EFFECT OF CO-FERMENTATION ON TOTAL PHENOLS, PHYTATE AND MINERALS IN CORN/COWPEA " OGI"

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Abstract

The effect of combined fermentation of maize and cowpea on total phenols, phytates and minerals in the preparation of ogi as complementary food for infants has been studied. While phytate decreased in the co-fermented product, proanthocyanidin reduced slightly just as P, K, Ca, Mg, Cu, and Zn content also reduced in the complementary food. The acceptability of the food also as a complementary feed in infants is discussed.

Introduction

The acceptability of fermented product is not only dependent on its flavor, color and texture after processing but also on its nutritional quality and the absence of anti-nutritional factor like phenolic compounds. According to Chavan and Kadam (1989), fermentation and or germination enhances the nutritional value of cereals by causing significant changes in the chemical composition and the elimination of anti-nutritional factors. The purpose and type of processing applied in preparing complementary foods for infants should induce the inactivation of anti-nutritional factors and reduction in the level of undesirable compounds.

Phytates (myoinositol hexaphosphate) are widely present in cereal and legume seeds, (Ferguson *et al* 1993) and it is the major factor in the low availability of calcium, Zinc, iron and magnesium from these foods. Phytates also interact negatively with protein, thus affecting digestibility. It complexes with proteins mono, and divalent- cations. Mensah *et al* (1991) reported that inhibitory effect of bran on iron could be completely removed by degrading phytic acid. It has also been reported that phytic acid can inhibit pepsin activity and alpha-amylase activity in- vitro, while the ability of phytic acid to inhibit the proteolytic enzyme pepsin has also been established. Reddy and Salunkhel (1980) observed that fermentation of rice for only 8hrs could result in complete hydrolysis of phytates.

Apart from phytates, phenolic compounds are also found in food crops usually as polyphenols. Polyphenols include three major classes of compounds: phenolic acids, flavanoids and condensed tannins like, proanthocyanidin. Phenolic compounds found in food crops are receiving attention as a result of their influence on their nutritional and sensory qualities, for instance; some adverse changes that occur during post-harvest storage and processing have been attributed to some reactions of phenolic substances. They (phenolic substances) reduce the availability of nutrients in food by forming indigestible complexes with other constituents, thus precipitating proteins in the gut, reducing digestibility or inhibiting digestive enzymes and minerals absorption; (Brune *et al* 1991), they also bind with carbohydrates, provoke a stringent reaction in the mouth and make food unpalatable thus affecting voluntary intake of food/feed due to astringency. Tannins, present in cereal may also interfere with the quality of fermented products. Tannins are structurally divided into two subgroups (1) condensed tannins synonymous with polymers of flavanols (catechins) and (2) hydrolysable tannins contain gallic acid or related compounds esterified to a carbohydrate.

Fermentation and/or germination, enhances the nutritional value of cereal by causing significant changes in the chemical composition and the elimination of anti-nutritional factors. (Chavan and Kadam 1989).

The Objectives of this Research Therefore are

(1) To study the level of phenols and minerals in maize-cowpea **"ogi"** consequent upon fermentation with a view to improve its nutritional quality as a complementary food in infants.
(2) To study the effect of fermentation process on the antinutritional factors in combined maize-cowpea "ogi"and
(3) To study the influence of polyphenols on the mineral content of the complementary food.

Materials and Methods Sample Preparation

The corn and cowpea grains were cleaned, and the pebbles were picked out from the grains . Seven hundred gram of corn was weighed with 300 gram of cowpea. Into this w was added 3 times volume of tepid water. The mixture was covered and left at 30°$_c$ for 72hrs.

After 72 hours the mixture was wet milled, mixed with more water, and sieved using cheesecloth. Re-washing and re-sieving of the residues was done, and left to further ferment for 24 hours. After 24 hours the clear water was decanted leaving the slurry. The slurry was dewatered leaving the" **ogi** "cake. The cake was later dried at 60°$_c$ for 48 hours, milled and sieved. The flour was kept in an airtight container in a dry place for analyses.

