Proximate Composition and Functional Properties of Rice (*Oryza Sativa L.*) Germinated-Cowpea (*Vigna Unguiculata L.*) Flour Blends

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Abstract

The study was conducted to investigate the proximate composition and functional properties of various blends of rice-cowpea flour that will provide convenient material base for food product development and processing. Rice Flour (RF) was blended with Germinated Cowpea Flour (GCF) at ratios of 100:0, 90:10, 80:20, 70:30, 50:50 and 0:100 to obtain six composite blends. Moisture Content (MC), ash, crude fibre, protein, carbohydrate (CHO), fat, methionine and lysine were determined for each blend. Functional properties namely; Water Absorption Capacity (WAC), Oil Absorption Capacity (OAC), bulk density, Least Gelation Capacity (LGC), dispersibility was determined. Results obtained showed that there were no significant ($p < 0.05$) differences in the MC, ash content, crude fibre and fat content. The protein content increased steadily with increase in cowpea flour addition from 7.58% in 100% RF to 19.57% in 50:50 (RF: GCF) while the carbohydrate content decreased. Lysine, the limiting amino-acid in rice increased significantly ($p < 0.05$) in the blends from 3.52% in 100% RF to 6.45% in 50:50 (RF: GCF) while methionine, the limiting amino-acid in cowpea, also increased from 1.21% in 100% CF to 2.38% in 90:10 (RF: GCF). The OAC increased significantly from 120% in 100% RF to 220.06% in 50:50 (RF: GCF). The blend of 50:50 (RF: GCF) gave the best improvement in terms of limiting amino-acid lysine and protein content. This study showed that significant stable probiotics, nutrient pockets and security as well as functional properties of rice were attained through the use of germinated cowpea flour in blend formulation.

Keywords: Chemical Composition; Functional property; Germinate cowpea-Rice Blend

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Introduction

Rice (*Oryza Sativa* L.) is a starchy food and a major staple food especially in Nigeria. It has many unique attributes such as ease of digestion; bland taste and hypoallergenic properties. However, rice has relatively low amounts of proteins and most of them are very hydrophobic and therefore resist swelling in water at neutral pH (Lumduwong and Seib, 2000). The low protein contents and absence of gliadin moiety in rice however make it an ideal food material to feed patients suffering from celiac sprue (gluten-sensitivity disease), other chronic diarrhea diseases, and conditions needing low protein diets (Hartsook, 1984). It is cholesterol, fat, sodium and gluten free

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(Hui, 1991). Rice varieties which include Rough, Brown, White, Parboiled and Rice Polish in the United states are classified for trading purposes, by grain size and shape into three types; long, medium and short grain, which have distinct cooking, eating and processing qualities (Hui, 1991).

Rice flour has a growing market with many diverse food applications (Bean and Nishita, 1985). Most rice flour is made from white rice, and major component is starch which finds uses in infant foods, extruded breakfast cereals and snack products. Rice flour (also rice powder) is a form of flour made from finely milled rice. It is distinct from rice starch, which is usually produced by steeping rice in lye. Rice flour may be made from either white rice or brown rice.

The cowpea (*Vigna Unguiculata* L.) is the most popular grain legume in tropical Africa. When grown for its dry seeds. It is also known as black-eyed bean, the black-eyed pea, the southern bean, the china pea, the kaffir pea and the marvel pea. Other varieties grown for their long immature pods, are the yard-long bean, the asparagus bean, the bodi bean and the snake bean. Apart from its high protein value, it is a good source of phosphorus, magnesium, potassium, calcium and iron. It contains a fair amount of the B vitamins and some trace elements (Kordylas, 1990). The development of flatulence upon consumption of cowpea is one of the constraints limiting the consumption of this legume seeds by human.

Nnanna and Philips (1990) reported that germination of seed for 24 h at 30^0C reduced the oligosaccharide responsible for flatulence to almost nil and permitted minimal protease and amylose activities as was desired. Altschull (1974) reported that cereals have low protein content and are in general lysine deficient but are adequate in sulphur containing amino acids. Legume protein rich in lysine but deficient in sulphur containing amino acids should complements the protein in cereal grains since the chemical and nutritional characteristics of legumes make them natural complements to cereal-based diets so as to achieve optimal nutritional level.

