Ethiopian Journal of Environmental Studies & Management 16(3): 386 – 395, 2023
ISSN:1998-0507 doi: https://ejesm.org/doi/v16i3.10
Submitted: June 19, 2023 Accepted: August 4, 2023

NATURAL ANTIMICROBIAL PROPERTIES OF AFRICAN WALNUT *(Tetracarpidium conophorum)* OIL AS COSMETIC PRESERVATIVE

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Abstract
This study investigated the use of African walnut oil to improve the physical, chemical, sensory and microbiological quality of cream during storage in the refrigerator at 4°C. Cream was prepared and divided into four equal parts; the first part without oil served as fresh sample (control), the second to fourth part had 0.5 %, 1.0 % and 1.5 % concentrations of African walnut oil added separately and stored for 15, 30 and 45 days in the refrigerator at 4°C. Physical and chemical properties of cream indicated that additions of African walnut oil in different concentrations resulted to increase in peroxide and refractive values and decrease in acidity, saponification and iodine values of cream during storage 15, 30 and 45 days. Sensory score results showed that cream with 0.5 % African walnut oil was most acceptable by the panelists. Microbial examination of cream samples when fresh and during storage with and without the addition of 0.5 %, 1.0 % and 1.5 % African walnut oil led to the non-detection of proteolytic and lipolytic bacteria during 15 and 30 days storage except 45 days. At 45 days storage period, the proteolytic bacterial count of fresh control cream was 10 x 10^1 and cream samples had 6 x 10^1, 9 x 10^1 and 8 x 10^1 while lipolytic bacterial count for fresh control cream was 9 x 10^3 and cream samples had 4 x 10^1, 7 x 10^1 and 3 x 10^1. Coliforms, yeasts and molds were not detected in all the cream samples.

Key Words: *African walnut oil, Cream, Physicochemical properties, Sensory properties, Microbiological examination*

Introduction

One of the underutilized plant species in Nigeria is the African walnut (*Tetracarpidium conophorum*- Mull. Arg). African walnut belongs to the family *Euphorbiaceae* (Edem *et al*., 2009). African walnut is a perennial creeping shrub that grows mainly in the Western region of Africa (Ayoola *et al*., 2011). The immature fruits are usually green in colour but turn dark brown to black as they reach maturity (Ayodeji and Aliyu, 2018). African walnuts are dry nuts which are encased in green pods. As walnut matures,

the outer covering dries and falls off leaving the segment tough black shell and the white seed (Ekwe and Ihemeje, 2013).

In Nigeria, ripe walnuts are mostly eaten whole often boiled, as dessert nuts or used in cakes, desserts and confectionery of all kinds and a large proportion is thrown away as a waste, whereas it is a rich source of nutrients and oils and can be used as a food (Ogunwusi and Ibrahim, 2016). African walnut has low oil content but is rich in essential fatty acids that supports growth and development in humans (Nkwonta, 2015) and reduces heart diseases as well as Type 2 diabetes in people (De Lorgeril and Salen, 2004).

Antioxidants are used as food preservatives that preserve food and militate against oxidative deterioration on storage and processing. The use of antioxidants in food preservation dates back to mid-20th century when scientists attempted to use it to elongate the shelf-life of foods. Therefore, as of today, antioxidants play significant role in slowing down lipid oxidation in foods, due to the presence of off-flavors and oxidized chemical compounds which are undesirable (López-Pedrouso *et al*., 2022). High amount of antioxidants are present in African walnut oil and other nuts (Bolling *et al*., 2010). The oil and extracts from pellicle and kernel of African walnut possess high antioxidant activity which has been attributed to the presence of high level of phenolic compounds such as ellargic acids (Jin *et al*., 2022). Other antioxidants present in African walnut are flavonoids and alkaloids (Jin *et al*., 2022).

