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Optimization of Antioxidant Extraction from Avocado Peel and its Antibacterial Properties and Potentials in Food Products

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Abstract

Avocado peel, with a high proportion of bioactive compounds, is usually discarded as waste. Preliminary study was carried out to determine the most suitable solvent (out of acetones, water, ethanol and methanol) for extraction of antioxidants from avocado peel. Free radical scavenging activity (DPPH), Ferric reducing antioxidant (FRAP) and total phenolic content (TPC) were evaluated as responses. The oxidative stability of cooked ground beef, fish, oil samples (soybean, palm, groundnut) treated with optimum concentration of avocado peel ethanol extract (APE) was evaluated over time using standard procedures. Although methanol had a significantly higher ($p<0.05$) TPC and FRAP, it was not significantly different ($p>0.05$) in DPPH from ethanol; hence ethanol was selected based on its known low-toxicity. Results indicated that process variables with. Optimum solvent concentration, extraction time and extraction temperature with the highest desirability index of 0.868 was 80 %, 90 min and 60 ^{o}C respectively. Cooked ground beef and fish treated with $\geq$ 0.3 % APE resulted in a more significant reduction ($p<0.05$) in pH values than the positive control (0.1 % BHT). However, there was no significant difference ($p>0.05$) in TBARS in both ground beef and fish samples treated with 0.5% APE and the positive control. Similarly, treatment with APE significantly reduced peroxide value (PV) in order groundnut oil>soybean oil> and palm oil and acid value (AV) in the order soybean oil> groundnut oil > palm oil used in this study. The minimum inhibitory concentration of APE was 20 mg/ml for *S. aureus*, 10 mg/mL for *S. typhi* and *E. coli.* The preservative effect of APE increased with increase in concentration of APE. Thus, its potentials in preservation of food products.

Keywords: Avocado Peel, Antioxidant, Extraction, Antimicrobial.

Introduction

Avocado (*Persea americana*) belongs to the family *Lauraceae*, genus *Persea* commonly known as avocado. It is an edible fruit from the Central America. Avocado is a known fruit that contain carbohydrates, protein, fibers and macronutrient necessary for humans including vitamins, minerals and polyphenols. Like other fruits, the peels of avocado fruit are generally discarded which are considered possessing low commercial value and their disposal into the environment without form of treatment poses environmental hazard due to their high biodegradability However, a recent study reported that the peels of avocado has high antioxidant content (Martinez-Gutierrez, 2023) other studies revealed that the avocado contains other classes of bioactive compounds with antioxidant properties and

that are equally beneficial to human metabolism, its peel contains significant amounts of mineral constituent such as (phosphorus, Magnesium and potassium) hydro and liposoluble vitamin's (vitamin E. B. C., β-carotene or *provitamin* A) (Rodriguez-Martinez *et al* 2021). Several studies have been conducted to unveil the antioxidant and antimicrobial activities of avocado (Ferreira and Santas, 2022). For example, the characterization of phenolic component and antioxidant of hydroethanolic extracts of avocado skin and seed revealed predominance of compounds belonging to the groups of flavonoids, proanthocyanidins and hydrocinnamic acid (Rodriguez-Martinez *et al*, 2021), Figuero *et al* (2021) also studied various polyphenols present in the peel among which were (+) – catechin and (-) - epicatechin, chlorogenic and protocatechuic acid. Martinez-Gutierrez, (2023) ascribed the high antioxidant activity exhibited by avocado peel extracts in various *invitro* assays to these phenolic compounds. Ferreira and Santas (2022) studied the antimicrobial activity of crude epicarp and seed extract from mature avocado fruit (*Persea americana*) of three cultivars (Hass, Fuerte and Shepard). They reported that ethanol extract showed antimicrobial activity toward both gram-positive and gram-positive bacteria. Extraction is the first and crucial step for studying natural antioxidants from plants. Many factors play important roles in the extraction efficiency, such as type and concentration of extraction solvent, extraction temperature, extraction time, and extraction pH. Among them, the solvent is one of the most influential factors (Tri Nhut *et al*., 2019). The selection of solvents is based on the chemical nature and polarity of antioxidant compounds to be extracted. Most of the phenolics, flavanoids and anthocyanins are hydrosoluble antioxidants. The polar and medium polar solvents, such as water, ethanol, methanol, propanol, acetone and their aqueous mixtures, are widely used for extraction (Fonmboh *et al*., 2020;). Phenolic compounds extracted using non-conventional technologies (such as microwave- assisted extraction, ultrasound assisted extraction) of avocado hass peel has been reported (Del Castillo- Liamosas *et al*, 2021). These technologies are expensive and require specialized equipment. Studies have been conducted using fixed extraction parameters and organic solvents such as 80% methanol, (Figueroa *et al,* 2018), 80% acetone (Figueroa, 2021), acetone/water 70:30v/v or methanol/water 70:30v/v (Rodriguez-Martinez *et al*, 2021). Melgar *et al* (2018) reported (227.9mg/g extract) of high recovery yield of phenolic compounds from Hass variety of avocado peel using conventional method of extraction, 80% ethanol as solvent. However, he also hypothesized that the concentration of ethanol, temperature and solvent/solid ratio extraction parameters were not evaluated. He also reported that increase in temperature of hass avocado peel over 60min period of extraction by maceration would increase the antioxidant capacity. Restrepo-Serna and Cardona-Alzate (2023) reported 60°C of 60 min as the conditions that improved the extraction of highest catechin 151.96mg and epicatechin 60.77mg per 100g Hass variety of avocado peel with 0.2mg/L of ethanol solvent

Antioxidant are often added to fat containing foods in order to delay the onset or slow the development of rancidity due to oxidation which has the ability to trap free radical thereby preventing undesirable changes in flavour, nutritional quality of foods associated with

various human diseases (Uzombah, 2022). The use of synthetic chemical such as Butylated Hydroxyl Anisole and Butylated Hydroxyl Toluene in combating or delaying oil/meat rancidity have been reported (Biasi *et al*, 2023). Although they are found effective at very low concentrations but they are not safe owing to their toxicity, carcinogenicity and mutagenicity (Biasi *et al*, 2023). This now necessitates the search for safer means of natural antioxidants of plant origin such as avocado peel extract that will have the same or higher potency with the synthetic antioxidants in preventing both oxidative and hydrolytic rancidity of edible oils and meat. With these, researchers now employed a variety of extraction procedures. This ranges from a few seconds to many hours. These variations include different ratios of solvent volume to sample weight, as well as different physical parameters, the fact that one single plant may contain up to several thousand secondary metabolites makes it necessary to develop high performance and rapid extraction methods one of the recent techniques being used to optimize the extraction process is Response Surface Methodology. Response Surface Methodology considers interaction among process parameters and optimize them to reasonable range, with the advantage of the relevant information in the shortest time with least numbers of experiments (Rodriguez *et al*., 2023). Thus, this study was aimed at optimizing the extraction condition on antioxidant potentials of avocado peel using response surface methodology and its application on food products.

Materials and Methods

Procurement of Raw Materials

Fresh, ripe and whole avocado pear (*Persea americana*) were purchased from Itam market and identified by a taxonomist at Department of Botany and Ecological Studies University of Uyo,Uyo, Nigeria. Fresh beef and fish, palm oil, soybeans and groundnut oil were bought from Itam market, Uyo, Akwa Ibom State. Strains of *Staphylococcus aureus, Escherichia coli* and *Salmonella typhimurium w*ere sourced from the Microbiology Laboratory, University of Uyo.

Preparation of Avocado Peel

Avocado (20 kg) was washed with tap water; the different parts of the fruit were separated manually into peel, pulp and seed. The peels were chopped into 1cm by 1cm and dried in an oven at 60 °C for 48 h and milled into fine powder which passes through 25µm mesh size. It was then stored in an airtight container and kept in a desiccator until needed for use (Sumathy and Sumathy, 2011).

Sample Extraction

In the first part of the study, solid-liquid extraction was performed by mixing 2 g of Avocado peel powder with 20 mL of each of the organic solvents (methanol, ethanol, acetone) and water at concentration of 90% ($^{V}/_{V}$) in an Erlenmeyer flask, which were duly covered to avoid

solvent loss and were heated for 30 min in a water bath with magnetic agitation at 60 °C. Thereafter, the resultant extracts were centrifuged (Centrifuge 80-2, Search Tech Instrument, British Standard) at 2500 rpm for 20 min and was filtered through 0.22 µm filters and used for the determination of the antioxidant properties of avocado peel extract (Wijekoon *et al*., 2011; Ballesteros *et al*., 2014).

Determination of the Total Phenolic Content of Avocado Peel Extract

Total phenolic content (TPC) of avocado peel extract was determined based on the method of Singleton *et al*., (1999) with slight modifications. -

Determination of Free Radical Scavenging Activity (DPPH)

The method of Indrianingsih *et al*. (2015) was adopted for Free radical scavenging activity of the extract using the radical, 2, 2 diphenyl1picryhydrazyl (DPPH).

Determination of Reducing Power of the Crude Extract

The reducing power of the extract was determined according to the method of Perumal and Klaus (2003).

pH Determination of the Cooked Beef

pH of the cooked beef and fish was determined by using a digital pH meter (Beckman Model 72, Japan) by homogenizing 10 g with different concentrations of avocado peel extract (0.1%, 0.3% and 0.5%) with 50 mL of distilled water in a homogenizer for 1 min. The pH values of the samples were monitored on 0, 3, 6 and 9 days of storage at 4 °C.

Thiobarbituric Acid-Reactive Substance

Thiobarbituric Reactive substance (TBARS) of meat and fish was determined by using the method described by Domenech *et al*. (2013) with some modifications.

Determination of Oxidative Stability of Soybean, Palm Oil and Groundnut Oil

The oxidative stability of soybean oil, palm oil and groundnut oil with different concentration of avocado peel extract was determined following the official method of AOCS (American Oil Chemists Society, 2009).

Determination of Acid Value

Acid values of soybean, palm oil and groundnut oil samples were determined according to the method of AOCS (American Oil Chemists' Society, 2009).

Determination of Peroxide Value

Peroxide values of samples were determined according to the method of AOCS (American Oil Chemists' Society, 2009).

Antibacterial Activity of Avocado Peel Extract

The disk diffusion method based on the method of Vanden and Vlietinck (1991) was used to determine the antimicrobial properties of avocado peel extract. Sterile petric dishes containing solid media were impregnated with each microorganism. Sterile paper disk (6mm diameter) made from filter paper (Whatman No.1) previously soaked in each extract having different concentrations, (5,10,15,20,25,100,150,200,250,300,350,400,450 and 500 mg/ml) were placed on the agar surface and incubated at 37 °C for 24 h The zones of inhibition were measured. Disk impregnated with ciprofloxacin (100 mg/ml) was used as positive control.

