peak areas arbitrary units are listed in Table 2. The odour descriptors associated with these compounds as found in databases (www.flavornet.org; www.pherobase.com) or in the literature are also shown (KTENIOUDAKI & al. [29], DONG & al. [19]).

Table 2 Volatile compounds of bread containing different levels of BSG.

Compound name	Odour perception	0% BSG	5% BSG	10% BSG	15 % BSG	20% BSG
Alcohols						
2-Methyl-1-propanol	Wine, solvent	37.83	43.02	75.70	104.71	137.83
2-Methyl-1-butanol	Alcoholic, green, malt	6.46	8.17	18.81	34.95	42.57
3-Methyl-1-butanol	Malty, alcoholic,	82.15	106.16	185.01	291.41	312.81

	fruity, whiskey, burnt					
Aldehydes						
2-Methyl-propanal	Wine, solvent, malty	10.32	7.68	10.22	82.96	79.61
2-Methyl-butanal	Malty, buttery, oily, cocoa	9.84	6.33	10.41	75.05	84.74
3-Methyl-butanal	Buttery, oily, dark chocolate, cocoa, almod	15.46	12.08	21.90	130.35	136.75
Hexanal	Green, grass, fat	4.08	4.03	5.83	12.69	15.30
Benzaldehyde	Almond, burnt sugar	3.30	4.55	4.77	5.82	5.58
Nonanal	Fat, citrus, green	0.00	0.00	0.00	2.03	1.12
Ketones						
2,3-Butanedione	Buttery, cheessey	5.48	3.53	6.61	7.71	9.06
2,3-Pentanedione	Cream, butter	0.00	0.00	0.00	3.58	4.27
Acetophenone	Must, flower, almond	2.39	2.89	1.68	3.46	1.44
Others						
2-Pentyl-furan	Green bean, butter	0.00	0.00	0.00	8.91	9.55
Limonene	Citrus, mint	0.00	2.46	6.00	19.02	18.20
Toluene	Pungent, caramel, ethereal, fruity, rubbery, solvent-like	10.35	10.37	9.08	0.00	0.00

The majority of the detected volatiles are typically found in wheat bread, being formed during fermentation, Maillard reaction or lipid oxidation. The predominant volatile constituents indicated by the peak areas obtained from GC-MS chromatograms are the alcohols (2-methyl-1-propanol, 3-methyl-1-butanol and 2-methyl-1-butanol) and the corresponding aldehydes (2-methyl-propanal, 3-methyl-butanal and 2-methyl-butanal). These compounds were found in three to eight times higher concentrations in the bread formulation containing 15% and 20% added BSG compare to the control sample. Their odour attribute has been generally described as malty flavour, suggesting that the differences in aroma of the bread formulations perceived by the panellists may be associated with the addition of BSG (COGHE & al. [31]).

Three volatiles, nonanal, 2-pentyl-furan and limonene, were identified only in the bread samples incorporating BSG flour. Nonanal and 2-pentyl-furan were detected only for bread formulations containing 15% and 20% BSG, while limone was found in all BSG bread samples, its concentration increasing with the amount of added BSG. Instead, toluene, a compound which odour attributes may vary from fruity, caramel to solvent-like, was found in relative high amounts in the 0%, 5% and 10% BSG breads and absent in the samples with 15% and 20% BSG added.

Usually, the concentration of detected volatile compounds increase with the amount of incorporated BSG. Nevertheless, there are certain compounds, like 2-methyl-propanal, 3-methyl-butanal and 2-methyl-butanal, which have a slightly lower concentration in 5% BSG formulation than in 0% BSG sample. Our findings corroborate with those of other authors and were explained by the factors that affect the release of the volatile compounds and by the changes in the bread samples microstructure induced by the addition of BSG (KTENIOUDAKI & al. [29], DONG & al. [19]).

The aromatic compounds listed in table 2 have a wide spectrum of pleasant and unpleasant odour descriptors, but the contribution of each compound to the aroma of the bread samples depends on its odour thresholds as well as on the product matrix. Also, the flavour imparted to the food by a certain volatile compound is directly related to its concentration, its release during mastication and the presence of other volatile compounds (KTENIOUDAKI & al. [29]).

Sensorial Analysis

In light of the above, the sensorial analysis was conducted for all five bread formulations samples. The sensorial quality of food products is a key factor in consumer's decision-making process. Hedonic testing is often used to determine consumer's attitude towards the food by measuring the degree of acceptance of a new product or improving the existing food product (MEILGARD & al. [32]).

It is very important that the organoleptic properties of bread enhanced with brewer's spent grain remained acceptable to consumers and the quality level similar to the current commercially available products. The results of sensorial evaluation of bread samples containing different level of BSG substitution compared to the control sample (100% wheat flour) are shown in the table 3.

