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Bioactive Compounds and Physicochemical Characteristics of Avocado Pear (*Peresea americana* Mill) Peel Oil Extracts and Microcapsules

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Authors' contributions

This work was carried out in collaboration among all authors. Authors MAO and NOA wrote original draft, reviewed the manuscript. Author MAO did data analysis. Authors MAO, NOA and OOA did Validation. Author TEA did formal analysis. Author TEA performed the methodology. Author TEA wrote original draft. Author TEA data collected & analysis. Author OOA conceptualized and experiment designed the research work. Author OOA supervised the study. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Fruit peels such as avocado peels are rich in bioactive compounds. Avocado peel is usually discarded after consumption of the pulp. This study was aimed at harnessing the rich antioxidant properties of the peel extract in the formulation of bioactive-rich microcapsule.
Study Design: The study design involved evaluating the microcapsules effectiveness and efficiency through the analytical evaluation of the physicochemical and microstructural properties.
Place and Duration of Study: Department of Food Science and Technology, Federal University of Agriculture Akure**.**
Methodology: Avocado peel oil was extracted using hexane and methanol and the extract with better bioactive activities was microencapsulated using corn and cassava starch cell wall materials. The bioactive contents and stabilities of the extracts and microcapsules were evaluated.
Results: While hexane extract had better physicochemical activities, the methanolic extract had better bioactive activities. The fatty acid profile of avocado peel oil was 87% saturated, 3.5% monounsaturated and 9.4% polyunsaturated. Corn starch was a better cell wall material for retaining total phenol, DPPH radical scavenging activities, $OH^{\bullet}$ scavenging ability and *in-vitro* starch digestibility than cassava starch cell wall. The FTIR indicated OH, C=H, C=O and C-H as the main functional groups.
Conclusion: This investigation proposes the utilization of avocado peel extract as a promising, low-cost source of bioactive compounds suitable for microencapsulation with high bioactive retention at 30 days.

Keywords: Avocado peel; bioactive activities; cell wall materials; corn starch; cassava starch; food nanotechnology; FTIR; microencapsulation.

1. INTRODUCTION

Many organic by-products generated in food industries and generally referred to as food wastes may serve as potential sources of antioxidant compounds [1]. Natural antioxidants, especially polyphenols, have many advantages such as decreasing the risk of inflammatory diseases and preventing lipid oxidation [2]. Nigeria is blessed with various plant food materials such as avocado pear which is a rich source of antioxidants.

Avocado pear (*Persea americana* Mill.), a tropical and subtropical fruit rich in oil, belongs to the *Lauraceae* family. The avocado fruit is made up of many varieties worldwide [3]. The fleshy pulp contains high-quality oil rich in palmitic and oleic acids with physicochemical characteristics similar to those of olive oil [4]. Avocado fruit is commonly regarded as a functional fruit because of its bioactive compounds which are useful to human health [5]. In addition, it contains significant levels of protein, fibre, vitamins and minerals.

"There is increasing research interest in the utilization of plant-based bioactive compounds for the development of nutraceuticals" [6]. Recently, "functional foods or nutraceuticals from plant sources are being used as viable alternatives to synthetic drugs in the treatment and management of neuro-degenerative and cardiovascular diseases since they are non-toxic and do not generally have negative adverse effects as compared to synthetic drugs" [7].

"Food nanotechnology is a vital tool for increasing the effectiveness of the use of nutraceuticals because it ensures bioavailability of the bioactive compounds at micro and nano scales, thereby promoting controlled release of the encapsulated nutrient to targeted sites" [8]. This study characterized the hexane and methanolic extracts of avocado peel oil for their physicochemical properties, bioactive and antinutritional composition and their potential for use as microcapsule.

2. MATERIALS AND METHODS

2.1 Raw Materials

Avocado pear (*Persea americana* Mill) was purchased from Owena market, Akure, Ondo State, Nigeria. The food material was authenticated at the Department of Crop, Soil and Pest Management, Federal University of Technology Akure, Nigeria. The chemicals for the analysis were of analytical grade [9].

2.2 Preparation of Avocado Peel Powder

The mature fruits were washed under running tap water until thoroughly clean, air-dried for 15 min and packed into an airtight black polyethylene bag for a period of 5 days to promote its ripening process for easy removal of the peel from the pulp. The peels (mesocarp) were manually removed from the flesh, washed thoroughly, sundried for 3 - 5 days, milled into fine powder using an attrition milling machine (Munson's Model SK-30-SS) and subsequently stored in refrigerator at 4^{o} C until further use [10].

2.3 Determination of Proximate Composition of Avocado Peel Powder

Avocado peel powder was analyzed for moisture, crude protein, crude fat, total ash and crude fibre using AOAC [11] standard methods. Moisture content was determined by drying in oven (Galenkamp, hot box, London, UK) at 105^{o} C, until constant weight was obtained. Total nitrogen was determined using micro Kjedahl method and a conversion factor of 6.25 was used to calculate crude protein. Crude fat was determined using 50% *n*-hexane for continuous solvent extraction for 4 h using the Lab Tech Scientific Works, LTSW-5 Soxhlet apparatus manufactured in Ambala, Haryana India. Total ash was determined by ashing at 550^{o} C for 3 h. Crude fibre was obtained by digestion and carbohydrate was determined by difference. The energy value (kcal/100 g) of the peel powder was estimated using the Atwater factors for protein (4.2 kcal/100g), carbohydrate (4.2 kcal/100 g), and fat (9 kcal/100 g) according to Iombor et al. [12].

2.4 Extraction of Avocado Peel Oil Extract by Soxhlet Extraction

The method described by Awolu et al. [13] with slight modification was used. Fifty grams (50 g) of avocado peel powder was tightly packed into a muslin cloth and placed inside the thimble of the soxhlet extractor. The solvents (*n* hexane and methanol) were used and the extraction carried out at the boiling temperature of each solvent for 3 h, according to Awolu and Manohar [14]. Excess solvent was removed in a rotary evaporator (Buchi R- 210, Flawil, Switzerland). The extracts obtained were stored in an air tight bottle in a laboratory refrigerator having a temperature of about 3 ^{o}C prior to further analysis. The percentage (%) yield was calculated using the equation:

$Percentage$ yield of oily extract =

$$\frac{\text{Weight of oil}}{\text{weight of sample}} \times 100 \qquad \text{Eq. (1)}$$

2.5 Determination of Peroxide Value (PV) of Extracts

Five grams of the oily extract was dissolved in 30 mL of glacial acetic acid:chloroform (3:2 v/v). About 0.5 ml of saturated potassium iodide (KI) was added and I_2 was liberated by the reaction with the peroxide. The solution was then titrated with standardized sodium thiosulphate using starch indicator [15].

$$PV\ (\text{Mq/kg}) = \frac{(S-B)\ X\ M\ X\ 1000}{Sample\ weight\ (g)} \qquad \text{Eq. (2)}$$

Where,

S = Sample titre value; B = Blank titre value; M = Molarity of $Na_2S_2O_3$.

2.6 Determination of Iodine Value (IV)

"About 0.1 M iodine monochloride in acetic acid was added to 0.2 mL of the oily extract dissolved in cyclohexane. The mixture was allowed to stand for 10 min to allow halogenation. About 0.1 M of KI solution was added to reduce excess iodine monochloride and to free iodine. The liberated iodine was titrated with a standardized solution of 0.1 M sodium thiosulphate using starch indicator" [15].

$$Iodine\ value = \frac{(B-S)\ X\ M\ X\ 1000}{Sample\ weight\ (g)} \qquad \text{Eq. (3)}$$

Where B =blank titre value; S = sample titre value; M = Molarity of $Na_2S_2O_3$; 12.69 = Conversion factor from Meq. $Na_2S_2O_3$ to gram iodine, molecular weight of iodine is 126.9 g.

