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Research Paper

The effect of ungerminated cowpea on the nutritional quality of germinated maize

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Abstract. Maize *(Zea mays)* shows qualitative and quantitative deficiency in protein content. This study made efforts to improve the nutritional quality of maize by germinating maize and co-fermenting with cowpea. Germinated maize grain was co-fermented with ungerminated cowpea grains (GM/CO) 700:300 w/w for 72 h. The control was co-fermented ungerminated maize/cowpea (UGM/CO). Using standard methods, products were analyzed for proximate composition, amino acids and gruels consistencies. Results indicated significant ($p < 0.05$) increase in protein content, consistency, cysteine, methionine, phenylalanine, leucine and valinein GM/CO over that of UGM/CO mixture. GM/CO had significant ($p < 0.05$) reduction in carbohydrate content compared to its UGM/CO. However, lack of germination enhanced some amino acids such as Isoleucine (28.0 to 18.0) and tyrosine (28.0 to 21). The essential amino acid index was higher in GM/CO than UGM/CO. Of the total amino acids enhanced by germination 40% were essential amino acids. Statistical analysis showed that all the linear correlation coefficient values were significant at $r = 0.05$ and n-2 degrees of freedom. Coefficient of alienation ranged from 20 to 50.7% while index of forecasting efficiency ranged between 49.30 to 65.0%. This study showed that germination and co-fermentation of maize with cowpea may improve the nutritional quality of maize and the increase in consistencies might increase the nutrient and energy densities.

Keywords: Co-fermentation, germination, consistency, cowpea, maize, nutritional quality.

INTRODUCTION

Spontaneous fermentation in Nigeria is a common traditional food processing technique employed in fermentation of cereals to improve its nutritional value and reduce the viscosity. According to Enechie (2009), germination and fermentation have been shown to be to be a convenient and effective food process in improving the nutritional quality of cereal based foods. The production of organic acids during cereals spontaneous fermentation process has been reported to be contributory to the texture, taste aroma and storability of fermented foods (Nout, 1993).

In Nigeria fermented maize (*Zea mays*) gruel is consumed in large quantities as infants complementary food. The maize grain is of good traditional acceptability due to its organoleptic properties and low cost. The protein content of maize is however, known to be deficient in lysine, threonine and tryptophan. Various legumes have been blended with fermented maize to improve maize nutritional quality (Agu and Alluyah, 2004; Oyarekua, et al. 2008). Hellard et al. (2002) reported that geminated maize porridge was more palatable than ungerminated one. Acidic foods like co-fermented cereals/cowpea slows gastric emptying and decrease absorption rate. So also, viscous food with soluble fibers, protein also delays gastric emptying (Dies, 2005). Kouakou et al. (2008) showed that the seeds of cereals during germination and fermentation develop a strong enzymatic activity (amylolytic). This observation can be advantageously utilized to improve the nutrition of infants and children, particularly in Nigeria where maize is consumed in large quantities.

However, not much had been done on germinated maize followed by co-fermentation with legume like cowpea. This study investigated the effect of germination and fermentation on maize/cowpea mixture. Co-fermentation was expected to provide energy and nutrient dense complementary gruel with minimal change in labour, maternal time and can be affordable for mothers.

MATERIALS AND METHODS

Sample preparation

Dried maize grains and cowpea seeds were sorted to remove stones and foreign particles. Maize grains were manually sorted and winnowed to remove stones, debris and defective grains. The cleaned grains were packaged in 10 L plastic buckets with cover. Maize/cowpea was divided into germinated maize and ungerminated cowpea and non-germinated maize/cowpea, co-fermented.

Reagents

All reagents ANALAR grade were purchased from BISLAB Scientific Company, Ado-Ekiti Nigeria.

Methods

Germination

Winnowed dried maize grains were moistened with distilled water, spread on wet insulin and layered by the window for air and sunlight for 3 days. The grains were allowed to sprout to a length of about 1 cm long after which the grains were oven dried at 55°C for about 12 h after which the sprouts and hulls were removed from the germinated grains by rubbing between palms and then winnowed

Fermentation

The mixture of about 700 g germinated maize with 700 g ungerminated cowpea were soaked in 3 L of distilled water and covered in a plastic container for 72 h. The same ratio of ungerminated maize and ungerminated cowpea was sampled to serve as control.

