

Article

Cubing Fabrication/Costing and Machine Performance on African Fermented Condiment Quality Attributes Compared with Commercial Bouillon Types

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Abstract: Cubing machines in food processing have evolved over the years, which have made products like Star[®], Knorr[®], and Chicken[®] bouillon cubes become commercially available today, even in many parts of Africa. On the other hand, the fermented mesquite seed “okpeye” food condiment, traditionally produced by well-trained artisans and widely utilised in Nigeria, requires further product development in order to compete with bouillon cubes. Quality comparisons between the cubed “okpeye” condiment and commercially available bouillon cube products have not yet been reported. Therefore, cubing fabrication/costing and machine performance on African fermented “okpeye” condiment quality attributes, compared with commercial bouillon types, were done. The processing of mesquite seeds into the “okpeye” condiment resembled those of artisans. Bouillon cube products involved the Star[®], Knorr[®], and Chicken[®] types. Quality attributes involved proximate, micronutrient, phytochemical, and microbial aspects. Results showed that the throughput capacity of a cubing machine increased with an efficiency of 48 condiment cubes/min, forming properly without separation. Across all studied samples, the protein, ash, moisture, crude fat, crude fibre, and carbohydrate contents were in the range of 1.45–42.50%, 5.29–6.75%, 8.50–12.29%, 2.56–18.54%, 2.45–3.19%, and 18.16–25.56%, respectively. The protein, fat, moisture, calcium, iron, magnesium, and manganese contents of “okpeye” condiment were significantly higher ($p < 0.05$) than those of bouillon cubes. Fair amounts of vitamins B1, B2, B3, and E were found, with the “okpeye” condiment higher in vitamin E. Besides the flavonoids (0.12–0.18%), alkaloids (0.08–0.15%), saponins (0.19–0.55%), and tannins (0.69–0.93%) present, the microbial loads were similar ($p > 0.05$) across all samples. Indeed, the “okpeye” condiment can be cubed, and by quality attribute, it competes favourably, and very promising substitute to commercial bouillon cubes.

Keywords: bouillon cubes; condiment; cubing machine; *Prosopis africana*; quality characteristics

1. Introduction

Cubing machines in food processing are not a new phenomenon and have progressed over the years with various promising patents. The earliest patent seems to be the apparatus used for cubing sliced material by Leibing [1], in which the cutting works were characterised by the nature of the cut, or through the rotating knives, to obtain the cubes.

Three decades after, there was a cheese cubing apparatus that used an actuated pressure plate mechanism, and an adapted screw continuously rotated in one direction with automatically released parts, which permitted a quick pressure plate retraction [2]. A decade later, there was a meat cubing apparatus patent by Soodalter [3], which involved, first, cutting to secure a slab from a piece of meat; second, cutting to sever a slab into elongated strips; and third, cutting to cut strips transversely into cubes. Besides the above-mentioned patents, Sokhansanj and Turhollow [4] described cubes as larger than pellets, in the form of a square cross section of chopped biomass, and less dense depending on a variety of factors, for example, cube size. By reviewing the typical cubing operation, Sokhansanj and Turhollow [4] demonstrated the schematic biomass flow that showed the position of the cuber and equipment layout for the biomass cubing. The commercial cube-making apparatus today, which probably emerged from the above-mentioned patents, are too expensive for small-scale food processors, especially in developing countries, to afford. Hence, there is a call to make an affordable cubing apparatus using locally available materials to help advance and develop agrofood products.

Condiments are preparation products supplemented in foods aimed at achieving specific desired changes in flavour or texture in the prepared food menu. African condiments, usually introduced during cooking in fairly small amounts, are largely of pungent flavour [5]. Besides, many African traditional menus constitute a combination of grain cereals, green leafy vegetables, fruits, legumes, and/or starchy tubers [6]. Legumes and their respective seeds, particularly when fermented, serve as strong condiments and are notable sources of minerals. Legume seeds equally account for about 80% of dietary protein and are the primary sources of protein for some groups [7]. Comparing the tropics and other parts of the globe, the utilisation of legume seed is underscored by its physical form and the processes employed especially in preparing various food menus, and is highly dependent on such factors as ethnic background, customs, and traditions [8]. Specifically and widely available in Africa, *Prosopis africana* (Guill and Perr) Taub., with common names like djembe, iron tree, Malinke, and African mesquite, is a flowering plant with legume seeds, which, after the fermentation process, make the well-known condiment called “okpeye” (also called either “okpehe,” “okpiye,” “daddawa,” or “kpaye”) [9–13]. In Nigeria, fermented leguminous seed condiments, largely utilised when preparing dishes, remain very key in enhancing flavours [14,15]. Besides the characteristic aroma (and flavour) that enhances the taste of traditional dishes and soups, the “okpeye” condiment is a rich source of protein with useful minerals and phytochemicals beneficial to human health [9–13]. Despite the popularity of the “okpeye” condiment as a flavouring agent particularly among many rural and urban people [12,13,16], there still remains some stigma associated with it, that is, being perceived as a low-cost (meat) substitute for poor (traditional) families [9,17].

The bouillon cube, also called broth or stock cube, is believed to have been introduced between the French and Germans. It is a dehydrated product and typically prepared from dehydrated vegetables, meat stock, a small portion of fat, monosodium glutamate (MSG), salt, and seasonings. It was in 1908 when “Maggi” commercialised bouillon cubes, followed by ‘Knorr’ in 1912 [18]. In many parts of Africa (including Nigeria), bouillon cube products like Star[®], Knorr[®], and Chicken[®] types are well known to occupy a very strong market base. On the other hand, there is the fermented mesquite seed (“okpeye”) food condiment, which is craft based and traditionally produced by well-trained artisans who largely learned this trade from their friends and relatives over time. Indeed, the commercial success of fermented mesquite seed (“okpeye”) food would strongly depend on the observance of good hygiene/manufacturing practices (GHPs/GMPs). Information regarding quality standards, especially in the production process of fermented mesquite seed “okpeye,” remains insufficient. Additionally, there continue to be (quality) inconsistencies in emergent output/product [19].

There appears to be a notion in the Nigerian populace that industrially commercially available bouillon cubes, like Star[®], Knorr[®], and Chicken[®] types, contain more nutrients than other traditionally handcrafted condiments, like the “okpeye.” Additionally, the

traditional (“okpeye”) condiment is yet to attain such widespread status like those commercial bouillon cube types (e.g., Maggi cube). Characteristic putrid odour, objectionable packaging materials, short shelf life, and stickiness are among the reasons mitigating the widespread status of “okpeye” condiment [20]. To improve this condiment product is, therefore, warranted to enable it compete favourably with commercially available bouillon cubes. However, there is paucity of relevant information about comparisons between the local “okpeye” condiment and commercial bouillon cube products. Specifically, quality comparisons between the “okpeye” condiment pressed in cube form and commercially available bouillon cube products have not yet been published. To supplement existing information, this work focused on determining the cubing fabrication/costing and machine performance of African fermented “okpeye” condiment quality attributes, compared with commercial bouillon types. The procedure for making the “okpeye” condiment is likened to those of artisans. The bouillon cube products include the commercially available Star[®], Knorr[®], and Chicken[®] types. Subsequent sections include the materials and methods, where all the processes used to implement the experimental program are explained. This is followed by the results and discussion, limitations and direction of future work, and conclusions.

2. Materials and Methods

2.1. Schematic Overview of Experimental Program

A schematic overview of the entire study, from the procurement of agrofood product (mesquite seeds + commercial bouillon cube) samples and cubing machine fabrication materials to the laboratory analysis of samples, is shown in Figure 1. The fabrication of a cubing machine and its testing forms the cubing machine design framework, which plays a key role in achieving the cubed “okpeye” condiment. For emphasis, this current research was designed to look into the cubing fabrication/costing and machine performance and examine how the quality attributes of the African fermented “okpeye” condiment turn out to be in comparison with the commercial bouillon types. The quality attributes include proximate, micronutrient, phytochemical, and microbial aspects, when comparing the African fermented “okpeye” condiment with commercial bouillon (Star[®], Knorr[®], and Chicken[®]) types.

