Incorporation of spent grains in bread: Chemical and Nutritional properties.

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Abstract

Spent grains was prepared from red sorghum malt (RSSG) and white sorghum malt (WSSG) and chemically compared with brewer’s spent grains (BSG). The composite bread was prepared by incorporating 5 to 15 % of each of the spent grains and the bread analyzed for nutrients and in-vitro starch digestibility. The results obtained for composite bread were compared with white bread (control). The results indicated that acid and neutral detergent fibre and hemicelluloses were mostly high (> 20%) and protein (19.00-23.37%) in spent grains compared to wheat flour. Unsaturated fatty acids were abundant in both spent grains with linolenic acid predominating. Total starch was highest in RSSG and lowest in BSG suggesting incomplete saccharification in the former. Reducing and total sugar contents were highest in RSSG followed by those of BSG and WSSG. Oxalate content of spent grains was highest (13.78 mg/g) and tannin lowest (0.2 mg/g lowest) in BSG. Conversely, tannin content was highest in RSSG (21.8 mg/g). Incorporating spent grain into bread lead to higher protein content (8-41%), fiber, and available lysine but lower starch content and glycemic index compared to those of control bread. The finding of this study concluded that spent grains have chemical and nutritional properties that could enhance nutritional quality of bread and could avail a healthier bread variety.

Keywords: spent grains, glycemic index, malting, starch hydrolysis

1. Introduction

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 brew house and improve the dietary attributes of different food formulations [1, 2].

Brewer’s spent grain (BGS) is a by-product of the brewing process, consisting of the solid residue remaining after mashing and lauterling. Of the original malted barley that goes into the mash tank, about 30% remains as spent grains; this means that there will always be an abundant supply as long as beer continues to be produced. It primarily consists of grain husks and other residual compounds not converted into fermentable sugars in the mashing process [3]. Traditionally this material is either discarded as waste or sold as animal feed [1]. Though it is a by-product in brewery, it can conveniently serve as a starting material in many production spheres, especially where there is need to boost fiber contents [4,5].

White bread is made from wheat flour in which about 70-80% of the initial nutrient content especially fiber was removed due to grinding and sieving processes. Starch in bread is rapidly digested and absorbed, producing high glycemic responses due to rapid carbohydrate breakdown in the digestive system which raises blood glucose...
Several technological approaches have been employed to increase fibre content and to modulate the GI of bread. Some of which are production of wholegrain bread [8]; Sourdough fermentation, addition of fiber, the use of resistant starch, and incorporation of non-wheat flour such as barley, cowpea and jackfruit seeds [9,10,6].

Some researchers have reported that brewer’s spent grains could serve as functional ingredient in improving the nutritional quality of wheat flour [1,2] but information is scarce on the effect of incorporation of spent grains on starch digestibility and glycemic index of composite bread. Hence, this study was designed to produce healthier bread with low glycemic load and high fiber content from composite flour of wheat flour and sorghum or barley malt spent grains. The study would produce avenue for adding value to brewers’ spent grains especially in Nigeria where barley malt and sorghum malt are used as inputs in the brewing process [11].

Therefore, in this study spent grain was prepared from red sorghum malt (RSSG) and white sorghum malt (WSSG) and chemically compared with brewer’s spent grain (BSG). Also the nutrient and anti-nutrient compositions as well as digestibility study of bread samples incorporating 5 to 15 % of spent grain were evaluated. This study will find added value for spent grains –‘a waste product’ as co-product that will help in maximizing the potentials of abundant quantities of agro-industrial by-products and help produce a healthier bread with low glycemic load and high fiber content.

2. Materials and Methods

2.1. Sample and sample preparation

Sorghum (sorghum bicolor L. Moench) grains (white and red species) were purchased from Central Market, Ile-Ife, the grains were subjected to malting [12] and mashing [11] processes and the spent grains were collected, drained of water and dried at 50º C in an oven for 48-72 h [13]. Brewer’s spent grains (BSG) were collected from International Breweries, Omi Asoro, Ilesa, Nigeria and dried as well.

The dried samples were milled using a locally fabricated mill (Lawood Metals, Osogbo, Nigeria). The milled samples were sieved using a local sieve (aperture size of 0.6 mm) to remove the coarser fragments. All the samples were milled as one batch, mixed thoroughly and sub-samples randomly taken from different parts of each milled sample, mixed together and stored in the freezer until analyzed.

