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Studies on the Production and Evaluation of Nutritional Contents of Traditional Couscous from Sprouted Maize, Fortified with *Glycine max* (l.) Soya Bean and c Pepo (Pumpkin Seeds)

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Abstract

Traditional Maize couscous was produced from sprouted Maize (*Zea mays*), Soya bean (*Glycine max*) and Pumpkin (*Cucurbita pepo*) seeds. The complementary couscous blends were compared with commercial couscous. The composite couscous blends produced comprises of raw (unprocessed maize, 100), blend 1 (sprouted maize mixed with soya bean and pumpkin seeds, 70:20:10), blend 2 (sprouted maize mixed with soya bean, 60:40) and blend 3 (sprouted maize mixed with pumpkin seeds, 60:40). Traditional maize couscous blends were fed to experimental albino rats of Wister strain weighing between (35g and 45g) for a period of 28 days. The nutritional and physiochemical analysis were determined using standard laboratory methods. The Statistical Package for Social Sciences (SPSS), version 20.0 was used to analyze the data collected which were expressed as means ± SE. One way analysis of variance (ANOVA) and Duncan's multiple range tests were used to compare the means obtained after each experiment. Differences were considered significant at $p < 0.05$. The results of anti-nutrients showed that sprouting decreased tannin and phytic acid, also supplementation with soya bean and pumpkin seeds have shown low level of tannin (3.11 mg/g to 3.49 mg/g) and phytic acid (0.47 mg/g to 0.57 mg/g), while commercial couscous recorded lowest tannin (0.62 mg/g) and high phytic acid (0.98 mg/g) when compared with the sprouted maize couscous blends. Processing (sprouting) also reduced the levels of some mineral elements and vitamins. Blending the sprouted maize couscous with soya bean and pumpkin seeds improved the chemical composition, mineral elements and vitamin contents of the sprouted maize couscous blends. Sprouting has also enhanced the *in vitro* protein digestibility at 1 hour (95.56% to 97.59%) and at 6 hours (97.24% to 98.98%) respectively. Results of *in vivo* studies showed high protein quality among the sprouted maize couscous blends when compared with the raw maize couscous. Blend 1 recorded high biological value (99.07%), apparent digestibility (97.69%), true digestibility (98.85%) and net protein utilization (99.60%), followed by blend 3 and then blend 1. In terms of sensory evaluation using hedonic method, blend 2 was most acceptable and was not significantly different with raw maize couscous but differ significantly ($p < 0.05$) with other sprouted maize couscous blends and the commercial couscous. These study hence, showed that sprouting reduced anti-nutrients, mineral elements, and vitamins, it also enhances digestibility, while supplementation with soya bean and pumpkin seeds greatly improved the nutritional quality of the sprouted maize couscous blends produced traditionally.

Keywords: *Maize (Zea mays), Soya Bean (Glycine max); Pumpkin (Cucurbita pepo)*

Introduction

Couscous is defined as a grits form of either *Triticum aestivum* L. (wheat), *Pennisetum glaucum* L. (Millet), *Sorghum bicolor L. moench* (Sorghum) and *Zea mays L.* (Maize), it usually comes in coarse form which is steam cooked and eaten as a full meal. Couscous is prepared

traditionally from mono cereals which lack adequate nutrients for adults and children, there is need for fortification/blending with plant legumes to meet up the nutrients requirement that may be loss during processing and production. Couscous is served with vegetables, fish or meat. It can also be absorbed in milk and sugar. Couscous is a staple food of North Africa and is very popular in West African countries. Wheat *(Triticum aestivum)* is the main raw material for the production of couscous commercially and traditionally. The grains were sprouted to reduce the anti-nutrients, improve protein digestibility and enhances the nutritional value of the couscous being produced. Fortification of couscous with *Glycine max* (Soya beans) and *Cucurbita pepo* seeds (Pumpkin seeds) will further supplement the deficient nutrients that were lost in the process of production. Maize is inadequate in terms of some of the essential amino acids (lysine, threonine and methionine). Mono cereal (Maize) can be enhanced by the addition of legume flour. Amino acids balance of legume flour is good and its protein content is high (Lee., *et al.* 1998). Today, in many countries including Turkey, couscous is made mechanically using extrusion technology and each couscous granule represents an aggregate of several semolina particles [1]. A comparison of the characteristics of traditional and commercial wheat couscous has been made by Guezlane., *et al* [2]. These authors found a higher elasticity in the traditionally prepared sample and a lower one in the commercially processed one.

It is widely processed into various types of products such as cornmeal, grits, starch, flour, tortillas, snacks, and breakfast cereals. Maize flour is used to make chapatis or flat breads which are eaten mainly in a few Northern states of India [3].

Soya bean is derived from seed *Glycine max (*L) merr of family-leguminosae or fabaceae. Soya bean is known as the "Golden bean" or "the super legume" of the twentieth century. It represents an excellent source of unsaturated fatty acids, high quality proteins and fibers. Soya bean contains very small amounts of saturated fatty acids but do not contain any trans-fatty acids. Both omega-6 and omega-3 fatty acids such as linoleic acid (56% total fat) and alpha linolenic acid (7 - 8% of total fat) are present in soya bean. Cooked soya bean are rich in Iron, Phosphorus, Magnesium, vitamin B_2 (Riboflavin) and Folate. It is one of the best vegetarian sources of total protein containing all essential amino acids required in the human diets. Common food preparations of soya bean include edamame (Whole soybean), soy flour, soymilk, tofu (Fermented soybean paste), soybean oil, soybean lecithin and soy sauce [4].

