Scientific Research Publishing

Journal of Agricultural Chemistry and Environment, 2024, 13, 142-168
https://www.scirp.org/journal/jacen
ISSN Online: 2325-744X
ISSN Print: 2325-7458

Extraction, Production and Quality Evaluation of Margarine from Oil Extracted from Waste Biomass Peels of Avocado and Virgin Coconut Oil, Using Chitosan from Reared Shells as Preservative

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How to cite this paper: Miyanui, S.-N.S., Ngwasiri, P.N., Muala, W.C.B., Ngangmou, N.T., Ambindei, W.A. and Ngassoum, M.B. (2024) Extraction, Production and Quality Evaluation of Margarine from Oil Extracted from Waste Biomass Peels of Avocado and Virgin Coconut Oil, Using Chitosan from Reared Shells as Preservative. *Journal of Agricultural Chemistry and Environment*, **13**, 142-168.
https://doi.org/10.4236/jacen.2024.131010

Received: November 17, 2023
Accepted: February 20, 2024
Published: February 23, 2024

Abstract

The production and consumption of avocado pears generates tons of wastes, mainly the pear peels which are usually discarded, although they have been reported to contain important phyto-chemicals with biological activities. The adverse health effect associated with the consumption of saturated lipid based foods has ignited research on reformulation of lipid based foods to eliminate Trans Fatty Acids (TFAs). This study was thus aimed at the extraction and characterization of oil from Avocado Peels (APO) and evaluation of the quality of margarine produced from it. Five verities of pear were used for oil extraction by soxhlet method and physiochemical, oxidative, functional and antioxidant characterization was done. Margarines were formulated using a central composite design using oil blends of APO and Virgin Coconut Oil (VCO) with an oil ratio of 10:90, 40:60, 70:30 respectively, varied blending speed, blending time, and chitosan concentration. Samples were characterized and the effect of process parameters on the physiochemical and functional properties of the margarine studied. Optimized conditions were used to produce samples for sensory evaluation. Color, spreadability, aroma, taste and general acceptability was evaluated using ranking difference test. The results showed that the yield, density, and iodine values of APOs oils ranged from 14.91 ± 0.18 to 11.76 ± 0.46; 0.93 ± 0.001 to 0.99 ± 0.1; 46.63 ± 1.70 to 52.4 ± 0.63, their acid values, TBA and PV values ranged from 1.42 ± 0.39 to

1.97 ± 0.5; 0.11 ± 0.002 to 0.18 ± 0.04; and 2.72 ± 0.14 to 4.43 ± 0.36 respectively, with Brogdon avocado peel variety having the overall best properties prepared blends of trans-free APO margarines showed that increase in APO ratio decreased melting point, increased oxidative stability and reduced moisture content of margarine samples. Chitosan addition leads to decrease moisture content and increase functional properties. VCO lead to increase in phenolic and flavonoid content of the margarines. Samples were spreadable and palatable with R20 being most palatable and the most accepted being R26 with a mean score of 7.07 ± 0.70. Decrease in color intensity increased acceptability. This study therefore demonstrated that avocado peel waste biomass can be valorized by using it as raw material for oil extraction, which can serve as good material for the production of trans-free margarines with good oxidative stability, functional and antioxidant properties.

Keywords

Food Waste, Avocado Peels, Extraction, Oil, Biological Activity, Margarine, Chitosan

1. Introduction

Increased demand for fat spreads and shortenings has caused an increase in margarine demand. Margarines are water-in-oil emulsions which were introduced as an economically viable alternative to butter [1]. Margarines consist of an aqueous phase dispersed as fine droplets in liquid oil stabilized within a network of solid fat crystals [2]. Unfortunately, most margarine fats are prepared from partial hydrogenation where trans-fatty acid (TFA) formation is inevitable [3]. Adverse health effects have been associated with the consumption of semi-solid fats containing trans-fatty acids from partially hydrogenated oils [3]. The WHO recommends the reformulation of lipid-based foods to promote the elimination of TFAs, increase PUFA, and decrease SFAs [4]. Given the current scenario involving nutritional issues with modified oils and fats, margarine has become the target of research to replace partially hydrogenated or interesterified vegetable fat with healthier fats without hydrogenation [5]. Most of the oils used in margarine production are low in bioactive compounds and little has been done is searching for alternative sources of oil with antioxidant properties and richer bioactive compounds.

Food waste and food-by-products can be used as source of oil and bioactive compounds extraction, which serves as functional food ingredients or nutraceuticals [6] as they have been reported as a valuable source of bioactive molecules and compounds [7]. Avocado is a significant tropical fruit with high amounts of bioactive components, and due to its health benefits, the consumption of avocado is increasing worldwide [8] [9]. Despite their proven bioactivity, these nonedible parts are commonly discarded. Annually, at least 1.6 million tons of avocado seeds and peels are estimated to be thrown away globally, turning them

into a remarkable source of environmental contamination. This waste can be valorized through extraction of oils which can be applied for production of healthy trans-free foods such as margarine.

According to Standards, salt is added to margarine at rates not exceeding 2%. This amount is not commonly sufficient to prevent spoilage of margarine. Chitosan exhibits antimicrobial and functional properties thus it can be used as an additive in the production of margarine. Chitosan is a partially deacetylated polymer of acetyl glucosamine (2 acetamido-2-deoxy b-1,4-D-glucan) obtained by the deacetylation of chitin, naturally occurring in the exoskeletons of crustaceans [10]. It has dietary health benefits owing to its ability to bind with fat, and therefore it is used to control obesity [11]. Chitosan exhibits several biological activities such as antioxidant [12], antimicrobial [11] and anticancer properties [13]. Chitosan and its derivatives are widely used in food and pharmaceutical fields [14] and have been approved for dietary use [15]. However, chitosan from reared snails have not been tested as preservatives in margarine production.

This research work was aimed at valorization of waste avocado peels for the extraction and characterization of oil and to evaluate the effect on the physicochemical, nutritional, functional and sensory properties of margarine produced from it. The margarine will be enriched with virgin coconut oil and chitosan from reared snail shells as preservative. The effect of process parameters on the properties of the margarine as well as on sensory quality and acceptability were evaluated.

2. Materials and Methods

2.1. Extraction of Oils from Avocado Waste Peels

Five species of avocado pears were bought from the bambui-Bamenda (Cameroon) market namely; Fuerte, Brogdon, Bacon, Pinkerton, and Russell and coded as Avocado peel sample A (APSA), sample B (APSB), sample C (APSC), sample D (APSD) and sample E (APSE). The pears were washed, sorted and tied in separate bags for ripening. The peels were removed, weighed, and oven dried at 50 degrees until a constant weight and the percentage moisture content was determined.

$$\text{Moisture content (\%)};\quad W = \frac{M_0 - M}{M_0} \times 100$$

where, W = moisture content, M = final weight of the dried peel, M_0 = initial weight of the peel.

The extraction of APO was done by Soxhlet extraction using n-hexane at boiling temperatures of 70˚C, using extraction time of 6 hours. The hexane was distilled off at 75˚C using a Rota evaporator (ML-E14-2050), the collected oil heated in an oven at 75˚C for 1 hour to enable the evaporation of any residual hexane. The yield of avocado peel oil was calculated as:

$$\text{Oil yield (\%)} = W_1 / W_0 \times 100$$

where, W_1 = Weight of oil and W_0 = Weight of ground peel before extraction.

2.2. Evaluation of Physical Parameters of Avocado Waste Peels Oil

2.2.1. Determination of Iodine Value

Iodine value (IV) was determined as described by the AOAC (1999) method using 0.3 g of oil, 25 ml of chloroform and 25 ml of Wij's solution and incubated in the dark for 1 hour. 20 ml of 10% aq KI was added and the solution turned orange. The mixture was titrated using 0.1N sodium thiosulfate with 1ml of 1% starch as an indicator and compared against a blank.

$$\text{IV (g/100g)} = (12.69*(\text{VB} - \text{VS})*N)/m$$

where m = weight of sample, VB = titre of blank, VS = titre of sample, N = normality of sodium thiosulfate.

2.2.2. Determination of Specific Gravity and Viscosity

Specific gravity (SG) was determined as described by the AOAC (2010) method using a pycnometer. The viscosity (V) was determined using Ostwald viscometer as described by Ismaili & Belghiti (2015). The Specific gravity and viscosity were calculated using the equations below.

$$\text{SG} = M_o - M_e / M_w - M_e$$

where, M_e =weight of empty pycnometer, M_o = weight of pycnometer + oil sample at 28˚C, M_w = weight of pycnometer + water at 28˚C

$$V \text{ of liquid}(n_2) = \rho_2 t_2 / \rho_1 t_1 * n_1$$

where, ρ_2 = density of oil, ρ_1 = density of water, t_1 = time taken for water to move from point A to B, t_2 = time taken for oil to move from point A to B, n_1 = viscosity of water at room temp (0.997Cp), n_2 = viscosity of oil sample.