2.7 Determination of Percentage Free Fatty Acids (% FFA)

Exactly 2 g of cooled oily extract sample was weighed into a 250 ml conical flask and 75 ml of freshly neutralized hot ethyl alcohol together with 1 ml phenolphthalein indicator solution were added. The mixture was boiled for 5 min and titrated hot against standard alkali solution [15],

$$Acid\ value = 56.1VN/W \qquad \text{Eq. (4)}$$

Where,

V = volume (ml) of standard KOH used,
N = Normality of the KOH solution
W = weight of the sample (g)

The percentage free fatty acid of the extracts was calculated using equation,

$$\%\ free\ fatty\ acid\ (oleic) = \frac{Acid\ value}{2} \quad \text{Eq. (5)}$$

2.8 Determination of Saponification Value (SV)

Two grams of the oily extract was added to excess alcoholic KOH. The solution was heated for 2 min to saponify the oil. The unreacted KOH was back-titrated with standardized 0.1 M HCl using phenolphthalein indicator.

$$Saponification\ Value = \frac{(S-B)\ X\ M\ X\ 56.1}{Sample\ weight\ (g)} \quad \text{Eq. (6)}$$

Where, S = Sample titre value; B = Blank titre value; M = Molarity of the HCl; 56.1 = Molecular weight of KOH

2.9 Determination of Specific Gravity

A clean and dry bottle of 100 mL capacity was weighed (W_0) and then the beaker was filled with the oily extract and reweighed to give (W_1). The oil was substituted with water after washing and drying the bottle and weighed to give (W_2). The specific gravity was calculated as:

$$Specific\ gravity = \frac{(W_1-W_2)}{W_2-W_0} \quad \text{Eq. (7)}$$

Where,

W_1= Mass of oil + Mass of beaker
W_o= Mass of beaker
W_2 = Mass of water + Mass of beaker

2.10 Determination of Thiobabituric Acid (TBA)

The method of Zeb and Ullah [16] was used. The standard solution of 4.0 mM of thiobarbituric acid (TBA) was prepared in glacial acetic acid. Standard stock solution (1 mM) of malondi-aldehyde tetrabutylammonium (MDA) was also prepared in glacial acetic acid from which different concentrations of 0.1, 0.2, 0.4, 0.6, and 0.8 mM were prepared. The standard MDA solution (1 mL) was mixed with TMA (1 mL) in a 10 mL test tube, and the mixture heated in a boiling water bath at 95^o C for 60 min, and later cooled to room temperature. The absorbance was measured at 532 nm using UV-visible spectrophotometer. Each standard for the calibration was repeated three times while a standard sample was prepared by replacing standard or sample with water. The extract of each sample (1 mL) was mixed with 1 mL TBA reagent and the above procedure was repeated five times (n = 5). The TBARS was calculated using the formula as μM/g of the sample:

$$TBARS\ (\mu M/g) = (Ac\ x\ V)\ /W \quad \text{Eq. (8)}$$

Where,Ac is the amount determined from the calibration curve and W is the weight of the sample taken while V is volume in mL or dilution factor of the total extract prepared.

2.11 Determination of the Total Phenolic and Total Flavonoid Content of the Avocado Peel Extract

The total phenolic content of the extracts was determined by the method described by Singleton et al. [17]. Exactly 0.2 mL of each sample extract was measured into a test tube and mixed with 2.5 mL of 10% Folin ciocalteau's reagent and 2 mL of 7.5% Sodium carbonate. The reaction mixture was subsequently incubated at 45^o C for 40 min, and the absorbance was measured at 550 nm using spectrophotometer (JENWAY 6305, United Kingdom). Gallic acid was used as standard phenol and total phenol was calculated and expressed as GAE.

The total flavonoid content was determined by aluminum chloride colorimetric assay [18]. About 500 µL of methanol was added to 10 mL flask containing 500 µL of aqueous extract. To this 50 µL 10% $AlCl_3$ and 50 µL of 1M CH_3COOK were added. The total volume was made up to 2500 µL with distilled water. The solution was then incubated at room temperature (28 ^{o}C) for 30 min. Absorbance was read against blank at 510 nm using spectrophotometer (JENWAY 6305, United Kingdom). Flavonoid content was calculated using quercetin as standard.

2.12 Determination of DPPH Radical Scavenging Ability of Oil Extracts

The method of Gyamfi et al. [19] was used for determination of DPPH radical scavenging

activity of the extracts. Exactly 1 mL of each extract was mixed with 1 mL of 0.4 mM methanolic solution of the DPPH in a test tube. The solution was allowed to stand for 30 min in the dark after which the absorption was measured, at 516 nm wavelength, with spectrophotometer (JENWAY 6305, United Kingdom). The percentage DPPH radical scavenging activity of the samples were determined using the equation:

DPPH radical scavenging activity (%) =

$$1 - \frac{A_{517\ of\ sample}}{A_{517\ of\ blank}} x\ 100 \qquad \text{Eq. (9)}$$

2.13 Chelation of Metal Ions

The ability of the extract to chelate Fe^{2+} was determined using a modified method reported by Puntel et al. [20]. $FeSO_4$ (150 mM) was added to a reaction mixture containing 168 mL of 0.1M Tris-HCl pH 7.4, 218 mL saline and extract and the volume was made up to 1 mL with distilled water. The reaction mixture was incubated for 5 min, before the addition of 13 mL of 1, 10-phenantroline and the absorbance was read at 510 nm.

2.14 Hydroxyl Radical Scavenging Ability

The hydroxyl radical scavenging activity of the extracted samples was determined according to the method reported by Girgih et al. [21]. Experimental samples, Glutathione and 1, 10 phenanthroline (3 mM) were separately dissolved in 0.1 M phosphate buffer (pH 7.4) while $FeSO_4$ (3.0 mM) and 0.01% hydrogen peroxide were separately dissolved in distilled water. An aliquot (50 µL) of samples was first added to a test tube, followed by 50 µL of 1, 10 phenanthroline and then 50 µL of $FeSO_4$. To initiate the Fenton reaction in the wells, 50 µL of hydrogen peroxide was added to the mixture, covered and incubated at 37° C for 1 h with shaking. The absorbance was measured using a spectrophotometer (JENWAY 6305, United Kingdom) at 536 nm at 10 min intervals for 1 hour. The hydroxyl radical scavenging activity was calculated using the reaction rate (DA/min).

OH radical scavenging activity (%) =

$$\frac{(\Delta A_{536}/min)\text{b} - (\Delta A_{536}/min)\text{s}}{(\Delta A_{536}/min)\text{b}} x\ 100 \qquad \text{Eq. (10)}$$

2.15 Ferric-Reducing Antioxidant Property (FRAP)

The ferric-reducing antioxidant power of each sample extract was determined by the method described by Pulido et al. [22]. Exactly 0.25 ml of each sample extract was measured into a test tube and mixed with 0.25 ml of 200 mM Sodium phosphate buffer pH 6.6 and then 0.25 ml of 1% KFC was added. The mixture was incubated at 50 ºC for 20 min and thereafter, 0.25 ml of 10% TCA was added and centrifuged at 2000 rpm for 10 min. Then, 1 mL of the supernatant was mixed with 1 mL of distilled water and 0.1% of $FeCl_3$. The absorbance was measured at 700 nm using spectrophotometer (JENWAY 6305, United Kingdom).

2.16 ABTS Radical Scavenging Activity

The ABTS scavenging ability of the extracts was determined according to the method described by Re et al. [23]. The ABTS was generated by reacting a 7 mM ABTS aqueous solution with $K_2S_2O_8$ (2.45 mM/L, final concentration) in the dark for 16 h and adjusting the absorbance at 734 nm to 0.700 with ethanol. About 0.2mL of the appropriate dilution of the extract was then added to 2.0 mL of ABTS solution and the absorbance was read at 732 nm after 15 min. The $ABTS^{+\bullet}$ scavenging activity was calculated.

$$ABTS^{*}\ scavenging\ ability\ (\%) =$$

$$\frac{Abs._{ref} - Abs._{sample}}{Abs._{ref}} \text{ x } 100 \qquad \text{Eq. (11)}$$

$$ABTS^{*}\ scavenging\ ability\ (mmol/g) =$$

$$\frac{Per._{sample}\ x\ Conc._{standard}}{Per_{\ standard}\ x\ Conc._{sample}} \text{ x } TMW \qquad \text{Eq. (12)}$$

2.17 Determination of Oxalate Content

The method described by Ukpabi and Ejidoh [24] was used for oxalate content determination. Avocado peel oily extract (2 mL) was digested with 10 mL 6 M HCl for 1 h and made up to 250 mL in a volumetric flask using a funnel. The pH of the filtrate was adjusted with concentrated NH_4OH solution until the colour of the solution changed from salmon pink to faint yellow. Thereafter, the filtrate was treated with 10 mL of 5% $CaCl_2$ solution to precipitate the insoluble oxalate. The suspension was centrifuged at 2500 x g, after which the supernatant was decanted. The precipitate was dissolved in 10 mL of 20%

(v/v) H_2SO_4 and the solution was made up to 300 mL. An aliquot (125 mL) was heated until near boiling point and then titrated against 0.05 M standardized $KMnO_4$ solution to faint pink which persisted for about 30 s after which the burette reading was taken and used to estimate the oxalate content.