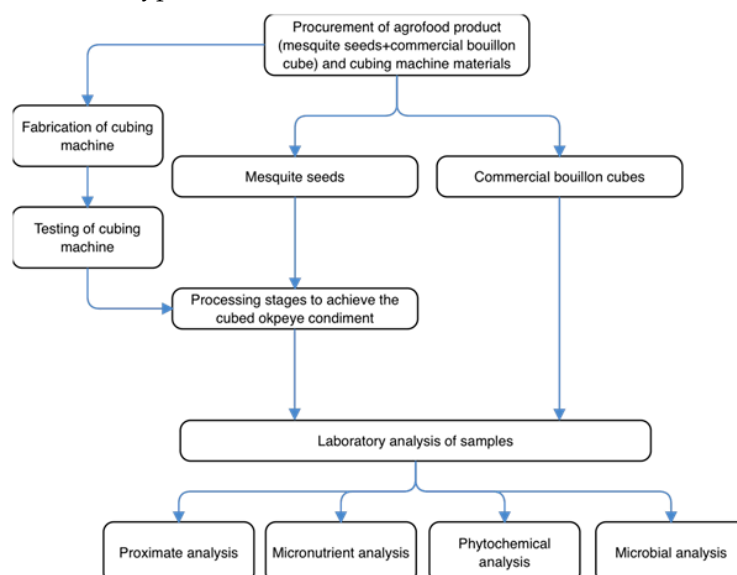


Figure 1. Schematic overview of the entire study, from the procurement of agrofood product (mesquite seeds + commercial bouillon cube) samples and cubing machine fabrication materials to the laboratory analysis of samples. The fabrication of the cubing machine is a key aspect of its design framework (Refer to Figure 2 for details of design framework). There are a number of processing stages required for the mesquite seed to achieve the cubed “okpeye” condiment (Refer to Figure 3 for details of processing stages).

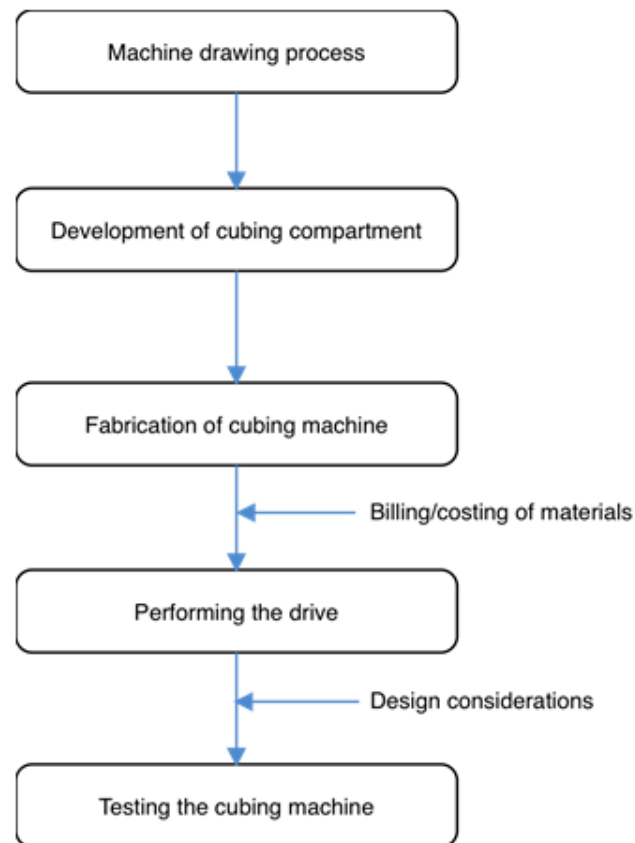


Figure 2. Design framework of the cubing machine. The machine drawing process entails the assembly drawing and its components (Refer to Figures 3 and 4, for the components). The testing of the cubing machine is an activity when the “okpeye” condiment gets cubed.

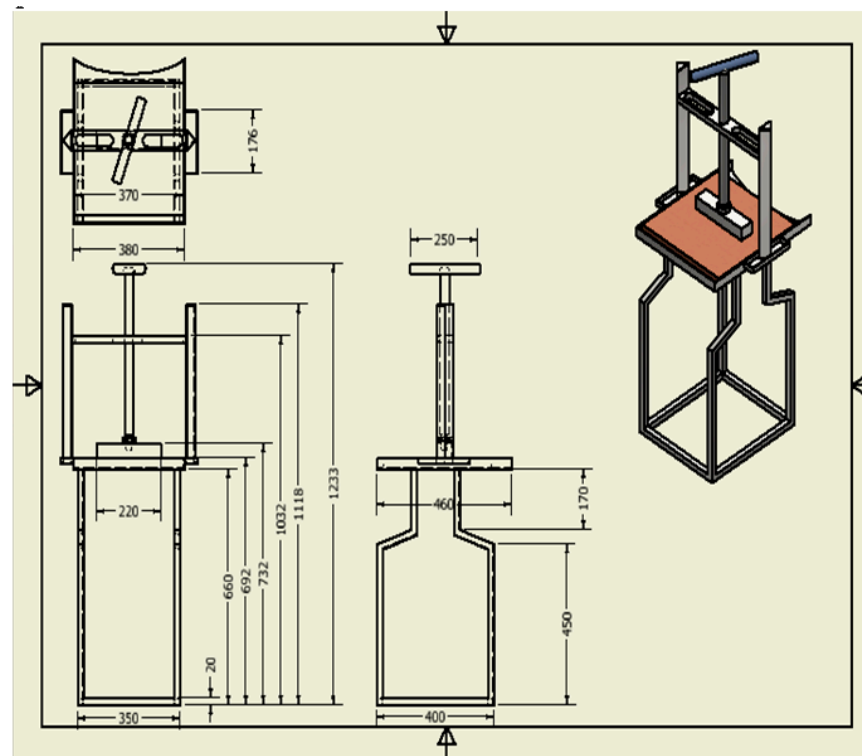


Figure 3. Assembly drawing of the component parts of a cubing machine. All dimensions are in millimetre (mm).

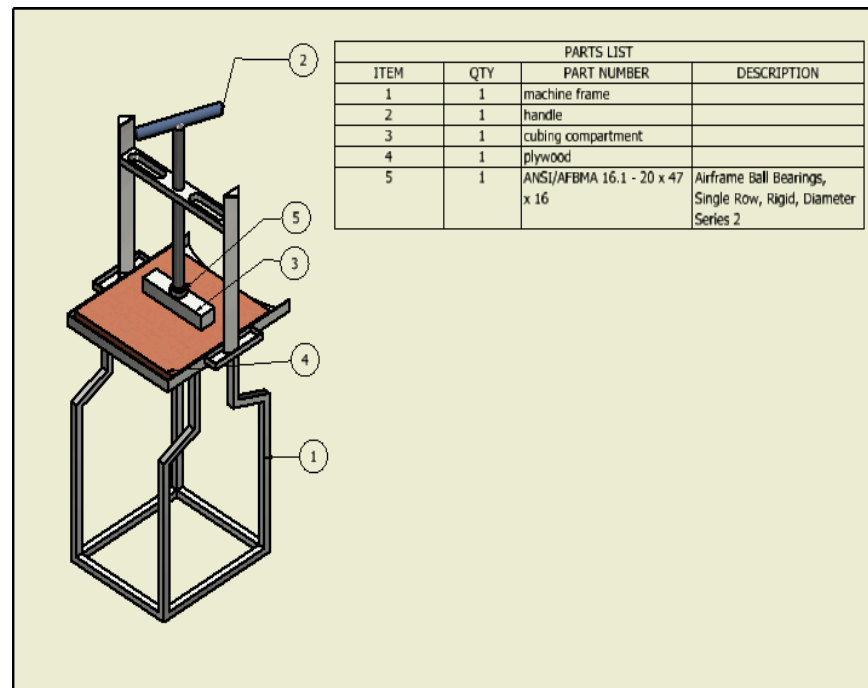


Figure 4. Component details of the cubing machine.

