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Physical and Microbiological Evaluation of Food Formulations From Malted and Fermented Maize (*Zea mays* L.) Fortified with Defatted Sesame (*Sesamun indicum* L.) Flour

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Abstract: The effects of malting and fermentation on some physical properties and microbiological composition and safety of maize/sesame food formulations were investigated. Malted and fermented maize flours were blended with defatted sesame flour, by material balancing, to give four food formulations (Un-malted maize + defatted sesame (UMS), malted maize + defatted sesame (MMS), un-malted fermented maize + defatted sesame (UFMS) and malted fermented maize + defatted sesame (MFMS)), which all contained 16 g protein and 9 g fat/100 g food. Viscosity, functional properties and microbiological composition of flours and gruels from the food formulations were evaluated using standard methods of analysis. Malting and fermentation significantly ($p<0.05$) decreased gruel viscosities from 318.00 to 210.70 cP, packed bulk density from 1.08 to 0.97 g/mL and swelling index from 4.43 to 2.93 mL/g; while increasing water absorption capacity from 3.30 to 4.07 g/g and reconstitution index from 5.02 to 6.13 mL/g in UMS and MFMS samples respectively. Total viable counts, yeasts and moulds all increased significantly ($p<0.05$) with malting and fermentation. *Enterobacteriaceae* and *Staphylococcus* species were predominant in the non-fermented products, while yeast dominated in the fermented products, whose gruels also inhibited growth of *Staphylococcus* in challenge tests.

Keywords: Challenge tests, functional properties, gruel, moulds, viscosity, yeast

INTRODUCTION

If a mixture of ground cereals and legumes is cooked with water into a porridge or gruel for use as complementary food, two problems may arise:

- The product will have high viscosity and bulk caused by starch gelatinization. This means that foods of acceptable viscosity will have low nutrient density and therefore, low energy value.
- The neutral pH (6.0-6.5) of such foods makes them highly susceptible to growth of spoilage and pathogenic micro-organisms (Ariahu *et al*., 1999a, b). Reduction of bulk and prevention of microbial infection are therefore of primary concern in the production of complementary foods from cereals/legumes.

Germination and fermentation are among the simple and easily adaptable technologies for reduction of bulkiness (high viscosity) and increasing shelf life of cereal and legume based food formulations (Sefa-Dedeh *et al*., 2001; Oluwamukomi *et al*., 2003; Gernah *et al*., 2011a). However, the incidence of diarrhea among children fed with this type of complementary food has been reported (AHRTAG, 1990). High counts of indicator pathogenic organisms (e.g., *Eschericia coli*) and spoilage organisms (e.g., *Bacillus cereus*) were found in stored non-fermented traditional Gambian complementary foods (Rowland *et al*., 1978); while severe contamination of Kenyan children's food with *Enterobacteriaceae* and *Staphylococcus aureus* was reported by Van Steenbergen *et al*. (1983). Challenge tests using food formulations from African breadfruit (*Treculia africana*) and soybean (*Glycine max*) gave growth of *Enterobacteriaceae* and *Staphylococcus* in non-fermented products, while the re-introduced bacteria died in the fermented products (Ariahu *et al*., 1999b). There is therefore need to evaluate locally produced complementary foods to ascertain their microbiological quality.

Though a lot of work has been done on the effect of malting and fermentation on the physic-chemical and microbiological quality of food formulations from other cereals/ legumes (Ariahu *et al*., 1999a, b; Sefa-Dedeh *et al*., 2001; Oluwamukomi *et al*., 2003; Obasi *et al*., 2009; Gernah *et al*., 2011b), information on maize/sesame food formulations is very scanty. Such information will be useful in expanding the horizons of usage of our locally available grains to improve the health of our growing population.