Oxalate (mg/g) =

$$\frac{(titre\ value\ X\ volume\ of\ KMNO_4\ X\ dilution\ factor)/5}{Sample\ size} \quad \text{Eq. (13)}$$

2.18 Determination of Phytate Content

The determination of phytate in the extracts was carried out using the method described by Abulude [25]. Eight millilitre (8 mL) of oily samples was dispersed in 200 mL of 2% HCl. Following extraction, the dispersion was filtered and 50 mL of the filtrate was mixed with 10 cm^3 of 0.3% ammonium cyanide (NH_4SCN) and diluted with 107 mL of distilled water. The extract was titrated against 0.00195 g/mL of Ferric chloride solution until a brownish yellow colour persisted. Phytate content was estimated from the expression:

$$phytate\ content =$$

$$(iron\ equivalent\ \times\ 1.95\ g\ of\ titre) \times 3.65g \quad \text{Eq. (14)}$$

2.19 Determination of Saponin Content

The method described by Obadoni and Ochuko [26] was used to "determine the saponin content of the avocado peel oily extract. Twenty millilitres of each extract was put into a conical flask and 100 cm^3 of 20% aqueous ethanol was added. This was heated over a hot water bath for 4 h with continuous stirring at about 55° C. The mixture was filtered and the residue re-extracted with another 200 mL 20% aqueous ethanol. The combined filtrate was concentrated to 40 mL with the water bath at about 90° C. The concentrate was transferred into a 250-mL separation funnel and 20 mL of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. This purification step was repeated. About 60 mL of n-butanol was added, the combined butanol extract was washed twice with 10 mL of 5% aqueous sodium chloride. The remaining solution was heated in a water bath for evaporation to take place and the samples dried at 100 °C in the oven (Jointlab STF-F 52 FALC; Italy) to a constant weight. Saponin content was calculated in mg/g".

2.20 Determination of Terpenoid

Terpenoid in the avocado peel oily extracts was determined according to procedure described by Sofowora [27]. About 0.5 ml of oil sample was measuured into a 50-ml conical flask, then 20 ml of chloroform: methanol (2:1) was added and the mixture was shaken thoroughly and allowed to stand for 15 min at room temperature. The suspension was centrifuged at 3000 rpm, and the supernatant was discarded while the precipitate was re-washed with 20 ml chloroform: methanol (2:1) and then re-centrifuged again. The precipitate was dissolved in 40 ml of 10% sodium dodecyl sulfate solution. About 1ml of 0.01M ferric chloride was added and allowed to stand for 30 min. Absorbance was read at 510 nm and compared with the standard (alpha terpenoid).

2.21 Determination of Steroid

A quantitative determination of steroid was determined by weighing 5 ml of the oily extract sample to 100 m conical flask and 50 ml of pyridine was added to it, and shaken for 30 min at room temperature. Thereafter, 3 ml of 250 mg/ml metallic copper powder or copper (1) oxide was added and allowed to incubate for 1 h in the dark. Absorbance was measured at 350 nm against reagent blank [27].

2.22 Determination of Glycosides

The procedure described by Sofowora [27] was used. About 10 ml of the oily extract was pipetted into a 250-mL conical flask and 50 ml chloroform was added and shaken on vortex mixer for 1 h. The mixture was filtered using Whatman No. 3 filter paper into 100 mL conical flask. About 10 mL of pyridine and 2 ml of 29 % of sodium nitroprusside were added and shaken thoroughly for 10 min. Exactly 3 ml of 20 % NaOH was added to develop a brownish yellow colour. Glycosides standard (Digitoxin) was used to prepare standard solutions with concentration ranging from 0 – 50 mg/ml and the absorbance was read at 510 nm.

2.23 Fatty Acid Profile of the Avocado Peel Oil Methanolic Extract

"The GC-MS of the methanol extracted oily extract was carried out using a Trace GC Ultra (Thermo, USA) gas chromatograph coupled with a DSQ II (Thermo) mass selective detector equipped mass spectrometer column. The mass-

selective detector was operated in electron-impact ionization (EI) mode with a mass scan range from m/z 40 to 550 at 70 eV. The oven temperature was maintained at 40° C for 3 min, and then raised to 100° C at a rate of 3 min, and to 180° C at a rate of 5 min, and finally to 300° C at a rate of 20 min. Helium was used as the carrier gas at a flow rate of 1 mL/min. A diluted sample (0.5 µL, 1/103 v/v, in dichloromethane) of extract was injected automatically and the GC split ratio used was 1:20. Kovats indices was calculated for all volatile constituents by use of a homologous series of C_5–C_{15} n-alkanes on the DB-5ms column" [28].

2.24 Encapsulation of Avocado Peel Extract

The method of Sangeeta et al. [28] was adopted for "encapsulation of the avocado peel extract. The extract obtained using 100% methanol was used for microencapsulation due to its high antioxidant potentials. Appropriate quantity of the extract, cell wall materials [corn starch (A) and cassava starch (B)], gum Arabic, Tween 80 and water were added into 250 mL plastic beaker as presented in Table 1. The samples were homogenized at 12,000 rpm for 7 min using a Lab GEN 700 Cole Parmer homogenizer. The homogenized samples were then freeze dried (frozen at – 20º C in a freezer for 24 h and then lyophilized in a freeze dryer). The freeze dried microencapsulates obtained were stored in an airtight container at room temperature".

2.25 Scanning Electronic Microscopy of the Microencapsulate

The SEM analysis of the microcapsules was performed by the use of a Quanta 400 thermal field environment scanning electron microscope (FEI instruments Co., Ltd., the Netherlands). The microspheres were examined and photographed at an accelerating voltage of 10 kV. The samples were coated with gold prior to observation.

2.26 Fourier Transform Infrared Spectroscopy (FTIR) of the Microencapsulate

The freeze dried microencapsulated samples with a high antioxidant profile for both corn starch (A2) and cassava starch (B2) wall material were characterized by Fourier Transform Infrared Spectroscopy (FTIR) using Infrared spectrometer (Varian 660 MidIR Dual MCT/DTGS Bundle with ATR). Before analysis, the samples were dried in an auto-desiccator for 24 h. Samples were directly applied to a diamante crystal of ATR and resulting spectra were corrected for background air absorbance. Potassium bromide (KBr) disks were prepared from powdered samples mixed with dry KBr in the ratio of 1:100. The spectra were recorded in a transmittance mode from 4000 to 500 cm^{-1} wavenumbers at a resolution of 4 cm.

2.27 *In vitro* Starch Digestibility of the Microencapsulates

In –vitro starch digestibility of microencapsulates were determined using pancreatic amylase and alpha glucosidase according to the method described by Singh et al. [29]. Each sample encapsulate (50 mg) were dispersed in 1 ml of 0.2 M phosphate buffer with pH 6.9 and 20 mg of the enzyme was dissolved in 50 mL of the same buffer. The mixture containing 0.2 ml of both the sample and enzyme; and 1 ml of DNSA reagent was heated for 5 min in a boiling water bath. After cooling, the absorbance of the solution was read at 540 nm against the blank containing buffer and maltose was used as a standard.

Table 1. Microencapsulation formulation of avocado peel extracts using corn starch wall (A) and cassava starch wall (B) materials

S/N	Avocado extract (mL)	Wall Material (Corn starch (A) g)	Wall Material (Cassava starch (B) g)	Gum Arabic (g)	Tween 80 (mL)	Water (mL)
A1	5.0	50	-	0	0.5	199.5
A2	4.9	49	-	1.0	0.5	199.5
A3	4.8	48	-	2.0	0.5	199.5
B1	5.0	-	50	0	0.5	199.5
B2	4.9	-	49	1.0	0.5	199.5
B3	4.8	-	48	2.0	0.5	199.5

2.28 Storage Stability of Avocado Peel Extract Microcapsules

The moisture content of the various microcapsules was carried out immediately after freeze drying and after 30 days, using the method described by AOAC [11]. This was meant to evaluate the moisture content of microcapsules during shelf storage at room temperature.

2.29 Total Phenol and Antioxidant Activities of Microencapsulate During Storage

Total phenol [17], DPPH [19] and metal Chelating assay [20] of the microencapsulates were determined immediately after freeze drying and 30 days after.

2.30 Statistical Analysis

All experiments were carried out in triplicate and errors were recorded as standard deviation from the mean. The optimization data was analyzed by ANOVA using Design Expert 6.0 software and some of the data were subjected to Duncan's multiple range tests at p=0.05 significance level using SPSS version 17 while some were subjected to T- test.

3. RESULTS AND DISCUSSION

The proximate composition of avocado peel powder is presented in Table 2. The value for moisture, crude fat, total ash, crude fibre, protein and carbohydrate were higher than the values (Moisture, total ash, protein, and carbohydrate 2.3, 2.0, 6.4, and 40.7 g/100g, respectively) reported by Morais et al. [30] except for crude fat, crude fibre and moisture content (4.7, 43.9, and 2.3 g/100g, respectively). Variation obtained in the result may be due to environmental factors such as geographical location. Values reported for crude fibre in the present study were also higher than 14.80 g/100g and 14.49 g/100g reported for pineapple and orange peels, respectively by Feuman et al. [31], thus, indicating that avocado peel is a good source of fibre. "Fibre has been reported to assist in satiation and the movement of food through the alimentary canal by aiding muscular action of the intestine, thereby reducing the incidence of constipation" [32]. The low moisture content of the avocado peel powder may help in ensuring prolonged storage stability by preventing mould growth, and other moisture-dependent deteriorative processes and biochemical reactions. The crude fat content of 6.71g/100g may indicate that avocado peel powder may be a good source of fat-soluble vitamins and its consumption may facilitate absorption of fat-soluble vitamins.

