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CHEMICAL AND MICROBIOLOGICAL EVALUATION OF COMMERCIAL COWPEA FLOURS SOLD IN ENUGU, ENUGU STATE, NIGERIA

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The proximate composition and microbiological qualities of commercial cowpea flours sold in three major markets in Enugu Southeastern, Nigeria were investigated. The home processed cowpea flours were randomly purchased from Ekeagbani, Ogbete and Aria markets, respectively and analyzed for proximate composition and microbial qualities using standard methods. The proximate composition of the flours showed significant ($p \leq 0.05$) differences in moisture, crude protein, carbohydrate and energy contents. The microbiological analysis of the flours showed that the mean microbial counts of the samples procured from Ekeagbani market were total aerobic bacterial count, $1.33 \pm 0.48 \times 10^5$ cfu/g, coliform count, $4.0 \pm 4.0 \times 10^4$ cfu/g, Escherichia coli count, $1.5 \pm 2.5 \times 10^4$ cfu/g and fungal count, $9.8 \pm 1.8 \times 10^4$ cfu/g. The mean microbial counts of the samples from Ogbete market were total aerobic bacterial count, $1.19+0.29 \times 10^5$ cfu/g, coliform count, $6.1 \pm 1.3 \times 10^4$ cfu/g, Escherichia coli count, $2.3 \pm 2.7 \times 10^4$ cfu/g and fungal count, $9.0 \pm 3.0 \times 10^4$ cfu/g. The mean microbial counts of the samples from Aria market were total aerobic bacterial count, $1.08 \pm 0.33 \times 10^5$ cfu/g, coliform count, $5.1 \pm 2.3 \times 10^4$ cfu/g Escherichia coli count, $1.8 \pm 2.2 \times 10^4$ cfu/g and fungal count, $1.5 \pm 1.5 \times 10^4$ cfu/g. The bacteria isolated from the samples were *Bacillus spp, Escherichia coli, Pseudomonas spp* and *Staphylococcus aureus* whereas the fungal genera isolated were *Aspergillus, Penicillium* and *Rhizopus*.

Keywords: Commercial cowpea flour, Proximate composition, Energy value, Microbial quality

INTRODUCTION

Legumes are common and popular classes of food consumed by many people in developing countries. Legumes which are otherwise known as the poor man's meat are the major source of protein used to complement cereal–based diets in most sub-Saharan African countries. Grain legumes have an advantage of containing twice as much protein as cereals and the nutritional value of their protein is generally of high quality. The high content of lysine in legumes make them suitable complements to cereals which are usually low in lysine (Okoye and Mazi, 2011). Cowpea (*Vigna unguiculata*) commonly called beans in Nigeria is an important grain legume in West Africa, other tropical countries and United States of America. Cowpea is one of the major cultivated *vigna* species among the 25-26 cultivated legumes selected from 600-700 genera of the *leguminoseae* family of plants. Cowpea generally contains 28-35% protein, 1-2% fat, 1.5-4% crude fibre, 3-4% ash and 55-58% carbohydrate, in addition to some vitamins and minerals (Lasekan *et al.*, 1987). These food components are distributed in the cotyledons, embryonic axis and hull or seed coat (Enwere, 1998). The nutritional quality of the cowpea protein depends

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on its essential amino acid content. Like soybean, cowpea is a good source of lysine, adequate in tryptophan but deficient in methionine and cystine (McWatters and Brantley, 1982). Cowpea is a unique food ingredient because it can be prepared into different food products, prior to consumption. The seeding, tender green leaves, seeds and pods that are fresh and immature can be cooked and eaten as vegetable. When dry and mature, it can be cooked as dehulled and undehulled seeds and eaten as soups, stews and with vegetables, fruits, maize, rice, millet, plantain, cocoyam, cassava and other foods (Ihekoronye, 1999). Cowpea seeds can also be processed into various products such as flour, paste, protein concentrate and isolate and extruded products which can be used for the preparation of a wide range of food products (Okoye and Okaka, 2009). Cowpea flour has been processed and used in many food preparations such as moin-moin, baby foods and baked products (Enwere and Uzombah, 1991; and Enwere, 1998). Of the legumes consumed in Nigeria, cowpea appears to be the most popular because they can be easily utilized in many food preparations. The objective of this study is to determine the proximate composition and microbiological qualities of commercial cowpea flours sold in three major markets in Enugu, Nigeria.