Pumpkin *(Cucurbita pepo)* is a cultivated plant of the genus Cucurbita. It yields varieties of winter squash and pumpkin, both a shrubby and creeping plant, ovoid or conical shape, pointed at the apex and with longitudinal grooves, thus resembling a spinning top (1 - 2). The mature or young fruit and the seeds of *C. pepo*, as well as to a lesser extent the flowers and young tips of the stems, are eaten in many parts of its native distribution area and in other regions of the world [5]. In Nigeria, the different parts of *Cucurbita pepo* are edible: the pulp, seed and leaves. They are used to prepare different types of dishes. Several studies have reported the chemical composition and oil characteristics of the pumpkin seed from different origins and varieties [6,7]. The four fatty acids presented in significant quantities are palmitic, stearic, oleic, and linoleic acids [6,7]. The pumpkin seed is a good source of potassium, phosphorus, magnesium, and also contains moderately high amounts of other trace minerals (calcium, sodium, manganese, iron, zinc, and copper) and these elements make pumpkin seed valuable for food supplements [6].

Material and Methods

Sources of raw materials

The sample, Maize, Soybean, Pumpkin seeds and Commercial couscous used for this study were obtained at the open market (Monday Market, and Gamboru Market). They were authenticated by a seed breeder at the Lake Chad Research Institute, Maiduguri, Borno State, Nigeria.

Sample preparation

The cereals Maize was sprouted for 3 days, the Soya bean was soaked overnight, drained, air dried, roasted, and grinded into coarse form and then steam cooked. The pumpkin seeds was also soaked in water, washed, drained, air dried in an open shed, roasted, grinded into coarse form and then steam cooked. The steam cooked soya bean and the pumpkin seeds were also air dried and packaged.

Sprouting

Ten kilograms (10 kg) of each cereal grains was sorted out and cleaned with water. The grains were then soaked overnight, in the morning the cereals were washed with water, drained, and then transferred onto a wetted jute bag and placed it in an air tight jar. The

various samples of cereal grains were sprouted on wet jute bags for 72 hours at room temperature. The samples were removed from the jute bag, air dried and grinded into fine grits.

Soaking and roasting

Ten kilograms (10 kg) of soya beans were soaked in tap water for 8 hours and washed with more water, air dried in an open shade for 48 hours and then roasted (Soaking and roasting were intended to remove the beany flavour). 10 kg Pumpkin seeds were also soaked in water for 2 hours, washed, air dried in an open shade and roasted. The soya bean and the pumpkin seeds were then grinded into fine grits; sieved and steam cooked for 20 - 30 minutes. They were then air dried in an open shade for 48 hours and then packaged.

Production of couscous

The cereal grains were sorted out and cleaned, sprouted at room temperature, air dried in an open shade, grinded into fine grits, sieved and then, water, a pinch of salt and small quantity of oil were added into the grits, mixed thoroughly with hand and then place in a steam cooking pot and steam cooked for 20 - 30 minutes at low temperature the result is the "traditional couscous" which is then air dried in an open shade for 48 hours, sieved and finally packaged in a container.

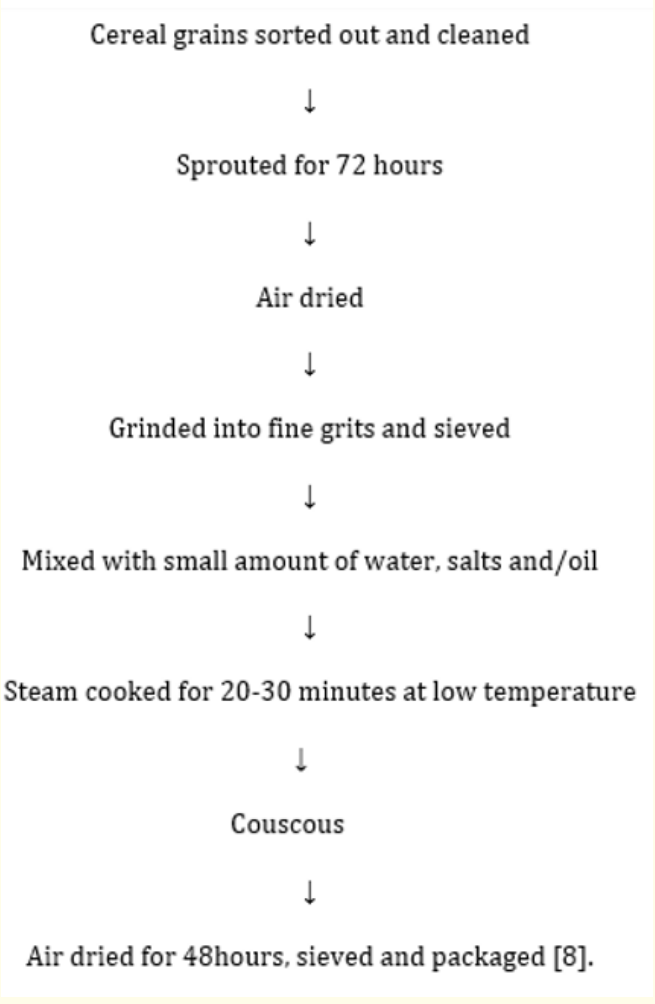

Figure 1: *Flow chart for production of traditional couscous.*

Formulation/blending (fortification) of couscous with soya bean and pumpkin seeds

The cereals were blended based on ratio 70: 20:10, (70g of the cereal was mixed with 20g of soya bean and 10 g of pumpkin seeds), 60:40 (60g of the cereal was mixed with 40g of soya bean without pumpkin seeds) and 60:40 (60g of cereal was mixed with 40g of pumpkin seeds without soya bean) respectively.