2.2. Cubing Machine Design Framework

The design framework of the cubing machine is shown in Figure 2, which comprises the major stages, from the machine drawing process to the testing of the cubing machine. The design framework demonstrates the steps followed, from the design concept to the final implementation of the cubing machine.

2.2.1. Machine Drawing

Machine drawing was the first step of the design framework, which involved two parts: (a) the assembly drawing (Figure 3); and (b) the component details (Figure 4) of the cubing machine.

2.2.2. Cubing Compartment

The cubing compartment is essentially made of wire mesh containing 50 square cubing holes with each cube measuring approximately 20 mm × 20 mm in cross section, designed to take the shape of the cubed condiment (Figure 5). The size of one of the holes in the mesh was determined using the expression [21] in Equation (1).

$$S = 6a^2 \quad (1)$$

where

A = equal length of sides (20 mm);

S = size of hole, meshing the cubing compartment (mm²);

$S = 6 \times 20^2$ (mm²);

$S = 2.4/10^3$ (m²).

Therefore, the size of each cubed mesquite seed condiment produced by the machine was 0.0024 m².

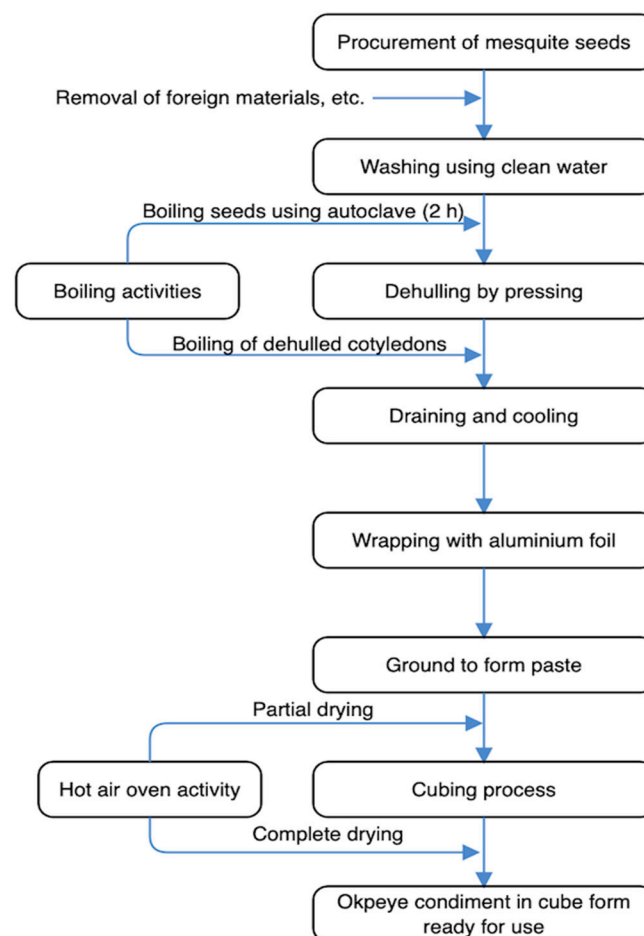


Figure 5. Processing of mesquite seeds into “okpeye” condiment cubes. Boiling activities are twofold, prior to and after dehulling, for seeds and dehulled cotyledons, respectively. Hot air oven activity is twofold, prior to and after the cubing process, for partial and complete drying, respectively. Besides the cubing process, the entire process is carried out to liken those of traditional artisans.

2.2.3. Fabrication of the Cubing Machine

The cubing machine was fabricated using the conventional workshop procedure. This involved bending, welding, cutting, and drilling. The handle of the machine was made of 1.5 mm thick mild steel material with a 250 mm × 150 mm cross section. The cubing compartment was made from a 2 mm thick stainless steel material, which has a cross section of 460 mm × 380 mm. The piston was made from 3 mm thick stainless steel material with a 440 mm × 220 mm cross section. The thread inside the piston was made from a 6 mm thick mild steel material with a 490 mm × 22 mm cross section. The shaft supporting the thread was made from 3 mm thick stainless steel material with a 210 mm × 20 mm cross section. Provisions were made in order to ease the dismantling of the cubing compartment using bolts and nuts. Thereafter, the fabrication processes were conducted.

2.2.4. Drive

The machine was manually operated to drive the piston. The human effort and speed generated are theoretically 0.075 kW and 60 rpm, respectively [22]. This effort was used to drive the piston against the connecting rod, and the pressure developed was transferred to the cubing compartment of the machine [23].

2.2.5. Testing of the Cubing Machine

The performance of the cubing machine was evaluated in terms of throughput capacity with respect to piston pressure for 1 h, and the pressure developed and kept constant at

21.5 kN/m² [22]. Approximately 500 g of the fermented condiment was introduced into the cubing machine. The machine was operated for ~5 min, and the mass of the output (cubed condiment) was measured and recorded. The procedure was repeated for a 1 h machine operation at an interval of 5 min. The throughput capacity was thereafter computed [24,25] using Equation (2):

$$Q = m/t \quad (2)$$

where

Q = throughput capacity (kg min⁻¹);

m = mass of the machine output (kg);

t = time of cubing (60 min at 5 min interval).

2.2.6. Machine Design Considerations and Material Selection

The designs of the machine and materials to be used were sought in order to build a cubing machine. The fabrication materials used to build the cubing machine were purchased locally from the markets within the Nsukka community. The machine was designed considering its adaptability for ease of operation and affordability within the capacity of the intended users. Additionally, the materials were selected on the basis of their availability, suitability, and viability in service among other considerations [24,25].

2.2.7. Billing/Costing of Cubing Machine Materials

As part of the objective of this study, the billing/costing of the cubing machine materials was documented. This was in terms of some description, quantity, rate, and amount. This would entail some details on the materials, quantity indicated by number, rate indicated by cost per unit, and amount revealed by eventual cost, which thereafter were totalled.

2.3. Processing of Mesquite Seeds into the “Okpeye” Condiment

The processing of mesquite seeds into “okpeye” condiment cubes is shown in Figure 5. The mesquite seeds (2 kg) were purchased from the Ogige market in the Nsukka metropolis of Enugu State, Nigeria. The seeds were manually sorted to remove foreign materials, such as broken and immature seeds, stones, tree branches, leaves, and sand [26]. The seeds were washed in clean water and boiled in an autoclave for 2 h. The boiled seeds were dehulled by pressing with fingertips to separate the cotyledons from the hulls. The dehulled cotyledons were boiled in an appropriate quantity of water for 30 min and drained through a sieve (1 mm pore size) [9]. The boiled cotyledons were allowed to cool, wrapped with aluminium foil, and allowed to ferment for 5 days. The fermented mash was ground to form a paste (10–50 µm particle sizes) using a seed grater. Thereafter, partial drying in a hot air oven at 60 °C was conducted to achieve an initial moisture content of about 39%. Thereafter, it was pressed into cubes with the help of the fabricated cubing machine. Thereafter, further drying in a hot air oven at 60 °C was conducted to achieve the desired final moisture content. With the exception of the cubing process, the entire “okpeye” condiment-making process was carried out in such a way to ensure it is consistent with those of traditional artisans. In addition, the initial partial drying prior to the cubing process, as well as the final drying, was to ensure that there was proper adhesion of the bean particles in the cubing process. Thereafter, the “okpeye” cubes were kept in a dry cool place under ambient temperature until required for laboratory analysis.