MATERIALS AND METHODS

Source and Preparation of Materials

The samples of cowpea flour used for the study were procured from Ekeagbani, Ogbete and Aria markets in Enugu, Enugu State, Nigeria. Four samples of the flour were randomly purchased from each of the three markets. Thereafter, the flour samples were subsequently packaged individually in an airtight plastic container and kept in a freezer until needed for analysis. The chemical reagents employed in the study were of analytical grade and were products of BDH Chemicals, Pooles, England. The microbiological media used were products of Oxoid and Defico Laboratories, England. The media used included nutrient agar used for the estimation of total heterotrophic bacteria, Sabouraud Dextrose Agar (SDA) used for the isolation of fungi and MacConkey agar used for the isolation of coliform bacteria.

Chemical Analysis

The moisture, crude protein, fat, ash and crude fibre contents of the samples were determined in triplicate according to the method of AOAC (2006). Carbohydrate was determined by difference (Onwuka, 2005). The energy content of the flours was calculated from the proximate composition using the Atwater factor 4xprotein, 9xfat, 4xcarbohydrate (Otunola *et al.*, 2004).

Microbiological Analysis

The total heterotrophic bacteria, fungi and coliform bacteria were enumerated according to the method of James (2003). Two grams of each sample of cowpea flour was serially diluted in ten folds. The total viable heterotrophic aerobic counts were determined in duplicate using the pour plate technique. The molten nutrient agar, Sabouraud dextrose agar and MacConkey agar were poured individually at 45 ^{o}C into the petri dishes containing one millilitre of appropriate dilution for the estimation of the total heterotrophic bacteria, fungi and coliform bacteria, respectively. The petri dishes containing the media and appropriate dilution in each case were swirled to mix and incubated at room temperature (30±2 ^{o}C) for 48 h. After incubation, the colony counts were taken in each case with the aid of Gallenkamp electronic colony counter and the mean values obtained were individually recorded.

Characterization and Identification of Isolated Microorganisms

The bacterial isolates were characterized and identified after studying their gram reaction and cell micro morphology. Other tests performed included spore formation, motility, oxidase and catalase production, citrate utilization, oxidative/fermentative (O/F) utilization of glucose, indole production, methyl red-Voges Proskaur reaction, urease and coagulase production, starch hydrolysis and sugar fermentation. These tests were performed according to the methods of James (2003) and Prescott *et al.* (2003). The microbial identification was performed by the use of the keys provided in the *Bergeys Manual* of *Determinative Bacteriology* (1994). The fungal isolates were examined macroscopically and microscopically using the needle mount technique. Thereafter, the fungal isolates were properly identified according to the method of Larone (1986).

Statistical Analysis

The data obtained were subjected to Analysis of variance (ANOVA) to detect significant differences ($p \leq 0.05$) among the sample means. The Turkey's Least Significant Difference (LSD) test was used where applicable in separating significant means (Okaka, 2010).

RESULTS AND DISCUSSION

The proximate composition of the cowpea flour samples are

shown in Table 1. The moisture content of the flours ranged from 9.12 to 11.94%. The values obtained in this study were lower than those (10.25-12.28%) reported by Eze *et al.* (2008) for commercial home prepared soybean flours. The low moisture content enhances the storage stability of legume and other flour products (Okaka, 2005). The crude protein content of the samples showed significant ($p \leq 0.05$) difference between the samples. The variation in the protein content of the flours could be due differences in processing treatments employed during processing (Hung *et al.*, 1988; and Enwere, 1998). The level of protein in all the samples of the flour is relatively high enough to make significant changes in the amount of protein intake of the consumers who buy the products and incorporate them in home or local food preparations. The fat content of the flours which ranged from 0.44 to 0.48% was generally lower than those (14.76-26.26%) reported by Udensi and Onuora (1996) for commercial soybean flours. The low fat content of the samples is an indication that the bean flours could be stored for a long period without the problem of peroxidation which is the major cause of fat instability (Ocheme *et al.*, 2008). The ash content of the samples ranged from 3.22 to 3.42%. The values obtained in this study were similar to those (3.32-46%) reported by Lawhon *et al.* (1992) for defatted soybean flour. The crude fibre content of the flours showed no significant ($p \leq 0.05$) difference between the samples. Fibre has been credited for promotion of increased intestinal distention and maintenance of normal peristaltic movement of the gastrointestinal tract in humans (Okaka *et al*., 2006). The carbohydrate content of the samples showed significant ($p \leq 0.05$) variation between the samples. The observed differences in carbohydrate content of the flours may be due to the addition of additives such as starch and sugar to the products by their processors during processing (Udensi and Onuora, 1996). This calls for the attention of the regulatory agencies such as National Agency for Food and Drug Administration and Control (NAFDAC) and Standard Organization of Nigeria (SON) to come up with standards and regulations for the packaging and labeling of cowpea flour and other home made or locally processed legume products in order to maintain their quality and protect the health of their consumers. The energy content of the flours ranged from 341.10 to 352.24 KJ/100 g. The differences could be attributed to variation in their protein and carbohydrate contents (Ngoddy *et al*., 1986). In effect, the result of the proximate composition of the commercial cowpea flours revealed that they have the potential to be used as nutritional supplements especially in developing countries where there is acute shortage of protein in order to meet the protein-energy needs of their populace.