Animal experimentation/nutritional studies

Eighty five albino rats of 35 - 45g were obtained from the small animal house unit of the Department of Biochemistry, University of Maiduguri. The rats were randomly assigned into four (100, 70:20:10, 60:40 and 60:40) dietary treatment groups of 5 rats per group. The rats in each group were housed together in standard plastic laboratory cages with stainless steel covers and were offered with their respective experimental diets and water *ad libitum* after one week of acclimatization period to the laboratory environment. The feeding trials lasted for four weeks (28 days).

The feed intake was determined as the differential between the quantity of feed served and the quantity of feed left over. The changes in weight were determined by weighing the rats at the commencement of the feeding trial and thereafter on a weekly basis until termination of the experiment. The faecal and urine of the rats were collected on daily basis for 7 days (week four) for determination of percentage Nitrogen using Kjeldahl method [9].

Another group of 5 rats with same weight of the initial eighty five rats were fed with protein-free diet (1% or 2%), also their faecal and urine samples were analysed for percentage nitrogen to calculate for endogenous and metabolic nitrogen in biological assay.

Measures of protein quality

Quality and digestibility of protein are two major determinants whether the dietary protein intake can furnish adequate level of essential amino acids to satisfy their function in the body.

Protein Efficiency Ratio (PER) was determined by the method described by Osborne., *et al.* [10]; Using the formula

$$PER = \frac{\text{gain in body weight (g)}}{\text{protein consumed (g)}}$$

Biological value (BV) was determined by the method described by Mitchell 1924 [11]; using the formula

$$BV = \frac{NI-(FN-MFN)-(UN-EUN) \times 100}{NI-(FN-MFN)}$$

Where NI = Nitrogen intake

FN = Nitrogen voided through faeces

UN = Nitrogen excreted through urine

MFN = Metabolic faecal nitrogen

EUN = Endogenous urinary nitrogen

Apparent digestibility (AD)

The nitrogen excreted in the faeces is subtracted from the amount ingested and the value expressed as a percentage intake.

$$AD = \frac{NI- FN \times 100}{NI}$$

True digestibility (TD) was determined using the formula

$$TD = \frac{NI-(FN-MFN) \times 100}{NI}$$

Net protein utilization (NPU) was determined by the method described by Bender and Miller 1953 [12]; using the formula

$$NPU = \frac{NI-(FN-UN) \times 100}{NI}$$

Proximate analysis

The determination of moisture content, ash, crude protein, fat, crude fiber, carbohydrate and energy (Kcal) were carried out according to AOAC [13].

Determination of *in vitro* Protein Digestibility was determined by Nills [14].

The nitrogen of the undigested samples was determined by Kjeldahl method [9].

$$\%\ \textit{in vitro}\ \text{protein digestibility} = \frac{CP_1 - CP_2 \times 100}{CP_1}$$

Where,

CP_1= Total protein of unprocessed couscous

CP_2 = Total protein after digestion with trypsin.

Determination of anti-nutrients contents

Determination of tannin contents

Assay by Vanillin-hydrochloric acid. Method: quantitative (Price., *et al*. 1978).

Calculation

$$\frac{Au}{Cu} = \frac{Astd}{Cstd}$$

$$Cu = \frac{Au}{Cu} \times Cstd = mg/g$$

Where

Au= Absorbance of unknown; Cu= Concentration of unknown

Astd = Absorbance of standard; Cstd = Concentration of standard

Tannin (% reduction)

$$\%\ \text{reduction} = \frac{CRS - CPS \times 100}{CRS}$$

Where CRS = Concentration of raw sample; CPS = Concentration of processed sample.

Determination of phytic acid was by the method of Davies and Reid [15].

Determinations of mineral elements by atomic absorption spectrophotometer (AAS) AA 6800 series shimazocorp was used for the determination of Ca, Na, K, Fe, Mg, F and Zn (Wittmas., *et al*. 1981).

Determination of Vitamin B1, B2, B6, Folate and C Contents, the method described by Angelika., *et al.* [16] was used.

Vitamin A was determined using HPLC [16].

Determination of percentage nitrogen

Percentage Nitrogen for Urine and Faecal of the test animals was determine as described by AOAC [9] the formula below was used to calculate:

$$\%N = \frac{(A-B) \times N \times F \times 100}{Mg \text{ of sample}}$$

Where; A= ml of acid for titrating the sample; B = ml of acid for titrating blank sample

N = Normality of acid used for titration; F = Factor (14.007)

Sensory evaluation

Fifteen panellists evaluated the sensory properties of cooked samples by using nine (9) hedonic scale from 9 (like extremely) to 1 (dislike extremely) for colour, texture, aroma, taste and overall acceptability of the couscous samples. Replication was achieved by the five different couscous samples being evaluated by fifteen panelists [17].

Statistical analysis

The Statistical Package for Social Sciences (SPSS), version 20.0 was used to analyze the data collected which were expressed as means ± SE. One way analysis of variance (ANOVA) and Duncan's multiple range tests were used to compare the means obtained after each experiment. Differences were considered significant at $p < 0.05$.

