Original Research Article

Synergistic effect of Lipophilic Antioxidants Extracted from Cloves (*Syzygium aromaticum*) with Vitamin E on the Stability of Cotton Seed Oil during Frying of Plantain Chips

ABSTRACT

Aims: This study was conducted in order to evaluate the synergistic effect of the ethanolic extract and powder of cloves buds (*Syzygium aromaticum*) with vitamin E in delaying cotton seed oil oxidation during frying of plantain chips.

Study design: Purchase of clove buds, cleaning, preparation of clove buds ethanolic extract and powder, supplementation of cotton seed oil enriched with vitamin E with the extract and powder at concentrations 0.30%, 0.60%, 1.00% and 1.30% and evaluation of oil stability during frying of plantain chips.

Place and Duration of Study: General Science Laboratory, School of Agriculture and Natural Resources, Catholic University Institute of Buea-Douala Campus, Littoral Region, Cameroon, from January to June 2019.

Methodology: In refined cotton seed oil (2.5 L) containing vitamin E as preservative was respectively added 0.30%, 0.60%, 1.00% and 1.30% of *Syzygium aromaticum* (SA) buds ethanolic extract and powder. After ensuring the good dispersion of the antioxidants added, oil samples were used for the deep-frying of plantain chips for 10 and 20 minutes respectively. Oil sample without clove served as control. The stability of the various oil samples was evaluated through the determination of peroxide, thiobarbituric acid, iodine and acid acid (% oleic) values.

Results: Results showed that the natural plant extract and powder increased the stability of the oil compared to the control. Oil samples supplemented with SA 0.30% and SA 0.60% presented good stability compared to the control, while the same extract at higher concentrations (1.00% and 1.30%) tend to behave as prooxidant. Concerning the powder it exhibited better good preservative properties on the oil and at all levels.

Conclusion: Clove ethanolic extract at concentration < 0.60% and its powder at concentration 0.30-1.5% can be used to delay cotton seed oil alteration during frying of plantain chips.

Keywords: Syzygium aromaticum, oxidative stability, Frying, Antioxidant, Synergistic effect

1. INTRODUCTION

Several scientific evidences suggest that over-production of free radicals and mostly reactive oxygen species might be the root cause of the damages caused by oxidative stress amongst which several chronic diseases such as cancer, cardiovascular diseases, neurodegeneration, and ageing [1]. Free radicals are compounds that are naturally produced in living organisms from metabolic and defense reactions [2]. They are not only bad but also have good properties as they contribute in the neutralization of pathogenic microorganisms. It is only when they are produce in excess that they become dangerous for the human body [3]. These radicals do not just affect living organisms; they also have urge consequences on food containing lipids, especially those rich in polyunsaturated fatty acids. They can affect the food sample by reducing their nutritional and sensory properties. They oxidize food samples containing unsaturated fatty acids and lead to the formation of toxic compounds such as aldehydes, ketones etc, which are harmful for the consumers and which procure rancid odor to foods. Throughout these reactions, essential amino acids can also be affected, same with proteins, which will see their bioavailability reduced [4]. The consequences of lipid alteration reactions are the economic losses in the food industry, due to the rejection of oxidized foods by consumers [5]. As solution, chemically synthesized and commercialized antioxidants have been used to preserve oil from oxidation during storage and processing. However, the demonstration of their side effects in many reports made them to be gradually avoided by consumers. In the view to provide solutions, researchers have been investigating and proposing natural sources of antioxidants that can be used to substitute synthetic ones [1]. Amongst the natural sources investigated, only one, rosemary has been approved for industrial use [4]. But the challenge is that geographically this plant is not available in all part of the world, especially in some African countries such as Cameroon. Previous reports carried out by [6]–[10], presented some Cameroonian plants as potential sources of antioxidants efficient in the stabilization of palm olein during accelerated storages. In their report, [6] demonstrated that Cameroonian spices amongst which *Syzygium aromaticum* were efficient in delaying soya beans oil oxidation during 24 days storage at 65 °C in an electric air-dried oven. However, there is almost no report on the effect of the synergetic action of this plant extract or powder from Cameroon and synthetic antioxidants on the stability of edible oils during processing. In this study, *Syzygium aromaticum* which the antioxidant properties of its extract, essential oil and powder is not more to be demonstrated was investigated for a synergistic action with vitamin E in cotton seed oil stabilization during frying. It is well known that spices in general are good sources of antioxidants. *Sygygium aromaticum*, commonly known as clove, is one of the most valuable spices that have been used for centuries as food preservative and for many medicinal purposes. It is a dried aromatic unopened floral bud from an evergreen tree of 10-20 m height belonging to the family of Myrstacae. It was demonstrated for having good biological activities such as, antioxidants, anti-inflammatory, antifungal, anti-cancer, antimicrobial properties etc.[11], [12]. Cotton seed oil known in Cameroon under the brand name Diamaor is one of the best polyunsaturated oil available in the market. The major fatty acids found in its composition are linoleic acid (52.89%) oleic acid (16.35%) and palmitic acid (25.39%) [13]. It is used in household for cooking, frying and seasoning. Diamaor is rich in vitamin E which contributes to its good stability. However, it is well known that vitamins are highly

unstable at high temperature and can easily get decomposed. Adding the natural extract and powder from *Sygygium aromaticum* buds for a synergistic effect with the vitamin E present in the oil might increase its stability during processing, reason why these investigations were carried out.

