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Utilization of sorghum, broken rice and white beans flours for producing high nutritional value and high quality gluten-free biscuits

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ABSTRACT

The aim of this study was to produce gluten-free biscuits from sorghum, broken rice flour with enriched white beans. The development of food products using composite flour has increased and is attracting much attention from researchers; especially in the production of bakery products .Six blends were prepared by homogeneously mixing sorghum flour and white beans flour in the following percentage proportions and used to make biscuits (80:0,70:10,60:20,50:30,40:40and30:50) (SB0,SB10,SB20,SB30 ,SB40 and SB50) , respectively. Broken rice flour was added by 20% level to all blends to improve the binding properties and the palatability of the produced biscuit. Control A (WB) was produced from 100% wheat flour as a superior quality biscuit consumed by all children. Control B (SB0) was produced from sorghum and broken rice flour as a biscuit consumed by the celiac disease children. The nutritional value and quality characteristics of produced biscuits were evaluated. Results showed that the SB40 biscuit had the highest score in overall acceptability among all the different blends of gluten-free biscuits. Hardness, spread ratio and spread factor of gluten-free biscuits increased with increasing the percentage of white beans flour. Protein, Fe, Ca, Zn, In-vitro protein digestibility (IVPD), total essential amino acids, chemical score and biological value were increased in gluten -free biscuits with increasing the level of white beans flour, meanwhile, total carbohydrate content and caloric value were decreased compared to control. It could be recommended that sorghum-broken rice and white beans composite flours could be utilized to improve nutritive value of gluten free biscuits and it is useful to be nutritious functional food.

Key words: gluten-free biscuits, sorghum, broken rice flour, white beans

Introduction

Biscuits are ready-to-eat, convenient and cheap snack that are consumed by all age groups in many countries (Bolarinwa *et al.,* 2016).

Composite flour refers to the mixture of different concentrations of non-wheat flours from cereals, legumes, roots and tubers with wheat flour or can be a mixture of flours other than wheat flour. Composite flours are recently manufactured not only to improve the desired functional properties of end product based on them but also to improve nutritional composition (Amir *et al.,* 2015).

Supplementation of sorghum with legumes has been advocated as a way of combating protein-calorie malnu- trition (PCM). Wheat, rice, sorghum and legumes grains were recommended as good ingredients for production of cereal-based baby foods *(*FAO, 1979).

Sorghum (*Sorghum bicolor* (L.) Moench) is the fifth most important cereal in the world after wheat, rice, maize and barley, in terms of production and is a major crop worldwide (FAO, 2009). Also, it's important for food security in Africa. According to Agriculture Directorates of Governorates statics (2015), the production of sorghum in Egypt was 720291 tons .It's called Milo or Jowar flour and available in red and white varieties. It has slightly sweet taste and rich in starch. It’s also high in fiber, phosphorous, potassium, vitamins B and phyotochemicals which making it a potential ingredient in the healthy food (Brou *et al.,* 2014). Absence of gluten makes sorghum highly favorable in the diet of gluten-intolerant populations (Kulamarva *et al.,* 2009).

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Dry beans (*Phaseolus vulgaris* L.) or common beans have been characterized as a nearly perfect food because of their high protein, fiber, prebiotic, vitamin B, and chemically diverse micro-nutrient composition. It is used throughout the world representing 50% of the grain legumes consumed as a human food source (Câmara, et al., 2013). According to Agriculture Directorates of Governorates statics (2015), the production of white beans in Egypt was 95970 tons.

The main phytochemicals found in Phaseolus vulgaris are enzyme inhibitors, tannins, lectins (phytohaemagglutinins), phytic acid (phytate), flatulence-causing α-galactosides and saponins . Flatulence-causing α-galactosides are oligosaccharides of the raffinose-series family which include raffinose, stachyose and verbascose. α-galactosides contribute to flatulence production in humans and mono-gastric animals due to a lack of the necessary α-galactosidase enzyme which helps to break down raffinose-series oligosaccharides during the consumption of dry beans. Hence, they are considered as unwanted components as a consequence of the accumulation of gas in the intestinal tract is discomfort, abdominal rumblings, cramps, pain and diarrhea after bean ingestion.(Admassu, 2008). However, various food processing and preparation techniques, such as decortications, soaking, cooking, germination and fermentation, are the major efforts made to reduce the amounts of anti-nutritional factors in foods (Mohamed *et al.,* 2011) .

Broken rice is a by-product produced during rice milling and polishing .It has been used in the manufacturing of rice flour and modified starches, with the advantages of low cost and great availability, as a value addition to various industrial utilization. Rice flour is a suitable ingredient to gluten-free products, because of its tender taste, white color, easily digestible carbohydrates, and hypoallergenic activities (Abdel-Haleem, 2016).

