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Storage Stability of Deep-fried Cowpea Products (AKARA) Incorporated with Soy-flour and *Aframomum danielli*

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Authors' contributions

This work was carried out in collaboration between both authors. Author CFA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author AF managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: To investigate the effect of incorporating soy-flour and *Aframomum danielli* on quality characteristics and keeping qualities of deep-fried cowpea products (akara).
Study Design: Two-way ANOVA.
Place and Duration of Study: Department of Food Science and Technology, Federal University of Technology, Akure, Ondo State, Nigeria, between January 2014 and November 2014.
Methodology: Four different samples of akara were prepared using 20% soy-flour and 3% *Aframomum danielli*. The choice of these levels was based on their acceptability during the preliminary trial. The fried akara samples were packaged in foil paper and stored at room temperature over a period of 4 days. Effect of soy-flour and *Aframomum denielli* on proximate composition and sensory quality of akara were evaluated. Changes in microbial load, free fatty acid

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value (FFA), peroxide value (PV) and thiobabarturic acid value (TBA) during the storage period were also determined.
Results: The protein content ranged from 23.5±0.4 to 26.2±0.3; ash content ranged from 3.1±0.2 to 3.57±0.3; crude fibre ranged from 3.27±0.3 to 3.63±0.4; crude fat ranged from 23.21±0.6 to 26.14±0.4 and carbohydrate ranged from 43.32±1.3 to 44.24±0.3. The results showed that at 20% substitution, soy-flour lowered the fat content of akara by 8.2% and increased the protein content by 10.7%. Addition of *Aframomum danielli* did not significantly (P≤0.05) affect the proximate composition of akara samples. Incorporation of soy-flour at 20% produced acceptable akara without affecting the sensory attributes of the akara while on the other hand, akara samples spiced with 3% *Aframomum danielli* were significantly (P≤0.05) rated lower than non- spiced samples in term of taste, colour, texture, aroma and overall acceptability. The results of storage stability of akara over a 4- day of storage period showed that akara samples spiced with 3% *Aframomum danielli* had lower microbial load, PV, FFA and TBA values when compared to non-spiced samples.
Conclusion: Acceptable akara with low fat content and high protein content were produced by substituting cowpea flour with 20% soy-flour. *Aframomum danielli* has the potential to be used as preservative ingredient in the production of akara. Hence, utilization of soy-flour and *A. danielli* in the production of akara would produce good quality akara with low fat content and elongated shelf life.

Keywords: Akara; soy-flour; Aframomum danielli; proximate composition; sensory attributes; storage stability.

1. INTRODUCTION

Akara, a deep-fried cowpea batter is the most consumed cowpea product in West Africa. Traditionally, the batter is made from fresh cowpea paste seasoned with bell or hot peppers, onion and salt and then deep-fried at 193ºC [1]. The production of fresh paste from cowpea is a major constraint in the preparation of akara [2]. This involves soaking, decortication and wet milling, which is tedious and time consuming [3,4].

Another major problem associated with akara is its susceptibility to various types of spoilage such as staling, rancidity and ropiness, soon after its production [5]. Akara starts to stale the minutes it leaves the fryer which makes its crumb to become firm, harsh, opaque and more crumbly. The poor shelf life of akara has been attributed to its fat content and high moisture content [5,6]. Associated with the fat content is lipid oxidation while high moisture content in the product predisposes carbohydrate and protein in it to fermentation and putrefaction respectively, causing ropiness by *Bacillus subtilis* [7,6].

Soybean is an important source of high quality but inexpensive protein and oil. Studies shown that soybean has the highest protein content of all legume crops with an average protein content of 40% and second to groundnut in term of oil with an average fat content of 20% [8]. Previous studies on soybean showed that addition of soybean flour to fried food formulations help to reduce the oil absorption capacity of the product during frying or the overall fat content [1,9].

Aframomum danielli, (Hook, F) K. Schum (family, Zingiberaceae) is a large, robust perennial plant 3-4m tall which grows in central and West African countries [10]. The seeds of this plant are used for flavouring traditional dishes and the essential oil is used in perfumery, flavouring and dye preparations. The nutritive status of *A. danielli* has been reported by past studies [11]. The duo activities of *A. danielli* as antimicrobial and antioxidant have been established. The spice has been found to inhibit the activities of some microorganism significantly with increased reduction in food spoilage [12]. The anti-oxidative activities of *A. danielli* and its preservative action on foods were reported by Ashaye et al. [13] and Adedeji and Ade-Omowaye [14].