2. MATERIAL AND METHODS

2.1 Materials

Refined cotton seed oil containing Vitamin E as preservative was purchased from Santa Lucia Super Market, Bonamoussadi, Littoral-Cameroon in March 2019. The chemicals and reagents used were of analytical reagent grade. Fresh plantains and dry *Syzygium aromaticum* (SA) buds were purchased from local markets in Douala.

2.2 Methods

2.2.1. Extraction of Syzygium aromaticum antioxidants

Syzygium aromaticum (SA) buds (dry) were cleaned and ground to pass through a 1 mm diameter sieve. 400 g of that powder was macerated at room temperature in 1000 mL of ethanol with regular shaking for 48 h. After filtration using the Whatman paper N° 1, the residues were again macerated in 800 mL of ethanol, this in order to maximize the extraction of phenolic antioxidants. The obtained filtrate was mixed to the previous one, before the elimination of the solvent on a rotatory evaporator at 40 °C under reduced pressure. The concentrated extract was stored in the refrigerator at 4 °C prior to further analysis.

2.2.2. Samples preparation

2.2.2.1. Preparation of Plantains

Fresh unripe plantains were peeled and sliced to a thickness of 2 mm using a mechanical slicer. They were kept submerged in distilled water at room temperature. They were then slightly blotted dry with tissue paper before weighing into 100 g batch for frying.

2.2.2.2. Addition of natural antioxidants in cotton seed oil and frying

The samples were prepared according to technique described by [14]. Frying was carried out in various systems that contained: refined cotton seed oil without additive or control (System I); cotton seed oil with 0.30% cloves extract (System II); cotton seed oil with 0.60% cloves extract (System III); cotton seed oil with 1.00% cloves extract (System IV); cotton seed oil with 1.30% cloves extract (System V); cotton seed oil with 0.50% cloves powder (System VI); cotton seed oil with 1.00% cloves powder (System VII); and cotton seed oil with 1.50% cloves powder (System VIII). Oil samples containing the cloves extract were stirred for 30 min to ensure dissolution of antioxidant. Those with the powders were macerated for 48h with regular shaking to facilitate the infusion of the lipophilic antioxidants in the oil before used for frying. The addition of cloves extract and powder to the oil enriched with vitamin E was to see if there can be a synergistic effect that can increase oil stability during processing.

2.2.3. Frying Process

Cotton seed oil (2.5 L) samples were individually put in a deep fat fryer (Type: ROWENTA). The temperature was brought up to 180 °C and the plantain (100 g) consecutively fried for 10 and 20 min respectively. The fryer was uncovered during the frying period. At the end of each frying (20 min), the fryer was switched off and the temperature was allowed to drop to 60 °C. Fryer temperature regulation was done by a thermostat. Oil samples were collected for analysis (60 g) in brown glass bottles at 60 °C. They were stored in the refrigerator at 4°C.

2.2.4. Analysis of oil quality

The following quality indexes were evaluated: Peroxide value, evaluated using the IDF standard method 74A: 1991 [15], thiobarbituric acid (TBA) value, evaluated using the method reported by [16], the iodine and acid values by the AOCS method [17].

2.2.5. Statistical analysis

Results obtained in the present study were subjected to one-way analysis of variance (ANOVA) with the Student-Newman-Keuls tests using Graphpad-InStat version 3.05, to evaluate the statistical significance of the data. A probability value at $p < 0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. Effect cloves ethanolic extract on the stability of cotton seeds oil during frying

3.1.1. Peroxide value

The changes in the peroxide value (PV) of cotton seeds oil samples during frying are presented in figure 1. A significant increase ($p < 0.05$) in PV was registered in almost all oil samples during the storage. At 10 and 20 min, it is clearly observed that the PV of the control, 0.30%, SA 0.60% and 1.00% SA keep increasing while that of oil with 1.3% SA significantly decreases ($p < 0.001$) from the 10th min. The increase rate of PV in the control was significantly lower ($p < 0.05$) compared to that of the other samples. The increase in peroxide value observed in almost all the samples during frying can be attributed to the formation of primary oxidation products mainly hydro peroxides. This is the mark of the primary oxidation of this oil [8], [14]. The constant evolution of the PV of the control during frying as well as the significant decrease of the PV of cottonseed oil supplemented with SA 1.30% can be related to the rapid transformation of hydro peroxides formed into secondary oxidation products such as aldehydes and ketones. Under frying conditions, a low or high peroxide value cannot really inform on the good quality of the oil due to spontaneous conversion of peroxides into secondary oxidation products. It is therefore important before giving any conclusion to quantify the secondary oxidation product. Similar observations were made by [9] during storage of palm olein under simulated frying conditions.

