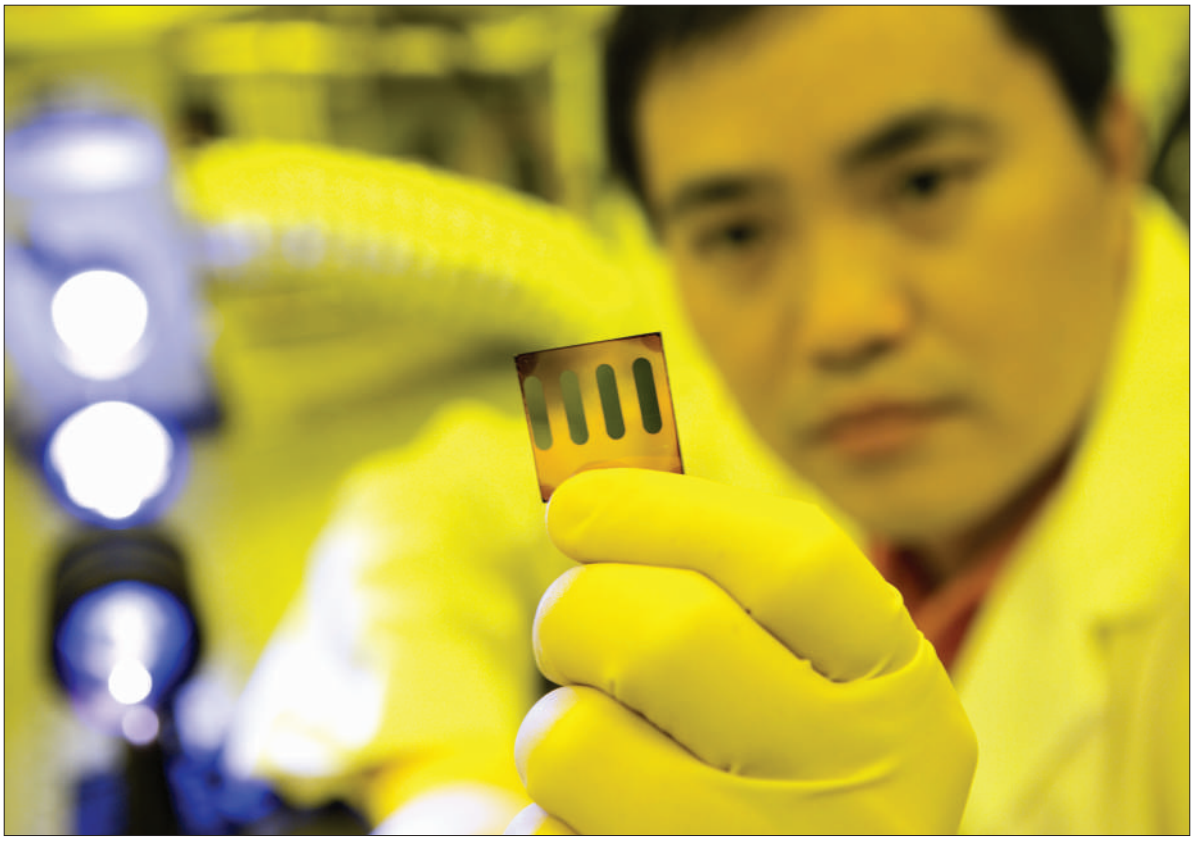

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Research Article
Effect of Fermentation Substrate Thickness on Physical, Chemical and Microbial Qualities of Fermented African Locust Bean Seeds (Daddawa)

Sulaiman Sani Kankara and Kabir Abdullahi Bindawa

Department of Biology, Faculty of Natural and Applied Sciences, Umaru Musa Yar'adua University, P.M.B. 2218 Katsina, Katsina state, Nigeria

Abstract

Background and Objective: Nutritional, organoleptic and chemical qualities of fermented African Locust Bean Seeds (ALBS) locally known as Daddawa is influenced by so many factors including fermenting substrates thickness. In this study, effect of fermenting substrate thickness (layers) on chemical, microbial, organoleptic and nutritional qualities of the product was investigated. **Materials and Methods:** A depulped, hydrothermally boiled, dehulled and parboiled good seeds of African Locust Beans (ALBS) was placed in clean absorbent plastic containers of about 25 cm long and fermented aerobically without stirring at 37°C in an incubator for two days (48 h) by random microbial inoculums. The fermented product were separated at 0-5, 5-10, 10-15 and 15-20 cm layers depth from top to bottom and analyzed for chemical, microbial, organoleptic and nutritional properties. **Results:** The result shows a statistically significant ($p<0.0001$) and organoleptically important variations in the different layers of the fermented substrate thickness on the product chemical, sensory and nutritional changes. Only substrates fermented within 0-5 cm thickness was organoleptically accepted, nutritionally high significantly and microbially potential. **Conclusion:** Therefore, for effective fermentation of ALBS, fermentation substrate thickness of 5 cm and below is recommended.