Optimization of Process Variables for Extraction of Avocado Peel

The best solvent obtained from the preliminary study was used for the optimization of the process variables for extraction of avocado peel. The experiment was carried out at 60-65 °C using different solvent concentration (25-80%$^{V}/_{V}$), extraction time (30-90 min). Six (6) grammes of avocado peel powder was blended with 20 mL of solvent concentration. This mixture was placed in a water bath by stirring at the required temperature and time specified by the experimental design (Table 1). At the end, it was cooled, centrifuged at 2500 rpm for 10 min, filtered and stored at -4 °C until needed for analysis. The optimum extraction time, solvent concentration and best temperature were determined by optimization.

Table 1: Essential Parts of Central Composite Ratable Design for Three Process Variables and Five Level Combinations Response Variables

Process Variable	-1.682	-1	0	+1	+1.682
Solvent Conc. (%) X_1	6.25	25	52.5	80	98.7
Extraction Time (min) X_2	9.54	30	60	90	110
Extraction Temp (°C) X_3	58.3	60	62.5	65	66.7

Where -1.682 = lower corner point; -1 = higher corner point; 0 = center point; +1 =lower star point; +1.682 = higher star point

Preparation of Beef Samples

Fresh lean beef and fish were bought from the market and transported immediately to the laboratory. They were washed with sterile water and then manually minced to pieces. Ten (10) grammes of meat and fish were weighed into sterile conical flask and different concentration of the avocado peel extract was also weighed (0.1%, 0.3% and 0.5%) and the weighed meat and fish (10) grammes each were poured into the already weighed extract. The various treatments were thoroughly mixed using sterile spatula for uniform distribution of the added extract. The control was beef + 0.1% of BHT and fish + 0.1% of BHT mixed separately. Each portion was cooked in the microwave (Panasonic genius, 1.2 Cu. Japan)

until the internal temperature reached 80 °C and was held for 20 min after which it was allowed to cool to room temperature. They were then divided and packaged separately into plastic bags sealed and stored at 4 °C for Thiobarbituric Acid – Reactive analysis on 0,3,6 and 9 days of storage (Ifesan *et al.*, 2009).

Statistical Analysis

A Central Composite Rotatable response surface design for three variables five levels of combinations coded -1, -1.682, 0, +1, +1.682 (Table 2.13) as reported by Nwabueze and Akobundu (2008). Data generated on avocado peel extraction were statistically regressed using statgraphic computer software to yield equations for optimizing avocado peel extraction responses. Models develop for each index was examined for lack of fit. Three-dimensional response surface plots were made from data generated. Analysis of variance (ANOVA) was employed and statistically significance accepted at 5% probability levels ($P \leq 0.05$). One-way ANOVA was performed to compare the differences between mean values using SPSS 21.0 software for Windows. Significant differences between means were determined by Turkey tests. A p-value less than 0.05 was considered statistically significant.

Result and Discussions

Effect of Extraction Solvent on Total Phenol Content and Antioxidant Properties of Avocado Peel

Effect of extraction solvent on the total phenol content and antioxidant properties of avocado peel is presented in Table 2.

Table 2: Effect of Extraction Solvent on Total Phenol Content and Antioxidant Properties of Avocado Peel

Solvent (%)	Total phenol (mgGAE/g)	%DPPH (%)	Reducing Power (µg/ml)
Water	$0.48^{d} \pm 0.02$	$23.27^{c} \pm 0.00$	$1.15^{d} \pm 0.02$
Acetone	$1.18^{c} \pm 0.04$	$52.27^{b} \pm 0.00$	$1.49^{c} \pm 0.02$
Ethanol	$1.39^{b} \pm 0.02$	$72.52^{a} \pm 0.00$	$2.18^{b} \pm 0.02$
Methanol	$1.40^{a} \pm 0.02$	$72.79^{a} \pm 0.00$	$2.45^{a} \pm 0.02$

Values are mean ± Standard deviation of triplicate determination. Samples with different superscripts within the same column were significantly ($p < 0.05$) different.

The result showed that the Total phenolic content of avocado peel extract (1.40 mgGAE/g) obtained using methanol as extractant was significantly higher ($p<0.05$) than the total phenolic content of extract (1.39 mgGAE/g) obtained using ethanol as extractant. This was followed by the phenolic content of avocado peel extract (1.18 mgGAE/g) obtained using acetone while the total phenolic content of aqueous extract (0.48 mgGAE/g) of avocado peel was significantly lower ($p<0.05$) than that obtained using every other extractants. The

effect of different solvents on the extraction of antioxidant compounds from avocado peel showed that Methanol, ethanol, acetone and water which are the most commonly used solvents for the extraction of phenolic compounds from different raw materials. This implies that solvent is an important factor that enables the extraction of bioactive components from plant sources. Methanol and ethanol solvents possessed higher polarity, good solubility and viscosity for extracting the phenolic component from plant materials (Wijekoon *et al*., 2011). It has been shown that phenolic compounds are often more soluble in organic solvents less polar than water has shown in table2 (Wijekoon *et al.,* 2011). It has been shown that the antioxidant activity of plant extract is mainly ascribed to the concentration of the phenolic compound present in the plant (Romelle *et al*., 2020). These results are similar to those of Tremocoldi *et al (*2018*)* who reported a considerable total phenolic content in avocado, mango, apple and banana peel.

% DPPH free radical scavenging activity of avocado peel extract obtained using methanol (72.79%) and ethanol (72.52%) as extractant were not significantly different ($p>0.05$) but were significantly higher ($p<0.05$) than the free radical scavenging activity (52.27%) of avocado peel extract obtained using acetone as extractant. The free radical scavenging activity (23.37%) of the aqueous extract of avocado peel was significantly lower ($p<0.05$) than that obtained from other extractants. All the solvent extracts tested possessed radical scavenging activity. However, methanol and ethanol were considered the most suitable solvents for the extraction of antioxidant from avocado peel, based on their DPPH activity. DPPH radical is a widely used method to evaluate antioxidant activities in a relatively short time compared with other methods (Chang *et al.*, 2002). These results are in agreement with other studies, which reported that organic solvents, particularly methanol and ethanol, are more efficient solvent to extract antioxidant phenolic compounds from different natural sources (Trabelsi *et al*., 2010; Wijekoon *et al*., 2011).

The reducing power activity of avocado peel extract (2.45µg/mL) obtained using methanol as extractant was significantly higher ($p<0.05$) than the reducing power of extract (2.18 µg/mL) obtained using ethanol as extractant. This was followed by the reducing power activity of avocado peel extract (1.49µg/mL) obtained using acetone while that of aqueous extract of avocado peel (1.15µg/mL) was significantly lower ($p<0.05$) than that obtained using other extractant. Antioxidant activities have been attributed to various mechanisms, among which are the prevention of chain initiation, binding of transition metal ion catalyst, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging (Yildirim *et al*., 2000). It may also be due to the presence of reductants as electron donors and are capable of converting them into a more stable products and terminating the free radical reaction.

Effect of Solvent Concentration, Extraction time and Extraction Temperature on Total Phenolic Content and Antioxidant Properties of Avocado Peel Extract

The total phenol content and the antioxidant properties of avocado peel extract as influenced by extraction variables is presented in Table 3. The result showed that the total phenol content of avocado peel extract was between 0.40 and 2.58 mg GAE/g. The least total phenol content (0.40 mg/GAE/g) was obtained using 52.50% solvent concentration, at 60 min and at 62.50°C (run 8,11,13,18) while the highest content (2.58 mgGAE/g) was obtained using solvent concentration of 80% for 90 min extraction at 60 °C (run 6).

%DPPH ranged from 1.24 – 89.8%. The least %DPPH (1.24%) was observed with solvent concentration of 6.25 % at 60 min extraction time and at extraction temperature of 62.5 °C while the highest %DPPH (89.8%) was obtained with solvent concentration of 80% at 90 min and 60°C extraction time and temperature respectively (run 6).

The reducing power of avocado peel extract ranged from 0.34 to 2.87µg/mL. The least reducing power (0.34 µg/mL) was obtained using a solvent concentration of 52.50% at extraction time of 60 min and extraction temperature of 62.50 °C (run 13) while the highest reducing power (2.867 µg/mL) was obtained using a solvent concentration of 80% for 90 min at temperature of 60 °C (run 6).As it is well known, the solvent concentration, extraction time and temperature are key factors in extraction processes, as they affect both the kinetic of phenolic compounds release from the solid matrix and the antioxidant activity of the extracts (Chirinos *et al*., 2007). Therefore, it is very important to define the conditions of these variables in order to maximize the extraction results.

Table 3: Total Phenol Content and Antioxidant Properties of Avocado Peel Extract as Influenced by Extraction Variables

Runs	Independent variables			Responses		
	X_1	X_2	X_3	$Y_{1(mg/GAE/g)}$	$Y_{2(\%)}$	$Y_{3\ (\mu g/mg)}$
1	52.5	60.0	62.5	0.42	81.9	0.35
2	25.0	30.0	65.0	2.38	53.9	2.59
3	98.7	60.0	62.5	1.54	82.2	1.59
4	52.5	60.0	66.7	1.83	62.0	1.82
5	25.0	60.0	65.0	1.21	58.2	1.14
6	80.0	90.0	60.0	2.58	89.8	2.87
7	25.0	30.0	60.0	1.85	67.2	1.94
8	52.5	60.0	62.5	0.40	81.9	0.35
9	52.5	9.55	62.5	0.47	77.2	0.89
10	80.0	30.0	65.0	1.13	65.1	1.47
11	52.5	60.0	62.5	0.40	80.9	0.35
12	52.5	60.0	62.5	0.41	79.9	0.35
13	52.5	60.0	62.5	0.40	81.9	0.34
14	25.0	90.0	60.0	2.13	61.3	1.55
15	80.0	90.0	65.0	2.49	55.9	2.76
16	52.5	110	62.5	1.87	56.9	1.80
17	6.25	60.0	62.5	1.18	1.24	1.28
18	52.5	60.0	62.5	0.40	80.9	0.35
19	80.0	90.0	60.0	2.13	57.9	2.83
20	52.5	60.0	58.3	1.80	66.1	1.774

X_1 = solvent concentration; X_2= time; X_3= temperature; Y_1 =Total phenol content; Y_2 =% DPPH and Y_3 = Reducing power activity.

From the result it has been reported that Since temperature increase improves the efficiency of extraction due to enhanced diffusion rate and solubility of the compounds in solvent (Dorta *et al.*, 2012), the extraction was performed between 60 and 65 °C.

