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Research Article

Effects of Natural Fermentation Time on the Nutritional, Functional and Objective Color Characteristics of Soybean Condiment (*Soy-Dawadawa*)

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Abstract

In this study, changes in the proximate, minerals, functional and color characteristics during the fermentation of decorticated and roasted soybean seed for the manufacturing of soybean-based condiment (soy-*dawadawa*) were evaluated for a period of 0, 24, 48, 72, 96 and 120 hours (hr). The results indicated that fermentation time significantly ($p<0.05$) increase protein content from 44.92% to 66.82% with non-significant impact on the moisture. However, Ca and Mg significantly ($p<0.05$) increase and Na, K and P decreased with increasing fermentation time. Water absorption capacity decreased during the first 24 hr from 63.41 to 63.25%, but gradually increased to 69.73% at the end of the fermentation time, oil absorption decreased significantly during the first 24 hr from 81.45 to 81.31% and gradually decreased to 78.62%. There was no significant difference ($p<0.05$) among the samples as a result of the changing fermentation time in terms of water solubility index. Increasing fermentation time was found to improve lightness (L^* = 49.50 to 53.88) of the product and reduced redness (a^* = 9.90 at 72 hr and 8.95 at 144 hr). There was no significant impact on the yellowness color attributes. Improved protein content suggests its possible use as potential source to improve the nutritional qualities of local soups. While the increased water absorption indicates suitability as soup thickener.

Keywords: Soybean, Condiment, Fermentation, Nutrients, Functional property, Color

Introduction

Traditional food condiments produced from plant seeds are important components of traditional culinary for centuries in several African communities. They have been consumed by most ethnic groups in Nigeria and provide nutritious non-meat protein as well as serving as condiments, flavoring and thickening agents in traditional soups (Achi, 2005). Majority of traditional Nigerian diet are produced from cereal grains and root/tuber crops, and eaten together with soup made from vegetables, legume seeds and nuts. The vegetable and seeds provide the nutrients required for normal growth, development and functional qualities that improve consumer acceptability.

Legume seeds have significantly contributed to the development of human nutrition (Nwosu and Ojimelukwe, 1993) as they account for about 80% of dietary protein supply and may be the only source of protein for low income class of the population. When cooked, legume seeds are eaten as meals or are commonly fermented and used as condiments to enhance the flavor of foods (Oniofiok *et al*., 1996). The process of converting legumes into semi-finished or finished products at

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both cottage and industrial levels, especially through fermentation, facilitates the transformation of the raw seeds into edible form, improve its digestibility, improve nutritional quality, and eliminate anti-nutritional factors, deactivate spoilage organisms and enzymes thus extending shelf-life and serves as sources of income.

Fermented foods have been defined as foods which have been subjected to the actions of microorganisms for a given period of time so that desirable physical and biochemical changes may occur that may cause significant modification of the foods (Campbell-Platt, 1987). In Nigeria, like in most tropical countries of the world, high temperature and humidity provide ideal conditions for fermentation to progress (Rolle, 1997); These changes may result in desirable and non-desirable changes that may affect nutritional, functional, color and other quality attributes of the final food products.

It is for these reasons that this study was designed and conducted with the aim of evaluating changes in physical, nutritional and functional characteristics of fermented decorticated roasted soybean used as local condiment (soy-*dawadawa*) for soup preparation. This study we believe will facilitate the definition of appropriate processing condition for the production of soybean based fermented condiment.

Materials and Methods

Materials and pre-treatments

Dried seeds of soybean were purchased from modern market Bida, Niger State. It was sorted and cleaned, roasted and decorticated. One kilogram of decorticated soybean was weighed into five places and each was cooked properly until completely soft to hasten fermentation. The time taken to achieve this was recorded as 140 min. The samples were labeled A, B, C, D, E and F corresponding to 0, 24, 48, 72, 96, 120 fermentation times respectively. The samples were drained and allowed to cool for 30 min after which they were wrapped in banana leaves and placed into polythene bags before placing into a fermentation vessel (locally fabricated) (Figure 1) to ensure an anaerobic condition and allow to ferment (Figure 2) for pre-determined times before harvesting and dried under the sun (32±2^{o}C) and packaged in plastic containers prior to analysis.