Rice, sorghum, and white beans flours formulations were used for the preparation of gluten free cookies. The blends of flour may reduce protein-energy malnutrition in the developing countries because of the expected improved nutritive value of the blend (Rai *et al.,* 2014).Therefore, the present investigation was carried out to produce gluten- free biscuits from sorghum-broken rice flour with enriched white beans and evaluate the nutritional value and quality characteristics of produced biscuits compared to wheat biscuit as a superior quality biscuit consumed by all children.

Materials and Methods

Materials

White sorghum (Dorado variety) grains and white bean (Nebraska variety) were obtained from Field Crops Research Institute, Agricultural Research Center, Giza, Egypt. Broken rice was obtained from Sakha Research Station, Agricultural Research Center, Kafrel-Shiekh, Egypt**.** Wheat flour (72% extraction rate), sugar, butter, table salt, baking powder, eggs and vanilla were purchased from local market, Cairo, Egypt. Standards of raffinose, stachyose, amino acids, catechin and vanillin were obtained from Sigma Co. Chemical, St. Louis, MO, USA). All other chemicals were of the analytical reagent grade.

Methods

Preparation of raw materials

Sorghum and white beans samples were carefully cleaned from impurities, and then washed with tap water. Sorghum grains were soaked in tap water for12h at room temperature (25˚C).After that, water was drained off and sorghum grains were dried in drying oven at 45˚C ± 5˚C/18 h. White beans seeds were soaked in water for 6 h at room temperature (25˚C). At the end of soaking period, the soaked water was discarded and hulls were removed manually, then, seeds were cooked in tap water at (100˚C) for15min until 50% of the seeds were softened when felt between the fingers according to Khattab and Arntfield, (2009). Cooked samples were dried in drying oven at 45˚C ± 5˚C/18 h.

All samples of dried sorghum, dried beans and broken rice were grounded into fine flour (315 micron) using an electric Brabender Duisburg roller mill, Germany and kept in polyethylene bags at refrigerator till using.

Preparation of biscuits

Biscuits were prepared according to Sayed, (2011).The formula used for biscuits preparation is shown in Table (1).Six blends were prepared by homo -geneously mixing sorghum flour and white beans flour in the percentage proportions, then used to make biscuits: 80:0,70:10, 60:20, 50:30, 40:40 and 30:50(SB0,SB10,SB20,SB30 ,SB40 and SB50) ,broken rice flour was added by 20% level to all blends to improve the binding properties of the produced biscuit. Control A (WB) was produced from 100% wheat flour as a superior quality biscuit consumed by all children, control B (SB0) was produced from sorghum and broken rice flours (80:20) as another biscuit consumed by the celiac diseases children. Butter and sugar were mixed using a Kenwood mixer at a medium speed until a light and fluffy creamy texture was formed, then eggs and vanilla were added and the mixing process were continued. Sorghum, white beans and rice flours were slowly added to the mixture, then the biscuits dough was sheeted, cut and baked at 160˚C for 15 min. Biscuit samples were cooled at room temperature for10 minutes after baking until sensory evaluation ,then kept in polyethylene bags at refrigerator till further analyses.

Table 1: Biscuits formula

Ingredients / Blends	Wheat flour (g)	Sorghum flour (g)	White beans Flour (g)	Rice Flour (g)	Sugar (g)	Butter (g)	Baking Powder (g)	Eggs (g)	Salt (g)	Vanilla (g)
WB Control A	100	0	0	0	30	30	3	15	0.5	1
SB0 Control B	0	80	0	20	30	30	3	15	0.5	1
SB10	0	70	10	20	30	30	3	15	0.5	1
SB20	0	60	20	20	30	30	3	15	0.5	1
SB30	0	50	30	20	30	30	3	15	0.5	1
SB40	0	40	40	20	30	30	3	15	0.5	1
SB50	0	30	50	20	30	30	3	15	0.5	1

WB (Control A) :Biscuit from wheat; SB0: Biscuit from 80 Sorghum flour +0 White beans flour(control B) ; SB10 : Biscuit from 70 Sorghum + 10 White beans flour; SB20: Biscuit from 60 Sorghum +20 White beans flour ; SB30 : Biscuit from 50 Sorghum flour +30 White beans flour;SB40 : Biscuit from 40 Sorghum+40 White beans flour; SB50: Biscuit from 30 Sorghum flourcc +50 White beans flour (w:w).

Sensory evaluation of biscuits:

Sensory evaluation of biscuit samples was conducted according to Larmond (1977), by ten panelists from the staff of Food Technology Research Institute, Agricultural Research Center, Giza, Egypt. Assigning scores for various qualities attributes such as: appearance, color, odor, texture, taste and overall acceptability on a nine- point hedonic scale (1= disliked extremely, to 9 = like extremely).

Physical characteristics of biscuits:

Hardness of biscuits were measured by using Brookfield Engineering Lab. Inc., Middleboro MA 02346-1031 USA (AACC, 2002).