Key words: Substrate thickness, fermentation, locust bean seeds, *Parkia biglobosa*, microbial potential

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Corresponding Author: Sulaiman Sani Kankara, Department of Biology, Faculty of Natural and Applied Sciences, Umaru Musa Yar'adua University, P.M.B. 2218 Katsina, Katsina State, Nigeria Tel: +2349036935227

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Parkia biglobosa (Jacq.) Benth is a perennial deciduous tree from the sub-family Mimosoideae and family Leguminosae[1]. The family Leguminosae is the third largest family of angiosperms[2]. Its products are collected and processed into economically essential products useful in various human endeavors including medicinal, handicraft, domestic, medico-magic, veterinary, cultural, food and commercial[3,4].

In African countries such as Benin, Burkina Faso, Niger, Togo and Nigeria, Fermented African Locust Beans Seeds (FALBS) called 'Daddawa', 'Iru', 'Ogiri' 'Soumbala', 'Netetu' comprised one of the essentially consumed traditional food based condiment serve primarily for seasoning sauces and soups[3,5]. The use of the fermented beans of African locust bean (Daddawa) dates back many centuries and was already described in the 14th century[6]. The production of fermented locust bean has remained a traditional family art in a cottage industry especially in the rural areas. Adisa *et al*.[7] reported that processors involved are middle-aged females with little or no formal education and are dominant in the processing activities.

The FALBS have nutritional, organoleptic and microbiological properties of immense importance in food technology. Nutritionally, it is rich in protein, lipid, carbohydrate, oils, mineral elements, vitamins, antioxidants and low level of antinutritional factors[8-14], sensorially and organoleptically, it is characterized by a sweet taste, smooth mouth feels, marshy texture and dark brown coloration[12,15]. Microbiologically, it is a rich harbor of many important micro-organisms responsible for its microbial and metabolic changes[9,16-18]. Alkaline solid substrate fermentation of African locust beans seeds is responsible for the nutritional, organoleptic and microbiological properties of FALBS. Fermentation of African Locust Beans Seeds (ALBS) is characterized nutritionally and chemically by increase in moisture content, protein and lipid profiles, antioxidants, mineral elements, digestibility, pH and also decrease in ash content, crude fiber and total carbohydrate, anti-nutritional factors[8,9,14], organoleptically and sensorially by increase in aroma, sweet taste, smoothness of mouth feels, textural marshness and darkening of brown color[12]; microbiologically by increase in microbial population, production of greyish and slimy mucilage[16-18]. However, it is associated with the microbial production of metabolites from carbohydrate and protein fermentation such as; alcohol, carbon dioxide, ammonia, etc.[8,19].

Among the challenges associated with the production of FALBS was fermentation failure after a couple of uncontrolled days of fermentation. This failure is mostly experienced when fermenting a large mass of substrate, during extreme hot and cold weather. Some recent studies revealed that optimum temperature, relative humidity and aeration have effects on the qualities of FALBS[17,19]. However, optimum substrate thickness has not been evaluated.

Therefore, this study is design to find out the effective substrates thickness with enhanced qualities and controlled fermentation for recommendation through analysis of the chemical, microbial, organoleptic and nutritional changes at different substrate layers (thickness) of the fermenting substrates.

MATERIALS AND METHODS

Study area: This study was conducted between May and September, 2019. Experiments were conducted at the Biology Laboratory, Department of Biology, Umaru Musa Yar'adua University, Katsina state, Nigeria.

Source of seeds: Good seeds of African Locust Beans (ALB) were purchased at Bindawa L.G. A weekly market throughout the period of study and these were used in the production of FALBS. The seeds were further sorted out in the laboratory to ensure only good seeds are used.

