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DOI: https://doi.org/10.64388/IREV10I2-1722167

Effects of Processing Methods on the Proximate and Microbial Quality of Cowpea (Vigna Unguiculata L. Walp) Flour

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Abstract- Cowpea (Vigna unguiculata), commonly known as black-eyed beans, is a nutritionally and economically important legume across Africa, Asia, and South America. Despite its value, whole seed consumption is often limited by digestive discomfort. The study investigated the effects of five (5) processing methods on the proximate and microbial quality of cowpea (Vigna unguiculata L. Walp) flour as limited data exist on how different processing methods affect the nutritive, anti-nutritive, sensory, and microbiological properties of cowpea flour. Black-eyed cowpea (Vigna Unguiculata L.) seeds were randomly purchased from dealers in Eke Market in Afikpo L. G. A. Afikpo Ebonyi State South-East Nigeria. All samples were processed, dried and milled into flour. Their Proximate quality and Microbiological quality were analysed following standard molecular methods. Results showed: Sprouting gave the lowest moisture (7.79%) and energy (307.12 kcal), highest crude fibre (2.98%) and ash (4.09%), but reduced fat (0.29%) and carbohydrate (65.14%). Organisms isolated included Bacillus safensis, Escherichia coli, Staphylococcus cohnii, Enterobacter spp. Aspergillus versicolor and Aspergillus frequens, Contamination was linked to handling, milling, and post-drying exposure. In conclusion, the proximate results showed that sprouting followed by drying improved the nutritional composition of the legume flour and also showed better microbial quality. The study concludes that sprouting cowpea before usage in the production of every other alternative products would present to the consumers more nutritious and healthier products. It is therefore, recommended that industries and domestic processors adopt sprouting as the major treatment in processing cowpeas into finished products.

Keywords: Sprouting, Dehull, Cowpea, Healthier, Alternative

I. INTRODUCTION

The existence of man is largely dependent on some factors including nutrients which may be of plant or animal origin (Obi and Okoye, 2017). According to Igwenyi and Akubugwo (2010), the choice of animals as a source of nutrient for man is often affected by cultural and religious beliefs, cost, health, food choice and preference hence plants play a significant role in human nutrition especially as a source of invariably all classes of food (Wagrovich, 2002). The usefulness of legume, Cowpea (Vigna unguiculata) commonly known as black eyed beans cannot be overemphasized as it has proven to be an important crop in many spheres of existence or living. Cowpea has shown several economic, agronomic environmental and therapeutic benefits and has contributed to improving the diet and income of peasant farmers across Africa, Asia and South America (Mida et al., 2002). Adeboye and Singh (2008) have reported that Vigna unguiculata L. Walp is a herbaceous annual crop of the family Fabeceae and yields favourably because of its adaptation and tolerance to stress (Mida et al., 2002). Despite its economic and nutritive value, people complain of discomforts after consumption. Alternative ways of eating Cowpea seeds can be adopted to avoid discomforts reported by individuals. This alternative ways will encourage and increase the daily intake of cowpea. A possible alternative way of consuming cowpea is by converting its seeds to flour to produce our local food, akara or moimoi (Affriftah et al., 2022). The possibility of converting beans to flour will also ameliorate the troubles of sourcing for the

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DOI: https://doi.org/10.64388/IREV10I2-1722167

conventional wheat flour for production of pastries. The preparation of cowpea flour is often by traditional methods at home or by street vendors; this could be mostly unhygienic considering that cowpea seeds pass through a lot of steps during processing which may expose it to pathogens or even loss in quality. More so, reports have been presented on the properties of cowpea seed, unfortunately, not enough work has been reported on the nutritive properties and microbiological quality of cowpea flour obtained from different processing methods. The main objective of this research is therefore centered on producing premium quality flour from bean seeds and providing information on consequent effect of various processing methods on the nutritional composition, sensory evaluation, anti-nutritive properties and microbiological quality of cowpea flour.

