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Technological and Nutritional Evaluation of Biscuits Fortified Amaranths

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ABSTRACT

Amaranth grain (*Amaranthus cruentus*) is a pseudo cereal consumed in various parts of the world with potential as a source of dietary nutrients. Amaranth grain is a good source of protein, crude fiber and vitamins and therefore is used largely for feeding children and the elderly. Therefore it can be used to produce biscuits from amaranth (flour, wholemeal and hull) with defatted soybean, sorghum and rice which play an important role in the diets for diarrhea patient. The chemical analysis, total phenolic, total flavonoids compounds were determined in raw materials. Whereas, the biscuits were prepared with 20% from amaranth fraction (flour, wholemeal and hull) and it was added separately to 50% sorghum, 20% rice mill and 10%defatted soybean to give three formulae. Sensory evaluation, color and texture analysis were characteristics of different biscuits. Biscuits made from different formulae were estimated using biological evaluation on diarrhea rat groups. The results showed that the defatted soybean was the highest in protein (51.6%). Meanwhile, amaranth fractions were the richest in crude fiber and total dietary fiber. Moreover, sorghum and rice mill were increased in total carbohydrates and the minerals content were the highest in all raw materials. Total phenolic and total flavonoids compounds were higher in defatted soybean and amaranth than other raw materials. The results of sensory properties indicated that no significant difference was found between control sample made from wheat flour 72% extraction and supplemented samples for all evaluated characteristics of biscuits contained 20% from amaranth. Color and texture analysis showed that the formulae made from raw materials and amaranth flour and wholemeal were nearly or equal control sample. Moreover the physical characteristics of different biscuits were determined. At the end of biological experimental the results reported that the protein efficiency ratio, net protein utilization and biological value were the lowest in the rats on basal diet described as control. Whereas, the protein values at all formulae were higher than control. The formula one had contained the highest (2.40, 89.31 and 91.12%) in protein efficiency ratio, net protein utilization and biological value in diarrhea rats followed by formulae 2 and 3, respectively. Moreover, the results occurred that the biscuits made from different formulae had improvement the kidney functions. This study achieved the objective of developing a complementary the biscuit product of adequate nutritive value that can be prepared using locally available resources and technology as sorghum, defatted soybean, rice mill and amaranth fractions to treat diarrhea patients. From the obviously results it could be recommended that the biscuits prepared flour and wholemeal and other components were acceptability. Moreover, the biscuits made from different formulae with amaranth fractions produced a ready nutritious product which improvement the kidney functions and treatment for diarrhea patient.

Key words: Amaranth, defatted soybean, sorghum and rice mill, diarrhea rat groups, kidney functions.

Introduction

The plant family Amaranthaceae consists of approximately 850 species in 65 genera, and is distributed world-wide, in both temperate and tropical regions. Many of these species are commonly used by man as medicinal herbs and as sources of both edible leaves and seeds (Marcone *et al*., 2003).

Amaranth is a plant which contained antihypertensive, antioxidant and cancer preventive peptides. Also there is evidence that amaranth has some hypoglycemic action; however, the antidiabetic potential and the effect upon body weight of the seed proteins have not been well characterized Velarde-Salcedo *et al*. (2012). In the last decade, the use of amaranth had broadened not only in the common diet (Berti *et al*., 2005). These pseudocereals seeds have high nutritional and functional values which are associated with the quality and quantity of their proteins, fats and antioxidant potential (Gorinstein *et al*., 2002; 2007 and Pas´ko *et al*., 2007). A new way in nutrition, in recent years, is the consumption of sprouts – the atypical vegetable, which have received attention as functional foods, because of their nutritive value including amino acid, fiber, trace elements and vitamins as well as flavonoids, and phenolic acids (Pas´ko *et al*., 2008). Moreover, Amaranth has become popular among patients with celiac disease because it does not cause allergic reactions in the intestinal mucosa. However, the high prevalence of diabetes mellitus among these patients is well known (Guerra-Matias and Areas, 2005).

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The soybean, (*Glycine max*) a grain legume, is one of the richest and cheapest sources of plant protein that can be used to improve the diet of millions of people, especially the poor and low income earners in developing countries because it produces the greatest amount of protein used as food by man (Liu, 2000). Defatted soy flour at 2-5% improves water holding capacity and sheeting process of dough. The enhanced sheeting strength produces better layering during the fat roll in process and more tender and finished product. Soybean(Glycine max) is one of the most important oil and protein crops of the world. Soybeans contain 30 to 45% protein with a good source of all indispensable amino acids (Serrem *et al*., 2011).

Sorghum (Bicolor L. Monech) is the fifth important cereal crop after wheat, rice, maize and barley in terms of production (FAO, 2005). The total world annual sorghum production is over 60 million tons from a cultivated area of 46 million ha. Sorghum is particularly adapted to drought prone areas hot, semi-arid tropical environments with 400 - 600 mm rain fall – areas that are too dry for other cereals. The sorghum genome is currently sequenced (Paterson *et al*. 2003). Sorghum nutritional quality is dictated mainly by chemical composition and the presence of antinutritional factors, such as phytic acid. The effects of phytic acid in human and animal nutrition are related to the interaction of phytic acid with proteins, vitamins and several minerals, and restrict their bioavailability (Elkhalil *et al*. 2001).

Rice (*Oryza sativa*) is one of the main cereal crops, as well as staple food for most of the world's population, especially Asian countries. Approximately 600 million tons are harvested worldwide annually Chen *et al*. (2012). Frequently, rice is eaten in cooked form by humans to obtain various nutrients, as well as to supplement their caloric intake Kim *et al*. (2011). The milling of paddy rice has nearly a 70% yield of rice (endosperm) as its major product, although there are some unconsumed portions of the rice produced, such as rice husk (20%), rice bran (8%) and rice germ (2%) Van Hoed *et al*. (2006).

