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Determination of Amino Acid, Fatty Acid, Mineral, Functional and Choking Properties of Germinated and Fermented Popcorn (*Zea mays everta*) Flour

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Research Article

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ABSTRACT

Background: Popcorn is cereal grains originated from a wild grass (*Zea mays everta).* Human consume popcorn as snacks. Popcorn provides a full complement of nutrition benefits, including dietary fibre, protein and essential micronutrient. Processing methods, such as, sprouting and fermentation improved the nutritional quality of cereals and legumes. In view of this, the present study, therefore, investigates the influence of germination and fermentation on nutrient composition, choking property and functional property of popcorn.
Methodology: The popcorn kernels were obtained from a local market in Akure, Nigeria. The popcorn kernel was divided into three portions. Two portions were subjected into germination and fermentation respectively, while the third portion was processed as raw sample. Each of the samples was milled, sieved and analysed for proximate, minerals, amino acids and fatty acids using standard methods. Also, the functional and choking properties of the processed flour were determined using standard methods.
Results: The result showed that the protein content of popcorn flour samples ranged between 12.13±0.56 - 14.37±0.52 g/100g; while the energy value was between 322.53±8.91 and 421.93±5.58 Kcal. The phosphorous, potassium, sodium, magnesium, iron and zinc content of germinated popcorn flour (GPF) were higher than fermented popcorn flour (FPF).

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The total amino acids content of the sample range between 13.52 - 26.55 mg/100g for essential amino acids, 28.08 - 40.57 mg/100g for conditional essential amino acids; while 20.90 - 23.71 mg/100g for the non-essential amino acids. The nutritional quality results were as follows: protein efficiency ratio (PER) range between 0.88 and 1.55, essential amino acid indices (EAAIs) was between 13.62% and 44.18%; while the biological values (BV) was between 3.15% and 36.45%. The overall dominant fatty acid in each of the samples was oleic acid (67.05501 mg/100g) for the raw popcorn flour (RPF), palmitic acid (50.42259 mg/100g) for GPF and linoleic (68.72825 mg/100g) for FPF; while the dominant polyunsaturated fatty acids in RPF, GPF and FPF samples was linoleic. For the functional property, the results showed that swelling capacity range between 4.224±0.005 and 4.958±0.020. Bulk density was between 0.783±0.001 and 0.821±0.012; while that of water absorption capacity was between 1.964±0.014 and 2.111±0.044. The protein solubility of the samples increased in both above and below pH 2 for RPF and GPF sample and pH 3 for FPF sample, i.e., at the isoelectric points. For sensory attributes, the FPF was significantly rated higher in the overall acceptability than GPF, but rated lower than the 'ogi' (a sweet corn gel).

Conclusion: The present study evaluates the amino acid profiles, fatty acids composition, choking property and functional property of RPF, GPF and FPF. The result showed that germination and fermentation processing techniques improved on the nutrient composition and also, eliminate the choking property of the processed popcorn flour. In view of this, the germinated or fermented popcorn flour may be used as traditional breakfast meal (ogi) or in the formulation of complementary foods.

Keywords: Germinated popcorn; fermented popcorn; amino acids; fatty acids; nutritional quality;

1. INTRODUCTION

Maize is the third most cultivated cereal after wheat and rice in the world. Maize is a cereal grain grown throughout Nigeria and thrives mainly in the savannah zone. The kernel is used both for human consumption and for livestock feed (Ikem 1991; Oyarekua and Adeyeye, 2009; Iken and Amusa, 2010). Maize is eaten either at the green stage, as boiled or roasted ears, or dried and prepared into a jelly-like "pap" or "eko" (Alika et al., 1988). Among the cereals, maize represents the staple food for most part of the population of Africa, Nigeria inclusive. Maize is grown throughout the world and is a staple food crop particularly in Latin America, the Southern and Eastern part of Africa. Nutritionally, maize is a relatively poor cereal when it comes to the quality of its protein, because it has limiting amounts of two essential amino acids, lysine and tryptophan (Azevedo et al., 1997). In their natural form whole grain cereals are also significant contributor of vitamins, minerals like manganese, zinc, copper and magnesium and considerable iron but its bio- availability is low. This results in incidence of iron deficiency anemia, however processes like fermentation has improved the chemical bio- availability of iron (Dada and Muller 1970). Corn kernels may have white, yellow, red, blue or variation of those colours based on its endosperm. However, all cultivated species grown for the food; feed or industrial purposes have been classified under *Zea mays*. The most important maize varieties are flint corn, dent corn, flour or soft corn and popcorn. Human consume popcorn as a versatile and nutritious snacks. Popcorn provides a full complement of nutrition benefits, including dietary fibre, protein and b vitamins (Donkeun et al., 2000).

The nutritional composition of popcorn showed that it contained 3.8–4.6% crude fat, 8.1–10.5% crude protein, 0.07–0.23% reducing sugars and 61.0–67.9% starch, in which 27.0–28.5% of the starch was amylase (Donkeun et al., 2000). Popcorn hybrids contained on an average approximately 12.6% palmitic, 2.0% stearic, 25.5% oleic, 58.4% linoleic and 1.5% linolenic acids, respectively (Donkeun et al., 2000). The major fatty acids in the popcorn hybrids were linoleic and oleic acids. The energy value (380kcal/100g) and other essential nutrients of the kernel were high when compared with other cereals like sweet corn and sorghum (Donkeun et al., 2000).

Processing methods, such as, sprouting and fermentation has been reported to improve the nutritional and functional properties of plant seeds (Jirapa et al., 2001; Yagoub and Abdalla, 2007). For instance, Sprouting or germination has been reported to improve digestibility, bioavailability of vitamins, minerals, amino acids, proteins and phytochemicals, and decrease anti-nutrients and starch of some cereals and legumes (Kylerand McCready, 1975; Asiedu et al., 1992; Camacho et al., 1992; Egli, 2001; Helland et al., 2002; Egli et al., 2004) and thereby improve protein and iron absorption and a-Amylase activity is also increased during germination of cereals, especially sorghum and millet. This enzyme hydrolyzes amylase and amylopectin to dextrins and maltose, thus reducing the viscosity of thick cereal porridges without dilution with water while simultaneously enhancing their energy and nutrient densities (Gibson et al., 1998). All over the world, fermented food provides an important part of human diet. It is evident that traditional food fermentation is capable of improving the nutrients of the food, preserve it by generating acidic condition, detoxify and reduce cooking time of the food (Zamora and Veum, 1979; Steinkraus, 1995). Also, it is evident that fermentation has the potential to enhance iron and zinc absorption (Teucher et al., 2004). In view of this, the present study therefore aimed at investigating the effects of germination and fermentation on the nutrient and antnutritional composition of popcorn.

