



Evaluation of proximate composition and the stability of alligator pepper (*Aframomum melegueta*) and Ethiopian pepper (*Xylopiya aethiopic*) extracts

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Abstract: This study investigated the proximate composition and the stability of the natural food flavouring agents present in *Aframomum melegueta* and *Xylopiya aethiopic* after Soxhlet extraction using: acidified ethanol; 1.5M HCl, acidified ethanol; 1% citric acid, 2% citric acid, acetone and hexane. Efficiency of solvent extraction was determined and four extracts with the highest yields were studied: for stability, the (pH) was monitored for 15 days period of storage and the titratable acidity was also determined over the period. Peroxide values were determined over the period of 5 weeks to monitor the shelf-life. Minor changes were observed in the peroxide values during the period of 5 weeks of storage. There was very little secondary oxidation of the extracts during storage. The nutritional potentials of these two medicinal and flavouring plants (seeds and fruits) and their extracts were evaluated through their proximate compositions. *Xylopiya aethiopic* had a higher crude fibre (15.20%) compared with *Aframomum melegueta* that was 8.55% Moisture contents of *Xylopiya aethiopic* and *Aframomum melegueta* were 7.35% and 7.47%, respectively. The total ash contents of *Xylopiya aethiopic* and *Aframomum melegueta* were 3.70% and 3.30%, respectively. Crude lipids of *Xylopiya aethiopic* and *Aframomum melegueta* were 30.80% and 8.85%, respectively while the total protein of *Xylopiya aethiopic* and *Aframomum melegueta* were 4.37% and 3.75%, respectively. Total carbohydrate of *Xylopiya aethiopic* was 38.58% and that of *Aframomum melegueta* was 67.80%. Alligator pepper seeds had higher titratable acidity values than Ethiopian pepper and their extracts have shown high oxidative stability over the periods of this study.

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Keywords: Alligator pepper, Ethiopia pepper, *Aframomum melegueta*, *Xylopiya aethiopic*

1. Introduction

Flavour is an important factor in the acceptability of food products and food quality can be adjudged on the basis of its flavour. People associate certain food with certain flavour (Delwiche, 2004; Oluwaniyi *et al.*, 2009) and the flavour of food can influence its demand.

Flavourings are defined generally as products that are: not intended to be consumed as such, but are added to food in order to impart or modify odour and/or taste. Made or consisting of the following categories: flavouring substances, flavouring preparations, thermal process flavourings, smoke flavourings, flavour precursors or other flavourings or mixture thereof. There are different types of flavouring products used in food, and these are further defined as follows: 'Flavouring substance' means a defined chemical substance with flavouring properties. 'Natural flavouring substance' means a flavouring substance obtained by appropriate physical, enzymatic or microbiological process from material of vegetable, animal or microbiological origin either in the raw state or after processing for human consumption by one or

more of the traditional food preparation processes (Abd El-Galeel, 2002).

Alligator pepper (*Aframomum melegueta*) which belongs to the family called '*Zingiberaceae*' is a spice in the ginger family with the common name; grains of paradise. Local Names: In Bini - *ehin-edo*; *ehie ado*; in Igbo - *Ose oji*; in Urhobo - *erhie*; in Yoruba - *oburo*; *ata*; *ata-ire* and in Hausas-*chitta mai ya' ya'*. It imparts peppery pungency and spicy aroma to classic West Africa soups. Alligator pepper is rich in essential oils that contribute to the pleasant flavour in soups (Ajaiyeoba and Ekundayo 1999). Alligator pepper is a spice that is utilized in medicine due to its antioxidant and antimicrobial properties (Adegoke *et al.*, 2002).

The spice from this pepper is used in West Africa for the purposes of alleviating stomach ache and diarrhea (Ilic *et al.*, 2010) as well as hypertension, tuberculosis and remedy for snake bites and scorpions (Oyedele *et al.*, 2002; Ajaiyeoba *et al.*, 2006). The seeds are also used for culinary reasons due to the

pungency of the seeds (Ilic *et al.*, 2010). They also tend to have general antimicrobial properties similar to many spices (Achinewu *et al.*, 1995) and helps in sexuality (enhances spermatogenesis) and aphrodisia (Kamtchouing *et al.*, 2002).

The aim of this study was to evaluate the proximate composition and the stability of the natural food flavouring agents present in *Aframomum melegueta* and *Xylopiya aethiopica*.

2. Materials and Methods

Two natural flavours: Alligator Pepper (*Aframomum melegueta*) and Ethiopia Pepper (*Xylopiya aethiopica*) fruits and seeds were purchased from Samaru Market in Zaria metropolis. The fruits and seeds were identified at the herbarium in the Department of Biological Sciences, Ahmadu Bello University, Zaria. Both seeds and fruits were properly dried under shade and pulverized using mortar and pestle, samples were stored in an air tight bottles and kept in the locker until extractions were carried out.