Consumption of foods rich in whole grains and cereal fibers has in epidemiological studies been shown to reduce the risk of chronic diseases such as diabetes, cardiovascular disease and certain cancers, as reviewed by Murtaugh *et al*. (2003) and shown by Larsson *et al*. (2005). Moreover, analogous carbohydrate was reported to be resistant for digestion and absorption in the human small intestine with complete or partial fermentation in the intestine. Dietary fiber includes polysaccharide, oligosaccharides, lignin and associated plant substances. Dietary fibers promote beneficial physiological effects including laxation and blood cholesterol attenuation (Anon, 2001). Furthermore, Harris (2000) noticed that dietary fibers may cause dietary starch to escape digestion in the small intestine and enter the large intestine. If these results from the dietary fibers reducing the gastro intestinal transit time those dietary fibers that's reduce this the most would be expected to cause the most starch to escape digestion.

The objective of the present study has been carried out to evaluate the different formulae made from amaranths fractions mill, defatted soybean, sorghum and riceto treat diarrhea inrats. The biscuits were prepared from different formulae which have rich contain in protein, polyphenol, minerals, dietary fiber and total carbohydrates. The different biscuits were used for feed the rats groups to treatment of diarrheaand prevent of benefit health.

Materials and Methods

Materials:

Amaranth light seeds (*Amaranth hypochondriacus* L.) were obtained from Horticulture Institute, Agricultural Research Center, Giza- Egypt.

Rice (*Oryze sativa* L.), variety Giza 175 (high amylose) was obtained from Rice Research and Training Center (RRTC) at Sakha, Experimental Station Kafr El-Sheik Governorate, Egypt.

Soybean (*Glycine max*) and Sorghum (*Sorghum bicolor* L. Moench) Giza 125 were purchased from Field Crops Research Institute, Agricultural Research Center, Giza- Egypt.

All seeds were milled in a Laboratory Mill Junior to give a fine power. The soybean powder was extracted oil using n-hexane (40-60) at room temperature for 48 h. The extracts were filtrated and soybean defatted was dried at 50±5^{o}C in an electric oven according to AOAC (2005).

Kits of measurement to different parameters were obtained from Bicon Diagnosemittel GmbH and Co.KG Hecke 8 made in Germany.

Methods:

Determination the chemical analysis of raw materials:

Proximate analysis including crude protein, crude lipids, ash, crude fibers and total carbohydrates were determined in the amaranths fractions mill, defatted soybean, sorghum and rice mill according to the methods of

AOAC (2005). Total dietary fiber (TDF), soluble and insoluble dietary fibers were determined according to the method described by Prosky *et al.* (1988).

Minerals content (K, Na, Ca, P, Cu, Fe and Mn) were determined in the amaranths fractions mill, defatted soybean, sorghum and rice using the Flam Photometer apparatus (Galienkamp, FGA330, England) and Perkin Elmer atomic absorption spectrophotometer (model 80, England) as described in AOAC (2005).

Extraction of antioxidant from dried raw materials powder:

Air dried amaranths fractions, defatted soybean, sorghum and rice were finely powdered as previously described and extracted with petroleum ether (40-60ºC) to remove fats and resinous materials. The residues were exhaustively separately extracted with 500 ml of methanol (70%). The extract was filtrated through Whatman no., 1 filter papers and the filtrates were evaporated to dryness under reduced pressure on a rotary evaporator (RE 300/MS) at 40ºC.

Determination of total phenolic content:

The total phenol content of the extracts was determined using the method reported by Xu and Chang (2007).A sample of methanolic extract (0.2 ml) was mixed with 1 ml of Folin– Ciocalteau reagent (ten folds dilution). The mixture was allowed to stand for 5 min at room temperature before adding 0.80 ml of 20% Na2CO3 and then mixed gently. The reaction mixture was incubated for 40 min and the absorbance measured at 760 nm in spectrophotometer. The total phenolic content was calculated using gallic acid as standard.

Determination of total flavonoid content:

The total flavonoid content was measured using the Aluminium chloride colorimetric method modified from the procedure reported by Woisky and Salatino (1998). Two ml of the extract was mixed with 100µl of 10 percent AlCl3, 100µl of 1 mol per liter potassium acetate and 2.8 ml water and allowed to incubate at room temperature for 30 min. Thereafter, the absorbance of the reaction mixture was subsequently measured at 415 nm.

Preparation of formulae and making biscuits:

Amaranths fractions mill, defatted soybean, sorghum and rice mill were used to prepare biscuits at different levels. The biscuits made from these ingredients were used to treat the diarrhea in rats, are given in Table (1).

Table 1: The ingredients used to biscuit

Formulae number	Sorghum	Amaranths			Rice mill	Defatted soybean
		Flour	Whole meal	Hull		
Formula 1	50	20	-----	--------	20	10
Formula 2	50	---------	20	--------	20	10
Formula 3	50	---------	--------	20	20	10

The control sample was prepared from 100% wheat flour 72% extraction. Three blends dough and control sample were prepared separately to make biscuits according to Omobuwajo, (2003).The biscuits were baked at 170- 180°C for 20 min in an electric oven and the sensory characteristics were determined by ten panelists according to AACC (2002).Chemical constituents in different biscuit formulae and control sample were determined according AOAC (2005).

Color analysis of biscuits:

Color measurement of biscuits was carried out using a Hunter Colorimeter fitted with optical sensor(Hunter Lab. Colorimeter model D 25) on the basis of L^*, a^*, b^* color system. L^*values measure black to white (0–100), a^* values measure redness when positive, and b^* values measure yellowness when positive according to Francis (1998).

Texture analysis profile(TPA)of biscuits:

The texture profile analysis (TPA) indices of different biscuits were determined using a texture analyzer (Cometech, B type, Taiwan). The conditions of texture analyzer were provided with software,35 mm diameter compression disc was used. Two cycles were applied at a constant crosshead velocity of 1mm/s, to 30% of sample depth, and then returned. From the resulting force-time curve the values for texture attributes, i.e. Hardness (N), gumminess (N), chewiness (N), adhesiveness (N.S), cohesiveness, springiness and resilieness were calculated from TPA graphic according to Bourne (2003). Gumminess and chewiness were determined from the following calculations: gumminess = hardness× cohesiveness; chewiness = hardness× cohesiveness× springiness.