2. MATERIALS AND METHODS

2.1 PROCESSING OF GERMINATED AND FERMENTED POPCORN

Raw: The popcorn seeds were obtained from a local market (Erekensan), Akure, Nigeria. The seeds were sorted and oven dried. It was milled in attrition mill and sieved through 0.4mm wire mesh. It was packed in plastic container sealed with aluminum foil and stored at room temperature prior to analyses. The flour was prepared according to the flow chart in figure 1.

Fermentation: The popcorn seeds were sorted and soaked in hot water and left for 7days to ferment. The fermented grains were washed, wet milled with attrition mill (locally fabricated grinding machine), sieved with muslin cloth, decanted and drained. The drained paste was oven dried in hot air oven at 60oc for 20 hours, re-milled, sieved and packed in plastic container sealed with aluminum foil and stored at room temperature prior to analyses.

Germination: The popcorn seeds were sorted and soaked in water for 4 hours. The grains were spread on trays lined with cloth and kept wet by frequent spraying of water at every morning and evening for 4 days. The germinated popcorn kernel was washed, oven dried at $60^{o}C$ for 20 hours, milled and sieved through 0.4mm wire mesh. The popcorn flour was packed in plastic container sealed with aluminum foil and stored at room temperature prior to analyses.

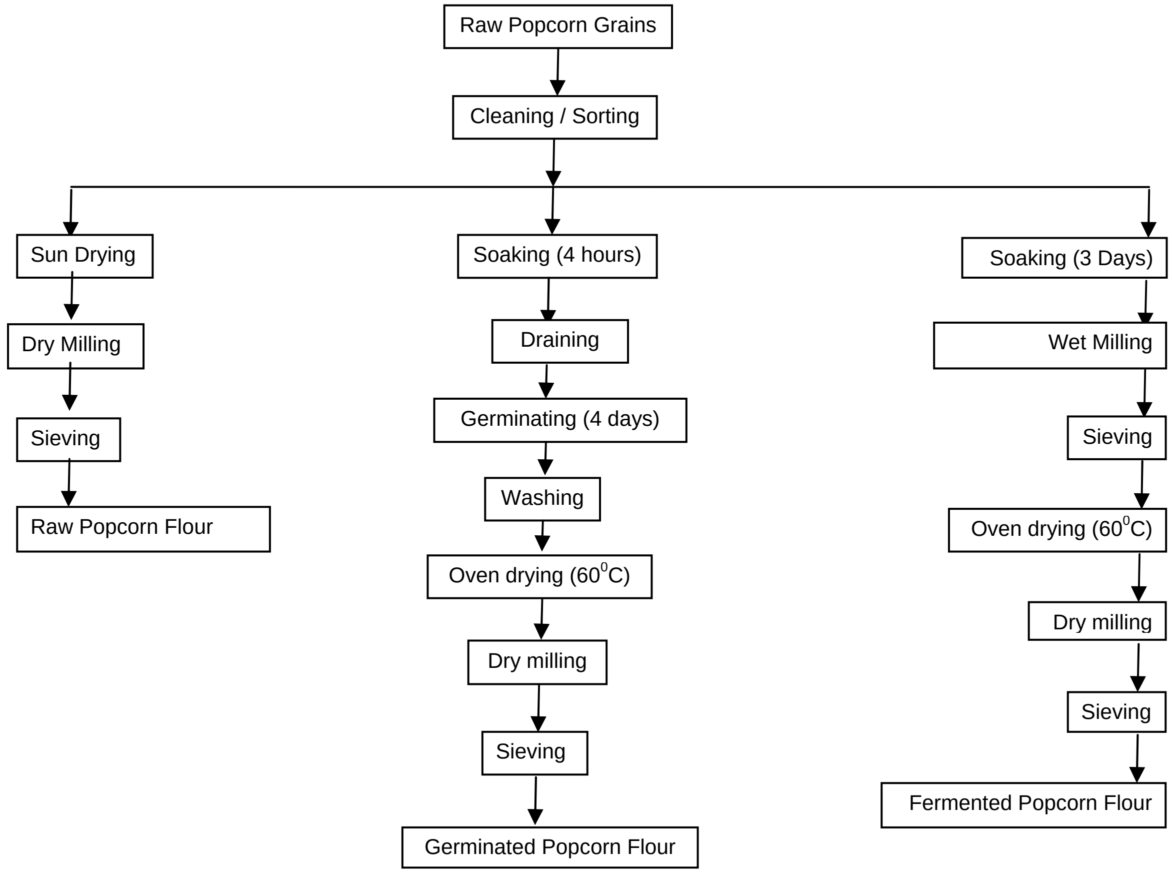

Fig.1. Production of raw, germinated and fermented popcorn flour

2.2 PROXIMATE ANALYSES

Proximate analyses for moisture, ash, fat and protein contents of the popcorn samples were carried out using Association of Official Analytical Chemists (AOAC) (2005) methods. Total carbohydrates content was determined by subtracting the ash, protein, and fat percentages from 100%. Estimation of energy value: The sample calorific value was estimated (in kcal/g) by multiplying the percentages of crude protein, crude lipid and carbohydrate with the recommended factors (2.44, 8.37 and 3.57, respectively) as proposed by Martin and Coolidge (1978).

2.3 MINERAL ANALYSES

The method described by Association of Official Analytical Chemists (AOAC) (2005) was used for mineral analysis. The samples were ashed at 550^{o}C. The ash was boiled with 10ml of 20% hydrochloric acid in a beaker and then filtered into a 100ml standard flask. This was made up to the mark with deionized water. The minerals were determined from the resulting solution. Sodium (Na) and Potassium (K) were determined using the standard flame emission photometer. NaCl and KCl were used as the standards (AOAC 2005). Phosphorus was determined calorimetrically using the spectronic 20 (Gallenkamp, UK) Kirk and Sawyer (1991) with KH_2PO_4 as the standard. Calcium (Ca), Magnesium (Mg) and Iron (Fe) were determined using Atomic Absorption Spectrophotometer (AAS Model SP9). All values were expressed in mg/100g.