2.2.3. Determination of Saponification Value (SV)

The SV was determined as described by Alajtal *et al.* (2018) [16] using 1.5 gm oil sample and alcoholic potassium hydroxide solution. After treatment of the oil, the condenser was wash with 10 mL of ethyl alcohol neutral to phenolphthalein. The excess potassium hydroxide was determined by titration with 0.5N hydrochloric acid, using 1.0 mL phenolphthalein as indicator.

$$\text{SV} = \left((B - S) * M * 56.106\right) / W_s \text{ in mgKOH/g}$$

where, B = titre value for blank, S = titre value for sample, M = molarity of of HCl, 56.106 = molecular mass of KOH, W_s = weight of sample.

2.2.4. Determination of Moisture Content (MC)

The MC was determined by the air-Oven Method [17] using 10 gm of oil, heated at 105˚C ± 1˚C for 1 hour and cooled in a desiccator containing phosphorus pentoxide

$$\text{Moisture and volatile} = W_1 * \frac{100}{W}$$

where, W_1 = Loss in weight (gm) of the material on drying, W = Weight in gm of the material taken for test.

2.3. Evaluation of Oxidative Quality Parameters

2.3.1. Determination of Peroxide Value (PV)

The PV was determined as described by the AOCS (2011) method, using 5 g of oil samples, 10 ml of chloroform, 15 ml of glacial acetic acid and 1ml of freshly prepared saturated KI. Incubation was done at room temperature for 10 mins. 25 ml of distilled water was added to test tubes followed by 0.5 ml of starch solution. This was titrated against 0.1N sodium thiosulfate until the blue grey color tuned a milky color and the titres recorded.

Calculation

$$\mathrm{PV}\left(\mathrm{meqO_2/kg}\right)=\left(\mathrm{VS}-\mathrm{VB}*N*F*1000\right)/m$$

where m = weight of sample, VS = titer of sample, VB = titer of blank, N = normality of sodium thiosulfate, F = factor of 0.01N sodium thiosulfate.

2.3.2. Determination of Acid Value (AV)

The AV was determined by the AOAC (2009) method using 10 g of sample, 99% ethanol and phenolphthalein as indicator. Neutralization was done using 0.1 N NaOH solution to a light pink color. The solution was then heated to boil until the sample dissolved in the ethanol completely. It was titrated with 0.1N solution using phenolphthalein indicator until a pale pink coloration is formed and the final end point noted.

$$\mathrm{AV}=\left(\mathrm{MW_{NaOH}}*N*V\right)/W_S$$

where, $\mathrm{MW_{NaOH}}$ = molecular weight of NaOH, N = Normality of NaOH, V = volume of titre, W_S = weight of sample.

2.3.3. Determination of TBA (Thiobarbiturate) Value

The TBAV analysis was done following the method described by Tarladgis *et al.* (1962) using 10 g sample mixed with 2.5 ml of 4 M HCl to reach a pH of 1.5 and then diluted to a total volume of 100 ml with distilled water. The sample was then distilled to obtain 50 ml distillate. To 5 ml of distillate, 5 ml of TBA reagent (0.2883 g TBA 100 $\mathrm{ml^{-1}}$ of 90% glacial acetic acid) was added and heated for 35 min until a red color solution was obtained. The sample was immediately cooled, and the absorbance was monitored at 538 nm using a Genesys 10S UV-Vis spectrophotometer. The TBAV was calculated using the equation:

$$\mathrm{TBA}\ =\frac{50\times\left(A-B\right)}{W\left(\mathrm{s}\right)\left(\mathrm{mg}\right)}$$

where, A = absorbance of sample, B = absorbance of blank, W = weight of sample, TBA is expressed in Mg MDA/Kg.

2.4. Determination of Functional Properties

2.4.1. Evaluation of Phenolic (TPC) and Total Flavonoid Content (TFC)

The TPC was determined using a spectrophotometer by the Folin-Ciocalteau assay method described by Ramde-Tiendrebeogo *et al.* (2012) and the TPC calculated from the gallic acid standard curve. The TFC was determined using the

Aluminum chloride colorimetric method and was calculated using the quercetin standard curve (quercetin concentration ranged from 0.015 to 2 mg/mL) and results were expressed as milligram equivalent quercetin per gram of quercetin. Quercetin served as a standard, and the results were expressed as mg QE/g DM.

2.4.2. Evaluation of Total Antioxidant Activity; DPPH and FRAP Analysis

1) DPPH analysis

The DPPH test samples were evaluated as described by Mensor *et al.* (2001). The percentages of antioxidant activity of each sample were calculated according to the formula below:

$$\%\ \text{antioxidant activity} = \frac{\text{Absorbance of DPPH}\left(\text{Absorbance of assey} - \text{Absorbance of blank}\right)}{\text{Absorbance of DPPH}} \times 100$$

Essay = sample + methanolic solution of DPPH

Blank = sample + methanol

The different percentages of antioxidant activity were used to determine the EC50 (the concentration of the sample being able to trap 50% of DPPH·).

2) FRAP analysis (Ferric reducing antioxidant power)

The reducing power of the samples was determined according to the protocol described by Benzie and Strain (1996).

2.5. Evaluation of the Effect of Process Parameters on the Physiochemical, Functional and Oxidative Stability Properties of APO Margarine

The extracted avocado peel oil was used in varying proportions with coconut oil extracted using cold press method as described by [18]. The proximate and functional properties of the extracted oil were analyzed. The ingredients used for the production of the various margarine samples were APO, virgin coconut oil VCO, Chitosan, salt, soy lecithin and water. The formulation was done according to the method described by [19] with some modifications, and according to the response surface methodology using statgraphics software. A central composite design (CCD) with 4 variables and 10 responses was used. The central composite design had 26 runs.

The factors were; X_1: APO percentage (10% - 70%), X_2: blending speed (4000 - 17,000 rpm), X_3: Blending time (4 - 10 minutes), X_4: Chitosan concentration (0% - 2%). The responses were; Y_1: Melting Point, Y_2: Iodine Value, Y_3: Moisture Content, Y_4: Peroxide Value, Y_5: Acid Value, Y_6: TBA, Y_7: TPC, Y_8: TFC, Y_9: FRAP, Y_{10}: Creaming Index. Table 1 presents the experimental design table with the 26 runs as on Table 1.

Formulation of margarine was comprised of 80% oil and 14% water. Ingredients were mixed in 2 phases; the aqueous phase and the oil phase in respective oil and aqueous phase ratios as per the experimental design. The oil phase comprised of the oil blends (80%) at 70˚C and soy lecithin emulsifier. The aqueous phase comprised of water, salt and chitosan held at 60˚C. The emulsions were formulated by mixing the both phases separately with an electrical hand blender

Table 1. Experimental design runs.

BLOCK	APO to VCO Oil Ratio (%)	Blending Speed (rpm)	Blending Time (Min)	Chitosan concentration (%)
	X_1	X_2	X_3	X_4
1	40	10,500	4	1
2	40	10,500	7	1
3	10	4000	4	2
4	70	4000	10	2
5	40	10,500	10	1
6	70	17,000	10	0
7	70	17,000	10	2
8	10	4000	4	0
9	40	10,500	7	0
10	70	10,500	7	1
11	40	4000	7	1
12	70	4000	10	0
13	40	10,500	7	2
14	10	10,500	7	1
15	70	17,000	4	0
16	70	17,000	4	2
17	10	4000	10	0
18	70	4000	4	2
19	40	17,000	7	1
20	40	10,500	7	1
21	10	17,000	4	0
22	10	4000	10	2
23	10	17,000	10	0
24	70	4000	4	0
25	10	17,000	4	2
26	10	17,000	10	2

(SOKANY R312 blender Model). The aqueous phase and the oil phase temperatures were brought down to 45˚C, combined and blended for emulsification under different blending speeds and times as mentioned in Table 1. The emulsions were cooled in a while under varying blending speeds and time in an ice bath at 10˚C containing 10% NaCl [19]. They were refrigerated for 24 hours for proper setting. All samples prepared were kept under refrigeration conditions prior to analysis.

2.6. Evaluation of Physiochemical Properties of APO Margarine Samples

2.6.1. Determination of Melting Point, Iodine Value and Creaming Index

The melting point was determined according to the method described by [19].

The iodine value was determined as described by the AOAC (1999) as described above while the emulsion stability was measured by the change in the height of the bottom serum phase (Hs) within a given time and was compared with the total height of emulsion (HE) according to the method described by Klinkerson *et al.*, 2004.

2.6.2. Evaluation of Oxidative Quality Parameters (Peroxide Value, Acid Value, TBA Values)

The peroxide value, acid value, TBA values were determined using the methods earlier described above.

2.7. Evaluation of Functional Properties

The functional properties including the TPC, TFC and FRAP were determined as earlier described above.