Physical analysis of biscuits:

Diameter of biscuits was measured by laying six biscuits edge to edge and again measuring the diameter of six biscuits (cm) and then taking average value. Thickness was measured by stacking six biscuits on top of each other and taking average thickness (cm). Weight of biscuits was measured as average of values of four individual biscuits with the help of digital weighing balance. Spread ratio was calculated by dividing the average value of diameter by average value of thickness of biscuits. Percent spread was calculated by dividing the spread ratio of supplemented biscuits with spread ratio of control biscuits and multiplying by 100 according to AACC (2002).

Nutritional experimental:

Male albins rats (30 rats), their weight ranged between 80-100 g and were obtained from Helwan Experimental Animal Station were used in this experiment. The basal diet which consisted of corn starch (70%), casein (10%); salt mixture (4%), vitamin mixture (1%) and cellulose (5%) was prepared according to AOAC (2005). Experimental rats were fed on basal diet for eight days. After that, the rats were divided to fifth main groups; the first main group fed on basal diet for four weeks and considerable as control negative. Whereas, the 24 rats were fed on basal diet using 3% castor oil plus 7% corn oil as alternative 10% corn oil in the basal diet for seven days to cause diarrhea in rats. The experimental rat diarrhea was divided randomly into four main groups. The second main group (6 rats) was fed on basal diet for another four weeks and considered as control positive rats. The third, fourth and fifth main groups (6 rats for each) were fed on 20% from biscuits made from formulae one, two and three, respectively as reported in obviously Table (1). The body weight and feed consumption were recorded every three days for four weeks. Also, biological value and protein efficiency ratio were estimated. At the end of experiment the blood samples were taken by drawing from the orbital plexus of each rat and were centrifuged at 3000 ppm to obtain the serum and kept of freezer until analysis. Kidney functions such as uric acid, urea and creatinine in serum were determined according to Bartels and Bohmer (1971); Tabacco *et al*. (1979) and Fosstti and Prencipe (1980).

Statistical analysis:

The obtained data were exposed to analysis of variance. Duncan's multiple range tests at ($P \leq 0.05$) level was used to compare between means. The analysis was carried out using the PRO ANOVA procedure of Statistical Analysis System (SAS, 2004).

Results and Discussion

Chemical analysis and polyphenolics compounds of raw materials

Chemical composition and polyphenolics (phenolic acid and flavonoids compounds) of amaranths fractions mill, defatted soybean, sorghum and rice mill were determined and the results are reported in Table (2). From the results, it could be noticed that the defatted soybean had contained the highest amount of protein (51.60%) followed by amaranths flour and wholemeal were 17.2 and12.5%. Meanwhile, sorghum, amaranths hull and rice mill were 11.5, 7.7 and 7.04%, respectively. Moreover, amaranths hull characterized by the highest content of crude fiber 21.7% followed by defatted soybean, amaranths wholemeal and rice mill were 10.7, 8.8 and 7.85%, respectively. Meanwhile, the amaranths flour and sorghum had the lowest content of crude fibers and the highest contained in total carbohydrates 80.7 and 77.0% followed by rice mill, wholemeal and hull amaranths (79.55, 65.10 and 59.3%, respectively) and also defatted soybean was the lowest in total carbohydrates. The amaranth proteins have a good digestibility, a majority of proteins belonging to the group of water-soluble albumins and salt-soluble globulins Gorinstein *et al*. (2002).

Drinah *et al*. (1990) found that the protein content, ether extract, ash and available carbohydrates of sorghum grain ranged from 9.9 to 15.8%, 1.8 to 5.3%, 1.49 to 4.16% and 58.2 to 84.7%, respectively. Whilst, Malleshi and Deskachar (1986) reported that sorghum and pearl millet contained 11.8 and 16.1% protein, respectively. Also, Lovis (2003) mentioned that the protein content, crude fat and total carbohydrate of sorghum grains were 11.3, 3.3 and 74.6%, respectively.

Total dietary fiber fractions as soluble and in soluble dietary fiber were determined in raw materials and the resultant showed that the amaranths hull had the highest in total dietary fiber and its fractions (34.8, 11.6 and 23.2%) followed by defatted soybean was 25.39, 8.73 and 16.66% and wholemeal amaranths had contained 14.35, 4.78 and 9.57%, respectively. Whilst, sorghum, rice meal and amaranths flour had the lowest amounted for total dietary fiber and its fractions.

Phenolic acid and flavonoids compounds as pigment and antioxidants were determined of amaranths fractions mill, defatted soybean, sorghum and rice mill and the results are reported in the same Table (2). The resultants illustrated that the sorghum, amaranths hull and defatted soybean had the highest contained amounts from phenolic acid compounds (116.5, 110.2and 110.0 mg/100g) followed by wholemeal amaranths, rice mill and amaranths flour. Meanwhile, the flavonoids compounds were arrangement from high to low as defatted soybean > amaranth hull > sorghum > wholemeal amaranth > rice meal > amaranth flour. Polyphenolic compounds have an important role in stabilizing lipid oxidation and are associated with antioxidant activity (Yen *et al*., 1993). The phenolic compounds may contribute directly to antioxidative action (Duh *et al*., 1999).

Table 2: Chemical analysis of raw materials g/100g dry weight

Chemical analysis	Sorghum flour	Amaranths whole meal			Defatted soybean	Rice mill
		Flour	Whole meal	Hull		
Protein	11.5	17.2	12.5	7.7	51.60	7.04
Ash	1.43	1.7	3.7	6.5	7.93	4.59
Lipids	3.55	2.3	6.0	3.8	5.22	0.97
Crude fiber	2.45	2.8	8.8	21.7	10.71	7.85
TC	81.07	77.0	65.10	59.3	24.54	79.55
TDF	7.59	4.8	14.35	34.8	25.39	5.21
TSDF	2.53	1.5	4.78	11.6	8.73	1.74
TIDF	5.06	3.3	9.57	23.2	16.66	3.47
*T. Phenolic	116.5	45.3	85.0	110.2	110.0	62.21
**T. Flavonoids	58.12	22.4	46.0	65.6	81.4	34.54