3.1 Percentage (%) Yield of the Avocado Peel Oil Extract

The mean percentage yield of avocado peel oily extract using 100% hexane (APOH) and 100% ethanol (APOM) is shown in Fig. 1. Yield obtained using hexane (25.15%) was significantly (P=05) higher than methanol yield (20.73%), an indication that 100% hexane is more effective for oil extraction from avocado peel than 100% methanol. Vega et al. [33] reported "soxhlet extraction to be more effective for extraction of anthocyanin in Renealmia Alpinia mass peel than agitation and power ultrasound. The high value observed for hexane in this study may be an indication that compounds present in the extract have more affinity for non-polar solvents (such as hexane) than for ethanol which has both polar and non-polar characteristics [33]. Hexane may also be considered a better extraction solvent because it has a lower boiling point (68^{o} C) than methanol (78^{o}C). Hence, this may imply that lesser energy will be utilized for extraction and recovery of avocado peel oil using hexane" [34].

Table 2. Proximate composition (g/100 g) of avocado peel powder sample

Sample	Avocado peel powder (%)	FAO/WHO Limit (1985)
Moisture	3.38 ± 0.87	10
Ash	2.47 ± 0.01	
Fat	6.71 ± 0.40	
Fibre	27.13 ± 2.52	
Protein	14.47 ± 0.25	16.70
Carbohydrate	45.84 ± 0.94	
Energy (Kcal)	403.69 ± 1.13	344

**The values present the Means (±SEM) of triplicate determination*

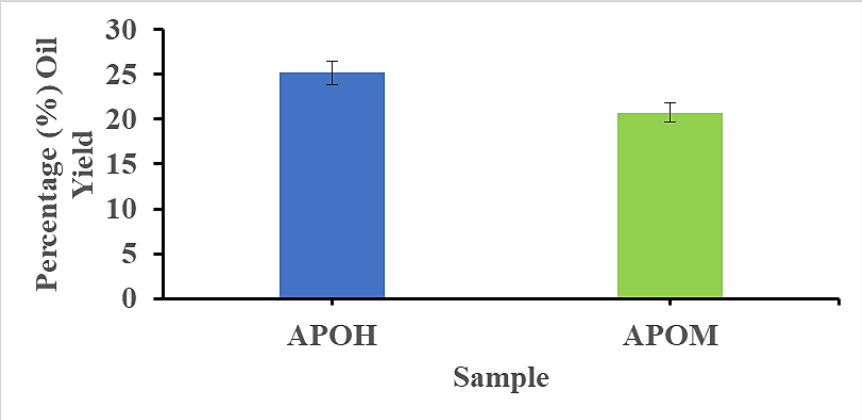

Fig. 1. Percentage (%) yield of avocado peel oil
APOH- Avocado Peel Oil extracted using Hexane Solvent.
APOM- Avocado Peel Oil extracted using Methanol Solvent

Table 3. Physicochemical characteristics of avocado peel oil

Parameters	APOH	APOM
Saponification (mgKOH/g)	30.05±0.00	23.61±0.00
Peroxide value (meq/kg)	0.96±0.03	0.93±0.00
Iodine value (g/100g)	7.33±0.13	2.53±0.06
FFA (%)	3.99±0.00	4.79±0.22
TBA (µM/g)	1.10±0.02	1.27±0.04
Specific gravity (kg/m^3)	0.88±0.07	0.88±0.09

**The values present the Means (±SEM) of triplicate determination*
APOH: Avocado Peel Oil extracted using Hexane solvent (APOH)
APOM: Avocado Peel Oil extracted using Methanol solvent (APOM)

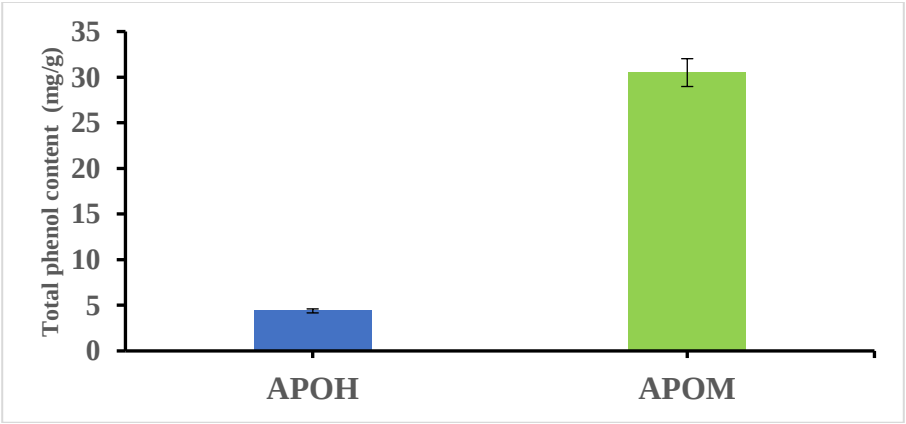

Fig. 2. Total phenol (mg/g) content of Avocado peel oil
APOH- Avocado Peel Oil extracted using Hexane Solvent.
APOM- Avocado Peel Oil extracted using Methanol Solvent

3.2 Physicochemical Properties of the Avocado Peel Oil

The physical and chemical parameters of the avocado peel oil extract using hexane and methanol solvents are presented in Table 3. APOH extract had higher saponification (30.05 mgKOH/g), peroxide (0.96 meq/kg), and iodine (7.33g/100g) values as compared to 23.61 mgKOH/g, 0.93 mg/kg and 2.53 g/100g, respectively, observed for APOM extract. Saponification value reported in this study was lower than 41.25 mgKOH/g reported for orange peel oil by Olabanji et al. [35]. High saponification

value indicates the presence of low molecular weight fatty acids in the extract. However, the low peroxide values observed for APOH and APOM [which are within the FAO/WHO Anon [36] recommended "acceptable range of ≤10.00 meq/kg] may be an indication that avocado peel oil may not decompose easily or be predisposed to rapid rancidity. Higher iodine value of APOM as compared to APOH may indicate presence of lower amount of double bond unsaturated fatty acid in APOM than in APOH, thus lowering the susceptibility of the oil to oxidative rancidity. However, edible oils with high iodine value (which infer high unsaturated fatty acid) are recommended for consumption in order to reduce incidences of cardiovascular disease".

"Free fatty acid (FFA) and Thiobabituric acid (TBA) values were higher in APOM than APOH. Free fatty acid contributes to rancidity either directly or by generating subsequent oxidation compounds" [37]. On the other hand, TBA measures lipid peroxidation products of oils and fats. Specific gravity was less than 1, which is similar for most oils and it implies that it is lighter than water, hence will be insoluble in water [38].

3.3 Total Phenol and Total Flavonoid Content of Avocado Peel Extract

The total phenol and total flavonoid contents of avocado peel extract are presented in Figs. 2 and 3 respectively. Total phenol content of APOH and APOM extracts were 4.38 and 30.50 mg/g, respectively, with a significantly higher value recorded for APOM. This may agree with the report of Chavan et al. [39] and Do et al. [40] that methanolic extract of Pardanthus chinensis and Limnophila aromatica fresh fruit pulp gave the highest total phenolic content. However, "the difference recorded in the present study may be attributed to variation in polarities of solvents, which selectively extract different hydrophobic or hydrophilic phenolic compounds in the sample. Higher phenolic content facilitates better bioactivity, meaning that methanolic extract (APEM) would possess better antioxidant activities" [41]. "Phenolic compounds are important plant constituents with redox properties responsible for antioxidant activity" [42].

"The values obtained for the total flavonoids of APOH and APOM extracts were 1.52 and 6.07 mg/g respectively. This result is in accordance with the findings of Dailey [43] who reported that extraction solvents significantly affects flavonoids contents. Flavonoids are secondary metabolites with antioxidant activity, the potency of which depends on the number and position of free OH groups" [44].

3.4 Antioxidant Activities of Avocado Peel Extract

Results of antioxidant activities (FRAP, metal chelating, hydroxyl radical scavenging, DPPH and ABTS radical scavenging abilities) of avocado peel oily extracts are presented in Figs. 4-8. Generally, sample APOM had higher antioxidant activity than sample APOH for all the antioxidant properties determined.