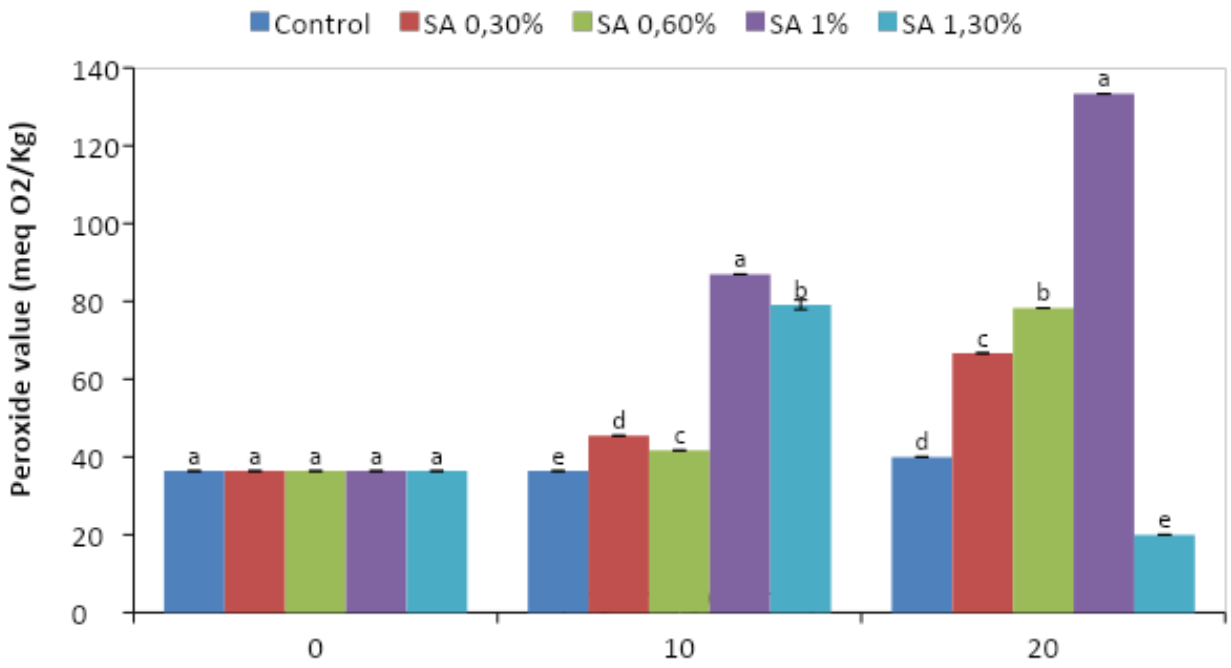

Fig. 1. Changes in peroxide value of Cotton seed oil samples during frying
Values are presented as Mean ± SD. [a-e]*Values of the same minute with different superscripts are significantly different (p<0.05)*

3.1.2. Thiobarbituric acid value

Figure 2 shows the changes in Thiobarbituric acid (TBA) value of cotton seed oil samples during frying of plantain chips. After 20 min frying, it can be observed that the TBA value of all oil samples significantly increased ($p < 0.05$) compared to the same parameter evaluated at 0 min. At 10th min frying, the highest TBA value was recorded in SA 1.00 % and SA 1.30 %. No significant difference was registered between the TBA value of SA 1.00 % and SA 1.30 %; and SA 0.30 % and SA 0.60 % respectively. At the 20th min, a drastic increase ($p < 0.001$) in TBA value was recorded with the control while the TBA value of SA 1.30 % significantly dropped ($p < 0.01$). The highest TBA value was registered with SA 1.00 % at the 20th min while that of SA 0.30 % and SA 0.60 % were significantly lower ($p < 0.05$). The significant increase in TBA value observed in processed samples compared to the initial, can

be attributed to the formation of secondary oxidation products (mainly malondialdehyde) which are the main products characterized by this test. The control which previously showed constant peroxide value compared to the initial, has exhibited a significantly higher TBA value at the 20th min of frying. In the same line, the oil sample supplemented with SA 1.30% which PV drastically decreased from the 10th to the 20th min, has also presented significantly higher TBA values. This result confirm the hypothesis previously stated with the peroxide value showing that the low PV of the control and the decrease in PV of the oil supplemented with SA 1.30%, was the consequence of the transformation of hydro peroxides into secondary oxidation products. The cotton seed oil enriched with SA 1.00% which previously presented a significantly higher peroxide value, has also exhibited a significant TBA value. This oil as well as the control and the oil supplemented with SA 1.30% were significantly altered. The fact that oil containing some natural antioxidants easily oxidized, has already been proven. It has been demonstrated that, the activity of an antioxidant can be affected by several factors such as the concentration of the antioxidant [18].

The low peroxide and TBA values of oil samples supplemented with SA 0.30% and SA 0.60% is the symbol of a good stability compared to the other oil samples. This activity can be related to the presence in this extract, of natural antioxidants such as eugenol [19] at an adequate concentration. These results are in accordance with the report of [20], which showed that some plant polyphenols can have both antioxidant and prooxidant effects. The results obtained in this study, are equally in agreement with those reported by [6], who pointed out that *Syzygium aromaticum* ethanolic extract is efficient in prolonging soya bean oil oxidation.