Sample preparation: The good sorted raw seeds of *Parkia biglobosa* were depulped manually in mortar with pestle, washed thoroughly with clean water. The depulped seeds were hydrothermally boiled for about 24 h until the cotyledons became softened. The hydrothermally boiled seeds were dehulled in mortar with pestle and the hulls/seed coats from the seeds cotyledons were manually separated by floatation mechanism using a perforated calabash until the cotyledons were cleaned. The cleaned cotyledons were parboiled for nearly 2 h and allowed to drain[12]. The cleaned cotyledons were placed in clean absorbent plastic containers of the same circumference and depth (about 25 cm long), then covered with a thin membrane of tissue paper and fermented aerobically without stirring at 37°C in an incubator for 2 days (48 h) by random microbial inoculums. The fermented product were separated at 0-5, 5-10, 10-15 and 15-20 cm layers depth from top to bottom for the assessment of visible microbial colony formation and homogenized for chemical (pH) analysis. The separated layers were oven dried to preserve the products for sensory assessment and nutritional analysis and kept in tight containers for immediate further analysis.

Samples analysis

Assessment of visible microbial colony: The formation and intensity of visible microbial colony of the fermented products at undisturbed states was observed, ranked assessed, recorded and photographically snapped.

pH determination method: The pH of the fermented seeds of *Parkia biglobosa* were measured directly in a mixture prepared with 10 g of sample and 30 mL of distilled water previously mixed in a stomacher at normal speed for 1 min. A pH meter was used for the measurements[19].

Sensory evaluation: Twenty panelists (10 males and 10 females) who are conversant with the condiment were selected and were briefed about the aim of evaluation and how it should be conducted. They were presented with the well dried samples each in label. The attribute of the samples evaluated by the panelists were: Taste, aroma, mouth feels, texture and color based on 3-scale rankings (Table 1). The evaluation was based on the highest consensus of the panelist. The results obtained were tabulated and presented. This adopted method is in accordance to Sadiku[12].

Proximate analysis of fermented African locust bean seeds: Proximate Analysis of each well dried samples of the fermented product was conducted in triplicate for Ash content, Crude protein (or Kjeldahl protein), Crude lipid, Crude fibre and Nitrogen-free extracts (digestible carbohydrates) using a standard methods of analysis by the association of analytical chemists[20]. The proximate analysis of the samples was determined at relatively uniform moisture content and carbohydrate determination was by difference.

Table 1: Scale of ranking for the determination of physical quality[12]

Parameters	1	2	3
Taste	Sweet	Sour	Bitter
Mouth feels	Very coarse	Coarse	Smooth
Colour	Creamy brown	Dark brown	Greyish brown
Texture	Slightly marshy	Marshy	Very marshy
Aroma	Very mild	Mild	Strong

Data analysis: The pH values and proximate results were analyzed using Graphpad Prism statistical software, version 7.03. The data were subjected to a normality test and Variance (ANOVA). The means were compared using Tukey's comparison test.

Data presentation: The mean pH values, sensory assessment scale values and the proximate compositions were presented in tabulated form, the formation of visible microbial colony was presented in graphical form.

RESULTS

Chemical and microbial changes during fermentation of ALBS: This study reveals that fermenting substrate significantly affects chemical and microbial qualities of ALBS. Table 2 shows a highly significant variation ($p<0.0001$) in pH from slightly alkaline medium to a more acidic medium from layer A-D down the substrate thickness. It is also observed at the first top most layer (layer A), the formation of greyish and slimy mucilage of heterogeneous microbial colony (Fig. 1) and the alkaline medium represented are good indicators of microbial activities and a successful solid substrate alkaline fermentation of the ALBS and which are absent or lost in all the other layers.

Physical qualities of the FALBS: Table 2 also revealed the effect of substrate thickness on the physical and sensory assessments of the fermented product. From the data, it can be seen that the sweet taste, the desirable aroma, the dark

Table 2: Effect of substrate layers/thickness on mean pH value, microbial colony and physical (sensory) attributes of fermented African locust bean seeds

Layers/thickness (cm)	Chemical changes(Ph)	Microbial activity (Formation of visible colony)	Physical (sensory) attributes					
			Taste (Ts)	Aroma (Ar)	Color (Cl)	Texture (Tx)	Mouth Feels (Mf)	OMQ
A (1-5)	8.41 ± 0.32^{a}	Present	1	2	2	2	2	Accepted
B (5-10)	5.47 ± 0.08^{b}	Absent	3	0	1	1	1	Rejected
C (10-15)	5.33 ± 0.15^{b}	Absent	3	0	1	1	1	Rejected
D (15-20)	5.12 ± 0.21^{b}	Absent	3	0	1	1	1	Rejected
Significance	$p<0.0001$							