2.4. Procurement of Commercial Bouillon Cube Products

Commercially available bouillon cube products, specifically Star[®], Knorr[®], and Chicken[®] types, were also purchased from a retailer within the Ogige market in the Nsukka metropolis of Enugu State, Nigeria. The batch purchased were those that had just/recently arrived at the retailer’s store. Permission was not given to use the name of the retail store. The quantity purchased was sufficient for the current study.

2.5. Determinations of Proximate, Micronutrient, Phytochemical, and Microbial Contents between the Fermented “Okpeye” Condiment and Commercial Bouillon Cube Products

2.5.1. Determinations of Proximate Contents

The proximate contents (moisture, ash, crude fat, crude fibre, and crude protein) of samples were determined using the AOAC method [27]. Briefly, moisture content was determined by drying in a hot air oven at 60 °C for 3 h. The ash content was determined with the help of a muffle furnace at 500 °C and maintained for 5 h. The crude fat content was determined by the extraction of an oven-dried sample with the help of petroleum ether via a Soxhlet unit (Tecator AB, Sweden) after the process of acid hydrolysis. The crude fibre content was determined by digestion of a sample with H₂SO₄ and NaOH, followed by incineration of residue with the help of a muffle furnace at 500 °C maintained for 5 h. The crude protein content followed the Kjeldahl method and was calculated by a multiplication of 6.25.

2.5.2. Determinations of Micronutrient Contents

Determination of Calcium, Iron, Manganese, Magnesium, Potassium, and Sodium

The calcium (Ca), iron (Fe), magnesium (Mg), manganese (Mn), potassium (K), and sodium (Na) of all samples was determined using the Atomic Absorption Spectrophotometer (AAS) (Shimadzu product, Model: Z-5010), following the AOAC method [27]. Approximately 3 g of sample was first digested with 30 mL of mixture of concentrated HNO₃ and HCl at a ratio of 1:3. The digested sample was filtered and made up to 50 mL with deionized water. The aliquots of the digested filtrate were used for AAS using filters that matched the different elements. The calibration curves were prepared for each element using the standard solution. Each mineral to be determined employed the hollow cathode lamp complementary to it and a particular wavelength. The lamp directed the wavelength to the sample, and the concentration of the mineral in the sample was determined with its corresponding value.

Determination of Thiamine (Vitamin B₁)

The thiamine content of samples was determined using the AOAC method [27]. Approximately 75 mL of 0.2N HCl was added to 2 g of sample, and the mixture boiled over a water bath. After cooling, ~5 mL of phosphate (enzyme solution) was then added to the mixture, and incubated at 37 °C overnight. The solution was placed in a 100 mL volumetric flask, and the volume made up with distilled water. The solution was filtered, and the filtrate passed through a silicate column. To approximately 25 mL of the filtrate in a conical flask, ~5 mL of acidic KCl, ~3 mL of alkaline ferricyanide, and 15 mL of isobutanol were added and shaken for ~2 min. The solution was allowed to separate, and the alcohol layer taken. About 3 g of anhydrous sodium sulphate was added to the alcohol layer. Approximately 5 mL of thiamine solution was measured into another 50 mL stoppered flask, followed by the oxidation and extraction of thiochrome, as carried out previously, were repeated using the thiamine solution, and ~3 mL of the 15% NaOH was added to the blank. The blank sample solution was poured into a fluorescence reading tube, and reading taken at this expression:

$$\% \text{ Thiamine} = \frac{X \times 25 \times 100}{Y \times 5 \times V \times W} \quad (3)$$

where, W = weight of sample; X = reading of sample–blank reading; Y = reading of thiamine standard–reading of blank standard; V = volume of solution used for the test on the column.

Determination of Riboflavin (Vitamin B₂)

The riboflavin content of samples was determined using the AOAC method [27]. Approximately 5 g of samples mixed with 50 mL of 0.2 N HCl was boiled and subjected to a water bath for ~1 h. The mixture was cooled to ~10 °C, and pH adjusted to ~6.0 with 15%

NaOH. The pH further lowered by adding 1N HCl, filtered into a 100 mL measuring flask and the volume marked up. To ensure minimal-to-zero interference, two tubes marked No. 1 and 2 were used. To tube No. 1, ~10 mL of filtrate and ~1 mL of water were added. To tube No. 2, ~10 mL of filtrate and ~1.0 mL of riboflavin standard were added. Approximately 1 mL of glacial acetic acid was added to each tube and mixed, and then 0.5 mL KMnO_4 solution was added. After ~2 min, ~0.5 mL of 3% H_2O_2 was added and mixed well. The fluorimeter was set to an excitation wavelength of 470 nm and an emission wavelength of 525 nm. The fluorimeter was adjusted to zero deflection against 0.1N H_2SO_4 . Fluorescence of tube number 1 was measured. To tube No. 1 and No. 2, ~20 mg of sodium sulphate was added, and fluorescence was measured within 10 s and recorded as blank readings. The riboflavin content in the sample was calculated as follows:

$$\text{Riboflavin} = \left(\frac{\text{mg}}{\text{g}} \right) = \frac{X}{Y - X} \times \frac{1}{W} \quad (4)$$

where

W = weight of sample; X = (reading of sample 1) – (reading of sample blank);

Y = (reading of sample + standard tube 2) – (reading of sample + standard blank).

Determination of Vitamin B₃ (Niacinamide)

The niacinamide content of samples was determined using the AOAC method [27]. Approximately 5 g of sample was macerated with 100 mL of distilled water and filtered using Whatman No. 1 filter paper (pore size ≈11 microns). Approximately 1 mL filtrate was pipetted into triplicate tubes, followed by ~0.5 mL of 5% ammonia and ~1 mL of bromine, ~1 mL of sulphanic acid, and ~0.5 mL of concentrated HCl. It was made up to ~10 mL with distilled water, and the absorbance was read at 430 nm by a spectrophotometer (UV BIOCHROM 4049, Cambridge, UK).

Determination of Vitamin E

The vitamin E content of samples was determined using the method described in Onwuka [28]. Approximately 1 g was placed into a 100 mL flask fitted with a reflux condenser. Approximately 10 mL of absolute alcohol and 20 mL of alcoholic sulphuric acid were added. The solution was refluxed for ~45 min and cooled. Then, it was transferred to a separating funnel and extracted with ~30 mL of diethyl ether. The extract was evaporated with very low heat, and the residue was dissolved with ~10 mL absolute ethanol. Aliquots of the solution and standards (0.3–3.0 mg vitamin E) were transferred to a 200 mL volumetric flask, and ~5 mL of absolute alcohol was added, followed by ~1 mL of concentrated nitric acid dropwise with swirling, after which the solution was cooled by running tap in a water bath. Thereafter, the absorbance was measured at 470 nm against a blank containing ~5 mL absolute alcohol and ~1 mL nitric acid.

2.5.3. Determinations of Phytochemical Content

Determination of Alkaloids

The alkaloid content of samples was determined using the gravimetric method described in Onwuka [28]. Approximately 5 g of sample was dispersed in 50 mL of 10% acetic acid solution in ethanol. The mixture was shaken and allowed to stand for ~4 h, after which it was filtered. The filtrate was evaporated to 1/4 of its original volume. Concentrated ammonium hydroxide was added in drops to precipitate the alkaloids. The precipitate was filtered using a weighed filter paper and was held with 1% NH_4OH solution. The precipitate in the filter paper was dried in an oven at 60 °C for 30 min and reweighed.

$$\% \text{Alkaloids}(\text{mg}) = \frac{w_1 - w_2}{w_1} \times 100 \quad (5)$$

where

W_1 = weight of the sample; W_2 = weight of the empty filter paper; and W_3 = weight of the filter paper and precipitate.