Table 1 shows the proximate composition of raw maize couscous, sprouted maize couscous, and sprouted maize couscous blends compared with commercial couscous. The moisture contents of raw maize couscous and sprouted maize couscous are 11.80% and 8.98% respectively. Sprouted maize couscous blends moisture content range from 6.94% to 8.49%, while commercial couscous had a moisture content of 9.16%. The ash content of raw maize couscous and sprouted maize couscous are 1.29% and 1.64% respectively. Sprouted maize couscous blends ash contents maize between 2.43% to 2.97% while commercial couscous ash content was 0.83%. Crude protein of raw maize couscous and sprouted maize couscous are 16.21% and 9.86% respectively. Sprouted maize couscous blends crude protein were 20.48%, 25.24% and 18.98% respectively commercial couscous had a crude protein value of 12.53%. Fat content of raw maize couscous and sprouted maize couscous are 3.16% and 5.75% respectively. The fat contents of sprouted maize couscous blends were 10.65%, 9.64% and 11.02% respectively. Commercial couscous recorded a fat content of 1.42%. Crude fiber of raw maize couscous and sprouted maize couscous are 0.47% and 1.06% respectively. Crude fiber of sprouted maize couscous blends range from 0.05% to 0.98% while the commercial couscous crude fiber was 0.97%. Total carbohydrate of raw maize couscous and sprouted maize couscous are 67.07% and 72.73% respectively. Sprouted maize couscous blends total carbohydrates range from 53.83% to 59.11% while the commercial couscous total carbohydrate was 75.10%. Energy (kcal) of raw maize couscous and sprouted maize couscous are 361.57 and 382.06 respectively. Sprouted maize couscous blends energy (kcal) range from 403.03 to 411.56, while commercial couscous energy (kcal) was found to be 363.20 kcal.

Table 2 shows mineral composition of raw maize couscous, sprouted maize couscous, sprouted maize couscous blends compared with commercial couscous. Raw maize couscous and sprouted maize couscous had sodium (Na) content of 21.31 mg/g and 11.24 mg/g respectively. Sprouted maize couscous blends Na content were 25.83 mg/g, 25.12 mg/g and 97.00 mg/g respectively. Commercial couscous Na content was 21.23 mg/g. Potassium (K) values of raw maize couscous and sprouted maize couscous are 4.74 mg/g and 1.91 mg/g respectively. Sprouted maize couscous blends K values were 2.43 mg/g, 2.44 mg/g and 0.42 mg/g respectively. While commercial couscous K value was 2.34 mg/g. Calcium (Ca) content of raw maize couscous and sprouted maize couscous are 1.53 mg/g and 2.49 mg/g respectively. Sprouted maize couscous blends Ca values were 2.93 mg/g, 4.21 mg/g and 21.02 mg/g respectively. Commercial couscous Ca content was 2.62mg/g. Zinc (Zn) values of raw maize couscous and sprouted maize couscous are 0.61 mg/g and 0.24 mg/g. sprouted maize couscous blends Zn values were 0.25 mg/g, 0.54 mg/g and 0.24 mg/g respectively the Zn value of commercial couscous was 0.56mg/g. Magnesium (Mg) content of raw maize couscous and sprouted maize couscous are 0.51 mg/g and 0.86mg/g respectively. Sprouted maize couscous blends mg content were 1.01 mg/g, 1.43 mg/g and 7.02 mg/g respectively. Commercial couscous Mg value was 0.78 mg/g. Iron (Fe) contents of raw maize couscous and sprouted maize couscous are 0.78 mg/g and 0.43 mg/g respectively. Sprouted maize couscous blends Fe contents were 0.19 mg/g, 0.47 mg/g and 0.62 mg/g respectively. Commercial couscous Fe value was 0.71 mg/g.

Sample	Raw	Sprouted	Blend 1	Blend 2	Blend 3	GPC
Moisture (%)	11.80 ± 0.03^{f}	8.98 ± 0.01^{d}	8.49 ± 0.01^{c}	8.27 ± 0.01^{b}	6.94 ± 0.01^{a}	9.16 ± 0.01^{e}
Ash (%)	1.29 ± 0.01^{b}	1.64 ± 0.01^{c}	2.43 ± 0.02^{d}	2.75 ± 0.01^{e}	2.97 ± 0.00^{f}	0.83 ± 0.02^{a}
Protein (%)	16.21 ± 0.02^{c}	9.86 ± 0.03^{a}	20.48 ± 0.03^{e}	25.24 ± 0.01^{f}	18.98 ± 0.01^{d}	12.53 ± 12.53 ± 0.01^{b}
Fat (%)	3.16 ± 0.02^{b}	5.75 ± 0.01^{c}	10.65 ± 0.02^{e}	9.46 ± 0.01^{d}	11.02 ± 0.01^{f}	1.42 ± 0.01^{a}
Fiber (%)	0.47 ± 0.01^{c}	1.06 ± 0.02^{e}	0.23 ± 0.01^{b}	0.05 ± 0.01^{a}	0.98 ± 0.00^{d}	0. 0.97 ± 0.01d
Carbohydrate (%)	67.07 ± 0.08^{d}	72.73 ± 0.07^{e}	57.72 ± 0.08^{b}	53.83 ± 0.04^{a}	59.11 ± 0.01^{c}	75.10 ± 0.04f
Energy (kcal)	361.57 ± 0.10^{a}	382.06 ± 0.03^{c}	408.66 ± 0.09^{e}	403.03 ± 0.08^{d}	411.56 ± 0.08^{f}	363.2 ± 0.11^{b}

Table 1: *Chemical composition of raw maize couscous, sprouted maize couscous, sprouted maize couscous blends compared with commercial couscous.*

Values are recorded as Mean ± SEM, n = 4.

Values on the same row with different superscript are significantly different (P < 0.05).

Blend 1 (Sprouted maize mixed with soya bean and pumpkin seeds, 70:20:10).

Blend 2 (Sprouted maize mixed with soya bean, 60:40).

Blend 3 (Sprouted maize mixed with pumpkin seeds, 60:40).

GPC (Golden penny couscous).