Proximate composition

The hydrogen ion concentration (pH), chemical proximate composition were determines using the standard methods of AOAC (2010). Carbohydrate value was calculated by difference.

The gruel consistencies were measured using Bostwick Consistometer. Each result was the mean of triplicates. The statistical calculations were made on the mean, standard deviation (SD), coefficient of variation (%CV). Consistency was determined using the method described by Mouquet and Treche (2001).

Statistical analysis

Calculations made were the mean, standard deviation (SD) and coefficients of variation in percentage (CV%). The summary of amino acid profiles in terms of factors A and B were also reported. The data obtained were also subjected to the determination of linear correlation coefficient (rxy), linear regression coefficient (Rxy) and the rxy calculated compared to rxy (Figure.9) at r = 0.05 and at n-2 degrees of freedom to evaluate the significant differences between the samples; both the coefficient of alienation (CA) and index of forecasting efficiency (IFE) were also calculated (Oloyo, 2001).

RESULTS AND DISCUSSION

Increase in consistency in Figure 1 might be due to starch degradation caused by the amylolytic activity of alpha and beta amylases generated during germination process. The flour of GM/CO at higher concentration can be used to have more reduced viscosity (increased consistency) than mixture with ungerminated maize. Reduced viscosity is indicative of higher nutrient density in agreement with Oyarekua (2012) and Moquet (1998). Therefore, the gruel from GM/CO might have a nutritional advantage in terms of reduced viscosity, nutrient density, and minerals this is in agreement with the findings of Dies (2005), Kayode et al. (2007) and kouakou et al. (2008).

There was a significant ($p < 0.05$) increase in consistency with the GM/CO and by implication it reduced viscosity and increased nutrient and energy densities, which was in agreement with results of Gibson et al. (1998). Adams et al. (1989) reported that germination reduced viscosity of barley, retain dry matter and fat. This reduction in viscosity could be due to starch degradation caused by the action of hydrolytic enzymes (α and β amylases), that developed during the germination process. As a result, hydrolyzing some of the starch into dextins and maltose, which do not swell when cooked. Flour prepared from GM/CO grains can therefore be used in greater amount to give the same viscosity as flour from un-germinated grains, thereby giving higher nutrient and energy density. Therefore, the porridge from GM/CO for children had a nutritional advantage: high energy density, intake of macronutrients and micro level (Kayode et al., 2007).

This study also showed that consistency is directly

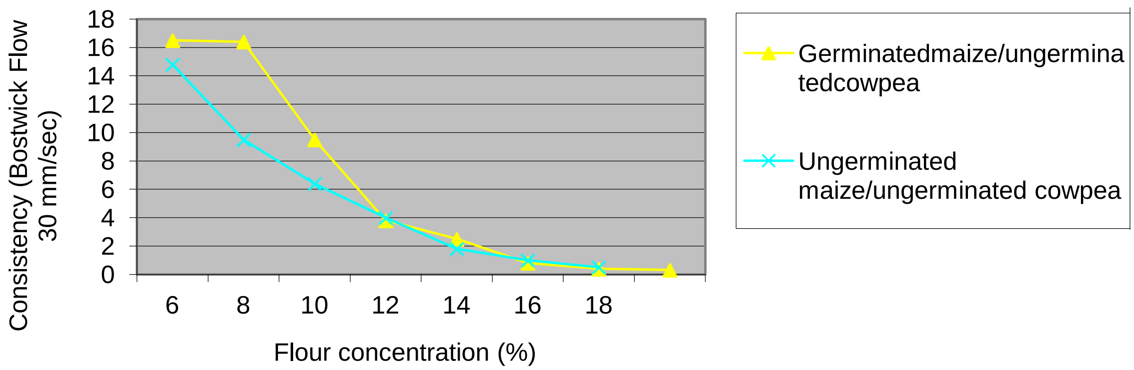

Figure 1. Effect of germination and co-fermentation on the consistency of GM/CO and UGM/CO gruels.

