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The Effect of Cooking and Fermentation on the Functional and Nutritional Properties of Walnut and Maize

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Abstract: Walnuts locally called 'asala'or 'awusa' are eaten after boiling and maize which is fermented in the production of gruels called 'ogi' a widely utilized complementary food, shows qualitative and quantitative deficiency in protein content. The objective of this work is to co-ferment raw maize and walnut and also cooked walnut and maize to get low cost infant complementary foods of improved nutritional quality which might address malnutrition in infants. The mixture was prepared by co-fermenting 300g cooked walnut with 700g raw maize w/w (CWM) for 72h at 30^{O}C. After 72h each product was wet- milled, sieved and dried at 60^{o}C. Resultant flour was analyzed for: Proximate composition, minerals, anti-nutrients, amino acids, fatty acids, phospholipids, sterols contents and consistency using standard methods. The pH of (RW/M) dropped more drastically from 6 to 5 at 12h to 72h than that of (CW/M). RW/M had higher values of ash (0.98), moisture (12.74), crude protein (10.72), crude fiber (4.63), ether extract (2.53), CHO (68.4) and energy (2824) than CWM. Cooking reduced the proximate composition. RWM was more enhanced in most amino acids, minerals, mystric, stearic, and linoleic acids. CW/M had more reduced values of oxalate, saponin, alkaloid, flavanoid and higher phytate. Phytin than RW/M. The values of total phenol in both samples were comparable. RW/M could serve as infant complementary food of improved nutritional quality. *Bacillus pumilus, Lactobacillus delbrueckii, Leuconostocmesenteroide and Saccharomyces cerevisiae*were isolated from the fermented foods.

Keywords: Maize, Walnut, Co-Fermentation, Nutritional Quality, Fatty Acids

1. Introduction

Adequate nutrition during the first two years of life is essential for optimal physical and mental development of infants. African walnut (*Tretracarpidim conoforum*), a less-utilized food legume in Nigeria harvested between June and September, mostly cultivated in south-west Nigeria, is eaten after boiling and locally called 'asala' or 'awusa'[1]. Walnuts are rich in protein, carbohydrate, B- vitamins, magnesium and copper [2]. It is predominantly composed of polyunsaturated fatty acids, rich in anti-inflammatory Omega-3 essential fatty acids in form of alpha linolenic acid (ALA), a precursor to Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Walnuts are also richer than other nuts in anti-oxidants which are useful in circulatory and cardiovascular systems. Walnuts are helpful in the treatment of type 2 diabetes, and act as a cancer chemo-preventive agent, due to the high phenolic and ellagic content [3;4].In the light of the nutritional values of Africa Walnut, the flour derived from the nut could serve as protein supplement in infant complementary food formulation. Maize is rich in carbohydrate but has lysine, threonine and tryptophan as limiting amino acids. Maize oil has a high content of polyunsaturated fatty acids mainly linoleic acid (24 %) and thereby a high n-6/n-3 fatty acid ratio. Developing countries depend on cereals like maize for supply of both energy and proteins [5]. In Nigeria, fermentation has been shown to be an effective and convenient process for improving the nutritional value and reducing the bulk and viscosity of maize food where the fermented product is made into gruel or pap called 'ogi' which is often fed to infants [6]. There is a growing interest in the formulation of food products, using fermented maize with other legumes as a way of improving nutritional quality [7]. There is therefore, need for more information on the biochemical changes associated with the processes of co- fermenting of maize with walnut on which beneficial infant complementary food can be obtained. This study was therefore designed to investigate the effect of co-fermentation on chemical, functional and nutritional properties

of co-fermented maize /walnut flour.

2. Materials and Methods

2.1. Sample Preparation

Mature nuts of African Walnut were purchased from Oja Oba market of Ekiti- State, Nigeria. The shells were removed and seeds were weighed.

2.2. Processing

Maize grains were sorted and winnowed. Unshelled walnuts were sorted and washed. They were divided into two; a section was soaked raw while the shelled walnuts of the other section were cooked before co-fermenting 300g cooked walnut with 700g raw maize w/w (CWM) for 72h at 30^{O}C. Co-fermented unshelled raw walnut with raw maize (RWM), served as control. Each mixture was soaked and left to ferment spontaneously for 72h each product was wet- milled, sieved and oven dried at 60^{o}C for 24h. Then ground into flourand passed through a 45-m mesh size sieve and stored in sealed polythene pending analyses.

2.3. pH Determination

The pH was determined at every 24h of fermentation time by using pH meter (Model 3505, England).

Solubility was determined by the method of [9].

2.4. Proximate Composition Determination

Crude Protein was determined according to micro- Kjeldahl Procedure according to method described by [8].Fat, carbohydrate, ash and moisture protein contents were determined using methods described by [8].Energy values were estimated and expressed in Kilo calories/100 kg and carbohydrate by difference

2.5. Minerals Content

The following minerals: Fe, Cu, Zn, Mg, K, Mn and Na, Ca were determined by atomic absorption spectrophotometer (Hitachi Z 6100, Tokyo, Japan), using the method described by [8].One gram of sample was weighed into each crucible and transferred into Muffle Furnace pre-set at 530oC for 120 minutes. The crucibles were cooled and weighed; this was done in triplicates. The percentage ash was calculated. Phosphorus was determined using spectrophotometer at 430nm[8].

2.6. Determination of Amino Acids

The amino acid profile in the cocoa samples was determined using methods described by[8] The samples were dried to constant weight. The mass was subsequently defatted, hydrolysed, filtered to remove the humins and evaporated to dryness at 400C under vacuum in a rotary evaporator. Each residue was dissolved with 5ml of acetate buffer (pH 2.0) and stored in a plastic specimen bottle kept inside the deep freezer pending subsequent analysis. The Technicon Sequential Multisample Amino Acid Analyser (TSM), Technicon Instruments Corporation, New York was used for the analysis. The principle is based on ion-exchange chromatography (IEC)]. The equipment is designed to separate free acidic, neutral and basic acids of the hydrolysate. The amount loaded for each sample was 5-10µl and about 76 minutes elapsed for each analysis. The column flow rate was 0.50ml/min at 60oC with reproducibility consistent within 3%. The net height of each peak produced by the chart record of the TSM was measured and calculated for the amino acid it was representing. All chemicals used were of analytical grade.