2.8. Evaluation of Sensory Attributes and General Acceptability of Optimized APO Margarines

Sensory evaluation of margarines was performed by a panel of 32 semi trained and trained judges using a fully randomized design. A ranking difference test was used to rank the margarine products in terms of the attributes; colour, spreadability, taste, aroma intensity, palatability and general acceptability on a 9 point scale.

2.9. Model Validation and Statistical Analysis

The coefficient of determination (R^2) greater than 70% and the P value (0.05) were used in validating the model. Pareto charts and the response surface curves were obtained using statgraphics and SIGMA plot respectively. Sensory evaluation was analyzed using Microsoft Excel and the results were presented in bar charts.

3. Results and Discussion

3.1. Characterization of Oils from Five Avocado Peel Varieties

3.1.1. Physicochemical Parameters of Oils from Five Avocado Peel Varieties

The physico chemical properties of oils extracted from the five varieties of avocado pear waste biomass peels are presented in **Table 2** with respect to the codes of the oil samples.

3.1.2. Density

The densities of the oils ranged from 0.93 ± 0.001 g/ml to 0.99 ± 0.1 g/ml. The density of oils are lower than the density of water. The Bacon specie (APSD) had the lowest density of 0.93 ± 0.00. This value is close to the value of avocado oil (0.91 ± 0.01) reported by [20]. Generally, the values are higher as compared to the 0.90 reported by [21]. This could be due to the geographical factors affecting the growth and properties of the avocado pears.

Table 2. Physicochemical properties of avocado peels oil varieties.

Sample	Density g/ml	SG	KVM m^2/s	IV MgKOH/g	Yield %	MC %	SV MgKOH/g oil
APSA	0.95 ± 0.0^{c}	0.86 ± 0.0^{c}	12.33 ± 0.67^{b}	46.63 ± 1.70^{a}	13.78 ± 1.43^{a}	0.20 ± 0.13^{e}	194.47 ± 1.32^{a}
APSB	0.99 ± 0.1^{e}	0.9 ± 0.0^{e}	44.99 ± 1.05^{d}	50.9 ± 1.06^{b}	14.91 ± 0.18^{a}	0.09 ± 0.05^{a}	177.57 ± 0.8^{a}
APSC	0.932 ± 0.0^{b}	0.85 ± 0.0^{b}	9.34 ± 0.34^{a}	51.03 ± 0.44^{b}	11.76 ± 0.46^{a}	0.18 ± 0.07^{bc}	149.6 ± 0.44^{a}
APSD	0.93 ± 0.001^{a}	0.84 ± 0.0^{a}	8.96 ± 0.70^{a}	52.4 ± 0.63^{b}	14.3 ± 0.84^{a}	0.20 ± 0.1^{cd}	168.3 ± 0.0^{a}
APSE	0.96 ± 0.0^{d}	0.87 ± 0.0^{d}	15.55 ± 1.03^{c}	51.18 ± 1.18^{b}	13.43 ± 1.02^{a}	0.17 ± 0.1^{b}	172.67 ± 0.39^{a}

All analysis were done in triplicates. Results were expressed as mean ± standard deviation. Means with the same superscript on the same column were not significantly different at $P < 0.05$.

3.1.3. Specific Gravity

The specific gravity of oils is employed to determine the purity of oils. For most fats and oil, this lies between 0.90 and 0.94 at 20 degrees [22]. The values for specific gravity of APO in this study ranged from 0.84 ± 0.0 to 0.9 ± 0.0 with APSB having the highest specific gravity. This is same as the value of specific gravity reported by [21]. The specific gravities of AO by Ogbaugu *et al.*, 2020 was reported to be 0.86. This indicates that the specific gravity of avocado peel oil is similar to that of avocado oil and it is less dense than water.

3.1.4. Viscosity

The viscosity of the investigated oils ranges from 8.96 ± 0.70 mm^2/s to 44.99 ± 1.05 mm^2/s. Oils with low viscosity values indicate that they are light and so probably highly unsaturated [23]. Thus, the oils were unsaturated.

3.1.5. Iodine Value

The iodine value (IV) indicates the degree of unsaturation of the oil [23]. This value could be used to quantify the amount of double bond present in the oil which reflects the susceptibility of oil to oxidation. The IV of the APO samples were generally low as compared to the values of IV of avocado oils as studied by Ogbaugu *et al.*, 2020 (127.40). The values ranged from 46.63 ± 1.70 to 52.4 ± 0.63. Thus, they are less susceptible to oxidation and can be used in the production of margarine with APSA being the most preferred.

3.1.6. Yield

With respect to oil yield, the oil content of avocado peels are generally low and vary from one specie to another. A study carried out by [24] on avocado peels portrayed a yield of 13.14 ± 0.20. The yield of APO in this study ranged from 11.76 ± 0.46 to 14.91 ± 0.18 with APSB having the highest yield. This is as a result of a variation in the properties of each specie.

3.1.7. Moisture Content

The maximum allowed moisture content in edible oils is 0.2% [25] higher moisture content indicate poor moisture refining process and higher susceptibility to spoilage. The mean moisture content of the APO samples in this study ranged from 0.09 ± 0.05 to 0.20 ± 0.31. Sample APSB had the lowest moisture content.

Thus, it will least favor spoilage microbes and is most suitable for application in further processing to margarine production. In comparison to AO, APO contains lesser moisture than AO studied by [22].

3.1.8. Saponification Value

Saponification value is a measure of oxidation during storage and it also indicates the deterioration of the oils [23]. The saponification values of the investigated APO were within the requirements of food domain, and varied from 149.6 ± 0.44 to 194.47 ± 1.32 mg KOH/g. generally, apart from sample APSA, the Saponification values of the APOs were lower than the results obtained by [21], who reported a value of 189.33 MgKOH/g in the extraction and characterization of APO.

3.2. Oxidative Stability Quality Parameters of Avocado Peels Oils

The results of oxidative stability of the five oils extracted are presented in **Table 3**.

3.2.1. Acid Value

The mean acid values of the APOs in this study ranged from 1.12 ± 0.24 mgKOH/g to 1.97 ± 0.54 mgKOH/g with sample APSB having the lowest acid value. The Codex maximum level of acid value for oils is 5 mg KOH/g oil so that the oils do not produce off-flavours and are also desirable for consumption [26]. Low acid value in oil indicates that the oil will be stable over a long period of time and protect against rancidity and peroxidation. This could be attributed to presence of natural antioxidants in the oils as well as other possible phytochemicals.

3.2.2. FFA

Free fatty acids (FFA) are hydrolysis products of triglycerides (TG) in vegetable oils. The FFA concentration in vegetable oils depends on multiple factors, namely the quality and variety of raw material, collecting conditions, processing, storage, the age of the oil and deterioration status [27]. The FFA values of APOs ranged from 0.56 ± 0.10 to 0.98 ± 0.22 with sample APSB recording the lowest

Table 3. Oxidative stability quality parameters.

Sample	ACID VALUE MgKOH/g	FFA MgKOH/g	TBA Mg MDA/Kg	PV Meq/kg
APSA	1.44 ± 0.27^{ab}	0.72 ± 0.111^{b}	0.18 ± 0.04^{b}	3.35 ± 0.31^{c}
APSB	1.12 ± 0.24^{a}	0.56 ± 0.10^{a}	0.15 ± 0.004^{ab}	2.72 ± 0.14^{a}
APSC	1.45 ± 0.25^{ab}	0.72 ± 0.1^{ab}	0.13 ± 0.003^{a}	3.03 ± 0.04^{ab}
APSD	1.97 ± 0.54^{b}	0.98 ± 0.22^{b}	0.17 ± 0.007^{b}	4.43 ± 0.36^{e}
APSE	1.42 ± 0.39^{ab}	0.71 ± 0.16^{ab}	0.11 ± 0.002^{b}	3.82 ± 0.19^{e}

Means with the same superscript on the same column were not significantly different at $P < 0.05$.

FFA value. The lower the FFA value, the higher the oxidative stability of the oil. In comparison with AO, APO has a lower acid value with respect to that reported in the characterization of AO by [20].

3.2.3. TBA

The extent of lipid oxidation is reported as TBA value, which corresponds to milligram of malonaldehyde equivalents per kilogram of sample or micromoles of malonaldehyde per gram of sample. The mean TBA values ranged from 0.13 ± 0.003Mg MDA/Kg to 0.18 ± 0.04Mg MDA/Kg with APSB having the lowest TBA value thereby indicating a higher degree of oxidative stability.

3.2.4. Peroxide Value

Peroxide value (PV) is the most common indicator of lipid oxidation/rancidity. Peroxides are formed when the triglycerides in the oil oxidize in the presence of moisture [28]. The PV of the oils studied ranged from 2.72 ± 0.14 meq/kg to 4.43 ± 0.36 meq/kg. High values of PV are indicative of high levels of oxidative rancidity of oils and also suggest absence or low levels of antioxidants. The CODEX (2005) stipulated a permitted maximum peroxide level of not more than 10 milli equivalent of oxygen/kg of the oils. Peroxide values higher than 10 to 20 meq/kg are commonly interpreted as rancidity. The peroxide values of the oil samples are all within the CODEX 2005 maximum limit 10 meq/kg with APSB having the lowest mean PV.