Various extracts obtained from plants have antioxidant and antimicrobial properties (Guerrini *et al*., 2020). In recent years, antimicrobial compounds and their effects against bacterial pathogens has been established. Antimicrobial compounds are of two main types: natural and synthetic. Natural antimicrobials are better than synthetic antimicrobial compounds because they are effective against a variety of diseases (Quinto *et al*., 2019). These are derived from plants, animals, bacteria, virus and algae (Quinto *et al*., 2019). The use of these natural antimicrobials as food preservatives are considered as good alternatives to other systems of preservation that could ensure the quality, and safety of food. Examples of such natural antimicrobials include plant extracts, essential oils, bacteriocins and organic acids (Teshome *et al*., 2022). The preservation of foods with chemical preservatives is often limited in use because of the biological accumulation of the chemical in humans resulting in deleterious toxicological effects. Hence there is the need to search for natural preservatives of plant origin (Guerrini *et al*., 2020). The aim of this study is to extend the antimicrobial knowledge of African walnut oil to focus on its possible use as a food preservative in improving the quality of cream and extending the shelf-life. This is the first paper to discuss the possible use of African walnut oil as food preservative.

Materials and Methods

Plant Material

Fresh African walnut (*Tetracarpidium conophorum*) was collected from walnut trees growing in Ogun State in September through October 2022 and a final sample of about 3 kg was randomly taken. The nuts were transported to the Microbiology laboratory of Olabisi Onabanjo University, Ago-Iwoye, Ogun State in a

paper bag where they were authenticated by a taxonomist.

Processing of Plant Material

African walnuts were washed with sterile distilled water to remove contaminants and other extraneous matter. After washing, the black husks were removed from the nuts and rinsed again. The cleaned nuts were cut into small pieces and sundried in shade for two weeks. Thereafter, the nuts were milled using Marlex Excella mixer/grinding machine (Amazon, UK), sieved and packed in air tight containers and kept in the refrigerator at 4°C for further processing.

African Walnut Oil Extraction

Oil was extracted from African walnuts with n-hexane using solvent extraction method described by Yangomodou *et al*., (2020). Prior to the extraction process, the milled nuts were kept in an oven at 105 ºC for 1 h to remove moisture. Then, sixty grams of the dried sample was wrapped in a white muslin cloth and put into a porous thimble of the Soxhlet extractor. Then, 200 mL of n-hexane of HPLC grade was added and heated with heating mantle for 8 h until a clear solvent was obtained. At the end of the extraction, the resulting mixture of the extracts was filtered with a 10mm Syringe-driven with a filter 0.45um to remove impurities. The solvents were further removed completely with a rotary-evaporator (Model N-1, Eyela, Tokyo Rikakikal Co., Ltd., Japan) to obtain a clear oil extract which was stored in white bottles and tubes under nitrogen at 4 °C until further use. After extraction, the amount of oil obtained was measured using an analytical balance and result obtained was used to calculate the percentage yield of the oil sample.

Production of Raw Cream

Five liters (5L) of raw farm fresh milk (4%) was obtained from healthy cows in Ayetoro, Yewa North, Local Government Area of Ogun State and poured into sterile 10 glass jar. The milk was left to rest for 24 hours to give the cream time to rise to the top of the milk and a ladle was dipped into the cream to scoop out the cream.

Standardization of Cream

Fat content of cream obtained after the separation of skim milk was estimated using Gerber method described by Kleyn *et al*., (2001). The standardization of cream fat to 40 % and 7.6 % solids not fat (SNF) was carried out using the calculation of Pearson's square method described by Chopde *et al*. (2005). Cream was then pasteurized at 74ºC for 10 min and divided into four (4) equal parts, the first part served as control with no oil added, while the second, third and fourth parts had 0.5%, 1.0 %and 1.5 % of African walnut oil added. The treated cream was stored in refrigerator at 4ºC and analyzed when fresh at 0 day, and after 15, 30 and 45 days.

Physical and Chemical Properties

Physical and chemical parameters determined were specific gravity, acidity value, peroxide value, refractive index, saponification value and iodine value. The procedures of Egan *et al*. (1981) and AOAC, (2000) were adopted for the estimation of all these properties in the cream samples.

Microbiological Examination

The method described by Elkassas *et al*. (2023) with minor modifications was used to examine the total bacterial count (TBC), coliform count, yeast and mold counts as well as proteolytic and lipolytic bacteria in all the cream samples.