Estimation of Proanthocyanidins

Extraction of proanthocyanidins has been found to be very inefficient and this has been partially attributed to binding of proanthocyanidins to cellular constituents such as proteins, polysaccharides, minerals and nucleic acids (Swain and Hills 1959). A direct sample extraction with acid-butanol extraction was used in this study.

Proanthocyanidin content was expressed as cyaniding chloride equivalent and was determined by using a calibration curve of the absorbance at 550nm, against the concentrations of standard solutions. The absorbance read at 550nm were subtracted from values obtained from unheated samples so as to get the true value of proanthocyanidin content.

Estimation of Total Phenols

Phenolic compounds embrace a very large group of chemicals of plant origin, which have functional groups of aromatic rings with one or more hydroxyl group. Polyphenols include three major classes of compounds: phenolic acids, flavonoids and condensed tannins. Tannins are structurally divided into two subgroups, condensed and hydrolysable tannins and are usually polymers of five to seven flavan-3-ols (cathechins). Condensed tannins have the property of precipitating protein in aqueous media. Via this mechanism, they interfere with protein utilization compounds and are usually extracted from freeze-dried plant material with methanol, ethanol, 1%HCL or water. The extraction with alcohol or water, especially when hot, may produce artifacts as a result of hydrolysis.

The Folin and Cioclteu method (Swain and Hills, 1959), which is expressed as chlorogenic acid equivalent, is therefore preferred and used in this study for the estimation of total phenols. The limitation however is that Folin 7 Ciocalteu reagent is not specific for phenols, as it can react with other constituents such as amino acids and proteins. (Lowry et al 1951). A standard curve of chlorogenic standard solution was plotted, and results were expressed as chlorogenic acid equivalent

Determination of Minerals

In most fermentation reports, there are little or no change in the individual mineral elements recorded Chompreeda and Fields (1981), observed 37.9% increase in phosphorus during natural fermentation of corn meal and observed that fermentation of corn plus soy blend decreased the solubility of phoshorus and iron but does not affect the solubility of magnesium, zinc or potassium.

Ash, phosphorus and minerals Determination

Dry ashing in furnace involves the burning of a required weight of samples to eliminate the organic matter and liberate the minerals present in the samples; after some molecules in the sample would have been combusted .The method of Egan *et al (*1981) was used in this study.

Some aliquots from each flask were stored in propylene tubes for colorimetric estimation of phosphorus. While the remaining was used for the determination of macro elements Ca^{2+}, Fe^{2+}, K^{+}, Na^{+}, and oligo elements Mg^{2+}, Cu^{2+}, Zn^{2+}, and Mn^{2+} using Atomic Absorptiomeric method.. Phosphorus: For the estimation of phosphorus the colorimetric measurement method (According to AOAC 1990),was used.

A standard curve was traced using solutions 10, 20, 40, and 60ug/ml by adding 1 ml of nitro-vanado-molybdic reagent to 1 ml of each standard solution and coloration read at 430nm on Spectrophotometer.

Estimation of Phytates (Myoinositol Hexaphosphate)

Gibson et al (1998), reported that Phytic acid accumulates under the bran of cereals and this is

significantly reduced during milling. They stated also that other traditional processes like fermentation and even soaking can activate phytases in cereals grains, which causes the degradation of phytic acid and thus the removal of the inhibitory effect of bran on iron absorption. The HPLC analytical technique differentiates phytate and its hydrolysis (phytic = inositol hexa- phosphate) unlike the other analytical techniques. (Obeleas 1983), which affects zinc bioavailability
Therefore, to understand the effect of phytates, in this study, the HPLC analytical method was emplored to estimate the phytic acid as %IP6 using the method of Talamond ***et al_(*** 1998).

