PRINT ISSN 1119-8362
Electronic ISSN 1119-8362

Full-text Available Online at
https://www.ajol.info/index.php/jasem
http://ww.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 24 (9) 1625-1630 September 2020

Effect of Fermentation, Germination and Combined Germination-Fermentation Processing Methods on the Nutrient and Anti-nutrient Contents of Quality Protein Maize (QPM) Seeds

***[1]ANAEMENE DI; [2]FADUPIN, GT**

**[1]Department of Chemical and Food Sciences, Bells University of Technology, Ota, Ogun State, Nigeria*
[2]Department of Human Nutrition, University of Ibadan, Ibadan, Oyo State, Nigeria
**Corresponding Author Email: dorisianaemene@gmail.com*

ABSTRACT: Cereals remain the major components of traditional complementary foods but possess antinutritional factors in addition to nutrients. High antinutrient content have been linked to poor quality complementary foods and high level of undernutrition among children less than two years. Hence, this study examined the effect of fermentation, germination and combined germination-fermentation processing methods on the nutrient and antinutrient contents of Quality Protein Maize (QPM). Maize seeds were fermented and germinated for 72 hours. A batch of the germinated seeds was further fermented for 24 and 48 hours separately. The raw and processed maize seeds were chemically analysed for proximate, mineral (calcium, iron and zinc) and antinutrient (phytate, tannin, oxalate, saponin, polyphenol and hemaglutinnin) composition. ANOVA was used to detect significant differences. Result showed that the crude protein content of raw QPM seeds increased significantly from 10.04% to 10.44% after fermentation while it decreased to 9.12% following germination (72 hours)-fermentation (48 hours). Crude fat content decreased significantly with the treatments (4.70-3.20%). Calcium (10.38-4.23mg/100g) and iron (3.70-1.90mg/100g) contents decreased significantly with all the methods. Germination and combined germination-fermentation reduced more antinutrients in maize compared to fermentation. Fermented seeds had the least tannin (28-27.5 mg/100g) and phytate (967.5-828.5 mg/100g) reduction while the oxalate (590-646 mg/100g), saponin (425-545 mg/100g) and hemaglutinnin (17.31-19.53 mg/100g) contents increased. Germination-fermentation (24 hours) decreased phytate content by > 90% and retained more iron (79 vs 61%) and zinc (80 vs 74%) than fermentation. Combined germination-fermentation (24 hours) was more effective in antinutrient reduction in Quality Protein Maize seeds.

DOI: https://dx.doi.org/10.4314/jasem.v24i9.21

Dates: Received: 10 August 2020; Revised: 16 September 2020; Accepted: 22 September 2020

Keywords: Maize, complementary foods, antinutrients, processing

Traditional complementary foods, mostly cereal based are regarded as inadequate (Serem *et al*., 2010) because of poor energy and nutrient density as well as bulkiness (Michaelsen *et al.,* 2003). Commercial complementary foods are expensive and beyond the reach of majority of mothers who depend on low cost food mixtures (Dewey, 2013). Numerous locally prepared complementary foods have been developed from various food groups which are believed to be superior to only cereal complementary food. However, locally formulated complementary food based on fermented cereal gruel still dominates because it is cheaper and perceived by mothers as easy to digest (Ibeanu and Okeke, 2001). Cereal remains a major component of complementary foods including commercial complementary foods. This implies that the quality of traditional complementary foods depends largely on the quality of the cereal used. Maize (*Zea mays*) is the most common cereal used in traditional complementary foods production in the developing countries (Ogunba, 2012;Temesgen, 2013). Nigeria is the largest African producer of maize with about 20 million tonnes (Udegbunem, 2019). Common maize has two major known flaws. It is low in protein quality as a result of low lysine and tryptophan. The protein content of maize is about 9g/100g dry weight (FAO 1992). In view of the deficit, Quality Protein Maize (QPM), an improved variety with high lysine and tryptophan was developed in 2004 (Vasal, 2006). The bulkiness caused by gelatinization of starch in cereals during cooking is another problem. The increased viscosity would require more dilution with water thereby reducing the energy density of prepared meal (Michaelsen *et al.,* 2003). Germination process and addition of amylase rich flours are some of the measures recommended to reduce the viscosity and bulkiness of cereal based gruels. The third salient issue is that cereals in addition to nutrients possess anti-nutritional properties which negatively affect the bioavailability of protein and micronutrients (Bora 2014; Tufa *et al*., 2016). Although anti-nutritional factors in cereals are lower than those in legumes, the high proportion of cereals in complementary food mix makes it considerable.