Total Bacterial Count

One gram of melted cream sample was transferred into a sterile test tube containing 9 mL of (0.1%) sterile peptone water (Oxoid, UK) which was then thoroughly mixed to prepare a $1:10^7$ dilution. Using the pour plate method, 1 mL of the aliquots was placed into petri-plates and 15-20 mL of sterile standard plate count agar (Oxoid, UK) was poured into each plate and mixed thoroughly. The plates were thereafter incubated at 30°C for 48 h after which colonies were counted using a colony counter.

Coliform Count

Presumptive test for determination of total coliforms was carried out using lauryl sulfate tryptone broth (LST) (Oxoid, UK). LST broth (9 mL) was put into test tubes with inverted Durham tubes for detection of gas. To this broth, 1 mL of previously prepared dilutions of samples $1:10^7$ was added and each plate was then incubated at 37ºC for 24 - 48 h. All LST tubes showing turbidity and gas formation within 48 h were recorded.

Yeast and Mold Counts

Total counts of yeast and mold was determined from previously prepared dilution of $1:10^7$. 1 mL of serially diluted samples was added to sterile plates and 15 mL of Sabouraud Dextrose Agar (SDA) was poured into each plate and they were all incubated for 5 days at 25ºC.

Sensory Evaluation

Panelists in the Food Science and Nutrition option from the Department of Home Science and Hospitality Management of Olabisi Onabanjo University conducted the sensory evaluation of cream samples. The panelists were requested to evaluate the fresh cream and refrigerated cream samples for a period of 15, 30 and 45 days interval of storage at 4ºC in a refrigerator. The panelists assessed the cream samples for flavour, texture, taste, appearance and general acceptability.

Data Analysis

All test analysis is expressed as the mean ± standard deviation (SD), and a one-way variance analysis was used to test for differences in the parameters examined. Duncan multiple range test was used to separate the means of each data.

Results and Discussion

Table 1 shows the percentage yield of oil from African walnut. African walnut powder contained 13.98% crude oil from its dry weight grounded sample of 60.08g.

Table 1: Oil content of African walnut

Properties	**Values**
Weight of grounded seeds (g)	60.08
Weight of oil extract obtained (g)	8.5
Percentage of African walnut oil obtained from sample (%)	13.98

Table 2 shows the effect of additions of African walnut oil on physical and chemical properties of cream. Knowing the effect was necessary to determine the effect of African walnut oil on keeping quality of raw milk. The changes in the physical and chemical properties of fresh control cream and cream samples containing 0.5%, 1.0% and 1.5% additions of African oil were taken as oxidation stability indices during a 45 days storage period in refrigerator at 4 °C. Result in table 2 showed changes in the FFA values of cream samples. It was observed that FFA values in all cream samples containing African walnut oil at different concentration decreased, compared with the fresh control samples during storage for 15 and 30 days. But at 45 days, a sharp increase in FFA value was observed in all cream samples which may be due to the appearance of mould on the surfaces of all samples. These results agree with those of El-Shazly *et al*. (2017) who reported decrease in FFA values of cream samples during 15 and 30 days and an increase in 45 days period of storage with addition of varying concentrations of pomegranate seed oil to cream.

The result in table 2, shows that the addition of different concentrations of African walnut oil resulted to an increase in peroxide value of cream samples mixed with African walnut oil, compared with PV of fresh control cream, but it was highly observed in cream containing 1.5% higher level of addition of African walnut oil. In addition, PV increased in all the cream samples during storage periods. The rate of increase in the PV was decreased with increasing addition of African walnut oil from 0.5% to 1.5% during the period of storage. The PV of cream samples was in the range of 1.50 to 7.09 meq O_2/kg. However, at 45 days period of storage, there was a sharp increase in PV of the cream samples. This value compares well with PV of 9 meq O2/kg oil recommended for fresh oils. This indicates that the African walnut oil used for the analysis was of good quality and has not undergone oxidative rancidity. Results agrees with the findings of El-Shazly *et al*. (2017) who reported the amount of PV for cream in the range of 1.40 to 7.29 meq O_2/ kg oil) with additions of pomegranate seed oil. Also, the refractive index values presented in table 2 indicated that the addition of varying concentrations of African walnut oil resulted to an increase in RI of cream samples mixed with African walnut oil, compared with PV of fresh control cream, and also PV increased in all the cream samples during storage periods. The RI of cream samples was in the range of 1.4482±0.13 to 1.4610 ± 0.22. The result showing that African walnut oil has lower refractive index than other plant oils are consistent with those of other studies (El-Shazly *et al*., 2017; Elama *et al*., 2019) and suggest that the oil is of good quality.

