

Lipid Oxidation: The Role of *Aframomum danielli* Antioxidant Extracts in Prevention

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Abstract: The effects of *Aframomum danielli* (*A. danielli*) anti-oxidant extract on lipid oxidation was studied. Mayonnaise samples were treated with antioxidant extracts and the anti-oxidant activities of *A. danielli* extracts from three different solvents; ethanol, n-hexane and diethyl ether and that of synthetic antioxidant butylated hydroxyl anisole (BHA) were evaluated using chemical parameters like peroxide value, pH and free fatty acid value. The mayonnaise sample treated with 200ppm concentration of the diethyl ether antioxidant extract of *A. danielli* was the best active in the control of lipid oxidation in mayonnaise with values of 0.40% free fatty acid (FFA), 2.60 mEq/kg peroxide value (PV) and 6.35 pH compared to synthetic antioxidant butylated hydroxyl anisole (BHA) with values of 0.80% FFA, 3.00 mEq/kg PV and 6.50 pH. The percentage antioxidant effectiveness on the 60th day of mayonnaise storage has shown *A. danielli* antioxidant extract (200ppm) concentration from diethyl ether, ethanol and n-hexane to be 83.30%, 80.33% and 78.72% respectively compared to 76.56% antioxidant efficiency of BHA making *A. danielli* extracts to be preferable to synthetic antioxidant extract BHA in the control of lipid oxidation in mayonnaise.

Keywords: Antioxidant activity, *Aframomum danielli* extracts, Mayonnaise, Lipid oxidation

I. Introduction

Peroxidation in a membrane or a system containing polyunsaturated fatty acids is due to the attack of a peroxy radical species which is capable of abstracting a hydrogen atom from a methylene group [1]. Oxidative deterioration of polyunsaturated lipids always result in a problem known as rancidity in food stuffs (e.g. mayonnaise, margarine and cooking oils) [2]. In cell membranes, lipid peroxidation causes the membrane to become less “fluid” with increasing loss of membrane integrity allowing ions such as Ca²⁺, which do not normally cross the membrane to do so [1]. Oxygen free radicals (OFR) play a significant role in the pathogenesis of many diseases like atherosclerosis, cancer, neuro-degeneration and inflammation [3]. Free radicals are produced endogenously during cellular metabolism and their production may be greatly enhanced by exogenous factors such as environmental pollutants, radiations, pathogens and metal ions [4][5]

Many synthetic chemicals such as BHA and butylated hydroxyl toluene (BHT), though very effective as antioxidants, have been known to have toxic and carcinogenic effects on humans [6][3]. Numerous studies have shown the antioxidant potentials of aromatic spices and medicinal plants [7][8][9]. The use of these plant materials as natural antioxidants for food, cosmetics and other applications become necessary because of food safety issues [6]. Natural antioxidants are being used as food additives for inactivation of free radicals because of their scavenging properties and are readily acceptable by consumers [10]. Different degrees of antioxidant activities have been reported from extracts of spices and herbs.

Aframomum danielli is a local spice found in tropical Africa. The spice has been reported to exhibit antioxidant properties in different oil systems [11][12]. This spice has been found to have preservative property in some food systems [13]. This study is therefore aimed at evaluating the antioxidant potential of *A. danielli* extracts from different solvents and that of synthetic antioxidant BHA in mayonnaise product.

II. Materials And Methodology

Aframomum danielli spices were obtained from Bode market, Ibadan, Nigeria. Ingredients for mayonnaise were obtained from Bodija market in Ibadan, Nigeria.

2.1 Spice Preparation:

The seeds of *A. danielli* were removed from the pods of the fruit and were cleaned of all extraneous materials and adhering particles. The seeds were air dried at 27 ± 2°C for 3 days due to its low moisture of about 10.9% determined by [14]. The seeds were then pulverized into a tiny powdery form in a hammer mill after drying and it was stored at 4°C until it was used.

2.2 Solvent extraction of *A. danielli* spice:

The method described by [7] was used for solvent extraction of *A. danielli* spice. 500ml each of diethyl ether solvent, ethanol solvent and n-hexane solvent was used to extract finely ground *A. danielli* spice under refluxing condition for 10 hours respectively. The filtrate of each extraction was freed of the solvent to recover the extract by evaporation of the solvent. The filtrate which was the spice extract was packaged and kept at 4°C until it was used.