2.7. Antinutritional Factors Determination

Estimation of total phenols: Standard method [10] in which total phenol is which is expressed as chlorogenic acid equivalent, was used for the estimation of total phenols. Antinutritional factors were determined by the chemical method described by [10]. The titration method was used to determine the oxalate content according to[11]. Phytate content was determined using the method described by [10].

2.8. Functional Properties Determination

Water absorption capacity (WAC) was determined as described by[12], in which 1gram of sample and 10 ml distilled water were added to 25 ml centrifuge tube, suspension was stirred on magnetic stirrer and allowed to stand for 30minutes at 25^{o}C. The volume of supernatant water on the suspension was measured.WAC was calculated as ml water.

2.9. Fatty Acid Methyl Esther Analysis

About 50mg of the extracted fat content of the sample was saponified (esterified) for five (5) minutes at 95^{0}c with 3.4 ml of the 0.5M KOH in dry methanol. The mixture neutralized by using 0.7M HCL. 3ml of the 14% boron triflouride in methanol was added. The mixture was heated for 5 minutes at the temperature of 90^{0}C to achieve complete methylation process. The Fatty Acid Methyl Esters were thrice extracted from the mixture with redistilled n-hexane. The content was concentrated to 1ml for gas chromatography analysis and 1 u1 was injected into the injection port of GC. The gas chromatography conditions of analysis are as attached in the analysis print out as described by [13].

2.9.1. Sterol Analysis

Sterol analysis was carried out by following the modified official method of [9]. The aliquot of the extracted oil was added to the screw-capped test tubes. The sample was saponified at 95^{0}C for 30 minutes, by using 3 ml of 10% KOH in ethanol, to which 0.20ml of benzene had been added o ensure miscibility. Deionised water (3ml) was added and 2ml of hexane was used in extracting the non-saponifiable materials (sterol, etc). Three extractions, each with 2ml of hexane, were carried out for 1 hour, 30 minutes and 30 minutes respectively, to achieve complete extraction of the sterols. The hexane was concentrated to 1 ml in the vial for gas chromatography analysis and 1 µl was injected into the injection port of GC. The gas chromatography conditions of

analysis are as attached in the analysis print out as described by [13].

2.9.2. Phospholipids Analysis

Modified method of [10] was employed in the analysis of the extracted oil phospholipids content determination. About 0.01g of the extracted fat was added to the test tubes. To ensure completes dryness of the oil for phospholipids analysis, the solvent was completely removed by passing the stream of the nitrogen gas on the oil. 0.40ml of chloroform was added to the content of the tube and it was followed by the temperature of 100^0C in a water bath for about 1 minute 20 seconds. The content was allowed to cool to the laboratory temperature and 5 ml of the hexane was added and the tube with its content shook gently several times. The solvent and the aqueous layers were allowed to be separated. The hexane layer was recovered and allowed to be concentrated to 1.0 ml for gas chromatography analysis using pulse flame photometric detector. The gas chromatography conditions of analysis are attached in the analysis print out as described by [13].

2.9.3. Consistency

Empirical measurement for consistency of each gruel was determined using Botswick Consistometer.(Laomat CEDEX) as described by [14]. Four to 18% (on dry matter basis weight *'ogi'* gruel cake: water) were mixed and stirred constantly on hot plate at 80 ^{0}C. At the first appearance of first appearance of first bubble, cooking was timed to 5 minutes after which were cooled to 45 ^{0}C. The first compartment was filled with 100ml of each co-fermented cereal/cowpea gruel. At time =0, the trigger was pressed to release the gate of the first compartment to allow gruel flow by gravity to the second compartment. The distance the gruel covered in 30seconds was measured in millimeters as the Bostwick Consistency reading (i.e flow in mm/30seconds). Dry matter of gruel was determined according to[8].

2.9.4. Microbiological Evaluations

Steep liquor (10ml portions) was aseptically removed every 24 hours over a 72hour period and serially diluted; using different sterile 1.0ml pipette 0.1ml of 10-1 to 10-8 dilutions. Ten serial dilutions of were done for each sample. The various samples were plated out by mixing with MRS agar medium in McCartney bottles and poured aseptically into sterile Petri dishes for lactic acid bacteria isolation. After solidifying, the Petri dishes were incubated in anaerobic jars using BTL Gas Park (hydrogen and carbon dioxide generators). The colonial morphology and cellular characteristic for the various colonies obtained were studied. The total viable counts were made on plate count agar (PCA). Yeasts and mould counts were determined on malt extract agar (MEA) containing 100ml – streptomycin and MacConkey agar for Enterobacteriacae. Plates were incubated at 30^{o}C for 24h. For the PCA 30^0C, for 48h for MRS, 37^0C for 24h for MacConkey and 4-5 days for the MEA medium. After the incubation period the plates were observed for bacterial growth and the colonies were randomly selected. Cultures were sub-cultured and repeated streaking was done on sterile MRS plates to obtain pure culture.

Characterization of Isolates

This was done by employing macroscopic, microscopic, and biochemical tests. Identification: Pure growth was heavily inoculated in the modified MRS broth containing the following sugar: glucose (plus Durham's tube) lactose, sucrose, salicin, mannitol, sorbose and xylose in MRS broth containing 2% glucose and in which the ammonium citrate has been replaced by 0.3% arginine for arginine hydrolysis, and in MRS broth containing 4% sodium chloride. These were incubated at 28^0C-30^0C for 3-4 days. Also, inoculated tubes of MRS broth were incubated at 15^0C and 45^0C. Identification was done as described by [15]

3. Statistical Analysis

All analyses were carried out in duplicates and the data collected were analyzed using Plot IT software [16]. Data were subjected to analysis of variance (ANOVA) and Turkey's test was used for comparison of means. Significance was accepted at $p = 0.05$.

4. Results

Fig. 1 shows that the pH of RWM dropped more drastically from 6 at 0hr to 4.0 at 12hr and rose to 6.0 from 24h to 72h. That of CWM was 6.0 at 0hr, 5.2 at 24h and 5.5 from 24hr to 72hr.