**Corresponding Author Email: dorisianaemene@gmail.com*

Cereals contain anti-nutrients such as phytate, tannin, trypsin and chymotrypsin inhibitors, cyanide, saponin, polyphenols, oxalates among others (Bora, 2014). The level of anti-nutrients in foods largely depends on the ingredients used in the preparation and methods of processing. Phytic acid is abundant in the seed portion of maize while in cereals such as wheat and rice it is more in the aleurone layer (Abdoulaye *et al*., 2011). Monogastric animals and humans lack phytase required to metabolize phytate (Singh *et al*., 2011). Phytic acid binds some minerals and inhibits the digestive enzymes necessary for protein starch degradation (Gupta *et al*., 2015). Roos *et al*. (2013) reported that the phytate contents of plant-based complementary foods offered to children in the developing countries ranged from 68 to 1536 mg/100g. Consumption of maize meals was also associated with reduced zinc absorption in children. Research has shown that processing methods such as soaking, boiling, germination and fermentation could reduce the level of antinutrients thereby improving nutritional qualities of produced complementary foods (Agostini *et al.*, 2010). Combination of processing methods may be more effective in eliminating or reducing anti nutrients than single techniques (Inyang and Zakari, 2008). During fermentation and germination, inherent phytases are activated which degrade the phytates (Temesgen, 2013). Reduction of anti-nutritional factors in cereals is very important in the formulation of complementary foods to ensure nutrient bioavailability. Extensive research has been undertaken on methods of reducing anti-nutrient content of various cereals used for complementary food formulation. However, there is limited information on the effect of processing methods on the chemical composition of Quality Protein Maize. Hence, this work examined the effect of fermentation, germination and combined germination-fermentation methods on the nutrient and anti-nutrient contents of QPM.

MATERIALS AND METHODS

Study design and sample collection: The study was experimental in design. Quality protein maize (ART 98/SW6-OB) seeds, a white maize variety with improved lysine and tryptophan content were obtained from the Institute of Agriculture, Research and Training, Ibadan (IAR&T), MOOR Plantation, Ibadan.

Production of fermented maize seeds flour: Two hundred grammes of white maize seeds were cleaned, washed and soaked in excess tepid water (1:3 w/v) and allowed to ferment naturally for 72 hours at 28^{o}C (Onweluzo and Nwabugwu, 2009). The fermented seeds were washed and oven-dried at 60^{o}C for 19-22 hours, partially milled, dehulled, milled and sieved with 450 µm pore sieve to obtain fermented maize flour.

Production of germinated maize seeds flour: Two hundred grammes of white maize seeds were cleaned, soaked in excess tepid water for 24 hours (rinsed at 12th hour and 24th hour), washed and spread on moist jute bags. The maize seeds were washed, drained and spread again at 24th hour and 48th hour of germination and allowed to continue to sprout at room temperature till 72 hours using a modified method of Onweluzo and Nwabugwu (2009). Water was sprinkled once a day during the germination period to encourage aeration. Half of the sprouted seeds were oven-dried at 60^{o}C for 18-21 hours. The rootlets and hulls were removed and seeds were milled, sieved with a 450 µm pore sieve and tagged as germinatedted maize flour.

Production of germinated fermented maize seeds flour: The remaining (half) of the sprouted maize seeds were divided into two and fermented separately in water (28^{o}C) for 24 and 48 hours. The sprouted fermented seeds were oven-dried at 60^{o}C for 20-22 hours, dehulled, milled and sieved with a 450 µm pore sieve into sprouted fermented (24) and sprouted fermented (48) maize flour (2 products). The flours obtained were stored in Zip-lock bags (26.5cm x 27.3cm, BJohnson) at 4^{o}C for analysis.

Nutrient and antinutrient analysis: The moisture, fat, crude protein (N x 6.25), crude fibre and ash contents of the raw and processed maize flour were determined in triplicates according to the approved procedures of the Association of Official Analytical Chemists (AOAC, 2005). The iron and zinc contents were determined using the atomic absorption spectrophotometer (Buck 200, Buck Scientific, USA), while calcium was determined using flame Jenway Digital Flame Photometer (PFP7, Bibby Scientific Limited, United Kingdom). The method of Onwuka (2005) was employed in the determination of oxalate while phytate, tannin, saponin and hemaglutinnin were determined by the method described by AOAC (2005).