Mineral elements (mg/g)	Raw	Sprouted	Blend 1	Blend 2	Blend 3	GPC
Na	21.31 ± 0.01^{c}	11.24 ± 0.01^{a}	25.83 ± 0.01^{e}	25.12 ± 0.01^{d}	97.00 ± 0.01^{f}	21.23 ± 0.01^{b}
K	4.74 ± 0.01^{e}	1.91 ± 0.01^{b}	2.43 ± 0.01^{d}	2.44 ± 0.01^{d}	0.42 ± 0.01^{a}	2.34 ± 0.01^{c}
Ca	1.53 ± 0.01^{a}	2.49 ± 0.01 ^{b}	2.93 ± 0.01^{d}	4.21 ± 0.01^{d}	21.02 ± 0.01^{e}	2.62 ± 0.01^{c}
Zn	0.61 ± 0.01^{c}	0.24 ± 0.01^{a}	0.25 ± 0.01^{a}	0.54 ± 0.02^{b}	0.24 ± 0.01^{a}	0.56 ± 0.01^{b}
Mg	0.51 ± 0.01^{a}	0.86 ± 0.01^{c}	1.01 ± 0.01^{d}	1.43 ± 0.01^{e}	7.02 ± 0.01^{f}	0.78 ± 0.01^{b}
Fe	0.78 ± 0.01^{f}	0.43 ± 0.01^{b}	0.19 ± 0.01^{a}	0.47 ± 0.01^{c}	0.62 ± 0.01^{d}	0.71 ± 0.01^{e}

Table 2: *Mineral elements of maize couscous, sprouted maize couscous, sprouted maize couscous blends compared with commercial couscous.*

Values are recorded as Mean ± SEM, n = 4.

Values on the same row with different superscript are significantly different (P < 0.05).

Blend 1 (Sprouted maize mixed with soya bean and pumpkin seeds, 70:20:10).

Blend 2 (Sprouted maize mixed with soya bean, 60:40).

Blend 3 (Sprouted maize mixed with pumpkin seeds, 60:40).

GPC (Golden penny couscous).

Table 3 present vitamins contents of raw maize couscous, sprouted maize couscous, sprouted maize couscous blends compared with commercial couscous. Vitamin A content of raw maize couscous and sprouted maize couscous are 15.91 µg/g and 15.23 µg/g respectively. Sprouted maize couscous blends vitamin A contents were 16.21 µg/g, 15.58 µg/g and 15.20 µg/g respectively. Commercial couscous vitamin A content was 14.23 µg/g. Vitamin B1 (thiamine) content of raw maize couscous and sprouted maize couscous are 0.63µg/g and 1.25µg/g respectively. Sprouted maize couscous blends vitamin B1 contents were 16.74 µg/g, 3.13 µg/g and 5.36 µg/g respectively commercial couscous vitamin B1 was 0.23 µg/g. Vitamin B2 (riboflavin) content of raw maize couscous and sprouted maize couscous are 2.00 µg/g and 0.99 µg/g respectively. Sprouted maize couscous blends vitamin B2 contents were 2.06 µg/g, 4.46 µg/g and 11.48 µg/g re-

spectively. Commercial couscous vitamin B2 was 0.64 µg/g. Vitamin B6 (pyridoxine) contents of raw maize couscous and sprouted maize couscous are 6.11 µg/g and 5.52 µg/g respectively. Sprouted maize couscous blends vitamin B6 contents were 17.71 µg/g, 25.54 µg/g and 29.68 µg/g respectively. Commercial couscous vitamin B6 was 0.33 µg/g. Folic acid contents of raw maize couscous and sprouted maize couscous are 15.04 µg/g and 6.63 µg/g respectively. Sprouted maize couscous blends folic acid contents were 19.06 µg/g, 16.56 µg/g and 29.55 µg/g respectively. Commercial couscous folic acid content was 5.54 µg/g. Vitamin C content of raw maize couscous and sprouted maize couscous are 26.69 µg/g and 13.37 µg/g respectively. Sprouted maize couscous blends vitamin C contents were 20.26 µg/g, 19.24 µg/g and 163.33 µg/g respectively. While the commercial couscous vitamin C contents was 13.37 µg/g.

Vitamin (µg/g)	Vit. A	Vit. B1	Vit. B2	Vit. B6	Folic Acid	Vit. C
Raw Maize	15.91 ± 0.01^{d}	0.63 ± 0.01^{b}	2.00 ± 0.04^{c}	6.11 ± 0.01^{c}	15.04 ± 0.01^{c}	26.69 ± 0.01^{d}
Sprouted Maize	15.23 ± 025^{b}	1.25 ± 0.02^{c}	0.99 ± 0.03^{b}	5.52 ± 0.02^{b}	6.63 ± 0.01^{b}	13.37 ± 0.01^{a}
Blend 1	16.21 ± 0.02^{d}	16.74 ± 0.01^{f}	2.06 ± 0.02^{c}	17.71 ± 0.01^{d}	19.06 ± 0.02^{e}	12.53± 20.26 ± 0.02^{c}
Blend 2	15.58 ± 0.01^{c}	3.13 ± 0.01^{d}	4.46 ± 0.02^{d}	25.54 ± 0.01^{e}	16.56 ± 0.02^{d}	19.24 ± 0.02^{b}
Blend 3	15.20 ± 0.02^{b}	5.36 ± 0.01^{e}	11.48 ± 0.01^{e}	29.68 ± 0.01^{f}	29.55 ± 0.01^{f}	163.83 ± 0.01^{e}
GPC	14.23 ± 0.01^{a}	0.23 ± 0.01^{a}	0.64 ± 0.02^{a}	0.33 ± 0.01^{a}	5.54 ± 0.01^{a}	13.37 ± 0.01^{a}

Table 3: *Vitamins contents of raw maize couscous, sprouted maize couscous, sprouted maize couscous blends compared with commercial couscous.*

Values are recorded as Mean ± SEM, n = 4.