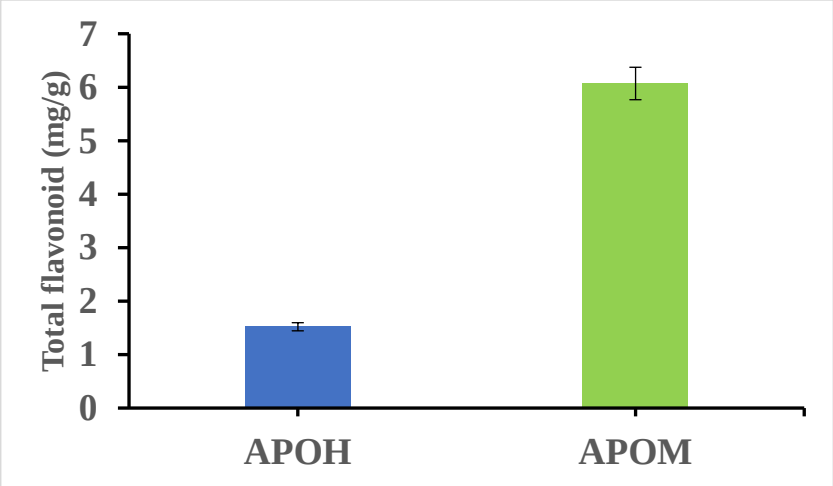

Fig. 3. Total flavonoid (mg/g) content of avocado peel oil
APOH- Avocado Peel Oil extracted using Hexane Solvent.
APOM- Avocado Peel Oil extracted using Methanol Solvent

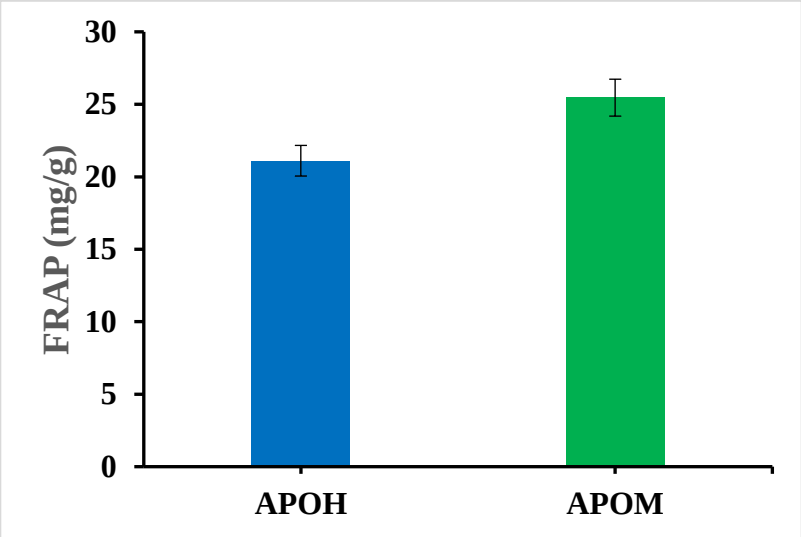

Fig. 4. FRAP Inhibitory activities of avocado peel oil
APOH- Avocado Peel Oil extracted using Hexane Solvent.
APOM- Avocado Peel Oil extracted using Methanol Solvent

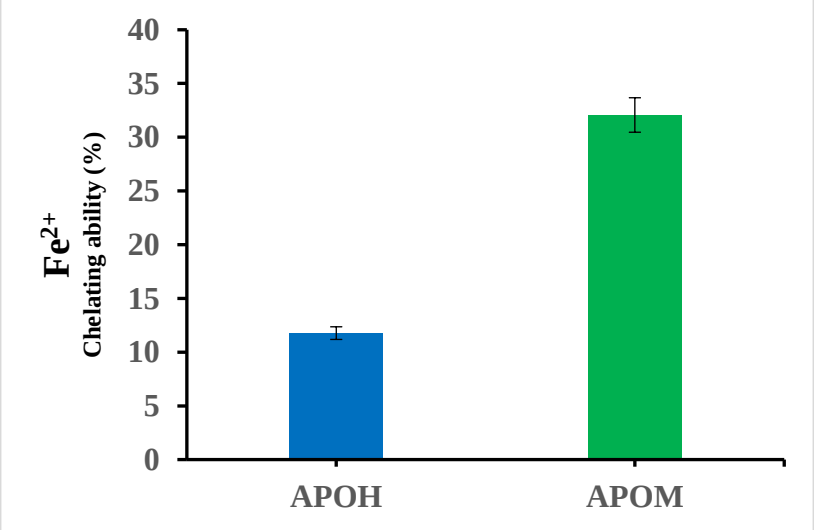

Fig. 5. Fe^{2+} Chelating activities of avocado peel oil
APOH- Avocado Peel Oil extracted using Hexane Solvent.
APOM- Avocado Peel Oil extracted using Methanol Solvent

Ferric reducing antioxidant power (FRAP) assay is based on the ability of antioxidant to reduce Fe^{3+} to Fe^{2+} ions. The ferric reducing antioxidant power activity of avocado peel extract using hexane and methanol were 21.11 and 25.46%, respectively. There is a high correlation between FRAP values and TPC/TFC; relatively APOM with higher TP and TF contents also showed a higher FRAP value. This suggests that the methanolic extract has higher electron-donating ability and may be a significant ferric reducer than the hexane extract. The values obtained were higher than values reported by Venkanna et al. [45] for *Albizia odoratissimal* leaf extract using methanol and hexane solvents (16.62 and 18.10%). Values of 31.84% and 13.47% were obtained for DPPH radical-scavenging ability of APOM and APOH extracts, respectively. A similar trend was observed in *Limophila aromatica* extract reported by Do et al. [40]. DPPH method may be utilized in aqueous and non-polar organic solvents and can be used to examine both hydrophobic and hydrophilic antioxidants [46]. APOM extract showed a better hydroxyl radical scavenging activity (79.43%) as compared to APOH extract (37.99%). This is

similar to the observation of Lozano et al. [47] who reported 66% hydroxyl radical scavenging activity of methanolic extract of *Lagenaria*. Hydroxyl radical scavenging ability is an extremely reactive free radical formed in biological systems and had been implicated as a highly damaging species in free radical pathology capable of damaging almost every molecule found in living cells.

"ABTS radical scavenging activity values obtained for APOH and APOM extracts were 0.52 and 2.78 TEAC/g, respectively. Evidently, APOM extract had better ABTS radical scavenging ability. The potent free radical scavenging ability may also be linked to its high phenolic content which is known to act as antioxidants owing to its ability to donate hydrogen or electrons required to neutralize the free radical" [48]. "A high $ABTS^{\bullet+}$ scavenging ability was reported for ethanolic extract of avocado peel than the other fruit parts i.e. pulp and seed [49]. The present result corroborates previous findings where methanolic extract fractions of avocado peel showed potent ability to scavenge DPPH and $ABTS^{+}$ radicals" [50].

Furthermore, APOM extracts had better capacity for iron chelating capacity (32.07%) compared to APOH extract (11.77%) as observed in this study. Chelating agents are effective as secondary antioxidants because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion [51]. Additionally, "antioxidants chelate heavy metal ions; the high radical scavenging and ferric reducing ability supports the fact that avocado peel extract possesses antioxidant properties. The high iron chelating ability as well as FRAP, especially in APOM is indicative of the ability of the extract to delay initiation of lipid peroxidation being that metal ions participate in initiation of lipid peroxidation. Iron chelators have been shown to promote formation of new blood cells and enhance wound healing in diabetic mice" [52].

3.5 Phytochemical composition of Avocado Peel Extract

Qualitative phytochemical screening of the avocado peel oily extracts of samples APOH and APOM are presented in Table 4. Extracts from APOH and APOM showed the presence of saponin, flavonoid, terpenoid, steroid and alkaloid while anthraquinone and phlobatannin were absent. The presence of cardiac glycoside in APOH and APOM extracts may indicate their potential usefulness in the treatment of heart diseases.

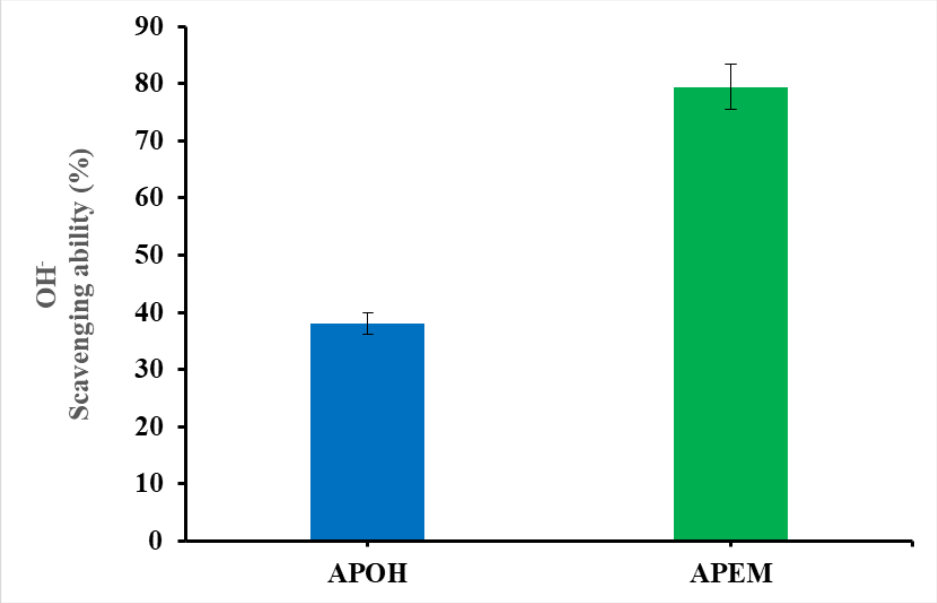

Fig. 6. Hydroxyl ion free radical scavenging ability of avocado peel extract
APOH- Avocado Peel Oil extracted using Hexane Solvent.
APOM- Avocado Peel Oil extracted using Methanol Solvent