Results Table 1

The Content of Anti-nutritional Factors in La)oratory Co-Fermentec Corn/Cowpea

Sample	*Proanthocyanidin (g)*	*Total Phenol (g)*	*Phytate %1P6*
Fermented Corn/Cowpea	***0.060 0.057***	***0.21 0.24***	***0.43 0.43***
Average	***0.059***	***0.22***	***0.43***
Unfermented Corn/cowpea ogi (Control)	***0.034 0.024***	***0.12 0.11***	***1.00 1.00***
Average	***0.029***	***0.12***	***1.00***

Table 2

Sample	Ash(g)	*P	*Na^+	*K^+	*Ca^{2+}	*Mg^{2+}	*Fe^{2+}	*Cu^{2+}	*Zn^{2+}
Fermented Corn/Cowpea ogi	0.74 0.74	156 156	10.01 9.91	96.3 100.1	28.5 28.5	20.7 21.3	16.08 16.00	0.47 0.42	2.44 2.63
Average	0.74	156	9.91	95.5	28.5	21.0	16.04	0.44	2.54
unfernented Corn/cowpea ogi (Control)	2.17 2.46	158 158	1.87 1.87	758.4 758.4	38.4 38.4	158.3 158.3	3.91 3.90	0.55 0.55	2.78 2.78
Average	2.31	158	1.87	758.5	38.4	158.3		0.55	2.78

KEY: * = milligrams.

Organpleptic Evaluation

The sensory evaluation is defined as a scientific discipline used in evoking measuring, analyzing and interpreting results of the characteristics of foods as perceived by the senses of sight, feincll, taste, touch and hearing; this indicates that sensory evaluation encompasses all senses and not (qsting alone. Weanling mothers totaling 50 were initially screened, out of these 25 mothers that were healthy, emotion free with normal taste and smell and with motivation for the exercise were chosen. The 25 panelists were refrained from smoking, eating, drinking or chewing gum 1 hour before the test. (Jcllinek 1985). Corn **"ogi"** corn/cowpea **"ogi"** was made into watery paste and put on hot plate at about 80°c. Gentle stirring to prevent lumping and uneven gelatinization followed this. At the appearance of the first bubble, the paste was cooked for 10 minutes. At the end of the cooking identical portions of the gruels labeled XYD and MZJ were immediately placed on white plastic soup bowls and allowed to cool to 45°c before evaluation.

Score sheets were given to each panelist of 10 members. Hedonic scale was used to evaluate sensory and physical characteristics of the -fermented corn (XYD) and co- fermented corn/cowpea (MZI) respectively on the basis of 5 parameters: color, texture, taste, aroma, and general acceptability. The data were analyzed using the T-test. (International Standard Organization. 1983).

Results and Discussion

Antinutritional Factors

In this study, (Table 1) there was a significant increase in the proanthocyanidin content of co-fermented corn/cowpea mixture (0.059) compared with the control.(unfermented corn/cowpea). (0.029). There was also a significant increase in the total phenol content of co-fermented com/cowpea mixture (0.22) when compared with a value of (0.12) for unfermented corn/cowpea mixture. This is in agreement with Goyal et al (1998) who reported a significant increase in total phenol content of rice- defatted soya flour fermented at35°c for 72 hours. However there was a significant decrease in the phytate content of co-fermented corn/cowpea (0.43) as compared with a value of 1.00 for unfermented corn/cowpea. This is in agreement with Khetarpaul and Chauvan (1998) who reported that single and mixed pure culture fermentation of pearl millet flour with yeasts and lactobacilli at 30°c for 72hrs brought about a significant reduction in phytic acid compared to autoclaved unfermented pearl millet.

Goyal ***et al*** (1998) reported that rice defatted soya flour fermented at $35°_c$ for 24hours in ratios 40/60, 50/50, and 60/40 reduced the phytic acid level to almost half in all blends and reported that higher temperature and longer period of fermentation reduced phytic acid content while total phenols increased significantly or remained constant. Anshu Sharma ***et al_{*** 1998) also reported reduction in phytic acid when fermented rice was incorporated with legume-whey blends.