2.4 AMINO ACID DETERMINATION

Amino acid composition of samples was measured on hydrolysates using amino acid analyzer (Sykam-S7130) based on high performance liquid chromatography technique. Sample hydrolysates were prepared following the method of Moore and Stein (1963). Two hundred milligrams of sample were taken in hydrolysis tube. Then 5 mL 6 N HCl were added to sample into the tube, tightly closed and incubated at 110°C for 24 hours. After incubation period, the solution was filtered and 200 mL of the filtrate were evaporated to dryness at 140°C for an hour. Each hydrolysate after dryness was diluted with one milliliter of 0.12 N, pH 2.2 citrate buffers, the same as the amino acid standards. Aliquot of 150 µL of sample hydrolysate was injected in a cation separation column at 130°C. Ninhydrine solution and an eluent buffer (The buffer system contained solvent A, pH 3.45 and solvent B, pH 10.85) were delivered simultaneously into a high temperature reactor coil (16 m length) at a flow rate of 0.7 ml/min. The buffer/ninhydrine mixture was heated in the reactor at 130°C for 2 minutes to accelerate chemical reaction of amino acids with ninhydrine. The products of the reaction mixture were detected at wavelengths of 570 nm and 440 nm on a dual channel photometer. The amino acid composition was calculated from the areas of standards obtained from the integrator and expressed as percentages of the total protein.

2.5 NUTRITIONAL QUALITY DETERMINATIONS

Nutritional qualities were determined on the basis of the aminoacid profiles. The Essential Amino Acid Index (EAAI) was calculated using the method of Labuda et al. (1982) according to the equation below:

$$EAAI = \sqrt[n]{\frac{100a \times 100b \ldots 100j}{av \times bv \ldots jv}}$$

where:
n = number of essential amino acids, a, b …..j = represent the concentration of essential amino acids (lysine, tryptophan, isoleucine, valine, arginine, threonine, leucine, phenylalanine, histidine and the sum of methionine and cystine) in test sample and av, bv ….. jv = content of the same amino acids in standard protein (%) (egg or casein) respectively. Biological value was calculated according to Oser (1959) cited by Mune et al. (2011) using the following equation:

BV = 1.09 x Essential amino acid index (EAAI) - 11.7

The Protein Efficiency Ratio (PER) was estimated according to the regression equations developed by Alsmeyer et al. (1974) cited by Mune et al. (2011)) as given below:
PER = -0.468 + 0.454(LEU) - 0.105(TYR)

2.6 FATTY ACIDS DETERMINATION

Fatty acid compositions of the samples were analysed using gas-liquid chromatography (with omega-wax capillary column Supelco, USA). The lipid classes were separated by thin layer chromatography on silica gel G 60 (Merck, Darmstadt), using n-hexane/ethylether/acetic acid (73/25/2/v/v/v) as developing solvent. The fatty acids of phospholipids and triglycerides were transformed with sodium methylate into methylesters.

2.7 FUNCTIONAL PROPERTIES

2.7.1 Water absorption capacity

Water and oil absorption capacities of the flour samples were determined by Beuchat (1977) methods. One gram of the flour was mixed with 10 ml of water or oil in a centrifuge tube and allowed to stand at room temperature (30 ± 2oC) for 1 h. It was then centrifuged at 200 x g for 30 min. The volume of water or oil on the sediment water measured. Water and oil absorption capacities were calculated as ml of water or oil absorbed per gram of flour.

2.7.2 Bulk density

A 50 g flour sample was put into a 100 ml measuring cylinder. The cylinder was tapped continuously until a constant volume was obtained. The bulk density (g cm-3) was calculated as weight of flour (g) divided by flour volume (cm^3) (Okaka and Potter, 1979).

2.7.3 Swelling capacity

This was determined with the method described by Leach et al. (1959) with modification for small samples. One gram of the flour sample was mixed with 10 ml distilled water in a centrifuge tube and heated at 80oC for 30 min. This was continually shaken during the heating period. After heating, the suspension was centrifuged at 1000 ×g for 15 min. The supernatant was decanted and the weight of the paste taken. The swelling power was calculated as: swelling power = weight of the paste / weight of dry flour.

2.7.4. Protein solubility

The protein solubility of wheat flour was studied using the Biuret method. 0.5g each of the samples was suspended in 10ml different salts concentrations. The solubility at natural pH was first determined, that is no acid or alkali was added and so solubility in this case was based on the normal pH of the sample in solution. The suspension was centrifuged at room temperature for 30 minutes at 3500rpm. The suspended obtained was filtered and the protein of filterate was determined by biuret method with standard Bovine Serum Albumin (BSA). The Biuret method is a convenient assay for large numbers of samples of relatively soluble protein unlike the Kjeldahl method which is not a rapid and convenient assay though useful for the determination of the amount of protein in crude mixtures. For the quantitative determination of standard protein in Biuret method, 1g of (BSA) was dissolved in 100ml distilled water in a volumetric flask. Five tubes were set up containing fractions of the BSA solution in order:- 0.0ml, 0.5ml, 1.0ml, 1.5ml, 2.0ml , and they were made up to 2ml adding 2.0ml, 1.5ml 10ml 0.5 and 0.00ml of distilled water by the addition of 8ml of Biuret solution. The tubes were left to stand for 30 minutes. The solution from the tube containing 2.0 distilled water and 8.0ml Biuret solution was used as the blank to standardize the UV spectrophotometer at 450nm (Spectronic 20 Bausch and Lomb). The absorbance of each of the other tubes was equally taken. A standard curve was drawn for absorbance against concentration. The determination of protein of the filtered supernatant in each sample was carried out in this way, 1.0ml of the filtrate was pipette into a test tube and 8ml of Biuret solution was added. The tube was allowed to stand for 30 minutes after which the absorbance was taken. The corresponding protein concentration was obtained curve earlier obtained. The obtained protein concentrations for the various salts were plotted.