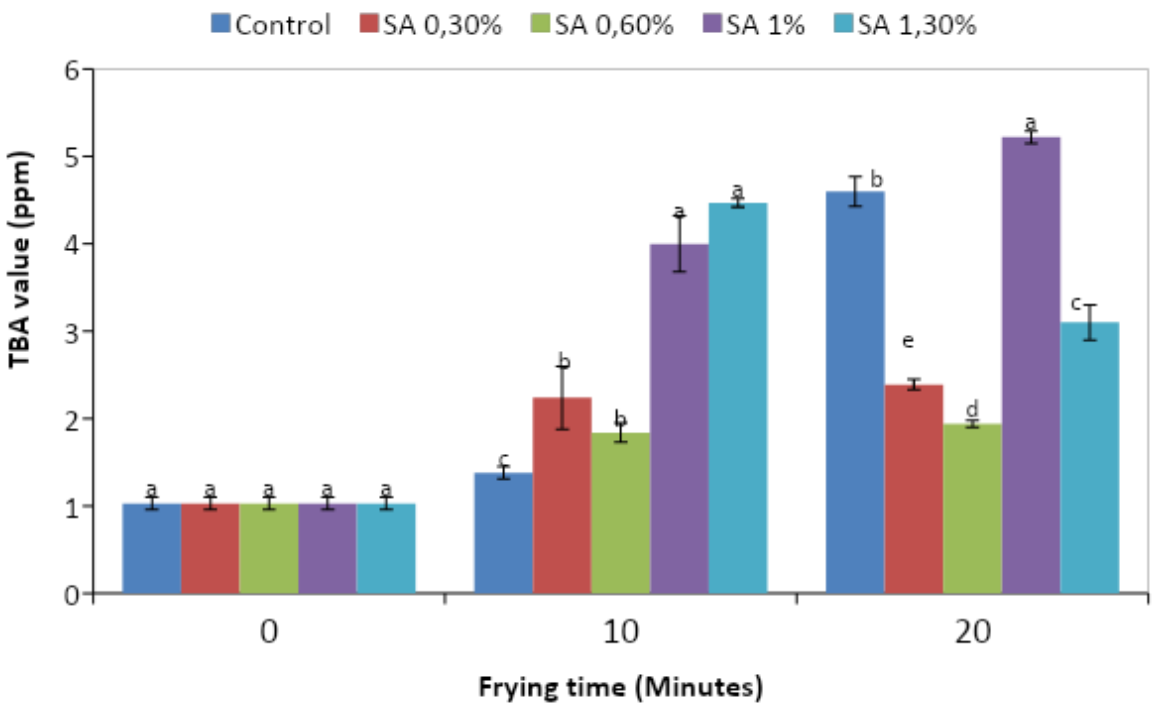

Fig. 2. Changes in TBA value of Cotton seeds oil samples during frying

Values are presented as Mean ± SD. [a-e]Values of the same minute with different superscripts are significantly differents (p<0.05)

3.1.3. Iodine value

The changes in iodine value of cotton seed oil samples during frying processes are shown in figure 3. Generally, the iodine value of all oil samples decreased with the frying time. The reduction in this parameter was significantly higher ($p < 0.05$) in the control followed by SA 1.00 % and SA 1.30 % after 20 min frying. The decrease in iodine value observed during processing can be attributed to the destruction of the double bond contained in polyunsaturated fatty acids by free radicals and ROS. It has been proven that when oils are processed at high temperature, the double bonds of their fatty acids are attacked leading to their destruction and the formation of conjugated bonds [21]. The highest decrease in iodine value registered with the control and oil samples supplemented with SA 1.00% and 1.30% at the 20th min of frying is the consequence of the destruction of the double bonds of the fatty acids catalyzed by heat and high concentrations of antioxidants which are now suspected as acting as prooxidants. The high iodine value registered in cottonseeds oil samples supplemented with SA 0.30% and 0.60% can be related to the efficiency of the antioxidant present at adequate concentration with the ability to delay the action of free radicals and ROS. From these observations, it is clear that these samples were more stable.

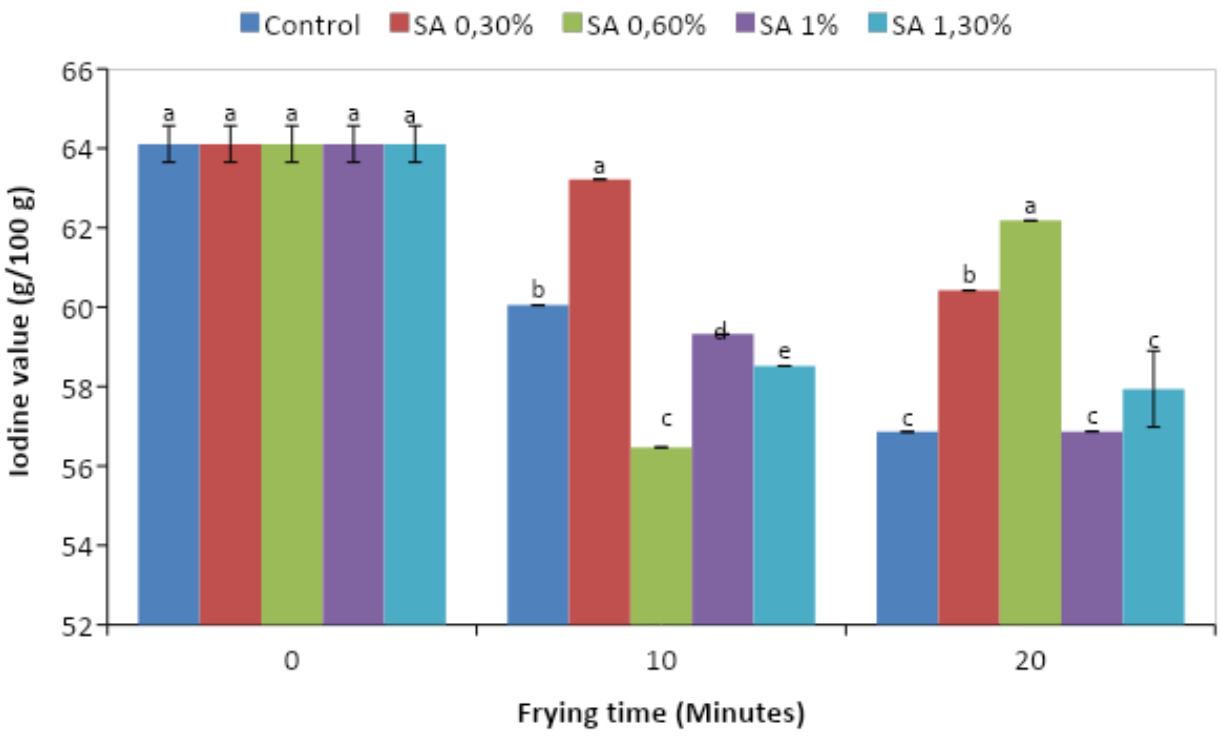

Fig. 3. Changes in iodine value of Cotton seeds oil samples during frying
Values are presented as Mean ± SD. [a-e]Values of the same minute with different superscripts are significantly differents (p<0.05)