Table 1. Proximate composition of co-fermented ungerminated maize/cowpea (UGM/CO) and germinated maize/ cowpea (GM/CO).

Parameter (%)	UGM/CO	GM/CO	Mean	STD	CV%
Ash	1.06	0.94	1	0.084853	8.49
Moisture content	9.78	12.14	10.96	1.668772	15.23
Crude protein	5.74	12.5	9.12	4.780042	52.41
Lipid	2.82	5.74	4.28	2.064752	48.24
Crude fibre	0.12	4.31	2.22	2.962777	133.46
Carbohydrate	80.48	64.37	72.43	11.39149	15.73
Energy	2584	2764	2674	127.2792	4.76

related to gruel composition, as the gruel thickens by increase in dry matter particularly in terms of starch contents. As dry matter increases, the viscosity reduces.

In Table 1, the decrease in carbohydrate content of GM/CO might be due to carbohydrate hydrolysis by germinating grains and microflora of the fermenting medium for their metabolic activities as reported by Sade (2009).

Changes in proximate composition during maize germination followed by co-fermentation with cowpea showed significant ($p < 0.05$) increase in protein content of 12.50% at 72 h in GM/CO over 5.74% in UGM/CO mixture. This could be as a result of the mobilization of storage nitrogen of the maize, synthesis of enzymatic proteins by germinating seeds and the superior protein quality of cowpea to produce the nutritionally high quality proteins needed by the young infants for development (WHO, 1998). Protein is needed to build and repair new tissues of skin, eyes, muscles hear lungs, brain, etc., manufacture of important enzymes, hormones antibodies and in regulation of body processes.

There was significant reduction in carbohydrate value of co-fermented germinated maize/cowpea compared to its (UGM/CO) ungerminated analogue. The utilization of carbohydrates by the germinating grains with concomitant production of carbon dioxide and utilization by microorganisms may account for the lower carbohydrate content this is in agreement with Sade (2009) who reported that fermentation reduced carbohydrate content.

The germination of maize for GM/CO in this study significantly ($p < 0.05$) increased the protein, but decreased ash and dry matter contents contrary to Oheme and Chinma (2009) while fat and energy values of GM/CO showed decrease. This was in agreement with the findings of Oheme and Chinma (2008). There is no recommended restriction of fat l for infants less than 2 years old (American Academy of Pediatrics, 1993). The increase in fat content is of particular interest because infants also require fat as a major source of energy, absorption of fat–soluble vitamins A, D, E, K insulator against body heat loss and for body organs protection. Fat plays a very important role in the proper development of brain cells in infants and children. However, germination/fermentation appeared to negatively affect the quantity of lipid.

Dietary fiber is found in legumes, whole grain foods, fruits and vegetables. Since breast milk contains no fiber so generally infants do not consume fiber in the first 6 months of life. As complementary food is introduced to the diet fiber intake increases. The level of crude fiber in GM/CO was comparable to the adequate 5grams/day recommended intake for 6 to 12 months old infants.

AA profile as shown in Figure.7

In infants, the histidine and arginine are essential amino acids for growth (Lourento and Camillo, 2002).

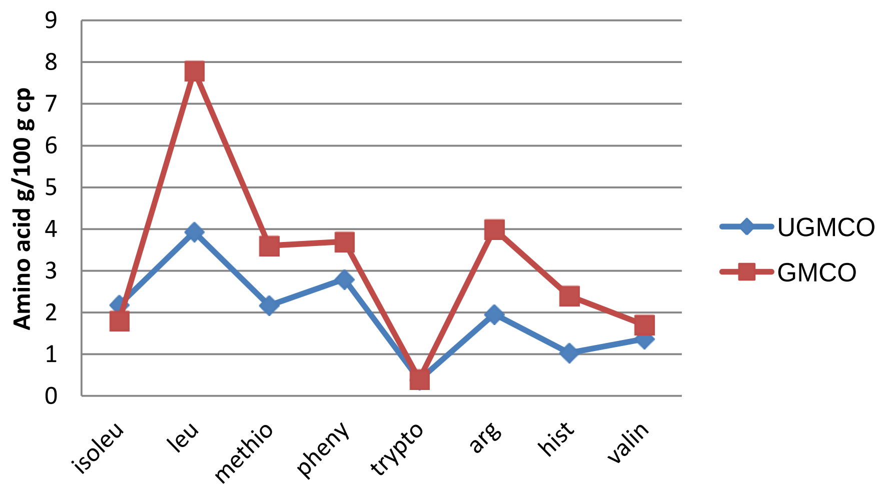

Figure 2. Essential amino acids of UGM/CO and GM/CO.