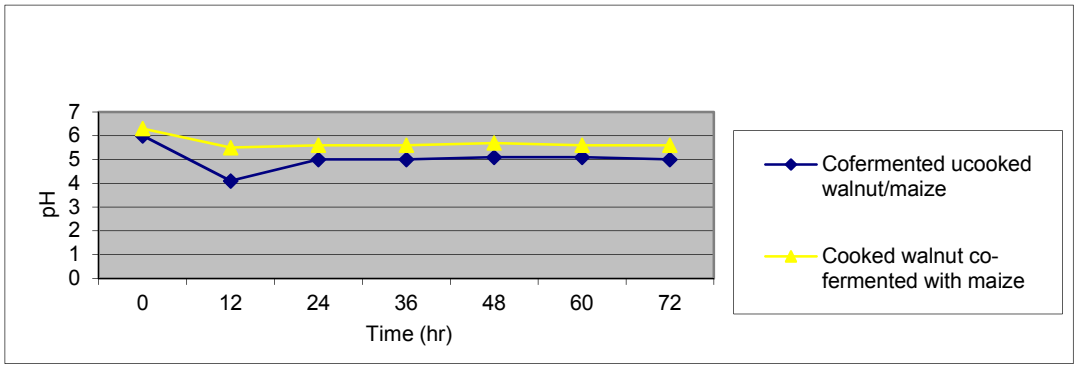

Fig. 1. *pH changes during co-fermentation of RWM and CWM.*

In chemical proximate composition, investigation into the proximate constituents of the samples as shown in Table 1 revealed that the ash and moisture content were 67.62% and 14% higher in CWM than RWM with 3.28 and 16.98% respectively.

However, the protein contents of the samples were also observed to be 3% higher in RWM than CWM. The moisture, crude protein and crude fat contents were comparable in both samples. The carbohydrate contents were recorded as 62.84% and 50.57% in RWM and CWM respectively.

Table 1. *The chemical proximate composition of RWM and CWM.*

Sample	Ash	Moisture	Crude protein	Fat	Crude fiber	Carbohydrate
RWM	3.28±0.057^{b}	4.33±0.011c	20.51±0.011^{e}	7.58±0.011^{d}	0.93±0.057^{a}	62.84±0.01^{f}
CWM	16.98±0.010^{d}	5.88±0.057^{b}	19.25±0.057^{e}	6.76±0.051^{c}	0.64±0.01^{a}	50.57±0.057^{f}

In Table 2, the functional properties, RWM had significantly (p0>0.05) higher Water absorption capacity,(WAC), oil absorption capacity (OAC) emulsion capacity ,(EC) , emulsion stability (ES) and least gelation (LG) compared to CWM. However CWM had foaming capacity (FC) which was observed to be 20% higher in RWM. The water absorption capacity was higher RWM with about 260% and the least gel efficiency was also found to be 14% higher in RWM than CWM. Nearly all the characteristics examined were observed to be higher in RWM than CWM.

Table 2. *Functional properties of RWM and CWM.*

Sample	WAC (%)	OAC (%)	FC (%)	FS (%)	EC (%)	ES (%)	LG (%)
RWM	260.25±0.0^{g}	158.5±0^{f}	4.16±0.02^{b}	2±0^{a}	51.69±0.58^{d}	60.23±0.058^{e}	8±0^{c}
CWM	85.63±0.05^{e}	95.54±0^{f}	6+0.0^{b}	2±0^{a}	40.52±0.01^{c}	45.31±0.01^{d}	6±0^{b}

Key: WAC=Water absorption capacity, OAC= Oil absorption capacity, FC= Foaming capacity, FS= Foaming stability, EC= Emulsion capacity, ES= Emulsion stability, LG= Least gelation .

In Fig.2, There was a significant increase in protein solubility of RWM from pH 1-11 but lower at pH12 than CWM. The protein of the RWM more soluble than that of CWM.

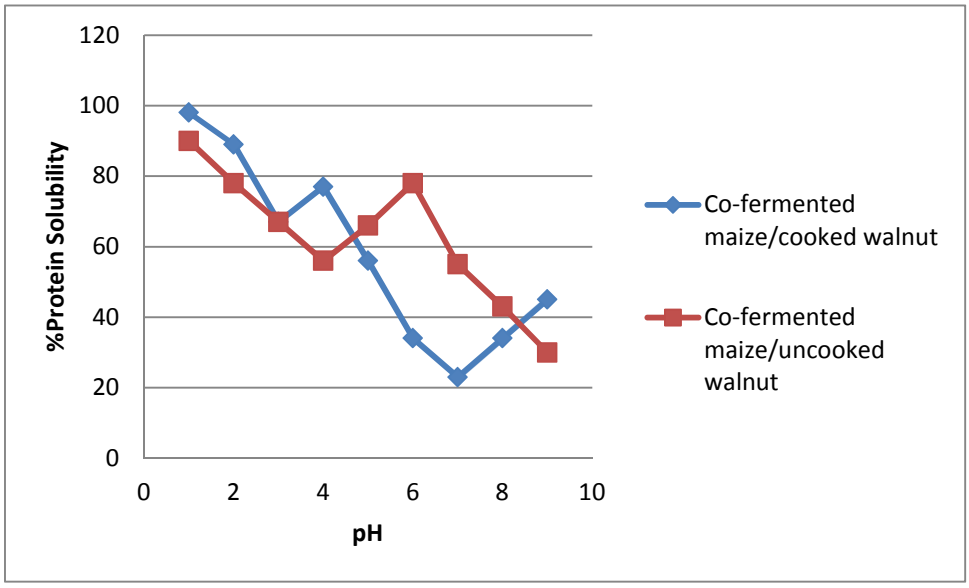

Fig. 2. *Effect of pH on protein solubility of RWM and CWM.*

Fig. 3 showed the amino acid profile. The total amino acids were 92% and 90.4% in RWM and CWM respectively. The levels of Histidine and Lysine in the two samples were observed to be 2.5 g/100gcp and 3.0 g/100gcp in CWM which were considered to be too low while Arginine and Leucine were also observed to be 9.8g/100gcp and 8.2 g/100gcp respectively. Mixture with RWM had higher values in: Glycine Serine, proline, leucine, aspartic acid and arginine while CWM had higher values in cysteine and valine than RWM. Both sample had comparable values in threonine, isoleucine, lysine, methionine, glutamate, phenylalanine, histidine and tyrosine. Total aminopacids (TAA) was higher in RWM with92.33% than CWM of 90.44% while total essential amino acids (TEAA) was higher in CWM than RWM.