RESULTS AND DISCUSSION

The row labeled Mo displays the proximate and mineral contents of raw white maize while the values after processing are displayed on the rows below it (Table 1). The table shows that the crude protein content of white maize (10.04 g/100 g) increased significantly ($p<0.05$) after 72 hours of fermentation (10.44 g/100 g), while it decreased significantly ($p<0.05$) to 9.12 g/100 g (9.2%) when germinated (72 hours) and fermented for 48 hours continuously. The

other treatment methods did not have significant effect ($p>0.05$) on the crude protein content of quality protein maize. Crude fat content of maize decreased significantly with all the treatments. The lowest value of fat (3.20 g/100g) was observed when the seeds were germinated for 72 hours. The ash content of maize was lowest (0.99 g/100g) when germinated-fermented for 48 hours. Carbohydrate composition of maize increased significantly ($p<0.05$), while calcium and iron content decreased significantly ($p<0.05$) with all the treatments (Table 2). Zinc contents decreased significantly ($p<0.05$) with all the treatments except for combined germination-fermentation for 48 hours where there was a slight insignificant increase (0.3%). The effect of fermentation, germination and germination-fermentation on the anti-nutrient contents of maize is presented in Table 3. It shows that the levels of phytate, oxalate, saponin, polyphenol and hemaglutinin reduced significantly ($p<0.05$) with germination and germination-fermentation methods. Germination and combined germination-fermentation methods were more effective in phytate reduction in maize (>90%) compared to fermentation (14%). The tannin contents of raw maize seeds reduced by only 1.8% when the seeds were fermented for 72 hours. Surprisingly, the oxalate, saponin and hemaglutinnin contents of maize increased with fermentation. Phytate levels decreased from 967.50 mg/100g (control) to 828.50 mg/100g (fermentation), 85.50 mg/100g (germination), 74.50 mg/100g (germination (72 hours)-fermentation (24 hours)) and 61.00 mg/100g (germination (72 hours)-fermentation (48 hours)). In summary (Table 4), fermentation of maize reduced phytate content by 14%, increased protein content by 4% and retained about 72% of calcium. Combined germination (72 hours)-fermentation (24 hours) of maize decreased phytate content of maize by more than 90% and retained more iron (79% vs 61%) and zinc (80% vs 74%) compared to fermentation. Germination although reduced above 90% phytate retained less calcium, iron and zinc.

Table 1. Proximate composition of fermented, germinated and germinated/fermented white maize flour (dry weight basis)

	Moisture (%)	Crude protein (%)	Crude fat (%)	Crude fibre (%)	Ash (%)	CHO (%)
M_O	$8.63^{a} \pm 0.02$	$10.04^{b} \pm 0.06$	$4.72^{a} \pm 0.02$	$1.41^{b} \pm 0.03$	$1.79^{a} \pm 0.02$	$74.82^{d} \pm 0.08$
M_1	$7.33^{d} \pm 0.02$	$10.44^{a} \pm 0.13$	$4.30^{b} \pm 0.02$	$1.18^{c} \pm 0.02$	$1.11^{d} \pm 0.01$	$76.81^{c} \pm 0.12$
M_2	$7.43^{c} \pm 0.03$	$10.04^{b} \pm 0.05$	$3.20^{e} \pm 0.03$	$1.20^{c} \pm 0.01$	$1.49^{b} \pm 0.02$	$77.84^{b} \pm 0.05$
*M_3	$7.47^{c} \pm 0.02$	$9.92^{b} \pm 0.13$	$3.40^{d} \pm 0.02$	$1.59^{a} \pm 0.02$	$1.41^{c} \pm 0.02$	$77.79^{b} \pm 0.14$
M_4	$7.74^{b} \pm 0.02$	$9.12^{c} \pm 0.12$	$3.80^{c} \pm 0.01$	$1.19^{c} \pm 0.02$	$0.99^{e} \pm 0.01$	$78.35^{a} \pm 0.12$

