

EFFECTS OF SOME CHEMICAL PRESERVATIVES ON THE STORABILITY AND SENSORY ATTRIBUTES OF AGBARATI-A MEAT SUBSTITUTE

Nwakaudu¹, A.A., Nwakaudu², M.S., Olawuni¹, I.A., Ofoedu¹, C.E., Adedokun³, A.O. and Ubaechu¹, F.

¹Department of Food Science and Technology, Federal University of Technology, PMB 1526 Owerri, Nigeria

²Department of Chemical Engineering, Federal University of Technology, PMB 1526 Owerri, Nigeria

³Nigerian Stored Product Research Institute, Federal Ministry of Agriculture & Rural Development, Port Harcourt, Nigeria.

ABSTRACT: *Agbarati, a melon seed based meat analogue was produced and treated with four different chemical preservatives; Sodium benzoate, Citric acid, Potassium sorbate and Ascorbic acid. The produced meat analogue samples were stored at room temperature for seven days and changes in their chemical attributes (Free Fatty Acids, Peroxide value and Iodine value) and sensory attributes were monitored to know if the chemical preservatives can extend the shelf life of the samples. At the end of the experiment, the result showed that Citric acid was adjudged the best of the four preservatives having the lowest increase in Free Fatty Acid value of 2.75% compared to the untreated sample of 4.18% in Free Fatty Acid. Similarly, the peroxide value increased to 6.41MeqKg⁻¹ in the untreated sample and 4.66MeqKg⁻¹ in Citric acid sample while the Iodine value reduced to 92.76 g/100g of oil in the untreated sample and 96.76g/100g of oil in the Citric acid sample. Result of sensory evaluation showed that panelists preferred most the Citric Acid preserved Agbarati.*

KEYWORDS: Agbarati-Meat Analogue, Chemical Preservative, Free Fatty Acid, Iodine Value, Peroxide Value

INTRODUCTION

Agbarati also known as "Usu" is an indigenous meat analogue produced mainly in the rural areas of South Eastern Nigeria. It is generally understood as melon cake. Melon cakes are sometimes mixed with cooked sliced fermented oil bean fruit called "Ugba" in Ibo and cooked with stockfish (Nwokoma, 2008). This local delicacy is a steamed product obtained after blending uniformly melon seeds (*Colocynthis citrullus*) and "Erousu" (*Sclerotus tuber-regium*) with the addition of other ingredients (Pepper, Onions, Salt, Warm water and Seasoning). It is eaten as a snack and is often added to vegetable soups as a meat substitute. In some rural parts of Nigeria, melon seed meal is compacted into patties that serve as a meat substitute (Ogunsua, 2000).

In Imo State, it is called "Agbarati", in Umuahia "Ngbam", in Onitsha, "Agbaghelu atui" and understood in the Ibo tribe as "Akpuruakpu Egusi". Melon seeds are pounded and used in the preparation of popular steamed snack known as "Agbaghelu atui" (Onitsha) (Nwokoma, 2008). They are molded into balls of various shapes (round, flattened or cylindrical) with the colour of its balls ranging from dark brown to light grey. Health wise, the meat analogue is important to the diet because they provide the body with proteins, fiber and other essential micronutrients

(Decker, 2004). David and Aderibigbe (2010) stated that its composition shows: Moisture content of 40%, Ash content of 14%, Crude fiber of 2.40%, 20% fat, 10.50% protein and Carbohydrate of 10.60%. It has a shelf life of 24hours at ambient temperature (Akobundu *et al.*, 1982; Nwakaudu, 2010).

The preservation of *Agbarati*-Indigenous meat analogue has been tried using such methods as drying and packaging material (Nwakaudu, 2010) but no work has been done on the use of chemical preservative methods to extend the shelf life of *Agbarati*. This product has a problem of deteriorating easily after 24 hours when kept under ambient temperature, it loses its taste, becomes slimy and mold infested. As a result of this problem, *Agbarati* has remained a remote product whose production has not been incorporated into an industrial scale. Its major raw material (melon seeds) has poor storage problems. At local levels however, stored melon seeds do not keep well possibly owing to poor processing and storage technique (Adegoke and Ndife, 1993). Therefore, there is need to extend the shelf life of melon seeds through processing and use of food grade chemical preservatives so as to make it available all year round. This would also help to reduce the high cost of melon seeds during off seasons having its product as a meat analogue (Decker, 2004). *Agbarati* is a good substitute to meat in vegetable soups especially for Vegetarians. Also, being a proteinous food, the success of its preservation will go a long way in boosting the Nigerian economy since its counterpart (animal protein) is very expensive.