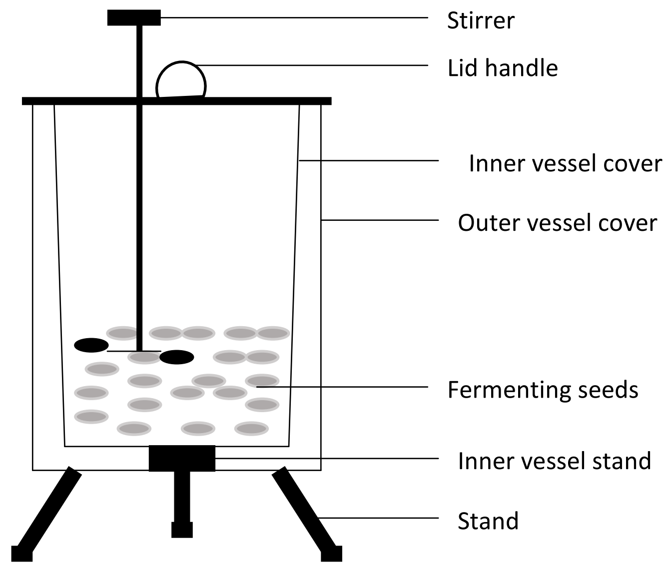

Figure 1: Schematic design of fermentation vessel

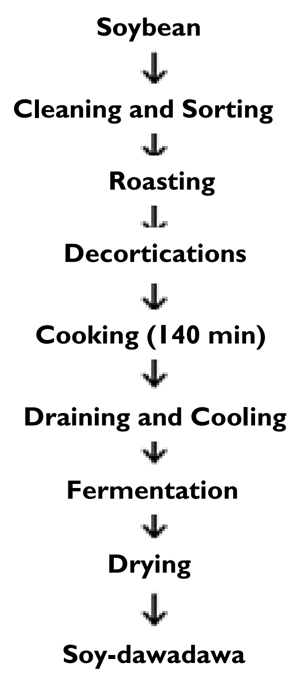

Figure 2: Flow chart for the production of soy-*dawadawa*

Chemical Analysis

Proximate composition analysis

Moisture content (MC) of samples was determined by weighing 5 g sample in a crucible and drying at 100^{o}C in an oven till a constant weight was attained. MC was then calculated as:

$$\mathrm{MC}\ (\%) = \frac{W_1 - W_2}{W_1 - W} \times 100$$

where W is the weight of empty crucible, W_1 weight of crucible plus sample and W_2 is the weight of crucible with dried sample.

Ash content was determined by following the method AOAC (1980) in which 2 g sample were incinerated in a furnace at 600^{o}C and the final weight determined. Ash content was calculated as:

$$\mathrm{Ash}\ (\%) = \frac{W_1 - W}{W_2 - W} \times 100$$

Where W is the weight of crucible, W1 weight of sample incinerated at 600^{o}C and W2 weight of crucible plus sample. The protein content was also determined by Micro-Kjeldahl Method where sample protein content was calculated after sample digestion and titration by the following equation:

$$\mathrm{Protein}\ (\%) = \frac{(A - B) \times 14 \times C \times 6.25}{D} \times 100$$

Where A is the volume of NaOH required for the blank to neutralize 20 ml of 0.1 N H_2SO_4, B is the volume of NaOH required for back titration, C is the normality of NaOH used for titration, and D is the weight of sample. The numbers 6.25 and 14 are the conversion factor for nitrogen to protein and atomic weight of nitrogen respectively. Soxhlet extraction technique was used for the determination of fat content. 5g of dried sample was weighed into an extraction thimble plugged with cotton wool before placing into a Soxhlet extraction apparatus containing anhydrous ether and extracted for 4 hr. After extraction, the ether was removed by evaporation and residues dried in an oven at 80^{o}C, cooled in a desiccators and weighed. Fat content was then calculated as:

$$Fat\ (\%) = \frac{W_2}{W_1} \times 100$$

Where W_1 is the weight of sample taken and W_2 is the weight of dried residue.

Carbohydrate was calculated by difference method (AOAC, 2003), where the amount of moisture, fibre, protein, fat and ash were subtracted from 100%, and the result considered as carbohydrate.

% Carbohydrates = 100 – (% Moisture + % Protein + % Lipid + % Ash).