2.2. Production of composite bread

The bread samples were produced at the bakery section of the Department of Food Science and Technology, Obafemi Awolowo University, Ile –Ife, Nigeria Wheat flour and each type of spent grains (industrial spent grains, red sorghum spent grains and white spent grains) were blended in the ratios of 95:5, 90:10, 85:15 with each sample weighed in triplicate using a weigh balance and a plastic spoon. The control bread sample was however made of 100 % wheat flour. The composite bread as well as control was subjected to sensory and organoleptic evaluations. The samples certified good for consumption by taste panelists were kept for further analysis.

2.3. Analytical procedures

Proximate composition of spent grains and composite bread: The moisture, ash, lipids crude fibre and protein content of the spent grains and composite bread samples were determined by AOAC methods [14]. Neutral detergent fiber and acid detergent fiber were analyzed using the method of Van Soest et al., [15] while hemicellulose content was calculated as the difference between neutral detergent fiber and acid detergent fiber. All determinations were made in triplicate.

Determination of Fatty Acid profile of spent grains

The fatty acid profile of the oil samples extracted from spent grains was determined by the AOAC method 965.33 [14] using Agilent 6890 gas chromatograph (Hewlett-Packard, Sunnyvale, CA, USA) equipped with an on-column automatic injector, flame ionization detector, HP-88 capillary column (100 m x 0.25 μm film thickness) and a Chemstation software.

The fatty acid methyl esters of the oil samples were identified by comparing their retention times to those of known standards and quantified using the principle of internal standardization. The coefficient of variation for the method was less than 4 %.

2.4. Determination of carbohydrate component

Determination of Total and Reducing Sugar content: The soluble sugar was extracted the spent grains and bread samples using the method described by Bambridge et al. [16].
The samples (2.0 g each) was placed in soxhlet extractor containing 85 % ethanol, the sample was refluxed for 2 h. The reducing sugar was determined on the extract using the ferricyanide method [17] Glucose was used as a standard and the glucose content of the sample was calculated using a linear equation \( y = 1.6216 - 0.001x \) \((R^2 = 0.972)\).

For total sugar, the extract (25 ml) in a beaker was added concentrated hydrochloric acid (5 ml) and left overnight. The sugar content was determined as done for reducing sugar.

**Determination of Total Starch:** The total starch content of spent grains and bread was determined by the method described by Bainbridge et al., [16]. The starch was measured as glucose using the ferricyanide method [17] Glucose was used as the standard and was calculated using a linear equation \( y = 1.6216 - 0.001x \) \((R^2 = 0.972)\). The glucose is the converted to starch by multiply with 0.9.

**Determination of Rapidly Digestible, Slowly Digestible and Resistance Starch:** The method of Han et al., [18] was used to estimate the rapidly digestible, slowly digestible and resistance starch of the bread samples. The rapidly digestible starch (RDS) is the fraction of starch hydrolyzed within 30 min of incubation, slowly digestible starch (SDS) is the fraction hydrolyzed between 30 to 180 min and the fraction that remained un-hydrolyzed after 180 min is regarded as the resistant starch.

**In-vitro starch hydrolysis:** The in-vitro starch digestibility of the bread samples was determined by multi-enzyme digestion method as described by Falade et al., [19]. Each sample (250mg) was digested with 20 mg pepsin (Sigma; CAS 2001/75-6, code 10132561), (prepared by adding 1.0 g of pepsin /10 mL of HCl - KCl buffer) for 1 h in a shaking water bath at 37 °C. The pH of the digestate was adjusted to 6.9 using Tris - maleate buffer (pH 6.9). Then 5.0 mL of (2.6 IU in 5 ml buffer pH 6.9) was added to the digestate which was incubated at 37°C in a shaking water bath. One ml of sample aliquots was collected at intervals of 30 min for 180 min. To these aliquots, 3 ml of 0.4 M sodium acetate buffer (pH 4.75) and 60 μl amyloglucosidase (Sigma, No;10105-5GF,70 ui/mg. Aspergillus niger) were added and incubated at 60°C for 5 min to hydrolyse the starch to glucose. The glucose released was determined using dinitrosalicylic acid. The concentration of glucose was calculated from the linear equation of glucose standard \((R^2 = 0.980)\) and glucose was converted into starch by multiplying with 0.9.