In this research work, it is expected that the oil extracted from the chemically treated *Agbarati* samples will be subjected to the basic rancidity analysis (Peroxide value, Acid value and Iodine value) and it is obvious that determination of these quality characteristics of *Agbarati* oil is important in ascertaining the shelf stability of the product on storage.

Therefore, the objectives of this work are to determine the effect of some chemical preservative on the storability of *Agbarati* and to find out which of the preservatives best extends the shelf life of the *Agbarati*.

MATERIALS AND METHODS

Materials

The raw materials used in this study included big mushroom i.e. "Erousu" (*Sclerotium tuber-regium*) and Melon seeds i.e. "Egusi" (*Colocynthis citrillus*) and other ingredients: salt, red pepper, onions and seasoning such as Star magi sauce were obtained from Ahiaohuru, a local market at Aba, Abia State. Plantain leaves used as "wrapping materials" were collected from the Horticulture plantation field of the National Root Crop Research Institute (NRCRI) Umudike, Umuahia in Abia State, Nigeria. Other facilities and materials used in this practical were obtained from the Central Laboratory Services Unit of the same Institute. The chemicals and equipment used were of analytical grade (ANALAR) and were supplied by the Central Laboratory of the National Root Crop Research Institute (NRCRI) Umudike, Umuahia, Abia State, Nigeria.

Production of *Agbarati* (Indigenous Meat Analogue)

400g mixture of ground "Egusi" and "Erousu" were weighed out at the ratio 60:40 respectively (240g "Egusi" and 160g "Erousu"). For each of the five (5) samples of *Agbarati* to be produced. The sample blend were thoroughly mixed in a clean dry mortar and kneaded together with the

ingredients (20g red pepper, 40g sliced Onions, ½ teaspoon of salt and 1 star magi cube). Warm water was added drop wise during kneading to facilitate molding. Kneading was continued until a thick moldable paste was obtained. For subsequent sample mixtures, preservatives were added at its permitted dosage in food; Citric acid (0.10%), Potassium sorbate (0.10%), sodium benzoate (0.10%) and Ascorbic acid (0.25%). These preservatives were added during the addition of the various ingredients after pounding. Each of the sample mixtures were molded into small balls and wrapped with the plantain leaves. They were tied using sack twines and steamed in boiling water for 90minutes. The steamed *Agbarati* were further dried in a Carbolite oven (Type PF 2i0) at 70°C for 5hours to a moisture content of 15%. The balls were then allowed to cool and stored in clean, dry airtight polyethylene containers labeled according to sample.

Extraction of Oil from Stored Agbarati Samples

Extraction of oil from the samples of "Agbarati" was done using cold solvent extraction method (Harbone, 1973). The solvent used was n-Hexane (B.P: 67.7-69.2)°C. Since oil should be extracted from the stored "Agbarati" samples on daily basis; 3 balls from each of the 5 samples were selected and crushed to obtain the fine *Agbarati* flour. Each crushed sample was transferred into a conical flask and was soaked in the solvent in their separate flasks. The flasks were corked well and allowed to stand overnight at room temperature. The next day, the soaked samples were agitated in a reciprocals shaker for 10minutes. The mixture was then filtered through a muslin cloth. After filtration, the filtrate was transferred into the soxhlet extractor for the oil extraction. The oil extracted was used to carry out the oil analysis.