2.1 Solvent extraction

Soxhlet extraction method was used; the dried and pulverized samples were weighed, 50 g of each sample was transferred into a thimble and extracted using each of the following selected solvents: ethanol acidified with 1.5M HCl (85:15, v/v), ethanol acidified with 1% citric acid. (85:15, v/v), acetone, 2% citric acid solution and hexane (Pouget *et al.*, 1990). The following extracts were obtained:

Alligator pepper Extracts:

- AA Ethanol + 1.50M HCl Extract
- AB Ethanol + 1% Citric Acid Extract
- AC Acetone Extract
- AD 2% Citric Acid Extract
- AE Hexane Extract

Ethiopia pepper Extracts:

- EA Ethanol + 1.5M HCl Extract
- EB Ethanol + 1% Citric Acid Extract
- EC Acetone
- ED 2% Citric Acid Extract
- EE Hexane Extract.

Heating mantle was used as the source of heat and the extract obtained from each was concentrated using a rotary evaporator into completely or partially purified forms and allowed to dry in a desiccator.

2.2 Solvent Efficiency

Five millilitres (5 ml) volume of the filtered extract was diluted to 100 ml with the extracting solvent. The colour intensity was measured at 520 nm for water and citric acid solution extracts and 535 nm for acidified ethanol using spectrophotometer while acetone and hexane were measured at 330 nm and 220 nm respectively. The total content was calculated using the following equation:

$$\text{Total pigment} = \frac{\text{absorption} \times \text{dilution factor}}{\text{sample weight} \times 55.9} \times 100$$

(Du and Francis, 1973).

2.3 Determination of Stability of the Flavouring Agent with Time

A dilute solution of four extracts with appreciable yield (10 ml in 250 ml of distilled water) were prepared and the stability of the flavouring agents of the extracts was monitored by measuring the pH at ambient temperature (27°C) for 15 days at 24 hours intervals. The titratable acidity was also determined by titrating 10 ml of each sample with 0.1 M NaOH solution using 1% phenolphthalein indicator. The titration was carried out on daily basis for a period of 15 days. The acid values were calculated using this equation: $56.1 \times M \times \frac{V}{m}$

Where V is the volume of sodium hydroxide (NaOH) solution used in (ml), M is the exact molarity and m is the mass in grams of the sample extract diluted (Saad *et al.*, 2007).

2.4 Determination of Oxidative Stability

This was carried out as described by AOCS (1992). 10 ml of the diluted extract was transferred into a 250 ml conical flasks using pipette, 10 ml acetic acid/chloroform mixture (3:2, v/v) was added and stirred for 2 minutes, 1 ml of saturated KI solution added into the solution and then stirred for another 2 minutes. The solution was heated and titrated with 0.10 M sodium thiosulphate, using a starch solution as indicator. A blank test was also conducted. The peroxide values were calculated using the equation 3.3 for the period of 5 weeks of storage after dilution. (Note: titrations were carried out on weekly basis)

$$\text{PV (meq/kg)} = \frac{(S-B) \times 1000 \times M}{W}$$

where S and B are the volumes of sodium thiosulphate solution consumed by sample and blank tests, respectively, M is the standardized molarity of sodium thiosulphate and W is the weight of sample (g) (Alhassane *et al.*, 2007).

2.5 Proximate Analysis

Proximate analysis was carried out to check the nutritional values as described by the method of AOCS (1990).

2.6 Moisture content determination

Moisture content was determined by weighing 1.5 g of each sample, oven dried at a controlled temperature, 80°C for four days. The loss in weight due to loss of moisture is calculated as a percentage of original weight of sample. This gives the percentage moisture content of the sample (Aremu *et al.*, 2006).

$$\text{Moisture Content (\%)} =$$

$$\frac{\text{Loss in Weight on Drying (g)}}{\text{Initial Sample Weight (g)}} \times 100$$

2.7 Percentage crude ash determination

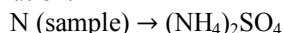
Percentage crude ash was determined by igniting 1.5 g of each of the samples in a crucible inside a

muffled furnace at a temperature of 600°C. The ashing was terminated on formation of the white ash from the sample. The ash was cooled in a desiccator for two hours and weighed. The weight after ashing was expressed as a percentage of the sample weight before ashing and this gives the percentage crude ash of the samples.

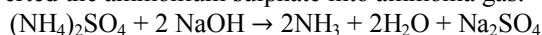
$$\text{Ash (\%)} = \frac{\text{Weight of ash (g)}}{\text{Dry Weight (g)}} \times 100$$

2.8 Crude protein determination

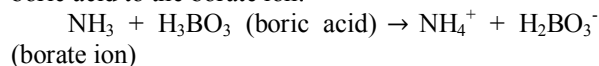
Crude protein content was estimated by Kjeldahl method. 5 g of each sample was digested with a concentrated sulphuric acid (H₂SO₄) using lithium sulphate as a catalyst. Digestion converted any nitrogen in the food (other than that which was in the form of nitrates or nitrites) into ammonia, and other organic matter to CO₂ and H₂O. Ammonia gas was not liberated in an acid solution because the ammonia was in the form of the ammonium ion (NH₄⁺) which bonded to the sulphate ion (SO₄²⁻) and thus remained in solution:



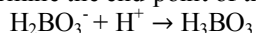
After the digestion has been completed the digestion flask was connected to a receiving flask by a tube. The solution in the digestion flask was then made alkaline by addition of sodium hydroxide, which converted the ammonium sulphate into ammonia gas:



The ammonia gas that was formed was liberated from the solution and moved out of the digestion flask and into the receiving flask - which contained an excess of boric acid. The low pH of the solution in the receiving flask converted the ammonia gas into the ammonium ion, and simultaneously converted the boric acid to the borate ion:



The nitrogen content was then estimated by titration of the ammonium borate formed with hydrochloric acid, using a suitable indicator to determine the end-point of the reaction.