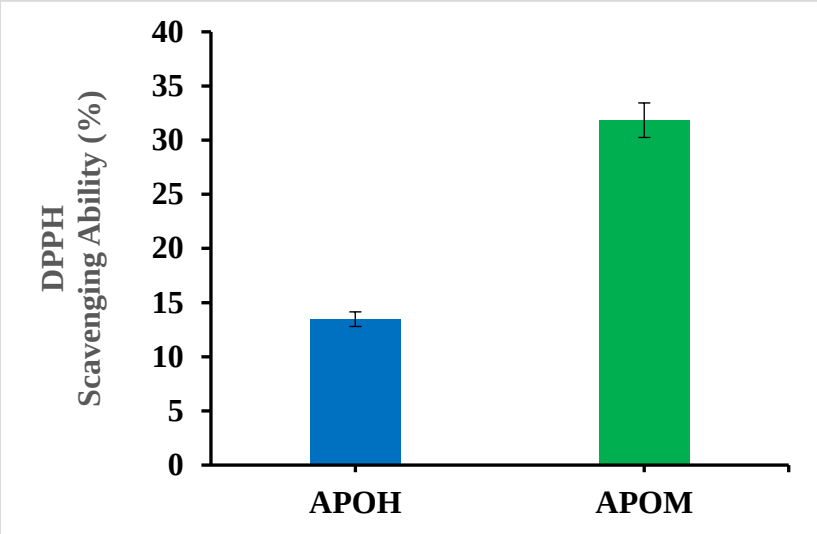

Fig. 7. DPPH$^{\bullet}$ radical scavenging ability of avocado peel oil
APOH- Avocado Peel Oil extracted using Hexane Solvent.
APOM- Avocado Peel Oil extracted using Methanol Solvent

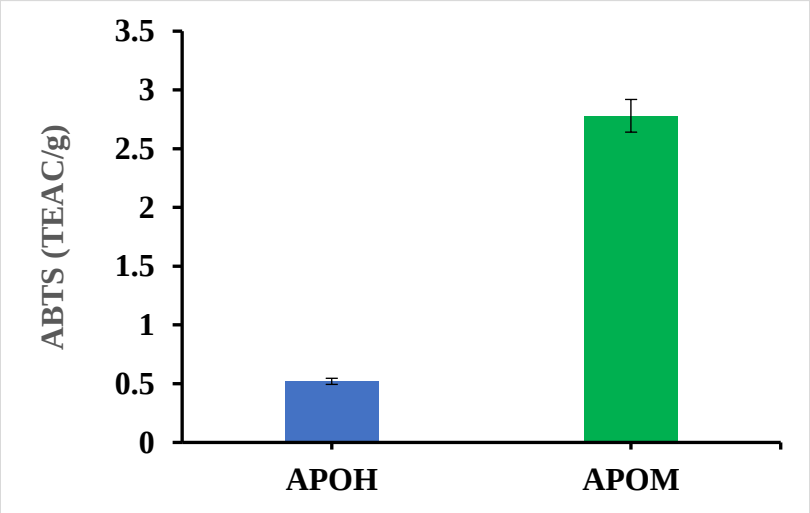

Fig. 8. ABTS$^{\bullet+}$ scavenging activity of avocado peel oil
APOH- Avocado Peel Oil extracted using Hexane Solvent.
APOM- Avocado Peel Oil extracted using Methanol Solvent

Phytochemical composition of avocado peel oily extracts as presented in Table 5 showed a significantly higher (P=.05) phytic acid content in APOM extract (4.53 mg/100g) as compared to APOH extract (1.65 g/100g) Phytic acid is considered an antinutrient as it interferes with the daily activities of human body like digestion and protein breakdown. It binds essential nutrients like iron, zinc and calcium, thereby diminishing their accessibility in human system (FAO [53] Soetan and Oyewole [54]). According to Hurrel [55], phytic acid intake of 4-9 mg/100g decreases iron absorption by 4-5 folds. Hexane extraction may therefore be preferable for production of oil from avocado peel with acceptable phytic acid content.

Tannin content of APOM extract was 5.27 g/100g, while APOH extract had 5.0 g/100g tannin content. It has been reported that tannin has characteristics similar to other polyphenols, serving as antioxidants in prevention of diseases [56,57]. However, negative effects of tannin include binding with other minerals leading to minerals non-bioavailability, especially reduction in iron absorption [58].

The oxalate levels in APOH and APOM extracts were 0.09 and 0.27 mg/g, respectively. The values were much lower than the estimated threshold of oxalate toxicity in humans (2-5 g/100g) on daily basis as reported by Jiménez-Martín et al*.,* [59]. This therefore suggests that consumers of the avocado peel extracts may not be exposed to oxalate toxicity. While APOH had a significantly higher (P=.05) saponin content of 37.19 g/100g, a lower value of 15.63 g/100g was observed in APOM. Saponin protects against microbial attack in plants and has been reported to be useful in the treatment of yeast and fungal infections [60]. "It also assists the immune system through its adjuvant activity and has ability to improve effective absorption of orally administered vaccines [61]. The cholesterol-lowering activity of saponin which has been reported in animals has been attributed to its inhibition of absorption of cholesterol from the small intestine or the reabsorption of bile acids" [62].

"Steroid content in APOH and APOM extracts were 9.92 and 4.37 mg/g. Consumption of fruits with high steroid lowers cholesterol level in the blood [63]. The glycosides contents in APOH and APOM oily extracts were 12.75 and 23.14 mg/g respectively. Terpenoid content was notably higher in APOH extract (20.64 mg/g) than APOM extract **(**7.97 mg/g).Terpenoid has been reported to have anti-inflammatory, anti-viral anti-malarial properties as well as exhibits inhibition of cholesterol synthesis" (Calle et al*.,* [64]; Olorunju et al*.,* [65]). It also has antibacterial properties (Wadood et al*.,* [66]). "The significant amount of terpenoids from this study show that avocado (*Peresea americana* Mill.) seed peel could be recommended as an effective source of anti-bacterial agent. In addition, terpenoid derived compounds have been known as potential bioactive compounds. They act as pigment for photosynthesis, attracting pollinators, involved in the protein N- glycosylation" (Santiago and Castro [67]).

Table 4. Phytochemical screening of avocado peel oil

Phytochemicals	APOH	APOM
Saponin	+	+
Tannin	+	+
Phlobatannin	-	-
Flavonoid	+	+
Steroid	+	+
Terpenoid	+	+
Alkaloid	-	-
Anthraquinone	-	-
Cardiac Glycoside	+	+
Legal test	+	+
Kellerkilliani test	+	+
Salkwwoski test	+	+
Lieberman test	+	-

APOH: Avocado Peel Oil extract using Hexane solvent (APOH)
APOM: Avocado Peel Oil extract using Methanol solvent (APOM)

Table 5. Phytochemical composition of avocado peel extract

Parameter	APOH	APOM	FAO (1985) (g/100g)
Saponin	37.19 **±** 0.64	15.63**±**0.54	
Steroid	9.92 **±** 0.01	4.37**±**0.07	
Terpenoid	20.64 **±** 0.06	7.97**±**0.04	
Glycosides	12.75 **±** 0.08	23.14±0.05	
Phytate	1.65 **±** 0.83	4.53**±**0.41	0.3
Oxalate	0.09 ± 0.00	0.27±0.00	5-6
Tannin	4.35 **±** 0.08	4.10**±**0.10	3

**The values present the Means (±SEM) of triplicate determination*
APOH: Avocado Peel Oil extract using Hexane solvent (APOH)
APOM: Avocado Peel Oil extract using Methanol solvent (APOM)
N.D: Not Detected

Table 6. Fatty acid profile of avocado peel methanolic extract

S/N	Compound	% Composition
A	**Saturated Fatty Acid (SFA)**	
1	Butyric Acid (C4:0)	1.84
2	Caproic (C6:0)	
3	Caprylic (C8:0)	
4	Capric (C10:0)	10.8
5	Undecylic (C11:0)	5.4
6	Tridecylic (C13:0)	8.34
7	Myristic (C14:0)	4.64
8	Pentadecylic (C15:0)	2.30
9	Palmitic (C16:0)	13.99
10	Margaric (C17:0)	9.32
11	Stearic (C18:0)	5.19
12	Arachidic (C20:0)	4.66
13	Heneicosylic (C21:0)	5.40
14	Behenic (22:0)	5.13
15	Tricosylic (23:0)	7.52
16	Lignoceric (24:0)	4.93
	∑SFA	89.46
B	**Monounsaturated (MUFA)**	
	Myristoleic (C14:1)	3.54
	∑MUFA	3.54
C	**Polyunsaturated (PUFA)**	
	Linolenic (C18:0)	9.45
	∑PUFA	**9.45**

3.6 Fatty Acid Profile of Avocado Peel Methanolic Extract

The fatty acid profile of APOM (Table 6) indicated the presence of 27 compounds. There were 87.1% saturated fatty acid, 3.5% mono-unsaturated fatty acid and 9.4% poly-unsaturated fatty acid in the APOM. Overall, palmitic acid, an unsaturated fatty acid, was the most abundant (13.99%), followed by capric acid (10.8%) and linolenic acid (9.45%). Linolenic acid, an essential fatty acid, has been reported to have cardiovascular-protective ability and other health benefits.