Biscuit diameter and thickness were measured according to AACC (2002).The diameter (D) was measured by placing six biscuits edge to edge to get an average diameter in millimeters. The Thickness (T) was measured by stacking six biscuits on top of one another and measuring them to get the average in millimeters. The spread ratio and spread factor were evaluated according to Manohar and Rao (1997), by using the following equations:

$$\text{Spread ratio (Sr)} = \text{Width} / \text{Height}$$

$$\text{Spread factor (Sf)} = \text{Spread ratio of sample} / \text{Spread ratio of control} \times 100$$

Water activity (a_w):

The water activity (a_w) of the biscuits was measured using Rotronic Hygrolab 3 CH-8303, Switzerland as mentioned by Cadden, (1988).

Chemical composition and minerals content:

Moisture, ash, protein, and fat were analyzed according to AOAC (2005). Total carbohydrates was calculated by difference from total gross chemical composition) on dry weight basis. Zinc, iron and calcium contents were analyzed by Perkin Elmer (Model3300, USA) Atomic Absorption according to the methods of AOAC, (2005).

Energy value of raw materials and biscuits:

Energy value of raw materials and biscuits were calculated from the following equation as reported by James, (1995).
Energy value (Kcal/100g) = 4 (g protein + g carbohydrates) + 9 (g fat).

Raffinose Family:

The levels of raffinose and stachyose are determined by HPLC according to Zielinski *et al.* (2014). An aliquot of 1.5 ml of diluted samples were injected into HPLC. Agilent packared (Series 1100) equipped with auto sampling injector and IR detector. The column (Aminex-carbohydrate.HPX-87C, 300 mm x7.8 mm) temperature was maintained at 35˚C.

Tannins content:

Tannins was determined by using the Vanillin-HCL method modified by Price *et al.* (1978). Catechin was used to prepare the standard curve.

In Vitro protein digestibility (IVPD):

In vitro protein digestibility (IVPD) was determined according to the method of Akeson and Stahmann (1964). After enzymatic digestion of samples with pepsin (37˚c/3h) and pancreatin (37˚c/24h), the protein in the resultant supernatant was estimated using the Kjeldahl method according to AOAC (2005). The percentage of protein digestibility was calculated by the ratio of protein in supernatant to protein in sample equation:

$$\text{Protein digestibility}\ \% = (\text{N in supernatant} - \text{N in blank} / \text{N in sample})\text{x}100$$

N= Nitrogen

Determination of Amino acids Profile of biscuits;

Amino acids content was determined using amino acids analyzer Biochrom 30 using the instruction manual according to AOAC, (2005).

Chemical score of biscuits:

Chemical score were calculated according to FAO/WHO (2007).
Chemical score %= (Essential amino acid of crude protein)/(Essential amino acid of FAO/WHO)×100

Biological value of biscuits:

Biological value of biscuits was calculated according to Eggam *et al.* (1979) as follows:

$$\text{Biological value}\ \% = 39.55 + 8.89 \times \text{lysin}\ \ (\text{g}/100\text{g protein})$$

Statistical analysis:

The obtained data were subjected to one-way analysis of variance (ANOVA) at P < 0.05. The results were separated using the Multiple Range Duncans test using the SAS (1987) statistical software.

Results and Discussion

Chemical Composition and minerals content of sorghum, broken rice and white beans flours.

The production of good quality biscuit would depend on selection the correct flour for each type and appropriate processes (Agu *et al.,* 2014).
Data in Table (2)showed that the white beans flour contained higher level of protein, ash, crude fiber and lower level of total carbohydrates compared to wheat, sorghum and broken rice flours. From the same Table, white beans flour was a rich source of calcium and iron. White beans is an excellent source of protein, fiber, ash, calcium, iron and zinc (Camara *et al.,* 2013; Brigide *et al.,* 2014).

Table 2: Chemical Composition (g/100g) and Minerals Content (mg /100g) on dry weight of raw materials.

Analysis / Raw materials	Ash	Crude fiber	Protein	Crude fat	T.C*	Ca	Fe	Zn
Wheat flour	0.66 c ±0.04	0.39 c ±0.05	11.36 b ±0.15	1.18 b ±0.09	86.41 b ±0.03	18.90c ±0.20	0.94c ±0.04	1.35 c ±0.05
Sorghum flour	1.73 b ±0.09	2.00 b ±0.02	10.65 c ±0.08	2.34 a ±0.06	83.28 c ±0.13	37.9 b ±0.10	5.4b ±0.30	4.01a ±0.11
White beans flour	2.98 a ±0.06	2.38 a ±0.26	21.91 a ±0.04	1.27 b ±0.13	71.46 d ±0.45	89.50a ±0.20	8.95a ±0.05	2.88 b ±0.08
Broken rice flour	0.43 d ±0.02	0.28 c ±0.02	7.95 d ±0.02	0.32 c ±0.02	91.02 a ±0.01	9.56 d ±0.03	0.56 d ±0.06	0.42 d ±0.02

T.C= Total carbohydrate calculated by deference*
Values are means (n=3) ±SD & Means with different letters are significantly different at P≤ 0.05

The highest fat and zinc contents were observed in sorghum flour. Moreover, the rice flour distinguished by the highest value of total carbohydrates. Chemical composition of raw materials is close to Seleem and Omran (2014); Abdel-Haleem and Hafez, (2015); Sayed, *et al.,* (2016) ; Krupa-Kozak and Drabinska, 2016).