The saponification value was in the range of 198.88±0.03 to 210.06±0.10 mg KOH/g. Result in table 2 indicated that at the beginning of storage and during the storage period at 4°C in the refrigerator for 15 and 30 days, the SV of cream samples containing different concentrations of African walnut oil were lower than in fresh control sample.

The iodine value of cream samples was almost similar at zero time. But it increased in fresh control cream compared with the cream samples containing varying concentrations of African walnut oil during refrigeration storage at 4 °C for 15, 30 and 45 days period respectively.

Table 2: Physical and chemical properties of cream with and without African walnut oil

Properties	Storage period (days)	Control	Oil concentration (%)		
			0.5	1.0	1.5
	Fresh	0.120±0.01[a]	0.198±0.05[c]	0.196±0.04[b]	0.194±0.03[b]
Acidity (%)	15	0.375±0.07[d]	0.343±0.06[c]	0.310±0.06[b]	0.229±0.04[a]
(FFA)	30	0.656±0.04[d]	0.489±0.03[c]	0.316±0.02[b]	0.302±0.04[a]
	45	4.25±0.10[a]	5.09±0.12[b]	6.05±0.12[c]	7.21±1.13[d]
Peroxide value (meq	Fresh	1.50±0.07[a]	1.55±0.06[a]	1.76±0.07[b]	2.74±0.12[c]
O_2/ kg oil)	15	2.56±0.08[a]	3.37±0.09[b]	4.75±0.11[c]	4.99±0.12[d]
	30	3.46±0.09[a]	4.44±0.13[b]	6.23±0.16[c]	7.09±1.03[d]
	45	1.26±0.02[a]	1.58±0.03[b]	1.63±0.03[c]	1.78±0.07[d]
Refractive index (at 25	Fresh	1.4482±0.13[a]	1.4488±0.13[a]	1.4488±0.13[a]	1.4582±0.21[b]
°C)	15	1.4487±0.15[b]	1.4489±0.15[b]	1.4492±0.14[b]	1.4617±0.22[c]
	30	1.4488±0.10[a]	1.4590±0.14[b]	1.4593±0.14[b]	1.4599±0.21[b]
	45	1.4489±0.13[a]	1.4591±0.20[b]	1.4593±0.21[b]	1.4610±0.22[c]
Saponification number	Fresh	198.88±0.03[c]	197.28±0.03[b]	196.88±0.02[a]	196.67±0.02[a]
(mg KOH/g)	15	198.67±0.05[c]	197.15±0.04[b]	199.05±0.04[d]	195.45±0.02[a]
	30	210.06±0.10[c]	197.13±0.03[a]	199.76±0.03[b]	196.93±0.03[a]
	45	198.02±0.03[b]	197.08±0.06[a]	200.33±0.06[c]	198.63±0.03[b]
Iodine (1_2/100gof oil)	Fresh	28.18±0.13[a]	28.15±0.13[a]	28.48±0.14[a]	28.03±0.12[a]
	15	28.43±0.12[c]	28.09±0.03[c]	27.66±0.03[b]	27.54±0.03[a]
	30	29.44±0.16[b]	28.76±0.18[a]	29.04±0.16[b]	29.18±0.16[b]
	45	25.44±0.12[a]	25.53±0.12[a]	26.66±0.12[c]	26.72±0.12[b]

Each data is mean ± Standard Deviation (SD) of three replicate readings.
Values with the same superscripts along the same row are significantly different.