Values are expressed as mean ±SD (n=3). Means in the same column with different superscripts are significantly different from each other at P< 0.05.M_O– raw maize; M_1- fermented maize (72h); M_2-germinated maize(72h); M_3- germinated/fermented (72h/24h); M_4-germinated/fermented (72h/48h), CHO-carbohydrate

Table 2. Mineral composition of fermented, germinated and germinated/fermented white maize flour (dry weight basis)

	Calcium (mg/100g)	Iron (mg/100g)	Zinc (mg/100g)
M_O	$10.38^{a} \pm 0.07$	$3.70^{a} \pm 0.53$	$2.28^{a} \pm 0.06$
M_1	$7.05^{b} \pm 0.14$	$2.25^{bc} \pm 0.35$	$1.68^{c} \pm 0.04$
M_2	$4.35^{d} \pm 0.07$	$1.90^{c} \pm 0.07$	$1.60^{c} \pm 0.07$
*M_3	$4.63^{c} \pm 0.11$	$2.93^{b} \pm 0.11$	$1.83^{b} \pm 0.03$
M_4	$4.23^{d} \pm 0.07$	$2.83^{b} \pm 0.11$	$2.38^{a} \pm 0.01$

Values are expressed as mean ±SD (n=3). Means in the same column with different superscripts are significantly different from each other at P< 0.05.M_O– raw maize; M_1- fermented maize (72h); M_2-germinated maize(72h); M_3- germinated/fermented (72h/24h); M_4-germinated/fermented (72h/48h)

Table 3. Anti-nutrient composition of fermented, germinated and germinated/fermented maize flour (dry weight basis)

Parameters	Tannin (mg/100g)	Phytate (mg/100g)	Oxalate (mg/100g)	Saponin (mg/100g)	Polyphenol (mg/100g)	Hemaglutinin (HU/100g)
M_O	$28.00^{a} \pm 1.41$	$967.50^{a} \pm 10.61$	$590.00^{a} \pm 84.86$	$425.00^{b} \pm 49.50$	$1110.00^{a} \pm 56.57$	$17.31^{b} \pm 0.04$
M_1	$27.50^{a} \pm 4.95$	$828.50^{b} \pm 2.12$	$646.00^{a} \pm 5.66$	$545.00^{a} \pm 35.35$	$715.00^{c} \pm 35.36$	$19.53^{a} \pm 0.07$
M_2	$20.00^{ab} \pm 1.41$	$85.50^{e} \pm 2.12$	$42.50^{b} \pm 4.95$	$195.00^{e} \pm 21.21$	$985.00^{b} \pm 35.36$	$13.65^{e} \pm 0.04$
**M_3	$20.50^{ab} \pm 4.95$	$74.50^{cd} \pm 4.95$	$31.00^{b} \pm 2.83$	$140.00^{e} \pm 14.14$	$795.00^{c} \pm 49.50$	$9.18^{d} \pm 0.04$
M_4	$17.00^{b} \pm 2.83$	$61.00^{d} \pm 1.41$	$29.50^{b} \pm 4.95$	$135.00^{e} \pm 35.35$	$745.00^{c} \pm 35.36$	$8.83^{e} \pm 0.05$

Values are expressed as mean ±SD (n=2). Means in the same column with different superscripts are significantly different from each other at P< 0.05. M_O– raw maize; M_1- fermented maize (72h); M_2-germinated maize (72h); M_3- germinated/fermented (72h/24h); M_4-germinated/fermented (72h/48h)

The crude protein values of raw and processed maize seeds ranged from 9.12-10.44%. These values agreed closely with the values reported by Tufa *et al*. (2016) for maize (7.34-10.61%). Fermentation increased the crude protein levels of maize as also observed by Tufa *et al*. (2016). In millet by Inyang and Zakari (2008)