3.7 Effect of Storage Period on the Bioactive Activities of the Microencapsulates

The total phenol content of the micro encapsulates is shown in Fig. 9. The highest phenolic content was 37.71 mg/g for sample A3: Corn Starch (48 g): Gum Arabic (2 g): Tween 80 (0.5 ml): peel extract (4.8 ml), and closely followed by 37.47 mg/g for sample B3: Cassava Starch (48 g): Gum Arabic (2 g): Tween 80 (0.5 ml): peel extract (4.8 ml). Sample B2: Cassava Starch (49 g): Gum Arabic (1 g): Tween 80 (0.5 ml): peel extract (4.9 ml) however, had the overall least phenolic content. The same trend was observed after 30 days storage at room temperature but with increasing phenolic contents. These high phenolic content indicate that the extract is a rich source of phenolic compounds. Since samples A3 and B3 were the highest, despite their lowest extract content in the formulation, it showed that other factors such as the cell wall materials and gum arabic may have contributed to total phenolic content. However, corn starch exhibited functionality as a better cell wall material than cassava starch owing to its higher phenolic content.

The DPPH radical scavenging ability of the micro encapsulates (Fig. 10) followed similar trend with total phenolic content (Fig. 9). Sample B2 was also the least, while sample A3 was the highest and closely followed by sample B3. Corn starch exhibited potential as a better cell wall material due to the high DPPH radical scavenging activity of its samples. In addition, gum arabic also contributed to the DPPH radical scavenging activities. The DPPH radical scavenging activities, however, decreases with storage (unlike it was observed in total phenolic contents). The DPPH radical scavenging activities of the samples were generally high with the exception of B2. Micro encapsulation

increased the total phenolic content and DPPH radical scavenging activities of methanolic extracts of avocado peel. Results showed over a 100% increase in DPPH• scavenging activity of encapsulate compared to methanolic avocado peel extract. The increase in radical-scavenging ability of micro encapsulated samples over the raw extracts could be as a result of the introduction of cell wall materials and polysaccharide (guar gum). However, encapsulates from corn starch (cell wall material) showed higher values than cassava starch. Micro encapsulation has been reported as a process which possesses the ability to preserve phenolic content and radical scavenging activities of encapsulates [68].

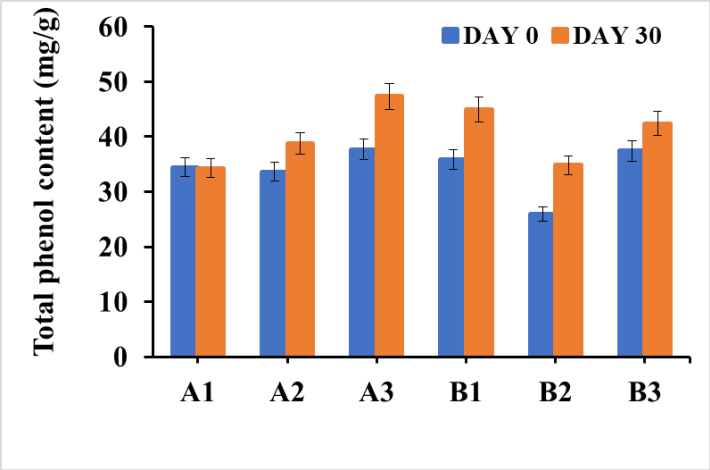

Fig. 9. Changes in total phenolic content of encapsulates from avocado peel extract during storage

Keys:
A1: Corn Starch (50 g): Gum Arabic (0): Tween 80 (0.5 ml): peel extract (5 ml)
A2: Corn Starch (49 g): Gum Arabic (1g): Tween 80 (0.5 ml): peel extract (4.9 ml)
A3: Corn Starch (48 g): Gum Arabic (2 g): Tween 80 (0.5 ml): peel extract (4.8 ml)
B1: Cassava Starch (50 g): Gum Arabic (0): Tween 80 (0.5 ml): peel extract (5 ml)
B2: Cassava Starch (49 g): Gum Arabic (1 g): Tween 80 (0.5 ml): peel extract (4.9 ml)
B3: Cassava Starch (48 g): Gum Arabic (2 g): Tween 80 (0.5 ml): peel extract (4.8 ml)

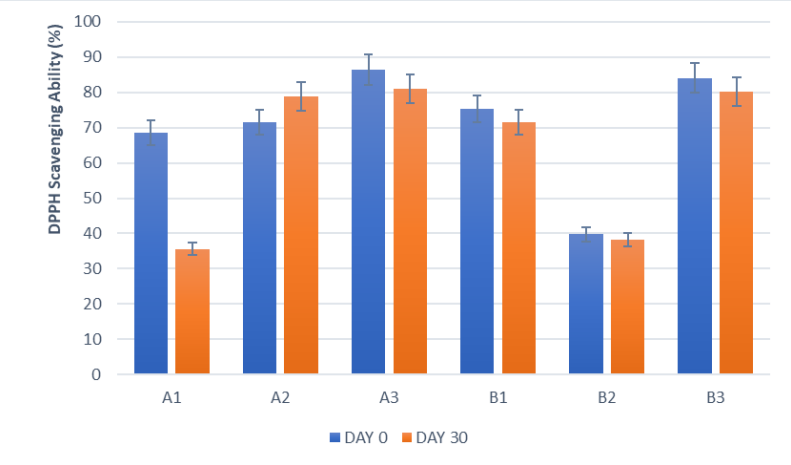

Fig. 10. DPPH radical scavenging ability of the encapsulates from avocado peel extract

Keys:
A1: Corn Starch (50 g): Gum Arabic (0): Tween 80 (0.5 ml): peel extract (5 ml)
A2: Corn Starch (49 g): Gum Arabic (1g): Tween 80 (0.5 ml): peel extract (4.9 ml)
A3: Corn Starch (48 g): Gum Arabic (2 g): Tween 80 (0.5 ml): peel extract (4.8 ml)
B1: Cassava Starch (50 g): Gum Arabic (0): Tween 80 (0.5 ml): peel extract (5 ml)
B2: Cassava Starch (49 g): Gum Arabic (1 g): Tween 80 (0.5 ml): peel extract (4.9 ml)
B3: Cassava Starch (48 g): Gum Arabic (2 g): Tween 80 (0.5 ml): peel extract (4.8 ml)

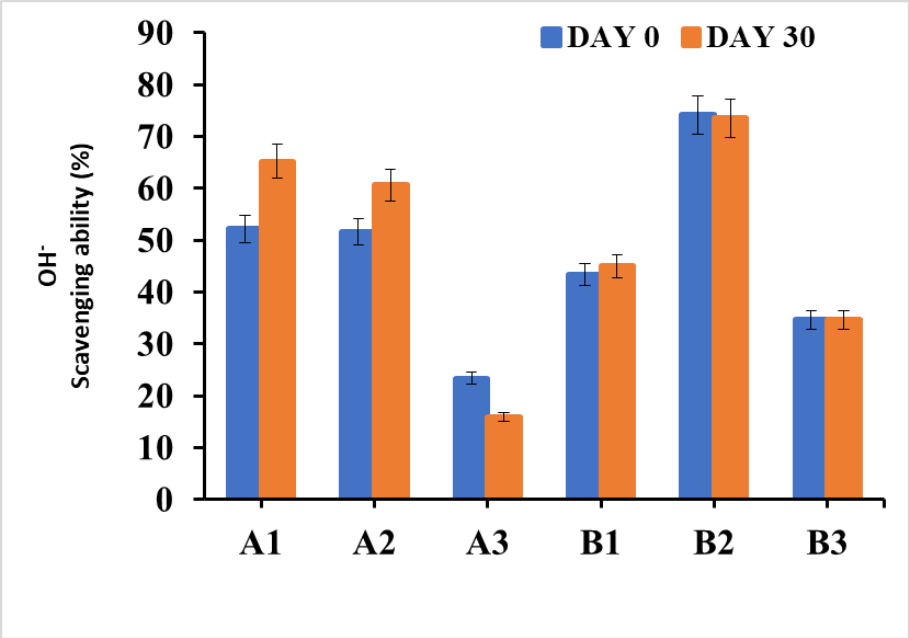

Fig. 11. Hydroxyl radical scavenging ability of Encapsulates from Avocado peel oily extract