The concentration of hydrogen ions (in moles) required to reach the end-point was equivalent to the concentration of nitrogen that was in the original food

(Equation 3.8). The following equation was used to determine the nitrogen concentration of the sample that was weighed using 1.5 M HCl acid solution for the titration: (Conklin-Brittain *et al.*, 1999).

Where v_s and v_b are the titration volumes of the sample and blank, and 14 g is the molecular weight of nitrogen N. A blank sample was run at the same time as the material being analysed to take into account any residual nitrogen which may be in the reagents used to carry out the analysis. The nitrogen content determined was converted to a protein content using the appropriate conversion factor: 6.25. Because the Kjeldahl method does not measure the protein content directly a *conversion factor (F)* is needed to convert the measured nitrogen concentration to a protein concentration. A conversion factor of 6.25 (equivalent to 0.16 g nitrogen per gram of protein) is used for many applications, however, this is only an average value (Conklin-Brittain *et al.*, 1999).

3. Results

The result for proximate analysis for Alligator pepper and its extracts is shown on Table 2 and indicates the nutritional values of the seeds and the extracts obtained. The result for proximate analysis for Ethiopia pepper and its extracts is shown on Table 3 and indicates the nutritional values of the fruits and the extracts obtained. The result for solvent efficiency for extraction is given in Figure 1. The Figure indicates the solvent efficiency for the various solvents used to carry out the extraction, with AA and EA having the highest followed by EC and AC. The result for the variation for the pH stability for flavourant sample is given in Figure 2 indicates the extracts (AA, AC, EA and AC) studied for the period of fifteen days at ambient temperature. Figure 3 shows the titratable acidity of the various extracts studied with time. These variations are indicated by the curve pattern AA, AC, EA and EC.

The result for the variation for weekly peroxide value of flavourant for Alligator and Ethiopia pepper is given in Figure 4. This indicates the weekly peroxide values for the four extracts studied for the period of five weeks. The curve pattern indicates the variation observed.

Table 1: Proximate Analysis of Flavourant Samples for Alligator Pepper

Flavourant samples	Proximate composition (%)					
	Moisture	Ash	Crude lipid	Crude protein	Crude fibre	Carbohydrate
Seeds	7.47	3.30	8.85	8.85	3.75	67.80
AA	1.13	0.67	3.03	1.04	0.00	49.72
AB	0.75	0.13	1.52	0.16	0.00	33.71
AC	0.55	0.83	3.27	1.25	0.00	38.63
AD	0.89	0.12	1.80	0.08	0.00	28.28
AE	0.81	0.12	2.20	0.10	0.00	31.5

(AA Ethanol + 1.50M HCl Extract; AB Ethanol + 1% Citric Acid Extract; AC Acetone Extract; AD 2% Citric Acid Extract; AE Hexane Extract)

Table 2: Proximate Analysis of Flavourant Samples for Ethiopian Pepper

Flavourant samples	Proximate composition (%)					
	Moisture	Ash	Crude lipid	Crude protein	Crude fibre	Carbohydrate
Fruits	7.35	3.70	25.80	9.37	15.20	38.58
EA	1.51	0.13	10.04	1.16	0.00	26.26
EB	1.12	0.08	7.98	0.45	0.00	17.87
EC	1.30	0.27	8.68	1.36	0.00	24.84
ED	0.59	0.05	6.68	0.13	0.00	21.85
EE	0.87	0.20	7.70	0.54	0.00	16.32

(EA Ethanol + 1.5M HCl Extract; EB Ethanol + 1% Citric Acid Extract; EC Acetone ED 2% Citric Acid Extract; EE Hexane Extract)