Cooking cowpea seeds (black eyed beans) can be time and energy consuming. More so, many complaints associated with the consumption of cowpea such as heart burns, bloated tummy and farting have been reported. As a result of these complaints, some individuals avoid eating cowpea meal but to encourage its consumption, a handful of people may opt for an alternative form of the cowpea diet such as akara (bean cake) or moimoi from street vendors. A lot of unhygienic processes are usually adopted by vendors who process this flour; this may expose consumers to diarrhea from food poisoning. Aside from consumers being infected, not enough information exists on the nutritional and anti-nutritional qualities of this processed bean flour used to make alternative diets. It is important to note that the method of cowpea flour production may lead to the loss of its quality and as well introduce microorganisms of serious health implications. Therefore, the need to evaluate the effect of the processing methods on cowpea seed flour becomes important.

II. MATERIALS AND METHODS

2.1 Collection and Preparation of samples for Analysis

Black-eyed cowpea (Vigna Unguiculata L.) seeds were randomly purchased from dealers in Eke Market in Afikpo L. G. A. Afikpo Ebonyi State South-East Nigeria. Latitude 5.89258° N and longitude 7.93534° E, with an elevation of 92 meters above sea level.

The cowpea seeds purchased were subjected to five (5) processing methods

i.) Dehull- Dry
ii.) Wash - Dehull- Dry
iii.) Soak (cold water 12hrs)- Dehull - Dry
iv.) Steam at 1000C for 5mims - Dry
v.) Wash - sprout at 25-30°C for 72hrs - Dry

After which they were all milled into bean flour, packaged in sterile Ziploc bags labelled M1 (control), M2, M3, M4 and M5, respectively in duplicate packs. A set was used for proximate composition at the Food Chemistry/Analysis Laboratory of the Department of Food Science and Technology of Akanu Ibiam Federal Polytechnic, Unwana Ebonyi State while the second set was aseptically transported to a Microbiology Laboratory in Yenogoa, Bayelsa State for microbiological examinations.

2. Proximate Composition of Samples

The proximate compositions of the samples were evaluated. The parameters evaluated included moisture content (MC), crude protein (CP), fat, crude fibre (CF), ash, carbohydrate (CHO) all in per cent and energy value (EV) in kilo calorie.

2.2 Isolation and Identification of organisms in samples

The associated microorganisms of the black eyed beans sample were isolated after a 1 in 10 fold serial dilution following the method described by Danladi, 2016. In this experiment, 1ml of 105 dilution of samples was plated on nutrient agar using pour plate method for total bacterial count while 0.1ml of 106 dilution was plated on Nutrient Agar (NA), MaCconkey Agar(MCA) and Sabroaud Dextrose (SDA) using the streak plate method for isolation and morphological identification of bacteria and fungi respectively. Inoculated media were incubated at 37°C for 24hrs for bacteria growth and 30°C for 5 days for fungi growth. Discrete colonies that developed on NA and MCA plates were counted, recorded (CFU/g) and sub-cultured for pure isolation after which they were sent for molecular identification and characterization

2.3 Molecular identification and characterization
The molecular identification of organisms involved three (3) steps.
The Amplification of the 16srRNA region of RNA genes in bacterial isolates which is the biological marker for all bacterial cells was achieved using the 27F (forward) and 1492R (Reverse) primers on ABI 9700 Applied Biosystem thermal cycler at a final volume of 50microlitres for 35 cyçles. For fungal isolates, The ITS region of the rRNA genes of the final isolate was amplified using the ITSIF Forward and ITS4 reverse primers on ABI 9700 Applied Biosystem thermocycler at a final volume of 50 microliters. The products were resolved on a 1% "agarose gel at 120 V for 15mins and visualized on a transilluminator.

Sequencing of the bacteria/Fungal isolates was done at final volume 1 microliters using the big dye terminator kit on a 3510 ABI Sequencer by Inqaba Technological, Pretoria South Africa.