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The objective of this study was therefore to investigate the effects of malting and fermentation of maize on the :

- Physical
- Microbiological composition
- Microbiological safety of maize food formulations fortified with defatted sesame flour

MATERIALS AND METHODS

Source of materials and preliminary treatments: This study was conducted in the laboratories of the Dapartment of Food Science and Technology, University of Agriculture, Makurdi, Nigeria. About 3.0 kg of white maize (TZW, 2005 harvest) was obtained from the Agronomy Department, University of Agriculture, Makurdi; while 4.0 kg of white sesame (variety E8, 2005 harvest), was obtained from the seed store of the National Cereals Research Institute, College of Agriculture, Yandev, Gboko. Most chemicals used for analyses were purchased from local stores in Nigeria and were of Analar grade (British Drug House chemicals, Poole England).

After manual sorting and winnowing to remove stones, debris and defective seeds, the clean maize and sesame seeds were packaged in tightly covered 10 and 5L plastic buckets respectively. All materials were stored in a household refrigerator and utilized for product formulation within 2 weeks.

Preparation of unmalted and malted maize flours: Malting was carried out using the method described by Ariahu *et al*. (1999a). Four hundred grams of raw maize grains were washed in 5% (w/v) sodium chloride (NaCl) solution to disinfect the grains. The grains were then soaked in tap water at room temperature (30+2ºC) using a ratio of 1:3 (w/v grain: water), in a plastic bucket. The steep water was changed every 4 h for a total steeping time of 12 h, followed by draining in a plastic basket and the grains were spread in a single layer on a moistened jute bag and allowed to germinate at room temperature (30+2º0C) for 72 h, while spraying with water at intervals of 12 h. The ungerminated and germinated grains were removed at 0 and 72 h respectively and dried in an air draft oven (Genlab Widnes, U.K, model T12H) at 100ºC to constant weight. The dried seeds were split in a disc attrition mill (Asiko A11, Addis, Nigeria) using a nip of about 3 mm, to detach testa and rootlets from cotyledons which were removed by winnowing. The cotyledons were then milled into flour using a bench top hammer mill (Brook Crompton, Series 2000, England) to pass through a sieve of 0.2 mm particle size. The resultant Unmalted Maize (UM) and Malted Maize (MM) flours were then packaged in low density dark-coloured polyethylene bags, stored in 500 mL plastic containers with airtight lids at room temperature (30+2ºC) and utilized for product formulation and analysis within 24 h.

Preparation of fermented maize flours: Fermented maize doughs were obtained by accelerated natural lactic acid fermentation using the method described by Ariahu *et al*. (1999b). In this process 120.0 g each of Unmalted (UM) and Malted (MM) maize flours were mixed with 80 mL of distilled water and subjected to natural fermentation in a covered 500 mL glass beaker at room temperature (30+2ºC) for 24 h. At the end of this period, 50% of the fermented mixture was used as starter culture for a new fermentation cycle. During this process, the pH and titratable acidity (an index of lactic acid bacteria activity) were monitored. The fermentation process was continued until the pH of the medium stabilized and remained constant. The fermented concentrates were dried at 80ºC in a fan driven electric oven (Genlab Widnes, U.K, model T12 H) to constant weight and milled in a disc attrition mill (Asiko A11, Addis Nigeria) to a particle size of 0.2 mm. The Unmalted Fermented Maize (UFM) and Malted Fermented Maize (MFM) flours were then packaged in low density dark-coloured polyethylene bags, stored in 500 mL plastic containers with airtight lids at room temperature (30+2ºC) and utilized for product formulation and analysis within 24 h.