3.1.4. Acid value (% Oleic acid)

The changes in acid value of cotton seeds oil samples during frying are presented in figure **4.** A significant decrease ($p < 0.05$) in acidity of all oil samples was registered after 10 min frying. However, at the 20th min, the acid value of all oil samples significantly increased ($p < 0.05$) compared to the same samples at the 10th and 0th min. The decrease in acid value observed in all oil samples at the 10th min can be attributed to the rapid conversion of the free fatty acids released from the hydrolysis of triglycerides into oxidized products. Here, one can suspect that the conversion rate of fatty acids is higher than the hydrolysis rate of triglycerides. The increase in acid value observed at the 20th min informs on the degree of acidity of the oil which is due to the high hydrolysis rate of triglycerides and low transformation of the fatty acids released.

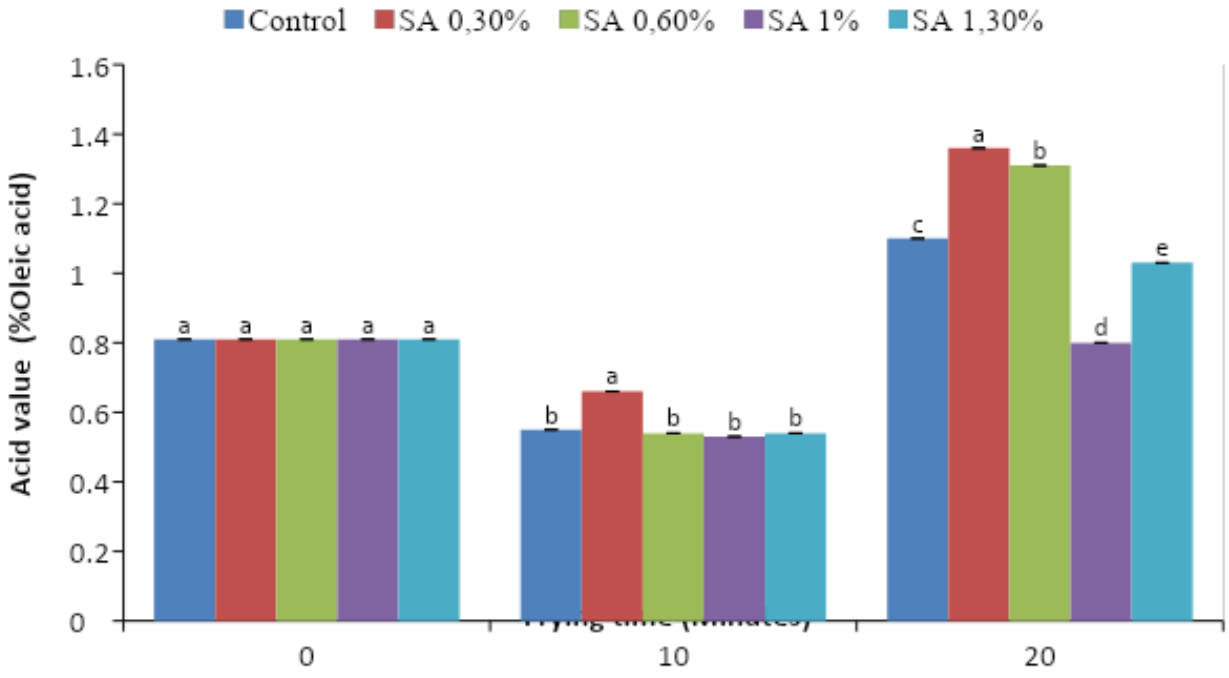

Fig. 4. Changes in acid value of Cotton seeds oil samples during frying
Values are presented as Mean ± SD. $^{a-e}$Values of the same minute with different superscripts are significantly differents ($p<0.05$)

3.2. Effect cloves powder on the stability of cotton seeds oil during frying

3.2.1 Peroxide value

The changes in peroxide value of cotton seeds oil samples during frying for given periods of time were evaluated and results are shown in figure 5. A significant increase ($p<0.05$) in

peroxide value in oil supplemented with SA extracts was observed at the 10th min of frying compared to 0 min. However, the PV of the control at the 10th min remains similar to that at the zero minute. At the 20th min, apart from the control and oil supplemented with SA 0.5%,whose peroxide value increased, the PV of cotton seed oil enriched with SA 1% and SA 1.5% have significantly reduced compared to that of 10th min. The increase in the peroxide value observed from 0-10 min for all samples, and from 10-20 min for the control and oil supplemented with SA 0.5% marks the formation of hydroperoxides, which are the main primary oxidized products [6], [7], [14]. It has been proven that at high processing temperature, the hydrogen with weakest bonds on the carbon of unsaturated fatty acids are removed, leading to the formation of free radicals that can react with the molecular oxygen to form peroxy radicals. In order to stabilize the structure, the peroxy radical can remove another hydrogen atom from an unsaturated fatty acid to form hydroperoxide [22]. The significant decrease in peroxide value observed with cotton seed oil supplemented with SA 1% and SA 1.5% can be the consequence of the breakdown of hydroperoxide into secondary oxidation products such as aldehydes, ketones, etc [23]. The low rate of hydroperoxide formation in control can be the proof of its low oxidation state compared to the other oils. However, under frying conditions, the peroxide value is not a good indicator for evaluation of oil quality due to the fact that the hydroperoxide formed could easily decompose into secondary oxidation products. If that is the case, high peroxide value can be an indicator of poor or good quality under such conditions. It is difficult to conclude directly based on this result on the efficiency of SA extract in preserving cotton seed oil. The secondary oxidation products have to be evaluated before any conclusion. It is important to note that the antioxidant activity of SA extract has already been demonstrated; [6] showed that SA formerly called *Eugenia carophyllus* is a powerful antioxidant and was efficient in prolonging the shelf life of soyabean oil during an accelerated storage of 24 days at 65°C.