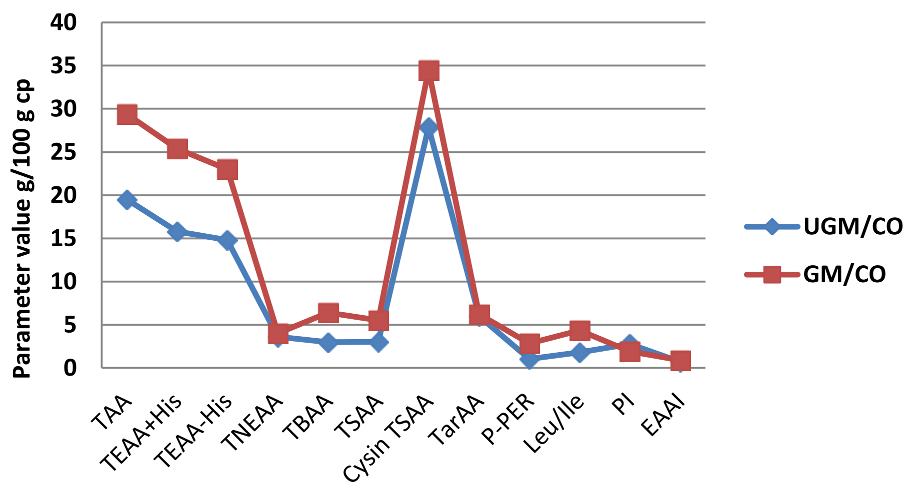

Figure 3. Quality parameter values of amino acids of UGM/CO and GM/CO.

The highest concentrations of amino acids were arginine and leucine. Of all the essential amino acid in both samples leucine had the highest concentration. The following amino acids arginine, hitidine, leucine, cystine, tryptophan, methionine, phenylalanine and valine were more enhanced in GM/CO than in UGM/CO (Figure 3).

Figure 3 shows the values (g/100 g on dry matter basis) of different classes of amino acids present in the samples. The total amino acid in GM/CO was close to the TAA value reported for African yam bean reported by Adeyeye (1997). The TEAA to TAA values were 81.02% in UGMO and 89.40% in GM/CO were significantly ($p > 0.05$) higher than the 39% RDA for infants (FAO/WHO/UNU, 1985); and than that reported for beach pea protein isolate by Chavan et al. (2001). However, the TarAA range of UGM/CO and GM/CO were close to 7.00 to 9.10 g/100 gcp (FAO/WHO/UNU, 1985). There was increase in sulphur amino acids, which might have positive effect on zinc absorption as reported by Mendoza (2002).

Figure 4 showing amino acids (%) quality parameter showed that the P-PER value in GM/CO was higher than 1.21 cowpea, millet and sorgum ogi as reported by Kadam (1989) and Oyarekua and Eleyinmi (2004) respectively. Belavardy (1995) reported that leucine/isoleucine ratio may balance excess leucine alone in regulating pellargra disease process. The Leucine/Isoleucine ratio was low in value.

Arginine, a precursor of nitric oxide, was significantly higher in UGM/CO mixture than mixture with GM/CO (Tejero et al., 2008). Histidine and arginine are essential amino-acids for infant growth because the metabolic pathways that synthesize these amino acids are not fully developed Lourenço and Camilo (2002). Cysteine, Cysteine,

