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EVALUATION OF CHEMICAL AND ANTI-NUTRIENT PROPERTIES OF BREWER'S SPENT GRAINS AND RICE BRAN BISCUIT

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Abstract

Utilization of brewer's spent and rice bran as composite flour for increased nutrient density of biscuit was investigated. The flour samples were blended in ratios: 90% wheat: 10% brewers spent as sample B; 90% wheat: 10% rice bran (C); 90% wheat: 5% brewers spent:5% rice bran (D) and 100% wheat (A) to produced biscuits and analysed using standard methods. Results obtained revealed the following ranges: Proximate composition; carbohydrate (71.81 - 75.40) %, protein (10.63 - 13.26) %, fat (1.77 - 3.93) %, fiber (1.22 - 2.96) %, ash (1.37 - 2.69) % and moisture (6.18 - 9.64) %. Physical properties: bulk density (0.66 - 0.67) g/cm^3, water absorption capacity (1.83 - 2.64g/g and apparent density (1.23 - 1.34) g/cm^3. The anti-nutrient factors: phytate (8.25 - 12.32) µg/ml and tannin (0.04 - 0.13) µg/ml while vitamin content of the products were vitamin A (0.75 - 1.77) mg/ml, vitamin B_1 (0.67 - 1.96) mg/ml and vitamin C (0.03 - 1.55) mg/ml. The microbiological properties: total viable count ranged from ($4.00x10^2$ - $1.20x10^2$) cfu/ml, Mould count and coliform count were ($0.00x10^2$ - $0.00x10^2$) cfu/ml. Sensory evaluation showed that sample D (90% wheat, 5% brewers spent and 5% rice bran) was the best formulation because it had a composition that combines optimal nutritive value with good characteristics for a biscuit that is nutritive and palatable.

Keywords: Biscuits, Wheat, Brewer's spent grain, Rice bran, composite flour.

Introduction

There is an increasing trend among children and adults to move from the traditional pattern of eating three times a day to just eating snacks. Different types of biscuits have the highest daily frequency of consumption among adolescent female students. Biscuits are therefore, convenient food items that can be fortified with proteins for people suffering from protein malnutrition (Mepba *et al.*, 2008). Biscuits are nutritive snacks produced from palatable dough that is transformed into appetizing products through the application of heat in the oven which reduces the moisture content, making them exceptionally crisp or brittle (Olaoye *et al.*, 2007).

A biscuit could be said to be small bread made with baking powder or baking soda as a chemical leavening agent rather than yeast (Anon, 2010). Biscuits are usually eaten as snacks and can contain fillings such as chocolate, jams, icing sugar, nuts, butter, etc. Biscuits are produced from the flour of wheat

which is a cereal. Cereal proteins are deficient in lysine therefore, to compensate the low protein content of wheat flour; it can be blended with brewers' spent grain (BSG) for biscuits production (Ajanaku *et al.*, 2011; Okaka *et al.*, 2006).

There is growing interest in the use of BSG in food product formulation for increase production of lipids, important Vitamin B in addition to cholesterol and protein enrichment (Ajanaku *et al.*, 2010; Vitali *et al.*, 2009; Stojceska *et al.*, 2008; Hassonal,1993; windell *et al.*,1974). Therefore, production of composite flour consisting of wheat and BSG will provide a flour meal that is high in carbohydrate and protein content; however there is still a need to fortify this composite flour with micronutrients, hence introduction of a cheap and readily available source of micronutrients such as rice bran will serve as an excellent channel through which nutritional security can be enhanced considering the fact that the chronic micronutrient deficiencies in Africa and Nigeria in particular.

Cereal bran is an excellent source of dietary fibre; for addition to food, it offers all the nutritional and neutraceutical benefits of whole grain. Rice bran a powerful source of vitamins, nutrients, protein and fiber. It contains a newly discovered complex of Vitamin E and tocotrienols as well as tocopherol, which is the compound generally called Vitamin E. Rice bran is also rich in protein, lipids, fibre, minerals (Mg, K, Fe and Mn) and an excellent source of choline and inositol (Hoffpauer *et al.*, 2005).