Based on this background, high quality characteristic of akara from cowpea flour could be produced by using a specified particle size distribution and adding soy-flour to reduce the fat uptake during frying. Incorporation of *A. danielli* in preparation of akara could serve as antioxidant and antimicrobial agents which may help to elongate the shelf life of the *akara*. This study is therefore carried out to determine the effect of soy-flour and *A. danielli* on the proximate composition, sensory properties and keeping quality of Akara.

2. MATERIALS AND METHODS

2.1 Materials

Cowpea (*Vigna unguiculata*), soybean (*Glycine max*), *A. danielli*, vegetable oil, table salt and pepper were purchased from Oba market Akure, Nigeria. All other chemicals were laboratory reagent grades.

2.2 Preparation of Cowpea Flour

The beans which were cleaned to removed dirts, stones, and metals were soaked for 30 minutes after which they were dehulled by rubbing with hands until the seed coat loosened. The loosed coats were floated off in water while the dehulled cotyledons were drained properly and dried in an oven at 60ºC for about 24 hours. The dried beans were milled in an attrition mill and then packaged in high density polyethylene bag and tightly sealed until needed.

2.3 Preparation of Soybean Flour

Properly cleaned soybeans were boiled for 20 minutes to remove the beany flavour and also enhance easy dehulling. The boiled beans were cooled, dehulled, drained and dried at 60ºC for about 24 hours. The dried beans were milled in an attrition mill and packaged in high density polyethylene (HDPE) tightly sealed until needed.

2.4 Flour Fractionation and Particle Size Blends

The particle size of the 100% cowpea flour (control) and the 100% soy flour samples were determined according to the ASAE [15] standard using Endecotts Test Sieve Shaker (model 1 MK11-11381, London, UK). A standard weight of 250 g of the flour sample (M) was placed on the topmost sieve of a four sieve Endecott test sieves of apertures 400, 240, 150 and 100 µm. Sieve shaker No 6247 was switched on for 20 minutes. Studies have shown that a minimum of 65% medium-sized particles (0.180–0.425 mm) is needed for proper functionality of cowpea meal used to make akara [4]. Therefore, the cowpea and soy-flour prepared for this study both had a minimum of 65% medium particles while the remaining 35% consisted of large (>0.425 mm) particles.

2.5 Preparation of Spiced and Non-spiced Akara Samples

A preliminary study was conducted to determine the percentage of soy-flour substitution that will produce the best quality akara which was found to be 20% soy-flour. Akara samples were then prepared using previous method of Olapade et al. [5] with slight modification. Briefly, blend of cowpea and soy-flour at 20% substitution with addition of 3% *A. danielli* (based on preliminary trial) was poured into the mixer. A predetermined amount of water was added gradually and then whipped properly inside the mixer at a speed of 3 for 10 minutes to a smooth paste consistency. Seasoning of salt, finely chopped red pepper and onion were stirred into the whipped batter which was then dropped in portions of about 12 g with a special deep hollow spoon into peanut oil at 140ºC and fried in a deep fryer for about 5-10 minutes until golden brown. The fried products were drained from the oil on absorbent paper. Some portions of akara from each sample or treatment were subjected to sensory evaluation immediately while the remaining portions were packaged inside foil paper and store at room temperature.

2.6 Proximate Composition of Spiced and Non-spiced Akara Samples

Proximate compositions of akara samples were determined using standard methods of AOAC [16]. The crude protein was obtained by multiplying the nitrogen content by 6.25 and the carbohydrate content was calculated by difference. All analyses were carried out in triplicate and results expressed on dry weight basis.

2.7 Sensory Evaluation of Spiced and Non-spiced Akara Samples

Sensory evaluation of akara samples was carried out as described by Olopade et al. [5]. The akara samples were coded and presented to a 10-person panel of judges who were familiar with the product for sensory evaluation. The 10-person untrained panel scored the taste, color, aroma, texture and overall acceptability of akara using 9-point hedonic scale, where 9 indicated 'liked extremely' and 1 indicated 'dislike extremely'

2.8 Microbiological Analysis of Spiced and Non-spiced Akara Samples

Standard methods were used to determine the total viable counts of the samples [7]. Briefly, a known weight of Akara sample was aseptically weighed using analytical balance into sterile laboratory mortar. The sample was crushed with the aid of sterile pestle in the mortar. The sample was diluted with distilled water and mixed thoroughly to give a homogeneous suspension with a final concentration of 1 g/ml. 1 ml of the suspension was aseptically withdrawn and added to 9 ml of sterile distilled water in a sterile tube to provide 10-1 dilution which was used to make further dilution up to 10-4 dilution. To determine the total viable count, 1 ml of each dilution was inoculated into a sterile nutrient agar by pour plate methods. The plates were incubated aerobically for 24 hours at 37ºC after which the viable colonies were counted.