Phylogenetic Analysis
Sequences obtained were edited via bioinformatics alogrithm trace edit and matched with data available on National Center for Biotechnology information (NCBI) using BLASTN. Evolutionary history was inferred using the Neighbor- joining method in MEGA 6.0 (Saitou and Nei, 1987).The evolutionary distance was computed using jukes- cantor method (Jukes and Cantor 1969)

III. RESULTS AND DISCUSSIONS

3.1 Proximate Composition

Table 1: Proximate composition of samples

Sample	MC (%)	CP (%)	Fat (%)	CF (%)	Ash (%)	CHO (%)	EV (kcal)
M_1	$7.98^{d}\pm0.06$	$16.91^{a}\pm0.03$	$1.28^{ab}\pm0.02$	$2.37^{b}\pm0.04$	$1.32^{b}\pm0.03$	$70.13^{c}\pm0.04$	$359.72^{a}\pm0.09$
M_2	$11.53^{a}\pm0.07$	$15.97^{d}\pm0.04$	$1.32^{d}\pm0.03$	$1.12^{a}\pm0.03$	$3.08^{a}\pm0.04$	$79.99^{a}\pm0.07$	$334.47^{d}\pm0.17$
M_3	$9.94^{c}\pm0.08$	$14.70^{b}\pm0.03$	$1.02^{c}\pm0.03$	$2.15^{c}\pm0.03$	$1.63^{d}\pm0.05$	$70.56^{b}\pm0.09$	$350.22^{b}\pm0.25$
M_4	$10.34^{b}\pm0.06$	$12.91^{c}\pm0.01$	$1.24^{b}\pm0.02$	$1.33^{d}\pm0.04$	$3.48^{b}\pm0.02$	$70.68^{b}\pm0.05$	$345.60^{c}\pm0.04$
M_5	$7.79^{e}\pm0.05$	$14.79^{b}\pm0.13$	$0.29^{a}\pm0.01$	$2.98^{a}\pm0.02$	$4.09^{c}\pm0.03$	$65.14^{d}\pm0.04$	$307.12^{e}\pm0.07$

Values are means of 2 replicates ± standard deviation. Means with different superscripts are significantly different ($p<0.05$).
M1 Dehull- Dry, M2 Wash - Dehull- Dry, M3 Soak (cold water 12hrs)- Dehull – Dry, M4 Steam at 100C for 5mims – Dry, M5 Wash - sprout at 25-30°C for 72hrs - Dry

The proximate composition of the test samples are displayed in Table 1 above. The table shows that the moisture content (MC) ranged from 7.79 (M5) to 11.53 (M2), this shows that sprouting has a way of reducing the moisture content of the sample. This position aligns with the findings of Waleed et al., (2024) that sprouted flour is drier than the samples of any other kind of treatments. The crude protein ranged from 12.91 (M2) to 16.91 (M1), this implies that increment in moisture content has a reductive effect on the protein content of the beans and this agrees with the position of Eze et al. (2008); Fat contents of the test samples ranged from 0.29 (M5) to 1.32 (M2) and this could be as a result of the utilization of fats during germination and agrees with the positions of Ekwe and Okorie (2017) and Khrisanapant et al. (2022) that sprouting has a diminishing effects on the fat contents of legumes. The Crude Fibre content was from 1.12 (M2) to 2.98 (M5) and confirms that sprouting increases the fibre content of legumes, which is the position of Malama et al. (2020). The per cent ash content ranged from

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DOI: https://doi.org/10.64388/IREV10I2-1722167

1.32 (M1) to 4.09 (M5) and confirms that sprouting increases the ash content is more concentrated as starch is used up. This finding agrees with the positions of Ekwe and Okorie (2017) and Khrisanapant et al. (2022). CHO 65.1 (M5) to 79.99 (M2) this evidenced by the understanding that the legumes burn carbohydrates in the course of germination and agrees with the position of Ekwe and Okorie (2017). The energy value (EV) results (307.12 (M5) to 359.72 (M1)] show that sprouting decreases the total energy value of legumes because according to Ekwe and Okorie (2017) and Khrisanapant et al. (2022), the seeds burn carbs and fat for fuel to grow.