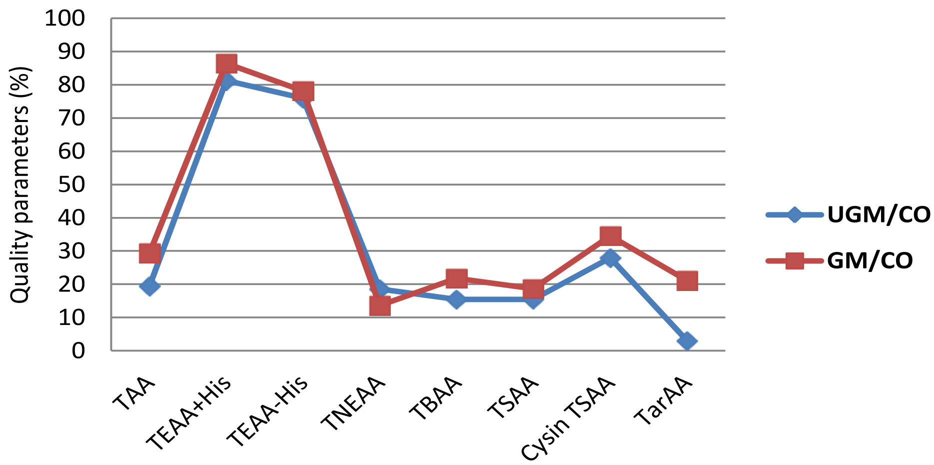

Figure 4. Quality parameters (%) of UGM/CO and GM/CO.

Table 2. Statistics Summary of Figures 1 to 6.

Compared parameters	rxy	rxy2	CA (%)	IFE (%)	TV	Remark
Amino acid profile	0.8065	0.6505	59.10	40.90	0.632	S
AA quality parameters	0.9709	0.9426	24.00	76.00	0.456	S
Amino acid scores- provisional	0.8617	0.7425	50.70	49.30	0.632	S
scores on pre-school child	0.7791	0.607	62.70	37.30	0.754	NS

Key: Rxy – linear correlation coefficient, Rxy2 – coefficient of determination, Rxy – linear regression coefficient, CA – coefficient of alienation, IFE – index of forecasting efficiency, * – significant r=0.05 at n-2 degrees of freedom, NS – not significant at r = 0.05.

histidine which are required for infants growth (FAO/WHO/UNU, 2007) are richer in GM/CO than in UGM/CO.

Tryptophan a precursor of the neurotransmitter serotonin Savelieva et al. (2008), had low but comparable values in both GM/CO (0.40) in UGM/CO samples (0.38). Tyrosine, a precursor of the neurotransmitter dopamine when too low, results in deficiency which can cause liver and kidney failure however GM/CO had a slightly lower value than UGM/CO. The UGM/CO mixture had a higher value of isoleucine than GM/CO mixture. GM/CO mixture was richer in phenylalanine which is the precursor of some hormones and melanin in the skin, eyes and hair. The values in both samples were comparable. Isoleucine is essential for infants and diet low in it can lead to brain damage and early death. The UGM/CO mixture had a higher value of isoleucine than GM/CO mixture.

Diet that is low in methionine leads to PEM, GM/CO mixture had a higher value thus its consumption might prevent PEM. Germination process in the GM/CO mixture may be responsible for the increase of some essential amino acids. From the amino acids profile in Table 2, high sulphur amino acid content is largely retained in GM/CO. The branched chain amino acids, valine and leucine, tend to be at a lower level in UGM/CO mixture but higher in GM/CO. Arginine and leucine had the highest concentrations among all the amino acids analyzed. Leucine constituted the highest single essential amino acid (EAA) in all the samples. The following AA was more concentrated in the GM/CO Arginine, Histidine, Leucine, Cystine, Methionine, Phenylalanine and Valine.

The most affected semi-essential AA by the processing method were arginine and histidine cystine which was reduced from 4.10 to 1.96 in GM/CO and UGM/CO mixtures respectively as shown in Figure 2.

The calculated parameters from amino acids were as shown in Figure 4. TAA, TEAA with or without histidine, TNEAA, TBAA, TSAA, cysteine in TSAA, TarAA, P-PER, leucine/ileleucine were all more concentrated in GM/CO than UGM/CO. The essential amino acid index was higher in GM/CO than UGM/CO while isoelectric point was higher in UGM/CO than GM/CO.