TC: Total carbohydrates TDF: Total dietary fiber TSDF: Total soluble dietary fiber TIDF: Total insoluble dietary fiber
**Total phenolic as mg gallic acid equivalent/100g*
***Total flavonoids as mg queractin equivalent/100g*

Minerals content of raw materials

Table (3) showed that the mineral content of amaranths fractions mill, defatted soybean, sorghum and rice mill were determined. The resultant reported that the raw materials were the highest amounts from potassium, sodium, calcium, phosphorus, cupper, iron and manganese, respectively. Bhat *et al*. (2015) reported that the mineral content in amaranths grain was revealed the presence of good amounts of essential minerals like magnesium (848 µg/g), calcium (519.3 µg/g), phosphorus (330 µg/g) and iron (65 µg/g).Amaranths have excellent nutritional value because of their high content of essential micronutrients such as b-carotene, iron, calcium, vitamin C and folic acid (Priya *et al*., 2007). Amaranthus hybridus contained the highest concentration of iron, zinc as well as phosphorus and calcium, respectively (Schönfeldt and Pretorius, 2011). Variations in mineral content are influenced by environmental conditions during plant growth and seed set, especially in soil mineral availability Alvarez-Jubete *et al*. (2009).

Table 3: Minerals content of raw materials mg/100g dry weight:

Minerals content	Sorghum flour	Amaranths whole meal			Defatted soybean	Rice mill
		Flour	Whole meal	Hull		
Potassium	264.5	110.9	326.8	230.7	360.0	320.0
Sodium	80.9	3.4	8.1	6.7	25.04	69.0
Calcium	33.1	85.4	190.7	180.9	220.0	133.0
Phosphorus	376.2	130.5	323.0	260.7	685.0	235.0
Copper	0.74	0.31	0.62	0.46	2.30	2.10
Iron	7.65	5.2	13.9	10.6	11.0	3.00
Manganese	1.98	7.85	22.0	15.41	2.80	1.80

Furthermore, taken into consideration that potassium depresses while sodium enhances blood pressure, thus, high amount could be an important factor in presentation of hypertension Yoshimura *et al*. (1991).Calcium and phosphorous are associated with each other for development and proper functioning of bone, teeth and muscles Turan *et al*. (2003). Iron deficiency according to World Health Organization (WHO) affect about 3.7 billion people out of which 2 billion people are anemic Meng *et al*. (2005).Moreover, iron is needed by infants for the proper growth and formation of healthy blood cells and prevention of iron-deficiency anemia. This mineral is a vital component of hemoglobin, the part of red blood cells that carries oxygen throughout the body; myoglobin, the part of muscle cells that stores oxygen; and many enzymes in the body. A child's growth and development depends on iron, and studies show that inadequate iron intake can have long-term consequences on learning, attention span and behavior (Krebs *et al*., 2006).

Calcium, magnesium and iron are minerals that are deficient in gluten-free products and in the gluten free-diet. The inclusion of these pseudo cereals, which are a good source of these and other important minerals, can assist to reduce this deficiency (Alvarez-Jubete *et al*. 2010).

Sensory characteristics of biscuits

Amaranths fractions mill, defatted soybean, sorghum and rice mill were used to produce biscuits. The biscuits were evaluated for their sensory characteristics and the results are reported in Table (4). From the results it could be clearly observed that there no significant difference between wheat flour biscuits (control) and samples supplemented with Amaranths fractions mill, defatted soybean, sorghum and rice mill for taste, color, texture and odor. Whereas, the formula (3) made from 20% amaranths hull, the biscuits product had significantly decreased than control sample and gave lower score in all sensory evaluation parameters.

The color characteristics of biscuits showed to be darker hull and wholemeal amaranths which enhanced consumer appeal up to 40 percent of incorporation. Similar trend to that of flavor scores was observed in case of color values of incorporated biscuits. Textural profile plays an important role in justifying the overall acceptability of biscuits, here in case of dietary fiber mixture incorporated biscuits, slight improvement in crispiness of biscuits were observed in samples made from 20% amaranths flour, hence secured better scores while in case of biscuits containing 20% of wholemeal amaranths, the panelists reported dryness of mouth hence secured least scores. This was contradicting with the result reported by Sindhuja *et al*. (2005) which revealed that the cookies containing 25% amaranth seed flour was found to be most acceptable by the panelists.

The results of statistical analysis indicated that no significant difference was found between control sample and supplemented samples for all evaluated characteristics of biscuits contained 20% from amaranth fractions.

Table 4: Effect of fortified amaranths fractions using different mixture dietary fiber sources on sensory evaluation of biscuit

Blends	Shape 20	Taste 20	Color 20	Texture 20	Odor 20	Over all acceptability 100
Control	18.6[a] ±0.56	18.7[a] ±0.13	19.45[a] ±0.66	18.9[a] ±0.11	19.2[a] ±0.80	94..85
Formula 1	18.53[a] ±0.60	18.4[a] ±0.39	17.0[ab] ±0.92	18.6[a] ±0.78	18.7[a] ±0.17	91.23
Formula2	17.13[b] ±0.23	18.1[a] ±0.12	16.2[b] ±0.28	18.2[a] ±0.48	18.7[a] ±0.19	88.33
Formula3	16.03[c] ±1.06	16.3[b] ±0.12	15.0[c] ±0.28	16.9[b] ±0.47	16.3[b] ±0.29	80.53

Control made from wheat flour 72% extraction.

Chemical analysis of different biscuit formulae

Table (5) showed that the chemical constituents of biscuits made from different formulae and compared with control sample made from wheat flour 72% extraction. The resultant showed that the biscuit made from 20% amaranth flour had the highest amount from protein 18.2% followed by biscuits made from equal weight (20%) wholemill and hull amaranth were 16.3 and 14.2%, respectively than control was 10.68%. The highest protein in biscuit may be caused the highest protein in defatted soybean (51.60%) was in recipe biscuits. The protein content of soybean is about 2 times of other pulses, 4 times of wheat, 6 times of rice grain, 4 times of egg and 12 times of milk. Soybean has 3% lecithin, which is helpful for brain development. It is also rich in calcium, phosphorous and Vitamins A, B, C and D, it has been referred to as "the protein hope of the future".