Result obtained revealed that experimental run 6 (solvent concentration of 80%, at extraction time of 90 min and temperature of 60 °C) gave the best yield (2.58 mg/GAE/g, 89.8%, and 2.87 µg/mg) in terms of total phenol, 2,2, diphenyl 1, picryhydrazyl and reducing power of the avocado peel extract. This may be due to different solubilities and polarities of different antioxidant compounds in the samples. It has been reported that increase in concentration of solvent led to recovery of more phenolic compounds from a particular sample (Wong *et al.*, 2014). Increase phenolic content with increase in extraction time and temperature had a significant effect on the extraction of phenolic compounds from plant sources (Harbourne *et al.,* 2009) Therefore higher temperature stimulate the solubility of phenolic compounds Thoo *et al.* (2010) reported extraction time of phenolic compound, which the key factors were based on the degrees of phenolic polymerization, solubility of

phenolic and interaction between phenolic compounds and sample extract. similar studies were determined by (Yolmeh *et al*., 2014)., Mohammed *et al* (2018) also reported increase in solvent concentration enhances increase in DPPH of *phaleria macrocarpa (scheff) Boerl* fruit.

Modelling of Effect of Solvent Concentration, Extraction Time and Extraction Temperature on the Total Phenol Content and Antioxidant Properties of Avocado Peel Extract

The ANOVA for response surface quadratic model for the effect of solvent concentration, time and temperature on the total phenol content of avocado peel extract as presented in Table 4.

Table 4: ANOVA for Response Surface Quadratic Model for the Effect of Solvent Concentration, Time and Temperature on the Total Phenol Content of Avocado Peel Extract

Source	Sum of squares	DF	Mean square	F Value	Prob> F
Model	9.61	9	1.07	3.89	0.0227
X_1	0.14	1	0.14	0.51	0.4903
X_2	0.41	1	0.41	1.49	0.2500
X_3	0.15	1	0.15	0.55	0.4767
X^2_1	2.79	1	2.79	10.15	0.0097
X^2_2	1.99	1	1.99	7.26	0.0225
X^2_3	5.19	1	5.19	18.29	0.0014
X_1X_2	10.40	1	0.40	1.47	0.2526
X_1X_3	0.059	1	0.059	0.21	0.6538
X_2X_3	0.015	1	0.015	0.056	0.8175
Residual	2.74	10	0.27		
Lack of fit	2.74	5	0.55	3.294 E+0.06	<0.0001
Pure error	8.33 E-0.07	5	1.667 E-0.07		
Cor total	12.36	19			
R^2	0.7778				
Adj R^2	0.5779				
Pred R^2	-0.8534				
CV (%)	38.76				
Adeq Precision	5.556				
Std Deviation	0.52				
Mean	1.35				

X_1 =Solvent concentration, X_2 = Time, X_3= Temperature R^2= Coefficient of determination. Cv= cofficient of variation, AD= adequate precision, Adj R^2=Adjusted R^2

From the result, Total Phenol Content is expressed by an equation = 9.61+ $2.79X_1^2$ $+1.99X_2^2+5.19X_3^2$.

The coefficient of determination (R^2) of 0.7778 suggests that 77.78% of variations in Total Phenol Content of avocado peel were explained by quadratic model. The co-efficient of variation (CV) value of 38.76% indicated less precision of the experiment but the results reliable in making predictions (Edem and Elijah, 2016). Furthermore, the adequate precision (5.556) which measures the signal to noise ratio indicate adequate signal, therefore the model can be used in navigate the design space.

The model lack of fit fitted significantly and coefficient of determination (R^2) indicated that the model was 77.78% adequate. The model (F=3.89; P = 0.0227) was significant (P < 0.05) and effective in describing the total phenol content of avocado peel. There was only 2.27% chance that an F-value this large could occur due to noise. The quadratic terms of solvent concentration (F = 10.15; P = 0.0097) time (F = 7.26; P = 0.0225) and temperature (F = 18.92; P = 0.0014) had significant (P<0.05) effect on total phenol content of avocado peel as shown in Fig (1 and 2). The positive coefficient indicates positive effect on the total phenol content of avocado peel. All variables affected total phenol content quadratically. The more each of the variables is doubled, the higher the total phenol content of the extract. That is increase in Temp> Solvent>Time increases the total phenol content of the extract. The effect of process variables on the total phenol content was better explained by response surface plots. Figure 1 and Fig 2 which shows the respective increase in total phenol content with increased solvent concentration, time and temperature.

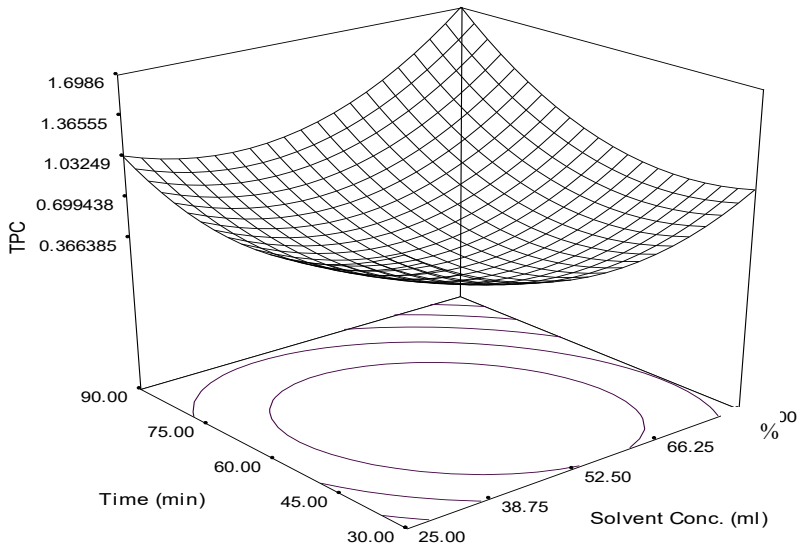

Fig.1: Effect of Time and Solvent Concentration on the Total Phenol Content of Avocado Peel

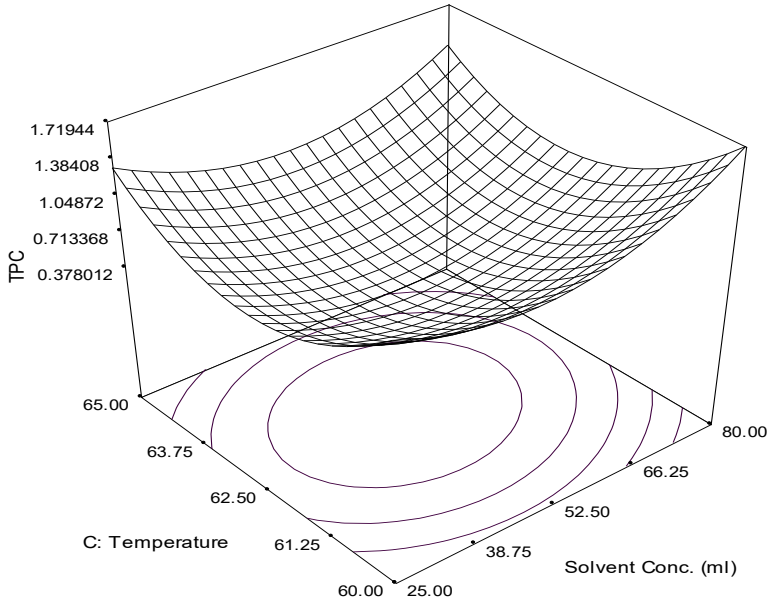

Fig.2: Effect of Temperature and Solvent Concentration on the Total Phenol Content of Avocado Peel

%

The ANOVA for Response Surface Quadratic Model for the Effect of Solvent Concentration, Time and Temperature on DPPH Scavenging Activity of Avocado Peel is presented on Table 5.

Table 5: ANOVA for Response Surface Quadratic Model for the Effect of Solvent Concentration, Time and Temperature on DPPH Scavenging Activity of Avocado Peel Extract

Source	Sum of squares	DF	Mean square	F Value	Prob> F
Model	5356.54	9	595.17	3.58	0.0296
X_1	1975.93	1	1975.93	11.90	0.0062
X_2	431.78	1	431.78	2.60	0.1379
X_3	180.56	1	180.56	1.09	0.3216
X^2_1	2216.95	1	2216.95	13.35	0.0044
X^2_2	170.40	1	170.40	1.03	0.3350
X^2_3	293.11	1	293.11	1.76	0.2135
X_1X_2	192.96	1	192.96	1.16	0.3064
X_1X_3	13.29	1	13.29	0.080	0.7831
X_2X_3	135.05	1	135.05	0.81	0.3884
Residual	1660.78	10	166.08		
Lack of fit	1657.44	5	331.49	496.20	<0.0001
Pure error	3.34	5	0.67		
Cor total	7017.32	19			
R^2	0.7633				
Adj R^2	0.5503				
Pred R^2	-0.7982				
CV (%)	19.20				
Adeq Precision	7.304				
Std Deviation	12.89				
Mean	67.13				

X_1= Solvent concentration, X_2= Time, X_3= Temperature R^2= Coefficient of determination, CV = coefficient of variation.

The result indicated that experimental data for 2,2 diphenyl-1-picryhydrazyl was fitted to response surface quadratic model. And is expressed with the equation:

$$\%DPPH = 5356.54 + 1.975593X_1 + 2216.95X_1^2$$

Analysis of variance (Table 5) shows that the response surface quadratic model (F = 3.58; P = 0.0296) was significant ($p< 0.05$) and effective in describing the percentage yield of 2,2 diphenyl 1 picryhydrazyl of avocado peel. Only solvent concentration had significance influence on DPPH activity whether linear or quadratic. The model fitted and was 76.3% adequate.

There was only a 2.96% error chance that an F-value this large could occur due to noise in addition, the goodness of fit of the model was ascertained by the coefficient of determination (R^2).

The goodness of fit of the model for the effect of solvent concentration, time and temperature on % DPPH was ascertained by the coefficient of determination (R^2) which should at least be approximately 80% (Lima *et al.*, 2010). However, R^2<0.8 has been reported by Gupta *et al.* (2014) and Edem and Elijah (2016), which indicated a fair fit of the model hence useful in making predictions. R^2 value of 0.7633 indicated that only 76.33% of the variations in % DPPH of the avocado peel were explained by the quadratic model.

Also result indicating solvent concentration (F = 11.90; P = 0.0062) and quadratic term of solvent concentration DPPH (F = 13.35; P = 0.0044) had significant (P<0.05) effect on the free radical (DPPH) scavenging activity of avocado peel extract. The coefficient of variation (CV) describes the extent to which the data were dispersed as well as the reproducibility and repeatability of the model (Firatligil – Durmus and Euranuz, 2010). A CV value of 19.20% implies that the experiment was less precise but reliable and the model considered reasonably reproducible. Adequate precision (7.304) which measures the signal to noise ratio indicates an adequate signal. A ratio greater than 4 is desirable. Therefore, the model was used to navigate the design spaces.

