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Studies on the Nutritional quality, anti-nutritional factors, amino acid profile and functional properties of co-fermented wheat/cowpea

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ABSTRACT

Nigeria was ranked 14th in global assessment of under-five mortality rate due to infants poor nutrition. (The Punch 2011) The Wheat, genus *Triticum*, tribe,*Triticeae* family, is limiting in lysine but has adequate amount of sulphur-containing amino acids (Adeyeye & Aye 2005).The cultivation of *Triticum durum* yields well in Nigeria. It is mainly consumed by adults who eatit in bread and other pastries (The Compass 2011).There have been cases of hypersensitive response to wheat gluten causing celiac disease which in children, prompts symptoms like bloating, vomiting, diarrhea or constipation (Nagano *et al*., 2003). During fermentation some microorganic enzymes hydrolyze and degrade wheat gluten by almost 80% making it hypoallergenic thus preventing the celiac disease (2007 www; mhtml fermented wheat protein and Gluten intolerance/Love to know Assessed on 6/10/2011). Cowpea (*Vignaunguiculata*(L) Walp.):the black-eyed variety called drum is widely cultivated in Nigeria. It is limiting in methionine. The dry beans is cooked without prior fermentation or dehullingand because the whole beans are unsuitable for infants, the broth or soup of the cooked beans may contain high levels of tannins and phytates extracted from the seed coats of colored beans during cooking thisis served the infants resulting in poor protein digestibility, diarrhea, as the liquid portion (Lyimo *et al*., 1992). Phytates and tannin are responsible for the low availability of calcium, zinc, iron magnesium and poor protein digestibility from cereals and some leguminous foods.

Introduction

Traditional cereal fermentation is a spontaneous process initiated by microflora of the raw materials. It reduces anti-nutritional factors, increases protein digestibility, positively enhance texture, aroma and improve the biological value of fermented foods (Adewumi and Odunfa 2009). The by-products of wheat fermentation have been reported to have better characteristics for food than those of maize (Borowicki*et al*., 2010). In fermentation process the enzyme a-amylase degrades the starch granules consequently lowers the viscosity of the gruel. (Moquet and Tréche 2001)Low viscosity gives gruel of high nutrient and energy densities which is desirable in infants' complementary foods. This study was conducted to draw attention to the nutritional value of wheat co-fermented with cowpea. The outcome of the findings might enhance the nutritional suitability of the product as an infant complementary food.

Materials and methods

Sample preparation

The grains of wheat (*T.durum*) and cowpea (*VignaUnguiculata*) were purchased from a local market in Ado-Ekiti, south-west Nigeria. Then grains were winnowed. For co-fermented sample the mixture (W/C) was wheat/cowpea (700:300 W/W). While for fermented wheat (W) 1000g was used. Each sample was soaked in water 1:3 w/v and left at room temperature of 25-27°C for 72 h. After 72 h fermentation period the sample was wet milled using Semotec mill Model 900, dispersed in water and sieved using Cheese cloth of about 30μm pore size. The sediment was left for further 24h fermentation; after which it was filtered.Filterate was dried at 60°C for 24h, milled and sieved. The resulting flour was stored at -4°C ahead of analyses .Values reported for each test were mean averages of triplicates.

Chemical proximate Analysis: Moisture, crude protein, fat fiber and ash were determined according to AOAC (2005).

pH:pH of fermenting samples were monitored at every 24 hr till 72hr of fermentation as described by (AOAC) Official Analytical Chemists (2005).

Amino acids (AA): About 5-10 μl of defatted and vacuum-dried residue of sample was analyzed using Technicon Sequential Multisample Amino Acid Analyser (TSM), (Technicon Instruments Corporation, New York) was used for the analysis. Analysis period was 76 min and column flow rate was 0.50 ml/min at 60°C with reproducibility consistent within ±3%. Net height of each peak produced by the chart record of TSM was measured and calculated for the amino acid it represented.

Protein Solubility: Was determined using the method of Oshodiand Ekperigin (1989).

Minerals: Cu, Zn, Mn, Mg and Fe was determined by spectophotometry using Atomic Absorption Spectrophotometer, (model703 Perkin Elmes, Norwalk, CT, USA). The standard curve for each mineral was prepared from known standards and the mineral value of samples estimated against that of standard curve. Sodium and potassium values were determined using Flame photometer (Sherwood Flame Photometer 410, Sherwood Scientific Ltd. Cambridge, UK).