Determination of Flavonoids

The flavonoid content of samples was determined using the method described in Onwuka [28]. Approximately 5 g of the sample was boiled in ~50 mL of 2 M HCl solution for ~30 min under reflux. It was allowed to cool and filtered through Whatman No. 1 filter paper. The extract was treated with equal volume of ethyl acetate drop by drop. The flavonoid precipitated was recovered by filtration using weighed filter paper. The weight difference was taken as the weight of flavonoid in the sample.

Determination of Saponins

The saponin content of samples was determined using the method described in Obadoni and Ochuko [29]. Approximately 10 g of sample was put into a 250 mL conical flask, and 100 mL of 20% aqueous ethanol was added. Then the flask was heated on a hot water bath for ~4 h with constant stirring at about 55 °C. The mixture was then filtered, and the residue was again extracted with another ~200 mL 20% ethanol. The combined extract was reduced to ~40 mL on a hot water bath at about 90 °C. The concentrate was transferred into a 250 mL separating funnel, and ~20 mL of diethyl ether was added, then vigorously shaken. The aqueous layer (lower layer) was recovered, while the ether layer was discarded. The purification process was repeated, after which ~60 mL of n-butanol was added for washing (two times), where in both cases, the upper layer was collected, while the lower layer discarded. The combined n-butanol extracts were washed twice with ~10 mL of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in an oven and weighed, and saponin content was calculated as a percentage.

$$\% \text{ Saponin} = \frac{\text{weight of extract}}{\text{weight of sample}} \times 100 \quad (6)$$

Determination of Tannins

The tannin of samples was determined using the method of Van-Burden and Robinson [30]. Approximately 500 mg of sample mixed with 50 mL of distilled water was shaken in a mechanical shaker for 1 h and filtered in a 50 mL volumetric flask. Approximately 5 mL of the filtrate was pipetted into a test tube and mixed with ~2 mL of 0.1 M FeCl_3 in 0.1N HCl and 0.008 M $\text{K}_4\text{Fe}(\text{CN})_6$ (potassium ferrocyanide). Absorbance was measured at 120 nm within 10 min. Absorbance was traced against concentration using a tannic acid standard graph. The percentage of tannin was calculated as follows:

$$\% \text{ Tannin} = \text{concentration} \times \text{dilution} \times 100 \quad (7)$$

That is,

$$\% \text{ tannin} = y/500 \text{ mg} \times 500 \text{ mg}/50 \text{ mL} \times 100;$$

$$y = (\text{reading of sample} + \text{standard tube 2}) - (\text{reading of sample} + \text{standard blank}).$$

2.5.4. Determination of Microbial Counts

Preparation of Ringer Solution

Following the method of Prescott, Harley, and Klein [31], a quarter-strength ringer solution was prepared by dissolving one ringer tablet in ~500 mL of distilled water. The clear solution formed was sterilised by autoclaving for ~15 min at 121 °C and 15 lb pressure. A ringer solution was then allowed to cool completely to 28 ± 2 °C.

Determination of Mould Count

The mould count determination followed the method described by Prescott, Harley, and Klein [31]. The Sabouraud dextrose agar served as medium. Approximately 1 g of sample mixed with 15 mL of Sabouraud dextrose agar in a petri dish was swirled to mix, allowed to set, and subsequently incubated at ~ 37 °C for ~ 48 h. After incubation, the number of colonies was counted in terms of colony-forming unit per gram (log cfu/g).

Determination of Total Viable Count (TVC)

The total viable count (TVC) determination followed the method described by Prescott, Harley, and Klein [31]. Approximately 1 g of sample and 9 mL of diluent were used to prepare serial dilutions of up to 10^{-3} , and thereafter pipetted into a petri dish, and 15 mL of prepared nutrient agar solution was added. The solution was swirled to mix and incubated at ~ 37 °C for ~ 24 h. After incubation, the number of colonies was counted in terms of colony-forming unit per gram (log cfu/g).

2.6. Statistical Analysis

One-way analysis of variance (ANOVA) was applied on the resultant data obtained from triplicate measurements. Results were expressed as means \pm standard deviation (SD). To separate the means, the Duncan multiple range test was applied. The level of probability was set at $p < 0.05$ (95% confidence interval). IBM SPSS software (version 22.0, IBM Corp., New York, NY, USA) was used to do the statistical analysis.

3. Results and Discussion

3.1. Cubing Machine Operation: Operating Principle, Performance Evaluation, and Billing/Costing

3.1.1. Operating Principle of the Cubing Machine

The operating principle is based on the piston-connecting rod arrangement within the cubing compartment. The handle of the cubing machine is equipped with a capacity to provide the required drive. Additionally, there is a crank-connecting rod–piston arrangement, which transfers the force from the handle in order to drive the piston rod. Through the connecting rod up to the crank end, the piston is pushed forward by the manual rotation of the handle [22]. During the cubing operation, the partially dried “okpeye” paste (~ 500 g) is spread on the aluminium foil and smoothed such that the paste obtains equal flatness. The plywood-made board already covered with the aluminium foil is to help prevent its contact with the “okpeye” paste. The board would then slide back, such that the cubing compartment is directly at the top. The reciprocating motion of the connecting rod is then transformed into the linear motion of the piston [24], which moves with a ~ 33 m/s speed to convey “okpeye” condiment into the cubing compartment [22]. Within the cubing compartment, the pressure developed by the moving piston delivers the force needed to push the condiment. The handle, equipping the cubing machine with the required drive, is moved to press the “okpeye” paste for ~ 1 min to generate the cubed condiment. This has been repeated at a 5 min interval. The product obtained has a measured approximate size of 20 mm \times 20 mm per cube. The cubed mesquite condiment is shown in Figure 6. After the cubing process, the cubes are further dried in the electric oven at a temperature of 60 °C for 12 h to achieve the desired moisture content.

3.1.2. Performance Evaluation of the Cubing Machine

The performance of the cubing machine in processing the “okpeye” condiment is shown in Figure 7. The throughput capacity (kg/min) is determined with cubing time (min) at a constant mass (0.5 kg). The throughput capacity of the cubing machine decreased as cubing time increased. During the first 20–25 min of operation, the machine performance increased, attributable to the dissipation of the reserved energy of the operator, who moved the piston-connecting rod arrangement to power the cubing compartment for 1 h. This specific scenario appears to agree with data published elsewhere [25,32–34]. The machine

performance, as driven by the operator(s) power output, would depend on the reserved energy required to power the cubing compartment [22]. Additionally, the natural adhesion property of the “okpeye” condiment might have decreased both throughput capacity and cubing machine performance as cubing time increased [22,35].



Figure 6. Cubed mesquite condiment.