Preparation of defatted sesame flour: Sesame seeds were dehulled using the method of Ramachandra *et al.* (1970). In this process the sesame seeds were cleaned and sorted by soaking jn water and removing the seeds that floated on top. The good seeds were then boiled in 0.6% NaOH solution for 1 min after which they were washed with excess cold water. Thereafter, the ruptured seed coats were separated by scrubbing between the palms and air dried to get rid of excess water. The dehulled seeds were then defatted by the screw press method described by Fasina and Ajibola (1989) as modified by Igyor *et al*. (2008). Sesame seeds (1.0 kg) were coarsely ground in a kitchen blender (Phillips, Holland model HR 1702), wrapped in a muslin cloth and placed in a screw press (Edwards and Jones, Meir, England). The handle of the screw press was turned until it reached maximum pressure (20 psi). The press was held at this pressure during which time the oil dripped into a holding tray and was collected. By varying the extraction time at intervals of 10, 20, 30 and 40 min (at maximum pressure) samples were collected for fat analysis until a fat content of 14-15% in the cake was obtained. The defatted sesame cake was dried at 80ºC to constant weight in an air draft electric oven (Genlab Widnes, U.K model T12H), after which it was milled to a particle size of 0.2 mm. The flour was then packaged in a low density dark-coloured polyethylene bag, stored in a 500 mL plastic container with airtight lid at room temperature (30+2ºC) and utilized for product formulation and analysis within 24 h.

Food products formulation: Four different food formulations were made by blending the different maize flours with the defatted sesame flour to obtain 16 g protein and 9 g fat/100 g food. This was achieved by material balancing from their respective proximate compositions (Smith, 2003). The four formulations were: maize + defatted sesame (UMS), malted maize + defatted sesame (MMS), unmalted fermented maize + defatted sesame (UFMS) and malted fermented maize + defatted sesame (MFMS). These were packaged in low density dark-coloured polyethylene bags and stored in 500 mL plastic containers with air tight lids in a household refrigerator from where samples were taken for diet formulation.

Preparation of gruels: Gruels were prepared from the food formulations using the method described by Uvere *et al*. (2002). A 5.0% (w/v) solution of each of the food formulations were used to prepare slurries. Gruels were then prepared by boiling the slurries for 10 min. All the gruels were cooled to 40-42ºC and used for determination of viscosity.

Analyses:

Physical properties: The apparent viscosity of slurries was determined by the method of Beuchat (1977) while that of gruels was determined by the method described by Uvere *et al*. (2002). Packed bulk density and Water absorption capacity were determined using the methods of Okezie and Bello (1980), while Swelling index and Reconstitution index were determined by the methods described by Flemming *et al*. (1974) and Banigo and Akpapunam (1987) respectively.

Microbiological composition: The microbiological quality of the flour-in water slurries was determined by the method described by Adegoke (2004); while the microbiological stability of gruels during the short term storage was assessed by challenge tests as described by Ariahu *et al*. (1999b). In this method, approximately 10.0 g of each sample was dissolved in 100 mL of sterile distilled water to give smooth slurries. The slurries were cooked for 10 min. at 90ºC, while stirring with a glass rod into a smooth paste in 250 mL glass beakers. The beakers were covered with aluminum foil immediately after cooking and allowed to cool, prior to incubation at 30ºC for 24 h. Microbial counts were carried out as earlier described by Adegoke (2004).

Statistical analysis: All results were subjected to Analysis of Variance (ANOVA) using a pre-packaged computer statistical software (MINITAB 15).

RESULTS

Physical properties: The effect of malting and fermentation on the physical properties of the different food formulations is presented in Table 1.

Viscosity: There was significant ($p<0.05$) decrease in viscosity of gruels from the food formulations with malting and fermentation. Viscosity values ranged from 318.00 cP for UMS to 303.00 cP for MMS, to 271.70 cP for UFMS to 210.70 cP for MFMS, at comparable slurry concentrations of 5%.

Effect of fermentation on viscosity: Gruels from fermented flours gave significantly ($p<0.05$) higher percentage reduction in viscosity compared to untreated and malted samples.

Effect of slurry concentration on viscosity: The effect of slurry concentration on the viscosity of gruels from the food formulations is shown in Fig. 1. There was corresponding significant ($p<0.05$) increase in gruel viscosity with increase in slurry concentration.