Cowpea flour is a convenient food ingredient with the potential to promote industrial utilization of cowpea. It could replace wet paste as the starting ingredient for some traditional West African cowpea foods (Henshaw, 2000). It could also become and ingredient in other food applications such as baked foods (bread, cake) extruded products (noodles, spaghetti) comminuted met products (sausage, ham) and weaning foods (Falcone and Phillips, 1988). According to Mc Watters (1986), the successful performance of legume flours as food ingredients depends on the functional characteristics and sensory quality they impart to end products and functionality of food ingredients is the sum total effect of the properties which affect utilizations such as foaming and emulsification properties (Poul-El, 1981).

In Nigeria, whole rice and cowpea grains mixture is prepared by boiling and this requires long time to cook and produce a softened product that can be eaten. This constraint tends to limit the regularity of combining rice and cowpea in meals. The availability of a ready-to-use flour blends from rice-cowpea would improve the nutritional profile and provide convenient material base for food product development and processing. Therefore, the objective of this study is to evaluate the chemical composition and functional properties of rice-cowpea flour blends.

Materials and Methods

Rice and cowpea grains were purchased from a popular market in Abeokuta, Ogun state, Nigeria.

Methods

The cowpea was processed according to the method of Nnanna and Phillips (1990). The cowpea grains were soaked in water at 30 ± 1^0C for 9h after cleaning. The hydrated grain was germinated in the dark cupboard for 24 h at 30 ± 1^0C. the germinated seeds was collected and dried at a low temperature of 50^0C in air oven for 12h, milled and passed through 210 Nm sieve to obtain germinated cowpea flour.

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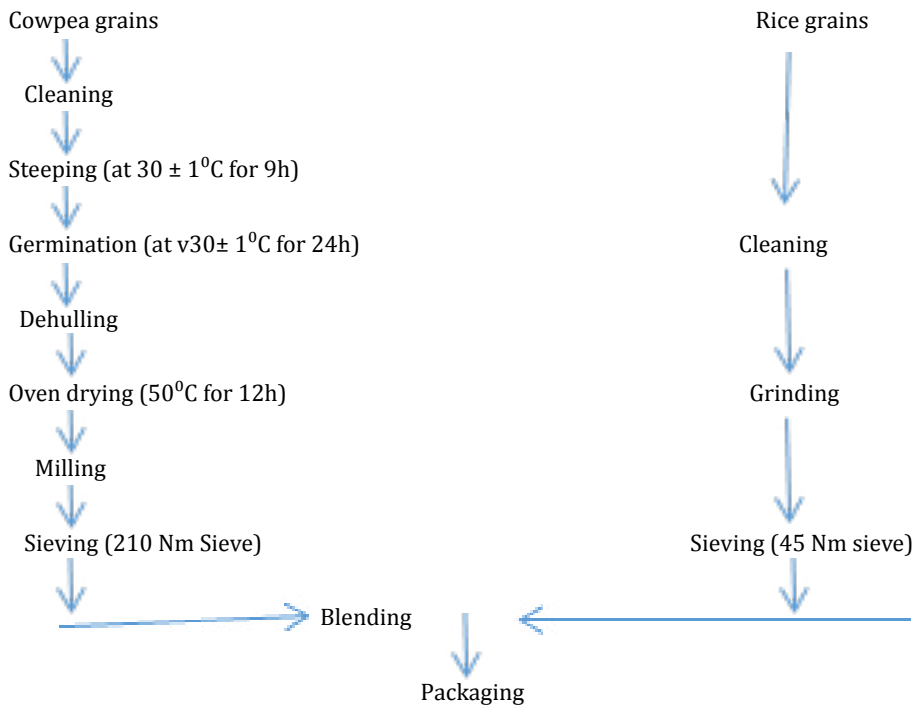**Processing method for rice flour**

The rice grain was processed according to the method of Abulude (2004). The rice grains were cleaned by handpicking to remove impurities, ground to pass through a 45 Nm sieve, packaged and stored for further analyses.

Flour Blending

The blends were prepared as put in Table 1

Samples	Rice Flour (%)	Germinated Cowpea Flour (%)
A	100	-
B	90	10
C	80	20
D	70	30
E	50	50
F	-	100

Table 1: *Ratios of Rice-Germinated Cowpea flour blends.*

Cowpea grains   Rice grains

↓

Cleaning

↓

Steeping (at 30 ± 1^{0}C for 9h)

↓

Germination (at v30± 1^{0}C for 24h)   Cleaning

↓

Dehulling

↓

Oven drying (50^{0}C for 12h)   Grinding

↓

Milling

↓

Sieving (210 Nm Sieve)   Sieving (45 Nm sieve)