Mensah ***el al*** (1991), also reported significant reduction, 20% in phytate of Ghanaian dough compared with unfermented maize.). The mechanism of degradation during the preparation of fermented foods is a mixture of endogeneous phytase activity in microbial phytase. Phytate interact negatively with protein thus affecting protein digestibility. It complexes with proteins as well as mono and divalent cathions , they have pH sensitive complexations and decreases the bioavailability, of minerals and solubility, functionality and digestibility of proteins. Phytates also inhibit pepsin activity and alpha-amylase aclivity in- vitro. Significant reductions have also been reported during fermentation due to hydrolysis. According to Svanberg and Sandberg (1991), and Svanberg, 1989); phytate concentration can be lowered by activation of plant endogeneous enzyme phytase which hydrolyse phytate to phosphate and myo-inositol- phosphate thereby increasing mineral availability. However, Davies and Warrington (1986), Sandstrom ***et*** a/_(1983) reported that the effect of phytate depend upon their relative levels, and that of calcium, and zinc as well as the amount and type of dietary protein. Ferguson ***et al*** (1993) affirmed that phytate:zinc molar ratio is a better predictor of zinc availability than phytate alone.

This study also observed little increase in the total phenol (0.22) in co-fermented and (0.12) in unfermented mixture. Slight increase in proantheyanidin (0.059) was also observed in co- fermented mixture when compared with unfermented mixture (0.029). This was contrary to the finding of Goyal et a) (1998). There was significant reduction in the ash content of might be due to the processes of milling and sieving of the co- fermented corn/cowpea mixture. This is in agreement with Ketiku and Ayoku (1984), and Akinyele and Fasaye (1988).

Ash and Minerals

There was general reduction in the content of phosphorus, potassium, calcium, magnesium, copper, and Zinc (0.74, 156, 95.5, 28.5,21.0,0.44, and 2.54 respectively) when compared with unfermented corn/cowpca (2.31, 158,758.4, 38.4, 158.3, 0.55 and2.78 respectively. (Table 2) This finding is in agreement with Hurell (1997) who reported that milling reduced ferrous, Zinc, Calcium, and magnesium. Fairweather et al (1996) also reported reduction in ferrous was due to soaking and sieving. This study also observed showed sharp decrease in calcium but increase in ferrous in co- fermented mixture compared with un- fermented mixture. Sharma and Khetapaul (1998) however reported an increase in calcium and ferrous availability when rice flour and dehulled legume were blended in a ratio of 70.30 compared to control products containing un- fermented blends.

Sensory evaluation

The results of chemical evaluation are usually correlated with the results of sensory evaluation so that acceptability of the food by the ultimate consumer can be correctly ascertained. Odum et aj (1981). For sensory evaluation to be meaningful, they must be interpreted statistically, as was done in this study. Corn "ogi" a fermented and cooked gruel was used as a control, while the co-fermented corn/cotvpe**a** was also cooked.

Color/Appearance

The flour and cake of corn/cowpea had a pale brownish color while that of corn **"ogi"** was brilliant white. However the cooked gruel of both were white. The T- test statistical analysis was carried out on the results of panelists. There was no significant difference (0.>05) between corn **"ogi"** and corn/cowpea **"ogi"** where T=0.75 as against calculated value of 2.064. Little information is available on the importance of color but in Nigeria, mothers prefer light color as weaning food for infants.(Odum etal 1981).

Texture

Corn/cowpea gruel had a slightly rough texture in comparison to corn **"ogi"** probably because of the shafts of corn and cowpea since whole grains were used in fermentation. The t-value of 1.043 was not greater than calculated value of 2.064. Therefore there was no significant difference (>0.05).

Taste: the corn/cowpea had a slight radish taste probably due to the taste of cowpea which might not half been properly done. The t value of 2.368 was greater than calculated value of 2.064. Therefore there was significant difference (<0.05). The co-fermentation of corn/cowpea may have also affected the taste.

Aroma

There was a slight sourer flavor in corn/cowpea "ogi"compared to corn "ogi". There was no significant difference between the two products where t-value of 1.587 was not greater than calculated value of 2.064. Therefore there was no significant difference (>0.05) in the aroma of the two products. Consistency: The corn /cowpea "ogi" was not as viscous as corn "ogi," during cooking the corn ogi gelatinized faster than corn cowpea "ogi" and at 45°c, the corn "ogi" solidified faster than corn/cowpea "ogi".