Anti nutrients analysis: Estimation of phytin phosphorus (phytin-P) was by the colorimetric procedure of Wheeler and Ferrel (1997).Phytic acid was calculated by multiplying phytin-P by a factor of 3.55 (Enujiugha and Olagunmoye 2001). Oxalate

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content was determined using the procedure of Day and Underwood (1986). Hydrogen cyanide (HCN) was done by according to the standard method of AOAC (2005). Saponin was determined using the method of Obadoni and Ochuko(2001).Flavanoidwas by the method of Boham and Kocipal-Abyazan (1994);alkaloid using the method of Harborne (1973).

Consistency measurements: Measurement for consistency of each gruel was determined using BoswickConsistometer (Laomart CEDEX). Four to 18% (on dry matter basis weight gruel cake:water) were mixed and stirred constantly on hot plate at 80°C . Cooking was timed to 5minutes after which the sample gruel was cooled to 45°C. The first compartment of the consistometer was filled with100ml of the gruel. At time 0, the trigger was pressed to release the gate of the compartment to allow gruel flow by gravity to the second compartment. The distance covered in 30 seconds was measured in millimetres as flow in mm/30secs. Dry matter was determined according to (AOAC, 2005).

Functional properties: Water absorption capacity (WAC): was determined by using the method described by Soulski (1962).Oil absorption capacity: the method of Sathe and Salunkhe (1981); Foaming capacity and foaming stability; according to the method of Coffman and Garcia (1977). The emulsion activity and stability was determined by the method of Beuchat *et al.*, (1975). Gelation was determined according to Coffman and Garcia (1977) as modified by Sathe and Salunkhe (1981).

Results

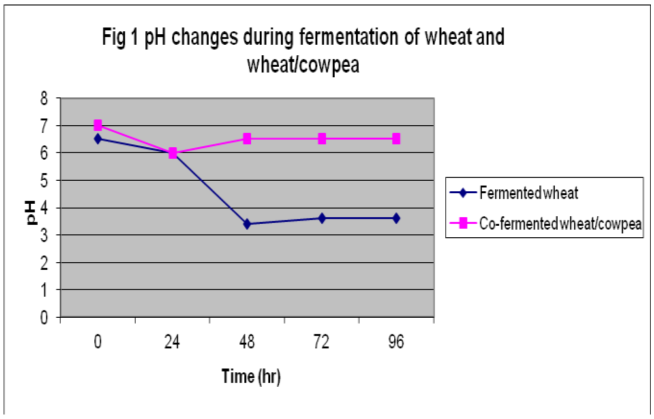


Fig 1 pH changes during fermentation of wheat and wheat/cowpea

Table 1 gives the percentage composition of the following parameters: Ash, Moisture, Crude protein, Mean, Standard deviation (STD) and CV% for the two samples studied

Parameter	W/C	W	Mean	STD	CV%
Ash	0.91	0.56	0.735	0.247487	33.672
Moisture Content	12.19	9.60	10.895	1.831407	16.809
Crude Protein	13.87	3.45	8.66	7.368053	85.081
Lipid	6.35	5.67	6.01	0.480833	80.005
Crude Fiber	4.47	1.14	2.805	2.354666	83.9453
CHO	62.22	83.45	72.835	15.01188	20.6108

Table 2 gives the percentage minerals composition mean Standard deviation (STD) and CV% for the two samples studied

Mineral	W/C	W	Mean	STD	CV%
Na	17.33	3.56	10.445	9.73686	33.671
K	58.91	7.23	33.07	36.54328	11.0502
Ca	12.35	7.00	9.675	3.783021	39.1009
Mg	8.54	4.48	6.71	2.588011	38.569
Zn	0.72	0.28	0.50	0.311127	62.225
Fe	0.74	3.80	2.27	2.163747	95.3192
Mn	0.02	0.30	0.16	0.19799	12.374

Table 3 gives the Mg/Cp Amino acids profile,Mean, Standard deviation (STD) and CV% for the two samplesstudied

Amino acid	Average W/C (g/16gN)	Fermented W (g/16gN)	Mean	STD	CV%
Arginine	80	2.10	5.05	4.17193	82.612
Histidine	48	1.14	2.97	2.588011	87.138
Leucine	158.5	3.84	9.845	8.492352	86.2605
Cysteine	36.5	1.02	2.335	1.859691	79.644
Tryptophan	11	0.34	0.72	0.537401	74.639
Methionine	87	2.90	5.8	4.101219	70.7106
Phenylalanine	82.5	3.10	5.675	3.6416	64.1692
Tyrosine	56	1.86	3.73	2.644579	70.9002
Isoleucine	55.5	2.20	3.875	2.199102	56.751
valine	51.5	2.04	3.595	2.199102	61.1711

