

STORAGE STABILITY OF DEEP-FRIED COWPEA PRODUCTS (AKARA) INCORPORATED WITH SOY-FLOUR AND *AFRAMOMUM DANIELLI*

ABSTRACT

Aims: To investigate the effect of incorporating soy-flour and *Aframomum danielli* on quality 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 danielli* on proximate composition and sensory quality of *akara* were evaluated. Changes in microbial load, free fatty acid 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 ($P \leq 0.05$) affect the proximate composition of the *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 ($P \leq 0.05$) rated lower than non-spiced samples in term of taste, colour, texture, aroma and overall acceptability. The results of storage stability of *akara* over four 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 could be 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*.

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 193°C [1]. The production of fresh paste from cowpea is a major constraint in the preparation of *akara* [2]. In order to simplify *akara* preparation,

efforts have been made to produce ready-to-use cowpea flour which can be hydrated to paste, thus reducing the time and labour of paste production [3]. Although ready-to-use cowpea flours that are commercially available in the market meet the requirement of being convenient, it was not well received due to its poor performance. The poor performance of the flour has been attributed to its particle size distribution [3]. In efforts to develop a dry-milling technology for the production of ready-to-use cowpea flour that will produce good quality *akara* similar to that of fresh cowpea paste, it has been recommended that cowpea flour should have 65% medium particle size (0.180-0.425 mm) and 35% large particle size (0.425-1.000 mm) [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 addition of soy-flour to reduce the fat intake. 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. MATERIAL 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.

65 2.2 Preparation of cowpea Flour

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

72 2.3 Preparation of Soybean Flour

73 Properly cleaned soybeans were boiled for 20 minutes to remove the beany flavour and
74 also enhance easy dehulling. The boiled beans were cooled, dehulled, drained and dried at
75 60 °C for about 24 hours. The dried beans were milled in an attrition mill and packaged in
76 high density polyethylene (HDPE) tightly sealed until needed.

77 2.4 Flour fractionation and particle size blends

78 The particle size of the 100% cowpea flour (control) and the 100% soy flour samples were
79 determined according to the ASAE [15] standard using Endecotts Test Sieve Shaker
80 (model 1 MK11-11381, London, UK). A standard weight of 250 g of the flour sample (M) was
81 placed on the topmost sieve of a four sieve Endecott test sieves of apertures 400, 240, 150
82 and 100 µm. Sieve shaker No 6247 was switched on for 20 minutes. Studies have shown
83 that a minimum of 65% medium-sized particles (0.180–0.425 mm) is needed for proper
84 functionality of cowpea meal used to make *akara* [4]. Therefore, the cowpea and soy-flour
85 prepared for this study both had a minimum of 65% medium particles while the remaining
86 35% consisted of large (>0.425 mm) particles.

87 2.5 Preparation of spiced and non-spiced *akara* samples

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

101 2.6 Proximate composition of spiced and non-spiced *akara* samples

102 Proximate compositions of the *akara* sample were determined using standard methods of
103 AOAC [16]. The crude protein was obtained by multiplying the nitrogen content by 6.25 and

the carbohydrate content was calculated by difference. All analysis were carried out in triplicate and result expressed on dry weight basis.

2.7 Sensory evaluation of spiced and non- spiced *akara* samples

Sensory evaluation of *akara* sample 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 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 matter 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 9ml 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 37 °C 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 $\times$ 2

Where: Acid value = (ml alkali $\times$ 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 weighted 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:

141 Peroxide value (Meq Peroxide/kg) = $[(S - B) \times M \times 1000] / [\text{Sample weight}]$

142 S = Sample titre

143 B = Blank

144 M = Molarity of sodium thiosulphate

145 **2.11 Thiobarbituric Acid value of spiced and non- spiced *akara* samples**

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

154 The TBA number was calculated as:

155 $\text{TBA} = 7.8 \times D$

156 Where: D = absorbance reading.

157

158 **2.12 Statistical analysis**

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

164 **3. RESULTS AND DISCUSSION**

165 **3.1 Proximate Composition of spiced and non-spiced *akara* samples**

166 The results of the proximate composition of *akara* samples are shown in Table 1. The
 167 moisture content of *akara* samples ranged from 26.86 to 29.70 %. Sample B has the lowest
 168 moisture content while sample A, C and D exhibited similar ($P \leq 0.05$) moisture content. No
 169 significant difference ($P \leq 0.05$) was observed in the ash, crude fibre and carbohydrate
 170 content of the *akara* samples. The fat content of *akara* samples ranged from 26.14 to 23.21
 171 %. The soy supplemented sample B and D have lower fat content when compared to those
 172 of 100% cowpea flour sample A and C. The protein content of *akara* ranged from 23.19 to
 173 26.19 %. Addition of soy-flour increased the protein content of the *akara* by 10.7% as evident
 174 in sample B and D. The result showed that at 20% substitution, soy-flour reduced the fat
 175 content of *akara* by 8.2% and increased protein content by 10.7%. The present result is
 176 similar to the results obtained by Plahar et al. [1], who showed that 20% substitution of soy-
 177 flour lowered the fat content of *akara* by 7.7% and increased the protein content by 28.7%.
 178 The observed values for proximate composition are within the range previously reported for