Functional properties: There was significant ($p<0.05$) decrease in the packed bulk density of the food formulations with a value of 1.08 g/mL for UMS, 1.04 g/mL for MMS, 1.06 g/mL for UFMS and 0.97 g/mL for MFMS. The un-malted flour had a swelling index of 4.43 mL/g, which was significantly higher ($p<0.05$) than the malted and fermented flours. Meanwhile, there was an increase in water absorption capacity which was significant ($p<0.05$), with malted and fermented flours absorbing more water, while the reconstitution index of MFMS (6.13 mL/g) and UFMS (5.79 mL/g) were also significantly ($p<0.05$) higher than the unfermented formulations.

Microbiological composition: The total aerobic, yeast, mould, *Enterobacteriaceae* and *Staphylococcus* counts of

Table 1: Effect of malting and fermentation on some physical properties of Maize/sesame food formulations

Parameter	UMS	MMS	UFMS	MFMS	LSD
Viscosity (cP)	318.00±0.90^a	303.00±1.04^b	271.70±0.88^c	210.70±1.11^d	2.65
Bulk density (g/mL)	1.08±0.01^a	1.04±0.01^b	1.06±0.02^b	0.97±0.01^c	0.03
Swelling index (mL/g)	4.43±0.02^a	3,72±0.03^c	4.02±0.04^b	2.93±0.02^d	0.12
Water absorp. capacity (g/g)	3.03±0.03^d	3.29±0.03^c	3.52±0.02^b	4.07±0.04^a	0.16
Reconstitution index (mL/g)	5.02±0.04^d	5.20±0.03^c	5.79±0.02^b	6.13±0.05^a	0.33

Values are means±standard deviations of triplicate determinations; Means with the same superscripts within the same row are not significantly different ($p>0.05$); UMS: Unmalted maize + defatted sesame; UFMS: Unmalted, fermented Maize + defatted sesame; MMS: Malted maize + defatted sesame; MFMS: Malted, fermented maize + defatted sesame; *LSD: Least significant difference

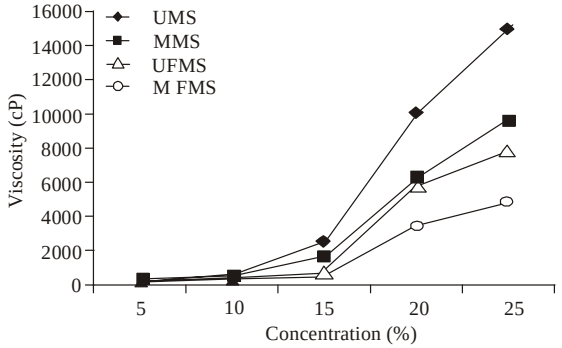

Fig. 1: Effect of slurry concentration on the viscosity of gruels from maize/sesame food formulations UMS: Unmalted maize + defatted sesame; UFMS: Unmalted fermented maize + defatted sesame; MMS: Malted maize + defatted sesame; MFMS: Malted fermented maize + defatted sesame

Table 2: Microbiological composition of the maize/sesame food formulations (CFU/g)

Parameter	UMS	MMS	UFMS	MFM
Total aerobic count	$1.2x10^4_d$	$2.2x10^4_c$	$3.0x10^7_b$	$2.4x10^8$
Yeasts/moulds	$4.8x10^3_c$	$2.3x10^3_d$	$2.8x10^4_b$	$2.4x10^5_a$
Yeast	$<30_c$	$<30_c$	$8.0x10^2_b$	$1.2x10^3_a$
Enterobacteriaceae	$3.0x10^2_a$	$1.2x10^2_b$	$<30_c$	$<30_c$
Staphylococcus	$2.5x10^2_a$	$1.0x10^2_b$	$<30_c$	$<30_c$

Values are means of triplicate determinations; Means with the same subscripts within the same row are not significantly different ($p>0.05$); UMS: Unmalted maize + defatted sesame; UFMS: Unmalted, fermented Maize + defatted sesame; MMS: Malted maize + defatted sesame; MFMS: Malted, fermented maize + defatted sesame

Table 3: Microbiological stability during storage and challenge tests of gruels from the maize/sesame food formulations (CFU/g)