↓

Blending

↓

Packaging

Figure 1: *Flow chart for Germinated Cowpea Flour and Rice Flour Production.*

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Sample Analysis

Moisture was determined by hot-air oven drying at 105°C to constant weight (AOAC, 2000). Ash, protein (microKjeldahl, N × 6.25), crude fiber and fat (Solvent extraction) were determined by the AOAC (2010) methods. Calorie was calculated using Atwater factors of 4 × % Protein, 4 × % carbohydrate and 9 × % fat and then taking the sum. Total energy value was determined by multiplying the percentage value obtained from protein, carbohydrate and fat by the value of 4, 4, 9, respectively. The calorie content was calculated as follows: Cal/100g = (Protein × 4) + (CHO × 4) + (Fat × 9).

Determination of pH and Titratable Acidity

The pH and Titratable acidity were determined using method described by Egan., *et al.* (1981). Ten gram of the sample were weighed and homogenized in 50 ml of distilled water for 8 min using a shaker. The pH was measured using a jenway pH meter. The homogenized samples were filtered; 25 ml of the filtrate was titrated with 0.1M NaOH using phenolphthalein as an indicator and the percentage total acidity expressed as lactic acid equivalent.

$$\% \text{ Lactic acid } = \frac{\text{Titre value} \times \text{Molarity} \times 90 \times 100}{1000 \times \text{Volume of sample}}$$

Determination of Methionine

Methionine was determined using method described by Lunder (1973). About 0.2g of samples were weighed into a 30 ml test tube. Trisbuffer reagent (10 ml, pH 7.2) was added with 0.1 mg papain and incubated at 55^0C for 18h overnight. It was removed from incubator and 4 drops of orthophosphoric acid (88 %) to stop enzymatic hydrolysis. The mixture was transferred into a 25 ml volumetric flask and made up to volume with distilled water. The solution obtained was shaken thoroughly and filtered through a Whatman No 4 Filter paper into a test tube. About 5 ml alcquot was pipetted out of this into another test tube to which 1 ml of 5N NaOH, 1 ml 1% aqueous glycine and 1ml 1% of sodium nitroprusside were added. The tubes were kept in the water bath at 40^0C for 10 min after thorough shaking. The contents were allowed to cool and 6ml of 88% orthophophoric acid added with thorough shaking to obtain a red colour. Methionine standard of range 0.5 to 2.5 mg/ml were prepared and treated as above. Absorbance of samples and standard were read on the spectronic 20 spectrophotometer at wavelength 520 Nm.

% Methionine or Methionine in g/100g protein is calculated using the formula:

$$\text{Methionine g/100g protein} = \frac{\text{Factor} \times \text{Absorbance} \times 100}{\text{Sample weight} \times \text{protein \% in sample}}$$

Determination of lysine

Lysine was determined using the method described by Jambunathan., *et al.* (1983). About 1g of samples were weighed into 50 ml centrifuge tube. Forty miles (40 ml) of acid orange dye solution was added and shaked for 1h. The mixture was filtered through a Whatman No 4 filter paper into a 50 ml volumetric flask and made up to mark with distilled water and incubated at 60^0C for 18h overnight. Standard lysine was also prepared from range of 0.35 to 0.85 mg/ml and treated with acid orange dye to develop colour. The absorbance for standard as well as sample was read on the spectronic 20 Spectrophotometer at wavelength 415 Nm.

% Lysine or lysine in g/100g protein is calculated using the formula:

$$\text{Lysine g/100g protein} = \frac{\text{Factor} \times \text{absorbance} \times 100}{\text{Sample weight} \times \% \text{ protein in sample}}$$

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Functional properties

Water and oil absorption capacity were determined as described by Hayta., *et al.* (2002). Bulk density was determined using the method of Akapapunam and Markakis (1981).Least gelation capacity was determined as described by Abulude (2001). Dispersibility was determined according to the procedure of Chukwu., *et al.* (1995). All determination was done in triplicates.

Statistical Analysis

All data were subjected to analysis of variance (ANOVA) to determine any significant difference at 5% level (Steel and Torrie, 1980) and was reported as means of three replicates. Means were separated by Duncan's multiple range tests to establish if there were significant differences between the samples (SAS, 1999).