Total lipid was the highest in biscuit formulae and control sample may be caused the butter was added to the recipe of making biscuit. Moreover, total carbohydrates were increased in control biscuit 69.71% followed by biscuit formula made from amaranth hull; wholemill and flour were 54.8, 52.2 and 49.9%, respectively. Higher lipid retention improves the mouth feel and retains the flavor of the biscuits. No definite trend in increase or decrease in crude fiber contents was observed. Total dietary fiber fractions were higher in formula 3 than other formula and control sample may be caused the highest total dietary fibers in hull amaranth.

Fiber is the carbohydrate fraction which is resistant to enzymatic digestion and absorption in the small intestine, and which usually undergoes full or partial fermentation in the large intestine. Dietary fiber is considered essential for optimal digestive health, and also imparts various functional benefits (Brownawell *et al*., 2012). Dietary fiber can promote satiety, reduce cholesterol and lipid absorption, modulate postprandial insulin response, promote endogenous cholesterol conversion to bile acids, improve intestinal microbiota, and reduce risk and severity of gastrointestinal infection and inflammation (Brownawell *et al*., 2012 and De Carvalho *et al*., 2014).

Table 5: Chemical constituents of different biscuit formulae on dry weight

Chemical analysis	Control	Formula 1	Formula 2	Formula 3
Protein	10.68	18.2	15.3	12.2
Ash	0.51	2.1	3.5	4.7
Lipids	18.2	25.2	23.7	20.1
Crude fiber	0.9	4.6	5.3	8.2
TC	69.71	49.9	52.2	54.8
TDF	3.71	6.5	11.7	17.3
TSDF	1.35	2.6	4.2	7.4
TIDF	2.36	3.9	7.5	9.9

Control biscuit made from wheat flour 72% extraction.

Color analysis of different biscuits

The color measurements of the composite biscuits substituted with different levels of amaranth flour are depicted in Table (5). From the results, it was noticed that the lightness (*L**) of the composite biscuits displayed a decreasing trend along with the addition of amaranth fractions (flour, wholemeal and hull).On the other hand, a reverse trend was noted for redness*) and yellowness (b*) in composite biscuit. The increase in a* and b* values was noticed in amaranth fractions meal in biscuit preparation. Chevallier (2000) suggested that protein content was negatively correlated with lightness of biscuits, indicating that the Maillard reaction played the major role in color formation. Maillard browning and caramelization of sugar is considered to produce brown pigments during baking (Laguna *et al*., 2011). The biscuit color is an important factor for the initial acceptability of food products by consumers.

Table 6: Color characteristics of different biscuits

Biscuits	L-value	a-value	b-value
Control	63.09 ± 0.21^{a}	6.31 ± 0.12^{b}	26.17 ± 0.14^{b}
Formula 1	62.57 ± 0.06^{a}	6.71 ± 0.17^{b}	26.71 ± 0.06^{b}
Formula 2	62.47 ± 0.08^{a}	7.17 ± 0.24^{a}	$27.45 \pm 0.19d^{a}$
Formula 3	59.59 ± 0.24^{b}	8.12 ± 0.16^{a}	28.45 ± 0.07^{a}

Texture analysis profile of different biscuits:

Texture result of the three types of biscuits prepared from blend of sorghum, defatted soybean and rice mill were added to amaranth flour, wholemeal and hullare shown in Table (7). Hardness differs significantly ($p < 0.05$) in all biscuits sample. The decrease in hardness in biscuits made from amaranths flour could be attributed to the changes in gluten content followed by the biscuits made from wholemeal amaranth was 7.24 N and the biscuit made from hull amaranth was the highest in hardness. The changes in total protein content were not as significant as the change in gluten content for the formation of composite matrix of biscuit dough (Chung *et al*., 2014). The continuous protein matrix in short-dough biscuits, such as sugar-snap biscuits, could be achieved mainly by gluten during baking (Chevallier *et al*., 2000).Whereas, the cohesiveness between 0.51 and 1.73, gumminess between 2.12 to 10.54 N, chewiness between 1.51 and 2.34 N, springiness between 0.60 to 0.69 and finely, resilience was between 0.45 to 0.52. The addition of amaranth hull was influenced the texture of biscuits may be caused amaranth hull was high in contained total dietary fiber. Consequently, the resulting gumminess and chewiness of the different biscuits followed the same general trend as the hardness of the different biscuits.

Table 7: Texture profile analyses of biscuits of different biscuits

Biscuits	Hardness	Cohesiveness	Gumminess	Chewiness	Springiness	Resilience
Control	4.29	0.51	2.12	1.51	0.60	0.45
Formula 1	5.32	0.79	5.24	1.86	0.64	0.47
Formula 2	7.24	1.25	8.36	2.11	0.67	0.49
Formula3	9.61	1.73	10.54	2.34	0.69	0.52

Physical characteristics of different biscuits

The physical properties of biscuits prepared from blend of sorghum, defatted soybean and rice mill were added to amaranth flour, wholemeal and hullare shown in Table (8). The diameter of biscuits made from flour, wholemeal and hull amaranth was found significantly lower than that of control biscuit ($p \leq 0.05$). The thickness of biscuits ranged from 0.79 to 0.86 cm. It increased with the incorporation of hull amaranth. Increase in thickness may be due to the decrease in diameter. The changes in diameter and thickness were reflected in spread ratio and percent spread of biscuit. The spread ratio and percent spread of control biscuits were 8.02 and100, respectively. Spread ratio and percent spread decreased with the addition of hull amaranth. Other research workers also reported reduction in spread ratio when soy flour and fenugreek flour were substituted for wheat flour (Hooda and Jood, 2005). Reduced spread ratios of hull amaranth fortified biscuits were attributed to the fact that composite flours apparently form aggregates with increased numbers of hydrophilic sites available that compete for the limited free water in biscuit dough (McWatters, 1978).The weight of biscuits increased as the concentration of hull amaranth increased in the blends. The range of biscuit weight was 10.73 to 12.00 g with maximum value in hull amaranth biscuits. The increase in biscuit weight was probably due to the ability of hull amaranth to retain oil during baking process (Rufeng *et al*., 1995). Finely, the blend of sorghum, defatted soybean and rice mill were added to amaranth flour, wholemeal and hull based biscuits as it has beneficial nutraceutical properties and its gluten-free nature can play important role in preventing celiac problem.