Table 3 shows the changes in the microbial analysis of cream as affected by additions of different concentrations of African walnut oil during storage. From the table, all cream samples with additions of different concentrations of African walnut oil had lower total bacterial counts compared to control cream when fresh and stored at 4 °C for 15, 30 and 45 days period respectively. However, it was observed that the total bacterial counts increased gradually to the end of 45 days. In addition, the proteolytic and lipolytic bacterial counts as well as yeast and mold counts increased gradually for fresh control cream during storage period for 15, 30 and 45 days. But additions of different concentrations of African walnut oil to cream led to the absence of bacteria, yeast and mold from all cream samples during storage days. Coliform was not detected in the control cream sample when fresh and throughout the storage periods due to strict hygiene. Therefore, results obtained in this study agree with those of other researchers like Mutlag and Hassan, (2008) who reported that bacterial populations in yogurt decreased with increase in essential oil concentrations. El-Shazly *et al*. (2017) who observed that the additions of different concentrations of pomegranate seed oil into cream reduced the counts of bacteria, yeast and moulds. Elama *et al*. (2018) and his colleagues who reported that total bacterial counts, yeast and mould counts decreased with

increase in concentration of different essential oils. Results indicated that African walnut oil improved the quality and shelf life of all cream samples.

Table 3: Microbial analysis of cream as affected by additions of different concentrations of African walnut oil during storage at refrigerator (4°C)

Properties	**Storage period (days)**	**Control**	**Oil concentration (%)**		
			0.5	**1.0**	**1.5**
Total count (CFU/gm)	Fresh	20×10^1	15×10^1	10×10^1	5×10^1
	15	9×10^2	7×10^1	8×10^1	7×10^1
	30	12×10^2	10×10^1	9×10^1	8×10^1
	45	20×10^6	13×10^6	14×10^6	10×10^{15}
Proteolytic bacteria	Fresh	ND	ND	ND	ND
	15	ND	ND	ND	ND
	30	ND	ND	ND	ND
	45	10×10^1	6×10^1	9×10^1	8×10^1
Lipolytic bacteria	Fresh	5×10^1	ND	ND	ND
	15	7×10^2	ND	ND	ND
	30	8×10^2	ND	ND	ND
	45	9×10^3	4×10^3	7×10^2	3×10^2
Yeast and molds	Fresh	2×10^1	ND	ND	ND
	15	5×10^2	ND	ND	ND
	30	7×10^2	ND	ND	ND
	45	9×10^3	ND	ND	4×10^2
Coliform	Fresh	ND	ND	ND	ND
	15	ND	ND	ND	ND
	30	ND	ND	ND	ND
	45	ND	ND	ND	ND

Each data is mean ± Standard Deviation (SD) of three replicate readings.
ND=Not detected

Table 4, shows the results for the scores of sensory evaluation for cream containing African walnut oil at different concentrations of 0.5, 1.0 and 1.5 % during refrigeration storage at 4°C for 0, 15, 30 and 45 days. The result showed that the control cream had the highest score when fresh and during storage periods. Furthermore, result in table 4 indicated that cream samples with 0.5% African walnut oil was the most acceptable and was not different from the fresh control cream. However, scores obtained for all the cream samples decreased with increased additions of African walnut oil and as the storage intervals progressed from 0, 15, 30 and 45 days. These results are in agreement with those of Nadeem et al., (2010) who reported that the incorporation of rape seed oil into ice cream improved its organoleptic properties and no adverse effect was observed. To El-Shazly *et al*. (2017) who mentioned that refrigerated cream sample containing 0.5 % addition of pomegranate seed oil was not significantly different from the control cream when fresh and stored for different days.

Table 4: Effect of additions of different concentrations of African walnut oil on sensory properties of cream during storage at refrigerator (4°C)