The water general conversion factor system was used to calculate calorie value where the energy values of 17 kJ/g (4.0 kcal/g) for protein, 37 kJ/g (9.0 kcal/g) for fat and 17 kJ/g (4.0 kcal/g) for carbohydrates was used for conversion. The conversion factors for joules and calories are: 1 kJ = 0.239 kcal; and 1 kcal = 4.184 kJ.

Mineral analysis

Mineral contents were determined by atomic absorption spectrometry and flame photometry according to the methods of (AOAC, 2003). 1 g of the sample was weighed into digesting glass tube. 12 ml of HNO_3 was added to the samples and mixture kept overnight at room temperature (30±2^{o}C). Then 4.0ml $HClO_4$ was added to this mixture and kept in a fumes block for digestion. The temperature of digester was increased gradually, starting from 50^{o}C to 250-300^{o}C. The process was completed in about 70- 85 min as indicated by the appearance of white fumes. The mixture was then left to cool and the contents of the tubes transferred to 100 ml volumetric flasks and the volumes of the contents made to 100 ml with distilled water. The wet digested solution was then transferred to plastic bottles labeled accurately and used it for mineral determination. All values were expressed in mg/ 100g.

Water absorption (WAI) and solubility indices (WSI)

Water absorption and solubility indices were determined according to the methods described by Anderson *et al*. (1969) as modified by Onwulata *et al*. (1998). 1 g sample was weighed into a centrifuge tube and 10 ml distilled water added. After standing for 1 hr and shaking every 5minutes interval, the samples were centrifuged for 30 min at 10,000rpm. The supernatant was decanted and the weight gain in the gel was recorded. WAI was calculated as the weight gain by the dry gel weight

$$WAI\ (g) = \frac{\text{Weight of sediment}}{\text{Weight of dry solid}}$$

The supernatant were dried overnight at 90^{o}C and WSI determined as weight of dried supernatant divided by the weight of dry sample.

$$WSI(g) = \frac{\text{Weight of disolved solid in supernatant}}{\text{Weight of dry solid}}$$

Color determination

The color characteristics of extruded snacks were estimated by the International Commission on Illumination (CIE, 1976) L*, a*, b* method which is often used in food research using Monilta Color Reader CR-10 (Minolta Co. Ltd., Tokyo, Japan). In Minolta color meter, the color of a sample is represented by L*, a*, and b*. The L* value gives a measure of product lightness, a chromatic color from absolute black (0) to absolute white (100); a* value means mixed red-to-green color of samples ranging from negative value on the red side to positive on the green side (a+ = 0-100 for red and a- = 0-(-100) for green); and b* value mean mixed blue-to-yellow color of samples ranging from negative to positive value (b+ = 0 - 100) for yellow and b- = 0-(-100) for blue. 10 measurements were taken for each sample.

Statistical Design and Analysis

The experimental set-up was a single factorial design, where fermentation time (hr) was the independent variable and the functional, proximate, minerals and color characteristics set as the response variables. At all point of the experiment, triplicate data were taken to achieve reliability and measure of experimental error. A completely randomized design (CRD) was used with the assumption that the experimental conditions were homogenous except when changed based on the experimental design. Analysis of variance (ANOVA) was performed on all data using Statistical Tool for Agricultural Research (STAR) version 2.0.1 (IRRI, 2013) and significant means ($p<0.05$) were separated using Turkeys's Honestly Significant Difference (HSD) test.