Values on the same row with different superscript are significantly different (P < 0.05).

Blend 1 (Sprouted maize mixed with soya bean and pumpkin seeds, 70:20:10).

Blend 2 (Sprouted maize mixed with soya bean, 60:40).

Blend 3 (Sprouted maize mixed with pumpkin seeds, 60:40).

GPC (Golden penny couscous).

Digestibility (%)	Raw	Sprouted	Blend 1	Blend 2	Blend 3	GPC
1 Hour	96.97 ± 0.01^{d}	90.38 ± 0.01^{a}	97.59 ± 0.01^{f}	95.56 ± 0.01^{c}	97.23 ± 0.01^{e}	95.17 ± 0.02^{b}
6 Hours	97.39 ± 0.02^{c}	90.89 ± 0.01^{a}	97.88 ± 0.01^{e}	98.98 ± 0.00^{f}	97.24 ± 0.01^{b}	97.64 ± 0.01^{d}

Table 4: *In vitro protein digestibility of raw maize couscous, sprouted maize couscous sprouted maize couscous blends compared with commercial couscous.*

Values are recorded as Mean ± SEM, n = 4.

Values on the same row with different superscript are significantly different (P < 0.05).

Blend 1 (Sprouted maize mixed with soya bean and pumpkin seeds, 70:20:10).

Blend 2 (Sprouted maize mixed with soya bean, 60:40).

Blend 3 (Sprouted maize mixed with pumpkin seeds, 60:40).

GPC (Golden penny couscous).

Table 5 shows the results of anti-nutrients contents of raw maize couscous, sprouted maize couscous, and sprouted maize couscous blends compared with commercial couscous. Tannin contents of raw maize couscous and sprouted maize couscous are 5.38 mg/g and 3.40 mg/g respectively. Sprouted maize couscous blends tannin contents were 3.49 mg/g, 3.11 mg/g and 3.23 mg/g respectively. Commercial couscous tannin and Phytic acid are 0.62 mg/g and 0.98 mg/g respectively. Phytic acid content of raw maize couscous and sprouted

maize couscous are 20.55 mg/g and 0.89 mg/g respectively. Sprouted maize couscous blends phytic acid were 0.57 mg/g, 0.53 mg/g and 0.47 mg/g respectively.

Anti-nutrients (mg/g)	Raw	Sprouted	Blend 1	Blend 2	Blend 3	GPC
Tannin	15.20 ± 0.02^{b}	5.36 ± 0.01^{e}	11.48 ± 0.01^{e}	29.68 ± 0.01^{f}	29.55 ± 0.01^{f}	163.83 ± 0.01^{e}
Phytic acid	20.55 ± 0.02^{f}	0.89 ± 0.01^{d}	0.57 ± 0.02^{c}	0.53 ± 0.01^{b}	0.47 ± 0.01^{a}	0.98 ± 0.01^{e}

Table 5: *Anti-nutrients contents of raw maize couscous, sprouted maize couscous, sprouted maize couscous blends compared with commercial couscous.*

Values are recorded as Mean ± SEM, n = 4.

Values on the same row with different superscript are significantly different (P < 0.05).

Blend 1 (Sprouted maize mixed with soya bean and pumpkin seeds, 70:20:10).

Blend 2 (Sprouted maize mixed with soya bean, 60:40).

Blend 3 (Sprouted maize mixed with pumpkin seeds, 60:40).

GPC (Golden penny couscous).

Table 6 shows protein quality of raw maize couscous and sprouted maize couscous blends. Raw maize couscous feed intake was 60.18g while sprouted maize couscous feed intake were 65.71g, 69.19g and 65.09g respectively. Weight gain of raw maize couscous was 227.67g while sprouted maize couscous blends weight gain were 244.67g, 218.43g and 255.96g respectively. Protein efficiency ratio (PER) of raw maize couscous was 3.78% while sprouted maize couscous blends PER were 3.72%, 3.16% and 3.93% respectively. Biological value (BV) of raw maize couscous was 97.64% while sprouted maize couscous blends BV were 99.07%, 98.95% and 98.96% respectively. Apparent digestibility (AD) of raw maize couscous was 97.85% while AD of sprouted maize couscous blends were 97.69%, 98.05% and 99.47% respectively. True digestibility (TD) of raw maize couscous was 99.53% while sprouted maize couscous blends TD were 99.85%, 99.68% and 99.95% respectively. Net protein utilization (NPU) of raw maize couscous was 99.09% while sprouted maize couscous blends NPU were 99.60%, 99.94% and 99.45% respectively.

Parameter	Raw Maize	Blend 1	Blend 2	Blend 3
Feed intake (g)	60.18 ± 0.02^{a}	65.71 ± 0.01^{c}	69.19 ± 0.01^{d}	65.09 ± 0.03^{b}
Weight gain (g)	227.67 ± 0.01^{b}	244.67 ± 0.01^{c}	218.43 ± 0.01^{a}	255.96 ± 0.01^{d}
Protein efficiency ratio (%)	3.78 ± 0.00^{c}	3.72 ± 0.00^{b}	3.16 ± 0.00^{a}	3.93 ± 0.00^{d}
Biological Value (%)	97.64 ± 0.01^{a}	99.07 ± 0.01^{c}	98.95 ± 0.01^{b}	98.96 ± 0.01^{b}
Apparent digestibility (%)	97.53 ± 0.01^{b}	97.69 ± 0.01^{a}	98.05 ± 0.01^{c}	99.47 ± 0.01^{d}
True digestibility (%)	99.53 ± 0.01^{a}	99.85 ± 0.01^{c}	99.68 ± 0.01^{b}	99.95 ± 0.01^{d}
Net protein utilization (%)	99.09 ± 0.01^{a}	99.60 ± 0.01^{c}	99.94 ± 0.01^{d}	99.45 ± 0.01^{b}

Table 6: *Protein quality of raw maize couscous and sprouted maize couscous blends.*

Values are recorded as Mean ± SEM, n = 4.