Protein deficiency has become a major dietary problem facing the world today, especially the underdeveloped and developing countries (Enweremadu *et al.*, 2008; Bi-yu and Chiou-Wenshy, 1998; Ahn, 1979). Animals protein which are of high biological value are expensive in a developing country like Nigeria, therefore it becomes necessary to find an alternative means of compensating for the low protein availability and also fortify the staple foods with vitamins and minerals. However, considering the nutritional, food potential and associated health benefits of consuming rice bran, the present investigation was planned to assess the physico-chemical, microbial, anti-nutrients and organoleptic qualities extent of defatted rice bran incorporated into wheat and brewers spent grain flours. This will help add variety to the existing biscuits and encourage the use of by-products of the brewing industries and rice production by highlighting the nutritional potentials of BSG and rice bran. It will also encourage more research work, production, marketing and consumption of the products.

Materials and Methods

The wheat flour, stabilized (3kg) rice bran and other ingredients used in this study was purchased from local retailers at King's market, Ikole, Ekiti State, Nigeria. The brewers' spent grain was obtained from International Brewery Ltd., Ilesa, Osun State.

Sample Preparation

Preparation of brewers' spent grain flour: The BSG was collected and sorted out to remove dirt, stones and other extraneous materials. It was spread under the sun to dry. After drying, it was milled into fine flour. The flour was put in a water tight polyethylene bag and kept in a cool dry place for analysis.

Preparation of the samples: Different samples of biscuits were produced by blending varying different proportions sample A(control sample 100% wheat; B 90% wheat,and10% brewers spent grain; C 90% wheat, and 10% rice bran,and D 90% wheat,5% brewers spent and 5%, rice bran The recipe for the biscuit production were: flour (500g), sugar (150g), fat (190g), baking

powder (2.5g), vanilla (5g), salt (5g), and water (125g)

Mixing and Baking Process: Creaming method was used by first mixing fat, sugar, salt, vanilla and baking powder together, the blend of composite flour was added and mixed for about 3 – 5 minutes. Water (125 ml) was measured out and gradually added to the mixing bowl, and blended using the continuous mixing method until a good texture and slightly firm dough was obtained. The barter was manually rolled into sheets and cut into shapes using the stamp cutting method. The shaped samples were transferred into a fluid fat greased tray and placed in the oven at a temperature of about 180^{0}C for 15 minutes. The biscuits were removed after baking, cooled and were subsequently packaged.

ANALYSIS

Proximate composition: Protein, moisture, ash, fiber and fat were determined using AOAC (2010), while carbohydrate was determined by difference.

Physical properties: Bulk density was calculated by measuring the actual dimension of the composite flour (Asare *et al.,* 2004), Apparent density (Onyangu *et al.,* 2004) while Water absorption capacity was determined by modified method of Anderson *et al.* (1989).

Anti-nutrient determination: Phytate was determined using Oberleas (1973) method while extraction of tannins was carried out using the modified methanol procedure by Price and Butler (1977).

Micro nutrients: Vitamin A was determined by Colometry method using triflux acid (TFA) of Snell and Snell (1953), Vitamin B_1 content was determined using the Calorimetric method, while ascorbic acid (Vit C) was carried out using Barakat *et al.* (1973).

Microbial Count was carried out using the method of Peterkin *et al.* (1998).

Sensory Evaluation: The biscuit samples were evaluated using 20 panelists on the bases of appearance, crust colour, crumb colour, flavour, taste, after taste, mouthfeel, texture, pore size and general acceptability of the product using a nine-point hedonic scale questionnaire (Ihekeronye and Ngoddy, 1985).

Statistical Analysis: Data analysis was carried out using one-way analysis of variance (ANOVA) based on completely randomized design; mean separation was by Duncan's New Multiple Range Test (DNMRT). Significance was accepted at $p<0.05$.

Results and Discussion

The results of proximate composition are shown in Table 1. Generally, significant ($p<0.05$) differences existed between the samples in almost all the parameters. The carbohydrate values ranged from 71.81 to 75.40% with sample B having the lowest value while sample A had the highest value. The low value obtained in sample B could be attributed to the conversion of BSG starches into sugars and extraction of the sugars for fermentation during beer production (mashing process).