Effect of Solvent Concentration, Extraction Time and Extraction Temperature on the %DPPH of Avocado Peel Extract

The regression for the effect of solvent extraction, extraction time and extraction temperature on %DPPH of avocado peel is presented in table 6 and the coded regression equation is given below:

$$Y_1 = +81.03 + 12.03X_1 - 12.4X^2_1$$

Where, Y_1 = %DPPH, X_1 = solvent concentration

Table 5: Estimated Regression Coefficient for % DPPH of Avocado Peel Extract

Factor	Coefficient Estimate	DF	Standard Error	P-value
Intercept	81.03	1	5.26	
X_1 – Solvent Conc.	12.03	1	3.49	0.0062
X_2 – Time	-5.62	1	3.49	0.1379
X_3 –Temperature	-3.64	1	3.49	0.3216
X^2_1	-12.40	1	3.39	0.0044
X^2_2	-3.44	1	3.39	0.3350
X_3^2	-4.51	1	3.39	0.2135
X_1X_2	- 4.91	1	4.56	0.3064
X_1X_3	-1.29	1	4.56	0.7831
X_2X_3	4.11	1	4.56	0.3884

X_1= Solvent concentration, X_2= Time, X_3= Temperature.

The estimated regression coefficient (Table 5.) shows that solvent concentration had a positive effect to reduce DPPH scavenging activity of avocado peel extract, and much more when quadratic or doubled.

Table 6: ANOVA for Response Surface Quadratic Model for the Effect of Solvent Concentration, Time and Temperature on Reducing Power of Avocado Peel Extract

Source	Sum of squares	DF	Mean square	F Value	Prob> F
Model	12.96	9	1.44	6.12	0.0045
X_1	0.77	1	0.77	3.26	0.1011
X_2	0.071	1	0.071	0.30	0.5955
X_3	0.096	1	0.096	0.41	0.5368
X^2_1	3.58	1	3.58	15.24	0.0029
X^2_2	3.23	1	3.23	13.73	0.0041
X^2_3	5.66	1	5.66	24.07	0.0006
X_1X_2	1.19	1	1.19	5.08	0.0479
X_1X_3	0.36	1	0.36	1.54	0.2427
X_2X_3	9.248 E-0.03	1	9.248E-0.03	0.039	0.8468
Residual	2.35	10	0.24		
Lack of fit	2.35	5	0.47	1.282 + 006	<0.0001
Pure error	1.833 E-0.06	5	3.667E-0.07		
Cor total	15.31	19			
R^2	0.8464				
Adj R^2	0.7082				
Pred R^2	-0.2354				
CV(%)	34.12				
Adeq Precision	7.456				
Std Deviation	0.48				
Mean	1.42				

X_1= Solvent concentration, X_2= Time, X_3= Temperature; R^2= Coefficient of determination, CV coefficient of variation.

From the result, Reducing power RP = $12.96+ 3.58X_1^2+ 3.23X^2_2+ 5.66X_3^2+ 1.19X_1 X_2$

The model fitted and contributed 84.64% adequacy. All the three variables affected the activity of the reducing power quadratically. However, combination of solvent concentration and time equally affected reducing power of avocado peel extract. The more each of them doubled the higher the reducing power. Also, solvent concentration and time together had positive effect on the reducing power. And the quadratic model (F= 6.12; P = 0.0045) was significant (P<0.05) and effective in describing the reducing power ability of avocado peel extract. There was only 0.45% chance that an F-value this large could occur due to noise. The interaction effect of solvent concentration and time (F = 5.08; P = 0.0479);

quadratic terms of solvent concentration (F = 15.24; P = 0.0029); time (F = 13.73; P = 0.0041) and temperature (F = 24.07; P = 0.0006) had significant (P < 0.05) effect on the reducing power ability. The R^2 value of 0.8464 indicated well fit of the model in describing effect of solvent concentration, time and temperature on the reducing power of avocado peel extract. As high as 84.64% of variations in the observed values of reducing power of avocado peel were explained by quadratic model. A CV value of 34.12% indicated reproducibility of the model while the adequate precision (7.456) indicate adequate signal because a ratio greater than 4 is desirable (Agarwal and Bosco, 2014). Thus, the model can be used to navigate the design space.

However, linear effects of solvent concentration, time temperature as well as interaction effects of concentration, temperature and time were not significant (P > 0.05) and were eliminated in order to develop a regression model that is statistically significant as presented in equation above. Positive coefficient in Table 6. Indicated positive effect.

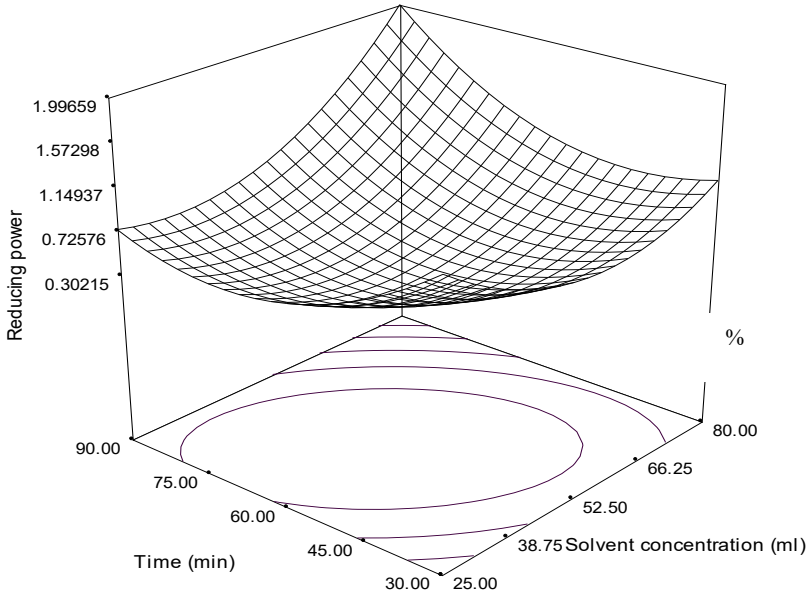

Fig 3: Effect of time and Solvent Concentration on the Reducing Power Activity of Avocado Peel Extract

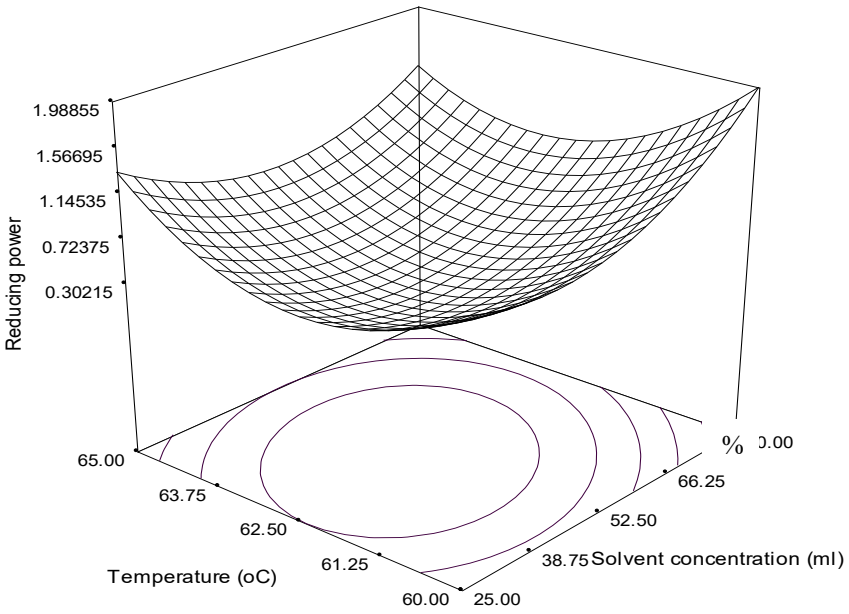

Fig 4: Effect of Temperature and Solvent Concentration on the Reducing Power Activity of Avocado Peel

Relatively, Figure. 3 revealed the interaction effect of solvent concentration and time which resulted in increased reducing power. Similarly, a positive coefficient of the quadratic terms of solvent concentration and temperature indicate positive effects on the reducing power, doubling these variables will also result in the increase in the reducing power and response surface plots (Fig 3and Fig 4.) having a concave shape.

Optimization of Process Variables

The level of responses for optimization of process parameters of Avocado peel extract is presented in Table 7.

Table 7: Levels of Responses Fixed for Optimization of Process Parameters of Avocado Peel Extract

Name	Goal	Lower Limit	Upper Limit
X_1(Solvent Conc.)	is in range	25	80
X_2(Time)	is in range	30	90
X_3(Temperature)	is in range	60	65
Total phenol content	Maximize	0.402	2.581
2,2 Diphenyl-1-picryhydrazyl	Maximize	1.24	89.80
Reducing power	Maximize	0.346	2.867

The process variable in Table 7 indicates respective levels of both independence and dependent variables responses using minimal independent variables.

The Solutions for the Optimum Conditions for Extraction of Avocado Peel is Presented in Table 8 Based on the chosen goals, the software generated seven (7) optimum solutions of process variables with predicted values shown in Table (7).

Solution having the highest desirability index of 0.868 (86.8%) of solvent concentration of 80.0%, time of 90 min and temperature of 60°C which gave optimal yield of responses was estimated as the best process variables. The optimal DPPH scavenging activity, reducing power and total phenol content were estimated to be 62.98%, 2.88 µg/mL and 2.45 mgGAE/g respectively.

Effects of Avocado Peel Extract on the Physiochemical Quality of Cooked Ground Beef and Fish Effects of Avocado Peel Extract on the of Cooked Ground Beef and Fish

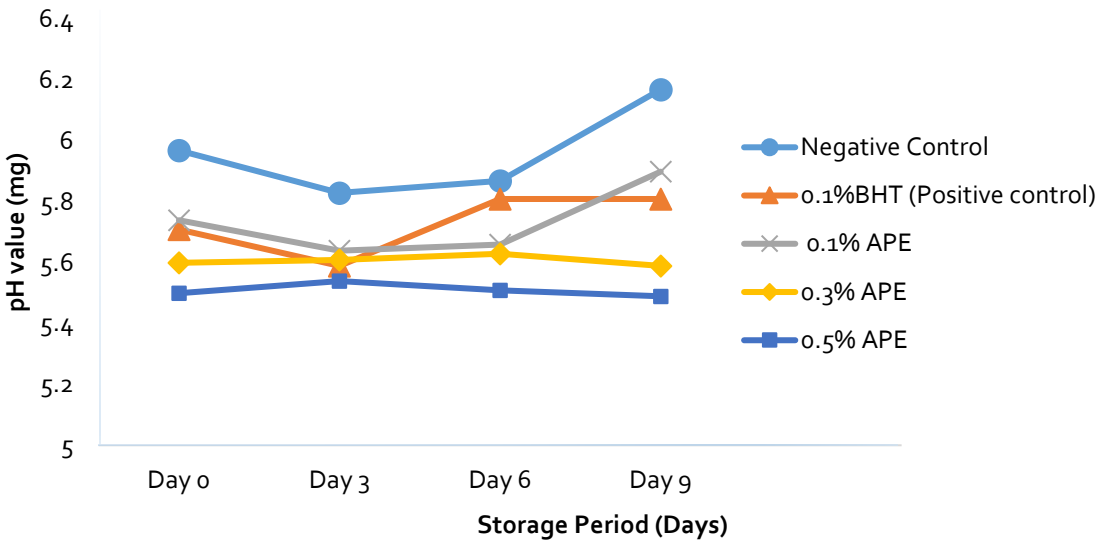

Fig 5: Effect of Avocado Peel Extract on pH Values of Cooked Ground Beef during Storage

The pH values of cooked ground beef and fish treated with varying concentration of avocado peel

The result showed that there were significant differences ($p<0.05$) in pH values of cooked ground beef sample throughout the cold storage period with the negative control recording a significantly higher pH value than other samples. There was a drop in the pH value of the negative control, positive control and sample containing 0.1% Avocado peel extract (APE) on the third day of storage and thereafter pH value gradually increases up till the 9th day of storage. However, there was no significant different ($p<0.05$) in the pH values of the samples treated with 0.3 and 0.5% APE as the storage period increased.