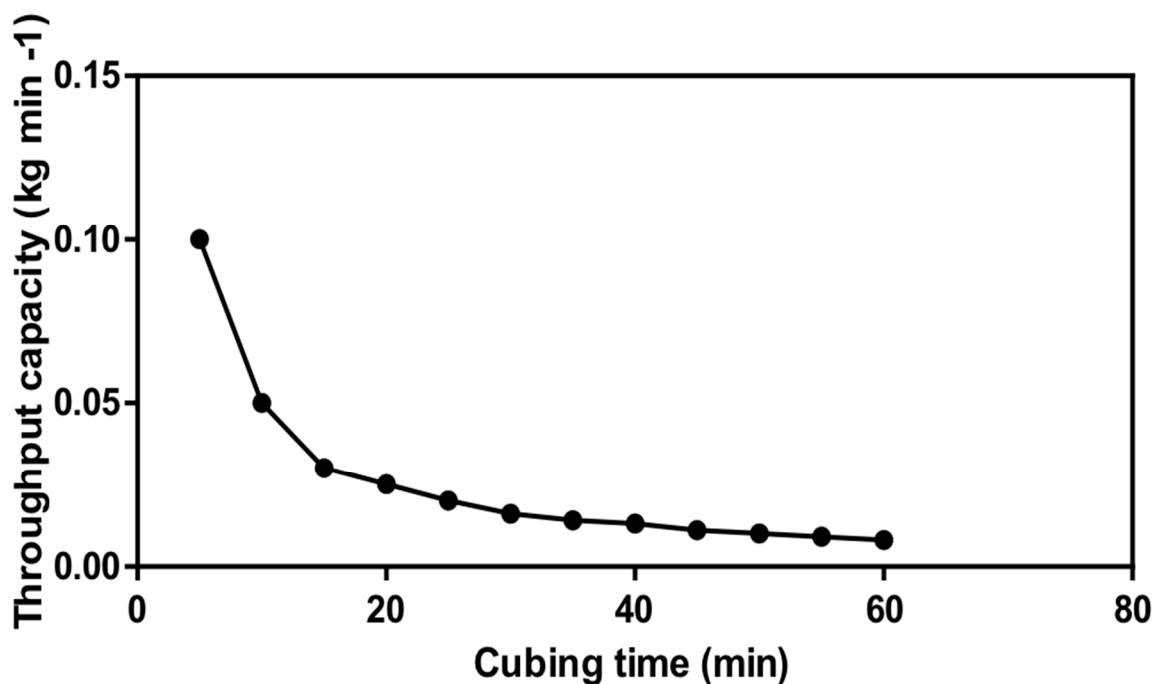


Figure 7. Throughput capacity (kg/min) against cubing time (min) (at a constant mass of 0.5 kg), where the time of cubing (1 min) has been determined at a 5 min interval. This helps to establish the performance of the cubing machine in processing the “okpeye” condiment.

3.1.3. Billing/Costing of Cubing Machine Materials

The billing/costing of cubing machine materials of the current study is shown in Table 1. The total cost of fabrication amounted to ₦45,740.00 at the time of construction/fabrication. Given the current living standard in Nigeria, the authors of the current

study deemed this total cost reasonably low, which would most likely be affordable to local/rural “okpeye” producers. In constructing a low-cost bean cubing machine, Yisa, Fadeyibi, and Hafeez [22] reported a total cost of ₦43,680.00, which appears to be roughly ₦2000 below the cost of the “okpeye” cubing machine of the current study. Developing a livestock feed cubing machine from locally sourced materials, Oyawale et al. [36] demonstrated the challenges rural farmers in Nigeria encounter in accessing cubing machines, notably because of the prohibitive costs as well as their unavailability. Food processing equipment, specifically US/EU- or Asian-based high-tech manufactured cubing machines, are well known to be very expensive. Therefore, developing and sourcing low-cost cubing machines very capable of actualising very promising quality (condiment) outcomes are imperative for the local/regional market.

Table 1. Bill of engineering measurement and evaluation (at the time of study).

Description	Quantity	Rate (₦)	Amount (₦)
Stainless steel plate	2 sheets	2300	4600
Sheets of 1.5 mm plate	1 sheet	2250	2250
Mild steel electrode	1 packet	1400	1400
1.5" × 1.5" angle iron	2 pieces	1300	2600
1.0" × 1.0" angle iron	1 piece	750	750
Mesh net	1 sheet	2450	2450
Mild steel plate	1 sheet	1580	1580
18 mm diameter rod	1 piece	1350	1350
Bolt	4 pieces	60	240
Barrel pipe	1 piece	6500	6500
Thread	1 piece	2400	2400
Stainless pipe	1 piece	7350	7350
Finger sheet	1 piece	4470	4470
Labour cost			7800
Total			45,740

₦ = Naira currency.

3.2. Comparisons of Proximate, Micronutrient, Phytochemical, and Microbial Aspects between the Fermented “Okpeye” Condiment and Commercial Available Bouillon Cube Products

3.2.1. Comparisons of Proximate Contents

The proximate contents of the “okpeye” condiment and commercial bouillon cube samples are shown in Table 2. The proximate composition (that is, ash, crude fibre, crude fat, crude protein, and moisture contents) showed significant ($p < 0.05$) differences across all tested samples. Further, the ash, carbohydrate, fat, fibre, moisture, and protein contents ranged 5.29–6.75%, 18.16–25.59%, 8.50–12.29%, 2.45–3.19%, 2.56–18.54%, and 1.45–42.50% across the tested samples, respectively. Specifically, the crude fat ($12.29 \pm 0.41\%$), crude protein ($42.50 \pm 0.13\%$), and moisture content ($18.54 \pm 0.07\%$) of “okpeye” condiment samples were significantly ($p < 0.05$) above Star[®], Knorr[®], and Chicken[®] commercial bouillon cubes. The fermentation process could increase the moisture content of “okpeye” condiment [9,12]. Food substances, especially of plant origin, with high moisture content will favour microbial proliferation that facilitates spoilage [37,38]. To enhance the shelf life, therefore, the moisture content of “okpeye” has to come down. Besides, free amino acids increases with a fermentation time during “okpeye” production [12], which makes the high crude protein value of “okpeye” ($42.50 \pm 0.13\%$) condiment not surprising, which appears above the (postproduction) dawadawa (crude protein) data range of 24.8–33.5% and 30.0–38.5%, respectively, reported by Gernah, Inyang, and Ezeora [39]. Besides, legume proteins have been recommended over cereal proteins because the former is deemed of better quality than the latter [40]. The high crude fat value of “okpeye” ($12.29 \pm 0.41\%$) might be because of the fermentation-led lipase activity [41,42].

Table 2. Proximate contents (%) of the “okpeye” condiment and commercial bouillon cube samples.

Samples	Crude Protein	Ash	Crude Fat	Moisture	Crude Fibre	Carbohydrate
A	42.50 ^d ± 0.13	6.04 ^{ab} ± 0.01	12.29 ^c ± 0.41	18.54 ^d ± 0.07	2.45 ^a ± 0.07	18.16 ^a ± 0.12
B	1.45 ^a ± 0.21	6.75 ^b ± 0.01	8.50 ^a ± 0.14	3.84 ^c ± 0.07	3.19 ^b ± 0.01	19.26 ^b ± 0.16
C	4.60 ^b ± 0.05	5.49 ^a ± 0.69	9.21 ^b ± 0.02	2.56 ^a ± 0.05	3.04 ^b ± 0.07	25.59 ^d ± 0.18
D	6.56 ^c ± 0.49	5.29 ^a ± 0.01	9.02 ^{ab} ± 0.02	3.05 ^b ± 0.07	3.02 ^b ± 0.09	20.05 ^c ± 0.07

Values are means ± standard deviation of duplicate determinations. Values in the same column with different letters are significantly ($p < 0.05$) different. The samples refer to A = “okpeye” sample; B = Star bouillon cube; C = Knorr bouillon cube; and D = Chicken bouillon cube.

Additionally, the carbohydrate of Knorr[®] ($25.59 \pm 0.18\%$) obtained significantly ($p < 0.05$) higher values compared with the “okpeye” condiment ($18.16 \pm 0.12\%$), Star[®] ($19.26 \pm 0.16\%$), and Chicken[®] ($20.05 \pm 0.07\%$) commercial bouillon cubes in the current work. Representative of the total mineral contents, ash content continues to remain part of proximate analysis for nutritional evaluation [43]. Processes like autoclaving and fermentation noticeably decrease the crude fibre contents in food mixtures [44]. Here, the ash content ($6.75 \pm 0.01\%$) and crude fibre ($3.19 \pm 0.01\%$) of Star[®] obtained significantly ($p < 0.05$) higher values compared with the “okpeye” condiment (ash content = $6.04 \pm 0.01\%$; crude fibre = $2.45 \pm 0.07\%$), Knorr[®] (ash content = $5.49 \pm 0.69\%$; crude fibre = $3.04 \pm 0.07\%$), and Chicken[®] (ash content = $5.29 \pm 0.01\%$; crude fibre = $3.02 \pm 0.09\%$) commercial bouillon cubes.