and in sorghum by Kinyua *et al*. (2016). The increase in protein level may be as a result of increased synthesis of certain amino acids by the fermenting seeds (Bora, 2014). Germination did not have any effect on the crude protein of maize (10.04%), while combined germination and fermentation methods decreased the crude protein contents of quality protein maize from 10.04% to 9.92%. This finding agrees with the report of Ikujenlola and Adurotoye (2014), but contrasted with the works of Inyang and Zakari (2008), and Kinyua *et al*. (2016). These researchers reported increase in crude protein contents of millet and sorghum with combined germination-fermentation and germination, respectively. The difference could be due to varietal and geographical variations as well as varying conditions of treatment and analysis. Total ash, iron, and zinc levels were reduced significantly in the maize flour with all the treatments. Similar results were reported by Ikujenlola and Adurotoye (2014) and Tufa *et al*. (2016). The loss in mineral content could be attributed to probable leaching of some volatile compounds to the fermenting medium and degradation of dry matter (Nnam and Obiakor, 2003). However, the losses were less in combined germinated-fermented maize. The reduced ash content for maize with fermentation did not agree with the finding of Mbaeyi-Nwaoha and Obetta (2016), who reported increased ash content of fermented millet flours. They also reported an increase in the zinc content of fermented millet while the iron level decreased. Carbohydrate content increased significantly ($p<0.05$) from 74.82% (control) to 78.35% in combined germinated-fermented maize (72/48 hours). The increased carbohydrate levels observed in maize contrasted with several works mentioned above, which reported decreased levels of carbohydrate with fermentation and germination. This could be due to reduced moisture content of the treated samples when compared to control as a result of the drying process. Fermentation, germination and combined germination-fermentation (72/48 hours) significantly decreased the crude fibre and crude fat levels in maize while combined germination-fermentation (72/24 hours) increased the crude fibre level from 1.41% to 1.59%. Ikujenlola and Adurotoye (2014) also observed reduction in the crude fibre of maize (5.08 to 4.51%) with germination but increased fat levels (1.80 to 2.35%), while Tufa *et al*. (2016) and Onweluzo and Nwabugwu (2009) reported increased fat levels of maize (4.35 to 4.70%) and millet (1.50 to 4.50%), respectively after germination process. Change in fat levels may be as a result of the increased activities of lipolytic enzymes which hydrolyse fats to fatty acid and glycerol (Mbaeyi-Nwoha and Obetta, 2016).

Table 4. Summary of effect of processing methods on nutrient retention and anti-nutrient reduction in maize

Processing methods	Increase	Decrease	No effect
Fermentation			
maize	Protein, fibre, Calcium, carbohydrate	Fat, ash, iron, zinc, phytate (14%)	
Germination			
maize	Fibre, carbohydrate	Anti-nutrients, ash, iron, zinc	protein
GerFer (72/24hrs)			
maize	Carbohydrate, fibre	Phytates (>90%), Protein(less),fat, ash, iron (less), Calcium (less), zinc	
GerFer (72/48hrs) Maize	Carbohydrate	Calcium, Iron, zinc, fat, protein, fibre, anti-nutrients	

Fermentation, germination, and combined germination-fermentation reduced the anti-nutrient contents of maize particularly phytate, tannin and oxalate contents (Tables 5). Similar findings were reported by several researchers including Kinyua *et al*. (2016) and Gbadamosi, *et al*. (2017). Germination of maize significantly reduced tannin, phytate, oxalate, polyphenol, and hemaglutinnin. However, the contents of ash, iron, and zinc were negatively affected. Combined germination (72hrs) -fermentation (24hrs) in addition to reducing above 90% of phytate in maize, retained more nutrients. Increased period of germination (Agostini *et al*., 2010) resulted in more phytate reduction. This reduction has been attributed to the activities of endogenous and microbial phytases in the germinating and fermenting seeds, respectively. Passive diffusion of water-soluble phytates could have contributed to the phytate reduction (Thompson and Amoroso, 2011). The natural lactic acid fermentation offers favourable pH conditions for the enzymatic degradation of phytates to organic phosphate, inositol, and other simpler forms. Germination and combined germination-fermentation methods were more effective in reducing the anti-nutrient content of maize compared to fermentation. High anti-nutrient content of foods is detrimental to health as it chelates vital minerals such as calcium, zinc, and iron including proteins to form complexes and make the unavailable.

Conclusion: Germination and combined germination-fermentation method reduces anti-nutrient content of maize compared to fermentation. More nutrients are

retained in maize after combined germination (72 hours) -fermentation for 24 hours. Therefore, combined germination (72 hours)-fermentation (24 hours) is the preferred processing method for maize. Mothers should be enlightened on the combined germination-fermentation of cereals. This should be part of the dietary diversity campaign. However, measures to ensure micronutrient sufficiency in processed complementary food must be part of infant feeding enlightenment programmes.

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