2.3 Production of Mayonnaise:

This was done according to the method described by [15]. 10.80g of egg yolk was mixed thoroughly with dissolved salt 1.50g, Sugar 2.50g, cinnamon 0.20g and vinegar 10.80g for about 2-3 minutes in a laboratory mixer(Kenwood Cheff model). Oil (soybean oil) was slowly added to the mixture to form an emulsion as the mixing proceeded. The addition of oil lasted for about 15 minutes. 10-15% of the oil was added slowly during the first five minutes of the mixing and 50% of the oil was added during the next five minutes of the mixing and then the remaining 35-40% of the oil was added gradually during the last five minutes of the mixing. After the production of the mayonnaise, it was packaged in transparent plastic materials, sealed and stored at room temperature.

2.4 Antioxidant Incorporation

The antioxidant extract of *A. danielli* spice extracted with diethyl ether, ethanol and n-hexane solvents were incorporated into different samples of prepared mayonnaise at 200ppm concentration by direct addition using the method of [16]. Synthetic antioxidant BHA was also incorporated into another mayonnaise sample at 200ppm concentration also using [16] method. Control sample which was a sample with no antioxidant extract was also obtained. Chemical tests were carried out on the five samples of mayonnaise.

2.5 Peroxide value (PV)

Peroxide value was determined by titrimetric method of [17]. 1g of the mayonnaise sample was weighed into a clean dry boiling tube to which 1g of powdered potassium iodide and 20ml mixture of glacial acetic acid and chloroform in the ratio 2:1 were added. The tube was held in boiling water for 30 seconds after which the contents were transferred into a 250ml conical flask containing 20ml of 5% potassium iodide solution. This was titrated against 0.002m sodium thiosulphate solution using 1ml of starch as indicator. A blank titration (without any sample) was also made and the results were reported as the number of 0.002m sodium thiosulphate per gram of sample.:

$$\text{Peroxide value} = \frac{(V-v_0)T}{M} \times 10^2 \text{ (mEq/kg)} \quad (1)$$

Where v = Titre value of sample, v_0 = Titre value of blank, T = molarity of sodium thiosulphate and M = mass of fat (gram)

2.6 pH

pH was estimated using the method described by [14] and pH was measured directly using Corning 220 pH meter. The pH of the samples were measured with electrode standardized using pH 4 and pH 7 buffer solution

2.7 Free fatty acid (FFA)

The FFA was determined by titrimetric method of [14]. Mixture of 25ml diethyl ether, 25ml alcohol and phenolphthalein solution (1%) was carefully neutralized with 0.1m sodium hydroxide. 1g of the mayonnaise sample was dissolved in the mixed neutral solution and was titrated with aqueous 0.1m sodium hydroxide. The mixture was shaken constantly until a pink colour that persisted for 15 seconds was obtained.:

$$\text{Acid value} = \frac{\text{Titration (ml)} \times 5.61}{\text{WtofSampleused}} \quad (2)$$

2.8 Determination of Antioxidant effectiveness

The percentage antioxidant effectiveness during storage test period was monitored using the method described by [18].:

$$\text{Antioxidant effectiveness} = \frac{\text{PVofcontrol} - \text{PVoftestsample}}{\text{PVofcontrol}} \times 100 \quad (3)$$

III. Result And Discussion

Table 1: Physical characteristics of *A. danielli* fruits

<i>Fruits</i>	<i>Characteristics</i>
Botanical Name	<i>Aframomum danielli</i>
Yoruba Name	<i>Atare oboro</i>
Family	<i>Zingiberaceae</i>
Shape	Oblong
Colour of seed	Reddish brown
Appearance of seed	Shiny
Average weight of fruit	2.76g to 3.10g
Percentage filled of seeds in the fruit	60.8% to 66.3%
Colour of the antioxidant extracts	Brownish black liquid with thick viscosity

Table 2: Yield of *A. danielli* Antioxidant Extracts

solvent	Vol. of solvent used (ml)	Quantity of <i>A. danielli</i> powder used (g)	Yields of <i>A. danielli</i> extracts (g)	(%) yield of <i>A. danielli</i> extracts
Diethyl ether	500	85	11.11	13.07
Ethanol	500	92	11.87	12.90
n-hexane	500	85	9.31	10.95

Table 3: Antioxidant effectiveness of extracts on mayonnaise samples

Samples	Antioxidant effectiveness (%) at day 20	Antioxidant effectiveness (%) at day 40	Antioxidant effectiveness (%) at day 60
A	60.61	77.24	83.30
B	59.09	74.80	80.33
C	56.06	72.68	78.72
D	54.55	71.54	76.56
E	Control		