Results and Discussion

Effects of fermentation time on proximate composition

The effects of fermentation time on the proximate composition of soybean are presented in Table 1. There was no significant difference ($p\leq0.05$) among the fermentation times in terms of moisture content ranging between 4.37 and 6.04%, however, scientifically the low moisture content in food samples has a tendency to increase the storage periods of the food products because high moisture content in foods encourage microbial growth; hence, food spoilage (Alozie *et al*., 2009; Assohoun *et al*., 2013). In terms of protein, there was a significantly rapid decrease from 63.13% to 44.92% within the first 24 hours before gradually increasing to 66.82% at the end of the fermentation period. The initial decrease in protein may be attributed to rapid microbial proliferation during the earlier hours of fermentation. The gradual increase in protein is in agreement with results of El-Tinay *et al*., (1979), Dirar (1985); and Omodara and Olowomofe (2013). The increased protein content of final product may be attributed to possible slight increase in nitrogen during fermentation (Dirar, 1985) as a result of microbial synthesis of protein during growth cycle (Zamora and Fields, 1979). There was no significant variation in ash content among the treatments during the fermentation period. Fat content significantly increased from 14.32% to about 17.90% during the first 24 hr of fermentation and gradually decreases to 13.53% at the end of 144 hr fermentation period (Table 1). The initial increase in fat content may be as a result of increasein activities of lipolytic enzymes that breakdown fat to fatty acids and glycerol. Obizoba and Atii, (1991) and Omodara and Olowomofe (2013) reported similar observations during fermentation of sorghum seeds.The crude fibre content of soybean was 2.59% and this value increased during the first 24 hr of fermentation and gradually decreased significantly ($p\leq0.05$) to 2.47% at the end of the fermentation time (Table 1). Similar trend was observed in the carbohydrate and energy value of the fermented soybean. Wang (1968) reported that fermentation may slightly alter the proximate composition of substrates. A slight increase in protein was noted in this study and this has reflected in the decrease of other constituents which the fermenting microbes might have consumed during growth.

Effects of fermentation time on mineral content

The mineral composition of soybean during the different fermentation periods in terms of Ca, Mg, Na, K and P are presented in Table 2. The results indicated significant reduction in the mean values of calcium and magnesium and increase in the means of Na, K and P. In most food processing conditions such heat treatment, minerals seems to stable possibly because of its resistance to heat. But as observed in this study, the reduction in the mean values of Ca and Mg may be as result of leaching into water during boiling before

fermentation or may be due to their utilization by fermenting organisms. The variation in Na (0.16 – 0.80), K (0.31-2.49) and P (0.09-0.71) may be due to the release of these elements from the organic compound. Increase in Na content Assohoun *et al*. (2013) reported for some fermented products was attributed to the release of this compound from its complex state which made it unavailable in fermented seeds. The magnesium Mg and the calcium Ca also decreased because of leaching during processing or because of the utilization of the compound by the fermenting organisms. Similar results were also observed by Assohoun *et al*. (2013).

Effects of fermentation time on functional properties

Fermentation significantly ($p<0.05$) decreased the water absorption capacity of soybean during the first 24 hr from 63.41 to 63.25%, but gradually increased to 69.73% at the end of the fermentation time. The increase in water absorption capacity could be attributed to the possible presence of high hydrophilic constituents as the fermentation time increased (Badifu and Akubor, 2001; Igbabul *et al.*, 2014). The values obtained in this study are greater than 11.2% for soybean, Adebowale *et al.* (2005) of 12-20% in mucuna. The oil absorption capacity of *dawadawa* decreased significantly during the first 24 hr from 81.45 to 81.31% (Table 3) and gradually decreased to 78.62% at the end of the production time (144 hr). These results are in disagreement with that of Elkhalifa *et al*. (2005) who reported increase in oil absorption capacity during the fermentation of sorghum flour. The decrease in oil absorption capacity in this study may be due to increase in fat content during fermentation process resulting in decreased hydrophobicity of the food system. There was no significant difference ($p<0.05$) among the samples as a result of the changing fermentation time in terms of water solubility index.

Effects of fermentation time on color characteristics

The effects of fermentation time on the color development in soybean based *dawadawa* are shown in Table 4. Color in food products are sensory quality attributes that is often overlooked but greatly influences flavor perception

(Hutching, 2003), while Schlosser (2001) demonstrated that color in foods had great effect on its acceptance. Generally, pigments in foods are unstable and therefore easily modified by harsh food processing methods. In this study, the chroma value for lightness (L*) significantly varies with fermentation time, increasing from 49.50 in unfermented sample to 53.88 at the end of 144 hr (Table 4). In the International Commission on Illumination (CIE) color rating, L* measures product lightness and ranges from absolute black (0) to pure white (100). This indicates that the color of the roasted soybean used for the production of *dawadawa* in this work gradually got lighter as the fermentation increased. The a* value represent redness of sample decreased from 9.70 in the non fermented sample to 6.40 at the end of 72 hr fermentation period, and start to increase again to 8.95 at the 144 hr. This result indicates that as fermentation time increases the color of the fermented soybean transverse away from red. There was no significant difference among the samples at the different fermentation time in terms of their yellowness (b*). It clear from this study, fermentation greatly affect the color characteristics of roasted soybean by improving it lightness and reducing redness with non-significant change in yellow color value.