2.9 Free Fatty Acids Value of Spiced and Non-spiced Akara Samples

Free Fatty Acid. Free fatty acid (FFA) was determined according to the method described by Chinma et al. [17]. Briefly, 25 ml diethyl ether was mixed 25 ml ethanol and 1 ml phenolphthalein indicator (1%), and carefully neutralized with 0.1 M sodium hydroxide. Five grams of the sample was dissolved in the neutral solvent and titrated with aqueous 0.1 M sodium hydroxide while shaking until a pink color that persisted for 15 s was obtained.

The FFA was calculated as:

FFA content = Acid value×2

Where: Acid value = (ml alkali x 5.6)/weight of sample

2.10 Peroxide Value of Spiced and Non-spiced Akara Samples

This was determined according to the method described by Young and Inengite [18]. Briefly, 5 g of samples was weighed into a 250 ml Erlenmeyer flask after which 30 ml of acetic acid–chloroform solution (2:1) was added and swirled to dissolve. A saturated solution of potassium iodide (5 ml) was added and allowed to stand for 1 min with occasional shaking. Then, 30 ml distilled water was added and titrated slowly with 0.1 M sodium thiosulphate with vigorous shaking until the yellow color almost disappeared. About 0.5 ml 1% starch solution (indicator) was added to this and titration continued until the blue color disappeared.

A blank was conducted and the peroxide value (PV) calculated as:

Peroxide value (Meq Peroxide/kg) = [(S – B) x M x 1000]/[Sample weight]

S = Sample titre
B = Blank
M = Molarity of sodium thiosulphate

2.11 Thiobarbituric Acid Value of Spiced and Non-spiced Akara Samples

Thiobarbituric acid value was determined using the method reported by Chinma et al. [17]. Ten grams of samples was macerated in 50 ml of distillation flask with 47.5 ml distilled water. About 2.5 ml of 4 M hydrochloric acid was added to bring the pH to 1.5. The flask was heated until 50 ml distillate was collected from the time boiling commenced. Five milliliters of thiobarbituric acid (TBA) reagent was added. It was shaken and heated in boiling water for 35 min. A blank was prepared using 5 ml of distilled water with 5 ml of reagent. The tubes were cooled in water for 10 min and the absorbance was measured against the blank at 538 nm.

The TBA number was calculated as:

TBA = 7.8×D

Where: D = absorbance reading.

2.12 Statistical Analysis

All analyses were carried out in triplicates. Means were tested for differences by Analysis of Variance (ANOVA) using Statistical Analysis System Software (SAS version 9.2, SAS institute, Cary, NC). Significant differences between mean values were determined by Duncan's Multiple Range Test and accepted at $P \leq 0.05$. Data are reported as mean±standard deviation from the mean.

3. RESULTS AND DISCUSSION

3.1 Proximate Composition of Spiced and Non-spiced Akara Samples

The results of the proximate composition of akara samples are shown in Table 1. The moisture content of akara samples ranged from 26.86 to 29.70%. Sample B has the lowest moisture content while sample A, C and D

exhibited similar ($P \leq 0.05$) moisture content. No significant difference ($P \leq 0.05$) was observed in the ash, crude fibre and carbohydrate content of the akara samples. The fat content of akara samples ranged from 26.14 to 23.21%. The soy supplemented sample B and D have lower fat content when compared to those of 100% cowpea flour sample A and C. The protein content of akara ranged from 23.19 to 26.19%. Addition of soy-flour increased the protein content of akara by 10.7% as evident in sample B and D. The result showed that at 20% substitution, soy-flour reduced the fat content of akara by 8.2% and increased protein content by 10.7%. The present result is similar to the results obtained by Plahar et al. [1], who showed that 20% substitution of soy-flour lowered the fat content of akara by 7.7% and increased the protein content by 28.7%. The observed values for proximate composition are within the range previously reported for akara [1]. The reduction in fat content of soy-substituted akara is beneficial because more consumers prefer low-fat or fat-free product to high-fat product as high fat food predisposes the consumers to different illness such as obesity and heart disease. Meanwhile, addition of spice (*A. danielli*) did not significantly ($P \leq 0.05$) affect the proximate composition of akara samples.