2.8 CHOKING PROPERTY DETERMINATION

Twenty male and female albino rats of the Wistar strain, weaned at 21 days, were obtained from the disease-free stock of the central animal house of College of Medicine, University of Ibadan, and reared on a balanced commercial stock diet (Pfizer Livestock Feed Ltd, Ikeja, Nigeria) until they were 30 days old, when they were weighed to the nearest 0.1 g and allocated on the basis of weight and litter origin to three groups of ten rats each. They were individually housed in perforated Perspex cages. The first two groups of animals were fed with pelleted germinated and fermented popcorn samples respectively while the third group (control) was fed with commercial stock diet for 60 days. The mean of survival periods were calculated for the groups of animals as follows:

$$\text{Mean of survival period} = \frac{\text{Cumulative number of survival albino rats for 60 days}}{\text{Number of albino rats per group (10)}}$$

2.9 SENSORY CHARACTERISTICS OF PROCESSED POPCORN

The processed popcorn flour was subjected to sensory test using 10 panellists. The products were rated in terms of taste, colour, aroma, texture and overall acceptability on a 9 - point hedonic scale ranging from 9-dislike extremely to 1-like extremely. Ogi, a traditional breakfast meal produced from fermented sweet corn, was used as the control food sample. The food samples were prepared in a slurry, puree or porridge form.

2.10 STATISTICAL ANALYSIS

The data were analysed using SPSS version 15.0. The mean and standard error of means (SEM) of the triplicate analyses were calculated. The analysis of variance (ANOVA) was performed to determine significant differences between the means, while the means were separated using the new Duncan multiple range test.

3. RESULTS AND DISCUSSION

3.1 MACRONUTRIENT COMPOSITION

The mean values of macronutrient composition of raw, germinated and fermented popcorn were presented in Table 1. The protein content of the germinated popcorn flour, GPF, (14.24 ±0.69`g/100g) was the highest when compared with the fermented popcorn flour, FPF, (14.37±0.52 g/100g) and raw popcorn flour, RPF, (12.13±0.56 g/100g) respectively. Nutritionally, the protein content of both germinated and fermented popcorn flour was significantly higher than the protein content of the raw (12.13g/100g) and that of ogi (12.3±0.62 g/100g), that is, a traditional meal produced from fermented sweet corn flour. Similarly, the energy value of germinated popcorn flour (421.93±5.58 kcal.) was higher when compared with fermented popcorn flour (322.53±8.91 kcal.).

Table 1. Mean (± SEM) of macronutrient composition (g/100g Dry weight matter) of raw, germinated and fermented popcorn flour

Nutrient/Sample	Raw popcorn flour (RPF)	Germinated popcorn flour (GPF)	Fermented popcorn flour (FPF)	Ogi (traditional food)
Protein	$12.13^{b}\pm0.56$	$14.24^{a}\pm0.69$	$14.37^{a}\pm0.52$	$12.32^{b}\pm0.62$
Fat	$6.86^{a}\pm1.59$	$6.39^{a}\pm1.14$	$5.85^{a}\pm1.63$	$6.13^{a}\pm1.28$
Ash	$1.49^{a}\pm0.08$	$1.39^{a}\pm0.11$	$0.87^{b}\pm0.07$	$0.70^{b}\pm0.06$
Fiber	$1.12^{a}\pm0.16$	$1.12^{a}\pm0.17$	$0.81^{a}\pm0.21$	$0.21^{b}\pm0.06$
Carbohydrate	$78.31^{a}\pm1.89$	$76.85^{a}\pm0.89$	$78.09^{a}\pm1.25$	$76.46^{a}\pm0.57$
Energy (Kcal.)	$424.38^{a}\pm7.75$	$421.93^{a}\pm5.58$	$322.53^{b}\pm8.91$	$332.23^{b}\pm2.73$

Mean values with the same superscript in a row are not significantly different (P>0.05)

Comparatively, the energy values of both germinated and fermented popcorn flour were insignificantly lower than that of the raw popcorn flour sample (424.38±7.75 kcal.), but they were significantly higher than the energy value of fermented sweet corn flour. The observed decreases in carbohydrate and oil contents with germination could be attributed to their utilization in the sprouting process as energy sources (Fasasi, 2009). The increase in respiration rate during germination brings about the release of energy from the breakdown of carbon compounds. Germination changes the stored insoluble nutrients in the cotyledons to soluble nutrients through the hydrolysis of macromolecules. Similarly, the reduction in carbohydrate and fat content of the fermented popcorn flour could be attributed to the activities of micro-organisms on these nutrients in utilizing them to synthesize protein for their growth. The increased protein content of the germinated and fermented popcorn kernel may be due to synthesis of enzymes or a compositional change following the degradation of other constituents. Several investigators have also observed significant increases in protein content with seed germination (Enujiugha et al., 2003; Fasasi, 2009).

Table 2. Mean (± SEM) of mineral composition (mg/100g) of raw, germinated, fermented corn flour and ogi (sweet corn gel)

Nutrient/Sample	Raw popcorn flour (RPF)	Germinated popcorn flour (GPF)	Fermented popcorn flour (FPF)	Ogi (a corn gel, traditional food)
Phosphorous	$143.550^{a}9.13$	$156.930^{a}\pm13.21$	$142.508^{a}\pm14.62$	$85.947^{b}\pm15.33$
Potassium	$101.985^{c}\pm6.62$	$141.243^{a}\pm3.12$	$122.592^{ab}\pm17.68$	$101.373^{c}\pm13.05$
Sodium	$11.326^{b}\pm0.07$	$13.715^{a}\pm0.65$	$14.071^{a}\pm0.45$	$14.511^{a}\pm0.42$
Calcium	$0.305^{c}\pm0.06$	$0.446b^{c}\pm0.03$	$0.850^{a}\pm0.07$	$0.683^{ab}\pm0.09$
Magnesium	$28.100^{c}\pm0.017$	$31.967^{b}\pm0.16$	$31.440^{b}\pm0,96$	$34.917^{a}\pm1.54$
Iron	$0.216^{b}\pm0.01$	$0.645^{a}\pm0.08$	$0.271^{b}\pm0.04$	$0.258^{b}\pm0.18$
Zinc	$0.200^{a}\pm0.08$	$0.560^{a}\pm0.25$	$0.570^{a}\pm0.32$	$0.083^{a}\pm0.01$
Ca/P	2.12	2.84	5.96	7.95
K/Na	9.00	10.3	8.71	6.99
Ca/Mg	1.09	1.39	2.70	1.96
Nickel	-	-	-	-
Copper	-	-	-	-
Manganese	-	-	-	-
Rubidium	-	-	-	-
Molybdenum	-	-	-	-
Cadmium	-	-	-	-
Bromine	-	-	-	-
Strontium	-	-	-	-
Astatine	-	-	-	-
Lead	-	-	-	-
Alumininum	-	-	-	-
Iodine	-	-	-	-