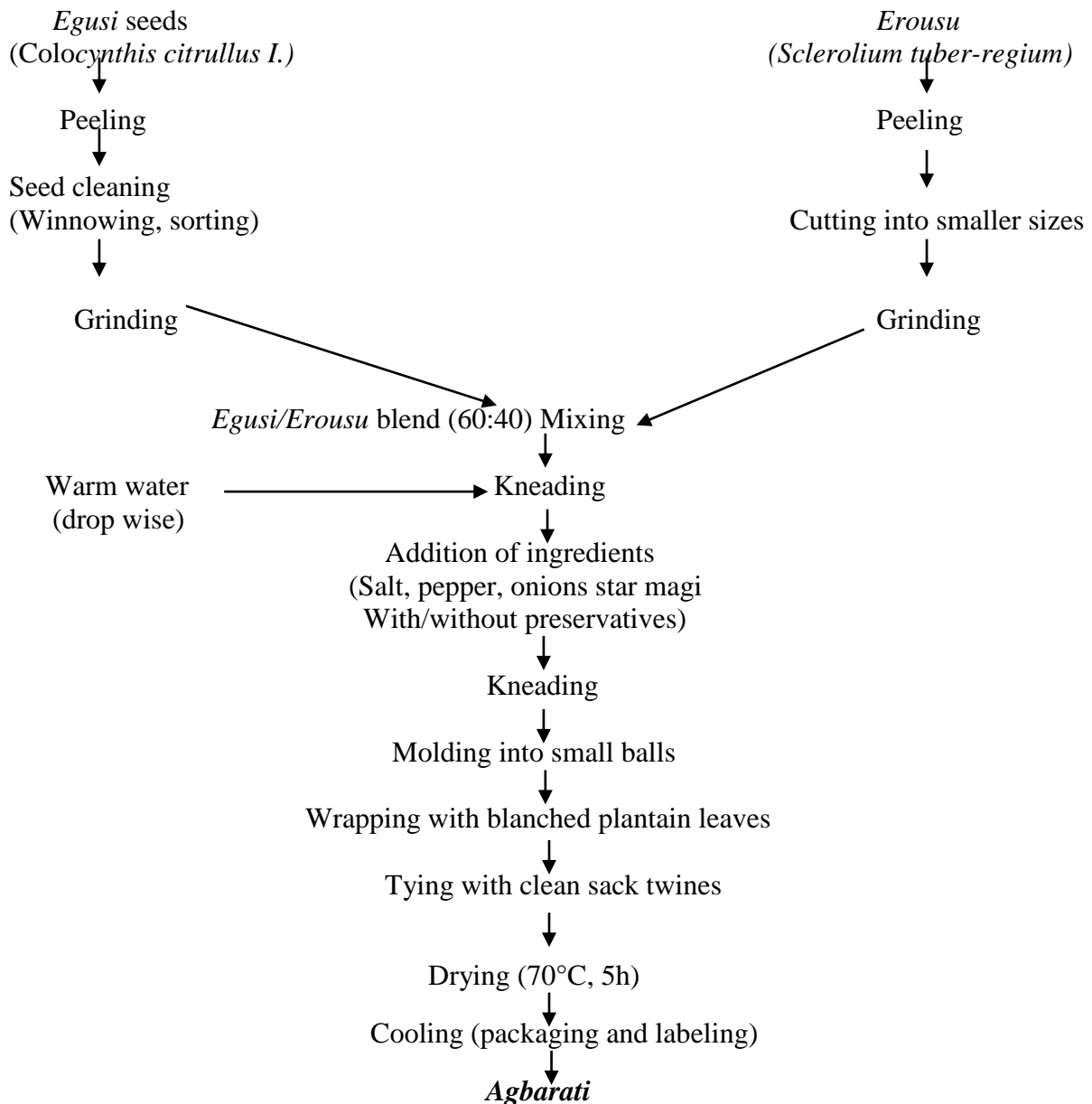


Figure 1: Flow diagram for the Production of Agbarati

Determination of Iodine value

The Wiji's titrimetric method (Pike, 2003) was used. A measured weight (0.2-0.5) g of the sample was dissolved in 10mls of carbon tetrachloride (CCl₄) in a conical flask. Twenty millimeters (20mls) of Wiji's Iodine solution was added to it and mixed well. It was allowed to stand in a dark cupboard at room temperature for 30mins. After that, 20mls of 10% potassium iodine solution was added to it followed by 100mls of distilled water and 2mls of 1% starch indicator. It was then titrated against 0.1M sodium thiosulphate solution until the characteristic iodine colour disappears. Meanwhile, a reagent blank was set up with 10mls of the carbon tetrachloride above and titrated as well. The Iodine Value was calculated as shown below:

$$\text{Iodine Value} = \frac{B-T \times 1.269}{W}$$

Where: B = Titre value of reagent blank

T = Titre value of sample

W = Weight of sample

Determination Peroxide Value

This was determined by the Thiosulphate titration method (James, 1995; Onwuka, 2005). One gramme (1g) of the sample (oil) was dissolved in a mixed solvent containing Chloroform and Acetic Acid in 2:1 ratio (v/v). The mixture was boiled under reflux for a minute (60 seconds) and 20 millimeters of 5% Potassium iodine solution followed by 50mls of distilled water. It was titrated against 0.002N Sodium thiosulphate solution using 2mls of 1% starch solution as indicator. Titration was done until the colour of the mixture cleared the Peroxide Value (PV) was calculated using the following formula:

$$\text{PV (Meq /kg)} = \frac{1000 \times N \times \text{Titre}}{W}$$

Where: W = Weight of sample

N = Normality of Titrant

Determination of Acid Value/Free Fatty Acid

This was determined using the alkaline titration method described by (Pearson, 1976; James 1995 and Pike, 2003). 1g of the sample was dissolved 50mls of neutral solvent containing 1:1 (v/v) mixture of ethanol and diethyl ether. A few drops of phenolphthalein indicator solution were added to the mixture and it was titrated against 0.1N Sodium Hydroxide (NaOH) solution. Titration was done to a pink end point colour which persisted for more than 15 seconds. The Acid value was calculated using the formula:

$$\text{AV} = \frac{\text{Titre} \times N \times 56.1}{W}$$

Where: N = normality of the titrant

W = weight of the sample

$$\text{FFA} = \frac{\text{AV}}{2}$$

Sensory Evaluation

Sensory evaluations of the *Agbarati* samples were carried out using a 20 man panel drawn from the Federal University of Technology, Owerri. The samples were scored for appearance, colour, aroma, taste, texture and overall acceptability using a 9-point hedonic scale.

A panelist was provided with enough privacy in order to avoid bias by other panelist and was to indicate the following:

- ❖ Age bracket (18-20, 21-30, 31-40, above 40 years) and gender (male or female)
 - ❖ Test for colour was carried by visual inspection
 - ❖ Test for aroma was carried out by sniffing twice or more of each sample provided
 - ❖ Taste for 1-5 minutes. Enough water was provided and the panelist was free to swallow or spit out the sample and rinse his or her mouth.
 - ❖ Test for texture was by touching or feeling the sample with the finger.
 - ❖ The panelists were to award a final score of overall acceptability.
 - ❖ The panelists were to give ratings based on the 9 - point hedonic scale as follows:
- | | | |
|---|---|--------------------------|
| 9 | - | Like extremely |
| 8 | - | Like very much |
| 7 | - | Like moderately |
| 6 | - | Like slightly |
| 5 | - | Neither like nor dislike |
| 4 | - | Dislike slightly |
| 3 | - | Dislike moderately |
| 2 | - | Dislike very much |
| 1 | - | Dislike extremely |

Statistical Analysis

This was done by analyzing the data obtained from the sensory evaluation using the means and analysis of variance (ANOVA) at $P < 0.05$. The separation of means was carried out using LSD (Least Significant Difference) and the significant difference and similarities of the samples were determined based on panelists' scores.