The carbohydrate content of the composite flour was the highest in terms of all proximate composition parameters determined. This was expected as the ingredients composed of mainly carbohydrate rich materials which are wheat, brewers spent grain and rice bran. The significant increases in all the proximate composition of samples B, C, and D is an indication that rice bran has the potential of being used in various foods to upgrade the nutritional and health promoting potentials. The low carbohydrates content of the samples B, C, and D would make it preferable to obsessed and diabetic patients.

The protein content increased from 10.63% to 13.26% with sample A having the least value

while sample D had the highest value. It can be deduced from the result that the addition of BSG and rice bran was found to increase the protein content of the composite flour. The relatively high protein content observed in sample B, C and D is an indication that the product will not only be of good value in man diet, but also contribute significantly to the daily protein requirement of human, particularly in developing countries where the cost of convectional protein sources is expensive.

The significant increase in the protein level of the samples will go a long way in providing most of the essential amino acids lacking in the diets of the people. Though, most plant proteins are reported to be limited in essential amino acids, the case of rice bran protein seems to be different. They have been reported to have a balanced composition of amino acids (Simmonds, 1978). Rice bran has been reported to be an excellent source of easily digestible food quality protein. It contains all the essential amino acids and threonine and isoleucine which are the limiting ones (Julliano, 1980).

Kimberly (2004) reported the anti-cancer action of rice bran and its oil. He also state that rice bran has the ability to regulate glycosylated haemoglobin levels in diabetes. It has also been reported to be a natural stress fighter. Addition of sulphur amino acids in the diets of the people through fortification with rice bran in biscuit will go a long way to reduce the pathological disorders associated with the sub-lethal doses of the cyanide over prolonged periods which are reported to be on the increase in cassava consuming countries like Nigeria, Brazil and India (Ekpechi, 1966; Balogopalan *et al.*, 1988).

Fat plays a significant role in the shelf life of food products. The fat content of the samples increased from 1.77% to 3.93% with sample A having the least value while sample C had the highest value. The fat content increased with the addition of BSG and rice bran. This is probably due to high fat content of BSG and oil retention ability of BSG during baking process (Rufeng *et al.*, 1995). Moreover, rice bran oil was responsible for the observed highest value of fat content in sample C. Rice bran oil in its natural state contains several constituents with great health benefits. Higher oil retention improves the mouth feel and retains the flavour of the biscuits. (Rufeng *et al.,* 1995). The fiber content of the samples ranged from 1.22% to 3.47% with sample A having the lowest value while sample C had the highest value. The increasing fiber content is probably due to the addition of BSG and rice bran which are rich in fiber (Quasem *et al.*, 2009; Singh *et al.*, 1999). Fibre is known to aid the digestive system of human indicating that BSG and rice bran could attract good acceptability by many people as well as health organizations.

The ash content of the biscuits increase from 1.37% to 2.69% with sample A having the least value while sample C had the highest value. The highest value observed in sample C was an indication that minerals in the rice bran were added to the biscuit. The increase in ash content could make the biscuit a good source of minerals as observed by De lemen *et al.* (2003).

The moisture contents of the biscuits decrease from 9.64% to 6.18% with sample D having the least value while sample A had the highest value. Water contributes significantly to the texture of food as a result of its physical interaction with proteins, polysaccharides, lipids and salts. The moisture content values obtained in this study, however, are minimal and this suggests reduced chances of spoilage by microorganisms and consequently increased shelf life.

The results of physical properties are shown in Table 2. Generally, significant ($p<0.05$) differences existed between the samples in almost all the parameters. The bulk density ranged from 0.66 to 0.67g/cm³. The bulk densities were not significantly ($p>0.03$) different from each other. Bulk density is linked with the expansion ratio in describing the degree of rising of the biscuit and also germane with regard to packaging. Therefore it is expected that under similar condition, as expansion index increases, the density would automatically decrease.

The water absorption capacity ranged from 1.83g/g to 2.64g/g with sample B having the least value while the sample A had the highest value. The highest water absorption capacity in sample A indicates higher affinity for water than other samples. For samples high in fiber, the water holding capacity increases with increase in fiber content (Abbey and Ibeh, 1998). The water absorption capacity of the biscuits were significantly ($p<0.03$) different from each other. Water absorption capacity has been generally attributed to the dispersion of starch in excess water or the water holding capacity of the sample. The dispersion usually increases by the degree of starch damage due to gelatinization. Water absorption depends relatively on the intact molecules which have the ability to bind and trap water. The ability of flour to absorb water was found to correlate with the starch content (Mgbofung *et al.*, 2006).