3.2.2. Comparisons of Micronutrient Contents

The mineral contents of the “okpeye” condiment and bouillon cube samples are shown in Table 3. The mineral contents, which include calcium (Ca), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), and sodium (Na), significantly differed ($p < 0.05$) with respective ranges of 6.65–18.68 mg/100 g, 0.38–10.67 mg/100 g, 5.82–21.28 mg/100 g, 2.17–12.32 mg/100 g, 0.08–2.03 mg/100 g, and 2.94–6.05 mg/100 g across all tested samples. The Ca (18.68 ± 0.02 mg/100 g), Fe (10.67 ± 0.04 mg/100 g), Mg (12.32 ± 0.01 mg/100 g), and Mn (2.03 ± 0.01 mg/100 g) contents of the “okpeye” condiment were significantly ($p < 0.05$) higher compared with those of the Star[®], Knorr[®], and Chicken[®] commercial bouillon cubes.

Table 3. Mineral contents (mg/100 g) of the “okpeye” condiment and bouillon cube samples.

Samples	Calcium	Iron	Potassium	Magnesium	Sodium	Manganese
A	18.68 ^d ± 0.02	10.67 ^d ± 0.04	5.82 ^a ± 0.03	12.32 ^d ± 0.01	2.94 ^a ± 0.04	2.03 ^c ± 0.01
B	6.65 ^a ± 0.02	9.57 ^b ± 0.04	15.48 ^c ± 0.02	4.28 ^c ± 0.02	5.64 ^c ± 0.05	0.09 ^a ± 0.01
C	8.02 ^b ± 0.03	9.67 ^c ± 0.01	21.28 ^d ± 0.02	2.93 ^b ± 0.01	5.46 ^b ± 0.01	0.08 ^a ± 0.01
D	9.97 ^c ± 0.03	0.38 ^a ± 0.02	9.33 ^b ± 0.04	2.17 ^a ± 0.04	6.05 ^d ± 0.01	0.32 ^b ± 0.01

Values are means ± standard deviation of duplicate determinations. Values in the same column with different letters are significantly ($p < 0.05$) different. The samples refer to A = “okpeye” sample; B = Star bouillon cube; C = Knorr bouillon cube; and D = Chicken bouillon cube.

The Fe (10.67 ± 0.04 mg/100 g) of the “okpeye” condiment seemed less than that in another (“okpeye”) data (24.17 – 25.77 mg/100 g) reported elsewhere [45], which might be attributable to the differences in fermentation time in those studies. In bean seeds particularly during the fermentation periods/times, it is believed that increases in mineral contents (for example, Ca, K, Mn, and P) could take place [46]. The Fe element, as a component of haemoglobin, helps to carry oxygen (O_2) via cells through the human body’s metabolic system for the effective delivery of the immune system to produce energy [45,47]. The Ca element is needed for strong bone/teeth development in humans, as well as needed in blood clotting [48]. The Mg element is well known to sustain various chemical processes in the human body, maintain both nerve and muscle functions, supporting a healthy immune system, keep the heartbeat steady, and activate enzymes in body metabolism.

The presence of the Ca, Fe, Mg elements in the “okpeye” condiment should be considered a great merit. Besides, the K content of Knorr[®] (21.28 ± 0.02 mg/100 g) obtained significantly higher ($p < 0.05$) values compared with that of the “okpeye” (5.82 ± 0.03 mg/100 g), Star[®] (15.48 ± 0.02 mg/100 g), and Chicken[®] (9.33 ± 0.04 mg/100 g) samples. The Na content of Chicken[®] (6.05 ± 0.01 mg/100 g) obtained significantly higher ($p < 0.05$) values compared with that of “okpeye” (2.94 ± 0.04 mg/100 g), Star[®] (5.64 ± 0.05 mg/100 g), and Knorr[®] (5.46 ± 0.01 mg/100 g). Both K and Na are well known to support the proper functioning of the heart. However, very small K and a large amount of Na can affect the heart [49].

The vitamin contents of the “okpeye” condiment and bouillon cube samples are shown in Table 4. Across all samples, the vitamins B1, B2, B3, and E ranged 0.12–0.70 mg/100 g, 0.17–0.85 mg/100 g, 0.11–0.75 mg/100 g, and 3.95–6.79 mg/100 g, respectively. The vitamin E of “okpeye” condiment (6.76 ± 0.01 mg/100 g) was significantly higher ($p < 0.05$) than that of the Star[®] (4.21 ± 0.01 mg/100 g) and Knorr[®]/Chicken[®] (3.95 ± 0.01 mg/100 g) commercial bouillon cubes. Whereas vitamin B1 of Star[®] (0.70 ± 0.01 mg/100 g) was significantly higher ($p < 0.05$) than those of “okpeye” condiment (0.12 ± 0.03 mg/100 g), Knorr[®] (0.50 ± 0.01 mg/100 g), and Chicken[®] (0.35 ± 0.01 mg/100 g) cubes, the vitamins B2 (0.85 ± 0.01 mg/100 g) and B3 (0.11 ± 0.01 mg/100 g) of Star[®] and Knorr[®] resembled ($p > 0.05$), respectively. Additionally, the vitamin E of Knorr[®] and Chicken[®] (3.95 ± 0.01 mg/100 g) resembled ($p > 0.05$). With the fermentation process progressing alongside microbial activities, as Olasupo, Okorie, and Oguntoyinbo [50] considered, the chemical constituents of raw materials could be transformed (which could apply to mesquite seeds, herein) to bring about potentially fortified products (which could apply to the “okpeye” condiment, herein) enriched with minerals and vitamins, supplemented with bioactive compounds.

Table 4. Vitamin contents (mg/100 g) of the “okpeye” condiment and bouillon cube samples.

Samples	Vitamin B1	Vitamin B2	Vitamin B3	Vitamin E
A	$0.12^b \pm 0.03$	$0.17^c \pm 0.01$	$0.13^b \pm 0.01$	$6.79^c \pm 0.01$
B	$0.70^{ab} \pm 0.01$	$0.85^b \pm 0.01$	$0.11^{ab} \pm 0.01$	$4.21^b \pm 0.01$
C	$0.50^a \pm 0.01$	$0.85^b \pm 0.01$	$0.11^{ab} \pm 0.01$	$3.95^a \pm 0.01$
D	$0.35^a \pm 0.01$	$0.55^a \pm 0.01$	$0.75^a \pm 0.01$	$3.95^a \pm 0.01$

Values are means \pm standard deviation of duplicate determinations. Values in the same column with different letters are significantly ($p < 0.05$) different. The samples refer to A = “okpeye” sample; B = Star bouillon cube; C = Knorr bouillon cube; and D = Chicken bouillon cube.

3.2.3. Comparisons of Phytochemical Composition

The phytochemical compositions of the “okpeye” condiment and bouillon cube samples are shown in Table 5. Across all samples, the flavonoids, alkaloids, saponins, and tannins ranged 0.12%–0.18%, 0.08%–0.15%, 0.19%–0.55%, and 0.69%–0.93%, respectively. Specifically, the alkaloids ($0.15 \pm 0.01\%$), saponins ($0.55 \pm 0.07\%$), and tannins ($0.93 \pm 0.01\%$) of “okpeye” condiment were significantly ($p < 0.05$) above those of the Star[®], Knorr[®], and Chicken[®] commercial bouillon cubes. Particularly, the flavonoids of the “okpeye” condiment ($0.18 \pm 0.01\%$) resembled ($p > 0.05$) that of Star[®] ($0.16 \pm 0.01\%$), both significantly higher ($p < 0.05$) than the Knorr[®] and Chicken[®] commercial bouillon cubes ($0.12 \pm 0.01\%$). The alkaloids in Star[®] ($0.09 \pm 0.01\%$) resembled ($p > 0.05$) that of the Knorr[®] ($0.10 \pm 0.01\%$) commercial bouillon cubes, herein.