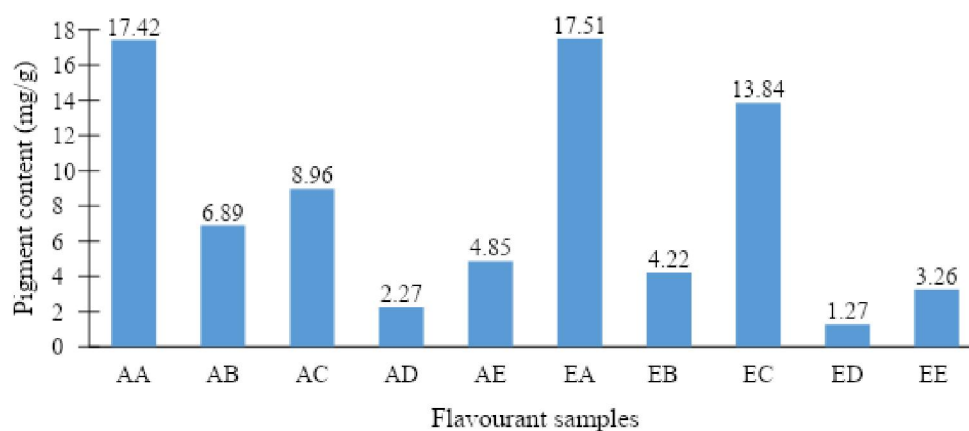


Figure 1: Efficiency of Solvent Extraction

(Alligator pepper= AA: Ethanol + 1.5 M HCl Extract; AC: Acetone Extract)
(Ethiopian Pepper= EA: Ethanol + 1.5M HCl Extract; EC: Acetone Extract)

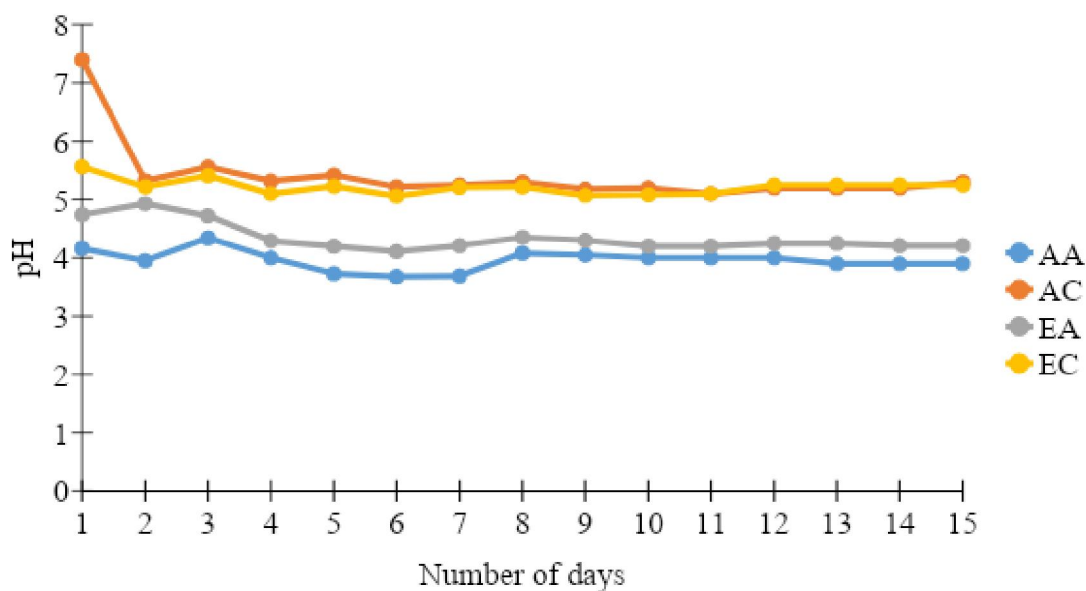


Figure 2: Variation for the pH Stability for Flavourant Samples

(Alligator pepper= AA: Ethanol + 1.5 M HCl Extract; AC: Acetone Extract)
(Ethiopian Pepper= EA: Ethanol + 1.5M HCl Extract; EC: Acetone Extract)

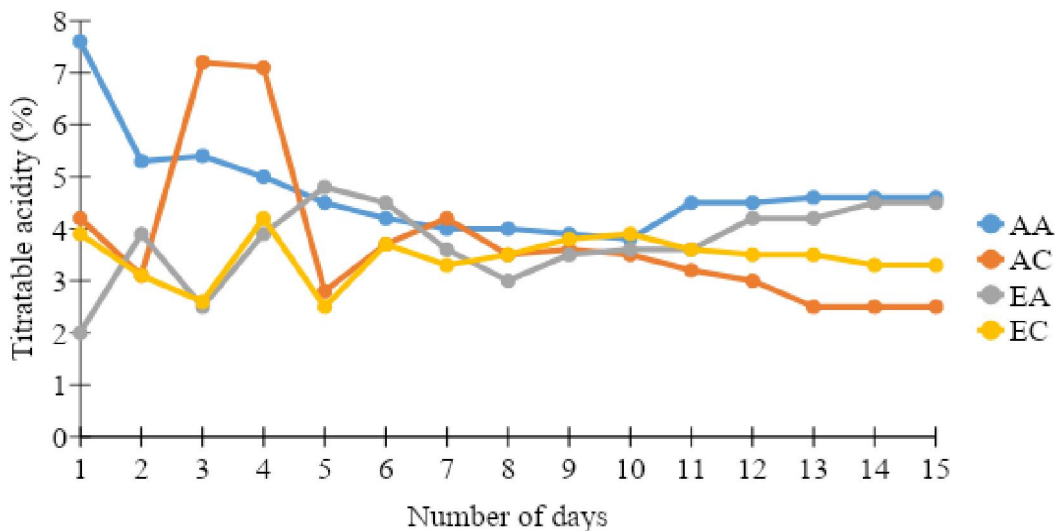


Figure 3: Variation for Titratable Acidity of Flavourant Samples
 (Alligator pepper= AA: Ethanol + 1.5 M HCl Extract; AC: Acetone Extract)
 (Ethiopian Pepper= EA: Ethanol + 1.5M HCl Extract; EC: Acetone Extract)