3.2 Microbiological Examination

Table 2: Microbiological quality of treated and untreated cowpea flours (CFU/g)

Sample	TBC	Coliform count	Fungal count
M_1	3.43×10^6	3.64×10^7	3.66×10^7
M_2	1.12×10^6	7.20×10^6	7.20×10^7
M_3	3.380×10^5	4.40×10^6	1.10×10^6
M_4	6.46×10^5	5.40×10^6	2.70×10^6
M_5	3.45×10^5	2.24×10^7	3.00×10^7

M1 Dehull- Dry, M2 Wash - Dehull- Dry, M3 Soak (cold water 12hrs)- Dehull – Dry, M4 Steam at 100C for 5mims – Dry, M5 Wash - sprout at 25-30°C for 72hrs – Dry

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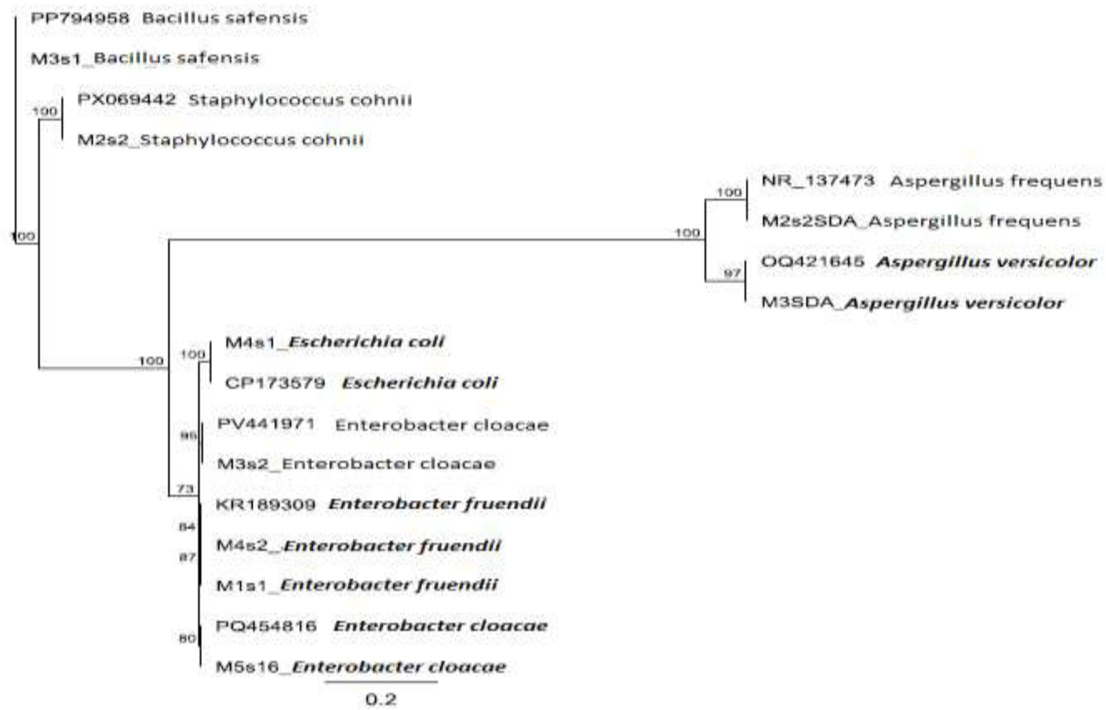

Figure 1: Phylogenetic tree showing the evolutionary distance between the bacterial and fungi isolates