Sensory evaluation of biscuits:

Sensory evaluation is considered a valuable tool in solving problems involving food acceptability. It is useful in product improvement, quality maintenance and more important in a new products development (Aly and Seleem, 2015).

The sensory characteristics of different biscuit samples are presented in Table **(3)**. It could be noticed that there were significant differences between the sorghum-white beans biscuits and control in all sensory parameters. Control A (WB) had the highest sensory scores relative to other biscuit samples. Furthermore, sensory scores of produced biscuits increase by increasing the addition of white beans flour.

Regarding taste score, results showed that increasing of white beans up to 50% affect significantly taste score of sorghum biscuits. However, significant increase in taste score was observed at SB40 compared with control B (SB0), it might be due to flavor associated with white beans and sorghum flour. The texture increased significantly with increase percentage of white beans.

This may be due to texture hardness caused by high protein contents of white beans flour. In addition, there were no significant differences between SB30, SB40 and SB50 in appearance, color, odor and texture.

Overall acceptability scores provide a general acceptability of the product based on all of the sensory parameters. SB40 had highest score in overall acceptability compared with other sorghum-white bean biscuit samples (Elobeid and Berghofer, 2014; Ciacci et al., 2007) reported that the sorghum derived cookies had an excellent palatability.

Table 3: Sensory evaluation of biscuits

Characteristics / Blends	Appearance 9	Color 9	Odor 9	Texture 9	Taste 9	Overall Acceptability 9
WB (Control A)	8.52±0.29 a	8.42±0.34 a	8.75±0.18 a	8.95±0.15 a	8.77±0.24a	8.78±0.18 a
SB0 (Control B)	7.29±0.09 c	6.88±0.09 c	7.51±0.44 c	7.74±0.32 c	7.39 ±0.19 c	7.49±0.03 d
SB10	7.48±0.12 c	7.29±0.64 c	7.53±0.17 c	7.92±0.09 bc	7.91±0.62bc	7.75±0.15 c
SB20	7.79 ±0.25b	7.87±0.08 b	7.77±0.15 bc	7.96±0.07 bc	7.72±0.22bc	7.94±0.19bc
SB30	8.02±0.03 b	7.98±0.09 ab	7.81±0.16 bc	8.01±0.02 b	7.87±0.14bc	8.04±0.13 b
SB40	8.08±0.03 b	8.04±0.05 ab	7.97±0.07 b	8.03±0.02 b	7.97±0.04 b	8.11±0.11 b
SB50	7.98±0.08 b	8.02±0.03 ab	7.90±0.10 b	8.05±0.04 b	7.84±0.06 bc	8.06±0.07 b

WB :Biscuit from wheat (control A); SB0: Biscuit from 80 Sorghum flour +0 White beans flour(control B) ;
SB10 : Biscuit from 70 Sorghum +10 White beans flour; SB20: Biscuit from 60 Sorghum +20 White beans flour ; SB30 : Biscuit from 50 Sorghum flour +30 White beans flour; SB40 : Biscuit from 40 Sorghum+40 White beans flour; SB50: Biscuit from 30 Sorghum flour +50 White beans flour .
Values are means (n=10) ± SD & Means with different letters are significantly different at $P \leq 0.05$.

Physical Characteristics of biscuits

The physical characteristics such as hardness, diameter, thickness, spread ratio, spread factor, water content and water activity of different biscuit samples are shown in Table (4).

Table 4: Physical characteristics of biscuits

Characteristics / Blends	Hardness H(N)	Diameter D(cm)	Thickness T (cm)	Spread Ratio (D/T)	Spread factor %	Water content %	Water Activity (a_w)
WB (Control A)	47.94 ± 0.27a	5.72± 0.1a	0.65± 0.01a	8.80± 0.10c	100± 0.00c	6.08± 0.01b	0.481± 0.02a
SB0 (Control B)	21.83± 2.42c	5.42± 0.07b	0.52± 0.03b	10.42± 0.01b	118.41±0. 20b	6.80± 0.14a	0.472± 0.01ab
SB40	43.35± 2.19b	5.46± 0.05b	0.44± 0.01c	12.42± 0.02a	141.02±0. 22a	5.81± 0.19b	0.451± 0.01b

WB =Biscuit from wheat (control A); SB0= Biscuit from 80 Sorghum flour +0 White beans flour (control B)
SB40 = Biscuit from 40 Sorghum+40 White beans flour,
Values are means (n=3) ±SD & Means with different letters are significantly different at $P \leq 0.05$

Results showed that hardness was increased in sorghum biscuits enriched with white beans, it could be attributed to high protein content of white beans. Sarabhai *et al.* (2015) and Ikuomola *et al.* (2017) stated that hardness of biscuits increase with increase in protein content of biscuits.