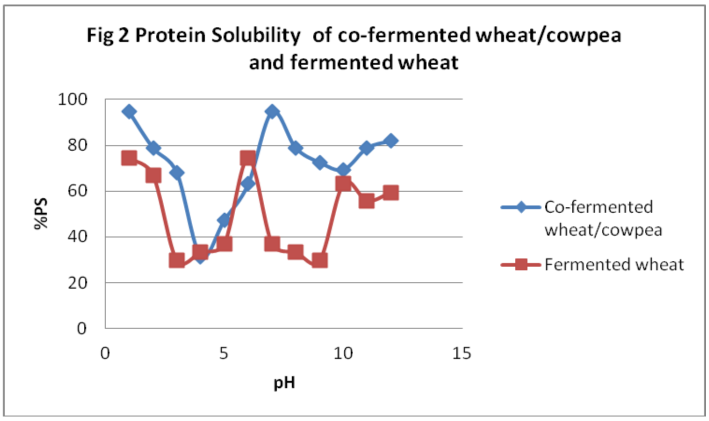


Fig 2 Protein Solubility of co-fermented wheat/cowpea and fermented wheat

Table 4 gives the Antinutritional properties, Mean, Standard deviation (STD) and CV% for the two samples studied

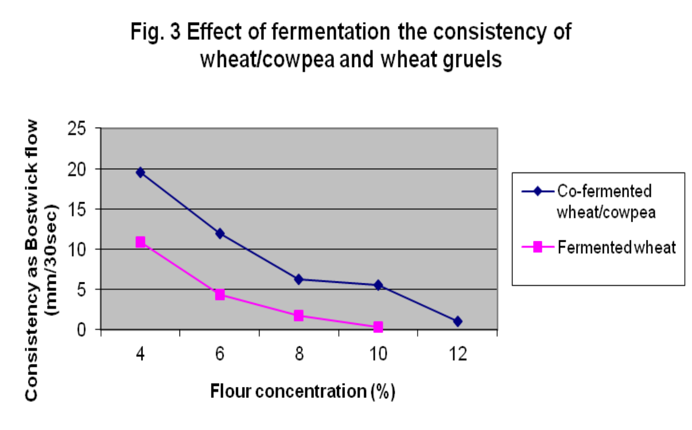
ANF (%)	W/C	W	Mean	STD	%CV
Total Phenol	3.25	0.35	1.80	2.051	11.39
Saponin	1.14	0.70	1.19	0.071	25.80
Alkaloids	2.78	1.24	2.19	0.834	26.8
Flavanoids	1.47	1.60	3.12	3.005	96.5
Mg/PA	0.49	4.76	2.62	3.005	114.9
Oxalate	1.40	1.60	5.50	5.515	100.3
Cyanide	2.60		2.00	0.848	42.4
Phytic		0.123			

Table 5 gives the Functional properties, Mean, Standard deviation (STD) and CV% for the two samples studied

Parameter	W/C	W	Mean	STD	%CV
WAC	260	34.78	147.79	159.113	107.9
OAC	223.4	65.91	144.66	111.362	77.0
FC	20.00	18.00	19.00	1.414	7.4
FS	4.00	8.00	6.00	2.828	47.1
EC	45.38	25.00	35.19	14.410	41.0
ES	50.00	75.00	62.50	17.678	28.3
LG%	4.00	2.00	3.00	1.414	47.1

Discussion

The pH of W gradually decreased from 0h 7.0 to 3.6 by 72h, while in W/C, the pH was 7.0 6.5 and 6.8 at 0,24 and 72 h respectively as shown in fig 1. The increase in W/C might be production of alkaline condition from the cowpea component (Harper and Collins 1992). This finding is contrary to the finding of Schaffner and Beuchat (1986) that the synergistic effect of multi-flora can increase titratable acidity and reduce pH. In table 1,the increase in moisture content of W compared with W/C may be due to contribution of more solids from cowpea component. However the generally low moisture content of both samples may give the products a longer keeping quality.



The higher protein value in W/C than in W might be from the protein contributed by the cowpea component. Protein is needed to build, maintain and repair new tissues in all organs of the body. They manufacture important enzymes, hormones and antibodies. Protein also plays specialized functions in regulating body processes. The value in W/C was significantly higher than estimated need from complementary foods by level of usual breast milk intake(low average and high) for 6-23 mo (WHO 1998) but this value meets the Dietary Reference Intake (DRI) recommended for 12-23 months based on 100g flour while the protein value of W meets DRI for 9-11 by level of average breast milk intake; and also meets the required value for fortified complementary foods per daily ration of 40g for 6-11months.The crude protein of W/C also compared favorably with that reported by (Lazos 1993, Masood *et al.*, 2011).Lipids are needed for energy, promotes accumulation of fats in the body , it serves insulator to reduce body heat loss, allow for absorption of fat soluble vitamins A,D,E,K and promotes fatty acids required for normal brain and eye development and helps in resistance to infection. The lipid (fat) value in W/C meets the required value in fortified complementary foods for 6-23 months based on 50g daily ration while that of W was lower. W/C had higher crude fibre than W this might be from the contribution of cowpea component.