The result of apparent density ranged from 1.23g/cm³ to 1.34g/cm³ with sample A having the least value while sample C had the highest value. Apparent density is the weight per unit volume of the final product. The apparent density increased with the addition of rice bran. This could be attributed to the high fat content of rice bran. The apparent density of the biscuits were significantly ($p<0.03$) different from each other.

The results of anti-nutrient content of the biscuits are shown in Table 3. Generally, significant ($p<0.05$) differences existed between the samples in almost all the parameters. The phytate (phytic acid) ranged from 8.25µg/ml to 12.32µg/ml with sample C having the least value while sample B had the highest value. The addition of rice bran must have lowered the phytic acid content of the biscuits while the addition of BSG increased the phytic content. The high baking temperature may have also contributed in reducing the phytic content of the product. Low phytate level will ensure availability of divalent cation (Quinteros *et al.,* 2003).

The phytic acid content of the biscuits was found to be within the acceptable level as it is not up to 1 - 6% of the product which according to Oke (1969) will over a long period decreases the bioavailability of mineral element in monogastric. It bounds to mineral elements to form complexes that are indigestible, thereby decreasing the bioavailability of these elements for absorption (Erdman, 1979). The result obtained in this study implies that the phytic levels present in the biscuits will pose no health risk.

The tannin content ranged from 0.04µg/m to 0.13µg/ml with sample C having the lowest value while sample B had the highest value. The tannin content of the products increased with the addition of BSG. Tannin impairs absorption of non-haem iron (Hallberg and Hulthen, 2002) Tannin precipitates proteins, inhibit digestive enzymes and affect the utilization of vitamins and minerals. The tannin content is within the acceptable range since 76 - 90kg DM^{-1} is regarded as high level of tannin and could be detrimental if consumed as reported by Aletor (1995). Low tannin content of the biscuits is an indication

that the products will provide the available protein.

The results of micro-nutrient content of the biscuits are shown in Table 4. Generally, significant ($p<0.05$) differences existed between the samples in almost all the parameters. From the result, vitamin A ranged from 0.75 to 1.77 µg/ml with sample A having the least value while sample C had the highest value. Vitamin B_1 ranged from 0.67 to 1.96 (mg/mol) with sample A having the least value while sample C had the highest value. with sample A having the least value while sample C had the highest value. Vitamin C ranged from 0.03 to 1.55 (mg/mol) with sample A having the least value while sample C had the highest value. The highest micro-nutrient values obtained in sample C must have attributed to the addition of 10% rice bran which increased the vitamin content of the biscuits.

Vitamin retention in baked product is affected by temperature and baking duration. However in this study, all four sample were subjected to heat treatment at the same temperature therefore variation in the vitamin content and retention may be attributed to the composition of the various raw materials used. Vitamin C is an acid derivative of a 6-carbon sugar, which acts as a powerful reducing agent (Belitz e*t al.*, 2009). It is evident that sample C had higher vitamin content than the other samples. All the samples meet the recommended daily vitamin A which is about 0.7 – 0.9 mg. However, samples A did not meet the recommended daily vitamin B_1 (thiamine) of about 1.1 – 1.2 mg. All the samples did not meet the daily recommended vitamin C (Loveleena, 2011) although sample C contains higher level of vitamin C than the others.

The results of microbiological properties of the biscuits are presented in Table 5. The total viable count (TVC, Cfu/g) ranged from $0.04\text{-}0.12x10^2$ cfu/g. The counts were minimal and are within acceptable limits (Fawole and Oso, 1988). Micro-organisms play significant role in the determination of shelf lives of food products. They are usually responsible for spoilage of many food items (Olaoye *et al.*, 2007). The result revealed that the microbial content of the final product was found to be relatively low probably due to duration and the temperature of baking. Mould and coliform were not detected in all the samples . This might be as a result of proper hygiene practices. The low microbial load of the biscuits may increase the shelf life of the product if stored under proper conditions.