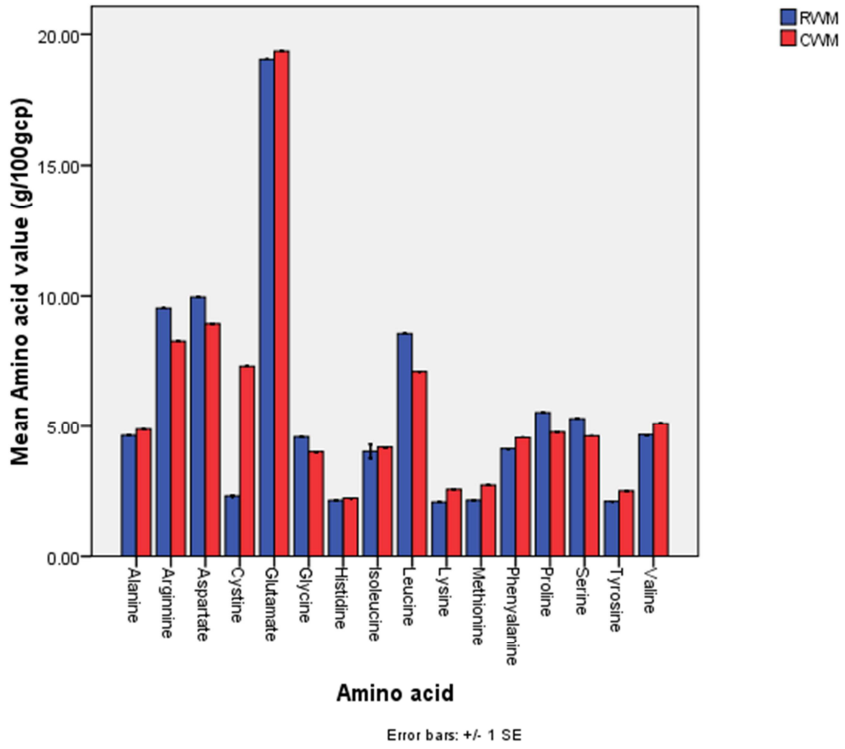

Fig. 3. *Amino acids profile (g/100gcp) of RWM and CWM.*

In Fig. 4, Cholesterol value was comparable in both samples. Cholestanol was higher in RWM than in CWM. Ergosterol and Savanesterol had comparable values in both samples. The values of Campesterol and stigmasterol were significantly higher(p0>0.5) in CWM than RWM. The total sterols was higher in sample with CWM (242.910 than that with RWM (136.9). Sitosterol was high in the two samples examined with values ranging from 110-185mg/100g in CWM and RWM respectively. The level of cholesterol was observed to be low in the two samples, lesser than 10mg/100g

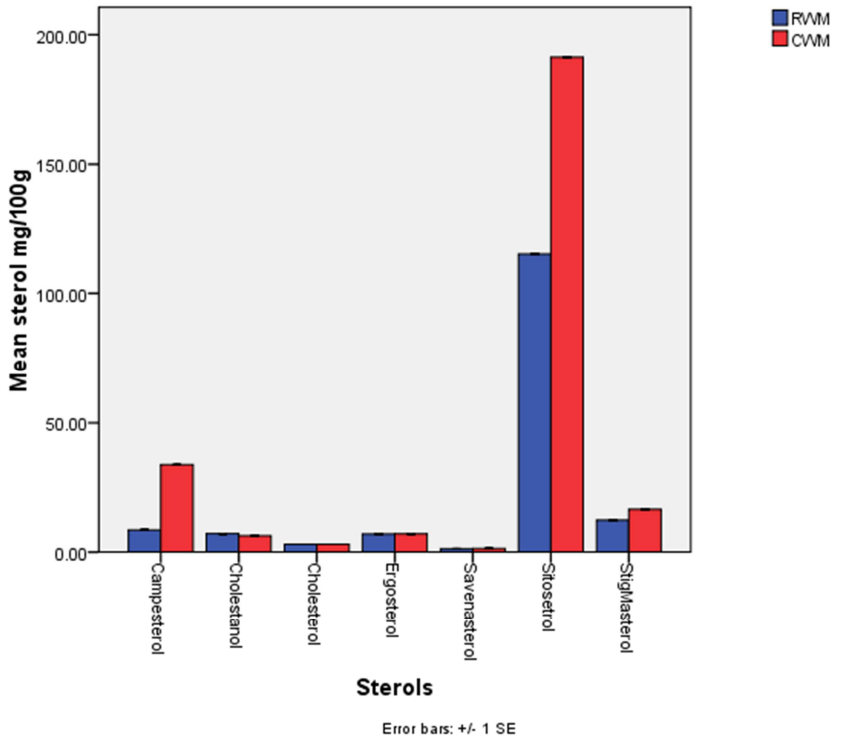

Fig. 4. *Steroids profile (%) of RWM and CWM.*

In Fig. 5 Both products are lacking in Caprylic (C8:0), Lauric acid (C12:0), Margaric acid (C17:0), arachidonic acid

(AA, C20:4n-6),Behehenic acid (C22:0(, Erucic (C22:1) and Lignoceric (C24:0). However, palmitic ,palmitoleic and linoleic acids were significantly higher (p0.>0.5) in CWM than RWM. Stearic, mistric and linoleic acids were higher in RWM than mixture than in CWM. The values of Oleic acids were comparable in both samples.