The rate of starch digestion was expressed as percentage total starch hydrolysed at different times. The hydrolysis index was calculated as relation between the AUC for the food and the AUC for the reference food (white bread) expressed as percentage. The glycemic index (GI) was estimated using the equation of Goni et al., [20].

**2.5 Statistical analysis**

Results were expressed as mean and standard deviation of triplicate analysis and the data were treated with one-way analysis of variance (ANOVA), and Duncan Multiple Range test was to separate the means at 5% probability level. Microsoft Excel package was used for the analysis of data.

**3. Results and Discussions**

**3.1. Chemical composition of the spent grains**

Table 1 presents the chemical compositions of spent grains compared with wheat flour. From the result, the protein (19.0 – 23.4 %), and the ash (1.1 – 3.8 %) contents in spent grains were significantly higher than wheat flour. The high concentration of protein in the spent grains could be as a result of the transformation of the seed starch into fermentable sugar during mashing, the process that will result in low starch content of the spent grains compared to wheat flour. Total starch was highest in RSSG and lowest in BSG suggesting incomplete saccharification in the former.

The available lysine ranged from 3.6 to 4.3 g / 100 g protein, the value is significantly \((P < 0.05)\) lower than that of wheat flour but the values compared with the 4.16 g / 100 g Protein reported for soymilk [21]. Available lysine is a term used to describe the unmodified lysine units that are absorbed in a form that can be potentially utilized for protein synthesis, catabolism and conversion [21] and it is an important factor in assessment of protein quality.

The acid detergent fibre, neutral detergent fiber and hemicelluloses content of spent grains were significantly \((P < 0.05)\) higher than wheat flour. Basically spent grains consist of husk – pericarp – seed coat layer that covered the sorghum or the barley grains. The coats are rich in cellulose and non cellulose polysaccharides and lignin. They are non-digestible oligo- and polysaccharides, known as
dietary fiber, the compounds are prebiotics because they are resistant to both digestion and absorption in the human small intestine and may suffer partial fermentation in the large intestine. The prebiotics also benefit the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon and thus improving host health [2, 22].

The contents of anti-nutrients, oxalate ranged between 2.5 and 13.8 mg/g and tannin 0.2 and 21.8 mg/g. Adewusi and Ilori [13] had earlier reported 7.5 and 1.0 mg/g tannin content for RSSG and WSSG and observed also tannin level of 15.4 and 1.2 mg/g for red and white sorghum grains, respectively. The low value recorded for the spent grains compared to sorghum grains could be due to steeping and malting processes. Both oxalate and tannin which are anti-nutrients were found below lethal levels in the spent grains.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>BSG</th>
<th>WSSG</th>
<th>RSSG</th>
<th>Flour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>12.1 ± 0.14*</td>
<td>11.7 ± 0.3b</td>
<td>11.3 ± 0.25c</td>
<td>10.6 ± 0.3d</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>3.8 ± 0.04a</td>
<td>1.1 ± 0.06b</td>
<td>1.5 ± 0.04b</td>
<td>0.5 ± 0.04a</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>23.4 ± 0.23a</td>
<td>19.0 ± 0.2c</td>
<td>21.7 ± 0.11b</td>
<td>9.6 ± 0.06a</td>
</tr>
<tr>
<td>ADF (%)</td>
<td>26.0 ± 0.48a</td>
<td>20.0 ± 0.9c</td>
<td>22.9 ± 0.9c</td>
<td>2.0 ± 0.20a</td>
</tr>
<tr>
<td>NDF (%)</td>
<td>49.1 ± 1.64a</td>
<td>36.0 ± 0.3b</td>
<td>29.4 ± 0.6c</td>
<td>3.0 ± 0.22a</td>
</tr>
<tr>
<td>Hemicelluloses (%)</td>
<td>23.1 ± 1.12a</td>
<td>15.9 ± 0.5b</td>
<td>6.1 ± 0.5c</td>
<td>1.0 ± 0.04a</td>
</tr>
<tr>
<td>Starch (mg/g)</td>
<td>98.6 ± 3.3c</td>
<td>117 ± 0.5b</td>
<td>114 ± 4.0c</td>
<td>295 ± 5.6a</td>
</tr>
<tr>
<td>Reducing Sugar (mg/g)</td>
<td>57.3 ± 1.5b</td>
<td>4.6 ± 0.2c</td>
<td>22.6 ± 1.0b</td>
<td>58.3 ± 0.9a</td>
</tr>
<tr>
<td>Total sugar (mg/g)</td>
<td>96.2 ± 7.0c</td>
<td>87.5 ± 0.2c</td>
<td>110 ± 0.2b</td>
<td>229 ± 1.0a</td>
</tr>
<tr>
<td>Oxalate (mg/g)</td>
<td>13.8 ± 0.33a</td>
<td>2.5 ± 0.12c</td>
<td>10.5 ± 0.13b</td>
<td>1.4 ± 0.13a</td>
</tr>
<tr>
<td>Tannin (mg/g)</td>
<td>0.2 ± 0.14d</td>
<td>2.8 ± 0.10a</td>
<td>0.4 ± 0.11b</td>
<td>4.3 ± 0.14a</td>
</tr>
<tr>
<td>Available lysine (g/100g P)</td>
<td>4.3 ± 0.14a</td>
<td>3.6 ± 0.15b</td>
<td>6.1 ± 0.24a</td>
<td></td>
</tr>
</tbody>
</table>