The pre-fermentation activities, like overnight soaking and washing with water, can substantially decrease the toxic phytochemical components, like alkaloids, flavonoids, saponins, and tannins, present in unfermented legume bean/seed [50]. Therefore, and considering the above proximate micronutrients and phytochemical contents, the “okpeye” condiment, even when used in small amounts, remains promising in boosting the human immune system. For instance, it can serve as an energy resource and contribute to repair cellular damage. Besides being potent water-soluble antioxidants and free radical scav-

engers, flavonoids can help to prevent oxidative cell damage [51,52], which when utilised in the required concentration, can provide health benefits through cell signalling pathways and antioxidant effects [53]. Moreover, the presence of different chemical compounds, like alkaloids, flavonoids, and saponins, in the *Prosopis* pods, prior to the fermentation process is believed to be the basis of the medicinal properties [54].

Table 5. Phytochemical composition (%) of the “okpeye” condiment and bouillon cube samples.

Samples	Flavonoids	Alkaloids	Saponins	Tannins
A	0.18 ^b ± 0.01	0.15 ^b ± 0.01	0.55 ^c ± 0.07	0.93 ^d ± 0.01
B	0.16 ^b ± 0.01	0.09 ^a ± 0.01	0.37 ^b ± 0.01	0.81 ^c ± 0.01
C	0.12 ^a ± 0.01	0.10 ^a ± 0.01	0.29 ^{ab} ± 0.01	0.75 ^b ± 0.01
D	0.12 ^a ± 0.01	0.08 ^a ± 0.01	0.19 ^a ± 0.01	0.69 ^a ± 0.01

Values are means ± standard deviation of duplicate determinations. Values in the same column with different letters are significantly ($p < 0.05$) different. The samples refer to A = “okpeye” sample; B = Star bouillon cube; C = Knorr bouillon cube; and D = Chicken bouillon cube.

3.2.4. Comparisons of Microbial Counts

The microbial counts of the fermented “okpeye” condiment and bouillon cube samples are shown in Table 6. At the end of the fermentation period, the TVC of the cubed “okpeye” condiment was 2.5×10^2 log cfu/g, which was not significantly different ($p > 0.05$) from those of the Star (1.9×10^2 log cfu/g), Knorr (7.1×10^1 log cfu/g), and Chicken (4.5×10^1 log cfu/g) commercial bouillon cube samples. Additionally, no moulds were detected at all the samples. In general, the TVC range between 1.9×10^2 and 4.5×10^1 log cfu/g across all samples herein, still appears lower than the bacterial load of a bouillon cube made from low-cost bean seeds (7.5×10^3 log cfu/g), the latter specifically obtained on nutrient agar [55].

Table 6. Microbial counts of the fermented “okpeye” condiment and bouillon cube samples.

Samples	Total Viable Count (log cfu/g)	Mould (log cfu/g)
A	2.5×10^2	ND
B	1.9×10^2	ND
C	7.1×10^1	ND
D	4.5×10^1	ND

log cfu/g = logarithm colony-forming units per gram; ND = not detected; The samples refer to A = “okpeye” sample; B = Star bouillon cube; C = Knorr bouillon cube; and D = Chicken bouillon cube.

Largely considered spontaneous and noncontrolled, the fermentation process that underpins the “okpeye” condiment has been understood as accompanied by a wide range of microbiological profiles, which would most likely corroborate with (fermentation) temperature(s) and time(s) [13]. For emphasis, the action of microorganisms during the fermentation process allows the hydrolysis of protein constituents in the mash to take place [9], which would enhance the release of amino acids [56]. Adding to the alkaline conditions given by protein hydrolysis, the fermentation process required by the “okpeye” condiment could help slow down the progress of microbial proliferation [57]. Such (fermentation) conditions, like increased fermentation time and drying temperature, probably brought about the relatively low TVC of “okpeye” condiment.

The Na that portrays salt present in the “okpeye” condiment might be helping to hold the progress of microbial proliferation. During the fermentation process, the heat generated can provide the ideal temperature condition(s) to optimise the proteolytic enzymes. To achieve a good-quality “okpeye” condiment product, the microbiota within the fermenting food matrix should align with factors such as the (respective) hygienic status of handlers, production environment, raw materials, and utensils used [50,56].

In Nigeria, there is the Food Safety and Applied Nutrition (FSAN) Directorate under the National Agency for Food and Drug Administration and Control (NAFDAC) that

guides the production of food by small to medium enterprises, prescribing good hygiene practices (GHPs) crucial during manufacturing, processing, and packaging, which are very applicable to both the “okpeye” condiment and commercial bouillon cubes. Ugwuanyi and Okpara [58] understood that, oftentimes, the hazard analysis and critical control point (HACCP) principles in the production and unit operations of indigenous fermented foods seem not adhered to, which could hinder the adoption of such products into the international market. Herein, the cubing process of “okpeye” condiment could help control the nature of processing techniques, which might help enhance the product’s microbiological quality.

4. Limitations and Direction of Future Work

Given that the cubing machine was fabricated using the conventional workshop procedure, the degree of accuracy and precision might likely be questioned, which could be considered a limitation. The idea here is to make a cubing machine, and to press “okpeye” into cube forms, then, to make some comparisons with commercial bouillon types. Another potential limitation could be the costing, which has two facets. One facet is the overall price fluctuations in Nigeria. The other facet is the price variations specific to the materials used. If this cubing machine were to be fabricated today, a higher bill might be realised. Another limitation could be the machine performance, largely dependent on the power output of operator, which decrease with increase in the operation time.

A direction of future work should be to increase the accuracy and precision of fabricating the cubing machine, which could help enhance the (cubing) process as well as (“okpeye”) product development. It would be useful if a business model is developed to elaborate on how a transition from artisanal to industrial production can take place. Additionally, it would be useful to have artisans assess the cubed “okpeye”, as this would help understand their opinion about the cubing machine. This could be carried out alongside the shelf assessment of the cube “okpeye” condiment, specifically evaluating different storage conditions/contexts, and adding to the parameters of the current study, to determine both colour and textural aspects, which would involve making comparisons with commercially available bouillon cubes, to help supplement existing information.

5. Conclusions

The cubing fabrication, costing, and machine performance on African fermented “okpeye” condiment quality attributes, compared with commercial bouillon types, were performed. The “okpeye” cube condiment measures 0.0024 m² approximately, while the cost of fabrication is considerably low (₦45,740.00) and can be afforded by local producers. The performance of this machine is largely dependent on the power output of the operator and decreases with the increase in time of operation.

The fermented condiment was significantly ($p < 0.05$) higher in protein, fat, and moisture as well as calcium, iron, magnesium, and manganese compared with the bouillon cubes. All the samples were fair sources of vitamins B1, B2, B3, and E, but the condiment was higher in vitamin E content. The very low Na content in “okpeye” can help to balance the Na/K ratio for heart pressure maintenance compared with the bouillon cubes. The microbial loads resembled ($p > 0.05$) across the tested samples.

Indeed, the regular use of this fermented “okpeye” condiment in food/soup preparations should be promoted, given its capacity to sustain consumer health and well-being. Importantly, we demonstrated in this current study that the “okpeye” condiment can be cubed, competes favourably in terms of quality attributes, and is a very promising substitute to commercially available bouillon cubes in food preparations.

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E.O.U., C.F.O., and N.N.U.; writing—review and editing, C.O.R.O., W.A.R., I.S., G.S., G.B., and M.K. All authors have read and agreed to the published version of the manuscript.

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