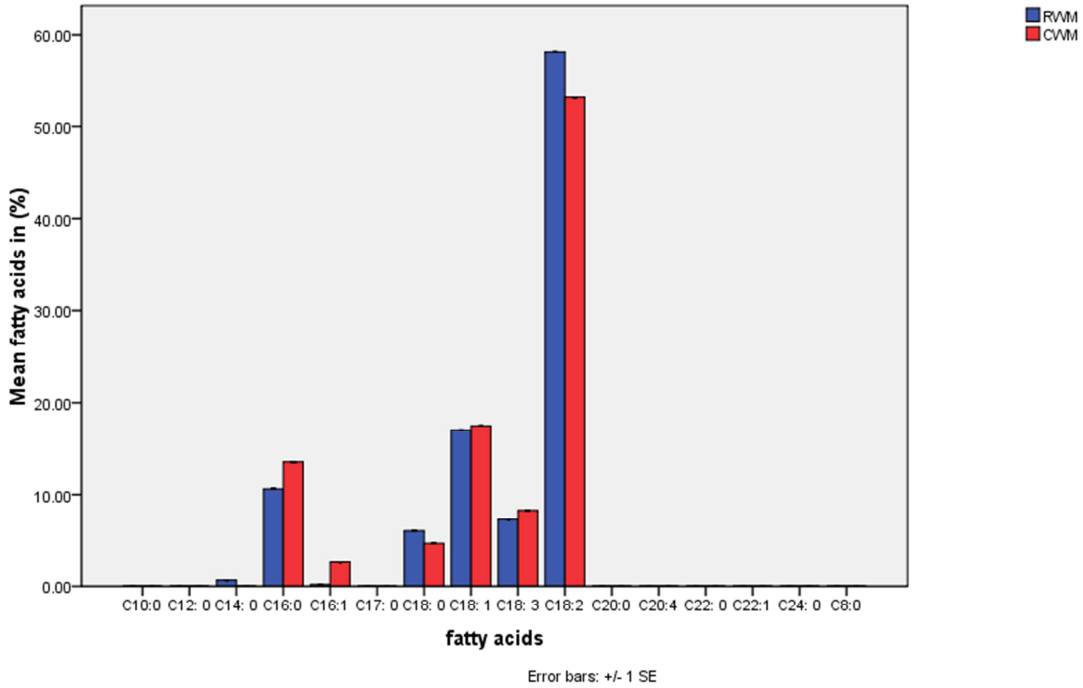

Fig. 5. *Fatty acids composition (Norm %) of RWM and CWM.*

Keys: Caprylic acid methyl ester (C8:0), Capric acid methyl ester (C10:0), Lauric acid methyl ester (C12: 0), Myristic acid methyl ester (C14: 0), Palmitic acid methyl ester (C16:0), Palmitoleic acid methyl ester (C16:1), Margaric acid methyl ester (C17: 0), Stearic acid methyl ester (C18: 0), Oleic acid methyl ester (C18: 1), Linoleic acid methyl ester (C18:2), Linolenic acid methyl ester (C18: 3), Arachidic acid methyl ester (C20 : 0), Arachindonic acid methyl ester (C20:4), Behenic acid methyl ester (C22: 0), Erucic acid methyl ester (C22:1) and Lignoceric acid methyl ester (C24: 0).

In Fig. 6, CWM had higher values in the following: phosphatdylethanolamine and phosphatidyllinsitol with the lipid level of the sample ranges from 7-330mg/100g in Phosphatidylinsitol and Lyso Phosphatidylcholine respectively in CWM while RWM lipid level also ranges from 10-220mg/100g in Phosphatidylinsitol and Phosphatidylserine respectively. However phosphatidylcholine and lysophosphatidylcholine were higher in RWM. The total phospholipids were higher in CWM (950.07804) than in RWM.

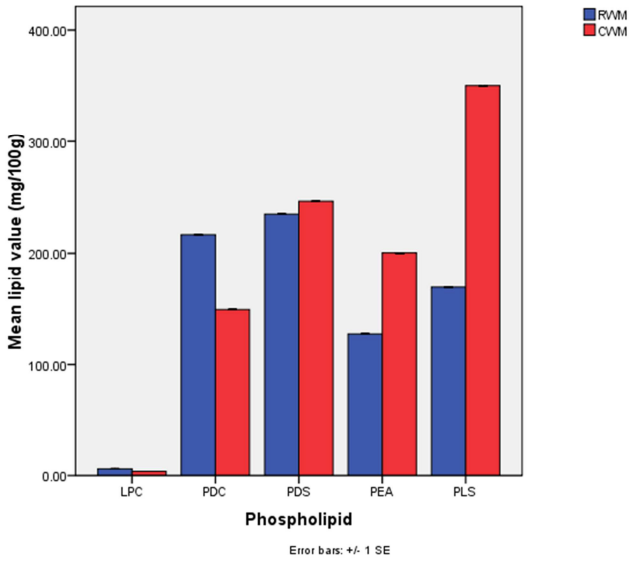

Fig. 6. *Phospholipids profile (%) of RWM and CWM.*

Key: Phosphatidylethanolamine (PEA), Phosphatidylcholine (PDC), Phosphatidylserine (PLS), LysoPhosphatidylcholine (LPC) and Phosphatidylinsitol (PDS).

In Fig 7. At 4% flour concentrations the consistencies of gruels of both samples were too watery for the Bostwick flow to be measured. At 6% and 8% CWM had higher Bostwick flow (hjgher consistency) than RWM. The thickness (lower consistency) was more pronounced at 6% flour concentration RWM with 3mm/30sec and 8mm/30sec for CWM. At 10% both samples consistencies were too thick and could not be measured.