Table 1: Proximate Composition of Cowpea Flours

Samples	Moisture (%)	Crude Protein (%)	Fat (%)	Ash (%)	Crude Fibre (%)	Carbohydrate (%)	Energy (KJ/100 g)
Em1	$9.86^{k}\pm0.32$	$27.34^{i}\pm0.10$	$0.48^{a}\pm0.02$	$3.40^{a}\pm0.36$	$3.32^{b}\pm0.35$	$58.92^{d}\pm0.60$	$349.36^{d}\pm2.42$
Em2	$9.68^{i}\pm0.28$	$28.42^{d}\pm0.11$	$0.46^{a}\pm0.02$	$3.34^{b}\pm0.34$	$3.30^{b}\pm0.35$	$58.92^{d}\pm0.60$	$349.36^{d}\pm2.42$
Em3	$9.58^{i}\pm0.26$	$26.62^{k}\pm0.09$	$0.44^{a}\pm0.01$	$3.22^{c}\pm0.34$	$3.32^{b}\pm0.35$	$60.14^{b}\pm0.62$	$351.00^{c}\pm2.46$
Em4	$9.42^{h}\pm0.24$	$28.64^{c}\pm0.11$	$0.48^{a}\pm0.02$	$3.32^{b}\pm0.35$	$3.32^{b}\pm0.35$	$58.16^{f}\pm0.58$	$351.34^{b}\pm2.50$
Om5	$9.12^{g}\pm0.19$	$26.04^{l}\pm0.9$	$0.46^{a}\pm0.02$	$3.42^{a}\pm0.37$	$3.28^{c}\pm0.34$	$60.94^{a}\pm0.62$	$352.24^{a}\pm2.52$
Om6	$10.18^{f}\pm0.18$	$27.44^{h}\pm0.10$	$0.46^{a}\pm0.02$	$3.24^{c}\pm0.34$	$3.32^{b}\pm0.35$	$58.68^{e}\pm0.59$	$348.62^{e}\pm2.40$
Om7	$10.14^{f}\pm0.16$	$28.22^{f}\pm0.12$	$0.47^{a}\pm0.02$	$3.34^{b}\pm0.35$	$3.28^{c}\pm0.34$	$57.92^{g}\pm0.58$	$348.43^{f}\pm2.38$
Om8	$10.24^{e}\pm0.20$	$26.82^{j}\pm0.09$	$0.44^{a}\pm0.01$	$3.26^{d}\pm0.34$	$3.18^{e}\pm0.28$	$59.22^{c}\pm0.60$	$348.12^{g}\pm2.36$
Am9	$10.68^{d}\pm0.38$	$27.86^{g}\pm0.10$	$0.46^{a}\pm0.02$	$3.26^{c}\pm0.34$	$3.20^{d}\pm0.28$	$57.78^{h}\pm0.59$	$346.34^{h}\pm2.35$
Am10	$11.22^{c}\pm0.42$	$29.22^{b}\pm0.14$	$0.48^{a}\pm0.02$	$3.24^{c}\pm0.34$	$3.26^{c}\pm0.34$	$55.84^{j}\pm0.54$	$344.56^{i}\pm2.32$
Am11	$11.46^{b}\pm0.46$	$28.32^{e}\pm0.11$	$0.44^{a}\pm0.02$	$3.42^{a}\pm0.37$	$3.22^{d}\pm0.34$	$56.36^{i}\pm0.54$	$342.68^{j}\pm2.30$
Am12	$11.94^{a}\pm0.50$	$30.22^{a}\pm0.17$	$0.46^{a}\pm0.02$	$3.36^{a}\pm0.36$	$3.38^{a}\pm0.36$	$54.02^{k}\pm0.50$	$341.10^{k}\pm2.27$

Note: Em1 - Em4 - Samples from Ekeagbani Market, Om5 - Om8 - Samples from Ogbete Market, Am9 - Am12 - Samples from Aria Market. Values are mean ± standard deviations of triplicate determinations. Means in the same column with different superscripts are significantly different ($p \leq 0.05$).