(-) Not detected, Mean values with the same superscript in a row are not significantly different (P>0.05)

In this present study, it was observed that popcorn has more nutritional advantage in terms of protein content and energy value over other cereals like sweet corn flour (ogi). Quite a number of studies have reported on the nutrient composition of popcorn (Donkeun et al., 2000); and also, on the advantages of germination and fermentation processing techniques on the nutrient composition of plant based materials such as cereals and legumes ((Jirapa et al., 2001; Yagoub and Abdalla, 2007). For instance, it is evident that germination and fermentation processing methods improved the digestibility and bioavailability of essential nutrients and also reduced the anti-nutrient composition of processed food samples, such as cereals and legumes (Kylerand McCready, 1975; Asiedu et al., 1992; Camacho et al., 1992; Egli, 2001; Helland et al., 2002; Egli et al., 2004).

3.2 MINERAL COMPOSITION

The mean values of mineral composition of raw, germinated and fermented popcorn flour are shown in Table 2. The mineral contents of the germinated and fermented popcorn flour were comparatively higher than the raw popcorn sample. For instance, phosphorous, potassium, sodium, magnesium, iron and zinc content of germinated popcorn flour were higher when compared with the fermented popcorn flour, however, both the germinated and fermented flour were significantly higher than the raw popcorn sample, but relatively comparable to fermented sweet corn flour (ogi) sample in virtually all the mineral composition. This finding showed that popcorn is a good source of these essential minerals, particularly for the iron and zinc which are of public health significant. For the heavy metals, like lead, aluminum, astatine, copper, nickel etc. these were not detected in the food samples; and this further enhanced the nutritional values of the popcorn products; hence, its utilization in the production of ogi (a traditional meal) or any other cereal-based meal products would not have any detrimental effects on the consumers. The Ca/P and Ca/Mg values ranged between 2.12 - 5.96 and 1.09 - 2.70 respectively. These values were very high when compared with the recommended values of 1.0 and 2.2 respectively (NRC, 1989). This observation showed that popcorn is a very good sources of calcium, magnesium and phosphorous, hence, its consumption would enhances teeth and bone formation in children and also, for the regulation of calcium in the blood (National Research Council, 1989). The K/Na value of the processed popcorn flour ranged between 8.71- 10.3. These values are greater than the recommended value of 1.0 (National Research Council, 1989), hence, popcorn consumption should be minimal particularly among the hypertensive patients.

3.3 AMINO ACID COMPOSITION

The amino acid composition of raw, germinated and fermented popcorn flour is shown in Table 3. The total essential amino acid profile of raw popcorn flour was 26.55 mg/100g, while fermented and germinated popcorn flour were 13.52 mg/100g and 13.61 mg/100g respectively. For the conditional essential amino acids, the value for raw popcorn flour was 40.57 mg/100g, while for the fermented and germinated popcorn flour were 28.08 mg/100g and 30.79 mg/100g respectively. The non-essential amino acid values showed that raw popcorn sample contained 23.705 mg/100g, fermented popcorn was 20.9 mg/100g and that of germinated popcorn was 21.27 mg/100g. Comparatively, the total values of essential, conditional and non-essential amino acid profiles of the fermented and germinated popcorn samples were lower than the raw sample. This observation could be as a result of leaching of nutrient during the fermentation processing and also part of the amino acids could have been used up during the germination processing. However, the essential, conditional and non-essential amino acids of germinated samples were higher than that of fermented flour sample. Several studies have reported that germination increased protein concentration and bioavailability of amino acid of plant based food products, particularly cereals and legumes (Camacho et al., 1992; Egli, 2002; Helland et al., 2002; Obasi et al., 2003; Egli et al., 2004; Gernah and Ingbian, 2011).

Table 3. Amino acid composition (mg/100g protein) of raw, germinated and fermented popcorn flour

Amino acids	Raw Popcorn	Fermented Popcorn	Germinated Popcorn
Non essential amino acids			
Alanine	3.395^{c}±0.025	5.845^{a}±0.015	4.270^{b}±0.050
Aspartic acid	8.205^{a}±0.015	7.210^{b}±0.020	4.335^{c}±0.035
Serine	3.0250^{a}±0.025	0.260^{b}±0.020	0.570^{c}±0.040
Glutamic acid	9.080^{b}±0.030	7.585^{c}±0.015	12.095^{a}±0.035
Total	23.705	20.900	21.270
Conditionally essential amino acids			
Proline	2.135^{a}±0.015	0.540^{c}±0.010	1.055^{b}±0.025
Glycine	4.180^{a}±0.030	0.355^{c}±0.015	2.030^{b}±0.070
Arginine	4.155^{a}±0.015	4.060^{a}±0.020	3.690^{b}±0.050
Cysteine	1.050^{a}±0.020	0.535^{b}±0.015	0.405^{c}±0.015
Tyrosine	3.245^{a}±0.025	1.135^{c}±0.015	1.325^{b}±0.045
Total	40.57	28.075	30.79
Essential amino acids			
Lysine	5.040^{a}±0.060	2.175^{c}±-.025	2.710^{b}±0.020
Threonine	2.580^{a}±0.030	1.230^{c}±0.010	2.030^{b}±0.020
Valine	4.090^{a}±0.030	1.375^{b}±0.015	1.595^{c}±0.035
Methionine	2.1750^{a}±0.045	0.560^{c}±0.020	0.805^{b}±0.025
Isoleucine	3.250^{a}±0.020	2.270^{b}±0.010	0.915^{c}±0.105
Leucine	5.185^{a}±0.025	3.780^{b}±0.040	3.275^{c}±0.045
Phenylalanine	4.230^{a}±0.010	2.130^{b}±0.020	2.280^{b}±0.080
Histidine	2.100^{a}±0.100	0.550^{c}±0.050	1.015^{b}±0.015
*Tryptophan	ND	ND	ND
Total	26.55	13.52	13.61
Nutritional quality			
TEAA+His+Arg/TAA%	32.8	18.1	18.3
TEAA/TAA%	39.6	32.5	30.7
TNEAA/TAA%	60.4	67.5	69.3
TSAA(Meth+Cys)	3.225	1.095	1.21
ArEAA (Phe+Tyr)	7.475	3.265	3.605
TEAA/TNEAA	0.65	0.48	0.44
PER (g/100g)	1.55	1.129	0.88
EAAI (%)	44.18	13.62	20.39
BV (%)	36.45	3.15	10.53

Mean values with the same superscript in a row are not significantly different (P>0.05), Total essential amino acids (TEAA). Total amino acids (TAA), Total non-essential amino acids (TNEAA), Total sulphur amino acids (TSAA), Aromatic essential amino acids (ArEAA), Protein efficiency ratio (PER), Essential amino acid index (EAAI), Biological value (BV).