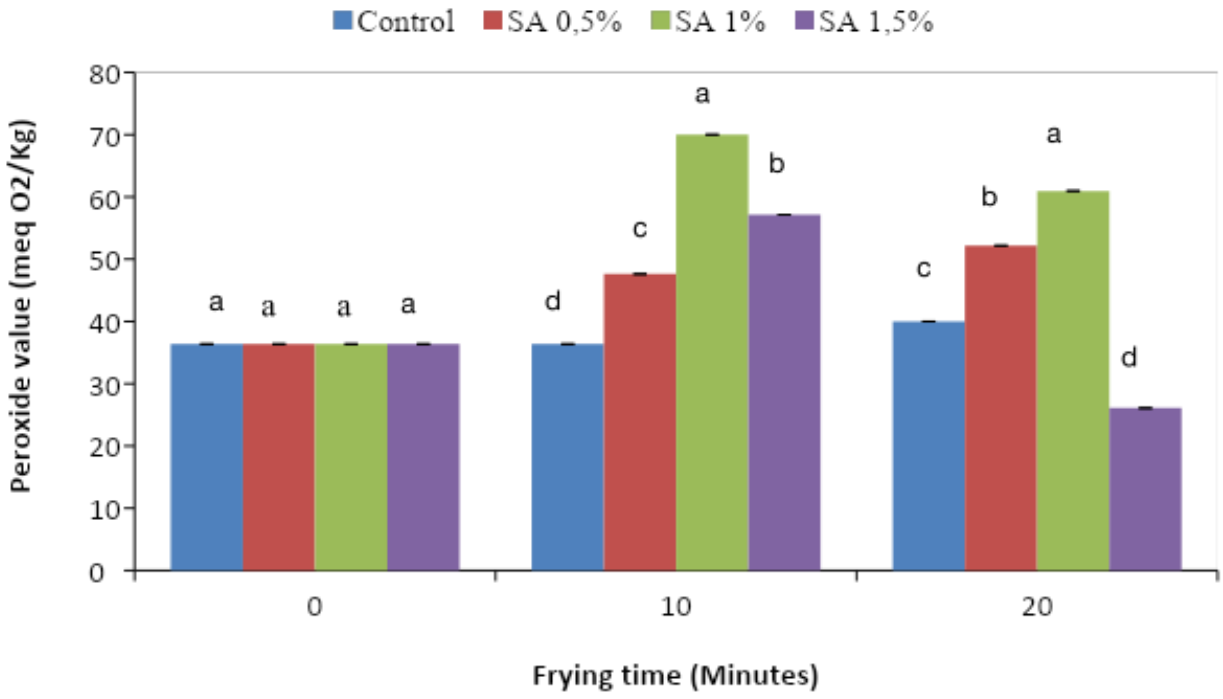

Fig. 5. Changes in peroxide value of Cotton seeds oil samples during frying

Values are presented as Mean ± SD. [a-d]Values of the same minute with different superscripts are significantly differents (p<0.05)

3.2.2 Thiobarbituric acid value

The thiobarbituric acid value is a condensation reaction between TBA and malondialdehyde which are the most predominant secondary oxidation products of fatty acid in polyunsaturated oils such as cotton seed oil. It is therefore considered as a good parameter used to check the quality of oils and fat [24]. The TBA value of cotton seeds oil supplemented with different concentration of SA powders is presented in figure 6. A significant increase in TBA values was registered in all the samples during frying; this is characteristic of the increase in concentration of malondialdehyde. The highest secondary oxidation was registered with the control and can be explained by the absence of antioxidants. The low TBA value recorded in the stabilized oil samples can be attributed to the presence in this oils of natural lipophylic antioxidants that have diffused from the powder to the oil. Here, we can have essential oils components such as eugenol, which is a phenolic antioxidant found in the essential oil of SA buds [19]. The fact that the powders from SA buds can delay the oxidation of given oils during processing has already been proven. Soyabean oil supplemented with 1000 ppm of spices powder had a longer shelf life than soyabean oil without antioxidant [6]. SA was part of these powders.