Figure 5 shows the concentrations of essential, non-essential, acidic, neutral, sulphur, aromatic, etc. (g/100 g crude protein) of co-fermented samples (dry matter of sample) based on hen's amino acids scoring pattern, the TAA here (66.8 to 85.9 g/100 g cp) were close to the TAA in the dehulled African yam beans (AYB) (Adeyeye, 1997). The percentage ratios of TEAA to TAA in the samples were 81.02 in UGM/CO and 89.40 for GM/CO which were well above the 39% considered to be adequate for ideal protein food for infants, 26% for children and 11% for adults (FAO/WHO/UNU, 1985). The TEAA/TAA percentage contents were also higher than to

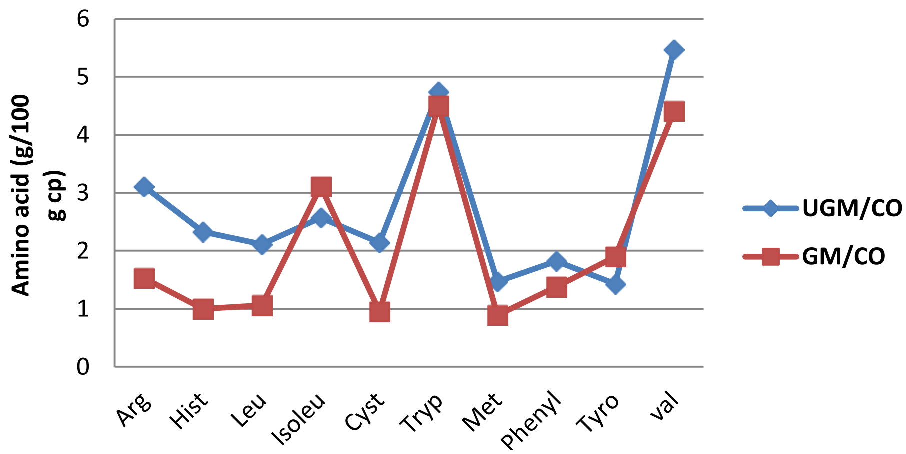

Figure 5. Amino acid g/100 g of UGM/CO and GM/CO based on whole hen's egg scoring pattern.

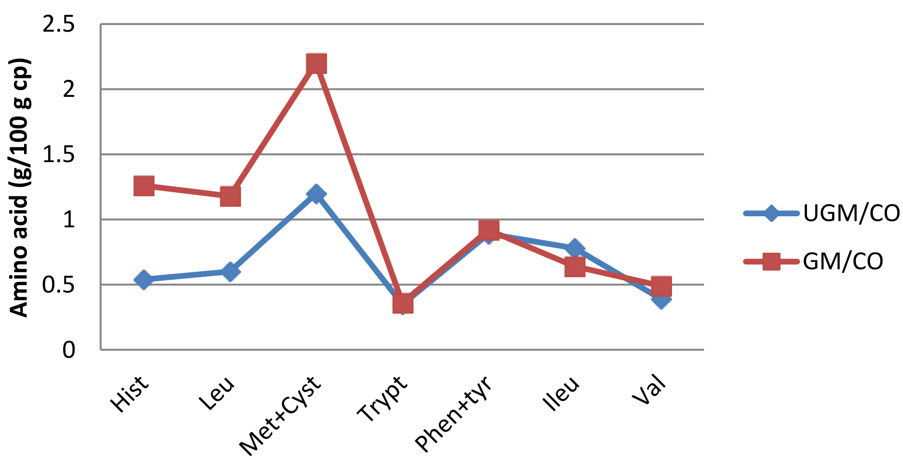

Figure 6. Amino acids of UGM/CO and GM/CO based on pre-school children scoring pattern.

that of egg (50%) (FAO/WHO, 1989), and for beach pea protein isolate (Chavan et al. (2001) and Luffa cylindrica seeds (Olaofe et al., 2008). The percent contents of TSAA (18.7 in GM/CO germinated mixture and 15.4 in UGM/CO were significantly ($p < 0.05$) higher than the 5.8 g/100 g cp recommended for infants (FAO/WHO/UNU, 1985).