RESULTS AND DISCUSSION

Chemical Analysis

The effect of chemical preservatives on the chemical changes of stored *Agbarati* is presented on Table 1. This Table reflects the changes in %FFA, PV (Meqkg^{-1}), and IV ($\text{g}/100\text{g}$) of *Agbarati* as these are the three major criteria for assessing chemical changes in stored oil. The table shows that the increase in the FFA of the chemically treated *Agbarati* samples differed significantly ($P < 0.05$) from the untreated sample (NP). NP had the highest FFA value while Citric Acid (CA) and Ascorbic Acid (AA) recorded the least FFA values. However, the values obtained for CA and AA, 2.75% and 2.97% respectively, were not significantly different from each other ($P > 0.05$). Therefore, on the basis of the FFA analysis, CA and AA were adjudged to be better than the other preservatives for *Agbarati* preservation. The effectiveness of CA and AA can be attributed to their antioxidant ability in retarding the process of oxidation. Linolenic acids (C18:3) which has been reported in Literature to be in higher amount in melon oil than Oleic is easily oxidized than oleic acid (Udah *et al.*, 1997). Changes which occur in stored oil

or oil foods in most cases lead to deterioration in terms of chemical and physical characteristics of such oils (Pike, 2003). One of the changes in lipid oxidation is that it plays important roles in shelf life of oils since it imparts rancidity and undesirable flavours. Spoilage of oil due to lipid oxidation has been associated with cardiovascular disorder as reported by Pezzuto and Park (2002).

Table 1: Effect of Chemical Preservatives on the Chemical Changes of Stored *Agbarati*

CPR	CHEMICAL CHANGES		
	% FFA	PV(Meqkg ⁻¹)	IV(g/100g)
NP	4.18 ^a ±1.91	6.41 ^a ±3.81	92.61 ^b ±4.78
SB	3.45 ^c ±1.85	5.57 ^a ±3.65	93.09 ^b ±4.35
CA	2.75 ^d ±1.58	4.66 ^b ±2.66	96.76 ^a ±1.38
PS	3.80 ^b ±1.93	6.22 ^a ±3.86	92.88 ^b ±5.15
AA	2.97 ^d ±1.68	4.75 ^b ±2.87	95.89 ^a ±2.31
LSD	0.31	0.80	2.03

Means within the same column not followed by same superscripts are significantly (P<0.05) different.

Key:

CPR: Chemical Preservative

IV = Iodine value

NP = No Preservative

CA = Citric Acid

AA = Ascorbic Acid

PV = Peroxide value

FFA = Free fatty Acid

SB = Sodium Benzoate

PS = Potassium Sorbate

The Peroxide value of CA and AA are not significantly different (P>0.05) from each other with the values of 4.66MeqKg⁻¹ and 4.75MeqKg⁻¹ respectively. The peroxide value of Potassium Sorbate (PS) and Sodium Benzoate (SB) showed a result that is not significantly different (P>0.05) from the untreated sample (NP); this shows that PS with a peroxide value of 6.22MeqKg⁻¹ is the least effective preservative that can be used for *Agbarati* among others used in this work. The increase in the Peroxide Value of the stored samples was found to be in agreement with previous work research findings by Raza *et al.*, (2009).

Furthermore, CA and AA had the highest iodine values of 96.76g/100g and 95.89g/100g of oil respectively which are not significantly different (P>0.05) from each other. The iodine values of SB and PS showed a result that is insignificantly different (P>0.05) from the untreated sample. The result of iodine values showed that the treated *Agbarati* contains appreciably high amounts of unsaturated bonds in its oil than the untreated sample. In view of the fact that drying oils have an iodine value above 100 (Duel, 1951), *Agbarati* oil can thus be characterized as a non-drying oil which can be used in paint industries (Enwere, 1998).

Table 2: Effect of Storage Duration on *Agbarati* treated with Citric acid.

Storage Duration (Days)	CHEMICAL CHANGES		
	% FFA	PV (Meqkg ⁻¹)	IV(g/100g)
0	0.49 ^h ±0.02	0.32 ^f ±0.05	98.56 ^a ±0.046
1	1.65 ^g ±0.62	2.93 ^e ±0.42	98.18 ^a ±0.31
2	2.42 ^f ±0.76	3.06 ^e ±0.44	96.82 ^{ab} ±0.84
3	3.11 ^e ±0.58	5.24 ^d ±0.27	95.60 ^b ±1.47
4	3.90 ^d ±0.49	5.96 ^{cd} ±0.44	94.58 ^b ±2.64
5	4.52 ^c ±0.45	6.77 ^c ±1.50	91.37 ^c ±3.31
6	4.98 ^b ±0.63	7.83 ^b ±1.24	90.27 ^{cd} ±3.37
7	6.34 ^a ±0.75	12.05 ^a ±1.77	88.58 ^d ±3.66
LSD	0.39	1.01	2.57

Means within the same column not followed by same superscripts are significantly (P<0.05) different.