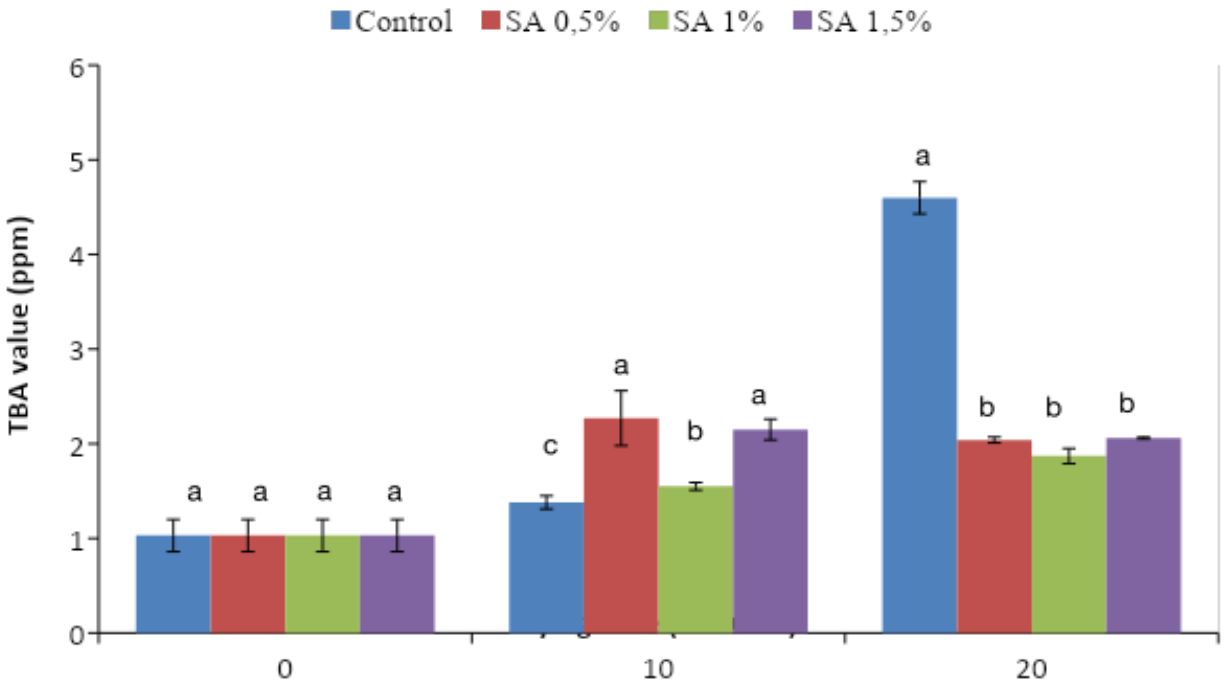

Fig. 6. Changes in TBA value of Cotton seeds oil samples during frying

Values are presented as Mean ± SD. [a-b]Values of the same minute with different superscripts are significantly differents (p<0.05)

3.2.3 Iodine value

The iodine value gives an idea on the degree of unsaturation of oils. When it decreases, it is generally a sign that the double bonds of unsaturated fatty acid are being broken [21]. The changes in iodine value of cotton seed oil samples are presented in figure 7. A significant decrease ($p<0.05$) in iodine value was observed in all the samples during frying. The highest degree was registered with the control. As previously mentioned, this reduction in the iodine value can be attributed to the destruction of the fatty acid double bonds in cotton seeds oil during frying. The highest values registered with the control might be due to the absence of antioxidant that can stop or delay the action of free radicals on the unsaturated fatty acid of the oil. It can therefore be suggested that the natural antioxidants present in SA buds powder are responsible of the slow decrease in their iodine value; this is due to the fact that they can limit the deteriorative effect of free radicals by donating hydrogen atoms for their stabilization. Similar observations were made by [6], who showed that the powder of some Cameroonian spices amongst which that of SA buds can delay the destruction of double bond in soya bean oil.

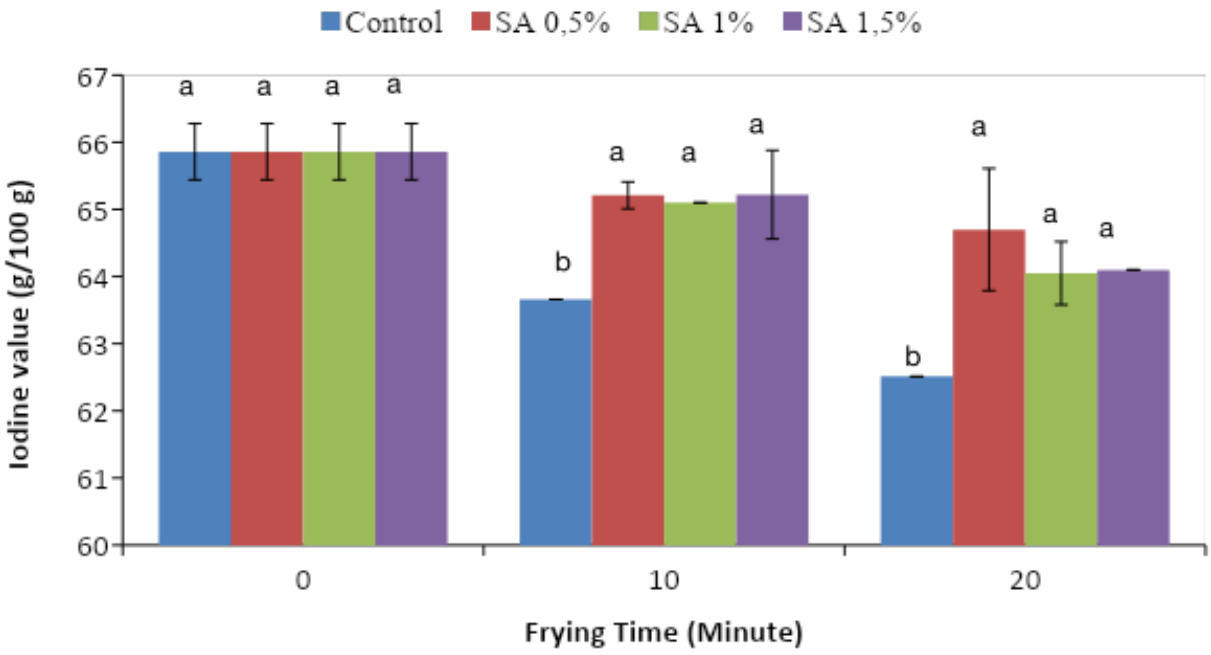

Fig. 7. Changes in iodine value of Cotton seed oil samples during frying
Values are presented as Mean ± SD. [a-b]Values of the same minute with different superscripts are significantly differents ($p<0.05$)

3.2.4. Acid value (% Oleic acid)

The acid value gives an idea of the amount of acidic components present in oil. This acidity is usually suspected as related to the presence of impurities such as; free fatty acids and other compounds. The free fatty acids generally released are products of triglyceride hydrolysis [25]. The changes in acid value of cotton seed oil samples supplemented with

different concentration of *Syzygium aromaticum* powder compared to the control are presented in figure 8.