Table 3 Results of Sensorial Evaluation

Bread sample	Colour	Aroma	Taste	Texture	Acceptability
0 %	7.9	7.58	7.58	7.9	7.8
5 %	7.9	7.5	7.63	7.9	7.88
10 %	7.88	7.68	7.53	7.78	7.83
15 %	7.48	7.2	7.3	7	7.33
20 %	7.7	7.1	7.03	6.75	7.35

A decrease in acceptability was observed when the levels of BSG were higher than 10%. The sample with 5% BSG substitution had the highest acceptability score (7.88) as well as for the other organoleptic characteristics. Also, it can be observed that the bread samples with 5% and 10% added BSG showed similar results to the control sample obtained from wheat flour only. An increase % of added BSG (15% and 20%) resulted in a lower scores for the overall acceptability characteristics (7.33 respectively 7.35).

The attributes that influenced the panellist's acceptability were predominantly taste and texture, with both 15% and 20% BSG containing samples receiving significantly lower scores when compared to the control and with the samples supplemented with 5% and 10% BSG. For all the bread samples the scores for texture decreased with increase in BSG substitution and simultaneously with the increase in fibre content. In general, the addition of rich fibre ingredients has led to an increase in the hardness of crumb by cross linking gluten proteins (AUTIO & al. [33], STOJCESKA & AINSWORTH, [23], AJANAKU & al. [30]).

The colour of the bread slices became visually darker as the level of BSG increased. Also, a darker colour of the crumbs was reported by HU & al. [34] to be directly related to the increase in fibre content.

The considerable increase in the amounts of volatile compounds with malty flavour in the case of 15% and 20% BSG bread formulations compare with 0%, 5% and 10% BSG samples, could be responsible for the poor scores that were obtained for those samples.

Finally, the sensorial evaluation revealed that breads with BSG substitution up to 10 % achieved higher score than the control white bread sample. A study performed by PRENTINCE & al. [35] demonstrated that BSG addition at a level of 15% was the upper limit for organoleptic acceptability. Instead, STOJCESKA & al. [16] found that at a level of addition of 30% BSG, the physicochemical characteristics remained acceptable. For the five tested bread formulations, the scores of the sensory acceptability are presented in figures 2 and 3.

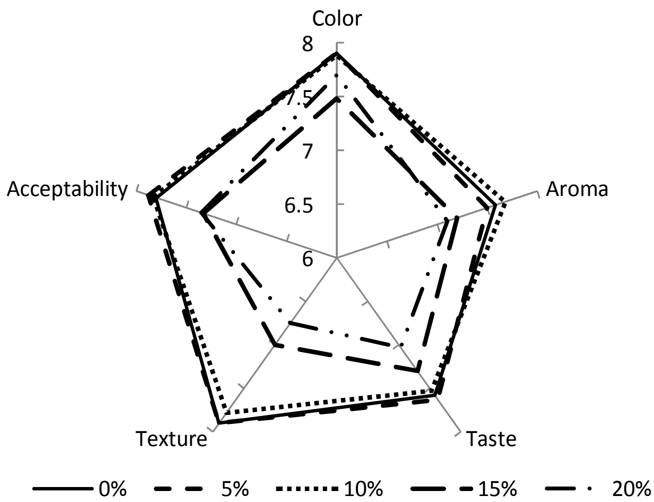

Figure 2 Acceptability scores of the bread containing different levels of BSG.

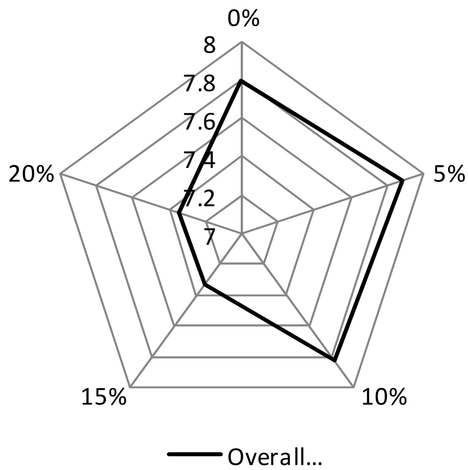

Figure 3 General acceptability scores of the bread containing different levels of BSG

4. Conclusion

Four formulations of bread supplemented with BSG were developed and analyzed in order to assess the contribution of BSG to the nutritional value of the fortified end-product. The substitution of wheat flour with 5-20% BSG resulted in bread formulations with enhance nutritional value (increased fibre, protein, fat and minerals content) and with pleasant flavour attributes imparted by the characteristic volatile compounds. The overall acceptability of the BSG enriched breads was performed by sensorial analysis, revealing good organoleptic attributes for the samples up to 10% BSG flour. The obtained results indicate the opportunity of using BSG as a low-cost source of dietary fibre in breadmaking in order to fortify the diet.

Acknowledgments: The research was financially funded by the program Innovation-subprogram Innovation Checks – PNII, CCCDI-UEFISCDI, project number PN-II-IN-CI-2013-1-0018.

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