Sensory properties	Storage period (days)	Control	Oil concentration (%)		
			0.5	1.0	1.5
Flavor (Cooked)	Fresh	6.56[b]	6.53[b]	5.32[a]	5.31[a]
	15	6.34[b]	6.32[b]	5.31[a]	5.28[a]
	30	6.30[b]	6.31[b]	5.30[a]	5.27[a]
	45	6.30[b]	6.30[b]	5.31[a]	5.26[a]
Texture by hand (Spreadability)	Fresh	6.15[c]	6.15[c]	5.15 [b]	4.14 [a]
	15	6.15[c]	6.14[c]	5.14 [b]	4.13 [a]
	30	6.15[c]	6.14[c]	5.13 [b]	5.12 [b]
	45	6.14[c]	6.13[c]	5.13 [b]	5.11 [b]
Taste (Sourness)	Fresh	5.30 [c]	5.30 [c]	4.28 [b]	4.27 [b]
	15	5.30 [c]	5.29 [c]	4.28 [b]	3.27 [a]
	30	5.29 [c]	5.28 [c]	4.27 [b]	4.26 [b]
	45	5.28 [c]	5.27 [c]	4.27 [b]	3.25 [a]
Appearance (Creamy white)	Fresh	4.10 [c]	4.10 [c]	3.49 [b]	3.29 [b]
	15	4.10 [c]	4.10 [c]	4.08 [c]	3.38 [b]
	30	4.09 [c]	4.05 [c]	4.09 [c]	2.37 [a]
	45	4.08 [c]	4.08 [c]	3.37 [b]	2.67 [a]
Acidity	Fresh	3.10 [c]	3.09 [c]	2.91 [b]	1.84 [a]
	15	3.02 [c]	3.04 [c]	2.37 [b]	1.67 [a]
	30	3.02 [c]	3.02 [c]	2.17 [b]	1.46 [a]
	45	3.05 [c]	3.06 [c]	2.56 [b]	1.35 [a]
General acceptability	Fresh	7.88[b]	7.53[b]	6.42[a]	6.31[a]
	15	7.34[b]	7.32[b]	6.35[a]	6.28[a]
	30	7.30[b]	7.11[b]	6.20[a]	6.27[a]
	45	6.21[b]	6.10[b]	5.31[a]	5.26[a]

Each data is mean ± Standard Deviation (SD) of three replicate readings.
Values with the same superscripts along the same row are significantly different.

Conclusion

In this study for the first time we showed that African walnut oil could be used as a natural compound to improve the quality of cream and extend its shelf life which is possible because of its antimicrobial and antioxidant properties. The addition of different concentrations of African walnut oil to cream inhibited the growth of coliforms, proteolytic bacteria and lipolytic bacteria as well yeasts and moulds which are main spoilage organisms of cream thereby reducing oxidative degradation of the cream samples. Furthermore, results obtained for sensory analysis, indicated that the use of African walnut oil in low concentration in cream is non-toxic and does not affect the quality of cream, so it's safe for consumption and can be used to preserve home-made creams. Therefore, with its good antimicrobial potentials it can be an alternative to chemical preservatives.

References

AOAC. (2000). Official Methods of Analysis. (7th Ed.). Association of

Official Analytical Chemists, Washington.

Ayodeji, A.E. and Aliyu, N. (2018). *Tetracarpidium conophorum* (African walnut) Hutch. & Dalziel: Ethnomedicinal uses and its therapeutic activities. *Journal of Medicinal Plants for Economic Development*, 2(1): 1-10.

Ayoola, P.B., Adeyeye, A., Onawumi, O.O. and Faboya, O. O. P. (2011). Phytochemical and Nutrient Evaluation of *Tetracarpidium conophorum* (Nigerian Walnut) Root, *IJRRAS*., 7(2): 197 – 202.

Bolling, B.W., McKay, D.L. and Blumberg, J.B. (2010). The phytochemical composition and antioxidant actions of tree nuts. *Asia Pacific Journal of Clinical Nutrition*, 19(1): 117.

Chopde, R.L., Chilgunde, S.N., Zanjad, P.N., Ambadkar, R.K. and Baig, M.I. (2005). Comparative studies on quality of Chhana prepared from buffalo milk and cow milk. *The Journal of Bombay Veterinary College*, 13(1and2): 87-90.

De Lorgeril, M., and Salen, P. (2004). Alpha-linolenic acid and coronary heart disease. Nutrition, *Metabolism and Cardiovascular Diseases*, 14(3): 162-169.

Edem, C.A., Dosunmu, M.I., and Bassey, F.I. (2009). Determination of proximate composition, ascorbic acid and heavy metal content of African walnut (*Tetracarpidium conophorum*). *Pakistan Journal of Nutrition*, 8(3): 225-226.