The same Table showed that there were slight differences in the diameter and thickness of biscuit samples. The highest diameter was observed in wheat biscuit (WB), while there no significant differences between sorghum biscuits (SB0) and sorghum biscuits enriched with white beans (SB40) in diameter.

Spread ratio is considered as one of the most important quality parameter of biscuits as it correlates with texture, grain finesse, bite and overall mouth feel of the biscuits. Biscuit spread ratio stand for a ratio of diameter to height (Bose and Shams-ud-din, 2010).The spread ratio significantly increase with adding of white beans flour .The highest spread ratio was observed in sorghum biscuit enriched with white beans (SB40) and the least spread ratio was observed in wheat biscuit (WB*).* Chauhan *et al.,* (2016) stated that biscuits having higher spread ratios are considered the most desirable dough with lower viscosity causes cookies to spread at a faster rate, so as a result viscosity of dough reduces as addition of white beans flour and increases the spread rate. It is noticed that the spread ratio increase with adding of white beans flour and with increase in the protein content of the biscuits and it could have been affected by absence of gluten .These results are in agreement with Singh *et al.* (2003) ;Chauhan et al. (2016). Omoba and Omogbemile, (2013) showed that the spread ratio could have been affected by the competition of ingredients and other functional properties of proteins and fat.

The spread factor are given in Table (4).Considering the spread factor of wheat biscuit (control) as 100, while it was increased to 118.41and 141.02% for sorghum biscuits(SB0) and sorghum biscuits enriched with white beans(SB40), respectively, The data close to Youssef, (2015).
From the same Table, results revealed that increasing the substitution levels with white beans in the biscuits slightly decreased the water activity of biscuits relative to control (A) 0.481.

Chemical composition, energy value and minerals content of biscuits

Chemical composition and minerals content of biscuits are presented in Table (5). Data revealed that biscuit sample SB40 had higher ash, crude fiber, protein, Ca, Fe and Zn contents than other biscuit samples (WB and SB0). It could be attributed to the effect of substitution with white beans flour. Considering Dietary Reference Intake (DRI, 2002/2005) each 100 g SB40 (as sample recorded the highest score of overall acceptability) provide 49.92%from daily intake of protein (based on 28g/day), 47.92% from daily intake of TC (based on 130g/day) and it represents 57.46 to 80.44% from daily intake of fat (based on 25-35% distribution range) for children 3- 10y.

Considering the Energy Requirements (ER), according to Torun (2005), SB40 will provide 24.27% from the ER for children 3-10y, doing light physical activity (calculated as 2000 Kcal/day).
From the same Table, significant fluctuations in Ca, Fe and Zn contents were observed. There was a significant increase in SB40 compared toSB0 and WB. Considering Dietary Reference Intake (DRI, 2001), each 100 g SB40 provide 9.93% from daily intake of Ca (based on 700 mg/ day), 93.92% from daily intake of Fe (based on 7.4 mg/d) and 69.29 % from daily intake of Zn (based on 5.6 mg/d) for children 3- 10y.

Camara *et al.* (2013) who reported that dry beans (*Phaseolus vulgaris* L.) or common beans, have been characterized as a nearly perfect food because of their high protein, fiber, prebiotic, vitamin B, and chemically diverse micronutrient composition.

From the above mentioned data about the chemical composition of biscuits, it could be demonstrated that the gluten free biscuits had reasonable amounts of the required nutrients for children particularly protein, fat, Ca, Fe, Zn and energy.

The Rafinose family, tannins content and in vitro protein digestibility

Raffinose family oligosaccharides (RFOs) are important constituents of a wide variety of grain legumes and are thought to be the major producers of flatulence. HPLC method is the most precise and sensitive technique to determin rafinose family. Raffinose family or flatulence factors (raffinose and stachyose) and total galactoside contents in (white sorghum and white beans) before and after processing methods (soaking,dehulling and boiling) are shown in Table (6). Stachyose was found to be the predominant sugar in white beans, while *raffinose* value was higher than stachyose in sorghum. Similar results were reported by Admassu, (2008) and Murty *et al.,* (1985). The results revealed differences between varieties of the raw seeds. Raffinose and stachyose concentrations of raw white sorghum before processing were 0.39 and 0.19 respectively. After soaking sorghum and discarding the water, the flatulence factors were decreased, these findings are in the line with Afify *et al.,* (2012). The values of raffinose and stachyose concentrations of raw dry white beans before proccesing was 0.48% and 0.96% ,respectively, these findings close to Muzquiz *et al.* (1999). Results indicated that the raffinose and stachyose reduced after soaking with discarding the water, dehulling and boiling white beans. Ramadan, (2012) mentioned that the raffinose, stachyose and verbascose are soluble in water, therefore, soaking and cooking beans in water and discarding the water will remove most of these sugars from beans.