On the other hand, there was a gradual increase in the pH value of cooked ground fish treated with 0.1- 0.5% APE within the first 3 days of storage before declining. A sharp

decline in pH value was observed in the positive control (0.1% BHT) within the first three days of storage before gradually declining till the experiment terminated. However, the pH value of the negative control was significantly higher ($p<0.05$) than that of other samples throughout the storage period.

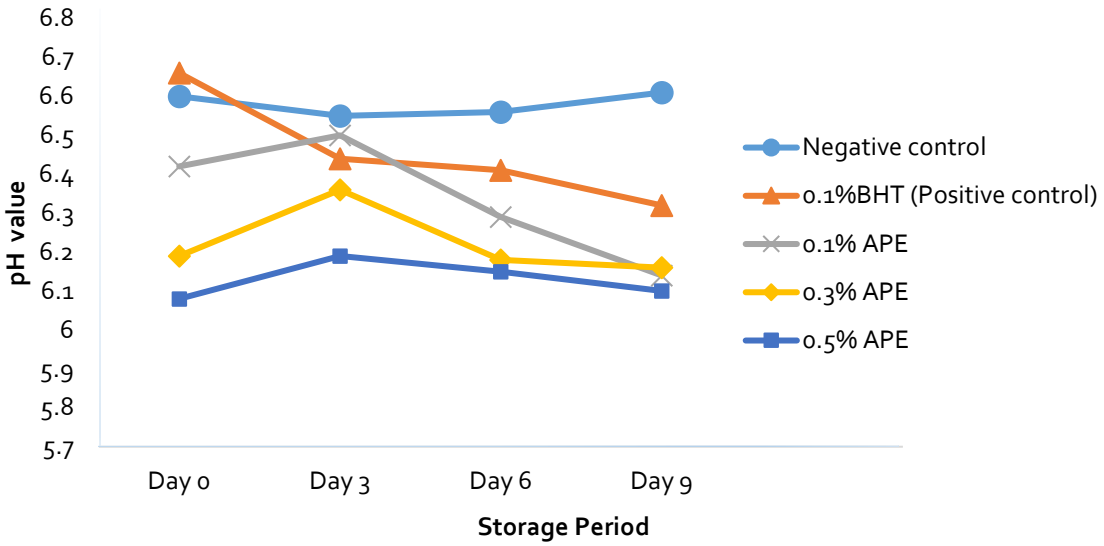

Fig 5: Effect of Avocado Peel Extract on pH Value of Cooked Ground Fish during Storage

The addition of avocado peel extracts to beef and fish patties resulted in a decrease in pH values of samples throughout the storage period when compared with the control sample, the crucial indicator of food stability is pH and this is greatly influenced by microbial and chemical interactions that contributes to food deterioration Higher pH values of untreated samples may be as a result of the degradation of beef and fish protein during cold storage resulting in the formation of some basic compounds (such as volatile nitrogen compounds, amines and hydrogen sulfide) leading to increase in pH value (Oroszvari*et al.*, 2006). The accumulation of organic compounds especially lactic acid resulting from microbial fermentation of stored beef and fish samples of avocado peel resulted in a decrease in the pH (pellissery *et al.*,2020) similar studies have been reported by Park and Chin (2010) who reported that addition of methanol extract of heated garlic acid to pork patties resulted in decrease the pH. Gaikwad *et al* (2020) also reported a pH value between 5.40-5.80 of keratin starch composite of Avocado Peel Polyphenolic rich extract coated beef.

Effects of Avocado Peel Extract on the Thiobarbituric Acid–Reactive Substances (TBARS) of Cooked Ground Beef and Fish

The TBARS Values of the Beef Sample Treated with different Concentrations of Avocado Peel Extract (APE) and BHT (Butylated Hydroxyl Toluene) is presented in Figure 6.

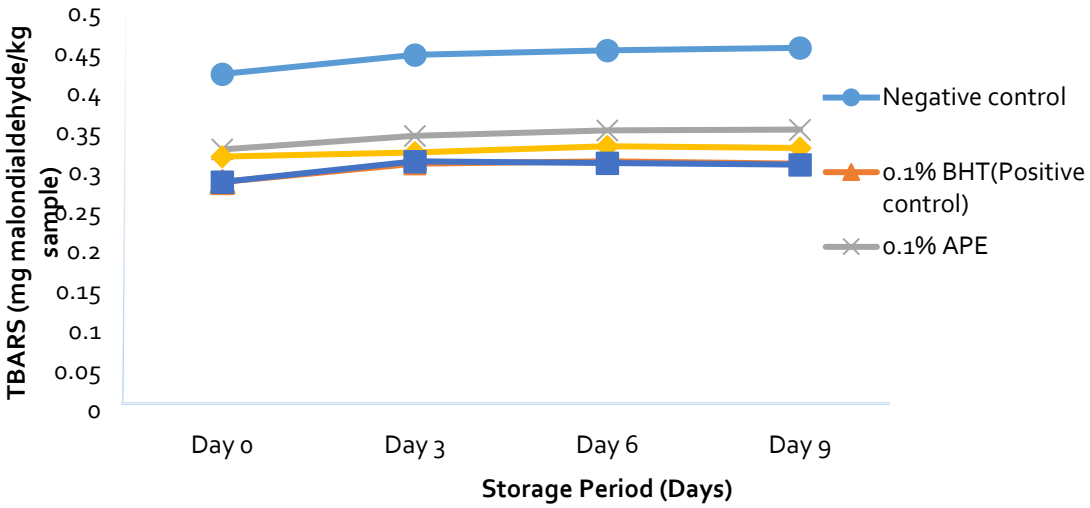

Fig 6: Effect of Avocado Peel Extract on TBARs (Thiobarbituricacid Reactive Substance) Values of Cooked Ground Beef during Storage

The result showed that the negative control sample had a significantly higher ($p<0.05$) TBARS than other samples throughout the experiment (Figure 6). Treatment with Avocado peel extract (APE) resulted in a significant decrease ($p<0.05$) in TBARS of the beef samples. The higher the % APE, the more significant the decrease in TBAR. There was no significant difference ($p<0.05$) between the positive control sample (0.1% BHT) and sample treated with 0.5% APE

The TBARS Values of the fish Sample Treated with different Concentrations of Avocado Peel Extract (APE) and BHT (Butylated Hydroxyl Toluene) is presented in Figure 6.

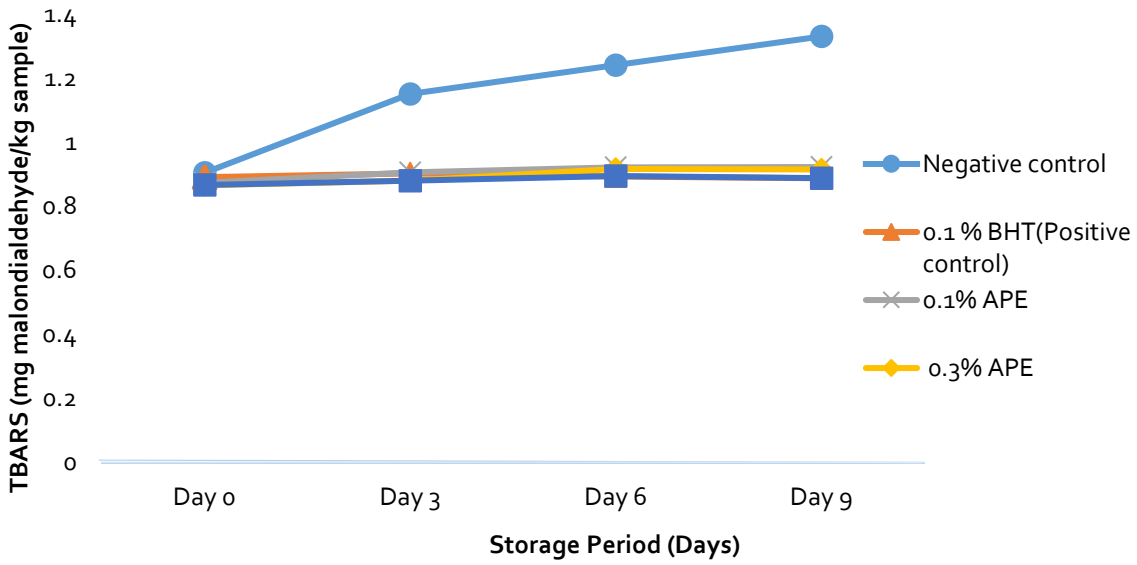

Fig 7: Effect of Avocado Peel Extract on TBARs (Thiobarbituric acid Reactive Substance) Values of Cooked Ground fish during Storage

Similarly, the negative control sample had significantly higher ($p<0.05$) TBARS than treated samples throughout the experiment (Figure 7). Treatment with Avocado peel extract (APE) also resulted in significant decrease ($p<0.05$) in TBARS of the fish samples with increasing effect as the concentration of APE increased while the TBARS of the negative control sample increased sharply as the storage period increased, TBARS of the treated samples increased slightly up to the 3rd day of storage before declining steadily. There was no significant difference in the TBARS of the fish samples treated with 0.1% BHT and the sample treated with 0.5% APE. The result also showed that the beef sample recorded less TBARS (0.288-0.424 mg malonaldehyde/kg) than the cooked ground fish (0.865-0.904 mg malonaldehyde/kg). TBARS is a reliable indicator used in measuring lipid peroxidation in food products. The initial TBARs values in cooked ground beef and fish showed that all treatments had significantly antioxidant effect compared with the control. This could be explained by the development of lipid oxidation from raw meat (Ahn *et al.*, 2009). There was a gradual increase in TBAR in the cold storage (Mc Carthy *et al.*, 2001). Cooking resulted in increase in TBARS; when meat cooked and exposed to atmospheric conditions; it oxidizes rapidly (Ahn *et al.*, 1993). This is also in agreement with Rhee *et al.* (1996) who reported that lipid oxidation of cooked meat product and their effect on meat flavours depends on the initial lipid oxidation in the raw meat. In addition, cooked meat is more susceptive to lipid oxidation than raw meat because the denaturation of antioxidant enzymes and the structural damages in membrane during heating can expose phospholipids to pro-oxidant environment (Gray *et al.*, 1996). Therefore, lipid oxidation in cooked meat increased faster,

and the variations between treatments were clear. The highest antioxidative effect was observed when 0.5% avocado peel extracts were added to the beef and fish samples compared with 0.1% BHA and the potency of avocado peel ethanol extract could have resulted from various polyphenols present in the peel among which are (+) – catechin and (–) – epicatechin (Soong and Barlow, 2004) and chlorogenic and protocatechuic acid (Guerrero *et al.*, 2008). Previous studies on this phenolic compound from avocado waste residue have been applied to pork porcine patties and have been shown to be effective in preventing oxidation and microbial growth (Rodriguez – Carpena *et al.*, 2011). Trujillo – Mayol also reported 0.5% APE reduces the Tbars of soy and beef burgers when compared with the control burgers. Permal *et al* (2020) also demonstrated the effectiveness of avocado peel powder as an alternative antioxidant addictive in inhibiting lipid oxidation in pork sausages.