3.3. Functional Properties of Avocado Peels Oils

Total phenol content (TPC) and the Total flavonoid content (TFC) of the oils from the avocado seeds are as presented on **Table 4**.

3.3.1. Total Phenol Content (TPC)

Results obtain for TFC (**Table 4**) revealed significant variation ($p < 0.05$) in the TPC of the 5 varieties. Peels of the APSB (Brogdon) variety contained the highest phenolic compounds with a mean value of 14.37 ± 0.67 (mg EAG/g of oil). In comparison to other studies, The TPC of peels were found to be 9.51 and 13.04 mg CE/g for Hass and Shepard varieties, of avocado peels respectively as reported by [29].

Table 4. Total phenol content (TPC) and the Total flavonoid content (TFC).

Sample	TPC (mg GAE/g oil)	TFC (mg QE/g oil)
APSA	11.36 ± 0.18	4.96 ± 0.12
APSB	14.37 ± 0.67	9.25 ± 0.56
APSC	8.31 ± 0.22	2.61 ± 0.33
APSD	6.87 ± 0.54	6.66 ± 0.43
APSE	6.69 ± 0.27	6.62 ± 0.56
Butylhydroxytoluene	426.85 ± 0.11	63.89 ± 0.97

Means (n = 3) ± standard deviations. Different letters in the same column represent significant differences at ($P < 0.05$).

3.3.2. Total Flavonoid Content (TFC)

The TFC of the APOs were relatively low with the values ranging from 4.96 ± 0.12 mg EQ/g oil to 9.25 ± 0.56 mg EQ/g oil as presented on Table 4. This could be as a result of choice of solvent used in the extraction of the oils from avocado waste peels, geographical origin and variety [30]. Sample APSB had the highest mean value of 9.25 ± 0.56 mg EQ/g oil while APSA recorded the lowest mean value of4.96 ± 0.12 mg EQ/g oil.

3.4. Antioxidant Parameters of Avocado Peels Oils

DPPH and the FRAP values of the oils from the avocado seeds are as presented on Table 5.

3.4.1. DPPH

The antioxidant capacity ranged from 934.11 ± 0.18 (mg/mL) to 69.82 ± 0.45 (mg/mL) as EC_{50} values required to lower the initial DPPH concentration by 50%. Previous studies carried out by [20] showed that, antioxidant capacity of AO was 32.4 mg/ml and the capacities of extra virgin olive oil, olive oil, corn oil, sunflower oil and soybeans oil were 15, 22, 52, 48, and 45 mg/ml respectively. Thus, from the results, all the 5 varieties of APO possess very high radical scavenging capacity than the aforementioned oils with APSD having the highest mean value of 198.11 ± 0.18.

3.4.2. FRAP

The FRAP in the extracts ranged from 39.8 ± 0.58 mmol $FeSO_4$/g to 35.26 ± 0.43 mmol $FeSO_4$/g as presented on Table 5. The highest FRAP was found in APSB 39.8 ± 0.58 mmol $FeSO_4$/g, followed by APSA mmol $FeSO_4$/g, whereas APSD showed the least value 35.26 ± 0.43 39.4 ± 0.33 mmol $FeSO_4$/g.

3.5. Physiochemical, Functional and Oxidative Stability of Margarines Made with APO and VCO

The physicochemical characterization and quality assessment of the edible oils studied showed a consistency in most of the parameters with CODEX/WHO values for edible oils, with APSB having better physical quality parameters, oxidative stability, functional and antioxidant capacity in most aspects.

Table 5. Antioxidant parameters.

sample	DPPH test EC50 (mg/mL)	FRAP test (mmol $FeSO_4$/g)
APSA	69.82 ± 0.45	39.4 ± 0.33
APSB	81.90 ± 0.56	39.8 ± 0.58
APSC	125.89 ± 0.76	35.64 ± 0.23
APSD	198.15 ± 0.18	35.26 ± 0.43
APSE	934.11 ± 0.48	35.71 ± 0.67
VIT C	2.36 ± 0.13	156.85 ± 0.55

Means (n = 3) ± standard deviations. Means with the same superscript on the same column were not significantly different at $P < 0.05$.

Physicochemical Properties of Margarine Samples

The results of the evaluation of the physiochemical properties of the margarine samples produced fusing oil sample APSB are presented in Table 6.

1) Melting point (MP)

The melting point of the experimental samples ranged from 19.73 ± 0.55 to 32.57 ± 0.28. The control sample had the highest melting point of 39.99 ± 0.19. Sample 18 had the lowest melting point while sample 5 had the highest melting point. There was no significant difference in the melting points of margarine samples made with the same oil ratio, the values obtained were in line with the findings of [31], who reported a decrease in melting point of trans free margarines made with different oil blends. Samples with low APO ratio and higher

Table 6. Physicochemical properties of margarine sample.

RUNS	MP	IV	MC	CI
1	32.57 ± 0.03	24.13 ± 0.03	15.77 ± 0.15	2.37 ± 0.23
2	23.33 ± 0.06	30.52 ± 0.06	15.67 ± 0.06	2.90 ± 0.10
3	26.53 ± 0.02	20.31 ± 0.02	17.77 ± 0.90	3.10 ± 0.69
4	21.03 ± 0.22	34.37 ± 0.22	14.27 ± 0.29	2.53 ± 0.15
5	32.57 ± 0.28	31.57 ± 0.28	15.27 ± 0.12	1.73 ± 0.21
6	20.67 ± 0.22	34.37 ± 0.22	14.37 ± 0.32	1.50 ± 0.26
7	21.20 ± 0.12	35.46 ± 0.12	14.10 ± 0.10	1.67 ± 0.06
8	26.17 ± 0.37	29.39 ± 0.37	18.07 ± 0.45	2.77 ± 0.15
9	23.17 ± 0.06	30.49 ± 0.06	15.43 ± 0.21	2.30 ± 0.17
10	20.33 ± 0.56	33.64 ± 0.56	14.50 ± 0.40	2.37 ± 0.06
11	21.73 ± 0.11	30.46 ± 0.11	15.50 ± 0.36	2.43 ± 0.29
12	19.47 ± 0.22	34.37 ± 0.22	14.27 ± 0.21	2.53 ± 0.12
13	24.03 ± 0.30	30.38 ± 0.30	14.73 ± 0.47	2.73 ± 0.06
14	25.23 ± 0.18	20.14 ± 0.18	17.67 ± 0.32	1.83 ± 0.06
15	20.87 ± 0.60	37.71 ± 0.60	14.67 ± 0.32	1.70 ± 0.06
16	20.60 ± 0.46	36.54 ± 0.46	14.17 ± 0.12	1.53 ± 0.12
17	25.57 ± 0.25	19.72 ± 0.25	17.80 ± 0.44	1.47 ± 0.12
18	19.73 ± 0.55	35.20 ± 0.55	14.67 ± 0.51	2.43 ± 0.06
19	19.73 ± 0.55	26.45 ± 0.46	14.93 ± 0.12	2.57 ± 0.42
20	21.60 ± 0.30	28.14 ± 0.30	15.60 ± 0.20	1.53 ± 0.23
21	22.37 ± 0.16	20.23 ± 0.16	17.53 ± 0.31	1.70 ± 0.20
22	24.40 ± 0.10	24.61 ± 0.10	18.00 ± 1.65	1.60 ± 0.17
23	26.10 ± 0.07	20.36 ± 0.07	18.00 ± 0.10	1.57 ± 0.21
24	24.93 ± 0.03	33.97 ± 0.03	14.93 ± 0.15	2.17 ± 0.12
25	24.77 ± 0.24	22.69 ± 0.24	17.17 ± 0.15	1.37 ± 0.06
26	24.33 ± 0.11	21.61 ± 0.11	17.03 ± 0.06	1.43 ± 0.21
Control	39.99 ± 0.19	33.99 ± 0.31	14.33 ± 0.23	0.05 ± 1.01

VCO ratio had higher melting point. This is because, VCO is solid at room temperature thus increase incorporation lead to increase in melting point.

2) Iodine value (IV)

From the Table 6, it can be seen that margarines made with APO and VCO had low IV. This is because the IV of APO was 50.9 ± 1.06 and the IV of VCO was lower. The IV of the margarine samples ranged from 19.72 ± 0.25 to 37.71 ± 0.60. Sample R17 had the lowest mean value for iodine while sample R15 had the highest mean value for iodine.