Table 5: Chemical Composition (g/100g), Energy value (Kcal/100g) and Mineral Contents (mg/100g) of Biscuits on dry weight.

Analysis / Blends	Ash	Crude fiber	Protein	Crude fat	T.C*	Energy value (Kcal)	Ca	Fe	Zn
WB (Control A)	1.20 c ±0.05	0.27 b ±0.05	10.65 b ±0.03	21.90 a ±0.25	65.98 a ±0.30	503.62 a ±0.93	19.55c ±0.15	0.97c ±0.07	1.15c ±0.05
SB0 (Control B)	1.98 b ±0.07	0.85 a ±0.11	10.60 b ±0.03	20.31 b ±0.16	66.26 b ±0.29	490.23 b ±0.43	32.21b ±0.2	4.66b ±0.06	2.99b ±0.09
SB40	2.87 a ±0.13	0.92 a ±0.03	13.80 a ±0.05	20.11 b ±0.07	62.30 c ±0.10	485.40 c ±0.97	69.48a ±0.08	6.95a ±0.05	3.88a ±0.02
FAO/WHO /UNU*** (3-10 years)	-------	25-30 g /day	28 g /day	25-35 g /day	130 g /day	2000 Kcal /day	700 mg /day	7.4 mg /day	5.6 mg /day

WB =Biscuit from wheat (control A); SB0= Biscuit from 80 Sorghum flour +0 White beans flour(control B) , SB40 = Biscuit from 40 Sorghum+40 White beans flour.
Values are means (n=3) ±SD & Means with different letters are significantly different at P≤ 0.05
T.C= Total carbohydrate calculated by deference.*
*FAO/WHO/UNU*** (2007), FAO =Food and Agriculture Organization of The United Nations.*
WHO =World Health Organization.,unu = The United Nations University.

Table 6: The raffinose family, tannins content and in vitro protein digestibility

Analysis / Samples	Raffinos	Stachyose	Total galactosides%	Tannins mg/100 g	*in vitro* protein digestibility %
Sorghum 1	0.39	0.19	0.58	2.19	48.75
Sorghum 2	ND	ND	-	1.87	61.97
White beans 1	0.48	0.96	1.44	211.00	65.83
White beans 2	0.21	ND	0.21	78.61	77.75
WB (Control A)	---	---	---	ND	71.57
SB0 (Control B)	---	---	---	1.06	37.57
SB40	---	---	---	21.43	66.69

Sorghum 1= before soaking, Sorghum 2 = after soaking, White beans 1 = before soaking, White beans 2 = after soaking, dehulling and boiling.
WB: Biscuit from wheat (control A); SB0: Biscuit from 80 Sorghum flour +0 White beans flour (control B); SB40 : Biscuit from 40 Sorghum+40 White beans flour.

Soaking (6 h/room temperature),dehulling and boiling (100˚C/15min) in water resulted in a loss of total α-galactosides to 85% in white beans flour compared to the corresponding raw sample. Jangchud and Bunnag, (2001) stated that soaking raw red kidney beans for 12 hours, then soaking water should be discarded prior to boiling and boiling for 15 min could decrease the raffinose and stachyose contents by 47% and 44%, respectively. Soaking and boiling / cooking, these processes improve the appeal and sensory properties of legume. Boiling is usually at 100° C for some minutes, It eliminates heat labile anti-nutritional factors (Subuola *et al.,* 2012). Nyombaire *et al.* (2006) reported that four hours of soaking resulted in greater than 76 and 84% of stachyose and raffinose reduction, respectively and they showed that it may not be necessary to soak beans for 12 hours if the aim is to reduce flatulence-producing oligosaccharides. Complete removal of flatus-producing oligosaccharides is not advisable as they have health benefits. The processing produced a higher reduction of total α-galactosides, in relation to the content of raw seeds. These reductions may have been due to leaching and solubility of sugars during soaking and boiling. The current results are in agreement with those reported by Oboh *et al.* (2000).

From the data presented in Table (6), it was apparent that tannin content of white sorghum and white beans flour decreased after soaking (for sorghum and white beans), dehulling and boiling(for white beans). Tannin reductions of 14.61% and 62.74% in sorghums and white beans, respectively, these results are in agreement with Awika and Rooney (2004); Chiremba *et al.* (2009).

Tannins in legumes, cereals and other foods were reduced by using one or combined methods, such as soaking, dehulling, cooking and soaking followed by cooking (Gulewicz *et al.* 2014). From data presented in Table 6 , biscuits had lower tannins content compared with raw flours(sorghum and white beans), these results agreed with Chiremba *et al.,* (2009) who showed that biscuit making caused substantial reductions of 95 and 96% in tannins .