Key:

PV = Peroxide value

IV = Iodine value

FFA = Free fatty Acid

The effect of storage duration on the chemically preserved *Agbarati* is given in Table 2.

Changes in the FFA values were observed to be significantly different (P<0.05) from each other on daily basis for the seven days of storage. Increase in the Fatty acid formation ranged from 0.49% at the beginning of storage to 6.34% at the end of the storage duration. Increase in the free fatty acids of oils was reported to be mainly due to lipolysis. Achi (2005) reported the lipolysis of legume which yielded different fatty acids such as Oleic, Linoleic and Linolenic acids.

The Peroxide value ranged from 0.32MeqKg⁻¹ at the beginning of storage to 12.05MeqKg⁻¹ after seven days storage duration. The results showed that from day 0 to day 1, the values were not significantly different (P>0.05) from each other thus the peroxides were formed at a slow rate up to day 5 and its formation was highest at the end of storage duration. Notwithstanding the increase in Peroxide Value, the result indicated that rancidity had still not set in, since the peroxide value after the storage duration had not yet reached 20-40MeqKg⁻¹ which is the range at which rancid taste was reported to occur in oils (Onwuka, 2005).

Iodine values ranged from 98.56g/100g of oil at the onset of storage to 88.58g/100g of oil after the seven days storage duration. It was observed that the iodine values from the day 0 to 3 were insignificantly different ($P>0.05$) from each other. Also, from day 4 to day 7, the results of iodine value indicated that there was no significant difference ($P>0.05$) for its values. This decrease in iodine value is indicative of increase in saturated fatty acids possibly due to auto oxidative deterioration which had set in slowly from day 4 to 7 of storage. According to Amoo *et al.*, (2004), Iodine value is an index for assessing the ability of oil to go rancid.

Sensory Analysis

The extent to which a particular food appeals to individual depends to a large extent on one's perception of the food based on the human senses of sight, taste, feel (touch) and so on. The acceptability of foods on the basis of such senses reveals the attributes of the food outside its nutritional consideration. This constitutes the sensory attribute of the test food.

The effect of chemical preservatives on the sensory attribute of *Agbarati* is presented in Table 3.

After the storage duration, SB showed the best result on colour (6.81) followed by CA and AA (6.73 and 6.73 respectively) which were not significantly different ($P>0.05$) from each other.

Results for taste and texture were not statistically different ($P>0.05$) from each other. This denotes that there was no change observed for the taste and texture attributes of the *Agbarati* samples for both treated and untreated samples.

Table 3: Effect of Chemical Preservative on the Sensory Attribute of *Agbarati*

Chemical	Sensory attribute				
Preservative Acceptability (CPR)	Colour	Taste	Texture	Aroma	Overall
NP	6.30 ^b ±1.16	5.78 ^a ±1.37	6.16 ^a ±1.25	6.32 ^c ±1.178	6.16 ^c ±1.12
SB	6.81 ^a ±1.01	6.19 ^a ±0.86	6.38 ^a ±0.87	6.65 ^b ±0.994	6.54 ^{ab} ±0.91
CA	6.73 ^a ±0.93	6.54 ^a ±0.94	6.55 ^a ±0.90	6.63 ^b ±0.824	6.65 ^a ±0.81
PS	6.41 ^b ±0.77	6.11 ^a ±1.35	6.24 ^a ±1.00	6.67 ^b ±0.99	6.40 ^b ±1.01
AA	6.73 ^a ±0.92	6.38 ^a ±0.88	6.42 ^a ±0.99	7.05 ^a ±1.19	6.67 ^a ±1.02
LSD	0.20	0.61	0.39	0.27	0.23

Means within the same column not followed by same superscripts are significantly ($P<0.05$) different.