3) Moisture content (MC)

The moisture content of the margarine samples ranged from 14.10 ± 0.10 in sample R7 to 18.07 ± 0.45 in R8. The water used in the preparation of the aqueous phase of all the margarine samples was constant. Thus, increase incorporation of APO in margarine samples produced desired moisture content as the standard for moisture content of margarine is 12% - 16%. The results obtained for moisture content are lower than that reported by [32] though samples with 10% APO ratio and 90% VCO ratio had MC higher than required. This may be due to defects in weighing in the preparation of the aqueous phase, or high moisture content of the VCO since high temperatures were not used.

3.6. Oxidative Stability

Results of oxidative stability of margarines made with APO are presented in the Table 7. The Peroxide value (PV), Acid value (AV), and TBA were used as parameters for evaluation of oxidative stability of the samples. In the current investigation, the PV, AV, and TBA of APO used in the formulation of the margarine samples were 2.72 ± 0.14 Meq/Kg, 1.12 ± 0.24 MgKOH/g, and 0.15 MgMDA/Kg respectively.

3.6.1. Peroxide Value

Peroxide values of margarines made with a blend of APO and VCO ranged from 0.45 ± 0.16 to 3.54 ± 0.02. The highest peroxide value was in sample R23, which was 10% APO, 90% VCO, and 0% Chitosan prepared with a blending speed of 17,000 rpm for 10 minutes. The lowest PV was recorded in the control sample. The Peroxide index is one of the most widely used index for oxidative stability of fats and oils [32]. The standard for margarine in terms of peroxide index is set at a maximum limit of 4Meq/kg. All the samples had PVs below the maximum limit hence they were of good stability.

3.6.2. Acid Value

The acid values of margarines made ranged from 0.42 ± 0.02 MgKOH/g to 0.85 ± 0.04 MgKOH/g. Sample R3 made with 10% APO, 90% VCO, 2% chitosan with a blending speed of 4000 rpm for 4 minute had the lowest AV while sample R24 made with 70% APO, 30% VCO, and 0% chitosan with a blending speed of 4000 rpm for 4 minutes. The maximum accepted AV for margarines as reports by [33] is 5 MgKOH/g. All the AVs of the margarine samples are lower than the

Table 7. Oxidative stability and functional properties of APO margarine.

RUNS	PV	AV	TBA	TPC	TFC	FRAP
1	2.98 ± 0.10	1.29 ± 0.01	0.14 ± 0.01	24.80 ± 0.10	16.01 ± 0.11	44.86 ± 1.14
2	2.95 ± 0.10	1.42 ± 0.04	10.13 ± 0.01	25.99 ± 1.07	17.07 ± 0.25	42.95 ± 1.19
3	4.02 ± 0.01	1.61 ± 0.02	0.12 ± 0.02	43.41 ± 0.25	34.30 ± 0.40	42.30 ± 1.79
4	2.57 ± 0.08	0.71 ± 0.07	0.15 ± 0.01	16.09 ± 0.24	10.53 ± 0.10	45.99 ± 1.00
5	1.43 ± 0.05	1.47 ± 0.03	0.11 ± 0.02	25.74 ± 0.38	16.78 ± 0.18	42.95 ± 1.19
6	2.88 ± 0.03	0.75 ± 0.08	0.15 ± 0.02	17.25 ± 0.25	8.99 ± 0.17	47.33 ± 1.05
7	2.47 ± 0.04	0.69 ± 0.04	0.12 ± 0.02	18.16 ± 0.11	9.97 ± 0.07	46.27 ± 1.60
8	1.35 ± 0.11	1.72 ± 0.03	0.09 ± 0.01	43.07 ± 0.28	33.35 ± 0.38	52.56 ± 0.38
9	2.20 ± 0.04	1.54 ± 0.04	0.11 ± 0.01	24.77 ± 0.22	15.73 ± 0.55	51.25 ± 1.24
10	2.98 ± 0.01	0.68 ± 0.09	0.13 ± 0.01	17.48 ± 0.09	9.31 ± 0.05	43.85 ± 0.83
11	1.31 ± 0.12	1.25 ± 0.01	0.11 ± 0.01	24.05 ± 0.12	15.30 ± 0.35	42.95 ± 1.19
12	2.10 ± 0.08	0.68 ± 0.04	0.13 ± 0.01	17.69 ± 0.15	9.51 ± 0.09	45.99 ± 1.00
13	1.32 ± 0.09	0.64 ± 0.01	0.11 ± 0.01	25.37 ± 0.25	17.23 ± 0.20	45.21 ± 2.19
14	2.92 ± 0.01	1.65 ± 0.04	0.12 ± 0.01	43.15 ± 0.15	34.00 ± 0.65	46.62 ± 1.63
15	2.85 ± 0.05	1.63 ± 0.04	0.10 ± 0.00	15.33 ± 0.08	7.20 ± 0.08	43.89 ± 0.79
16	2.46 ± 0.04	0.60 ± 0.05	0.10 ± 0.01	19.66 ± 0.14	11.57 ± 0.10	45.99 ± 1.00
17	3.16 ± 0.04	1.73 ± 0.04	0.12 ± 0.02	42.26 ± 0.19	33.03 ± 0.10	53.91 ± 5.18
18	2.14 ± 0.05	0.61 ± 0.06	0.10 ± 0.02	18.62 ± 0.14	10.51 ± 0.03	48.12 ± 0.87
19	1.38 ± 0.14	0.64 ± 0.03	0.12 ± 0.01	24.95 ± 0.14	16.82 ± 0.33	52.46 ± 0.63
20	1.33 ± 0.14	0.65 ± 0.04	0.12 ± 0.01	25.77 ± 0.10	16.62 ± 0.01	52.14 ± 0.63
21	3.12 ± 0.03	1.73 ± 0.03	0.14 ± 0.00	42.18 ± 0.09	32.95 ± 0.01	51.56 ± 3.01
22	3.00 ± 0.02	0.69 ± 0.03	0.13 ± 0.02	43.29 ± 0.58	34.10 ± 0.03	50.13 ± 2.33
23	3.54 ± 0.02	1.70 ± 0.03	0.13 ± 0.01	41.56 ± 0.89	32.36 ± 0.04	53.24 ± 1.49
24	2.19 ± 0.07	0.65 ± 0.04	0.12 ± 0.01	18.18 ± 0.25	10.03 ± 0.09	52.19 ± 0.24
25	3.07 ± 0.01	1.63 ± 0.03	0.11 ± 0.01	43.88 ± 0.54	34.15 ± 0.01	48.59 ± 0.52
26	3.13 ± 0.16	1.65 ± 0.03	0.10 ± 0.01	43.74 ± 0.42	34.59 ± 0.11	48.59 ± 0.72
control	1.14 ± 0.12	0.51 ± 0.02	0.10 ± 0.00	4.01 ± 0.33	2.19 ± 0.45	13.41 ± 0.33

maximum limit, thereby indicating high oxidative stability. Samples containing higher APO ratio show higher AVs than samples with higher VCO ratio. Thus, it can be seen that the AVs are influenced by the initial AV or the raw materials.

3.6.3. TBA Value

The results of the TBA analysis of the margarine samples show that the TBA values ranged from 0.10 ± 0.00 to 0.15 ± 0.02 MgMDA/Kg. Sample R15 had the lowest TBA value while sample R6 had the highest mean value for TBA. It can be seen that an increase in the ratio of APO used in the formulation of the margarines lead to a decrease in the TBA. The maximum limit for TBA in margarines

is 1 MgMDA/Kg. therefore it can be seen that blending of different vegetable oils namely APO and VCO lead to a decrease in TBA hence increase stability of margarine samples. This is in line with the findings of Basury 2014. All prepared blends were safe for human consumption in terms of oxidation as stated by CODEX 200.

3.6.4. TPC, TFC and FRAP

TPC, TFC, and FRAP of the experimental margarines were far higher than that of the control samples. This is because the virgin coconut oil and the oil extracted from avocado waste peels were good sources of flavonoid and other phytochemicals as well as bioactive compounds. This is in line with the findings of Ramos-Aguilar *et al.* 2021 [34] who stated high levels of phytochemicals and bioactive compounds present in oil extracted from avocado waste peels as well as Nurah *et al.* 2017 [35] who reported high phytochemicals and bioactive compounds in virgin coconut oils. The control sample was very low in TPC, TFC and FRAP. This proves that margarines produced using oil extracted from avocado waste peels, virgin coconut oil and chitosan are highly beneficial to human health. Increase in phenolics lead to increase in antioxidant activities [35].

3.7. Effect of Process Parameters on the Properties of APO Margarines

3.7.1. Effect of Process Parameters on the Iodine Value of APO Margarines

The general equation for the effect of process parameters on the iodine value of APO margarine was;

$$\begin{aligned} IV = {} & 23.3147 + 0.295X_1 - 0.00042X_2 + 0.369X_3 - 7.231X_4 - 0.0016X_1^2 \\ & + 0.0000049X_1X_2 - 0.00047X_1X_3 + 0.00334771X_1X_4 + 2.08447E-9X_2^2 \\ & + 0.0000083X_2X_3 + 0.000063X_3X_4 - 0.057X_3^{\,2} + 0.3321X_3X_4 + 2.069X_4^2 \end{aligned}$$

The result is illustrated in the Pareto chart in Figure 1.