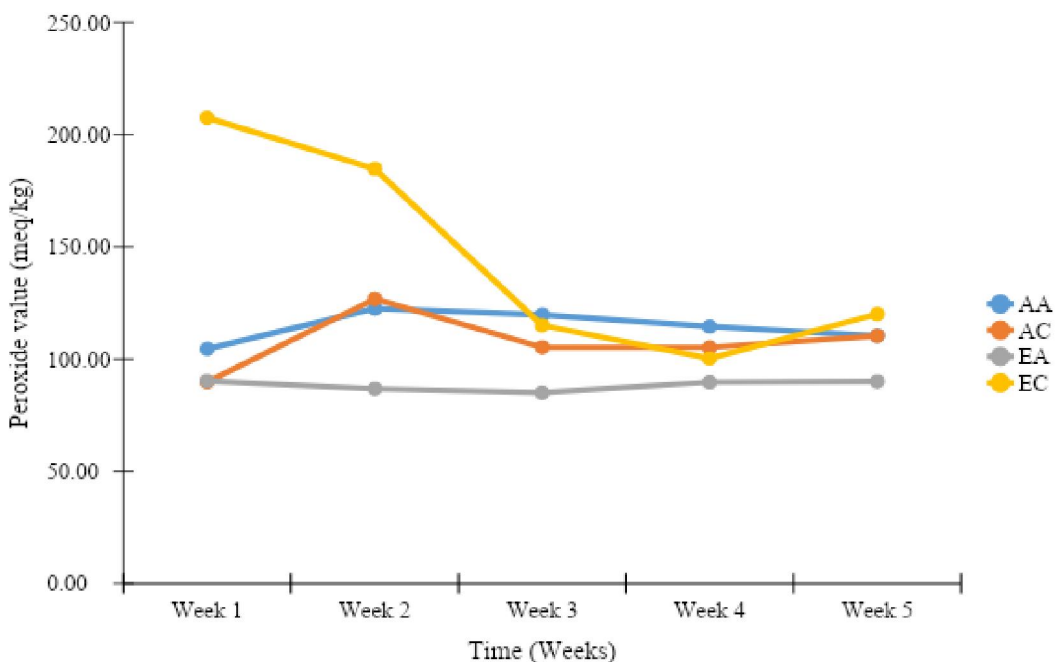


Figure 4: Variation for weekly peroxide value of Flavourant for Alligator and Ethiopian pepper.
 (Alligator pepper= AA: Ethanol + 1.5 M HCl Extract; AC: Acetone Extract)
 (Ethiopian Pepper= EA: Ethanol + 1.5M HCl Extract; EC: Acetone Extract)

The correlation between titratable acidity and pH of the test samples is given on Table 3 and it indicates very low positive and negative correlations. Figure 5 shows the variation of peroxide values for Alligator pepper. The two extracts were from the same pepper and the variation between them are shown by the curve pattern. Figure 6 shows the variation of peroxide

values for Ethiopia pepper. The two extracts were from the same pepper and the variation between them are shown by the curve pattern. The result of variation of peroxide values for Alligator and Ethiopia pepper using the same solvent is given in Figure 7. The two extracts (AA and EA) were from different pepper and

the variation between them are shown by the curve pattern.

The result of variation of peroxide values for Alligator and Ethiopia pepper using the same solvent

(Acetone) is given in Figure 8. The two extracts (AC and EC) were from different pepper and the variation between them are shown by the curve pattern.

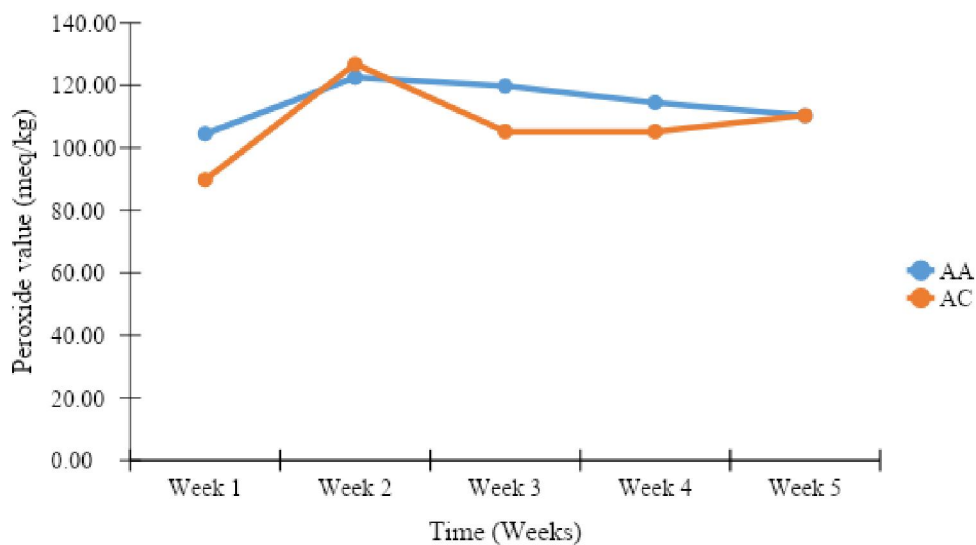


Figure 5: Variation of Peroxide Values for Alligator pepper (AA: Ethanol + 1.5 M HCl Extract; AC: Acetone Extract)