Results are expressed as Mean±SD of triplicate determinations, Same letters indicate mean values that are not significant different on the same column, [Formation of visible colony: P: Present, A: absent], 1, 2, 3, 4, 5,6: Order of increasing visible colony formation, Ts: Taste, Ar: Aroma, Mf: Mouth feels. Tx: Texture, Cl: Color, OMQ: Objection ability for the major qualities (i.e., Taste and Aroma), Taste (1 = Sweet; 2 = Sour; 3 = Bitter), Aroma (1 = Very mild; 2 = Mild; 3 = Strong), Mouth fees (1 = Very coarse, 2 = Coarse, 3 = Smooth), Texture (1 = Slightly marshy, 2 = Marshy; 3 = Very marshy), Color (1 = Creamy brown, 2 = Dark brown, 3 = Greyish brown), 0 = Absence of the quality

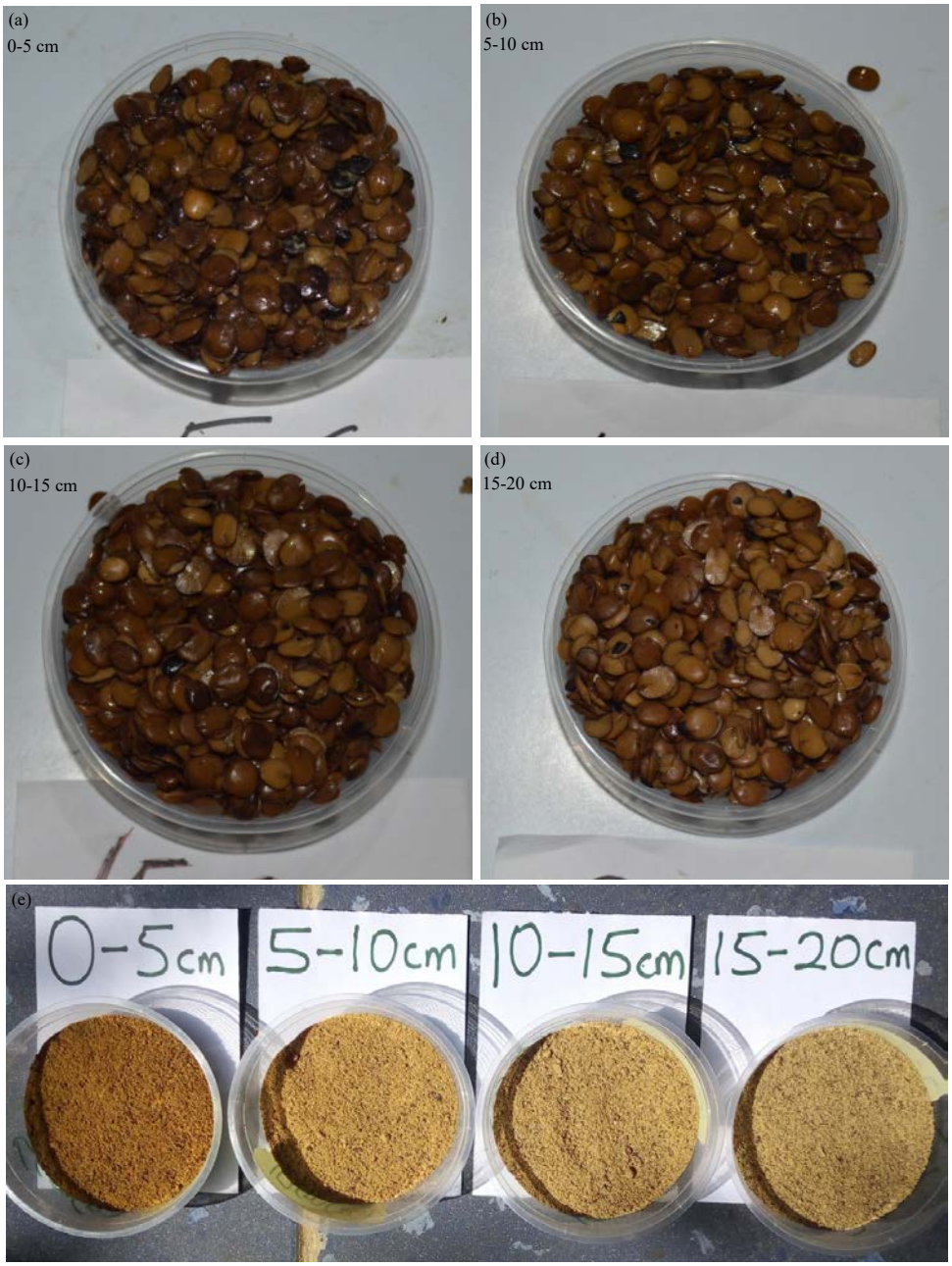

Fig. 1(a-e): Formation of visible microbial colony and color variations at different substrates layers or thickness of the fermented African locust bean seeds