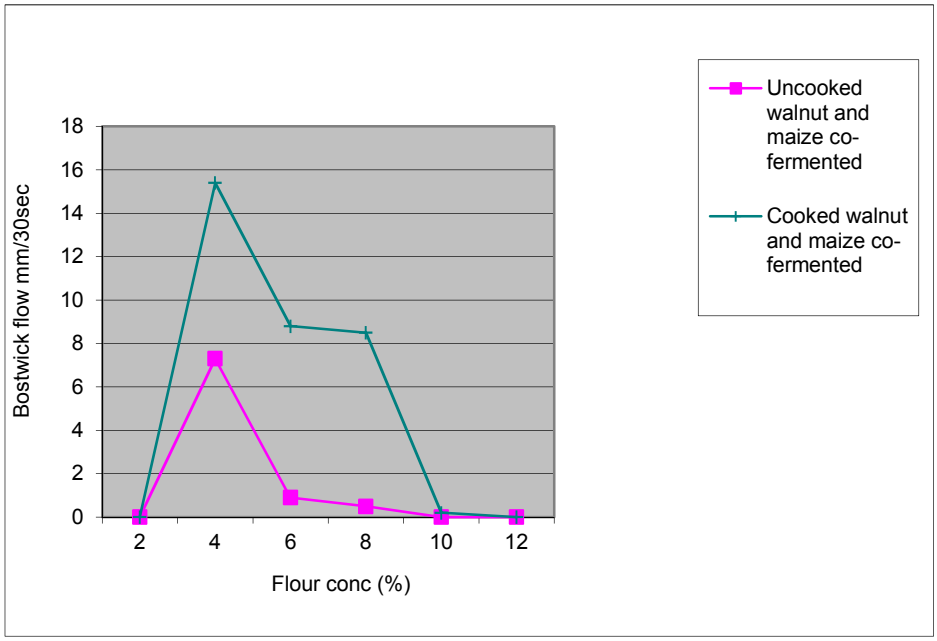

Fig. 7. *Effect of cooking and fermentation on the consistency of co-fermented walnut and maize.*

Table 3. *Minerals content of RWM and CWM (mg/100g).*

Minerals	RWM	CWM
Na	15.65 ± 0.01^{e}	12.69 ± 0.02^{e}
K	32.29 ± 0.01^{f}	20.48 ± 0.0^{f}
Ca	81.58 ± 0.05^{h}	65.54 ± 0.05^{i}
Mg	163.17 ± 0.005^{i}	108.16 ± 0.005^{j}
Zn	70.34 ± 0.05^{g}	58.36 ± 0.01^{g}
Fe	2.34 ± 0.06^{c}	1.22 ± 0.05^{d}
Mn	0.33 ± 0.006^{b}	0.55 ± 0^{c}
Cu	0.01 ± 0^{a}	0.03 ± 0^{b}
P	8.34 ± 0.057^{c}	3.08 ± 0.017^{d}
Pb	0 ± 0^{a}	0.06 ± 0.06^{a}

Table 3 shows that RWM had significantly (p0>0.05) higher values of most of the minerals examined except in manganese and copper in which CWM had higher values. The value of lead was comparable in both samples. However, the quantity of Copper and lead observed in the two samples were too low.

In Table 4.Phytate level was high in the two samples with an approximately value of 11.5 and 13.9 for RWM and CWM respectively. The phenol level was observed to be insignificant both samples with an average value of 0.19mg/100g

Table 4. *Antinutritional factors of RWM and CWM.*

Sample	Tannin mg/100g	Phytate mg/g	Phytin - phosphorus	Oxalate mg/g	Saponin %	Alkaloids %	Flavonoids %	Total Phenol mg/100g
RWM	0.24 ± 0.0^{b}	11.53 ± 0.005^{h}	3.25 ± 0.005^{g}	0.99 ± 0^{d}	2.63 ± 0^{f}	1.31 ± 0^{e}	0.73 ± 0.11^{c}	0.17 ± 0^{a}
CWM	0.3 ± 0^{a}	13.9 ± 0.17^{e}	3.94 ± 0^{d}	0.72 ± 0^{b}	1.34 ± 0^{c}	0.51 ± 0.44^{a}	0.52 ± 0^{a}	0.21 ± 0.06^{a}

B .pumilus, L. delbrueckii, L. mesenteroide and S. cerevisiae were observed to be the predominant culture in the samples examined with RWM containing all the cultures while the CWM samples was found to contain *L. delbrueckii, L. mesenteroide and S. cerevisiae only.*

Table 5. *Micro flora of RWM and CWM.*

Organisms/ Food	*B. pumilus*	*L. delbrueckii*	*L. mesenteroide*	*S.cerevisiae*
RW/M	√	√	√	√
CW/M	X	√	√	√

5. Discussion

In Fig. 1.The observed reduction in pH of CWM and RWM might be due to inactivation of some microbes or microbial metabolic activity by heat during the cooking of the walnut. The sharp drop of pH at 12h might be due to the activity of lactic acid bacteria. The later increase in pH might be due to amine production from the protein component of walnut which can make the fermenting medium to become alkaline in nature.

The proximate chemical composition in (Table 1) of RWM and CWM revealed that the ash and moisture content were

higher in CWM than RWM and because the ash content is an indication of mineral elements present in the flour, the reduction in the ash content of RWM may arise as a result of usage of minerals by increased metabolic activities of inherent microorganisms (Table 1) [17] reported that walnuts contain high-quality protein that can substitute for meat. However, the increase in RWM (12.74g/100g) compared favorably with the value of 11.4% reported for canephor nut flour [18], and higher than 8.57% reported for jackfruit seed flour [19]; but significantly ($p>0.05$) lower than 22.8% value reported for raw walnut by [20].

Table 1. The crude protein is significantly higher in both samples than 6-11g/100g required for 6-23months based on average, medium and high breast milk intake. This indicates that a reduction in walnut/maize ratio might give adequate protein content of the mixture to be adequate for these age ranges. A decrease in the carbohydrate content of CWM compared to RWM may be due to an increased in the process of breaking down of starch to sugar by increased metabolic activities of microorganisms containing enzyme amylase; this can enhance the use of CWM in food formulation exploration in agreement with[21].

Investigation showed that some of the functional characteristics of RWM were higher than CWM (Table 2). The water absorption capacity was 260.25% and 85.6% for CW/M and RW/M respectively; these values are lower than water absorption capacity of 340% reported for raw conophor flour by [18], while that of RWM was higher than 170% reported for African yam bean [21]. This result suggests that any of the co-fermented walnut/maize flour may find application in the production of some baked products. The lower oil absorption capacity of CW/M as compared to RW/M might be due to low hydrophobic proteins which show superior binding of lipids[22]; indicating that any of the products in this study may be a lower flavor retainer in agreement with [23].