In vitro Protein digestibility (IVPD) is an important factor for evaluation of protein quality as well as an indicator for protein bioavailability in food (Chinma *et al.,* 2011).

Data from Table 6 showed that the method of processing, before preparing sorghum flour and white beans flour (soaking, dehulling and boiling), increased the IVPD. Hassan *et al.* (2005) estimated the in vitro-protein digestibility of lupin seeds and processing methods(soaking, cooking, dehulling) of lupin seeds improved protein digestibility. IVPD for sorghum biscuits (SB0) increased with added white beans flour.At SB40, IVPD was 77% higher than biscuits from sorghum only (SB0).These findings agree with the results of Serrem et al. (2011). Nakitto *et al.* (2015) stated that Overall, combined processing of beans (dehulling, soaking, sprouting, steaming and roasting were eliminated phytates and tannins and increased protein digestibility. In vitro protein digestibility and mineral (iron and zinc) extractability were negatively correlated with tannin and phytate content.

In generally, soaking, dehulling and cooking resulted in a loss of raffinose, stachyose and tannins, compared to the corresponding raw seeds. These processing methods on the other hand led to an increasing in protein digestibility (IVPD). These results are in the line with Wang *et al.* (2009); Hefnawy (2011).

Amino acids profile, Chemical Score and Biological value of Biscuits

Amino acids of food product are important indicators of protein quality. The essential amino acids are necessary for tissue maintenance and required for growth of children.

Results presented in Table (7) showed essential and non essential amino acids content of biscuit samples. Results indicated that WB sample had the lowest total levels of all amino acids, while accepted sample (SB40) showed higher levels of the amino acids, when compared with recommended values of FAO/WHO/UNU, (2007) for children between 3-10 years. Therefore, white beans protein could be a very well complement those protein sources that are low in lysine, tryptophan, leucine, total sulfur amino acids, threonine and valline. The lysine content of sorghum- white beans biscuits (SB40) was markedly improved by 89.83%, compared to sorghum biscuits (SB0). The increase due to that white beans protein having higher lysine than the cereals (Serrem *et al.,* 2011).

The chemical scores is one method for assessing dietary protein quality, it is determining by the ratio of a gram of the limiting amino acid in a test diet to the same amount of the corresponding amino acid in a reference diet multiplied by 100.Consequently, the biscuit protein lysine scores (chemical scores) were improved from 51.04% in SB0 to 96.67% in SB40 (Serrem *et al.,* 2011).

The increase can be explained by the higher lysine contained in white beans flour. Considering Dietary Reference Intake (DRI 2002/2005) each 100 g SB40 provide 96.67%from daily intake of lysine (based on 4.8g/day) for 3 to 10 year old children. Similar results were reported by Serrem, (2010). From the same Table, the chemical scores in all biscuit samples showed that the limiting amino acid was lysine. Besides, chemical score of sorghum- white beans biscuit (SB40) was higher for all essential amino acids. Protein digestibility is an important factor to estimate the protein availability for intestinal absorption after digestion reflecting on the efficiency of protein utilization on diet FAO/WHO/UNU, (2007).

Table 7: Amino acids profile, chemical score and biological value of biscuits (3-10 years).

	WB			SB0			SB40			
Essential Amino Acid	Amino acid g/100 sample	Amino acid g/100 protein	Chemical score %	Amino acid g/100 sample	Amino acid g/100 protein	Chemical score cc%	Amino acid g/100 sample	Amino acid g/100 protein	Chemical score %	FAO/[1] WHO/ UNU
Leucine	0.52	4.88	80	0.76	7.17	117.54	1.08	7.83	128.36	6.1
Valine	0.36	3.38	84.5	0.43	4.05	101.25	0.71	5.15	128.75	4.0
Lysine	0.19	1.74	36.25*	0.26	2.45	51.04*	0.64	4.64	96.67*	4.8
Isolucine	0.28	2.63	84.84	0.31	2.92	94.19	0.53	3.84	123.87	3.1
Phenylalani +	0.39	3.66	156.43	0.41	3.87	166.19	0.71	5.15	212.38	AAA 4.2
Tyrosine (AAA)	0.31	2.91		0.33	3.11		0.52	3.77		
Methonine +	0.21	1.97		0.20	1.89		0.25	1.81		SA A
Cystine **(SAA)	0.28	2.63	191.67	0.16	1.51	141.67	0.31	2.25	169.17	2. 4
Theronine	0.21	1.97	78.80	0.24	2.26	86.92	0.45	3.26	130.40	2.5
Hisitidine	0.17	1.59	99.38	0.19	1.79	111.88	0.33	2.39	149.38	1.6
Tryptophan	ND	ND	-	ND	ND	-	ND	ND	-	6.6
Total E.A.A	2.92	27.36	77.73	3.29	31.04	88.19	5.53	40.09	113.89	35.20
Chemical score[2] (Lysine score)	36.25%			51.04%			96.67%			
Limiting Amino acids	Lysine			Lysine			Lysine			
*IVPD	71.57			37.57			66.69			
[3]PDCAAS	0.26			0 .19			0.65			
[4]B.V.%	55.02			61.33			80.80			