Values on the same row with different superscript are significantly different (P < 0.05).

Blend 1 (Sprouted maize mixed with soya bean and pumpkin seeds, 70:20:10).

Blend 2 (Sprouted maize mixed with soya bean, 60:40).

Blend 3 (Sprouted maize mixed with pumpkin seeds, 60:40).

Table 7 shows sensory scores of raw maize couscous sprouted maize couscous blends compared with commercial couscous. In terms of all the attributes tested, blend 2 was most acceptable by the panelist and was significantly different (P < 0.05). In all, the samples were accepted by the panelist with exception of blend 3 which recorded lowest values.

Sample	Raw Maize	Blend 1	Blend 2	Blend 3	GPC
Colour	7.46 ± 0.02^{a}	7.88 ± 0.01^{b}	8.29 ± 0.02^{d}	7.46 ± 0.01^{a}	7.98 ± 0.02^{c}
Texture	7.66 ± 0.01^{c}	5.68 ± 0.01^{a}	8.12 ± 0.02^{d}	6.54 ± 0.01^{b}	8.19 ± 0.01^{e}
Aroma	8.03 ± 0.02^{c}	7.74 ± 0.01^{a}	8.41 ± 0.01^{e}	7.88 ± 0.01^{b}	8.32 ± 0.01^{d}
Taste	8.34 ± 0.01^{d}	8.03 ± 0.03^{c}	8.35 ± 0.02^{d}	7.66 ± 0.01^{b}	7.02 ± 0.02^{a}
Overall acceptability	8.54 ± 0.01^{d}	7.21 ± 0.01^{b}	8.53 ± 0.00^{d}	5.69 ± 0.02^{a}	7.32 ± 0.01^{c}

Table 7: *Sensory scores of raw maize couscous, sprouted maize couscous blends compared with commercial couscous.*

Values are recorded as Mean ± SEM, n = 15.

Values on the same row with different superscript are significantly different (P < 0.05).

Blend 1 (Sprouted maize mixed with soya bean and pumpkin seeds, 70:20:10).

Blend 2 (Sprouted maize mixed with soya bean, 60:40).

Blend 3 (Sprouted maize mixed with pumpkin seeds, 60:40).

GPC (Golden penny couscous).

Discussion

Proximate composition of raw maize couscous, sprouted maize couscous, sprouted maize couscous blends compared with commercial couscous

The raw maize couscous have higher moisture content than the sprouted maize couscous, the moisture content of the sprouted maize couscous blends were significantly different (P < 0.05) than the commercial couscous. This moisture contents provide products conservation for a longtime [18]. Ash content shows a great significance difference (P < 0.05) between raw maize couscous and sprouted maize couscous and also among sprouted maize couscous blends and the commercial couscous. The high contents of crude protein and fat could be as results of protein and fat contents of soya bean and pumpkin seeds. Sprouting have increased fiber contents of the sprouted maize couscous while addition or fortification of sprouted maize couscous with soya bean and pumpkin seeds reduced the fiber contents of the sprouted maize couscous blends and there was no significance difference between blend 3 and commercial couscous fiber contents. The low carbohydrate content in the couscous blends could be as a result of high protein and low carbohydrate in soya bean and pumpkin seeds used as supplements. The energy (kcal) varies as results of the variation in the composition or ratio of the food formulation used.

Mineral elements of raw maize couscous sprouted maize couscous, sprouted maize couscous blends compared with commercial couscous

The Na content of raw maize couscous and sprouted maize couscous range from 21.31 mg/g and 11.24 mg/g respectively. Sprouting had reduced the Na content while addition of soya bean and pumpkin seeds had improved the Na contents of the sprouted maize couscous blends. Blend 3 had the highest Na value (97.00 mg/g) followed by blend 1 (25.83 mg/g) and then blend 2 (25.12 mg/g), while the commercial couscous Na Content (21.23 mg/g) was found to be the least among the sprouted maize couscous blends produced. Potassium (K) content of raw maize couscous (4.74 mg/g) was higher than the sprouted maize couscous (1.91 mg/g). K contents of blend 1 and blend 2 are in close range, while blend 3 had least k value (0.42 mg/g) while commercial couscous had a K value of 2.34mg/g and there was significant difference (P < 0.05) between the sprouted maize couscous blends and the commercial couscous. Calcium (Ca) content of raw maize couscous (1.53 mg/g) was lower than the sprouted maize couscous (2.49 mg/g). Blend 3 had the highest Ca content (21.02 mg/g) followed by blend 2 (4.21 mg/g) and then blend 1 (2.93mg/g) while the commercial couscous Ca content was 2.62mg/g which was lower than the Ca contents of the sprouted maize couscous blends produced. The Zinc (Zn) content of raw maize couscous (0.61 mg/g) was higher than the sprouted maize couscous (0.24 mg/g). Blend 1 and blend 3 Zn values are in close ranged while blend 2 Zn value was higher than blend 1 and blend 2 and was in close range with commercial couscous (0.56 mg/g) and there was no significant difference between blend 1 and blend 3 and also between blend 2 and commercial couscous (P > 0.05). Magnesium (Mg) content of raw maize couscous (0.51

mg/g) was lower than the sprouted maize couscous (0.86 mg/g). Blend 3 (7.02 mg/g) had the highest Mg contents followed by blend 2 (1.43 mg/g) and the least was blend 1 (1.01 mg/g), while the commercial couscous Mg content was 0.78 mg/g which was lower than the sprouted maize couscous blends produced and there was significant difference ($P < 0.05$) between the sprouted maize couscous blends and the commercial couscous. Iron (Fe) content of raw maize couscous (0.78 mg/g) was higher than the sprouted maize couscous (0.43 mg/g), sprouting have reduced the Fe content and addition of soya bean and pumpkin seeds had enhanced the Fe content of sprouted maize couscous blends. Blend 3 had the highest Fe content (0.62 mg/g) followed by blend 2 (0.47 mg/g) and lastly blend 1 (0.19 mg/g) while the commercial couscous Fe content was (0.71 mg/g) which was higher than the sprouted maize couscous blends and there was significant difference ($P<0.05$) among the sprouted maize couscous blends and the commercial couscous.