179 *akara* [1]. The reduction in fat content of soy-substituted *akara* is beneficial because more
180 consumers prefer low-fat or fat-free product to high-fat product as high fat food predisposes
181 the consumers to different illness such as obesity and heart disease. Meanwhile, addition of
182 spice (*A. danielli*) did not significantly ($P \leq 0.05$) affect the proximate composition of *akara*
183 samples.

184

185 **Table 1: Proximate composition of spiced and non-spiced *akara* sample**

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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

187 Values in the same column with different superscript are significantly different ($P \leq 0.05$). Means ±
188 standard deviations of triplicate samples

189 Key: A= 100% cowpea flour without *A. danielli*; B = 80% cowpea flour + 20% soy-flour without *A. danielli*;

190 C = 100% cowpea flour with *A. danielli*; D = 80% cowpea flour + 20% soy-flour with *A. danielli*

191 3.2 Sensory evaluation of spiced and non-spiced *akara* samples

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

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214 **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

215 Values in the same column with different superscript are significantly different ($P \leq 0.05$). Means ± standard deviations of
 216 triplicate samples.

217 Key: A= 100% cowpea flour without *A. danielli*; B = 80% cowpea flour + 20% soy-flour without *A. danielli*;

218 C = 100% cowpea flour with *A. danielli*; D = 80% cowpea flour + 20% soy-flour with *A. danielli*

219 **3.3 Microbiological analysis of spiced and non-spiced *akara* samples**

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

232 **Table 3: Total plate count of spiced and non-spiced *akara* samples over 4 day of storage**

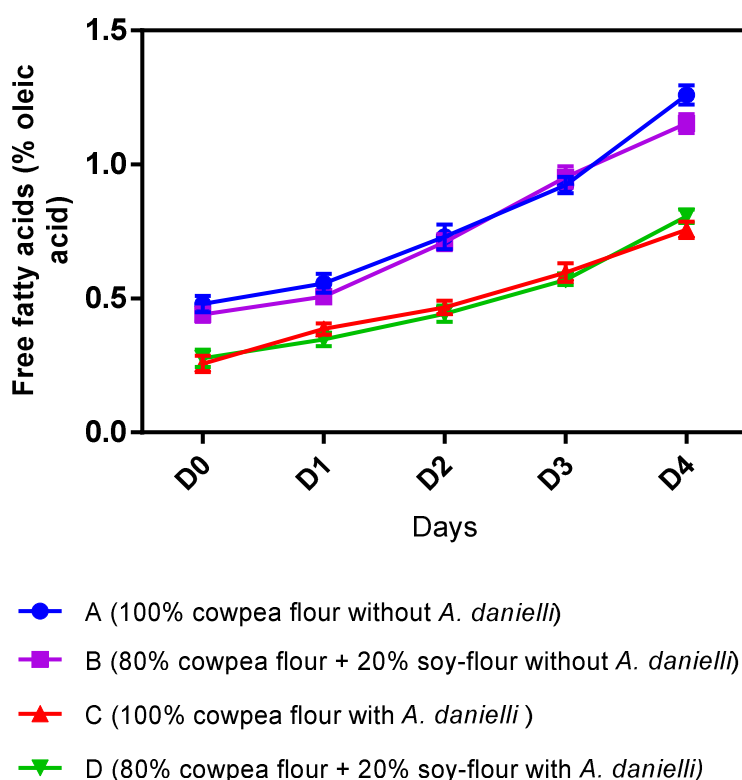
Samples	Total viable count (cfu/g)				
	D0	D1	D2	D3	D4
A	1.3×10^2	3.5×10^4	4.1×10^5	6.2×10^6	8.4×10^6
B	1.9×10^2	3.4×10^4	4.6×10^5	7.2×10^6	9.0×10^6
C	47	71	3.2×10^3	3.8×10^5	4.9×10^5

D 56 72 3.2×10^3 4.0×10^5 5.1×10^5

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

235 3.4 Changes in free fatty acid value of spiced and non-spiced *akara* sample during storage