The TarAA range from 5.98 (UGM/CO) to 6.20 (GM/CO) were close to that suggested for ideal infant protein from 7.00 to 9.10 g/100 g cp (FAO/WHO/UNU, 1985). Increasing the concentration of sulphur-containing amino acids in staple foods showed that Cysteine had positive effect on zinc absorption (Mendoza, 2002; Sandstrom et al., 1989). The P-PER value in GM/CO was higher than 1.21 (cowpea), 1.82 (pigeon pea) (Salunkhe and Kadam, 1989), 1.62 (millet ogi) and 0.27 (sorghum ogi) (Oyarekua and Eleyinmi, 2004).

The present Leucine/Ilelucine ratios were low in value. The EAAI can be useful as a rapid tool to evaluate food formulations for protein quality. The EAAI for soy flour is 1.26 (Nielsen, 2002), which is better than the current result of 0.69 for mixture of UGM/CO and 0.87 for mixture with GM/CO.

In amino acid score figure 5 based on hens egg essential amino acid scoring pattern, UGM/CO mixture was more enhanced in the following amino acids than its GM/CO analogue: Cystine (2.14 to 0.95), Methionine (1.47 to 0.89), Phenyalaninel (1.82 to 1.38), and Valine (5.47 to 4.41). Arginine (3.11 to 1.53), histidine (2.33 to 1.00), leucine (2.11 to 1.06), cystine (2.14 to 0.95), but GM/CO had enhanced values in isoleucine (3.11 to 2.57) and tyrosine
(1.90 to 1.43), while tryptophan values were comparable (4.74 to 4.50).

However, lack of germination enhanced some AA such as Isoleucine (28.0 to 18.0) and Tyrosine (28.0 to 21). Of the total AA enhanced by germination, four or 40% were EAA. From the present result, germination/fermentation

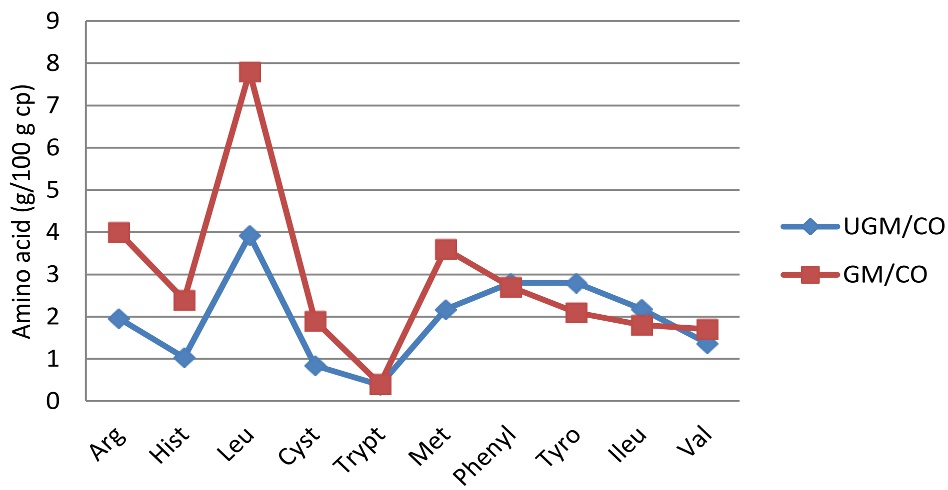

Figure 7. Differences in amino acids profile of UGM/CO and GM/CO.