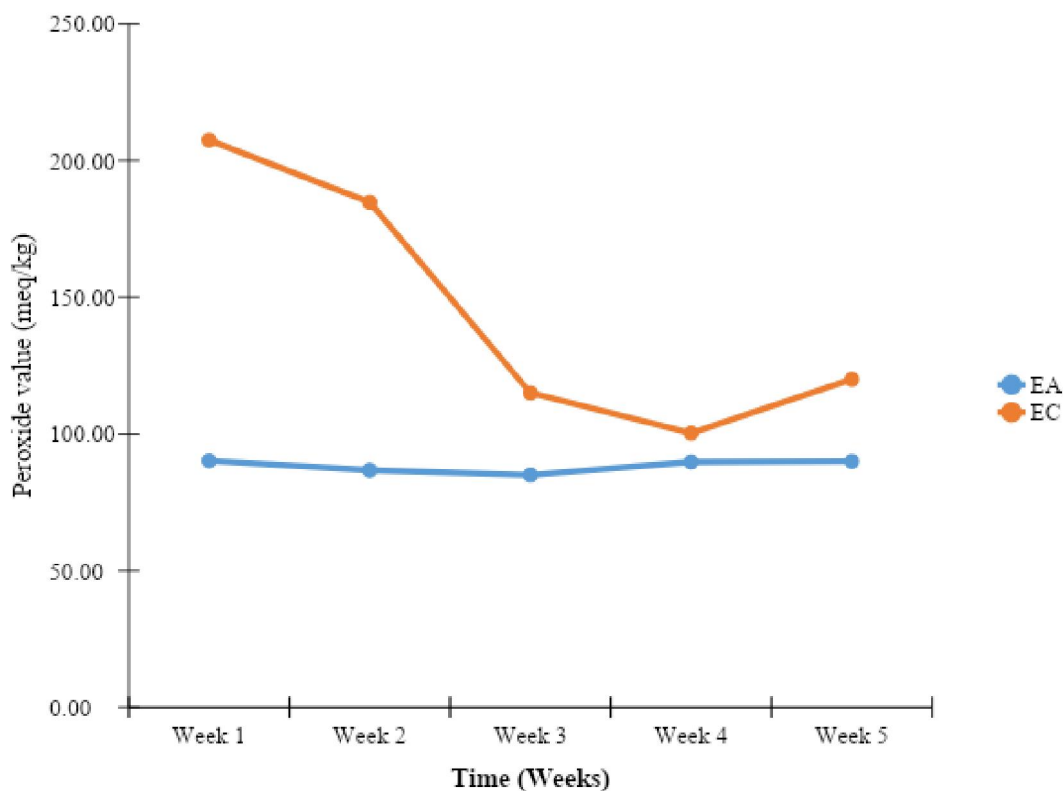


Figure 6: Variation of Peroxide Values for Ethiopia pepper (Ethiopia Pepper: EA Ethanol + 1.5M HCl Extract; EC Acetone Extract)

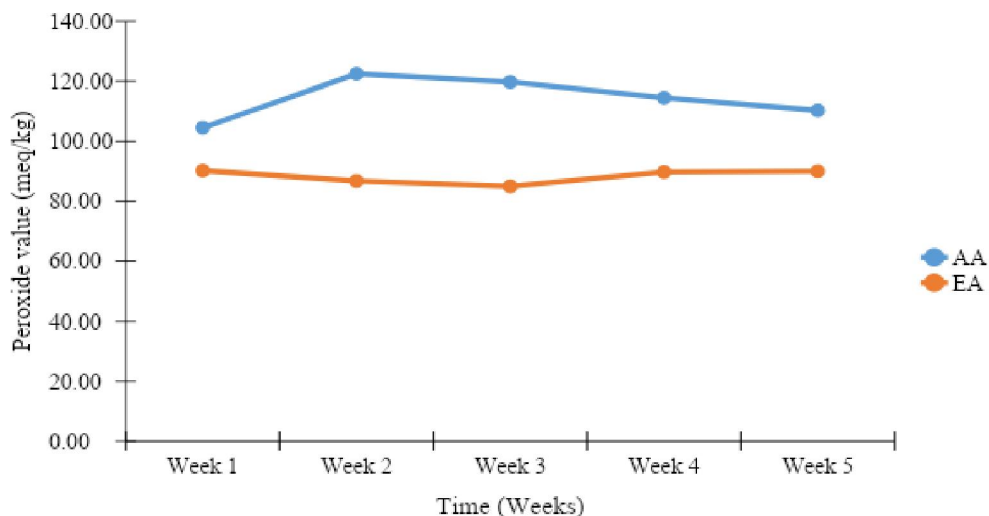


Figure 7: Variation of Peroxide Values for Alligator and Ethiopia pepper (AA Ethanol + 1.5 M HCl Extract Alligator Pepper; EA Ethanol + 1.5M HCl Extract Ethiopia Pepper)