Degree of Preference

The mean of means for corn/cowpea was **6.95** and that of corn **"ogi"** was **6.75,** therefore corn/cowpea was preferred to corn **"ogi".**

Organoleptic Evaluation Review: The difference between the rating of the two products ws mainly because corn **"ogi"** is the familiar product to the panelists; this might have played an integral part in the panelists response to corn/cowpea **"ogi"** which is the new product. Therefore the judgment of the new product was largely based on comparison with corn **"ogi ".**

Conclusion

Co-fermentation process reduced slightly, the level of antinutrional factors,Goyal et al (1995), reported slight decrease or no change in total phenol content of rice-defatted soy-blends. However polyphenols like total phenol have favorable anti oxidative effects. (Besancon (1999). Cofermentation also reduced the mineral content of the mixture probably due to the fermentation process and the due to the decrease in phytic acid content because there was a negative correlation between the phytic acid and essential dietary minerals. According to Besancon 1999, phytates reduce the bioavailability of ferrous, calcium, manganese copper and zinc, which can lead to loss of bioavailability of some of these minerals and might result in anaemia. In this study soaking and lactic fermentation during which phyase - producing microorganisms develop.Phytate is hydrolyzed by enzyme phytase. Also, there was a relationship between the mineral contents of fermented and unfermented corn/cowpca mixtures.(Table 2). However the content of iron, zinc, and copper of the cofermented product meets the minimum requirement to mention the product as "enriched with minerals" 6-12 months. (Tchibindat and Treche (1999).

If corn/cowpea **"ogi"** is cooked longer than 10 minutes, the taste might be acceptable. However, the rating of corn/cowpea **"ogi"** was still higher than corn **ogi,** despite being a new product. Thus corn/cowpea **"ogi"** is more acceptable than corn **"ogi ".**

Co fermentation, of corn /cowpea is acceptable for removal of antinutritional factors and also enhances the organoleptic acceptability of the co-fermented mixture. This product can be acceptable as an infant complementary food.

 References

Akinyele, I. O., Fasaye O. A. (1988):Nutrient Quality of Corn and Sorghum Supplemented with Cowpea (V. Unguiculata) in the Traditional Manufacture of ogi -***Journal of Food Science*, 53, (6) 1750-1755**

Besancon, Piere (1999) Safety of Complementary Foods and Bioavailability of Nutrients. In: Complementary Feeding of Young Children in Africa and the Middle East.WHO/NHD99:3, WHO/AFRO/Nut 99:4.

Brune, M., Halberg, L., and Skanberg A. B-(1991) Determination of Iron Binding Phenolics Groups *in foods* .-Journal of Food Science, ***58, 128-131.***

Chavan, J. K. and Kadam S.S. (1989)Nutritional Improvement of Cereals by Fermentation—*Critical* Review in Food Science and Nutrition ***vol. 28 issue (5).***

Chompreeda, P.T. and Fields M.L. (1981): Effect of Heat and Fermentation on Extractability of Minerals from Soybean Meal and Corn Blends.- *Journal of Food Science,* 49; 566.

Davies, N.T. and Warrington S.(l 986) The Phytic Acid ,Mineral, Trace Element, Protein and Moisture Content of U.K Asian Immigrant Foods—*Human Nutrition Applied Nutrition* 40A 49- 59.

Egan, H, Kirk R, and Sawyer R. (1981) General Chemical Methods, ash and Mineral Matter. Pp.11- 12. In: Pearson's Chemical Analysis of Foods. 8th edn. Harlow, Uk: Longman Scientific and Technical.