The sensory evaluation scores of biscuits produced from wheat, BSG and rice bran blends is shown in Table 6. Lower scores recorded in the blended samples for appearance, taste, and general acceptability compare to control sample is similar to the findings of Jiamyagyuen *et al;* (2005). The decrease values obtained also similar to the findings of Jiamyagyuen *et al*;(2005), in terms of liking scores of colour, taste, odour, texture, and overall acceptability of bread supplemented with rice bran protein. The highest score (7.38) for flavour recorded in sample C is an indication that the flavour of the biscuit was significantly enhanced by addition of rice bran (90% wheat, 10% rice bran).

Sample B was rated very low (6.04) in terms of texture probably due to its sharp deviation in attributes compared to control and the remaining samples. This is could be due to increase addition of BSG which affected the texture. The scores for general acceptability decreased from 8.84 for sample A to 7.53 for sample C which was the least. The result showed that samples A and D were not significantly ($p>0.05$) different from each

other in terms of overall acceptability. However, sample A was rated highest and most accepted probably due to panelists being used to biscuit produced from 100% wheat flour.

Table 1: Proximate Composition of Wheat Flour, BSG and Rice Bran Blends

Proximate (%)	Sample A	Sample B	Sample C	Sample D
Carbohydrate	75.40 ± 0.84^{a}	71.81 ^{b} ± 0.07	71.94 ± 0.04 ^{b}	72.33 ± 0.04^{b}
Protein	10.63 ± 0.05^{d}	12.65 ± 0.06 ^{b}	11.47 ± 0.06 ^{c}	13.26 ± 0.03 ^{a}
Fat	1.77 ± 0.03^{d}	2.55 ± 0.03^{c}	3.93 ± 0.02^{a}	3.32 ± 0.02^{b}
Fiber	1.22 ± 0.03^{d}	3.47 ± 0.03 ^{c}	2.96 ± 0.03 ^{a}	2.69 ± 0.04 ^{b}
Ash	1.37 ± 0.02^{d}	1.81 ± 0.06 ^{c}	2.69 ± 0.02 ^{a}	2.24 ± 0.03 ^{b}
Moisture content	9.64 ± 0.06 ^{a}	6.62 ± 0.02 ^{c}	6.98 ± 0.02 ^{b}	6.18 ± 0.02 ^{d}

Value represents mean ± SD (n = 3). Values bearing the same superscripts within the same row are not significantly (p>0.05) different from each other. Key: A = 100% wheat (control), B = 90% wheat, 10% BSG, C = 90% wheat, 10% Rice bran, D = 90% wheat, 5% BSG, 5% Rice bran

Table 2: Physical Properties of Flours from Wheat, BSG and Rice Bran Blends

Physical Properties	**A**	**B**	**C**	**D**
Bulk density (g/cm^3)	0.66 ± 0.03^{a}	0.66 ± 0 .02^{a}	0.67 ± 0.02^{a}	0.67 ± 0.02 ^{a}
WAC (g/g)	2.64 ± 0.04^{a}	1.83 ± 0.02 ^{d}	2.47 ± 0.03 ^{b}	2.27 ± 0.03^{c}
Apparent Density (g/cm^3)	1.23 ± 0.02 ^{d}	1.24 ± 0.02^{c}	1.34 ± 0.02^{a}	1.27 ± 0.02 ^{b}

Value represents mean ± SD (n = 3). Values bearing the same superscripts within the same row are not significantly (p>0.05) different from each other. Key: A = 100% wheat (control), B = 90% wheat, 10% BSG, C = 90% wheat, 10% Rice bran, D = 90% wheat, 5% BSG, 5% Rice bran.

Table 3: Anti-Nutrient Contents of Flours from Wheat Flour, BSG and Rice Bran

Anti-nutrients	**Sample A**	**Sample B**	**Sample C**	**Sample D**
Blends				
Phytate (µg/ml)	11.49 ± 0.03^{b}	12.32 ± 0.03^{a}	8.26 ± 0.04 ^{d}	9.46 ± 0.01^{c}
Tannin (µg/ml)	0.05±0.02^{c}	0.13±0.02^{a}	0.04±0.01^{d}	0.07±0.02^{b}

Value represents mean ± SD (n = 3). Values bearing the same superscripts within the same row are not significantly (p>0.05) different from each other. Key: A = 100% wheat (control), B = 90% wheat, 10% BSG, C = 90% wheat, 10% Rice bran, D = 90% wheat, 5% BSG, 5% Rice bran.