Among the functional properties of proteins, solubility is probably the most critical function. Protein solubility characteristics are influenced by factors such as origin, processing conditions, pH, and the presence of other ingredients [24]. In Fig. 2.The significant increase in protein solubility of RWM from pH 1-11 than CWM might be due to the higher proteolytic activity during co-fermentation of RWM which might lead to an increase in the protein solubility resulting from hydrolysis of the storage proteins.

Amino acid score should not be less than 70% of that of casein [25]. In Fig 3, both samples had comparable values of Arginine, met., phenylala, isoleucine, leucine and histidine. Histidine and lysine which are essential amino acids for child's growth while both values were lower than 6.3g/100g of reference egg protein and RDA value of (6.6g/100g) while arginine is higher than RDA value. Cystine, which acts as a sparring partner in methionine synthesis has positive effect on zinc absorption thus making consumption of CWM mixture desirable. Leucine constituted the highest single essential amino acid (EAA) in all the samples this finding agrees with the finding that leucine content had highest values compared to other amino acids in raw, roasted and cooked groundnut (Arachishypogaea) seeds [26] Methionine + cysteine are the limiting amino acids in legumes CW/M had significantly ($p0>0.5$) higher values of Met+Cyst. High values glutamate in both products are by-products of microbial fermentation which might affect the flavour of the products.

The total amino acids (figure 3) were 92% and 90.4% in RWM and CWM respectively. The ArAA of RW/M 122.56 and CW/M 135.65mg/g cp was close to range suggested for ideal infant protein (68-118mg/ g cp). The ArAA are precursors of epinephrine and thyroxin. The percentage ratios of TEAA to TAA in the samples were 26.23% RWM and 24.73% in CW/M respectively these values are lower to 39% considered to be adequate for ideal protein food for infants. The percentage ratios of total essential amino acids (TEAA) to total amino acids (TAA) in the samples were 26.23% and 24.73% in RW/M and CW/M respectively these values are lower to 39% considered to be adequate for ideal protein food for infants.

In Fig 4.Cholesterol levels are very significant in the body because they moderate the fluidity of membranes. Cholesterol prevents the crystallization of fatty acyl chains by fitting itself between them. Cholesterol is a major component of nerve membranes and it is needed for cell growth. Dietary cholesterol also affects the maturation of high-density lipoprotein as reported by [27].

A profile of phytosterols (Plant sterols) of 30-65% -sitosterol, 10-40% campesterol, 6-30% stigmasterol and a total of 5% other phytosterols, based on total sterol content (w/w), was reported to be acceptable[27] . The major phytosterols are sitosterol, campesterol, stigmasterol and savenasterol CWM had higher values of these phytosterols than RWM. Phytosterols are lypophilic naturally occurring compounds that are structurally related to cholesterol. Phytosterols are not endogenously synthesized in humans and are derived solely from diet. It has been recently reported that phytosterols have been reported to significantly reduce cholesterol absorption in humans and blood cholesterol to a small degree [28]. The consumption of high doses of plant sterols significantly reduces the blood levels of carotenoids and, to a lesser extent, of other essential fat-soluble nutrients. In Cholestanol, RWM had a slightly higher level than CWM. This shows that cooking reduced cholestanol content. The CWM also had higher total sterols than RWM.

In Fig. 5, Linoleic acid is the most enhanced fatty acid in both samples in this study. RWM had a higher content of this acid than CW/M. However CW/M had a higher content of linolenic acid. Walnuts are rich in anti-inflammatory Omega-3 essential fatty acids in form of alpha linolenic acid (ALA), a precursor to Eicosapentaenoic acid (EPA) and decosahexaenoic acid (DHA). EPA and DHA are efficiently used for tissue incorporation and specific body function.

The fat content by implication the energy density, is low in traditionally plant based diet therefore, increasing the content of fat of such diet might increase nutrient density. In this study, fatty acids is reported in (Norm %) as shown in Fig. 5, [29] reported that Myristic acid on serum cholesterol and lipoprotein concentrations should not exceed 20% of the total fat contents for it has a greater effect in raising cholesterol levels than did palmitic acid. In this study, RWM had a higher value of palmitic acid which could raise cholesterol than CWM.

Erucic acid is reported to have no known nutritional benefits therefore its value of 0.0000 in all the samples were therefore desirable. Cooking enhanced palmitic acid as seen in the value of CWM than RWM. In Palmitoleic acid value CWM had significantly (p0.>05) higher than RWM, thus cooking increased palmitoleic fatty acid. Stearic acid has been reported to have neutral effects on blood cholesterol and lipoproteins but with possibility of thrombogenicity RWM had higher stearic acid content than CWM.

In this work, LA value of RWM was slightly higher than that of CWM. Small amounts of LA and ALA must be provided in the diet [30]. Linoleic acid deficiency may develop as secondary conditions like protein energy malnutrition and fat malabsorption. [30], reported that when ratios of AL: ALA are low, there would be modest improvement in some n-3 LCPUFA levels. The presence of sterols and lipids (figure 4 and 6) revealed that the food can present virtually all nutrients essential for optimal growth for infant. Cholesterol plays an important role in the absorption of fatty acids from the intestine and in their consequent transportation in the blood or haemolymph.

Phospholipids as shown in Fig.6.CWM had higher values in the following: phosphatdylethanolamine and phosphatidyllinsitol while phosphatidylcholine, lysophosphatidylcholine were higher in RWM. The total phospholipids were higher in CWM (950.07804) than in RWM. Phospholipids results as (mg/100g).CWM had significantly (P 0.>05) higher value of Phosphatidylethanolamine. According to[2] Choline in food exists in either a free or esterified form that is choline is bound within another compound, such as lipid-soluble phosphatidylcholine which bypasses the liver once absorbed. The cation appears in the head groups of phosphatidylcholine is abundant in cell membranes. Choline deficiency may play a role in liver disease, atherosclerosis, and possibly neurological disorders[2].Choline is the precursor molecule for the neurotransmitter acetylcholine, which is involved in many functions including memory and muscle control. RWM had double the content of phosphatidylcholine than CWM, while the value of lysophosphatidylcholine was higher in CWM than in RWM. However, CWM had samples had significantly (p0>0.5) higher value of Phosphatidylinsitol than RWM. This shows that cooking might have enhanced the availability of Phosphatidylinsitol. All membranes whether they are at the cell surface or form part of an intracellular organelle such as a mitochondrion are composed of phospholipid bilayers as reported by [32]. Phosphatidylserineand phosphatidylethanolamine are the major ones containing phospholipid in neural membrane of the cerebral grey matter. Certain phospholipid solubilizes lipophilic compounds and acting as a source of long chain polyunsaturated fatty acids. Phospholipids is also described by [32] as indispensable components of cholesterol in lipoprotein synthesis and metabolism.