Egan, R.S. and Suryer R.C. (1981). Pearsons Chemical Analysis of Foods. 8th Edn., Churchill, Edinburgh.

Ekwe, C.C. and Ihemeje, A. (2013). Evaluation of physiochemical properties and preservation of African walnut (*Tetracarpidium conophorum*). *Academic Research International*, 4(6): 501.

Elama, C., Alayoubi, M., Jazzar, M. and Al-Rimawi, F. (2019). Effect of different essential oils on the shelf life of concentrated yogurt. *Annual Research and Review in Biology*, 30(6): 1-9, 2018; Article no. ARRB.47

Elkassas, W.M., Yassin, S.A. and Taksira, D.M. (2023). Effect of Adding Propolis on Quality Standards of Raw Milk and Yoghurt. *Current Research in Nutrition and Food Science Journal*, 11(1): 231-245.

El-Shazly, H.A., Mohamed, E.S. and Abdelatif, S.H. (2017). Effect of Pomegranate Seed Oil on Certain Food Spoilage Microorganisms and its Role in Shelf Life of Cream. Journal of Food and Dairy Sciences, 8(4): 173-178.

Guerrini, A., Burlini, I., Lorenzo, B. H., Grandini, A., Vertuani, S., Tacchini, M., and Sacchetti, G. (2020). Antioxidant and antimicrobial extracts obtained from agricultural by-products: Strategies for a sustainable recovery and future perspectives. *Food and Bioproducts Processing*, 124: 397-407.

Jin, F., Wang, Y., Huang, R., Li, B., Zhou, Y., and Pei, D. (2022). Phenolic extracts from colored-walnut pellicles: antioxidant efficiency in walnut oil preservation. *International Journal of Food Properties*, 25(1): 1458-1471.

Kleyn, D.H., Lynch, J.M., Barbano, D.M., Bloom, M.J., Mitchell, M.W. and

Collaborators: Cooper LS Cusak E Fick M Hanks T Hesen MK Johnson J Kleyn DH Mercer F Monahan D Peat B Petit M. (2001). Determination of fat in raw and processed milks by the Gerber method: collaborative study. *Journal of AOAC international*, 84(5): 1499-1508.

López-Pedrouso, M., Lorenzo, J.M. and Franco, D. (2022). Advances in natural antioxidants for food improvement. *Antioxidants*, 11(9): 1825.

Mutlag, A.O. and Hassan, E.D. (2008). Improvement of the quality and shelf life of concentrated yoghurt (labneh) by the addition of some essential oils. *African Journal of Microbiology Research*, 2(7): 156-161.

Nadeem, M., Abdullah, M. and Ellahi, M. Y. (2010). Effect of incorporating rape seed oil on quality of ice cream. *Mediterranean Journal of Nutrition and Metabolism*, 3(2): 121-126.

Nkwonta, C.G., Medina, A., del Carmen, A.M. and Terry, L.A. (2015). Impact of postharvest processing on the fungal population contaminating African walnut shells (*Tetracarpidium conophorum Mull.Arg*) at different maturity stages and potential mycotoxigenic implications. *International Journal of Food Microbiology,* 194: 15-20.

Ogunwusi, A.A. and Ibrahim, H.D. (2016). Industrial potential of underutilized plants in Nigeria. *Journal Natural Science Research*, 6, 20-28.

Quinto, E.J., Caro, I., Villalobos-Delgado, L.H., Mateo, J., De-Mateo-Silleras, B. and Redondo-Del-Río, M.P. (2019). Food safety through natural antimicrobials. *Antibiotics,* 8(4): 208.

Teshome, E., Forsido, S.F., Rupasinghe, H.P. and OlikaKeyata, E. (2022). Potentials of Natural Preservatives to Enhance Food Safety and Shelf Life: A Review. *The Scientific World Journal,* 2022.

Yangomodou, O.D., Akinhanmi, Y.U., Solana, O.I., Olugbemi, M.T., Ferry, N. and Hadfield, J.A. (2020). Physical properties, chemical composition and antimicrobial activity of oil and extracts from *Tetracarpidium conophorum* (African walnut). *KIU Journal of Social Sciences*, 6(2): 293-305.