3.2 Sensory Evaluation of Spiced and Non-spiced Akara Samples

The results of sensory evaluation of akara samples are shown in Table 2. The taste of non-spiced akara samples was more acceptable than that of spiced samples. Addition of soy-flour did not affect the taste of akara samples as evident in sample B. The texture of sample D (containing both soy-flour and *A. danielli*) was rated lower when compared to other samples. In term of colour and aroma, 100% cowpea flour akara without spiced was most preferred of all the samples. Although there was no significant difference ($P \leq 0.05$) between the colour and aroma of 100% cowpea flour akara without spice and 80% cowpea flour akara without spice which indicates that the addition of soy-flour did not affect the taste and aroma of the akara samples. In term of overall acceptability, non-spiced akara samples were more acceptable than that of spiced samples. Addition of 20% soy-flour produced acceptable akara without affecting the sensory attributes of the *akara*. The result is consistent with the previous result obtained by Plahar et al. [1] who reported that acceptable akara was produced at 20% soy-flour substitution. In contrast, addition of 3% *A. danielli* affected the sensory attribute of akara as evident in taste, colour, texture, aroma and overall acceptability.

3.3 Microbiological Analysis of Spiced and Non-spiced Akara Samples

The result of microbiology analysis of akara samples over the 4-day storage period is shown in Table 3. At the first day, sample B has the highest microbial load of 1.9 $x10^2$ cfu/g while the lowest microbial load of 47 cfu/g was observed in sample C. There was increase in the microbial load of all the samples over the storage period. At four day of storage, the highest microbial load was observed in sample B followed by sample A. The microbial loads of samples A and B at second day of storage were higher than the recommended limit of 10^5 cfu/ml for ready to eat food by International Commission on Microbiological specifications for food at ambient temperature. Moreso, at day 3 samples C and D also exceeded this recommended limit. The lower microbial load observed in sample C and D could be attributed to the spice (*A. danielli*) present in them. The results indicate that *A. danielli* has the ability to reduce the spoilage rate of *akara*. The result is consistent with the previous result reported by Ikya et al. [6] who showed that addition of *A. danielli* reduced the microbial load of akara during the storage time. The increase in microbial count during the storage of akara could be attributed to its high moisture content and the availability of nutrients for the growth of microorganisms.

3.4 Changes in Free Fatty Acid Value of Spiced and Non-spiced Akara Sample during Storage

The amount of free fatty acid in food product indicates the extent of fatty acid deterioration due to hydrolysis of fatty acid double bonds. Hence, formation of free fatty acid could enhance rancidity of food. Fig. 1 showed the changes in free fatty acid of spiced and non- spiced akara samples during storage. Although both spiced and non-spiced akara samples showed increase in free fatty acid during the storage period but the highest increase was observed in non-spiced akara while the lowest increase was observed in spiced samples. The low free fatty acid observed in spiced akara showed the stability of fat presence in the food product during the storage. The result showed no effect on the free fatty acid value of akara samples with addition of soy-flour.

Table 1. Proximate composition of spiced and non-spiced Akara sample

Samples	Moisture	Ash	Crude fibre	Fat	Protein	Carbohydrate
A	29.2^{a}±0.2	3.6^{a}±0.3	3.6^{a}±0.3	25.5^{a}±0.7	23.5^{b}±0.4	43.8^{a}±0.9
B	26.9^{b}±0.8	3.1^{a}±0.2	3.6^{a}±0.4	23.2^{b}±0.6	25.8^{a}±0.4	44.2^{a}±0.3
C	29.4^{a}±0.9	3.4^{a}±0.2	3.3^{a}±0.3	26.1^{a}±1.1	23.8^{b}±0.6	43.3^{a}±1.3
D	29.7^{a}±0.5	3.1^{a}±0.2	3.3^{a}±0.3	23.6^{b}±0.8	26.2^{a}±0.3	43.8^{a}±1.0

Values in the same column with different superscript are significantly different (P ≤ 0.05). Means ± standard deviations of triplicate samples; Key: A= 100% cowpea flour without A. daniell; B= 80% cowpea flour+20% soy-flour without A. danielli; C=100% cowpea flour with A. danielli; D=80% cowpea flour+20% soy-flour with A. danielli