Key:

CPR: Chemical Preservative

PA = Potassium Sorbate

NP = No Preservative

AA = Ascorbic Acid

SB = Sodium Benzoate

CA = Citric acid

The overall acceptability however showed that AA, CA and SB were the most accepted preservatives as regards to the panelists' views and this was reflected in their values (6.67, 6.65 and 6.54 respectively). The values showed that the product was liked slightly on an overall acceptability view.

Table 4: Effect of Storage Duration on the Sensory Attribute of Agbarati treated with Citric acid.

Storage		Sensory attribute			
Duration (Days)	Colour	Taste	Texture	Aroma	Overall Acceptability
0	7.70 ^a ±0.21	7.29 ^a ±0.24	7.55 ^a ±0.16	7.58 ^a ±0.59	7.55 ^a ±0.13
1	7.48 ^a ±0.25	7.20 ^a ±0.25	7.18 ^a ±0.14	7.58 ^a ±0.21	7.35 ^b ±0.12
2	7.29 ^b ±0.22	6.83 ^{ab} ±0.22	7.04 ^a ±0.12	7.41 ^{ab} ±0.16	7.27 ^c ±0.17
3	7.01 ^c ±0.22	6.68 ^b ±0.28	6.89 ^b ±0.14	7.23 ^{bc} ±0.30	6.95 ^d ±0.19
4	6.70 ^d ±0.34	6.25 ^{bc} ±0.37	6.52 ^b ±0.22	7.03 ^c ±0.54	6.62 ^e ±0.30
5	6.30 ^e ±0.29	5.91 ^c ±0.59	6.06 ^c ±0.20	6.37 ^d ±0.36	6.28 ^f ±0.34
6	5.50 ^f ±0.32	5.07 ^d ±0.57	5.14 ^c ±0.32	5.51 ^f ±0.29	5.39 ^g ±.32
7	4.79 ^g ±0.22	4.34 ^e ±0.45	4.44 ^d ±0.32	4.53 ^g ±0.19	4.52 ^h ±0.34
LSD	0.26	0.47	0.26	0.34	0.03

Means within the same column not followed by same superscripts are significantly (P<0.05) different.