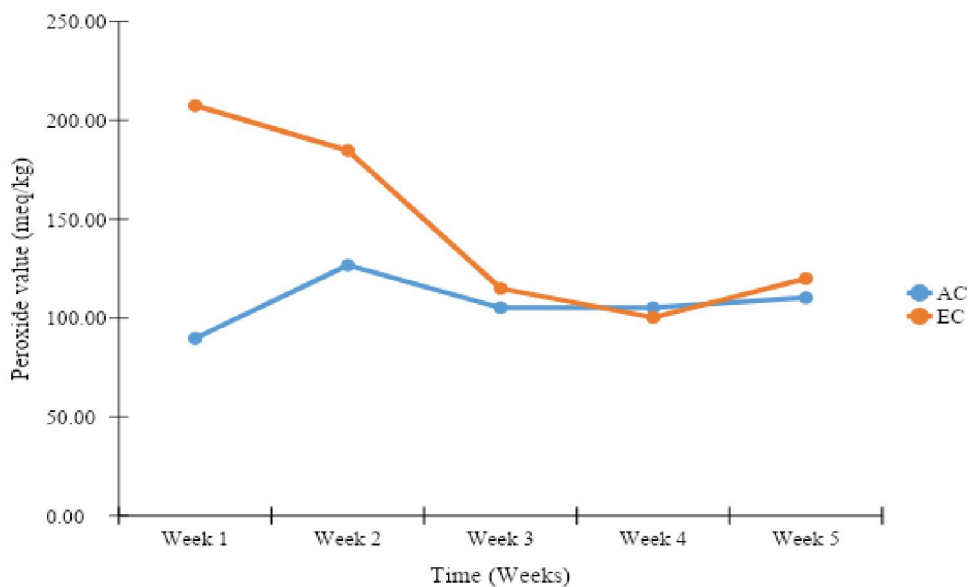


Figure 8: Variation of Peroxide values for Alligator and Ethiopia pepper using the same solvent (Acetone) (AC: Acetone Extract Alligator Pepper; EC: Acetone Extract Ethiopia Pepper)

	<i>AApH</i>	<i>ACpH</i>	<i>EApH</i>	<i>ECpH</i>	<i>AATA</i>	<i>ACTA</i>	<i>EATA</i>	<i>ECTA</i>
AApH	1							
ACpH	0.3760	1						
EApH	0.5887	0.5356	1					
ECpH	0.4641	0.7846	0.6112	1				
AATA	0.4540	0.9015	0.6996	0.8124	1			
ACTA	0.4900	0.1898	0.3257	0.1406	0.2794	1		
EATA	-0.7600	-0.6537	-0.6082	-0.5516	-0.5573	-0.5089	1	
ECTA	0.0600	0.1373	-0.2271	-0.3293	0.0265	0.0887	-0.1673	1

Table 3: Correlation between Titratable Acidity and pH of the test samples Values presented in tables are correlation (R) values.

4. Discussion

4.1 Titratable acidity and pH Stability

The results obtained for the pH values of the samples showed that the pH for all samples are relatively stable i.e. the solutions became more slightly acidic as the time progressed (Figure 2). A sharp increase was observed after 24 hours for EC and AC. AA and EA had similar pH values from day to day and the values ranged between 4.2 and 5.6. The results of titratable acidity are shown in Figure 3. After 24 hours, the titratable acidity of AA decreased up to 10th day and became relatively stable from 11th day to the 15th day. Low acidity values were observed in AC, in general, all the four samples showed a similar relationship; they tend to be stable from day 9 to 15. Samples with high pH values had low titratable acidity and as the pH increased, the acidity decreased. AC with the highest pH values also had the least titratable acidity values.

This study has shown that the flavouring agents of these two peppers were relatively stable during the period of study. From day seven (7) pH stability was observed up to the fifteenth day of storage as shown on Figure 2 and from day eight (8) that of acidity was observed as indicated in Figure 3. This observation agrees with research that percentage total titratable acidity and pH are inversely related (Egbera *et al.*, 2007; Nwafor and Ikenebomeh, 2009).

The test of correlation between titratable acidity of AA and its corresponding pH showed that there was positive correlation ($r = 0.4540$) between the titratable acidity and pH though the correlation was not statistically significant. Similarly, there was positive correlation (0.1898) between the pH of AC and its titratable acidity but the relationship was not significant.

The pH of EA on the other hand showed a significantly strong negative correlation (-0.6082) with its corresponding titratable acidity; this implies that as the pH value of EA increases, the titratable acidity decreases. Also, there was a negative correlation (-0.3293) between the pH of EC and its titratable acidity. Unlike the EA, the correlation between pH and titratable acidity of EC was weak and insignificant (Table 1).