Table 4: Micro-nutrient Content of Flours from Wheat Flour, BSG and Rice Bran Blends

Micro-nutrient	Sample A	Sample B	Sample C	Sample D
Vitamin A (mg/ml)	0.75 ± 0.02 ^{d}	0.78 ± 0.02 ^{c}	1.77 ± 0.02^{a}	1.45 ± 0.02^{b}
Vitamin B_1 (mg/ml)	0.67 ± 0.02 ^{d}	1.74 ± 0.02 ^{c}	1.96 ± 0.02^{a}	1.84 ± 0.02^{b}
Vitamin C (mg/ml)	0.03 ± 0.02 ^{d}	0.04 ± 0.02^{c}	1.55 ± 0.03^{a}	1.23 ± 0.02^{b}

Value represents mean ± SD (n = 3). Values bearing the same superscripts within the same row are not significantly (p>0.05) different from each other. Key: A = 100% wheat (control), B = 90% wheat, 10% BSG, C = 90% wheat, 10% Rice bran, D = 90% wheat, 5% BSG, 5% Rice bran

Table 5: Microbiological Qualities of Flours from Wheat Flour, BSG and Rice Bran Blends

Parameters	Sample A	Sample B	Sample C	Sample D
Total viable count (cfu/ml)	$1.00x10^2$	$1.2x10^2$	$4.00x10^2$	$7.00x10^2$
Mould count (sfu/ml)	-ND-	-ND-	-ND-	-ND-
Coliforms (cfu/ml)	-ND-	-ND	-ND-	-ND-

Value represents mean ± SD (n = 3). Values bearing the same superscripts within the same row are not significantly (p>0.05) different from each other. Key: A = 100% wheat (control), B = 90% wheat, 10% BSG, C = 90% wheat, 10% Rice bran, D = 90% wheat, 5% BSG, 5% Rice bran. ND-Not Detected

Table 6: Sensory Evaluation Scores of Biscuits Produced from Wheat Flour, BSG and Rice Bran Blends

Attributes	A	B	C	D
Appearance	7.41±0.25^{a}	6.65±1.32^{c}	6.46±1.25^{d}	6.73±1.63^{b}
Crust colour	7.58±0.72^{a}	6.85±1.24^{c}	6.75±1.83^{d}	7.34±0.68^{b}
Crumb colour	7.42±0.75^{a}	6.59±0.92^{c}	6.41±1.74^{d}	6.78±0.63^{b}
Flavour	6.88±1.46^{c}	6.84±1.31^{d}	7.38±0.52^{a}	7.26±0.86^{b}
Taste	8.43±1.31^{a}	6.70±1.35^{c}	6.62±1.37^{d}	7.83±0.74^{b}
After taste	8.15±1.42^{a}	6.65±1.32^{c}	6.45±1.44^{d}	7.73±0.63^{b}
Mouthfeel	7.48±1.35^{a}	6.85±1.64^{c}	6.38±1.63^{d}	7.32±1.47^{b}
Texture	7.69±1.32^{a}	6.04±1.26^{d}	6.23±1.42^{c}	7.48±1.36^{b}
Crumb size	7.62±1.13^{a}	6.81±1.23^{c}	6.44±1.35^{d}	7.42±1.22^{b}
General acceptability	8.84±0.57^{a}	8.46±1.29^{b}	7.53±1.57^{c}	8.82±0.42^{a}

Value represents mean ± SD (n = 3). Values bearing the same superscripts within the same row are not significantly (p>0.05) different from each other. Key: A = 100% wheat (control), B = 90% wheat, 10% BSG, C = 90% wheat, 10% Rice bran, D = 90% wheat, 5% BSG, 5% Rice bran

Conclusion

Sample D had the best composition that combines optimal nutritive value with good characteristics for a biscuit. It contains an appreciable amount of protein (13.25%), low moisture content (6.18%) and moderate carbohydrate (72.33). The anti-nutrient (phytic acid and tannin) contents and microbiological (total viable count, mould and coliforms counts) qualities of the biscuits were found to be within the acceptable level.

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