Table 8: Physical properties of different biscuits

Biscuits	Diameter (cm)	Thickness (cm)	Spread ratio	Weight (g)	Spread %
Control	6.34±1.20[a]	0.79±0.33 [b]	8.02±0.67 [a]	10.70±0.23 [b]	100.0
Formula 1	6.31±1.45 [a]	0.79±0.33 [b]	7.99±0.25[bc]	10.73±0.34 [b]	99.62
Formula 2	6.21±1.45[b]	0.81±0.67 [b]	7.67±0.67 [b]	10.82±0.08 [b]	95.63
Formula 3	6.02±1.00[c]	0.86±0.58 [a]	7.00±0.05 [c]	12.00±0.22 [a]	87.28

Biological evaluation of different biscuits

Biscuits made from different formulae were estimated using biological evaluation on diarrhea rat groups and the results are reported in Tables 8and 9, respectively. At the end of experiment (four weeks) the results showed that the weight parameters and protein values are recorded in Table (9). The results illustrated that the gain body weight in rats fed on biscuits made from formula one was the highest (40.1 gram) whereas, the gain body weight in rats fed on formulae 2 and 3 were significantly near with formula one (38.2 and 34.2 g) respectively. The gain body weight of the rats fed on basal diet (as control negative and positive), was the lowest (32.5 and 15.7g) compared with other formulae. The results did not reveal any significant differences between different formulae.

Table 9: Effect of the biscuits made from different formulae on weight parameters and protein values in diarrhea rat groups

Groups	Initial weight (g)	Final weight (g)	Body gain weight (g)	Protein efficiency ratio	Biological value
Control negative	105.1± 3.3	137.6±5.72	32.5±1.91	3.20±0.30	83.60±0.65
Control positive	104.5±3.21	120.24±2.48	15.74±0.68	1.5±0.05	65.70±0.38
Formula 1	104.6± 3.54	144.7±7.71	40.1±0.97	4.00±0.27	92.02±0.51
Formula 2	104.8±3.65	143.0±4.02	38.2±0.85	3.80±0.26	89.91±0.27
Formula 3	104.3±3.82	138.5±3.81	34.2±0.72	3.40±0.25	85.70±0.22

The results in the same table showed the protein efficiency ratio and biological value in diarrhea rats fed on control diet substituted different formulae. From the results it could be noticed that the protein efficiency ratio and biological value were the lowest in the rats on basal diet described as control positive were 1.5 and 65.7%. The formula one had contained the highest (4.0 and 92.2%) in protein efficiency ratio and biological value in diarrhea rats followed by formulae 2 and 3, respectively. It is important to estimate that the kidney functions which include serum urea, uric acid and creatinine were determined to evaluate the disorders which may be occurred as a result of diarrhea and the obtained data recorded in Table (10). From the resultant it could be noticed that the biscuits made from different formulae had improvement the kidney functions. This biscuits made from different formulae were treatment the diarrhea rats groups may be caused amaranth fraction had contained a sweetener and polysaccharides that will be metabolite and absorbed as glucose which consequently increases sodium absorption.

Table 10: Influence of using biscuits made from different formulae on kidney functions in rat diarrhea mg/100 ml

Groups	Urea	Uric acid	Creatinine
Control	59.60± 7.54[b]	4.50± 0.75 [b]	0.86± 0.07 [b]
Control	63.42±5.78[a]	5.84±0.62 [a]	0.95±0.11 [a]
Formula 1	51.13± 7.83[c]	3.12± 0.45 [c]	0.69± 0.10 [c]
Formula 2	53.77± 5.61 [c]	3.60± 0.76 [c]	0.72± 0.04v
Formula 3	57.13± 3.97 [b]	3.86± 0.52 [b]	0.79± 0.07 [b]

Diarrhea is loose, watery stools. Having diarrhea means passing loose stools three or more time a day. Acute diarrhea is a common problem that usually lasts 1 or 2 days and goes away on its own (Ramaswamy and Jacobson, 2001). Moreover, diarrhea is a leading cause of illness and death among children in developing countries, where an estimated 1.3 thousand million episodes and 3.2 million deaths occur every year in those under 5 years of age (NCDDP, 1996).Jan *et al*. (1997) reported that appropriate feeding practices have an important impact on diarrhea disease management in developing countries. Moreover, rice and potatoes were the most likely items to be retained during diarrhea at all ages if he or she had been receiving these foods before coming ill. Rice was found to be well to relate and digest during acute diarrhea (Molla *et al*. 1982).

From these data, it could be noticed that the rats fed on all formulae to use biscuits were reduced diarrhea compared with rats fed on basal diet. It may be given to the high contents of minerals as sodium and potassium in ingredients of all blends are essential for regeneration of intestinal epithelial cell (Curran and Barness, 2001).

From this study it could be concluded that defatted soybean as a source of protein, and sorghum is increased in crude fiber and protein and rice mill was the highest in total carbohydrates. Amaranths fractions are good sources for protein, minerals and soluble dietary fiber. From the obviously data it could be noticed that the biscuits made from formula no. one and two are the best for reduction of diarrhea, safety and healthy followed by blends no., three, respectively.

References

AACC, 2002. American Association of Cereal Chemist. Approved Method, Published by American Association of Cereal Chemists, Ins. St. Poul, Minesota, USA.

Alvarez-Jubete, L., E.K. Arendt and E. Gallagher, 2009. Nutritive value and chemical composition of pseudo cereals as gluten-free ingredients. Int. J. Food. Sci. Nutr., 60 (suppl 4): 240-257.

Alvarez-Jubete, L., M. Auty, E.K. Arendt, E. Gallagher, 2010. Baking properties and microstructure of pseudo cereal flours in gluten-free bread formulations. Eur Food Res Technol., 230: 437-45.

Anon, Y., 2001. The definition of dietary fiber. Cereal Food World, 46(3): 112-135.