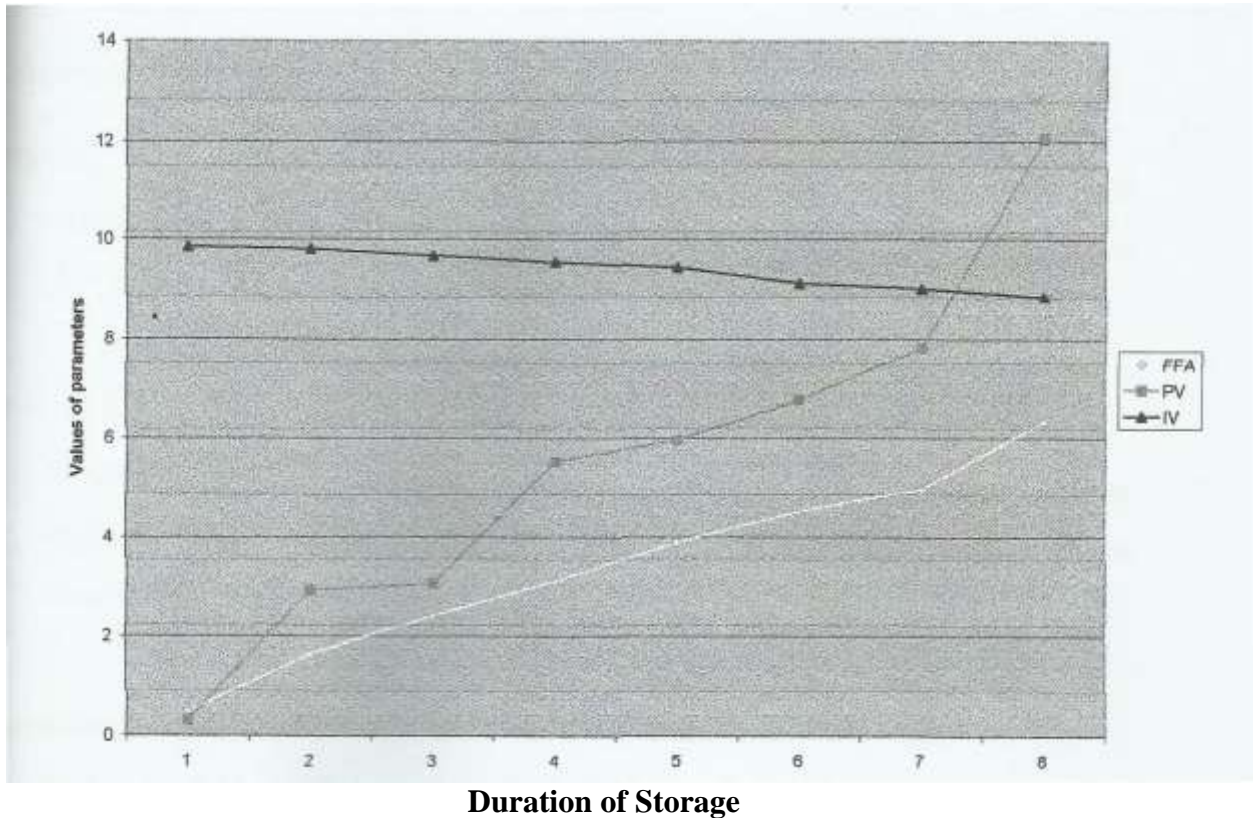


Figure 2: Changes in %FFA, Peroxide value (Meq/kg) and Iodine value ($\times 10^{-1}$ g/100g of oil) of *Agbarati* treated with chemical preservatives.

Table 4 showed the effect of storage duration on the sensory attribute of *Agbarati*. Changes in colour attribute ranged from 7.70 (moderately liked) at the beginning of storage to 4.79 (slightly disliked) at the end of storage duration. The values show significant difference ($P < 0.05$) in colour attribute from day I to 7 of storage. These changes could be attributed to oxidation which results in lighter or darker colours in food depending on the substrate; fats and oils tend to darken (Siegal and Fawcett, 1976).

The taste attribute showed no significant difference ($P > 0.05$) from day 0 to 5 but the sixth and seventh storage days showed significant differences ($P < 0.05$) in taste scores.

The texture varied according to storage days. Day 0 to 2 showed no significant differences ($P > 0.05$). Similar but distinct results were also recorded for all the days 3 to 4 and 5 to 6 respectively. However the result obtained for the seventh day varied significantly ($P < 0.05$) from the previous storage days.

However, the overall acceptability attribute showed that there was significant difference ($P < 0.05$) for all the storage days. Therefore on the basis of storage duration, there was constant variation in the sensory attributes of *Agbarati* samples on storage.

Generally therefore, from the findings recorded in the results as discussed above, Citric Acid was adjudged the best preservative followed by Ascorbic Acid and Sodium Benzoate as it affects the chemical characteristics and sensory attributes of *Agbarati* on seven days storage.

In contrast, Potassium Sorbate was the least effective of the four preservatives as its result was always in accordance to that of untreated *Agbarati*. However, a study of the mechanism of preservation or mode of action was considered to be beyond the scope of this study.

CONCLUSION

In conclusion, this experiment showed that the preservatives were able to offer some retardation in the spoilage process of the *Agbarati* samples with time. This was recorded in reduced rancidity in the form of Iodine Value, Peroxide value and Free Fatty Acid. Also the sensory evaluation showed varying range of preference for the different samples. The colour, taste, texture and odour, all reduced during storage which indicated reduced acceptability with storage time for all samples.

However, citric acid treated samples preserved much better than all other samples. This was manifested in the level of rancidity recorded and relative preference in scores for sensory attributes.

RECOMMENDATION

The use of chemical preservative in controlled concentrations was considered adequate to prolong shelf life of the *Agbarati* sample relative to the unprotected or untreated control samples. Altogether, citric acid proved to be the best preservative hence its use as a choice chemical preservative was recommended. This is so since it has no adverse effect on the quality and sensory attributes of the stored *Agbarati* samples. Finally, at ambient temperature, it is recommended that stored *Agbarati* was still relatively nutritious and palatable within a seven-day period.

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