Parameter	Storage time (hours)	UMS	MMS	UFMS	MFMS
Total aerobic counts	0	$2.0x10^2_a$	$1.0x10^2_b$	$<30_c$	$<30_c$
	24	$2.6x10^7_a$	$1.4x10^8_b$	$1.8x10^4_c$	$1.6x10^4_d$
Yeasts	0	$<30_a$	$<30_a$	$<30_a$	$<30_a$
	24	$2.0x10^2_d$	$3.0x10^2_c$	$7.0x10^2_b$	$1.4x10^3_a$
Moulds	0	$<30_a$	$<30_a$	<30	$_a<30_a$
	24	$<30_a$	$<30_a$	$<30_a$	$<30_a$
Enterobacteriaceae	0	$<30_a$	$<30_a$	$<30_a$	$<30_a$
	24	$<30_a$	$<30_a$	$<30_a$	$<30_a$
Staphylococcus	0	$<30_a$	$<30_a$	$<30_a$	$<30_a$
	24	$2.3x10^4_a$	$1.6x10^4_b$	$<30_c$	$<30_c$

Values are means of triplicate determinations; Means with the same subscripts within the same row are not significantly different ($p>0.05$); UMS: Unmalted Maize + Defatted Sesame; UFMS: Unmalted, Fermented + Defatted Sesame; MMS: Malted Maize + Defatted Sesame; MFMS: Malted, fermented maize + defatted sesame

the maize/sesame food formulations are presented in Table 2. Malting and fermentation resulted in significant ($p<0.05$) increase in microbial load of the food formulations, with the total aerobic counts, ranging from $1.2x10^4$ CFU/g in UMS to $2.8x10^8$ CFU/g in MMS.

Microbiological stability: The relevant microbiological counts from the gruels during storage and challenge tests are presented in Table 3. There were very low levels of viable micro-organisms in the gruels immediately after cooking. After 24 h of storage, the bacteria counts increased significantly ($p<0.05$) in those made from non-fermented products, with gruels from the fermented products having much lower levels. The yeasts, moulds and *Enterobacteriaeae* counts were very low (<30 CFU/g) in all the gruels even after 24 h of storage at 30ºC. After 24 h storage, *Staphylococcus* was able to re-establish itself in the non-fermented gruels, but could not grow in the fermented gruels.

DISCUSSION

Viscosity: The significant reduction ($p<0.05$) in viscosity with malting and fermentation could be due to breakdown of macromolecules such as polysaccharides and polypeptides to smaller units, such as dextrins and peptides respectively by the enzymes mobilized during the germination and fermentation process (Gernah *et al*., 2011a).

Effect of fermentation on viscosity: The higher percentage reduction in viscosity of fermented gruels compared to untreated and malted samples could be due to the breakdown of complex molecules by enzymes and microorganisms, which may have resulted in less viscous soluble matter, including sugars and short chain dextrins as observed by Uvere *et al.* (2002). It also suggests that the unfermented flours had higher quantities of gel-forming components and that a combination of fermentation and gelatinization makes the starch granules susceptible to hydrolysis by amylases. Gopaldes *et al*. (1986) also suggested that the effect of fermentation on the apparent viscosity of gruels could be due to two factors: the method of fermentation and the length of the lag phase of fermenting microorganisms. The method of fermentation determines the rate of production of more soluble substances utilizable by the micro-organisms and hence contributes to the length of their lag phase, which in turn affects the reduction in apparent viscosity.

Fermentation further decreases the total amount of carbohydrates and other nutrients, since microbial activity requires energy and nutrients (Akpapunam and Sefa-Dedeh, 1995). This is nutritionally advantageous, since for equal volumes germination and fermentation would permit the addition of higher quantities of food solids to the gruels in comparison with the UMS product. These observations are in conformity with earlier reports on cereal/l egume based gruels (Marero *et al*., 1989a; Frias *et al*., 1996). The lowered starch complexity resulting from the dextinogenic (viscosity-reducing) effect of enzymic modification of starch and the partial digestion by enzymes during germination, therefore, helps in its utilization (Oluwamukomi *et al*., 2003).