Table 2: Mean Microbial Counts of Cowpea Flours

Samples	Total Aerobic Bacterial Count (cfu/g)	Coliform Count (cfu/g)	*E. Coli* Count (cfu/g)	Fungal Count (cfu/g)
Em	$1.33\pm0.48 \times 10^5$	$4.0\pm4.0 \times 10^4$	$1.5\pm2.5 \times 10^4$	$9.8\pm1.8 \times 10^4$
Om	$1.19\pm0.29 \times 10^5$	$6.1\pm1.3 \times 10^4$	$2.3\pm2.7 \times 10^4$	$9.0\pm3.0 \times 10^4$
Am	$1.08\pm0.33 \times 10^5$	$5.1\pm2.3 \times 10^4$	$1.8\pm2.2 \times 10^4$	$1.5\pm1.5 \times 10^4$

Note: Em - Ekeagbani Market, Om - Ogbete Market, Am - Aria Market.

Table 3: Microorganisms Isolated From Cowpea Flours and Their Percentage Occurrence

Organisms	% Occurrence
Bacteria	
Bacillus	30.8
Pseudomonas	15.4
Escherichia coli	15.4
Staphylococcus aureus	38.5
Fungi	
Aspergillus	28.6
Rhizopus	57.1
Penicillium	14.3

Table 2 shows the mean microbial counts of the cowpea flours. The high microbial counts observed indicate the exposure of the samples to air and other atmospheric conditions, which are responsible for their contamination. The main sources of food contamination include water, air, soil, dust, humans, sewage, utensil, processing equipment, handling and storage conditions, insects and rodents (Benchatu, 1996; Benwart, 2002; and James, 2003). Furthermore, the result also showed that various types of microorganisms were present in cowpea flours and these organisms were mostly bacteria and mould. Generally, both gram-positive and gram-negative organisms were isolated from the samples. Table 3 shows the microorganisms isolated from the bean flours and their percentage occurrence. It has been reported that the primary causative agents of microbial spoilage of foods are bacteria, yeast and mould (James, 2003; Agwung *et al.*, 2006). The isolation of these microorganisms from the cowpea flours clearly indicates their involvement in the contamination and spoilage of the products through their proteolytic and lipolytic activities (Eze *et al.*, 2008). The organisms isolated from the samples were *Bacillus spp*, which are gram-positive and spore-forming organisms. The spores of these organisms are able to withstand high temperature and pH, hence, they can easily germinate fully on food products. Most of the members of the genus are saprophytic organisms that are prevalent in the soil, water, air and on vegetation. *Bacillus cereus* and *Bacillus subtilis* are the most encountered in this group. *Bacillus cereus* when grown on food causes food poisoning by the production of an enterotoxin (Thomas, 1994; Brooks *et al.*, 2005). The presence of *Escherichia coli*, which is a true enteric pathogen, indicates faecal contamination of the samples. This may be attributed to improper sanitary condition and use of unsterilized utensils during the preparation of the flour. The presence of *Escherichia coli* in food causes gastroenteritis in infants and young children (Brooks *et al.*, 2005). *Staphylococcus aureus* is a normal flora of the body and its presence in food is an indication of contamination from handlers. The organism can enter into food during harvesting, processing and even storage. The consumer is at risk of acquiring food-borne diseases. *Staphyloccus* is the major causative agent of food poisoning known *staphylococcal* food poisoning. This is caused by the ingestion of enterotoxin produced by the organism and is characterized by diarrhoea and vomiting (Frazier and Westhoff, 2004). The fungal genera isolated could be traced to the harvesting period. The organisms produce spores which may have been attached to the grains during preparation and because of their resistance to heat and other environmental conditions, they can be retained in the finished products (James, 2003; Frazier and Westhoff, 2004).

CONCLUSION

The study showed that the nutritional and microbial qualities of home processed commercial cowpea flours can

be greatly improved by the use of appropriate processing condition and treatment followed by packaging and labeling of the products with the main components clearly stated on the label. The observation from the present study also indicated that some producers of commercial cowpea flours dilute their products with starch and this practice has been found to have a reduction effect on their protein content. It is therefore recommended that regulatory agencies such as the National Agency for Food and Drug Administration and Control (NAFDAC) and Standard Organization of Nigeria (SON) should come up with standards and regulations for the preparation, processing, packaging, labeling, marketing and distribution of cowpea products in order to ensure that their nutritional and microbial qualities are properly maintained if they are not in place.

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