According to Romero-Arenas et al., 2013, Blacked eyed cowpea are a basic component for vegetable diet and represent a great source of elements needed for human nutrition. The microbial analysis of black eyed beans flour samples as shown in table 1 revealed a total bacterial count (TBC) of 3.43×106 for untreated flour sample M1 (control) and a TBC range of between 3.45×105 to 1.12×106 for treated bean flour samples with M2 recording highest TBC and M5 recording lowest TBC. The total coliform count (TCC) was recorded to be highest for untreated M1 (control) 3.64 ×107 followed by M5 2.24×107 and M3 4.46×106 for treated samples whereas In addition, a total fungal count of 3.66×107 was observed to be highest for untreated M1 (control) and for treated samples 3.08×107 for M5 and lowest fungal count M3 at 1.1× 106 was recorded. Although processing reduced the microbial content of black eyed bean flour, the total bacterial count and coliform count for all processed and unprocessed bean flour were high when compared to the acceptable limit by ICMSF (1998), which should be 1×106 CFU/g. The high microbial count observed could have been as a result of exposure to the atmosphere filled with numerous microorganisms as well as indiscriminate handling such as touching with bare hands and other contaminating materials during milling and packaging. Results obtained partly agrees with Okoye et al ., 2017; who had similar values for total aerobic bacterial count but less values for fungal and coliform count and Uko et al., 2017 who obtained close but lower coliform values for both treated and untreated cowpea flour.

The phylogenetic tree in Figure 1 also showed results of a variety of bacteria and fungi species .According to James 2003; Awung et al., 2006, primary microbial agents of food spoilage are mostly bacteria and fungi and the isolation of these agents from the black eyed cowpea flour clearly indicates their involvement in the contamination and possible spoilage of food products through proteolytic and lypolytic activities (Eze et al., 2008) The organisms isolated from the black eyed cowpea flour samples were Bacillus safensis which are saprophytic in nature, gram positive and spore forming. Their spores are able to withstand high temperature and pH and can easily germinate on food products. They are known to produce entertoxins which could cause mild food poisoning just like their counterparts Bacillus cereus (Thomas 1994; Brooke et al., 2005), Escherichia coli is an enteric pathogen which can be responsible for enterogastritis in infants and young children when in food (Brooke et al., 2005) The presence of E. coli may be attributed to improper sanitary conditions and use of unsterilized utensils during the preparation of the flour. Staphylococcus cohnii, which is a coagulase negative organism and commonly found on human skin and mucous membrane and can be opportunistic causing a range of infections, from endocarditis to sepsis especially in immunocompromised individuals. Its survival in low water activity could have promoted it's survival in flour sample. (Hennekinne et al 2013; Finn et al., 2013) Its presence in food can be an indicator of contamination from handling. The organism can enter into food from harvesting, processing and even storage and consumers are often at risk of acquiring foodborne diseases through enterotoxins produced by the organism (Frazier and Westhoff, 2004)

Most Enterobacter sp possess high dedication resistance. They are able to persist in dry processing environments and survive in powdered food products for extended periods of time. Investigation of contamination routes has indicated that contamination most likely occurs after the drying stage and emanates either from nonsterile dry-mix ingredients or from the processing (Laura ViÃuela GonzÃ¡lez., 2022). Enterobacter cloccae and Enterobacter freundii formerly citrobacter freundii were observed in this study in processed and unprocessed cowpea flour. Isolation of species Escherichia coli, Staphylococcus sp, Bacillus sp. Enterobacter sp. from the samples as observed in this study, have also been observed in other studies.(Uko et al., 2017; Odu et al.,2019). The fungal species Aspergillus versicolor and Aspergillus frequens isolated could be traced to the harvesting period. They produce spores and toxins too which may have been attached to 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).

IV. CONCLUSION

The study concludes that sprouting enhanced the nutritional content of cowpea and though the process of sprouting could have increased the microbial load but the drying processes further reduced the microbial load, making is safer than the flours from the un-malted samples. This suggests that sprouting should be encouraged for adoption in the industry for the production of high nutritive foods. When flours are processed from malted cowpea, stricter good manufacturing practices (GMPs) should be engaged to produce healthy and safe foods for the consumers.

V. ACKNOWLEDGMENT

We are thankful to the Tertiary Education Trust Fund (TETFUND) of the Federal Government of Nigeria for funding this research through her Institution Based Research (IBR). We also acknowledge the supports of the Management of Akanu Ibiam Federal Polytechnic, Unwana under the leadership of Dr. F. U. Attah during the course of this study.

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