Fairweather-Tait, S. and Hurell R. F. (1996) Bioavailability of Mineral and Trace Elements *-Nut.* Research Review ***9; 295-324.***

Ferguson, E. L., Gibson R.S. ,Opare-Obisaw C., Osei-Opare , Stephen A.M., Lehrfeld J. and Thomson L.U.(1993) The Zinc , Calcium, Copper, Manganese, Non-starch Polysaccharide and Phytate Content of Seventy-eight Locally Grown and Prepared African Foods—*Journal of Food* Composition and Analysis ***.6, 87-99.***

Gibson, R.S., Ferguson E.L, Lehrfeld J (1998) Complementary Food for Infannt Feeding in Developing Countries: their Nutrient Adequacy and Improvement - European *Journal of Clinical Nutrition,* 52,764-770.

Goyal - Rakhi, Khetarpaul-Neeman,Goyal R. and Khelarpaul N. (1995) Changes in the contents of phytic acid and polyphenols of fermented rice-defatted soy blends. *Journal of Dairying food-and Home Science* 14: 1-2, 17-24.

Hurell R. F. (1997) Bioavailability of iron .—*European Journal of Clinical Nutrition*$_x$ 51 Suppl. 1 54- 58.

International Standard Organization. 5495 (1983) ***Sensory Analysis. Methodology. Paired*** Comparison test.

Ketiku, A., Ayoku S. (1984) Nutritional Studies of Nigerian Multimix Weaning Food -Apapa Multimix ***Nigerian Journal of Nutrition*** Sci. 5:39-45.

Jellinek, G. (1985) Sensory Evaluation of foods. Theory and Practice. Chichester U.K. Ellis Horwood.

Khetarpaul, N. and Chauhan B.M (1989) Effect of Fermentation by Pure Culture of Yeasts and Lactobacilli on Phytic acid and Polyphenol Content of Pearl Millet- ***Journal of Food. Science,*** 54 ,(3), 780-781

Lowry O.H, Ronsenbrough N.J., Farr A. L., and Randall R.J. (1951) Protein Measurement with the Folin Phenol Reagent -***Journal of Biol. Chem.*** 193 265-276.

Mensah P., Tomkins D.M., Drasar B. S. and Harrison T. D. (1991) Antimicrobial Effect of Fermented Ghanian Maize-dough.- ***Journal of Applied Microbiology,*** 70, 203-210.

Oberleas, D. (1983) Phytate Content in Cereals and Legumes and Methods of Determination.—***Cereal Foods World,***28, 352-357.

Odum, P. K., Adamson L. A. Morange L. and Edwards C. H.(1981) A Weaning food from locally Grown Grains in Nigeria. Formulation and Organoleptic Evaluation. ***Nutrition Reports International*** 23 1005-1019.

Reddy, N. R. and Salunkhel D. K. (1980) Effect of Fermentation on Phytate Phosphorus and Mineral Content of Black gram, Rice, and Black gram Rice -Blends. -***Journal of Food Science,*** 49, 1780.

Sandstrom B., Cederblad A. and Lnnnerdal B. (1983) Zinc Absorption from Human Milk, Cow Milk and Infant formulars. ***American Journal of Disease of Children*** 137: 726-729.

Svanberg, U. (1987) Dietary Bulk in Weaning Foods and its Effect on Food and Energy Intake. In: Almvick, D,, Moses S. Schmidt O.G. eds. Improving young Child feeding in Eastern and Southern Africa. Nairobi, New York, Stockholm : IDRC, UNICEF, SlDA:272-287.

Svanberg, U. and Sandberg A. S. (1991) Improved Iron Availability in Weaning Foods Using Germination and fermentation—I.NIST CNRS.

Sharma N., Kheitapaul N., (1998) Development of Products Incorporating Fermented Rice-legume whey Blends:Effect on Phytic acid Content and Availability (in-vitro) of Calcium and Iron.- Journal of Ecology and Food Nutrition, **36 (6) 491-500**

Swain ,.!. and Hills W.E. (1959) The Phenolic Consistuents of Pumus Domestica 1 . The Quantitative Analysis of Phenolic Consistuents . ***Journal of Science Food Agric,*** 10:63.

Talamond, P. Gallon G. Guyot J.P. Mborne Lape , Treche S. (1998) Comparison of High Performance ion Chromatography and Absorptiometric Methods for the Determination of Phytic acid in Food Samples. -***Analysis*** 26, 377-381.