Table 2. Sensory evaluation of spiced and non-spiced Akara samples

Samples	Taste	Texture	Colour	Aroma	Overall acceptability
A	6.7^{a}±0.9	6.2^{a}±0.9	6.0^{a}±1.4	6.0^{a}±1.2	6.6^{a}±0.7
B	6.0^{a}±1.1	6.0^{a}±1.0	5.1^{ab}±1.1	5.6^{ab}±1.3	6.0^{a}±0.8
C	4.7^{b}±1.0	5.8^{a}±1.1	4.4^{b}±0.8	4.7^{b}±0.9	5.4^{b}±0.5
D	4.2^{b}±0.9	4.6^{b}±1.0	4.2^{b}±1.0	4.6^{b}±0.8	4.9^{b}±0.7

Values in the same column with different superscript are significantly different (P ≤ 0.05). Means ± standard deviations of triplicate samples; Key: A=100% cowpea flour without A. daniell; B=80% cowpea flour+20% soy-flour without A. danielli; C=100% cowpea flour with A. danielli; D=80% cowpea flour+20% soy-flour with A. danielli

Table 3. Total plate count of spiced and non-spiced Akara samples over 4 day of storage

Samples	Total viable count (cfu/g)				
	D0	D1	D2	D3	D4
A	1.3x10^{2}	3.5x10^{4}	4.1x10^{5}	6.2x10^{6}	8.4x10^{6}
B	1.9x10^{2}	3.4x10^{4}	4.6x10^{5}	7.2x10^{6}	9.0x10^{6}
C	47	71	3.2x10^{3}	3.8x10^{5}	4.9x10^{5}
D	56	72	3.2x10^{3}	4.0x10^{5}	5.1x10^{5}

Key: A=100% cowpea flour without A. daniell; B=80% cowpea flour+20% soy-flour without A. danielli; C=100% cowpea flour with A. danielli; D=80% cowpea flour+20% soy-flour with A. danielli

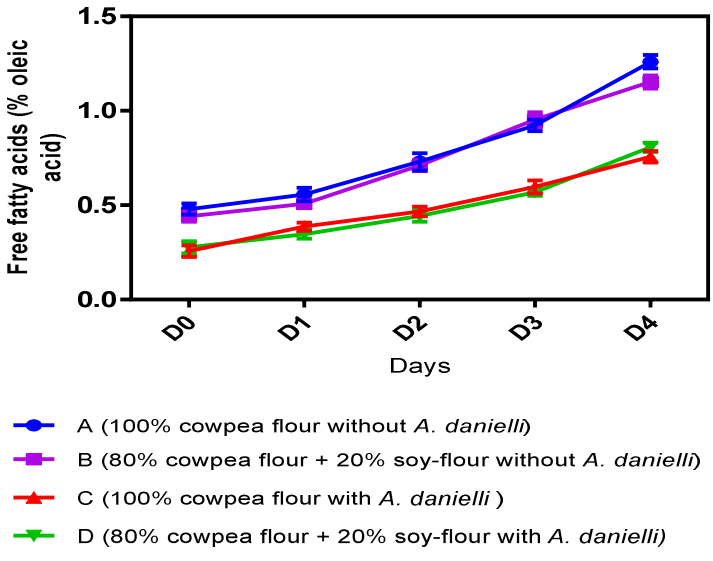

Fig. 1. Changes in free fatty acid value of spiced and non-spiced Akara sample over the 4-day storage period

3.5 Changes in Peroxide Value of Spiced and Non-spiced Akara Samples during Storage

Increase in peroxide value during storage period indicates formation of peroxide due to lipid oxidation. The active oxygen combines with double bonds of the fatty acids in the triglycerides to produce hydroperoxide which are useful indicators of the early stages of rancidity [19]. However, PV alone is not a suitable parameter to assess the extent of fat and oil deterioration in food; this is because as deterioration continues, the hydroperoxide can decompose to form carbonyl and aldehydic compound causing peroxide value to decrease [20]. Changes in peroxide value of spiced and non-spiced akara samples are shown in Fig. 2. There is increase in peroxide value of all the akara samples as the storage period increases. The rate of increase in peroxide value during storage was lower in spiced akara when compared to non-spiced samples. The low PV observed in spiced akara sample indicates that *A. danielli* could be used as food preservative by limiting fat peroxidation. Food with high PV could be harmful to human health due to the free radicals that can be generated in the oxidative process [21].