Key:

A = mayonnaise sample treated with extracts of *A. danielli* from diethyl ether solvent

B = mayonnaise sample treated with extracts of *A. danielli* from ethanol solvent

C = mayonnaise sample treated with extracts of *A. danielli* from n-hexane solvent

D = mayonnaise sample treated with synthetic antioxidant BHA

E = mayonnaise sample treated with no antioxidant (Control)

figure 1: Peroxide values of mayonnaise treated with antioxidant extract

figure 2: Free fatty contents of Mayonnaise treated with antioxidant extracts

Generally, antioxidants are widely used to prevent lipid oxidation in processed foods, raw materials or fats and oils based foods[19]. The potency of the antioxidant is aimed at preventing or delaying rancidity in foods.

3.1 Peroxide Value

The peroxide value is a measure of the peroxides contained in the oil. Peroxides are primary reaction products formed in the initial stages of oxidation, and therefore give an indication of the progress of lipid oxidation [17]. During storage, peroxide formation is slow at first during an induction period that varies with time and temperature of the environment.

From Fig. 1, the highest increase in peroxide value was found in the control sample E, this was because there was no antioxidant extract incorporation into the sample. This result confirms the result of [20] on their study on the use of antioxidants to minimize rancidity in pressurized cooked chickens slurries, their results showed that samples with rosemary extracts had effects on their pressurized samples compared to samples without any antioxidants. Sample “A” which was sample of mayonnaise with extracts of *A. danielli* from diethyl ether solvent was the least and the best in terms of peroxide value as shown in Fig. 1. It means that lipid oxidation was slowest in this sample due to the work of the antioxidant, it could also be stated that diethyl ether solvent was able to extract the active component from *A. danielli* that was responsible for the work of anti-oxidization in mayonnaise. This further confirms the fact that natural antioxidants are better antioxidant agents compared to synthetic antioxidant BHA in this case (sample D) [21].

Table 4: Changes in pH of mayonnaise samples after 60days

<i>Sample</i>	<i>pH initial</i>	<i>pH after 60 days</i>	<i>Decrease in pH</i>
A	6.35	6.30	0.05
B	6.40	6.30	0.10
C	6.70	6.36	0.34
D	6.50	6.30	0.20
E	7.10	5.54	1.56

3.2pH

From table 4, the pH of all the samples decreased or tended towards acidic region. The reduction in pH of the samples indicates microbial and enzymatic activities during the storage of the samples and thus resulted in the production of acidic compounds. The decrease in pH was highest in the control sample (E), because there was no antioxidant to control or Inhibit the activity of enzymes and microorganisms.

Sample A, extracts of *A. danielli* antioxidant using diethyl ether solvent had the least decrease in pH showing that microbial and enzymatic activity was really slowed in this sample compared to synthetic antioxidant which was sample D, this also confirms the facts that natural antioxidants possess antimicrobial activity[22].

3.3Free fatty acid (FFA)

From Fig. 2, the changes in FFA of mayonnaise samples during sixty (60) days of storage can be seen. Increase in FFA was highest in sample E and least in sample A. the order of increase was as follows: sample E > sample D > sample B > sample C > sample A. Rancidity is often accompanied by FFA formation, it means that

Sample A was able to inhibit the formation of FFA better than the other samples, this further confirms the fact that diethyl ether extracts of *A. danielli* antioxidant was able to retard lipid oxidation better than synthetic antioxidant BHA (sample D) and other extracts [21].

3.4 Antioxidant Effectiveness

Table 3 shows the results of antioxidant effectiveness of extracts on mayonnaise samples during the 60 days of storage. The antioxidant effectiveness increased with increase in days of storage. Sample A had the highest antioxidant efficiency (83.30%) compared to synthetic antioxidant sample D with BHA (76.56%) antioxidant efficiency. This further confirms that natural antioxidants are better substitute to synthetic antioxidants in the control of lipid oxidation [23].

IV. Conclusion

The result of this study has shown that at 200ppm concentration, *Aframomum danielli* antioxidant extracts can be used to reduce lipid oxidation in mayonnaise thereby extending the shelf life of the product. It was also seen that natural antioxidant (*A. danielli*) is better than synthetic antioxidant (BHA) in inhibiting lipid oxidation in mayonnaise.

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