Effect of Avocado Peel Extract on the Oxidative Stability of Different Vegetable Oil

Effect of different concentrations of ethanol extract of avocado peel on acid value of Soybeans, palm oil and groundnut oil during storage.

Effect of Different Concentrations of Ethanol Extract of Avocado Peel on Acid Value of Vegetable Oil during Storage

The acid value of soybean oil treated with different concentration of ethanol extract of avocado peel under accelerated storage is presented in Fig 8.

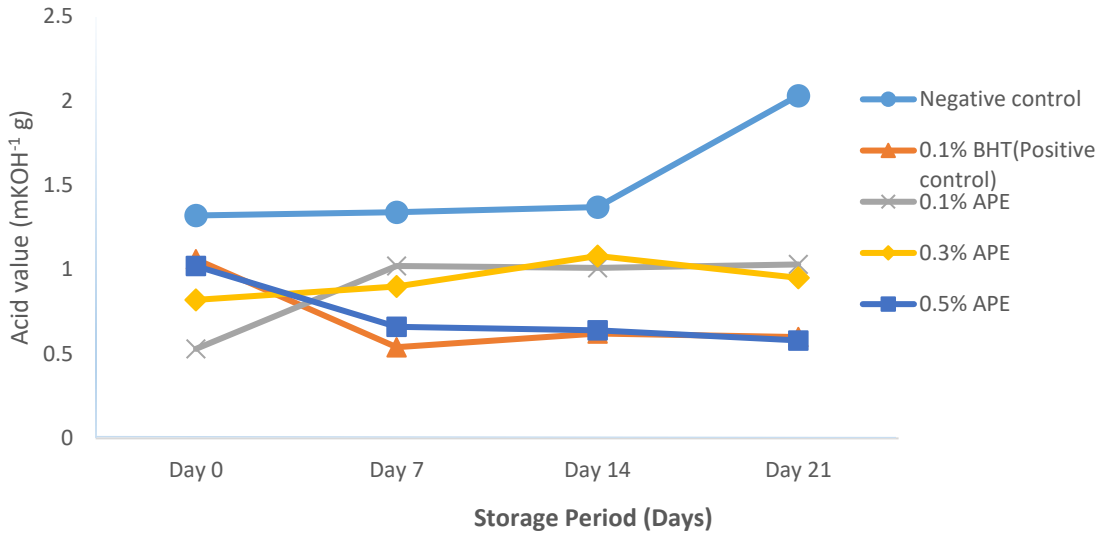

Fig 8: Effect of Different Concentrations of Ethanol Extract of Avocado Peel on Acid Value of Soybean oil during Storage.

The result (fig 8) showed that there was a significant decrease ($p<0.05$) in the acid value of soybeans oil sample treated with Avocado peel extract (APE) as well as the positive control (treated with 0.1% BHT). There was a sharp decrease in acid value of sample treated with 0.5% APE and the positive control sample on the 7th day of storage, before it increased gradually up to day 14th of the storage and then stabilized until day 21 when the experiment was terminated. Except on day 7, there was no significant difference ($p>0.05$) between the acid value of the samples treated with 0.5% APE and the positive control.

However, there was a steady increase in the acid value of the sample treated with 0.1and 0.3% APE as well as the untreated sample (negative control) which recorded a significantly higher ($p<0.05$) acid value than the other samples throughout the storage period.

The acid value of palm oil treated with different concentration of ethanol extract of avocado peel (APE) under accelerated storage is presented in Fig 9.

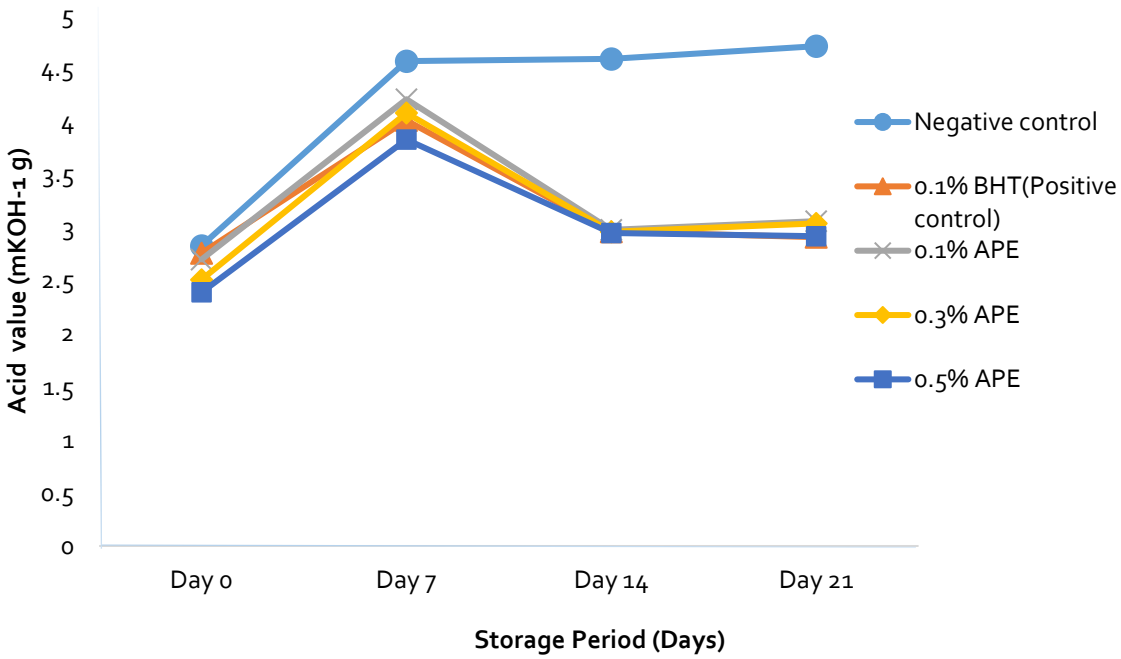

Fig. 9.: Effect of Different Concentrations of Ethanol Extract of Avocado Peel on Acid Value of Palm Oil During Storage.

The result (fig. 9.) showed that there was a significant decrease ($p<0.05$) in the acid value of soybeans oil sample treated with Avocado peel extract (APE) as well as the positive control (treated with 0.1% BHT). There was a sharp decrease in acid value of sample treated with 0.5% APE and the positive control sample on the 7th day of storage, before it increased gradually up to day 14th of the storage and then stabilized until day 21 when the experiment

was terminated. Except on day 7, there was no significant difference ($p>0.05$) between the acid value of the samples treated with 0.5% APE and the positive control.
However, there was a steady increase in the acid value of the sample treated with 0.1and 0.3% APE as well as the untreated sample (negative control) which recorded a significantly higher ($p<0.05$) acid value than the other samples throughout the storage period.
The acid value of palm oil treated with different concentration of ethanol extract of avocado peel (APE) under accelerated storage is presented in Fig. 9.

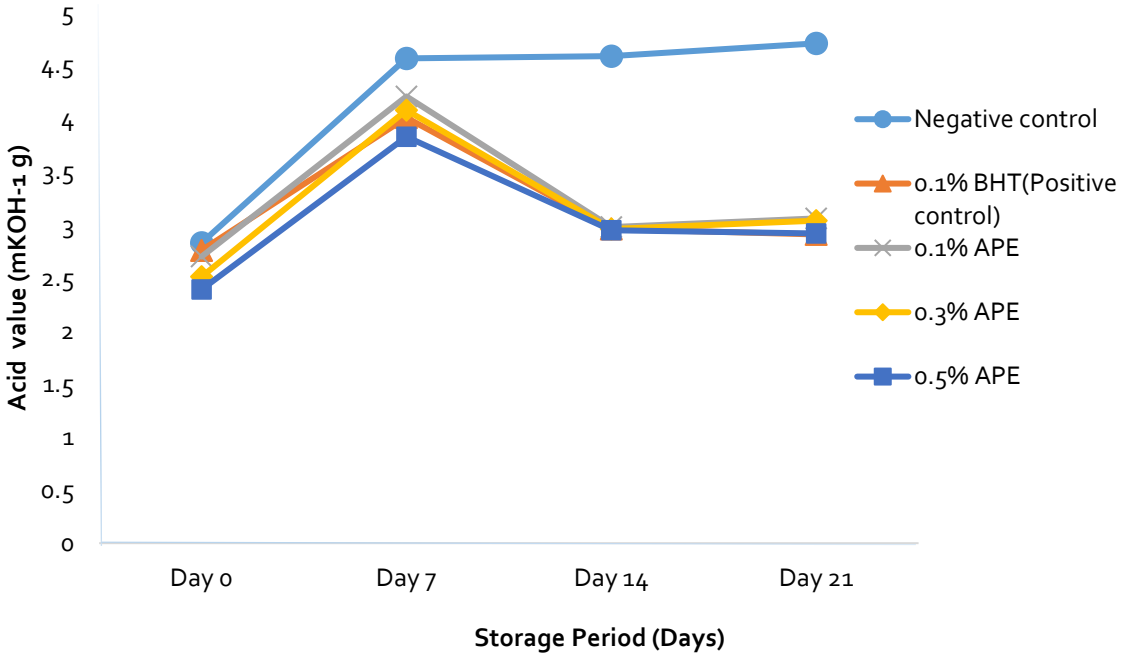

Fig. 10.: Effect of Different Concentrations of Ethanol Extract of Avocado Peel on Acid Value of Palm Oil During Storage

The acid value of palm oil treated with Avocado peel extract (APE) and the positive control increased sharply as the storage period increased and peaked on day 7 before declining and seemed to stabilize from day 14 till the end of the experiment (fig 10). The untreated sample (negative control) had significantly higher ($p<0.05$) acid value throughout the storage period. However, there was no significant difference ($p<0.05$) between the positive control and sample treated with up to 0.5% APE. The result also showed that the higher the concentration of APE, the more effective the extract in reducing the acid value.
The acid value of Groundnut oil treated with different concentration of ethanol extract of avocado peel (APE) under accelerated storage is presented in fig. 10.