3.6 Changes in Thiobarbituric Acid Value of Spiced and Non-spiced Akara Samples during Storage

Peroxidation of fatty acids can cause deleterious effects in foods and living tissues by forming complex mixtures of secondary break-down products of lipid peroxide. Further intake of these foods can cause a number of diverse effects including toxicity to mammalian cells [22,23]. Thiobarbituric acid test is used as a measure of lipid peroxidation or oxidative rancidity in fat-containing foods. Thiobarbituric acid reactive substances (Malondialdehydes) are formed as a by-product of secondary lipid peroxidation which can be detected using thiobarbituric acid as reagent. Fig. 3 showed changes in thiobarbituric acid value of spiced and non-spiced akara samples during storage. TBA value increased with increased in storage time, which was higher in non-spiced akara *s*amples when compared to spiced samples. The results of TBA value were consistent with that of PV and FFA value. These results are in agreement with the previous results reported by Chinma et al. [17], who showed increase in FFA, PV and TBA value of Akara-Akpu store at ambient temperature as the storage time increases. The low TBA value observed in spiced akara samples indicates that *A. danielli* has ability to reduce oxidative rancidity in fat-containing food.

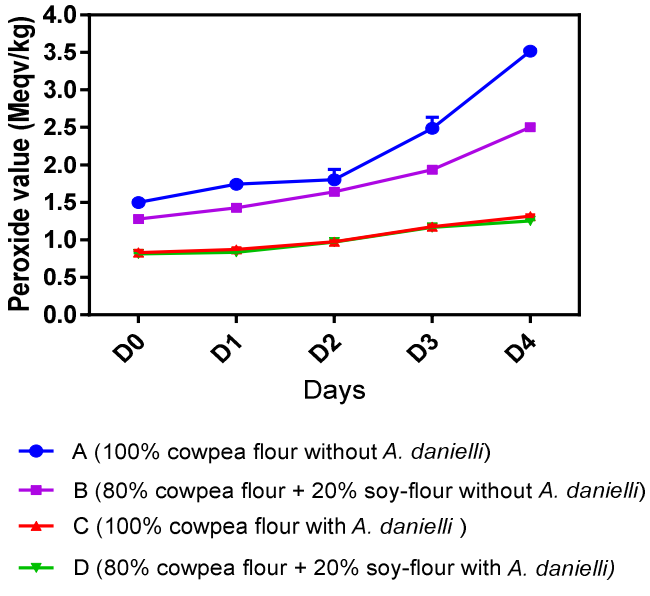

Fig. 2. Changes in peroxide value of spiced and non- spiced Akara samples over the 4-day storage period

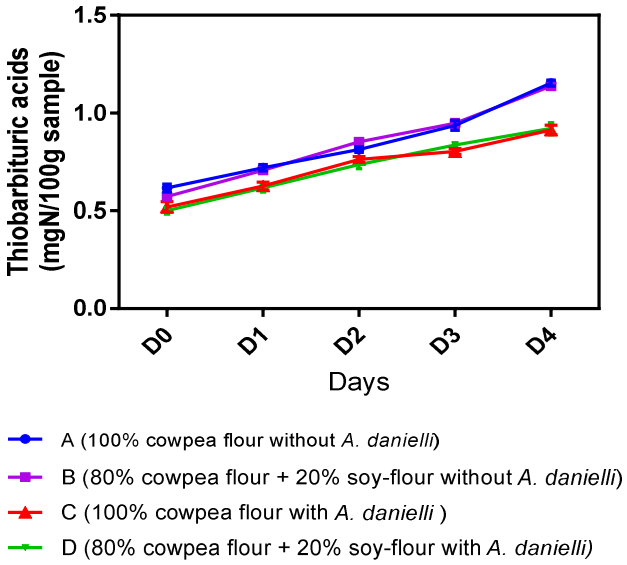

Fig. 3. Changes in thiobarbituric acid value of spiced and non-spiced Akara samples over the 4-day storage period

4. CONCLUSION

The results of the study indicate that substitution of cowpea flour with 20% soy-flour produced acceptable akara with low fat and high protein content. Addition of 3% *A. danielli* in production of akara elongated the shelf life of akara up to three days. *A. danielli* has the potential to be used as preservative ingredient in the production of akara. Utilization of soy-flour and *A. danielli* in the production of akara would produce good quality akara with low fat content and elongated shelf life.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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