The ratio of total essential and non-essential amino acids showed that the raw popcorn sample was 0.65, fermented popcorn sample was 0.48 and that of germinated popcorn sample was 0.44. The values of protein efficiency ratio of the samples were 1.55 g/100g, 1.13 g/100g and 0.88 g/100g for the raw, fermented and germinated popcorn respectively. The essential amino acid indices (EAAIs) of the raw, fermented and germinated popcorn flour were 44.18%, 13.62% and 20.39% respectively; and while the biological values (BV) of the food samples were 36.45% for the raw popcorn, 3.15% fermented popcorn and 10.53% germinated popcorn.

Table 4. Comparison of FAO/WHO amino acids recommendation and the germinated and fermented popcorn amino acids composition

EAA	FAO/WHO	RPF	FPF	GPF
Arginine	2.0	4.155	4.060	3.690
Histidine	1.9	2.100	0.550	1.015
Isoleucine	2.8	3.25	2.270	0.915
Leucine	6.6	5.185	3.780	3.275
Lysine	5.8	5.040	2.175	2.710
Methionine	2.2	2.1750	0.560	0.805
Phenylalanine	2.8	4.230	2.130	2.280
Threonine	3.4	2.580	1.230	2.030
Tryptophan	1.1	ND	ND	ND
Valine	3.5	4.090	1.375	1.595
TSAA(Meth+cystein)	2.5	3.225	1.095	1.210
TArAA (Phenyl+Tyro)	6.3	7.475	3.265	3.605
TEAAs	33.9	26.55	13.52	13.61

Source: FAO/WHO 1991. ND (Not Detected), Total sulfur amino acids (TSAAs), Total aromatic amino acids (TAAAs), total essential amino acids (TEAAs)

Generally, a protein material is said to be of good nutritional quality when its biological values (BV) is high (70-100%) and also when the essential amino acid index (EAAI) is above 90% and to be useful as food when the values is around 80% and to be inadequate for food material when below 70% (Oser, 1959). From the present study it is observed that the BV and EAAI values were generally low; and these could be attributed to the fact that popcorn, a cereal, is low in protein content compared with the legumes. Investigations have shown that cereals, including maize, are generally low in protein content, for instance, maize is deficient in some essential amino acids, notably lysine and tryptophan (Okon, 1998). Therefore, the traditional cereal-based food products, e.g. ogi (a fermented corn gel), are generally of poor nutritional quality for human; and this could have been the reason for the high prevalence of protein malnutrition among weaning aged children in developing countries where cereals are solely used as complementary foods (Okoye 1992; Devlin 1997). In view of the low protein content of maize several efforts have been geared towards supplementation of maize products with legumes or animal proteins (Badamosi et al., 1995; Oyarekua, 2010). The essential amino acids of both raw and processed popcorn were compared with the FAO/WHO (1991) reference standard (Table 4), it was observed that the amino acids composition of the germinated and fermented popcorn flour were lower than the reference standard, with the exception of arginine. This means that before popcorn could be suitable to supply all the necessary nutrient requirements for its consumers, particularly infant, it must be complemented with other food materials like legume or animal based food (Mehta and Singh, 1989).

3.4 FATTY ACID COMPOSITION

The fatty acid compositions of raw, germinated and fermented popcorn flour are presented in Tables 5. Results show the presence of saturated and unsaturated fatty acids. The overall dominant fatty acids in the processed popcorn kernel are oleic acid (67.05501 mg/100g for the raw, palmitic acid (50.42259 mg/100g) for germinated acid and linoleic (68.72825 mg/100g) for the fermented popcorn flour. The dominant monounsaturated fatty acid in both raw and processed popcorn were oleic acid with the following values 67.05591mg/100g) for the raw, 34.49436 mg/100g) germinated and 16.82802 mg/100g for fermented popcorn sample, while the dominant polyunsaturated fatty acids in raw, germinated and fermented popcorn samples was linoleic with the following values 19.37746 mg/100g, 2.46934 mg/100g and 68.72825 mg/100g respectively.

Table 5. Fatty acids composition (mg/100g protein) of raw, germinated and fermented popcorn flour

Fatty acids	Raw popcorn	Germinated popcorn	Fermented popcorn
Capric acid	Trace	0.00002	-
Lauric acid	0.00003^{b}	0.00037^{a}	0.00002^{b}
Myristic acid	0.00969^{a}	0.00189^{b}	0.00667^{a}
Palmitic acid	2.32885^{c}	50.42259^{a}	9.62110^{b}
Palmitoleic acid	0.14651^{b}	0.85462^{a}	1.11352^{a}
Stearic acid	1.98614^{b}	2.74345^{a}	3.53573^{a}
Oleic acid	67.05501^{a}	34.49436^{b}	16.82802^{c}
Linoleic	19.37746^{b}	2.46934^{c}	68.72825^{a}
Linolenic acid	8.92132^{a}	0.00524^{b}	0.00885^{b}
Arachidic acid	0.17446^{b}	9.00632^{a}	0.15478^{b}
Behenic acid	0.00054^{b}	0.00087^{b}	0.00305^{a}
Lignoceric acid	Trace	0.00094^{a}	0.00002^{b}
Saturated Fatty acids (SFA)			
Myristic acid	0.00969^{a}	0.00189^{b}	0.00667^{a}
Palmitic acid	2.32885^{c}	50.42259^{a}	9.62110^{b}
Stearic acid	1.98614^{b}	2.74345^{a}	3.53573^{a}
Arachidic acid	0.17446^{b}	9.00632^{a}	0.15478^{b}
Behenic acid	0.00054^{b}	0.00087^{b}	0.00305^{a}
Total	4.49967^{c}	62.17512^{a}	13.1940^{b}
Poly unsaturated fatty acids (PUFA)			
Linolenic acid	8.92132^{a}	0.00524^{b}	0.00885^{b}
Linoleic	19.37746^{b}	2.46934^{c}	68.72825^{a}
Arachidonic acids	-	-	-
Docohexanoic acid	-	-	-
Total	28.29878^{b}	2.47468^{c}	68.7371^{a}
Mono unsaturated fatty acid (MUFA)			
Palmitoleic acid	0.14651^{b}	0.85462^{a}	1.11352^{a}
Oleic acid	67.05501^{a}	34.49436^{b}	16.82802^{c}
Total	67.20152^{a}	35.34898^{b}	17.94154^{c}
P:S	6.2891	0.0398	5.2097