Mean ± SD – Mean and standard deviations of triplicate analysis
Means along the same rows with same superscript are not significantly different P < 0.05

<table>
<thead>
<tr>
<th>FATTY ACID</th>
<th>RSSG</th>
<th>WSSG</th>
<th>BSG</th>
</tr>
</thead>
<tbody>
<tr>
<td>C14:0</td>
<td>0.6</td>
<td>ND</td>
<td>0.10</td>
</tr>
<tr>
<td>C15:0</td>
<td>ND</td>
<td>ND</td>
<td>0.07</td>
</tr>
<tr>
<td>C16:0</td>
<td>17.6</td>
<td>15.5</td>
<td>40.4</td>
</tr>
<tr>
<td>C17:0</td>
<td>ND</td>
<td>ND</td>
<td>0.09</td>
</tr>
<tr>
<td>C18:0</td>
<td>2.70</td>
<td>4.07</td>
<td>3.8</td>
</tr>
<tr>
<td>C20:0</td>
<td>0.40</td>
<td>ND</td>
<td>0.9</td>
</tr>
<tr>
<td>C22:0</td>
<td>ND</td>
<td>ND</td>
<td>0.81</td>
</tr>
<tr>
<td>C24:0</td>
<td>0.10</td>
<td>ND</td>
<td>1.07</td>
</tr>
<tr>
<td>C14:1</td>
<td>0.11</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>C16:1.cis</td>
<td>0.32</td>
<td>ND</td>
<td>0.23</td>
</tr>
<tr>
<td>C17:1.cis</td>
<td>ND</td>
<td>ND</td>
<td>0.11</td>
</tr>
<tr>
<td>C18:1.cis</td>
<td>26.7</td>
<td>28.4</td>
<td>11.4</td>
</tr>
<tr>
<td>C20:1.cis</td>
<td>0.04</td>
<td>ND</td>
<td>0.5</td>
</tr>
<tr>
<td>C18:2.cis</td>
<td>32.4</td>
<td>48.8</td>
<td>33.5</td>
</tr>
<tr>
<td>C18:3.cis</td>
<td>1.37</td>
<td>2.65</td>
<td>2.92</td>
</tr>
<tr>
<td>Unknowns</td>
<td>17.6</td>
<td>0.68</td>
<td>4.05</td>
</tr>
<tr>
<td>Total SFA</td>
<td>21.45</td>
<td>20.32</td>
<td>47.25</td>
</tr>
<tr>
<td>Total MUFA</td>
<td>27.16</td>
<td>28.36</td>
<td>12.25</td>
</tr>
<tr>
<td>Total PUFA</td>
<td>33.77</td>
<td>51.44</td>
<td>36.44</td>
</tr>
<tr>
<td>% oil content</td>
<td>2.82</td>
<td>4.02</td>
<td>12.32</td>
</tr>
</tbody>
</table>

CV is less than 4 %
ND – Not detected
3.2. Nutrient content of spent grain incorporated bread

From the result of organoleptic assessment of composite bread, the bread made by incorporating red sorghum was rated low in preference scale and was not analyzed further. The use of spent grains as additive or as a replacement for wheat in bread production could cause alteration of bread properties; the texture, the colour and the taste. The spent grains are brownish in colour when moist and can only be used for off-white products. This could be a major limitation in the use spent grains as food.