From Anova, oil ratio (A) has a positive significant effect on IV ($P < 0.05$) whereas blending speed and time had negative insignificant effect on the IV of

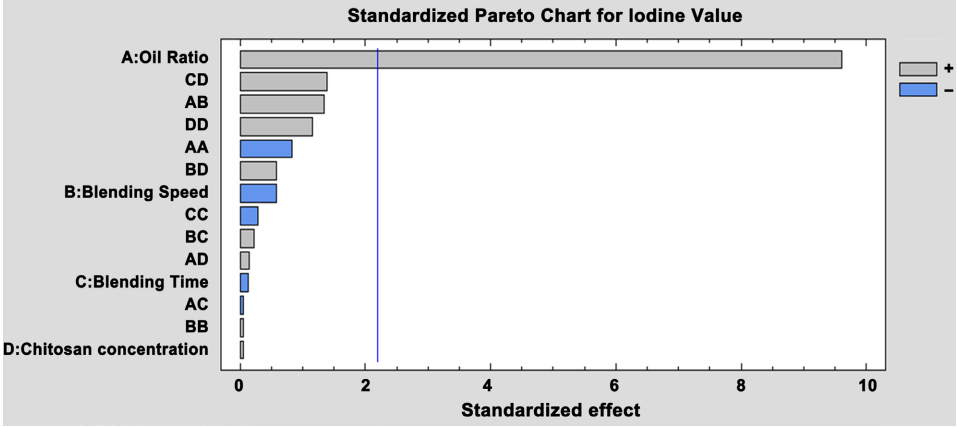

Figure 1. Standardized pareto chart for Iodine value.

the margarine at ($P < 0.05$). From the general equation, the new equation was plotted as seen below

$$IV = 22.3605 + 0.3131X_1 - 5.650X_4 - 0.002X_1^2 + 0.0034X_1X_4 + 2.069X_4$$

Blending speed = 4000; Blending time = 4; Oil ratio = X_1; Chitosan concentration = X_4.

Figure 2 shows that there is a steady increase in IV of margarine as APO ratio increases. This is because APO has a higher IV than VCO. This is similar to the findings of Nnaji & Okereke, 2016 [19] who detected better stability and IV of margarine with substituted fats from avocado oils. The results are also in line with the findings of [36] who proves that an increase in unsaturation leads to an increase in IV. From the results, optimum IV of 37.437 is derived from an oil ratio of 70% APO with 30% VCO with a blending speed of 17,000 rpm and time of 10 minutes with 2% chitosan concentration.

3.7.2. Effect of Process Parameters on the Peroxide Value (PV) of APO Margarines

The general equation of the fitted model for the effect of process parameters on PV of APO margarine was:

$$\begin{aligned} PV = {} & 5.587 - 0.123X_1 + 0.0002X_2 - 0.665X_3 + 0.519X_4 + 0.0012X_1^2 \\ & + 6.82692E-7X_1X_2 + 0.00072X_1X_3 - 0.0013X_1X_4 - 1.14184E-8X_2^2 \\ & + 0.0000035X_2X_3 - 0.000023X_2X_4 + 0.0419524X_3{}^2 - 0.02317X_3X_4 \\ & - 0.067X_4^2. \end{aligned}$$

From Figure 3, only the oil ratio had a negative significant effect ($P < 0.05$) on the PV of margarine. From the general equation, a new equation was plotted which was:

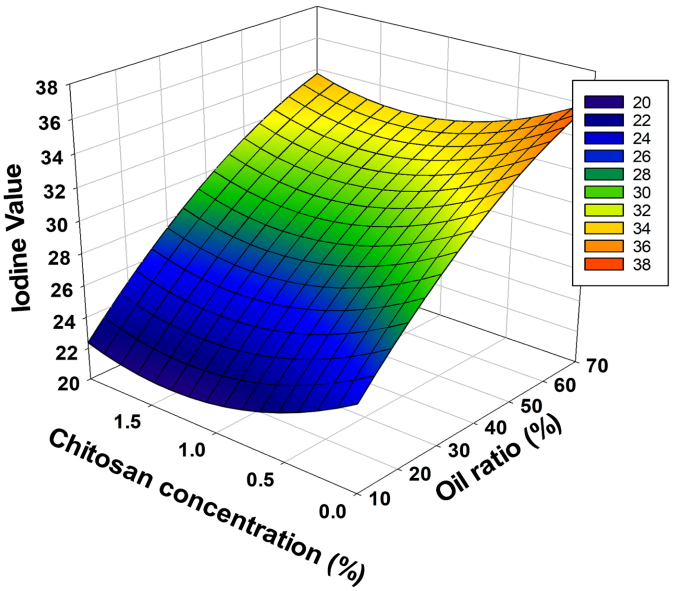

Figure 2. Response surface curve on the effect of process parameters on the Iodine value of APO margarines.

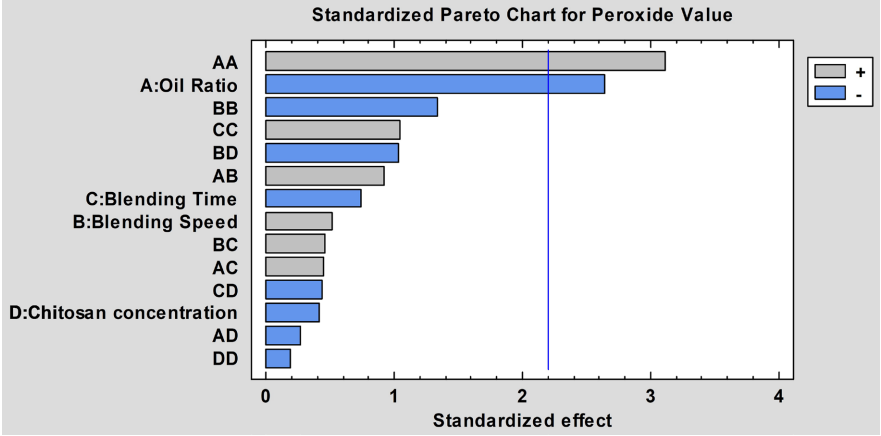

Figure 3. Standardized Pareto chart for peroxide value.

$$PV = 5.587 - 0.123X_1 + 0.52X_4 + 0.0013X_1X_1 - 0.0013X_1X_4 - 0.067X_4^2$$

As the ratio of APO increases, there was an increase in the PV of the margarine. This is because APO has a higher PV than VCO. This is in correspondence to the findings of Sonwai and Lauanginpong, [31] who attributed a decrease in PV of trans free margarines with increase in substitution with VCO. The response surface chart also shows that there is an invert relationship between chitosan concentration and PV where an increase in the chitosan concentration will cause a decrease in the in the PV of the margarine. This is because chitosan concentration reduces moisture content thereby leading to a decrease in PV, and also contains antioxidant properties, as reported by [15] (**Figure 4**).

3.7.3. Effect of Process Parameters on the TPC Value of APO Margarines

The general equation of the fitted model for the effect of process parameters on the TPC of APO margarine was,

$$\begin{aligned} TPC = {} & 52.5189 - 0.888546X_1 + 0.000068X_2 - 0.497X_3 + 0.188X_4 + 0.0059X_1^2 \\ & + 1.57051E-7X_1X_2 - 0.0008X_1X_3 - 0.0024X_1X_4 - 1.17532E-8X_2^2 \\ & + 0.0000123 * X_2X_3 + 0.0000857X_2X_4 + 0.0304X_3^2 - 0.0836X_3X_4 \\ & + 0.0735X_4^2 \end{aligned}$$

Figure 5 shows that oil ratio had a significant negative significant effect ($P < 0.05$ while chitosan concentration (D) had a positive significant effect ($P < 0.05$) on TPC of the margarine produced. From the general equation, a new equation was plotted which was:

$$TPC = 52.519 - 0.889X_1 + 0.188X_4X_4 + 0.0059X_1^2 - 0.0024X_1X_4 + 0.0735X_4^2$$

From **Figure 6**, as the ratio of APO used in margarine formulation increases, it is seen that the TPC of the margarine reduces. This is because VCO had higher phenolic content than APO. The phenolic compounds in VCO have been determined by [37]. They are mainly protocatechuic, vanillic, caffeic, syringic, ferulic, and p-coumaric derivatives, which strongly contribute to the antioxidant capacity of the VCO. Due to the beneficial effects of phenolic antioxidants and

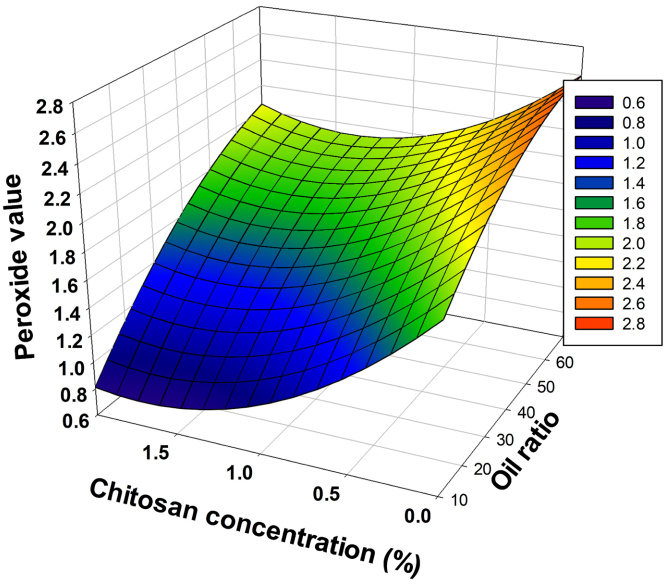

Figure 4. Response surface curve of the effect of process parameters on the peroxide value of APO margarines.