	WB		SB0		SB40	
Non-Essential Amino Acid	Amino acid g/100 sample	Amino Acid g/10 protein	Amino acid g/100 sample	Amino acid g/100 protein	Amino acid g/100 sample	Amino acid g/100 protein
Aspartic	0.48	4.51	0.65	6.13	1.17	8.48
Serine	0.27	2.54	0.30	2.83	0.55	3.98
Glutamic	2.10	19.72	1.28	12.07	1.99	14.43
Glycine	0.27	2.54	0.23	2.17	0.43	3.12
Alanin	0.28	2.63	0.56	5.28	0.73	5.29
Argnine	0.32	3.00	0.38	3.58	0.69	5.00
proline	0.72	6.76	0.43	4.05	0.57	4.13
Total Non –E.A.A	4.44	41.7	3.83	36.11	6.13	44.43

WB: Biscuit from wheat (control A); SB0: Biscuit from 80 Sorghum flour +0 White beans flour (control B); SB40 :Biscuit from 40 Sorghum+40 White beans flour

Most limiting amino acid- AAA=aromatic amino acids.,**SAA= sulfur amino acids., *** (IVPD) =In vitro protein digestibility.*

[1]Amino acid reference patterns for children 3-10 years adapted from (WHO, 2007),

[2]Lysine score= mg lysine in1g protein of test sample/48 mg lysine in requirement pattern for children 3-10 year; (W H O, 2007).

[3]PDCAAS = [3]Protein Digestibility Corrected Amino Acid Score (lysine score x IVPD/100).

[4]B.V. =Biological value (%) = 39.55+8.89 x lysine (g/100g protein)

The in vitro protein digestibility (IVPD) assay is a widely used method to determine the digestibility parameter and it mimics conditions simulated by the digestive processes occurring in the human gastrointestinal tract through proteolytic enzymes (i.e. pepsin-pancreatin enzyme system or papain system), measuring the percentage of proteins which is hydrolyzed by such enzymes .This method is faster, more affordable, and equally effective than in vivo assays (Almeida *et al.,* 2015). Compositing with white beans substantially increased the IVPD of the sorghum biscuits (Table 7). IVPD was higher 77% for (SB40) than the sorghum only biscuit (SB0). These results agree with the results of Serrem *et al.,* (2011). As a consequence of the dramatic improvement in lysine score in the sorghum biscuits and improvement in the IVPD of the sorghum biscuits as a result of compositing with white beans.

Protein Digestibility Corrected Amino Acid Score (PDCAAS) is the internationally accepted measure of food protein quality and is used to assess the protein quality of both dietary mixtures and individual protein food sources. The highest possible PDCAAS is 1.0. A score of 1.0 means that after digestion of the food, it provides 100% or more of the recommended amount of essential amino acids per unit of protein (World Health Organization, 2007). PDCAAS was three times higher in biscuits (SB40), compared to control B (SB0).

The biological value (B.V) of a protein is a number from 100 down to 0, that describes how well it is absorbed by the body. More precisely, it is a measure of the percentage of the protein that is actually incorporated into the proteins of the human body (World Health Organization, 2007). Biological value (BV) of WB, SB0, and SB40 biscuits is shown in Table (7). Results indicated that white beans flour increased the biological value to SB40 compared with WB and SB0 (55.02, 61.33 and 80.80%, respectively). There was a positive correlation between biological value and lysine 100 g^{-1} proteins (Eggam *et al.,* 1979). Higher lysine content was associated with increasing in biological value (Kohajdova *et al.,* 2011).

In generally, it could be noticed that, the total EAA and total non-EAA were highest amount in SB40 compared to WB and SB0.

Conclusion

Based on our results, it could be concluded that gluten free flours (sorghum, broken rice and white beans) composite could be used to produce good quality biscuits with acceptable sensory, physical qualities and nutritional values. This study demonstrated the possibility of incorporating white beans flour into sorghum biscuits processing. This product could be used to improve child nutrition, alleviate protein-energy malnutrition (PEM) and celiac patient due to its high amount of protein and provides calories and a good source of amino acids and Zn, Fe, and Ca and this product had good *in vitro protein* digestibility, good stability and extending shelf-life and it had no raffinose family contents and low level of tannin. Sensory evaluation shows that the characteristics of biscuit sorghum-white beans at 40% level are close to the characteristics of wheat biscuit. Therefore, utilization of white beans flour into sorghum biscuits improved sensory characteristic, nutritional value and could be useful for locally product white beans at bakeries industry.

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