Table 3: Effect of fermentation substrate layers/thickness on the nutritional qualities of FALBS

	Proximate compositions				
Layers (cm)	Ash	Crude fibre	Crude lipid	Crude protein	Total carbohydrate
A (1-5)	3.22±0.04[a]	4.16±0.03[a]	33.77±0.62[a]	38.42±0.14[a]	20.43±0.65[a]
B (5-10)	3.75±0.18[b]	4.20±0.06[b]	27.86±0.60[b]	33.28±0.06[b]	30.90±0.35[b]
C (10-15)	4.08±0.03[c]	4.23±0.02[c]	27.47±0.64[b]	31.14±0.09[c]	33.09±0.54[c]
D (15-20)	4.17±0.06[c]	4.34±0.06[c]	27.29±0.17[b]	30.68±0.50[c]	33.52±0.54[c]
Significance	p<0.0001	p = 0.0019	p<0.0001	p<0.0001	p<0.0001

Results are expressed as Mean±SD of triplicate determinations, Same letters indicate mean values that are not significant different on the same column

brown color and the textural marshness have drastically reduced or are completely absent along the substrate and the coarseness of mouth feel is increased with substrate thickness. Figure 1 shows the reduction in the dark brown color of the fermented substrates at different layers or thickness.

Nutritional qualities of the FALBS: The results of proximate analysis of the treated layers are presented in Table 3. The data shows that highest level of proximate chemical compounds and a highly significant variation ($p<0.0001$) was recorded at the first top most layer. As substrate depth increases, the crude protein and crude lipid decreased while total carbohydrate, crude fiber and ash content are also increased.

DISCUSSION

In this study, it was found that chemical and microbial changes in ALBS were significantly influenced by the thickness of fermenting substrate. To achieve an even fermentation of all the substrate irrespective of its thickness, a periodic stirring of the fermenting substrate is required[20]. However, prolonged fermentation produces unpleasant and undesirable changes that immensely affect the product qualities[1,21]. Ouoba *et al.*[22] earlier reported that the desired state of fermentation of condiments is indicated by formation of mucilage and overtones of ammonia produced and alkaline pH of the fermented substrate as a result of breakdown of amino acids during fermentation. Therefore, somewhere at a distance of 5 cm from the top moving down to the bottom layers, alkaline protein fermentation was not achieved and this may be due to low pH value, accumulation of heat and reduced oxygen for aerobic fermentation by aerobic micro-organisms. It can also be seen that the normal characteristics of the unfermented seeds of ALBS are retained because of low pH value, poor establishment of desirable microbial activities and accumulation of heat. Thus, if there is no establishment of desirable fermentation, then no desirable quality changes. Ikenebomeh[15] reported that the well fermented product has a sweet nutty aroma, soft in texture and easy to mash between the fingers while the poorly fermented product is relatively hard in texture, not easy to mash between the fingers and with a beany aroma. In this study, it is also established that thickness of fermentation substrate significantly affects nutritional qualities of FALBS, whereby upper most layer ($\leq$5 cm) was found to be the best layer for optimum nutritional qualities. The decrease in the above mentioned compounds along the layers is probably due to low pH and little or no microbial activities at less substrate depth of less than 5 cm. Several studies have shown that fermentation increases moisture, crude protein, crude lipid and decreases total carbohydrate, crude fiber and ash content[9-11, 23, 24].

CONCLUSION

This study reveals that fermentation substrate significantly ($p<0.0001$) affects mean pH value, organoleptic properties and potential microbial content. Fermenting substrates thickness of not more than 5 cm was found to be effective. Further studies aimed at examining the activity of extracellular enzymes in fermenting African locust bean seeds for Daddawa production is highly recommended.

SIGNIFICANCE STATEMENT

This study established that fermentation substrate thickness significantly affects desirable qualities of FALBS. Fermentation substrate thickness of not more than 5 cm was found to be effective for optimum chemical composition, microbial quality, physical attributes as well as nutritional values of Fermented African Locust Bean Seed (Daddawa). Results of this study would be useful to the producers of this condiment as it will go a long way in reducing losses incurred by the producers as a result of poor fermentation resulting in undesirable features of the product due to very thick fermentation substrate.

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