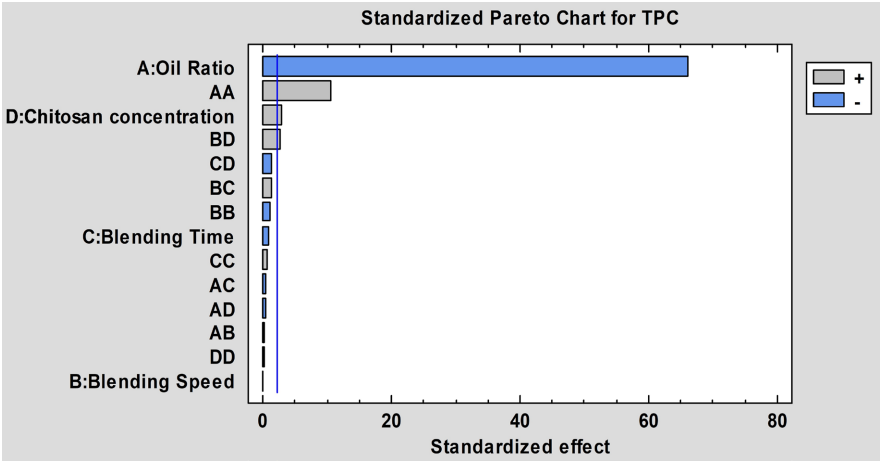

Figure 5. Standardized Pareto chart for TPC.

their high content in VCO the optimum TPC value of 43.359 g/ml was obtained with 10% APO, blending speed of 17,000 rpm for 10 minutes. Also as the chitosan concentration increases, the TPC also increases, with optimum TPC is obtained with 2% chitosan addition.

3.7.4. Effect of Process Parameters on the TFC of APO Margarines

The general equation of the fitted model for the effect of process parameters on the TFC of APO margarine was,

$$\begin{aligned}\text{TFC} = {} & 42.167 - 0.867X_1 - 0.0000182X_2 - 0.1038X_3 + 0.219X_4 + 0.006X_1^2 \\ & + 1.37821\text{E}-7X_1X_2 - 0.0012X_1X_3 - 0.003X_1X_4 - 7.43983\text{E}-9X_2^2 \\ & + 0.000013X_2X_3 + 0.000087X_2X_4 + 0.00223X_3^2 - 0.086X_3X_4 + 0.107X_4^2\end{aligned}$$

In Figure 7, the oil ratio (A) and chitosan concentration (D) are seen to have

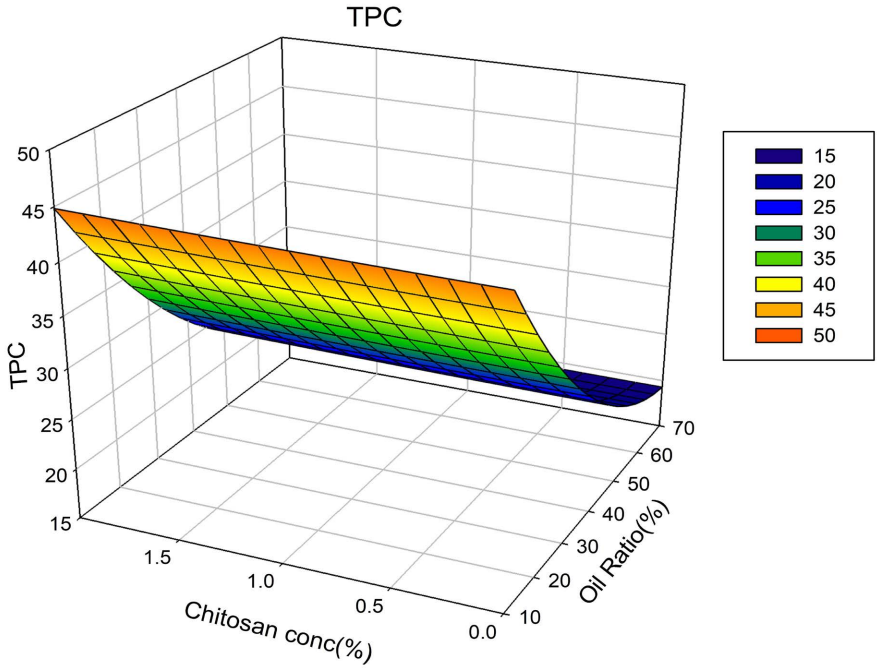

Figure 6. Response surface curve on the effect of process parameters on the TPC of APO margarines.

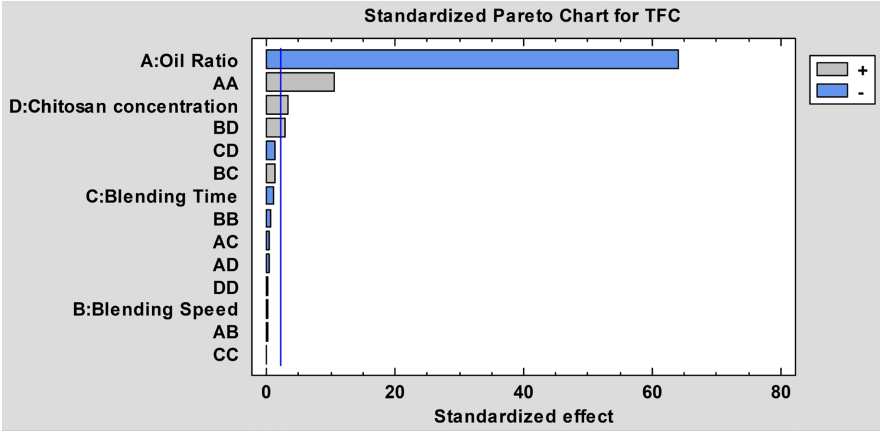

Figure 7. Standardized Pareto chart for TFC.

a negatively and positive significant effect ($P < 0.05$) on TFC respectively. On the other hand, blending speed (B) and time (C) both had negative effects on TFC however; this effect is not significant at ($P < 0.05$). From the general equation, a new equation was plotted which was:

$$\text{TFC} = 42.167 - 0.866X_1 + 0.218X_4 + 0.00587X_1^2 - 0.0028X_1X_4 + 0.1057X_4$$

Figure 8 shows that an increase in the concentration of APO ratio will lead to a decrease in TFC content whereas an increase in the chitosan concentration will have little or no effect on the TFC content. Generally, the TPC and TFC of APO margarines varied with respect to their oil ratio and chitosan concentration. An increase in the amount of APO leads to a decrease in the amount of TPC and

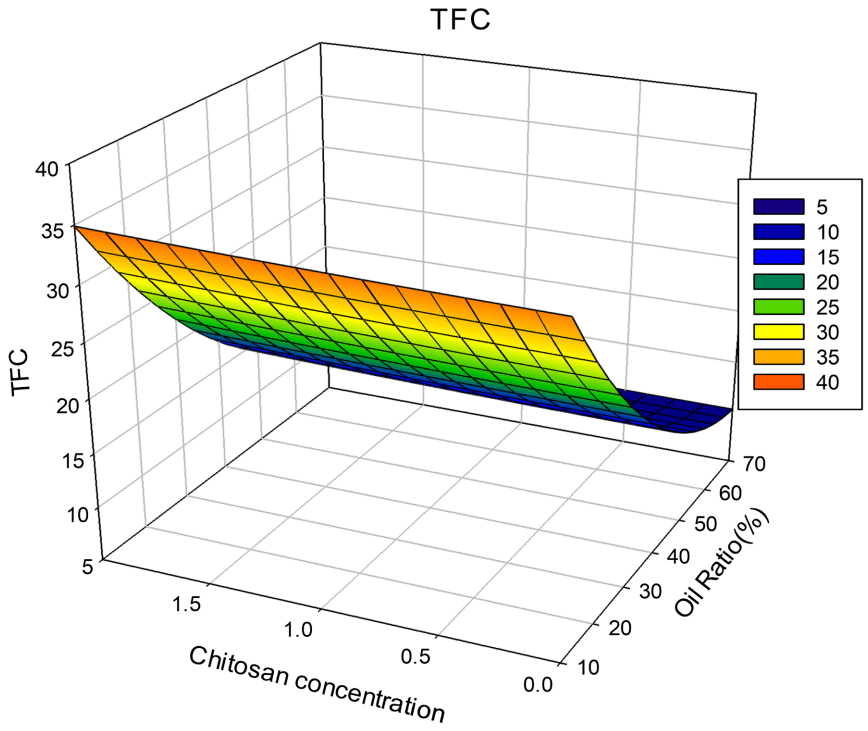

Figure 8. Response surface curve on the effect of process parameters on the TFC of APO margarines.