In vitro protein digestibility of raw maize couscous, sprouted maize couscous, sprouted maize couscous blends compared with commercial couscous

In vitro protein digestibility of raw maize couscous at 1 hour and 6 hours (96.97% and 97.39%) showed high digestibility than the sprouted maize couscous at 1 hour and 6 hours (90.33% and 90.89%), this could be as a result of the leaching out of some nutrient due to processing method (sprouting). Sprouted maize couscous blends shows digestibility range of 95.56% to 98.98%, while commercial couscous percentage digestibility was within the range of sprouted maize couscous blends and there was significant difference ($P < 0.05$).

Protein quality of raw maize couscous, compared with sprouted maize couscous blends

Raw maize couscous feed intake (60.18g) was lower than the feed intake of sprouted maize couscous blends, blend 2 had high feed intake (69.19g), followed by blend1 (65.71g) and then blend 3 (65.09g). Weight gained of raw maize couscous (227.67g) was lower than the weight gained in blend 1 (244.67g) and blend 3 (255.96g) but higher than blend 2 (218.43g) and there was significant difference ($P < 0.05$). Protein efficiency ratio (PER) of raw maize couscous (3.78%) was higher than the PER of blend 1 (3.72%) and blend 2 (3.16%) but lower than the PER of blend 3 (3.93%). Biological value (BV) of raw maize couscous (97.64%) was lower than the BV of sprouted maize couscous blends, blend 1 had highest BV (99.07) followed by blend 2 (98.95%) and blends 3 (98.96%) but there was no significant difference between the BV of blend 2 and blend 3. Apparent digestibility (AD) of raw maize couscous (97.85%) was higher than the AD of blend1 (97.69%) but lower than blend 2 (98.05%) and blend 3 (99.47%). True digestibility (TD) of raw maize couscous (99.53%) was lower than the TD of the sprouted maize couscous blends, blend 3 (99.95%) had the highest TD followed by the blend 1 (99.85%) and then blend 2 (99.68%). Net protein utilization (NPU) of raw maize couscous (99.09%) was lower than the NPU of sprouted maize couscous blends, blend 2 (99.94%) had the highest NPU followed by blend 1 (99.60%) and then blend 3 (99.45%) and are statistically significant ($P < 0.05$).

Sensory evaluation

Fifteen panelists evaluated the sensory properties of cooked couscous samples by using Nine (9) hedonic scale from 9 (like extremely) to 1 (dislike extremely) for colour, texture, aroma, taste and overall acceptability of the couscous samples. Replication was achieved by the five different couscous samples being evaluated by fifteen panelists (Penfield and Campbell, 1990).

Conclusion

This study has shown that complementary couscous of acceptable quality can be produced from composites grits of maize, soya bean and pumpkin seeds. Sprouting significantly reduced the levels of antinutrients, mineral elements and vitamins while supplementation of mono cereals with soya bean and pumpkin seeds have improved the nutritional value of the sprouted couscous blends produced traditionally.

The results of proximate composition showed significant increase in ash contents, crude protein, fat content and low carbohydrate contents in the sprouted mono cereal Maize couscous blends produced traditionally. Commercial couscous recorded lowest proximate values compared to sprouted couscous blends.

Results of mineral elements studied (Na, K, Ca, Zn, Mg and Fe) showed a significant decrease in some mineral elements, while supplementation of sprouted mono cereal with soya bean and Pumpkin seeds have significantly increased levels (Na, Ca, Mg, and Fe), with blend 3 of all the sprouted mono cereal recorded higher values of the mineral elements.

Results of vitamins analyzed (Vit. A, vit.B1, vit.B2, vit.B6, folic acid and vit.C) showed a significant difference ($P > 0.05$) among the sprouted mono cereal couscous blends compared with commercial couscous. Blend 3 of all the mono cereal couscous blends recorded high levels of vitamins (vit. B2, vit B6, folic acid and vitamin C). Commercial couscous vitamin contents were lower or within a close range with the mono cereal couscous blends.

In vitro protein digestibility of each of the sprouted mono cereal couscous blends occurred at a range of 76% to 99% at 1 hour and 6 hours respectively.

The results of anti-nutrients showed that raw mono cereals contained higher tannin and phytic acid, while sprouting reduced the levels of these anti-nutrients and supplementation with soya bean and pumpkin seeds significantly enhanced the nutritional values of the sprouted mono cereal couscous blends produced. Commercial couscous recorded lowest tannin content (0.6 ± 0.02^{a}).

Biological assay (*in vivo* studies) showed high protein quality in raw and sprouted mono cereal couscous blends. Blend 1 if each of the sprouted mono cereal couscous blends recorded high PER, BV, AD, TD, and NPU.

In term of sensory score evaluation provides acceptability therefore, nutritious and acceptable complementary couscous blends can be produced from sprouted mono cereals fortified with soya bean and pumpkin seeds. The cost of producing traditional couscous blends is cheaper than commercial couscous.

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