The consistency of gruels prepared might be due to interaction of cereal starches with protein which can influence gelatinization and retrogradation of starches. There is a strong negative relationship between flour dry matter and Bostwick flow of gruel R value of -0.83 to -0.97. The higher the consistency values, the less the dry matter values as seen in Fig.7. This is in agreement with [33]. It was noted that as gruel cools, the gruel consistency decreased irrespective of the concentration. The most ideal temperature of gruel for an infant's mouth is 45^{o}C.

The lower value of RW/M might be due to greater thixotropic behavior suspensions of swollen starch granules, present more in RW/M in agreement with [20]. The higher consistency values of CW/M means reduced viscosity and by implication more energy and nutrient densities. The consistency of gruels prepared (figure 7) might be due to interaction of cereal starches with protein which can influence gelatinization and retro-gradation of starches. There is a strong negative relationship between flour dry matter and Bostwick flow of gruel R value of -0.83 to -0.97. The higher the consistency values the lesser the dry matter values. This is in agreement with [33]. It was noted that as gruel cools, the gruel consistency decreased irrespective of the concentration. The most ideal temperature of gruel for an infant's mouth is 45^{o}C. The lower value of RW/M might be due to greater thixotropic behavior suspensions of swollen starch granules [20], which might be more present in RWM. The higher consistency values of CWM means reduced viscosity and by implication more energy and nutrient densities.

Table 3.shows the mineral contents of both samples. The Magnesium deficiency interferes with protein utilization and increases the risk of developing potassium depletion. In Table 4, the value of magnesium was higher in RWM than CWM, the value in both samples meet that of 6-23mo (80-120mg/100g). Magnesium complements calcium in the body for bone health, heart muscle support; it is enzyme co-factor in red blood cell formation and support energy levels [2].Copper support collagen formation for joints and bones, red blood cell formation and support energy levels. The values of calcium found in the flours, 81.58mg/100g and 65.54 mg/100 g are adequate for bone and teeth development in infants [34].

According to [35], the daily ration of a fortified complementary food should contain 4–5 mg of zinc. The value in this work also exceed the RDA of 3 mg/100g making both products desirable in terms of zinc content and is justified because of the lower bioavailability of Zinc in cereal-based diets typical in developing countries. RW/M was richer in zinc and relatively lower in anti nutritional constituents (Table 4) therefore could be of nutritional advantage in enhancing iron absorption. Phosphorus is an essential component of hydroxyapatite, the main structural bone mineral, deficiency of phosphorus is common in malnourished children and severe hypophosphatemia is associated with increased mortality in kwashiorkor [36].RW/M had higher P, Na, K, Ca, Mg, Zn and Fe than CWM. Thus adding RW/M to infant diet might boost intake of these minerals.

In Table 4.Fermentation process can reduce phytate by almost 90%, due to activation of endogeneous phytase in cereal and legume being prosessed. The pH of RW/M and CW/M by 72h 5.0 and 5.5 respectively (fig.1) were optimal for phytases activities[37].

As reported by [38], phytic acid is a strong inhibitor of iron absorption in both infants and adults, inhibits pepsin, amylase

and trypsin necessary for hydrolysis of proteins and starch in the small intestine. [38] also reported improvements in absorption of iron, zinc, and calcium in cereal-based foods prepared with a reduced phytate content. RWM had higher phytate and phytin content.

Phenols and tannins complex with iron and zinc and inhibit enzymatic activities of pectinase, amylase, lipase, proteolytic enzymes, β-galactosidase and those microbial enzymesinvolved in fermentation of cereal grains like maize while tannins in legumes like walnut reduce ionisable iron absorption by acting as a natural iron chelating agent. Flavonoids adds astringent taste to food to form complex formation with metals. According to [29], the complex formation immobilizes nutrients for digestion and absorption and thereby reduces the nutritional value of the food. Soaking in water prior to cooking has a significant effect on reducing the tannin content (37.5-77.0%), provided the water used for soaking is discarded .

Both samples had comparable tannin values. This was also obvious in their comparable proline content. Proline bind more tannin than other amino acids. Lower value of saponin and alkaloid (both of which were reduced by 50%) in CW/M may be due to the effect of cooking of walnut in this mixture and this might make the product less astringent and bitter in taste. While the higher value of saponin in RW/M might aid in the absorption of important minerals and cause lesser irritating effect on digestive system but might also have negative effects on the permeability of the small intestinal mucosa and have been found to impair active nutrient transport in animal and cell models[29] . The higher content of saponin might also make RW/M to be beneficial in the control of blood cholesterol levels, promotion of bone health, building up of the immune system and helping in heamolysis.

In Table5.RWM and CWM had *L. delbrueckii,L. mesenteroide and S. cerevisiae* inherent microorganisms however in addition, RWM had *B. pumilus.* However, the increase in the microbial load in the form of single cell proteins could also be responsible for increase in protein content of RWM [29].

6. Conclusion

Walnut flour is a rich source of oil and contains moderate amount of protein. Co- fermentation of maize with walnut can also be used to improve the nutritional content of this seed. Investigation of food processing methods like fermentation can reduce the risk of microbial contamination in the food after preparation in the home.

Recommendation

Further work needs to be carried out on the molecular characteristic of microorganisms associated with the fermentation especially as fermentation hours progresses and to develop starter culture to ferment this seed and probably see changes on the resultant flour for usage in food formulation.

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