Results and Discussion

Table 2 shows the percentage moisture of Rice-Germinated Cowpea flour blends samples. There was significant difference ($p < 0.05$) among the samples. Moisture content ranged between 8.30%-12.70%. The observed values for the blends are within the value (12%-15%) designated as sample grade under U.S. standard whereas 12% is recommended for long-term storage (Spadaro., *et al.* 1980). This implies that the blends may have long shelf life. From this study, high ash content was recorded in [Table 2] by 100% Germinated-cowpea flour (2.83 %), suggesting that germination process increases the mineral content. The values reported are consistent with the values reported by Agu and Aluyah (2004) for maize, soybean and fluted pumpkin seed flour. The fibre content of the blends increased with increasing level of germinated-cowpea flour substitution. The fibre content of 100% germinated-cowpea flour (2.16%) was higher than 100% rice flour with the least crude fiber content (1.56%). The amount of crude fibre in the flour samples may influence the digestibility of menu or diets prepared from the products, and may also help to maintain the normal internal distention of the intestinal tract and thus, aid peristaltic movements (Edem., *et al.* 1984).

Samples	Moisture Content (%)	Ash Content (%)	Crude Fibre (%)	Protein (%)	Fat (%)	Carbohydrate (%)
A	12.70[d]	2.13[c]	1.56[a]	7.58[a]	2.00[d]	75.18[d]
B	12.40[d]	1.60[a]	1.60[b]	12.53[b]	1.15[b]	70.10[c]
C	12.20[d]	1.70[b]	1.64[b]	16.70[c]	1.07[a]	66.99[b]
D	11.40[c]	1.73[b]	1.76[c]	18.50[d]	1.06[a]	66.34[b]
E	9.37[b]	2.33[c]	1.82[d]	19.57[e]	1.03[a]	66.04[b]
F	7.84[a]	2.83[d]	2.16[e]	22.30[f]	1.42[c]	60.70[a]

A = 100% Rice Flour, B = 90% Rice Flour; 10% Germinated-Cowpea Flour, C = 80% Rice Flour; 20% Germinated-Cowpea Flour, D = 70 % Rice Flour; 30% Germinated-Cowpea Flour, E = 50 % Rice Flour; 50% Germinated-Cowpea Flour, F = 100 % Germinated-Cowpea Flour.

Table 2: *Proximate composition of Rice-Germinated Cowpea Flour Blends.*

The protein content of the blends increased as germinated-cowpea flour substitution increased. The highest value was observed in sample F (Germinated-cowpea flour) and lowest in sample A (100% Rice flour). Since legumes, including cowpeas are low in sulphur amino acids but high in lysine (Okoh., *et al.* 1981) the proteins in cowpeas flour complement that of rice and thus improve nutritional quality of the blends. In a similar study, Achi (1999) observed a steady increase in the protein content of the yam flour supplemented by soy flour in the blends. In this study, such significant increases were observed both at 0% and 50% rise in cowpea flour content.

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The highest value was observed in sample A (100% Rice flour) with and lowest in sample E (50:50 blends). The value (2.00%) obtained by sample A is in accordance to that reported by King and Puwastein (1987), but low when compared with the value reported for selected seafood (2.4-4.7 %) by Ogunlade., *et al*. (2005) and 2.29 - 5.1% for maize, soybean and fluted pumpkin seed flour (Agu and Aluyah, 2004).

The Carbohydrate level in 100% Rice flour (75.18%) was significantly ($p < 0.05$) higher than 100% Germinated-Cowpea flour (60.70%). This also might be an indication of increase in the level of carbohydrate in Rice flour. The value found in germinated cowpea flour was similar to the findings of Akpapum and Achienewhu (1985) for germinated cowpeas.

Samples	Energy (kcal/100g)	pH	TTA (%)	Methionine (g/100g protein)	Lysine (g/100g Protein)
A	337.28[b]	6.17[b]	0.03[b]	3.29[d]	3.52[a]
B	342.55[c]	6.01[b]	0.02[a]	2.38[c]	6.04[b]
C	343.83[c]	6.21[c]	0.03[b]	2.33[c]	6.25[c]
D	346.13[c]	6.27[c]	0.04[b]	2.13[b]	6.33[c]
E	355.60[d]	6.60[d]	0.06[c]	2.09[b]	6.45[cd]
F	329.25[a]	5.45[a]	0.11[d]	1.27[a]	6.58[d]

A = 100% Rice Flour, B = 90% Rice Flour; 10% Germinated-Cowpea Flour, C = 80% Rice Flour; 20% Germinated-Cowpea Flour, D = 70% Rice Flour; 30% Germinated-Cowpea Flour, E = 50% Rice Flour; 50% Germinated-Cowpea Flour, F = 100% Germinated-Cowpea Flour.