The manner in which protein interact with other components like water, ions , fats carbohydrates, vitamins, colour and flavour constituents directly or indirectly affect processing applications, food quality and ultimate acceptance.(Akintayo, *et al.*, 1998). Protein solubility had been reported to be modified by fermentation (Witoon *et al.*, (1997). A careful study of fig 2 shows highest protein solubility at acidic (73.28%) and basic (63.14%) regions for W/C. This product may therefore be useful in formulating acid foods. The result of the pH effects on protein solubility of W/C in both acidic and basic regions of the pH. Highest solubility at acidic region was 94.66% (pH1) while it was82.04% (pH12) at basic region. For W, highest solubility was 74.28%(pH1) and 63.14 % (pH 10). The fact that the samples were soluble at both acidic and basic regions meant that the mixture might be useful in formulation of acids foods such as protein rich carbonated beverages and milk analogue products (Adeyeye and Aye 2005). In Table 2, generally Na, K and Ca contents were higher than other minerals in both samples as seen in table 2. W/C had higher Na, Ca, Mg K, and Zn than W which had significantly (p>0.05) higher Fe content. Fe and Ca requirements increase by about 10-50% from early infancy through 2 years of age. Calcium is believed to be critical in determining the course of fermentation process and it plays important role in bone and tooth development, blood clotting and maintenance of healthy nerves and muscles. Ca deficiency can cause rickets in infants. The mg/100g values of Na, K, Ca, Fe and Mg in both samples were significantly lower (p0<0.05) for DRI and WHO recommended values for fortified complementary foods for 6-23mo and the needs from complementary foods by level of usual breast milk intake (low, average and high) for 6-23 months old infants. The value of Zn in W/C meets the DRI(IOM 2000) and WHO recommended for fortified complementary foods for 12-23mo, and meets the WHO recommended values for fortified complementary foods for 6-23 mo WHO (1998) while the value in W was too low as shown in table 2. Zinc helps in the formation of protein in the body thus helping in wound healing, blood formation, growth and maintenance of all tissues and taste perception and healthy immune system components of many enzymes. The Mn value in W/C meets the WHO recommended value for fortified complementary foods for 6-8months based on Safe Nutrient Intake from British Dietary Reference Values (WHO 1998).Manganese is needed by infants for proper functioning of the spinal cord because manganese deficiency leads to defective growth of central nervous systems.

Non-heme iron is found in cereals and legumes and infants receive most of iron as non-heme iron. Iron is required for proper growth, formation of healthy blood cells and prevention of iron deficiency anemia. It is a vital component of heamoglobin and part of myoglobin and many enzymes in the body. Iron deficiency leads to anemia, mal-absorption of foods, pallor, irreversible behavioral abnormalities and abnormal functioning of the brain. W had a significantly(p0>.05) higher value of iron than W/C. Sodium is required for water, balance regulation of blood volume and ensure proper functioning of cell membranes and other body tissues. The sodium content was significantly (p0>0.05) higher in W/C (17.33mg/100g) than in W(3.56) but Na values in both samples were significantly (P <0.05) lower than DRI and WHO for 6-23months.Magnesium is required growth, maintenance of bones and proper formation of nerves and muscles. High fiber foods are high in magnesium thus W/C with higher fiber content was also higher in Mg compared with W. Because magnesium is relatively high in cereals and legumes, the deficiency is not common in infants.

Anti-nutritional factors. The study of table 4 showed W/C had more total phenol, saponin alkaloids and flavanoids than W this showed that co-fermentation of wheat and cowpea did not effectively reduced these anti nutrients.