Mean values with the same superscript in a row are not significantly different (P>0.05)

In comparison, the total saturated fatty acid in germinated popcorn flour was significantly higher than raw and fermented samples; while the total polyunsaturated fatty acid of fermented sample (68.72825 mg/100g) was significantly higher when compared with the germinated and raw popcorn sample respectively ($p<0.05$).

In this present study, the finding showed that fermentation improved on the nutritional quality of fatty acid. Quite a number studies have reported that high polyunsaturated fatty acid levels in dietary intakes are desirable because of their potential health benefits (Bonvehi and Coll, 1993; Cunnane et al., 1993; Zwarts et al., 1999).With the current emphasis on lowering consumption of saturated fats, minimizing or eliminating trans fat, and increasing polyunsaturated and monounsaturated fats intake the consumption of popcorn would improve the integrity of cardiovascular system.

3.5 CHOKING PROPERTIES

The mean survival period of albino rats fed with germinated and fermented popcorn flour is shown in Fig. 2. The result showed that the mean survival of the albino rats fed with fermented popcorn flour was higher than the germinated popcorn flour, however, there was no significant difference between the proportion of albino rats fed with fermented and germinated popcorn flour ($p>0.05$).

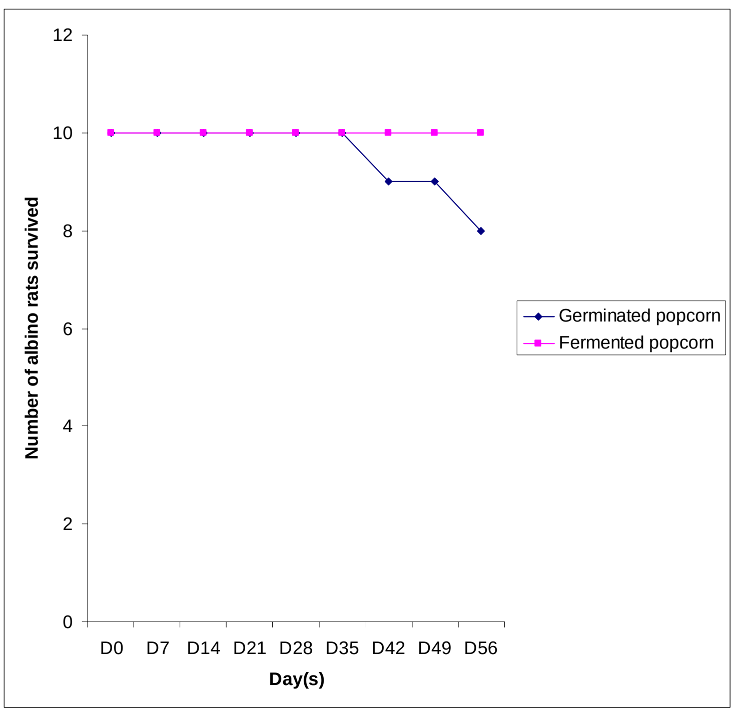

Fig. 2. Mean survival of albino rats fed with processed popcorn flour

This observation showed that germination and fermentation processing methods eliminate the choking properties of popcorn, however, the two causalities recorded against the germinated popcorn sample might not have been as a result of the choking property. It is evident that germination and fermentation enhance the nutritional value of legumes and cereals by causing significant changes in chemical composition and elimination of antinutritional factors (Bau et al., 1997; Nkama and Ikwelle, 1998; Traore et al., 2004; Abdelrahaman et al. 2005; Mohamed et al., 2007; Inyang and Zakari, 2008; Onwuka et al., 2009; Mugendi et al., 2010).

3.6 FUNCTIONAL PROPERTIES

The functional properties of raw, germinated and fermented popcorn flour are shown in Table 6. Results showed that the swelling capacity of the popcorn flour samples ranged between 4.224±0.005 and 4.958±0.020 for germinated and fermented sample respectively. Bulk density ranged between 0.783±0.001 and 0.821±0.012 for germinated and raw popcorn flour respectively; while the water absorption capacity ranged between 1.964±0.014 and 2.111±0.044 for germinated and fermented popcorn flour respectively. The functional properties of the popcorn flour samples were compared with ogi flour sample (a corn flour and traditional breakfast meal). It was observed that there was significant difference between the swelling capacity of ogi flour and the popcorn flour ($p<0.05$). However, for the bulk density and water absorption capacity the ogi flour was significantly lower when compared with the popcorn flour ($p>0.05$).

The functional properties of the food materials are very important for the appropriateness of the diet, particularly, for the growing children (Omueti et al., 2009). The consistency of energy density (energy per unit volume) of the food and the frequency of feeding are also important in determining the extent to which an individual will meet his or her energy and nutrient requirements (Omueti et al., 2009). The bulk density value is of importance in packaging (Snow, 1974). The lower loose bulk density implies that less quantity of the food samples would be packaged in constant volume thereby ensuring an economical packaging. However, the packed bulk densities would ensure more quantities of the food samples being packaged, but less economical. Nutritionally, loose bulk density promotes easy digestibility of food products, particularly among children with weak digestive system (Osundahunsi and Aworh, 2002; Gopaldas and John1991**)**. The water absorption capacity is an index of the maximum amount of water that a food product would absorb and retain (Marero et al., 1988; Mosha and Lorri, 1987**)**. With respect to water absorption capacity, Giami and Bekeham (1992) reported that the microbial activities of food products with low water absorption capacity would be reduced. Hence the shelf-life of such product would be extended. The swelling capacity is an important factor used in determining the amount of water that diets would absorb and the degree of swelling within a given time. The present study showed that the swelling capacities of the ogi sample were higher when compared with the popcorn flour samples. The high values of swelling capacity imply that more of the ogi sample would be needed for reconstitution when compared with the popcorn flour samples.