Table 3: *Chemical composition of Rice Germinated-Cowpea Flour Blends.*

From table 3, it can be deduced that the high energy observed in germinated cowpea flour samples may be due to the breakdown of starch, which is broken down into simple sugar, which are used to generate energy for germinating seeds. The pH of the flour blends ranged between 5.45-6.60 in table 3 agrees with the findings of pearson (1976) who reported pH range of flours of 6.0-6.8. The lysine contents of the blends increase with increasing level of cowpea flour substitution while reversal effect was observed for methionine value. Both the lysine and methionine value of the samples was in the range (4.32 and 2.30g/100g protein) reported by Ene-Obong and Carbovale (1992) for cowpea and 3.9 and 3.0g/100g protein by Oyenuga (1968) for rice respectively, though germinated cowpea flour samples were observed to be high.

Samples	WAC (%)	OAC (%)	Bulk Density (g/ml)	LGC (%)	Dispersibilty (%)
A	225.03[d]	120.80[b]	0.79[a]	22.00[d]	64.67[a]
B	189.43[a]	103.67[a]	0.81[ab]	16.00[b]	85.70[b]
C	191.70[ab]	158.20[c]	0.82[ab]	18.00[c]	86.63[b]
D	206.70[bc]	160.00[c]	0.83[ab]	18.00[c]	86.80[b]
E	211.40[c]	220.60[d]	0.83[ab]	18.00[c]	87.93[bc]
F	199.60[b]	210.20[d]	0.85[b]	15.00[a]	89.27[c]

A = 100% Rice Flour, B = 90% Rice Flour; 10% Germinated-Cowpea Flour, C = 80% Rice Flour; 20% Germinated-Cowpea Flour, D = 70% Rice Flour; 30% Germinated-Cowpea Flour, E = 50 % Rice Flour; 50% Germinated-Cowpea Flour, F = 100% Germinated-Cowpea Flour.

Table 4: *Functional Properties of Rice-Cowpea Flour Blends.*

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Table 4 shows the water absorption capacity (WAC) of the flour blends. The WAC was observed highest in sample A (225.03%) and lowest in sample B (189.43%). Water absorption capacity or characteristics represent the ability of a product to associate with water under conditions where water is limited (Singh, 2001). The highest WAC of potato flour could be attributed to the presence of higher amount of carbohydrates (starch). Aletor., *et al.* (2002) reported that the WAC ranging from 149.1% to 491.5% is considered critical in viscous foods such as soups and gravies. Thus, the flours might find use as functional ingredients in soups, sausages, dough and baked product.

Oil absorption capacity (OAC) has been attributed to the physical entrapment of oil. This is important since fat acts as flavour retainer and increases the mouth feel of foods (Eke and Akobundu, 1993). The highest value was observed in sample E (50:50 blends) and lowest in sample B (90:10 blends). Germination increased the OAC of the cowpea flour. The values reported for 100% Rice flour (120.80%) were close to the report of rice flour (89.75%) by Chandra and Samsher (2013) and quinoa flour (84.6%) by Ogungbenle (2003).

The highest bulk density was observed for 100% germinated-cowpea flour (0.85 g/ml) and lowest for 100% rice flour (0.79 g/ml). The high bulk density of flour suggests their suitability for use in food preparations. On contrast, low bulk density would be an advantage in the formulation of complementary foods (Akpata and Akubor, 1999).

Least gelation capacity is a measure of the minimum amount of flour or blends of flours that is needed to form a gel when mixed with water and heated. The higher the least gelation capacity, the higher the amount of flour needed to form a gel (Adebowale, 2002). The values obtained were similar and lower than the fermented locust bean (30%) reported by Ikpeme., *et al.* (2003) but higher than the value (10%) observed by Sathe., *et al.* (1982) for great northern bean and (14%) for lupin seed flour.

The higher the dispersibility, the better the flour reconstitutes in water (Kulkarni., *et al.* 1991). An increased in values was observed for the blends but higher values was observed in germinated cowpea flour. However, the values are relatively high and the flours will reconstitute easily to give fine consistent pastes (Kulkarni., *et al.* 1991).

Conclusion

This work has shown that significant improvement in the chemical composition and functional properties can be attained through the use of germinated cowpea in a blend formulation. Substitution up to 50% (50% Rice Flour and 50% Germinated-Cowpea Flour) is recommended, since the blend gave the best improvement in terms of limiting amino acid lysine and protein content.

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