Effect of slurry concentration on viscosity: The increase in viscosity with increase in slurry concentration could be due to the increase in dry matter which led to increase in the thickness of the medium and is in agreement with the findings of Ariahu *et al*. (1999b). The implication in infant nutrition is that, for low nutrient density foods, increase in concentration of dry matter in the gruel to meet the recommended nutrient levels, will lead to gruels that are too viscous for the infants to manage.

Functional properties: The decrease in packed bulk density could be because malting and fermentation tend to soften the seeds, thus making milling easier, with smaller particle sizes than un-malted grains, hence the reduction in bulk density. The significance of this is that the less bulky flours will have higher nutrient density, since more flour can be packaged in the same given volume (Iwe, 2003). Conversely, the higher swelling index for the un-malted sample was expected. The swelling of starch granules leads to disruption of some of the intermolecular hydrogen bonds, thus allowing more water to enter and enlarge the granules (Ihekoronye and Ngoddy, 1985). Adeyemo *et al*. (1992) also reported the implication of fibre and starch in this phenomenon. The malted and fermented flours, whose starches had already been dextrinized, could not swell as much. Swelling capacity could also be an index of stickiness of the resultant product (Uvere *et al*., 2002).

The increase in water absorption capacity and reconstitution index with malting and fermentation could be due to increased solubility as a result of the increase in amount of soluble sugars present in the malted and fermented flours. This means that the malted and fermented formulations, which had better water absorption capacity, were easier to reconstitute in water when needed. Microbiological Composition

The significant increase in microbial load observed with malting and fermentation is consistent with the findings of Nout (1991) that germination gives rise to enormous increases in moulds, fungi, yeasts and bacilli as well as potential pathogenic and toxinogenic species. Acid fermentation also uses acid producing bacteria, yeast and fungi which grow rapidly within 18-36 h (Nout, 1991).

Microbiological Stability: The very low levels of viable micro-organisms in the gruels immediately after cooking were expected, with the few survivors being most probably heat resistant bacteria (Ariahu *et al.*, 1999b). The significantly ($p<0.05$) increase in bacteria counts in gruels made from non-fermented products, with gruels from the fermented products having much lower levels after 24 h storage could be as a result of inhibition of growth of aerobes, due to their higher acidity (lower pH) compared to the non-fermented gruels. ICMSF (1996) reported that the optimum pH for growth of *Enterobacteriaceae* is in the range of 6.0-8.0. The very low (<30 CFU/g) counts of yeasts, moulds and *Enterobacteriaeae* in all the gruels even after 24 h of storage at 30ºC was an indication of lack of recontamination or ability of these micro-organisms to grow in the gruels.

The fact that *Staphylococcus* was able to re-establish itself in the non-fermented gruels, but could not grow in the fermented gruels after 24 h storage could be a potential advantage of lactic acid fermentation, to help prevent re-contamination of gruels from utensils or food handlers, who commonly prepare and store gruels at ambient temperatures for intermittent feeding of infants.

However, the total viable counts of non fermented products were within the acceptable limits of 10^7 CFU/g as given by ICMSF (1978) for flours, while those of fermented products were over the limit at $3.0x10^7$ CFU/g for UFMS to $2.4x10^8$ CFU/g for MFMS. *Enterobacteriaceae* and *Staphylococcus* counts of all the food formulations were within ICMSF (1978) specifications of $4.0x10^2$ CFU/g and 10^3 CFU/g respectively for flours. Therefore the food formulations were considered safe for human consumption.

CONCLUSION

Malting and fermentation significantly ($p<0.05$) decreased gruel viscosities, leading to improved nutrient density. There was also significant ($p<0.05$) reduction in packed bulk density and swelling index, while water absorption capacity and reconstitution index increased.

Natural lactic fermentation significantly ($p<0.05$) affected the microbiological composition and enhanced microbiological safety of the food products by increasing the dominance of lactic acid bacteria and inhibiting growth of pathogenic micro-organisms.

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