The results of nutritional composition of composite bread and control bread are presented in Table 3. The moisture content of composite bread ranged between 29.78 % in 5 % WSSG and 27.98 in 15 % BSG, while control bread sample was 30.21 %. This is in concord with the moisture content of cassava incorporated bread [24]. The results showed that composite bread had higher ash and fibre content [25]. Consumption of foods high in dietary fibre provides many health benefits. Their frequent intake reduces risk of developing some diseases including coronary heart disease certain gastrointestinal disorders, hypertension, diabetes obesity and stroke [4, 26].

Incorporation of spent grains significantly (P < 0.05) increased the protein content of composite bread, an increment of between 8 and 41 % in protein was observed. The higher the level of incorporation of spent grains, the higher the protein increment.

The results of nutritional contents of spent grains also indicated high content of fat in brewers’ spent grain (12.3 %) saturated fatty acids from varied from 20.5 to 47.3 %, monounsaturated fatty acid ranged from 12.3 to 28.4% and polyunsaturated fatty acids varied between 33.8 and 51.4 %. Palmitic acid (C16:0), linoleic acid (C18:1) and linolenic acid (C18:2) were the most abundant. Linolenic acid is an essential fatty acid needed for the synthesis of long chain fatty acid such as eicosanoids [23]. The high fat content could be of advantage in the improvement of taste and crispiness of component bread if incorporated.
Similar observation has also been reported earlier by Kissel and Prentice [27]. Bread rich in protein will be of great advantage especially among Nigerian populations where kwashiorkor, marasmus and protein malnutrition is endemic.

Although incorporation of spent grains increased the level of anti-nutrients, but the increment could not cause any significant health problem because the level is below lethal. Recommended daily oxalate intake limit is 50 - 60 mg per day [28].

The starch, total sugar and reducing sugar content of bread were 293 mg/g, 213 mg/kg and 65.3 mg/g, respectively (Table 3), the content of starch decreased in bread in dose dependent fashion with the addition of spent grains to bread. The inverse relationship between the levels of spent grains incorporation and starch or sugar could be advantageous in reducing the amount of energy released to the body from bread consumed. Carbohydrate is an energy giving food and consumption of high energy food has been reported to lead to overweight and obesity [29].

The result of starch component (Table 4) showed that 15% BSG and 5% WSG bread had rapidly digestible starch that is significantly (P < 0.05) lower than control, whereas resistance starch was higher than control in all bread samples and this could be adduced to fiber content of spent grains. The high resistant starch recorded by the composite bread compared is desirable in that the bread will be of greater health benefits in ameliorating problems of diabetics and pre-diabetic subjects and to fight human obesity [7, 30].

In order to obtain more quantitative information on digestibility properties of the composite bread, in-vitro digestion modeling kinetics was performed over a period of 180 min and the starch hydrolysis curves are presented in Figure 1. The reaction followed a first order model which has been found applicable for in – vitro starch digestion of raw and processed food and feed [20, 31]. The hydrolysis parameters (Table 4) indicated that C∞ which is the maximum starch hydrolysis over the experimental period ranged from 43.8 to 52 %, the rate constant (K) ranged from 0.02 to 0.05, the hydrolysis and glycemic indices ranged from 73.4 to 90.9 and 79.8 to 89.6 %, respectively. The kinetic constant (K) of amylosis has been proposed as a reliable index of the inherent susceptibility of flour to starch hydrolysis, the K-values obtained for the composite bread was lower than control, which means the composite bread could release glucose slowly compare to control which recorded higher k-value. Both the hydrolysis and glycemic indices of composite bread were lower than control, an indication that the presence of spent grains could positively affects the glycemic index of wheat products.

![Figure 1. Starch hydrolysis curves of spent grains incorporated bread compared to white bread (control)](image)

From this study, it was evident that there are some desirable chemical characteristics such as high fiber, protein and essential fatty acids. Incorporation of spent grain into bread was found to positively enhance the nutritional values of bread by causing the increase of essential nutrients while it also helps in modulating the glycemic index of bread.

It is noteworthy to point it out that consumption of brewer’s spent grains may not pose any nutritional hazard because the brewing process uses ingredients approved for human consumption; hence it has the potential for developing new products that can meet full health regulatory approval [32].

4.Conclusion

Incorporation of spent grains in bread at various blend levels could avail a healthier bread variety and also help in maximizing potential uses of abundant quantities of Agro-Industrial by-products.

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Compliance with Ethics Requirements. Authors declare that they respect the journal’s ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards.

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