TFC in the margarines. This could be as a result of the method of oil extraction used and the choice of solvent used in the extraction of APO. Studies have shown that polyphenols are heat liable [38]. The predicted optimum value for TFC was 42.856 to be obtained with 1.8% chitosan concentration with 0.006 APO ratio. The optimum response obtained from the samples was 34.75 in R26 with 10% APO ratio and 90% VCO ratio and 2% chitosan.

3.8. Presentation of Sensory Evaluation Results

Sensory evaluation test was carried out using difference ranking test [39] and the attributes tested were; appearance, spreadability, taste, aroma, palatability and overall acceptability. The sensory codes and composition of each sample is presented in **Table 8**.

From data obtained, the mean scores and standard deviation of the sensory attributes of the margarines made with APO, VCO and chitosan are illustrated in **Figure 9**.

The mean values for appearance ranged from 3.67 ± 1.79 to 7.73 ± 1.03 with R6 having intense green color and R25 having the least intensity. The intensity of the color was due to the dark green nature of the oil extracted from avocado peels, which signifies a high chlorophyll content. This is in line with the findings of [34] who reported a high chlorophyll content in APO leading to intensity of a green color on the oils.

The mean values for spreadability on the APO margarines ranged from 6.67 ±

Table 8. Composition and codes of sensory analysis samples.

response	Optimum value	Sample code	APO ratio (%)	Blending speed (rpm)	Blending time (mins)	Chitosan concentration (%)
CI	3.1	R20	47	4000	6	2
PV	1.011	R6	70	4000	4	0
FRAP	54.86	R19	10	17,000	7	2
MP	26.689	R25	10	4000	4	0
TFC	43.355	R23	10	4000	10	2
TPC	43.755	R26	10	17,000	10	2
IV	26.867	R13	70	17,000	10	2
AV	0.8	R9	40	7000	10	2
TBA	0.145	R7	70	7000	10	1
Control	0	R91	X	X	X	X

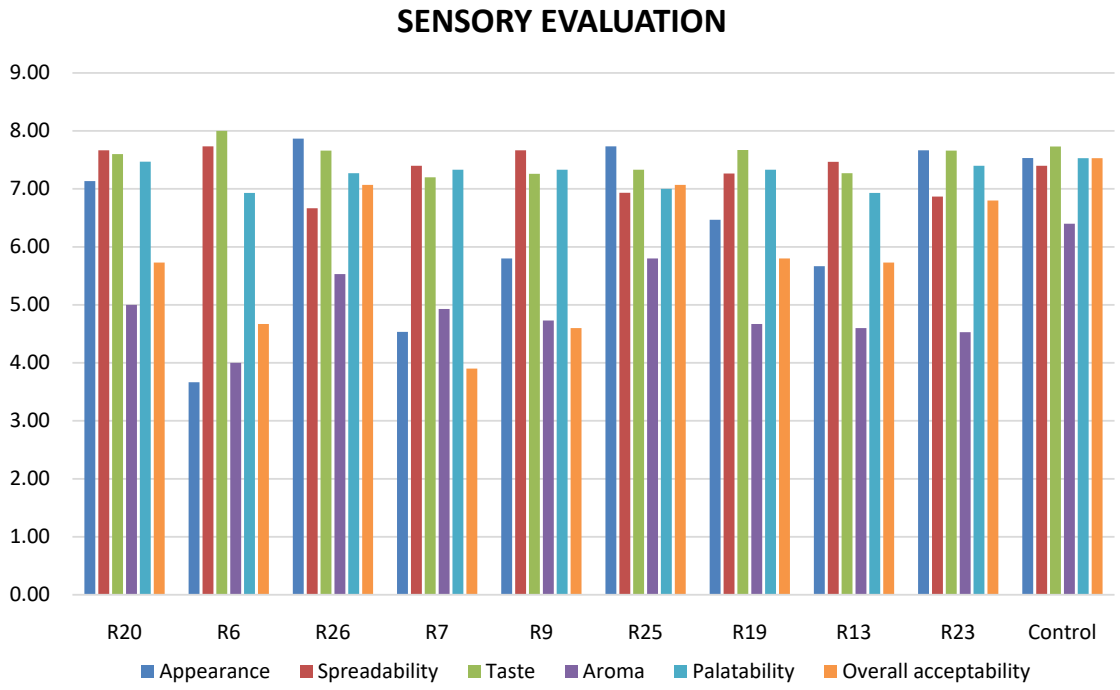

Figure 9. Mean scores of sensory attributes of the APO margarine samples. APO ratio per sample; R20, 40:60, R6, 70:30, R26, 10:90, R7, 70:30, R9, 40:60, R25, 10:90, R19, 40:60, R13, 70:30, R23, 10:90.

0.35 to 7.73 ± 0.79. R6 made with 70% APO and had the highest value for spreadability while R23 made with 10% APO had the lowest mean value for spreadability. This is because of the difference in the physical properties of the oils. APO is liquid at room temperature and has a lower melting point than VCO due to the high level of saturation in them. This leads to improvement in texture and little resistance to shear force thus increasing spreadability properties. This is in line with the findings of [40] who relates spreadability of margarines made

without hydrogenation to have texture attributes affected by the melting point of the oils.

The mean values for taste ranged from 8.0 ± 1.0 to 7.20 ± 1.47 the taste was close to the taste of the control sample, which had a mean value of 7.73 ± 0.45. The similarity in the taste of the various samples could be due to the fact that the salt content was constant.

Mean values for aroma ranged from 4.07 ± 1.4 to 5.8 ± 1.3, and 6.4 ± 1.3 for the control sample. It was seen that an increase in the percentage of APO in the margarines lead to a decrease in aroma intensity, while an increase in substitution with VCO lead to an increase in aroma intensity, making it to smell more like coconut. This is in line with the findings of [31] who produced zero trans margarine using a blend of coconut oil and palm stearin.

All the APO margarine samples were described to be highly palatable. They were easy to melt in the mouth without leaving a greasy sensation or feeling. The control sample had the highest mean value of 7.53 ± 1.30 followed by R20, which composed of 47% APO with a mean palatability value of 7.47 ± 0.51.

The results from the sensory evaluation with regards to general acceptability showed that, samples with high percentage of APO were least preferred and samples with the least percentage of APO were more acceptable. The control sample had the highest acceptability with a mean value of 7.53 ± 0.51 followed by R26 made using 10% APO and 90% VCO, which had a mean value of 7.07 ± 0.70. Based on the general remarks, the colour and aroma of R26 was much appreciated.

4. Conclusion

The main objective of this study was to valorize avocado waste biomass peels through oil extraction, and to further apply the extracted oils in the production of margarines. Five varieties of avocado waste peels were used in the study and the findings revealed that all the oils were of good quality with respect to their physical parameters, oxidative stability, functional and antioxidant capacity. The overall quality of the Brogdon variety (APSB) outweighed the other four varieties, with it having low moisture content, peroxide value, acid value, density, and high oxidative stability. The oil from this variety was further applied for the production of trans-free margarine. Analysis carried out on the margarine samples produced using different oil ratio, blending speed, blending time and chitosan concentration revealed that the percentage of APO had a significant effect on the melting point, moisture content and oxidative stability of the margarines. Chitosan had a significant effect on the moisture content and functional properties of the margarines. Also, variation in the oil ratio, blending speed and blending time affected the creaming index of the margarine samples. The total polyphenol, flavonoid and DPPH radical scavenging activity of the samples were far greater than those of commercial margarines used as control, proving that this is a healthier functional product. Sensory evaluation of the margarines revealed

that adding a high percentage of APO lead to an increase in the green color intensity of the samples and a decrease in the degree of liking as the color was more intense and unusual as compared to common available margarines. Samples made with 10% APO and 90% VCO were more appreciated for the colors and aroma. All the margarines had good spreadability and mouth melting properties. This study therefore demonstrated that avocado peel waste biomass can be valorized through oil extraction and the oils can be considered as raw material for the production of trans-free margarines with good oxidative stability, functional and antioxidant properties thereby recovering valuable properties in the waste and reducing the problem of agricultural food spoilage and waste disposal management.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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