AOAC, 2005. Official Methods of Analysis of the Association of Official Analytical Chemists, 18th ed., Washington, D.C.

Bhat, A., G. Satpathy and R. K. Gupta, 2015. Evaluation of Nutraceutical properties of Amaranthus hypochondriacus L. grains and formulation of value added cookie. Journal of Pharmacognosy and Phytochemistry, 3(5): 51-54.

Bartels, H. and M. Bohmer, 1971. Creatinine standard and measurement of serum creatinine with picric acid. Clin. Chem. Acta., pp: 32-81.

Berti, C., P. Riso, A. Brusamolino and M. Porrini, 2005. Effect on appetite control of minor cereal and pseudocereal products. British Journal of Nutrition, 94: 850-858.

Bourne, M.C., 2003. Food texture and viscosity: Concept and measurement. Elsevier Press, New York/ London.

Brownawell, A.M., W. Caers, G.R. Gibson, C.W.C. Kendall, K.D. Lewis, Y. Ringel and J.L. Slavin, 2012. Prebiotics and the health benefits of fiber: current regulatory status, future research, and goals. J Nutr., 142(962–974).

Chen, M.H., S.H. Choi, N. Kozukue, H.J. Kim and M. Friedman, 2012. Growth-inhibitory effects of pigmented rice bran extracts and three red bran fractions against human cancer cells: relationships with composition and antioxidative activities. J Agric Food Chem, 60: 9151-9161.

Chevallier, S., P. Colonna and G. Della Valle, 2000. Contribution of major ingredients during baking of biscuit dough systems. Journal of Cereal Science, 31: 241-252.

Chung, H.J., A. Cho and S.T. Lim, 2014. Utilization of germinated and heat-moisture treated brown rice in sugar-snap cookies. LWT-Food Science and Technology,57: 260-266.

Curran, J.S. and L.A. Barness, 2001. Vitamins deficiencies and excesses, vitamin A deficiency. In Nelsn Text Book of Paediatries Edition, Behrman R. E., Kliegman R. M. and Jenson H. B. 16th edition. W.B. Saundres Co. Philadelphia. London, Toronto, Montreal, Sydney, Tokyo, 176: 179.

De Carvalho, F.G., P.P. Ovidio, G.J. Padovan, A.A. Jordao Junior, J.S. Marchini and A.M. Navarro, 2014. Metabolic parameters of postmenopausal women after quinoa or corn flakes intake – a prospective and double-blind study. Int J Food Sci Nutr., 65(3): 380-5.

Drinah, B.C., B. Nyirend and P. Vohra, 1990. Nutritional improvement of tannin-containing sorghum by sodium bicarbonate. Cereal Chem., J. 67: 533-537.

Duh, P.D., Y.Y. Tu and G.C. Yen, 1999. Antioxidant activity of water extract of Harng Jyur (*Chyrsanthemum morifolium* Ramat). Lebensmittel- Wissenschaft und Technologie, 32: 269-277.

Elkhalil, E.A.I., A.H. El- Tinay, B.E. Mohamed and E.A.E. Elsheikh, 2001. Effect of malt pretreatment on phytic acid and in vitro protein digestibility of sorghum flour. Food Chem., 72: 29-32.

FAO (Food and Agricultural Organization), 2005. The world sorghum and millet economics. Cited from http:/ faostat. fao. Org / faostat/.

Fossatti, A. and M. Prencipe, 1980. Standard Methods of Clinical Chemistry, Edited, Selingson, D. Clin. Chem.J., 26: 227-231.

Francis, F.J., 1998. Color analysis in S. S. Nielson (ed), Food Analysis Maryland: Chapman and Hall.

Gorinstein, S., E. Pawelzik, E. Delgado-Licon, R. Haruenkit, M. Weisz and S. Trakhtenberg, 2002. Characterisation of pseudo cereal and cereal proteins by protein and amino acid analyses. Journal of the Science of Food and Agriculture, 82: 886-891.

Gorinstein, S., O.J.M. Vargas, N.O. Jaramillo, I.A. Salas, A.L.M. Ayala, P. Arancibia-Avila, F. Toledo, E. Katrich and S. Trakhtenberg, 2007. The total polyphenols and the antioxidant potentials of some selected cereals and pseudocereals. European Food Research and Technology, 225: 321-328.

Guerra-Matias, A.C. and J.A.G. Areas, 2005. Glycemic and insulinemic responses in women consuming extruded amaranth (*Amaranthus cruentus* L.). Nutrition Research, 25: 815-822.

Harris, P.J., C. Jones and I. ferguson, 2000. Effects of two contrasting dietary acid production and transit time in rats. J. Sci. Food. Agric., 80: 2089-2095.

Hooda, S. and S. Jood, 2005. Organoleptic and nutritional evaluation of wheat biscuits supplemented withuntreated and treated fenugreek flour. Food Chemistry, 90: 427-435.

Jan, A.S., Z.A. Mustafa, Thoban and S.H. Ruddin, 1997. Evaluation of Dowdo (wheat-milk) in children with acute diarrhea. J. Pak. Med. Assoc., pp: 47-56.

Kim, S.P., J.Y. Yang, M.Y. Kang, J.C. Park, S.H. Nam, 2011. Composition of liquid rice hull smoke and anti-inflammatory effects in mice. J Agric Food Chem, 59: 4570-4581.

Krebs, N.F., J.E. Westcott, N. Butler, C. Robinson, M. Bell and K.M. Hambidge, 2006. Meat as a first complementary food for breastfed infants: Feasibility and impact on zinc intake and status. Journal of Pediatric Gastroenterology and Nutrition, 42: 207-214.

Laguna, L., V. Paula, S. Ana, S. Teresa and M.F. Susana, 2011. Balancing texture and other sensory features in reducedfat short-dough biscuits. Journal of Texture Studies, 43: 235-245.

Larsson, S.C., E. Giovannucci, L. Bergkvist and A. Wok, 2005. Whole grain consumption and risk of cholesterol, cancer: a population-based cohort of 60.000 women. Br. J. Cancer, 92: 1803-1807.

Lovis, L.J., 2003. Alternative to wheat flour in baked goods. Cereal Food World, 48(2): 61-63.