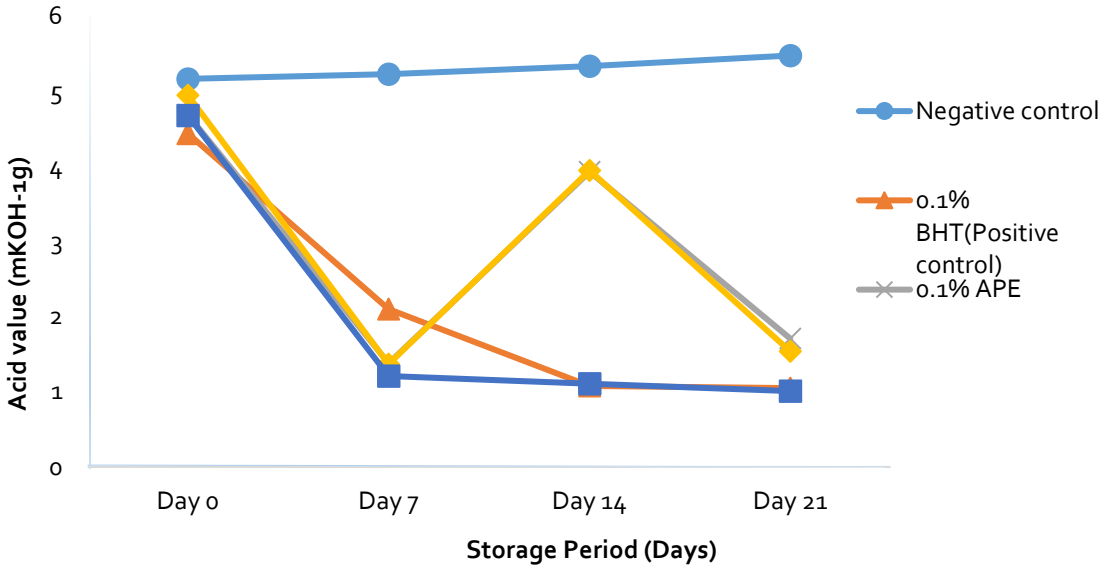

Fig 10.: Effect of Different Concentrations of Ethanol Extract of Avocado Peel on Acid Value of Groundnut oil during Storage

The effect of different concentrations of Avocado peel extract (APE) on the acid value of the groundnut oil during storage is presented in figure 10. The result showed that there was a sharp decrease in the acid value of the treated samples and the positive control within the first 7 days of storage. This decrease was more pronounced in the groundnut oil samples treated with APE. Untreated sample (negative control) had significantly higher ($p<0.05$) acid value than other sample throughout the storage period while sample treated with 0.5% APE had a significantly lower ($p<0.05$) acid value than other samples. In general, while the acid value of groundnut oil was significantly higher ($p<0.05$) at the onset of the experiment, followed by that of palm oil and then soybean oil, the extract appeared to have been more effective in reducing acid value in the order soybean oil> groundnut oil > palm oil.

Acid value is as an indicator of oil hydrolysis. It is formed due to the hydrolysis of triglycerides in oils and is important indicator in oil rancidity (Keefe and Pike, 2010)

Production of free fatty acids and increase in peroxide values are the best predictors of fat deterioration, which could be used to monitor the extent of oil spoilage (Chang *et al.*, 2016). Development of rancidity in oil (soybean, palm oil and groundnut oil) was affected by storage time.

Addition of avocado peel extracts at varying concentrations to vegetable oils lowered the amount of acid formed when compared to the control. Acid value of any lipid is a measure of hydrolytic rancidity (Rehab, 2010). The higher the acid values of any lipid, the higher the degree of hydrolytic rancidity that set in (Arawande and Amoo, 2009). It is also used to

measure the extent to which glyceride in the oil has been decomposed by lipase and other actions such as light and heat (Demian, 1990).

The acid value of soya bean oil sample without any additives was higher than that of the soya beans oil treated with varying concentrations of avocado peel extract. The acid value of treated soya bean oil with 0.5% of avocado peel extract was slightly lower than those treated with 0.1% BHT. This finding confirmed the finding of Abdel-aal and Hussein (2010) who reported that soya bean oil treated with ethanol extract of orange peel at concentration of 800, 1200 and 1600 ppm showed lower acid value than the soya bean oil treated with synthetic antioxidant (BHT, 200 ppm) at the end of the storage period. Sample treated with 0.5 % of avocado peel extract had similar effect with the positive control in inhibiting acid value of soyabeans on the 21 days of storage, while acid value of groundnut oil was significant ($p<0.05$) lower that than the 0.1% BHT. This may be due to the phenol compounds in the extract (Rodriguez-Carpena *et al.*, 2011).

The acid value content is the most widely used criterion for determining the quality of palm oil (Almeida, *et al.,* 2013). Codex 210 (codex Alimentarius, 2013) and Brazilian Legislation (Brasil, 2005) established a maximum concentration value of acid value up to 5.0% for crude palm oil in oleic acid. Thus, according to these norms, all palm oil treated with varying concentrations of avocado peel extract were within the set limit. Result showed that the Acid value in the treated oil samples were within the standard range proceeds**.** Thus, finding may indicate that the palm fruit were handled gently and processed rapidly after harvest and sterilized with steams in order to limit lipase activity (Vincent *et al.,* 2014). Tagoe, *et al.,* (2012) demonstrated that processed fresh palm fruit oil had initial acidity of 0.5% and 3% after 12 months of storage. Increase in the said oil 4.57 mg $NaoHg^{-1}$ on day 7 in acid value could be caused by endemic species of microorganism that were introduced into the oil during various stages of processing and transport within the plant (Nkpa *et al.,* 1990).

Palm oil tends to have higher moisture content and increased microbial load as storage time increases, allowing the hydrolytic reactions responsible for the formation of free fatty acid (Tagoe *et al.,* 2012).

The acid value of groundnut oil was quite high. This may be due to impurities that could cause the hydrolysis of esters linkages (Nkafamiya *et al.*, 2006) but increase in concentration of avocado peel extract gradually reduced the acid value. Hence there was no significant difference ($p<0.05$) between the treated and positive control samples.

Effect of Different Concentrations of Ethanol Extract of Avocado Peel on Peroxide Value of Vegetable Oil During Storage

The peroxide value of soybean oil treated with varying concentration of Avocado peel extract (APE) under accelerated storage is presented in figure 11.

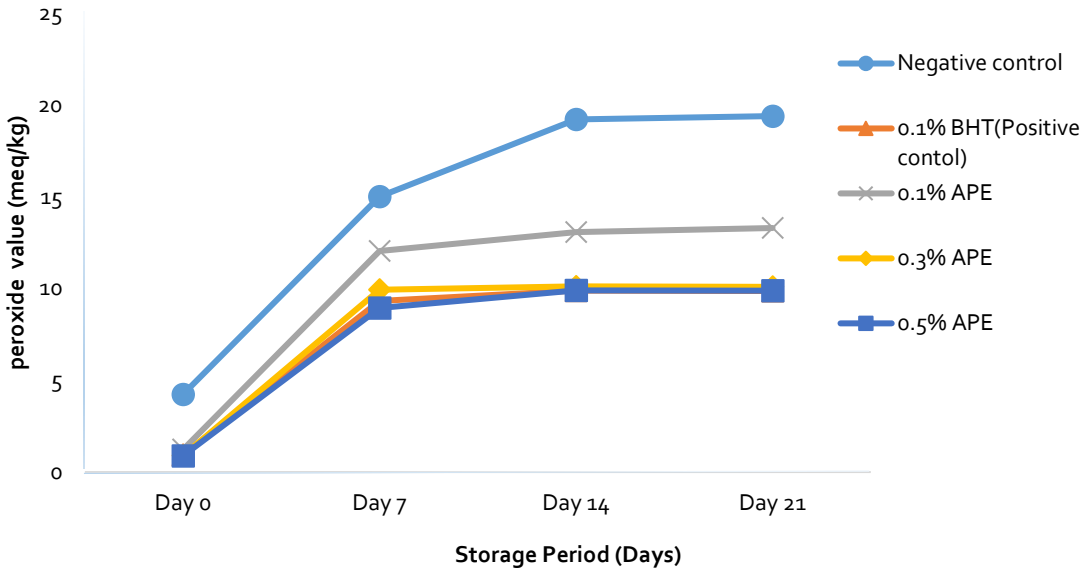

Fig 11.: Changes in Peroxide Value of Soybean Oil Treated with Varying Concentration of Ethanol Extract of Avocado Peel during a 21-day Storage Period.

The result showed that there was a sharp increase in the peroxide value of all samples within the first 7th day of storage but this increase became less thereafter. The untreated sample had significantly higher ($p<0.05$) peroxide value throughout the storage period while peroxide value decreased the more as the concentration of APE increased. There was no significant difference ($p<0.05$) in peroxide value between the positive control and the sample treated with 0.5% APE from day 14th of storage.

The peroxide value (PV) of palm oil treated with varying concentration of Avocado peel extract (APE) during a 21-day storage period is presented in figure 12.

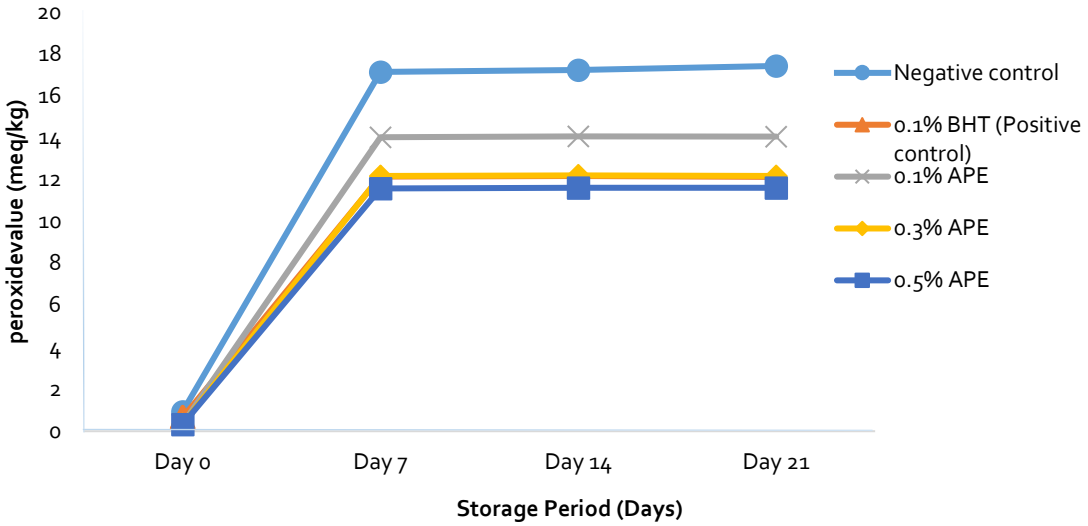

Fig. 12. Changes in Peroxide Value of palm oil Treated with Varying Concentration of Ethanol Extract of Avocado Peel during a 21-day Storage Period.