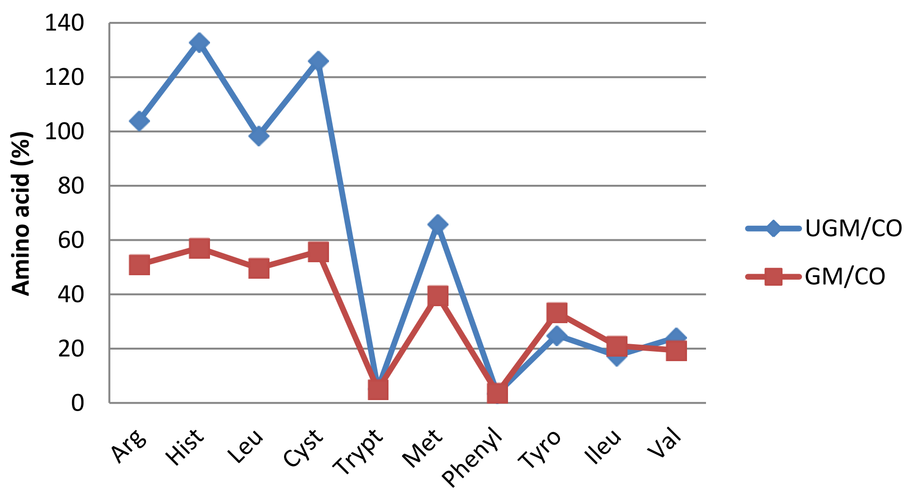

Figure 8. Percent difference in amino acids of UGM/CO and GM/CO.

reduced the total AA.

The suggested patterns of total EAA requirement from literature (mg/gcp) were Histidine: 460 (infant) (FAO/WHO/UNU, 1985), whereas the values of both samples in this study were lower with Histidine were 233 mg/g cp in UGM/CO and 100 mg/cp in GM/CO. Valine was high in our samples; 54.7 mg/g in UGM/CO and 44.1 in GM/CO mg/g.

However according to scores in figure 6 based on pre-school children (2 to 5 years) amino acid scoring pattern GM/CO was more concentrated in histidine, leucine, methionine + cysteine, valine, isoleucine, and valine GM/CO was more concentrated than UGM/CO, while tryptophan phenylalanine+tyrosine and valine had comparable
values in both samples (Figure 5).

Table 2 depicts the statistical summary of the data in Figures 1 to 8. The rxy levels were mostly positively high (0.8065 and 0.9798) whereas the Rxy ranged between 0.6505 and 0.9601.

All the rxy values were significant at r = 0.05 and n-2 degrees of freedom. The coefficient of alienation (CA) ranged from 20 to 50.7% whereas the corresponding index of forecasting efficiency (IFE) ranged between 49.30 and 65.0%. Figure 9 is actually a comparative analysis between GM/CO and its UGM/CO analogue. For proximate composition shows a high rxy value of 0.999958, rxy^2 = 0.999954. Coefficient of alienation CA = 0.006797% index of forecasting efficiency IFE = 99.32. The index of forecasting efficiency (IFE) was high at 99.32.

High IFE means forecasting of relationship was high. The IFE is actually the reduction in the error of forecasting efficiency; the higher the IFE, the lower is the error in the

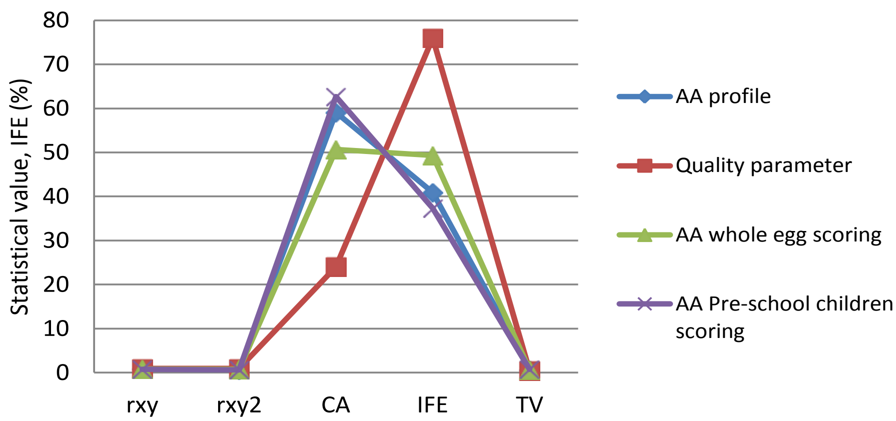

Figure 9. Comparative amino acids statistical data of UGM/CO and GM/CO.

forecasting efficiency. For CA of 1.00, it made the IFE high thereby giving a reduction of 99.0% in the error of prediction of relationship between raw seeds and roasted seeds amino acid profiles.

CONCLUSION

This study shows that maize germination followed by co-fermentation with cowpea enhances the nutritional value of maize and reduces the viscosity which makes it suitable as infant complementary food.

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