A significant decrease ($p<0.05$) in acid value was registered with the control after 10 min frying followed by a significant increase ($p<0.05$) at the 20th min. The acid values of oil samples enriched with the natural antioxidant at the 10th min were similar to that of minute zero. Similar observation was recorded with cotton seed oil supplemented with SA 1% at the 20th min. However, a significant increase in this parameter was observed in the oil sample supplemented with SA 1.5%. The acid value of oil supplemented with SA 0.5% was constantly increasing with the frying time. The decrease in acid value observed in some samples can be attributed to the rapid transformation of the free fatty acid released into oxidized products. Here, we can suspect that the conversion rate of fatty acid into oxidized products was higher than the rate of hydrolysis of triglycerides. The significant increase in acid value observed in some samples suggests that the hydrolysis rate of triglycerides is higher than the transformation rate of free fatty acids. The constant acid value observed in some samples after 10 and 20 min can be related to the fact that the transformation rate of free fatty acid is equal to the hydrolysis rate of triglycerides. The acid value is not a parameter that can clearly dictate the oxidation state of oils at very high temperature, due to the spontaneous transformation of free fatty acids. Globally, the highest acid value obtained in this study was 1.6%, which is significantly lower than 4% recommended by [26].

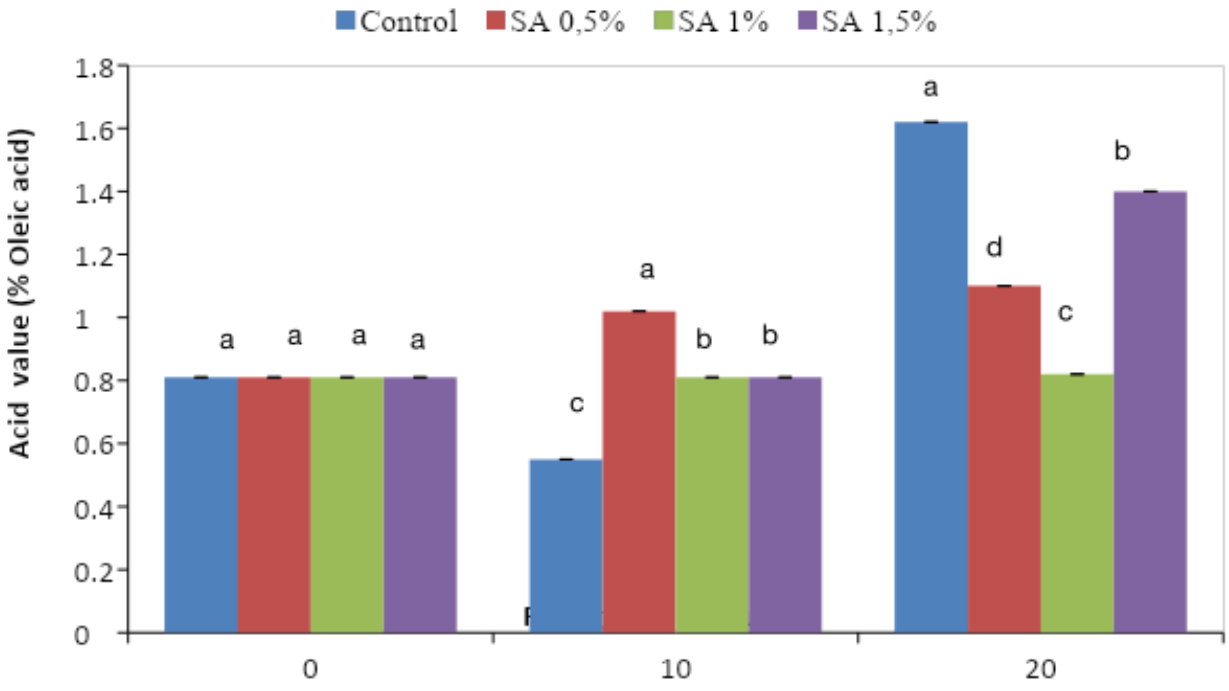

Fig. 8. Changes in acid value of Cotton seed oil samples during frying
Values are presented as Mean ± SD. [a-d] *Values of the same minute with different superscripts are significantly differents (p<0.05)*

4. Conclusion

The aim of this work was to assess the synergistic effect of clove buds extract, powder and vitamin E on the oxidative stability of cotton seed oil during the frying of plantain chips. Results showed that the addition of these natural plant extract and powder increases the stability of the oil compared to the sample containing only vitamin E as preservative. Oil samples supplemented with SA 0.30% and SA 0.60% presented good stability compared to the control, while the same extract at higher concentrations (1.00% and 1.30%) tend to act as prooxidants. As far as the powder is concerned, they exhibited good antioxidative properties at all levels. This is quite interesting as people in rural areas who extract crude oils can use such cloves powder for their preservation.

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