Table 6. Functional properties of raw, germinated and fermented popcorn flour

Functional attributes/Sample	Raw Popcorn flour	Germinated popcorn flour	Fermented popcorn flour	Ogi
Swelling capacity	4.626^{c}±0.002	4.224^{d}±0.005	4.958^{b}±0.020	6.475^{a}±0.001
Bulk density	0.821^{a}±0.012	0.783^{b}±0.001	0.797^{ab}±0.004	0.732^{c}±0.011
Water absorption	2.061^{a}±0.005	1.964^{b}±0.014	2.111^{a}±0.044	1.818^{c}±0.008

Mean values with the same superscript in a row are not significantly different (P>0.05)

Figure 3 shows the protein solubility for the raw, germinated and fermented popcorn seed samples. For the three samples, lowest and highest solubility were recorded at pH 2 and pH 10, respectively. Cereal proteins are known to be soluble in dilute salt solutions, except at their isoelectric points. The present study shows increased in the solubility both above and below pH 2 for raw and germinated popcorn sample and pH 3 for the fermented popcorn sample, These pH values are the isoelectric points, that is, the point at which the net charge on protein is zero (positive and negative charges are equal) . The isoelectric point of a protein is an important property because it is at this point that the protein is least soluble, and therefore unstable. It should be noted that both below and above the isoelectric point (isoelectric pH) the protein will be soluble. This behaviour is similar to many other seed flours reported by Akobundu et al. (1982). Germination and fermentation brought about an increase in protein solubility both at acid and alkaline pH. In comparison, the protein solubility of germinated popcorn flour was highest at pH 6 (>80%) while that of fermented was at pH 10 (>90%). This observation showed that as the pH increases the protein solubility of germinated and fermented popcorn flour increases, hence, alkaline pH supports the greater extraction of soluble protein (Padilla et al., 1996). This observation shows that the functional quality of popcorn seed flour can be improved by germination and fermentation prior to processing.

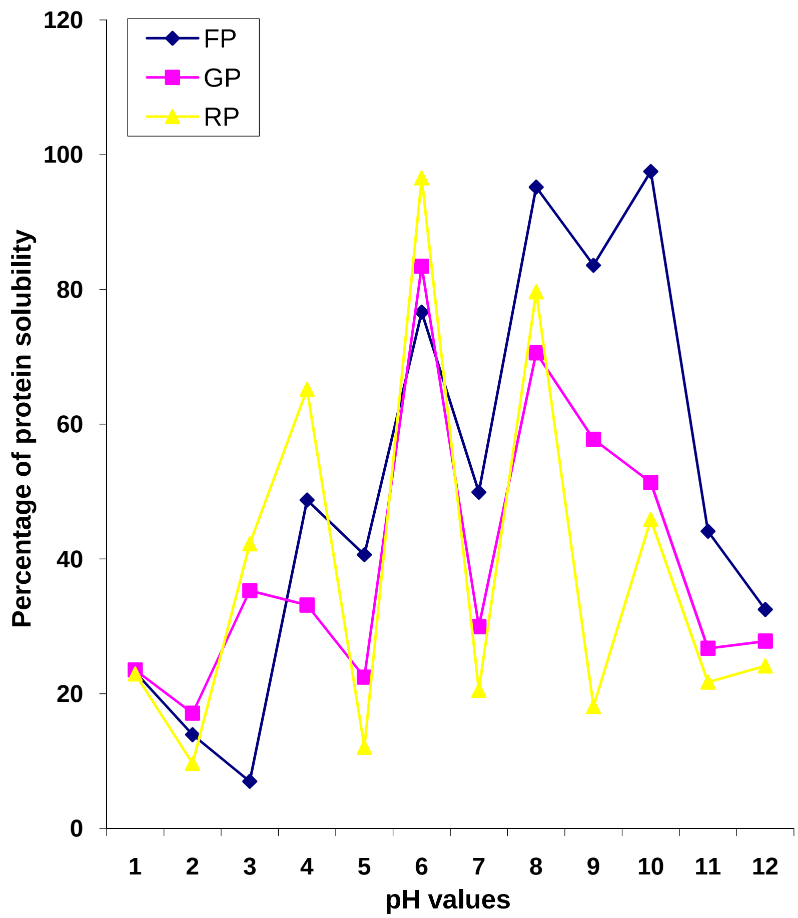

Fig. 3. Protein solubility of raw, germinated and fermented popcorn

3.7 SENSORY ATTRIBUTES

The comparison of sensory attributes of constituted germinated and fermented popcorn flour and ogi (traditional corn gel) is presented in Table 7. The result showed that the fermented popcorn was significantly rated higher in terms of taste, colour, mouthfeel, aroma and overall acceptability when compared with the germinated popcorn (p<0.05). However, the traditional ogi (a corn gel) was significantly rated higher when compared with the fermented and germinated popcorn in all the parameters respectively. This observation could be due to the fact that the panelists have been familiar with the 'ogi' (a traditional fermented sweet corn-gel); and this could have also been the reason why fermented popcorn was rated next to the traditional ogi sample.

Table 7. Comparison between sensory attributes of germinated and fermented popcorn and 'ogi' (fermented sweet corn gel, a traditional Nigerian meal)

Samples	Taste	Colour	Mouthfeel	Aroma	Overall acceptability
Fermented popcorn	3.45^b	4.35^a	4.15^b	3.55^b	3.9^b
Germinated popcorn	1.35^c	2.60^b	1.50^c	2.75^c	1.9^c
Ogi (corn-gel)	4.9^a	4.65^a	4.75^a	4.4^a	5.0^a

Mean values with the same superscript in a row are not significantly different (P>0.05)

4. CONCLUSION

The present study evaluates the amino acid profiles, fatty acids composition, choking property and functional property of germinated and fermented popcorn flour. The result established that processing techniques, such as, germination and fermentation, improved on the mineral, amino acid and fatty acid composition; and also, eliminate the choking property of the processed popcorn flour. In view of this, the germinated or fermented popcorn flour may be used as traditional breakfast meal (ogi) or in the formulation of complementary foods.

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