The result that the PV of both treated and untreated samples increased sharply between dayo and day 7th of the storage and there after seemed to stabilize till the end of the experiment. A similar trend was observed in the positive control. The untreated sample had a significantly higher ($p<0.05$) PV value than other samples throughout the storage period. Treatment with APE significantly reduced ($p<0.05$) peroxide values in palm oils. As the concentration of APE increased, reduction in PV of the sample became more. Sample treated with 0.5% APE had a significantly lower ($p<0.05$) PV than other sample (including positive control) from day 7th of storage.

Changes in peroxide value (PV) of groundnut oil treated with varying concentration of Avocado peel extract (APE) during a 21-day storage is presented in figure 12.

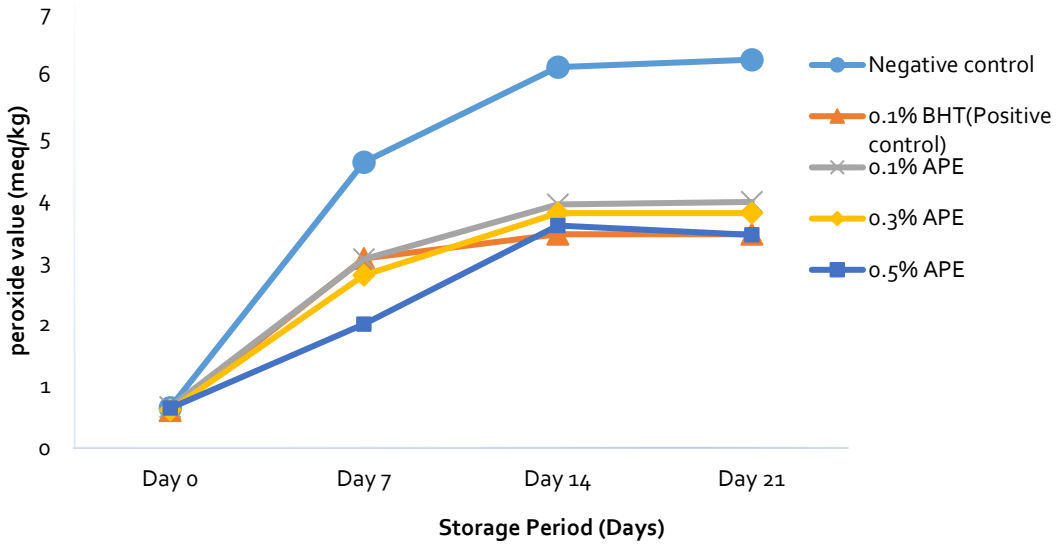

Fig 12.: Changes in Peroxide Value of groundnut oil Treated with Varying Concentration of Ethanol Extract of Avocado Peel during a 21-day Storage Period.

The result showed that the PV increased in both treated and untreated samples as well as the positive control as the storage increased. Untreated groundnut oil (negative control) had a significantly higher PV than other samples throughout the storage period. Treatment with Avocado peel extract (APE) significantly reduced ($p<0.05$) PV in groundnut oil. Reduction in PV was more pronounced as the concentration of APE increased. There was no significant difference ($p<0.05$) between the PV of the sample treated with 0.5% APE and the positive control at the end of the experiment. In general, the PV of soybeans oil was higher (0.89-4.24 meq/kg) at the beginning of the experiment, followed by groundnut oil (0.61-0.65 meq/kg) and then palm oil (0.33-0.94 meq/kg). However, at the end of the experiment the extract appeared to have been effective in reducing PV in the order of groundnut oil followed by soybean oil and palm oil. Peroxide value is the most common method in measuring the oxidative deterioration in oil (Arawande *et al.*, 2009). Peroxides are the main primary oxidation products, since high amounts of peroxides consist of low oxidative stability (Yang *et al.*, 2016). Significance difference ($p<0.05$) in peroxide value was observed between the control and the oil treated with avocado peel extract which slowed the rate of peroxide formation. However, inhibition of oxidation rate of palm oil, soybean oil and groundnut oil by avocado peel extract, was slightly dependent on concentrations. Increase in PV value within the 7 and 14-day storage showed that soybean oil palm oil and groundnut oil which demonstrated the oxidation reaction was transformed to intermediate or advanced oxidation stage, and a large number of hydroperoxides were generated because of many free radical and intermediates. A decline was observed in all treatments after reaching their peak. This may be due to the fact that the primary products of oxidation

(hydroperoxides) formed during the accelerated storage can be degraded into low molecular weight compounds and also volatile substances such as aldehydes which were unstable and highly susceptible to further breakdown into secondary oxidation products (Ruiz *et al*., 2001). The effectiveness of the avocado peel extract in reducing the peroxide value in treated oil sample may due to the bioactive compounds in the extract (Guerrero *et al*., 2008). Similar studies have been reported by Shahid *et al*., (2018b) where cinnamon extract exhibited antioxidant effect on palm oil stability. However, similar studies were reported by Kosinska *et al*. (2012) on the flavonoid and catechin compound in avocado seed on the antioxidant effect on soybean oil. The impact of potato peel extract on the oxidative stability of sun flower oil, comparing it with synthetic antioxidant (BHA&BHT) was studied, result indicated a decrease in 48% in peroxide value and 0.55% in acid value (Saeed *et al.*, 2022).

Antibacterial Activity of Avocado Peel Extract

The antibacterial activity of ethanol extract of avocado peel is presented in Table 9. The result showed that avocado peel extract inhibited the growth of *Staphylococcus aureus, Salmonella typhii and E. coli* at varying degrees. As for minimum inhibitory activity, the result showed that the inhibitory effect of avocado peel extract on *staphylococcus aureus* was effective from 20 mg/ml, showing 12 mm zones of inbition, *Salmonella typhii* 10 mg/ml with 10 mm zone and *Escherichia coli* 10 mg/ml with 10 mm zone. The extract significantly inhibited *Staphylococcus aureus* more followed by *Salmonella typhii* and then *Escherichia coli.* There was a significant difference ($p<0.05$) in the zone of inhibition diameter of *S. aureus* as the concentration of the extract increased from 100-500 mg/ml. Similarly, there was a significant difference in the zone of inhibition diameter of *S. typhii* as the concentration of the extract increased from 100-500mg/ml and that of *E. coli* as the extract concentration increased from 100-500 mg/ml. However, the test samples (*Staphylococcus aureus, Salmonella typhii and Escherichia coli*) had a significantly lower ($p<0.05$) inhibition zone diameter than ciprofloxacin (100 mg/ml) which was used as control.

Table 9: Minimum Inhibitory Concentration and Antibacterial Activity of Avocado Peel Extract against Pathogenic Organism

Conc. (mg/ml)	*Staphylococcus aureus*	*Salmonella typhii*	*Escherichia Coli*
	Inhibition zone Diameter (mm)		
5	0.00 ± 0.00^{g}	0.00 ± 0.00^{f}	0.00 ± 0.00^{e}
10	0.00 ± 0.00^{g}	10.0 ± 0.00^{e}	10.0 ± 0.01^{d}
15	0.00 ± 0.00^{g}	10.0 ± 0.00^{e}	10.0 ± 0.00^{d}
20	12.0 ± 0.01^{f}	10.0 ± 0.00^{e}	10.0 ± 0.00^{d}
25	14.0 ± 0.00^{e}	10.0 ± 0.00^{e}	10.0 ± 0.00^{d}
100	15.0 ± 0.01^{d}	12.0 ± 0.01^{d}	12.0 ± 0.00^{c}
150	15.0 ± 0.00^{d}	12.0 ± 0.00^{d}	12.0 ± 0.00^{c}
200	15.0 ± 0.01^{d}	12.0 ± 0.00^{d}	12.0 ± 0.00^{c}
250	17.0 ± 0.00^{c}	12.0 ± 0.00^{d}	12.0 ± 0.01^{c}
300	17.0 ± 0.00^{c}	14.0 ± 0.00^{c}	14.0±0.00^{b}
350	17.0 ± 0.01^{c}	14.0 ± 0.01^{c}	14.0±0.00^{b}
400	17.0 ± 0.00^{c}	14.0 ± 0.00^{c}	14.0±0.00^{b}
450	18.0 ± 0.00^{b}	15.0 ± 0.00^{b}	14.0± 0.00^{b}
500	18.5 ± 0.00^{b}	15.0 ± 0.00^{b}	14.0 ± 0.00^{b}
Ciprofloxacin	19.5 ± 0.00^{a}	17.0 ± 0.00^{a}	17.05± 0.00^{a}

Samples with different superscripts within the same row were significantly (p<0.05) different. Ciprofloxacin was used as positive control

The antibacterial activity of ethanol extract of avocado peel with varying concentrations showed inhibitory effect on *Staphylococcus aureus, Salmonella typhii* and *Escherichia coli* this may be due to phenolic compounds in the peel (Singh *et al*, 2019). Plant extracts are sources of variety of antimicrobial compounds. Studies have been conducted in order to evaluate characteristics of avocado peel extracts, which can be used for the treatment of disease, due to their antimicrobial, antifungal, analgesic and anti-inflammatory activities (Rodriguez-Carpena *et al*., 2011). There was a significant effect (P<0.05) among the tested microorganism (*Staphylococcus aureus, salmonella typhii and Escherichia coli*). The leaf, stem, fruit and peel of avocado have biological activities. Proven studies with seed demonstrated antioxidant and antimicrobial activities against *Bacillus cereus, staphylococcus aureus, listeria monocytogenes, Escherichia coli and pseudomonasSpp* Rodriguez *et al.* (2011). Vinha *et al*. (2013) have reported on the bioactive compounds (phenolics, flavonoids, carotenoids, ascorbic acid and vitamin E) of edible and non-edible part (pulp, seed and peel) of avocado variety. Similar studies by Rikomah *et al (*2019) reported higher concentration of avocado leaf extract of 70% produces an effective inhibition zones against *Esherichia Coli.*

Conclusion

Avocado peel utilization as a source of antioxidant for food preservation holds great promise for both environmental and economic perspectives. The use of response surface methodology (RSM) enhanced the release of phenolic compounds at it high concentration, thus preventing and exhibiting antioxidant potentials. We investigated the optimum concentration, time and temperature for extracting the avocado peel. The most effective concentration was 80% ethanol, 90min and temperature 60 °c. The incorporation of avocado peel extract at >3% enhances the oxidative stability of meat and oil samples tested thus enhancing the food storage within a period of 21 days when compared to positive control BHA. Avocado peel ethanol extract exhibited antibacterial properties against *S. typhi* from 20-500 mg/ml, *E. coli* and *S. aureus* at a concentration of 10-500 mg/ml.

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