Conclusion

Fermentation of decorticated and roasted soybean for the manufacture of soybean based *dawadawa* was found to have significant changes in nutritional composition, functional behavior and color of the final products. Improved protein of soy-*dawadawa* suggests its possible use as potential source to improve the nutritional qualities of local soups. The increasing water absorption of the condiment is an indication of its suitability as soup thickener. Wh ile increasing fermentation time have been found to improve lightness of the product and reduces redness and has little or no impact on the yellowness color attributes.

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Table 1. Effect of fermentation time on the proximate composition of soybean condiment

Fermentation time (hr)	Proximate composition (%)						
	Moisture	**Protein**	**Ash**	**Fat**	**Fibre**	**CHO**	**Calorie (Kcal/100g)**
0	6.04±1.63[a]	63.13±0.17b	3.90±0.50a	14.32±0.34b	2.59±0.12b	8.62±0.62e	186.38±0.66b
24	5.41±0.89[a]	44.92±0.38e	5.303±0.18a	17.90±0.64a	3.48±0.35a	23.11±0.15b	274.67±0.50e
72	5.51±0.53[a]	52.14±0.70d	5.44±0.64a	11.75±0.14c	1.36±0.12d	22.87±0.12b	221.89±0.79d
96	5.24±0.21[a]	52.21±0.25d	5.54±1.83 a	18.46±0.28a	2.21±0.13c	27.37±0.51a	292.08±0.14f
120	5.37±0.88[a]	58.85±0.13c	4.49±0.64a	11.68±0.33c	3.31±0.13a	15.41±0.45c	176.15±0.36a
144	4.37±0.03[a]	66.82±0.10a	4.77±0.53a	13.53±0.60b	2.47±0.05bc	12.90±0.16d	194.99±0.29c

Mean in the same column followed by the same letter are not significantly different ($p<0.05$), CHO = Carbohydrate

Table 2. Effect of fermentation time on the mineral composition of soybean condiment

Fermentation time (hr)	Mineral composition (%)				
	Ca	**Mg**	**Na**	**K**	**P**
0	0.47±0.14a	0.65±0.21d	0.16±0.14d	1.19±0.09d	0.44±0.04c
24	0.22±0.01cd	0.25±0.28c	0.80±0.14a	1.31±0.03cd	0.52±0.03c
72	0.19±0.01d	0.42±0.21a	0.19±0.01d	2.18±0.21ab	0.69±0.09ab
96	0.33±0.01b	0.29±0.01bc	0.38±0.30c	2.49±0.67a	0.09±0.09d
120	0.25±0.06cd	0.34±0.05b	0.19±0.05d	1.54±0.06bcd	0.58±0.02b
144	0.27±0.21c	0.33±0.04bc	0.51±0.05b	2.02±0.03abc	0.71±0.05a

Mean in the same column followed by the same letter are not significantly different ($p<0.05$)

Table 3. Effect of fermentation time on the functional characteristics of soybean condiment

Samples	Functional properties		
	Water absorption capacity (%)	**Oil absorption capacity (%)**	**Water solubility index**
0	63.41±0.63a	81.45±1.07c	0.21±0.19a
24	63.25±0.45d	81.31±0.26ab	0.11±0.11a
72	67.72±0.02d	80.32±0.86ab	0.17±0.11a
96	67.01±0.16bc	82.89±0.49bc	0.13±0.01a
120	68.59±0.49c	80.72±0.89a	0.28±0.24a
144	69.73±0.47ab	78.62±0.74b	0.33±0.25a

Mean in the same column followed by the same letter are not significantly different ($p<0.05$)

Table 4. Effect of fermentation time on the color characteristics of soybean condiment

Fermentation time (hr)	Color characteristics		
	L*	**a***	**b***
0	49.50±3.25ab	9.70±0.85ab	19.10±0.28a
24	55.45±0.49a	6.90±0.00a	16.00±0.85a
72	53.95±1.63b	6.40±0.57bc	15.95±0.49a
96	51.30±0.17ab	6.90±1.27c	18.50±1.84a
120	53.40±3.25ab	8.40±1.13bc	19.20±3.39a
144	53.88±2..12ab	8.95±1.34abc	17.65±4.03a

Mean in the same column followed by the same letter are not significantly different ($p<0.05$)