4.2 Oxidative Stability

The research work was conducted to determine the extent of oxidative deterioration of selected sample extracts under light and ambient temperature condition. The results revealed that oxidation of flavouring agents decreased with increasing storage period up to five weeks of storage. Natural antioxidants decreased the peroxide value in the extracts studied and this supports the findings that these peppers' extracts are used as preservatives in food industries. The initial peroxide value in sample AA was $105.00 \text{ meq kg}^{-1}$ and

in sample AC was $90.00 \text{ meq kg}^{-1}$. At the expiry of the 5th week experimental period, the peroxide values increased from week one to three and then decreased in week four and five: $105.00, 123.00, 120.00, 115.00$ and $110.00 \text{ meq kg}^{-1}$ for AA and increased slightly in AC from week two through week five: $90.00, 127.00, 105.00, 105.00$ and $110.00 \text{ meq kg}^{-1}$ as indicated in figure 4.4. This showed there was no significant deterioration of the flavouring agents in the samples.

The same trend was observed for samples EA and EC in peroxide values: $115.00, 90.00, 87.00, 85.00, 90.00, 90.00 \text{ meq kg}^{-1}$ and $110.00, 210.00, 185.00, 115.00, 100.00, 120.00 \text{ meq kg}^{-1}$ respectively. The flavouring agents in the two plants have high oxidative stability, hence an appreciable shelf-life during storage.

4.3 Proximate Analysis

The result of nutritional composition shows that alligator pepper and Ethiopia pepper have similar moisture content, both peppers have a significant amount of protein content 4.37% and 3.75% respectively. The same trend was also observed in their ash content, 3.70% and 3.30% . The protein and ash content of alligator pepper is similar to the values reported by (Odeunmi *et al.*, 2008).

This work is aimed at using the proximate analysis as well as the percentage compositions of some mineral elements to assess the nutritional potentials of the plant parts in relation to the different purposes they are meant for. From the results obtained, *Xylopiya aethiopicum* and *Aframomum melegueta* are high in the amount of total carbohydrate and this is beneficial since carbohydrate constitutes a major class of naturally occurring organic compounds that are essential for the maintenance of plant and animal life and also provide raw materials for many industries (Ebun-Oluwa and Alade, 2007). *Xylopiya aethiopicum* is also high in percentage compositions of total fat and crude fibre. Because of the high contents of total carbohydrate, total fat and crude fibre contents of *Xylopiya aethiopicum*, it was particularly recommended to women who have newly given birth as a tonic in Ivory Coast (Burkill, 1985). The total fat in *Xylopiya aethiopicum* if further analysed may contain essential fatty acids as well as vitamins. Nutritionally, this is of beneficial effect since it had been reported that food fibre aids absorption of trace elements in the gut and reduce absorption of cholesterol (Le Veille and Sanberlich, 1966).

The various extracts of these peppers have shown a significant nutritional values as well as the seeds and fruits. The high amount of lipid observed in Ethiopia pepper and its various extracts may be due to the fruits unlike alligator pepper which only the seeds were used.

The nutrient composition of *Xylopiya aethiopicica* fruits differs relatively from what has been reported by other researchers. Abolaji *et al.* (2007) had earlier reported 2.10±0.25 % protein, 9.55±2.10 % fat and 12.14±0.70% ash in the fruits of *Xylopiya aethiopicica*. Another worker also reported 55.80±4.26% carbohydrate, 7.20% crude fat and 10.00 % ash by weight of dried powdered sample of *Xylopiya aethiopicica* and 9.00% crude protein. The findings of Abolaji *et al.* (2007) are quite different from what is being reported in this present study. The varying composition reported by various researchers may imply that the nutrient composition of these peppers vary with season, environment and/or condition or time of evaluation.

Natural antioxidants are found in many fruits and vegetables, which include ascorbic acids, α -tocopherole, β -carotene, chlorogenic acids and flavonols. Consumers are becoming increasingly conscious of the nutritional value and safety of their food and its ingredients. Natural antioxidants are believed to be safer than artificial ones (Papadoulos *et al.*, 1991; Tian and White, 1999). It is hoped that the findings will help a lot to the vegetable oil and ghee industries as well as oil seed growers. Therefore, the present study was carried out to determine the extent of deterioration of flavouring agents in Alligator pepper and Ethiopia pepper under ambient temperature.

5. Conclusion

This study has drawn attention to the stability of the flavourants, with particular attention on the acidity of the Alligator pepper as indicated by AA and AC which may make it unsuitable for individuals with a history or tendency of stomach or peptic ulcer. From this research, Ethiopia pepper may be preferred to be used by individuals with the above mentioned health challenges. However, it can be concluded that when considering bio-assimilation, bio-digestibility and easy elimination, both peppers are preferred over the synthetic ones. The higher presence of nutrients and antioxidants in the natural flavours also support their choice over synthetic flavours. Therefore, depending on the usage target or goal, any of the peppers can be used. Based on the nutritional analysis carried out and the elemental analysis, these two peppers can serve as good food supplements and they can be used to produce peppery flavours with an appreciable shelf-life.

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