Liu, K., 200). Expanding soybean food utilization. J. Food Technol., 54(7): 46-47.

Ma, Y.S., J.A. Griffith, L. Chasan-Taber, B.C. Olendzki, E. Jackson, E.J. Stanek, J. Li, S.L. Pagoto, A.R. Hafner and I.S. Ockene, 2006. Association between dietary fiber and serum C-reactive protein. Am. J. Clin. Nutr., 83: 760-766

Malleshi, N.G. and H.S.R. Deskachar, 1986. Studies on cereals malting characteristicsof som tropical cereals and millets. J. of the Inst. Of Brewing, pp: 92-176.

Marcone, M.F., F. Jahaniaval, H. Aliee and Y. Kakuda, 2003. Chemical characterization of *Achyranthes bidentata* seed. Food Chemistry, 81: 7-12.

Meng, F., Y. Wei and X. Yang, 2005. Iron content and bioavailability in rice. J. Trace Element Med. Boil., 18: 333-338.

McWatters, K.M., 1978. Cookie baking properties of defatted peanut, soybean and field pea flours in baking powder biscuits. Cereal Chemistry, 55: 853-863.

Molla, A.M., H.M. Sanker and W.B. Ough, 1982. Rice power electeolyte solution as role therapy in diarrhea due to vibrocholera and E. coli. Lancat, pp: 1-1317.

Murtaugh, M.A., D.R. Jacobs Jr, L.M. Steffen, B. Jacob and L. Marquart, 2003. Epidemiological support for the protection of whole grains against diabetes. Proc. Nutr. Soc., 62: 143-149.

NCDDP., 1996. National Control of Diarrheal Disease Project. Ministry of health, Cairo, Egypt.

Omobuwajo, T.O., 2003. Compositional characteristics and sensory quality of biscuits, prawn crackers and fried chips produced from bread fruit. Innovative Food Sci. Emerging Technol., 4: 219-225.

Pas´ko, P., H. Barton, M. Fołta and J. Gwizdz, 2007. Evaluation of antioxidant activity of amaranth (*Amaranthus cruentus*) grain and by-products (flour, popping, cereal). Roczniki Pan´stwowego Zakładu Higieny, 58: 35-40.

Pas´ko, P., M. Sajewicz, S. Gorinstein and Z. Zachwieja, 2008. Analysis of the selected phenolic acids and flavonoids in *Amaranthus cruentus* and Chenopodium quinoa seeds and sprouts by HPLC method. Acta Chromatographica, 20(4): 661-672.

Pateron, A.H., J.E. Bowers, G.J. Peterson, J.C. Estill and B. Chapman, 2003. Structure evolution of cereal genomes. Curr. Opin. Gen Dev., 13: 644-650.

Priya, V.P., V.A. Celine, C. Gokulapalan and L. Rajamony, 2007. Screening amaranth genotypes (Amaranthus spp.) for yield and resistance to leaf blight caused by Rhizoctonia solani Kuhn. Plant Genet Res Newsl 147: 1-4.

Prosky, L., N.G. Asp, T. F. Schweizer, J.W. Devries and I. Furdal, 1988. Determination of insoluble and soluble and total dietary fiber in food products: Interlaboratory study. J. Assoc . Off . Anal. Chem, 71: 1017-1023.

Ramaswamy, K. and K. Jacobson, 2001. Infectious diarrhea in children. American J. of Gastroenterology, 30(3): 611-624.

Rufeng, N., L. Enqi, C. Chuangji and Z. Jiangping, 1995. A study of the production of healthy biscuit made with Tartary buckwheat grown in North China. Current advances in buckwheat research, pp: 861-865.

SAS, 2004. Statistical Analysis System. SAS User's Statistics SAS Institute IncCary, NC.

Serrem, C., H. Kock and J. Taylor, 2011. Nutritional quality, sensory quality and consumer acceptability of sorghum and bread wheat biscuits fortified with defatted soy flour. Int. J. Food Sci. Technol., 46: 74-83.

Schönfeldt, H. and B. Pretorius, 2011. The nutrient content of five traditional South African dark green leafy vegetables—a preliminary study. J Food Compos Anal., 24(8): 1141-1146.

Sindhuja, A., M.L. Sudha and A. Rahim, 2005. Effect of incorporation of amaranth flour on the quality of cookies. European Food Research Technology, 221: 597-601.

Tabacco, A., R.L. Searcy and J.E. Reardon, 1979. Standard Method of Clinical Chemistry, Edited Seligson, D. Clin. Chem., 25: 336-341.

Turan, M., S. Kordali, H. Zengin, A. Dursun and Y. Sezen, 2003. Macro and micro minerals content of some wild edible leaves consumed in Eastern Anatolia. Acta Agric. Sccandinavica, Section B, Plant Soil Sci., 53: L129-137.

Van Hoed, V., G. Depaemelaere, J. Vila Ayala, P. Santiwattana, R. Verhé, 2006. Influence of chemical refining on the major and minor components of rice bran oil. J Am Oil Chem Soc., 83: 315-321.

Velarde-Salcedo, A.J., de Mejía, E.G. de la Rosa and A.P.B., 2012. In Vitro Evaluation of the Antidiabetic and Antiadipogenic Potential of Amaranth Protein Hydrolysates. Hispanic Foods: Chemistry and Bioactive Compounds, Chapter., 12: 189-198.

Woisky, R and A. Salatino, 1998. Analysis of propolis: some parameters and procedures for chemical quality control. J. Apic. Res., 37: 99-105.

Xu, B.J. and S.K.C. Chang, 2007. A Comparative Study on Phenolic Profiles and Antioxidant Activities of Legumesas Affected by Extraction Solvents. Journal of Food Science, 72: S159-S166.

Yen, G.C., P.D. Duh and C.L. Tsai, 1993. Relationship between antioxidant activity and maturity of peanut hulls. J. Agric. Food Chem. 41: 67-70.

Yoshimura, M., H. Takahashi and T. Nakanishi, 1991. Role of sodium, potassium, calcium and magnesium on blood pressure regulation and antihypertensive dietary therapy. Jap. J. Nutr., 49: 53-62.