A higher phytic acid content in W fermented sample can be attributed to phytate hydrolysis during fermentation by enhanced activity of the enzyme phytase and that of alpha-amylase leading to the release of Ca ion chelated with phytic acid making phytic acid more available. Ca ion is important for the activity of alpha-amylase (Eiman*et al.*, 2008).Cyanogenic glucoside on hydrolysis yields toxic hydrocyanic acid HCN. Cyanide ions inhibit several enzymes systems; depress growth by interference with certain amino acids (Soetan and Oyewole 2009).Lethal dose of oxalates is between 200-500mg/100g. Saponins are phyto-chemicals. The part of sapogenin is either a triterpene or steroid. The steroid type is found in most of beans and herbs. Saponin comprises of polycylicaglycones. Sapoxin is the toxic type that causes lysis of the red blood cells. There is an emetic effect when saponins are taken in bulk. Saponins control of blood cholesterol levels help in bone health and building of immune system and absorption of important minerals. It is lethal in excess of 0.71%. the values in W/C was 1.14% while that of W was 0.70%.Phyatates, oxalates and phenols bind important minerals thus resulting to their low availability. Oxalates and phenols are easily vaporized at 22°C to 26°C therefore substantial part must have evaporated during the fermentation temperature of between 25-27°C. Lethal dose of oxalate is between 200-500mg/100g.In doses in excess of 20mg/100g of sample, alkaloids cause gastrointestinal and neurological disorders. (Soetan and Oyewole 2009)Alkaloid value in W/C was twice as high as that of W. In this study, anti-nutritional factors reduced more in W than co-fermented W/C, this might be from the cowpea component. However since the toxic effect of anti-nutrients can be avoided if the food is fermented and cooked before consumption; the combination effect of fermentation(Mustafa *et al.,* 2002) and cooking may drastically reduced the effect of anti-nutritional factors to safe levels in both samples especially in W/C which appeared to have improved nutritional value .

According to Silveira and Badiale-Furlong (2009), wheat gluten form a cohesive and visco-elastic mass suitable for bread making; because the gluten proteins are water compatible and thus will swell and interact. In this study as shown in table 5the value of WAC in W/C was significantly higher than that of W, the high value of WAC in W/C might be from the cowpea component which is more hydrophilic than lipophilic in processes like fermentation (Witoon*et al*., (1997). OAC in W/C was also significantly (p0>0.5) higher than that of W and thatreported for wheat and soy flours(Lin *et al.*, 1974, Adeyeye and Aye 2005).This meant that W/C may be a better flavor retainer than W since oil acts as flavor retainer and increases mouthfeel of foods (Oyarekua and Adeyeye 2008). The low FC and Foaming Stability in both products will make the products not to be suitable as whipping toppings agents especially in cake production.

The best least gelling property was in W/C this might make the product to be a better gelating agent than W this might be due to the ratio of different constituents of lipids, proteins and carbohydrates contributed by cowpea in W/C. However, the generally low LG of both products might lead to good setting of stews prepared from them. Decrease in the oil emulsion stability W/C over that W might be due to increased contact between the two components leading to coalescence which might thereby reduce stability this is in agreement with the report of Adeyeye and Aye (2005).The Oil Emulsion stability in both samples was better than 11.0% reported for wheat flour and 18% for soy flour (Lin *et al.,* 1974); thus both products might be useful in the production of soups.

Fig 3 shows that at 10% flour concentration, W/C had a higher consistency value of 5.8mm/30sec than W of 0.4mm/30sec the higher the consistency the lower the viscosity This shows that W/C gruel at 10% might be easy for the infant to swallow and might contain more nutrient and energy densities than W at this concentration. This study showed that the incorporation of 30% cowpea was capable of increasing the consistency contrary to the finding of Schaffner and Beuchat (1986) who reported that the synergistic effect of multi-flora can increase viscosity.

Fermentation process may increase the bioavailability of some essential amino acids. From the amino acids profile in table 3, high sulphur amino acid content is largely retained in W/C d product. The branched chain amino acids valine, leucine, and isoleucine tend to be at a lower level in W but higher in W/C. Phenyalanine is the precursor of some hormones and melanin in the skin, eyes and hair. W/C had higher value of phenylalnine. Tyrosine, when too low, results in deficiency which can cause liver and kidney failure. W/C had significantly (p0>0.5) higher value of tyrosine. Isoleucine is essential for both infants and diet low in it can lead to brain damage and early death. The W/C had significantly higher value of isoleucine than W. Diet low in methionine leads to PEM thus consumption of W/C might prevent PEM.Histidine which is essential for is significantly (p <0.05) higher in W/C than in W. Histidine is essential for infant growth. However isoleucine in W/C was higher than W NAS (1980) reported that wheat is low in isoleucine. Cysteine, tyrosine and arginine are required by infants and growing children (FAO/WHO/UNU 2007). Thus W/C product is rich in essential amino acids.

Conclusion

It is apparent from this study that wheat/cowpea would meet most of the nutritional needs of infants also the combination effect of fermentation and cooking may further drastically reduced the anti-nutritional factors to safe levels in both samples especially in W/C which appeared to have improved nutritional value .

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