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



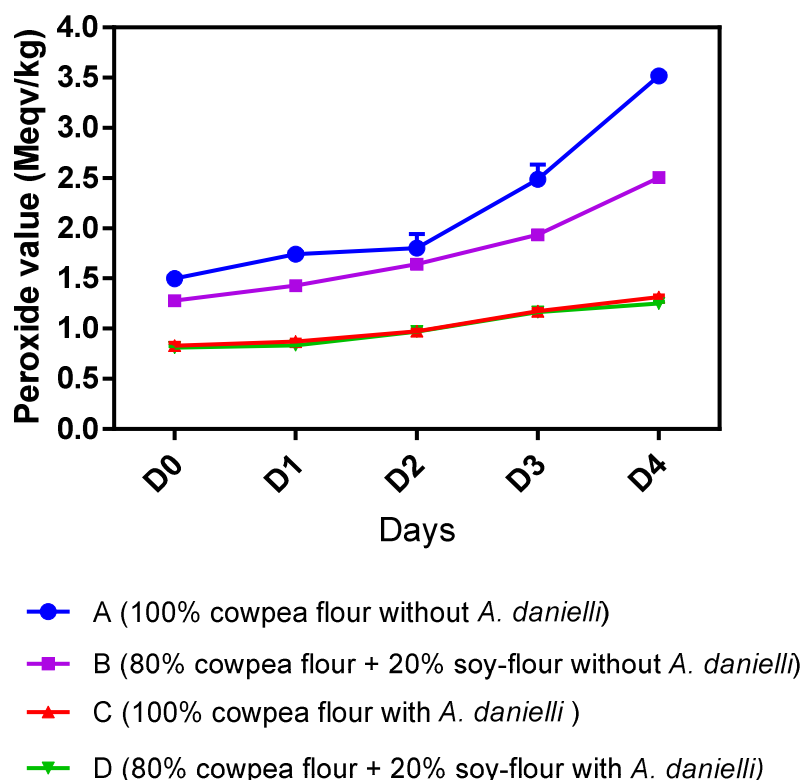
244

245 **Figure 1: Change in free fatty acid value of spiced and non-spiced *akara* sample over 4 day storage**
246 **period**

247 3.5 Changes in Peroxide value of spiced and non-spiced *akara* samples during storage

248 Increase in peroxide value during storage period indicates formation of peroxide due to lipid oxidation. The
249 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]. Change in peroxide value of spiced and non-spiced *akara* samples are shown in Figure 2. There is increase in peroxide value of all the *akara* samples as 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].



259

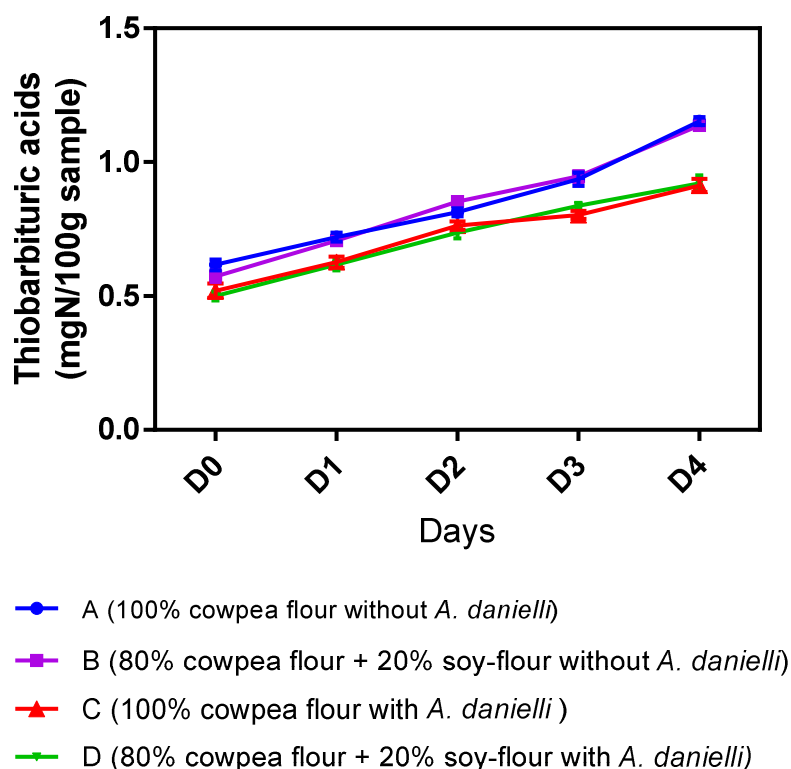
260 **Figure 2: Change in peroxide value of spiced and non- spiced *akara* samples over 4**
 261 **day of storage.**

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263 **3.6 Changes in thiobarbituric acid value of spiced and non-spiced *akara* samples during** 264 **storage**

265 Peroxidation of fatty acids can cause deleterious effects in foods and living tissues by forming complex
 266 mixtures of secondary break-down products of lipid peroxide. Further intake of these foods can cause a
 267 number of diverse effects including toxicity to mammalian cells [22, 23]. Thiobarbituric acid test is used as a
 268 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. Figure 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* samples 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.



277

278 **Figure 3: Change in thiobarbituric acid value of spiced and non-spiced *akara* samples over 4 day of**
 279 **storage**

280 4. CONCLUSION

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

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