Keys:
A1: Corn Starch (50 g): Gum Arabic (0): Tween 80 (0.5 ml): peel extract (5 ml)
A2: Corn Starch (49 g): Gum Arabic (1g): Tween 80 (0.5 ml): peel extract (4.9 ml)
A3: Corn Starch (48 g): Gum Arabic (2 g): Tween 80 (0.5 ml): peel extract (4.8 ml)
B1: Cassava Starch (50 g): Gum Arabic (0): Tween 80 (0.5 ml): peel extract (5 ml)
B2: Cassava Starch (49 g): Gum Arabic (1 g): Tween 80 (0.5 ml): peel extract (4.9 ml)
B3: Cassava Starch (48 g): Gum Arabic (2 g): Tween 80 (0.5 ml): peel extract (4.8 ml)

However, for hydroxyl radical scavenging ability, sample B2 had the highest value, followed by samples A1: Corn Starch (50 g): Gum Arabic (0): Tween 80 (0.5 ml): peel extract (5 ml) and A2: Corn Starch (49 g): Gum Arabic (1g): Tween 80 (0.5 ml): peel extract (4.9 ml), while sample A3 had the least OH radical-scavenging activity (Fig. 11). There was significant increase in samples A1, A2 and B1: Cassava Starch (50 g): Gum Arabic (0): Tween 80 (0.5 ml): peel extract (5 ml) increased after 30 days' storage. On the other hand, sample A3 showed significant decrease in hydroxyl radical scavenging ability after 30 days, while sample B3 was not affected. Encapsulation did not cause a significant increase in $^{\bullet}OH$ scavenging ability as the methanolic extract exhibited a 79% scavenging ability whereas the encapsulate showed a 16 to 73% scavenging ability. Encapsulates with lowest volume of extract and amount of cell wall material (corn and cassava starch), i.e., samples A3 and B3, had the lowest hydroxyl radical scavenging ability. Notwithstanding, the phenolic content and significant radical scavenging ability (DPPH$^{\bullet}$, $^{\bullet}OH$) of the prepared encapsulates may suggest their high antioxidant capacity.

3.8 Moisture Content of Micro Encapsulate from Avocado Peel Oily Extract after Storage

Moisture content of samples A3 and B3 at day 1 and 30 is shown in Fig. 12. At day 1, the moisture ranged from 3.0 to 3.7%. The freeze-drying process at temperature of -50^{o} C could have contributed to the low moisture content observed. After 30 days storage on the laboratory shelf (28^{0}C – 30^{0}C), the samples had absorbed moisture from the environment and increased significantly to 4.3 to 5.7%. The samples were stored inside a low-density polyethylene. However, the moisture content was within the acceptable range and lesser than the recommended standard value of <15.5% (CODEX STAN 152-1985). The low moisture content obtained may enhance the storage stability and prevent mold growth and biochemical reactions in the samples.

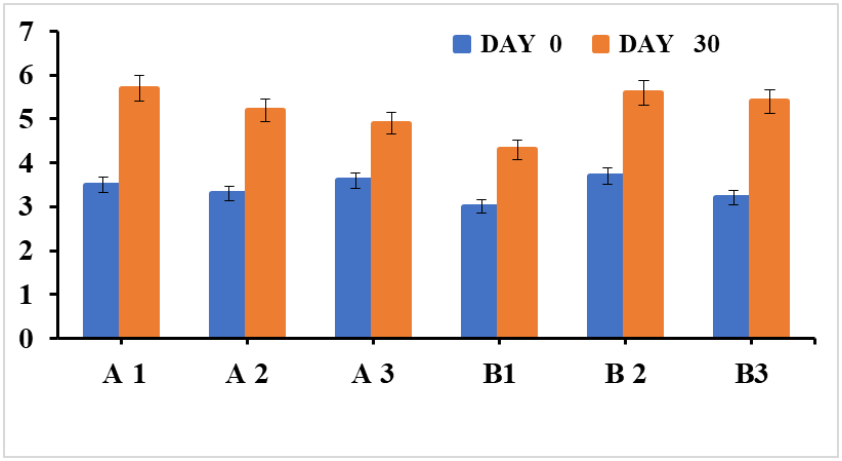

Fig. 12. Moisture content of encapsulates from avocado peel oily extract

Keys:
A1: Corn Starch (50 g): Gum Arabic (0): Tween 80 (0.5 ml): peel extract (5 ml)
A2: Corn Starch (49 g): Gum Arabic (1g): Tween 80 (0.5 ml): peel extract (4.9 ml)
A3: Corn Starch (48 g): Gum Arabic (2 g): Tween 80 (0.5 ml): peel extract (4.8 ml)
B1: Cassava Starch (50 g): Gum Arabic (0): Tween 80 (0.5 ml): peel extract (5 ml)
B2: Cassava Starch (49 g): Gum Arabic (1 g): Tween 80 (0.5 ml): peel extract (4.9 ml)
B3: Cassava Starch (48 g): Gum Arabic (2 g): Tween 80 (0.5 ml): peel extract (4.8 ml)

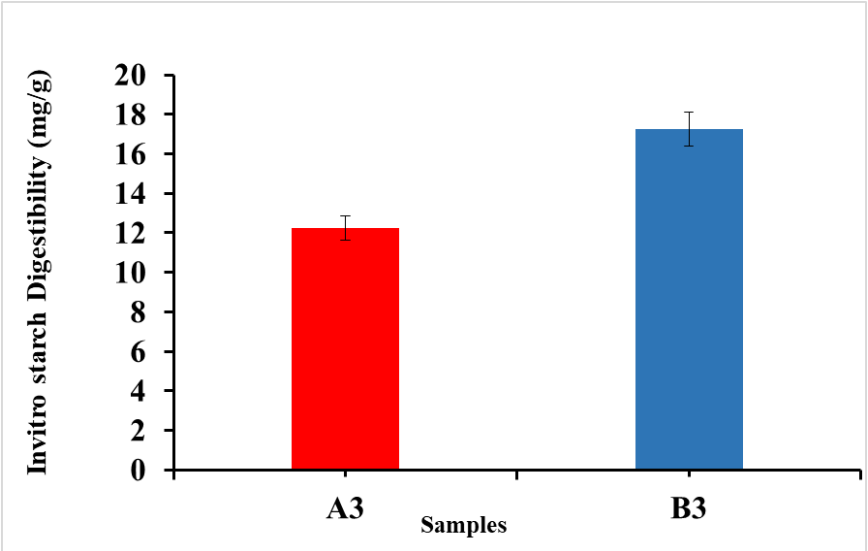

Fig. 13. *In vitro* starch digestibility of avocado peel oily extract encapsulate

Keys:
A3: Corn Starch (48 g): Gum Arabic (2 g): Tween 80 (0.5 ml): peel extract (4.8 ml)
B3: Cassava Starch (48 g): Gum Arabic (2 g): Tween 80 (0.5 ml): peel extract (4.8 ml)

3.9 *In vitro* Starch Digestibility of the Microencapsulate

In vitro starch digestibility of sample A3 and B3 (samples with the highest total phenolic content and DPPH radical scavenging activities) is shown in Fig. 13. While sample A3 had a lower *in vitro* starch digestibility (12.34%), sample B3 had a higher value of 17.50%. Factors that may have contributed to this variation may include amylose content, particle size, type of cultivar processing and storage condition. According to Trindade and Grosso [69], cassava starch is known to have smaller granules when compared to corn starch. Furthermore, there is an inverse relationship between starch granule size and its digestibility such that larger granules result in smaller surface area. Thus, promoting better absorption or

digestion in the small intestine. This may have contributed to the increase in the digestibility of starch in sample B3 (cassava wall material).

3.10 Fourier Transform Infrared Spectroscopy (FTIR) of the Micro Encapsulate

Results of FTIR analysis of samples A3 and B3 (which had the best antioxidant activities) are shown in Figs. 14-15. The basic functional groups in sample A3 were OH, CH_2 and C=O representing alcohol, alkane and carboxyl groups, respectively. Similarly, sample B3 had OH, CH_2 and C=O. Other minor functional groups observed were cyanide (CN), alkene (C=C) and thiol (S-H). The difference in the functional groups could be as a result of the wall materials used for the sample. Corn starch produced better and clearly defined spectrum, while cassava starch had several other functional groups probably due to the cassava.

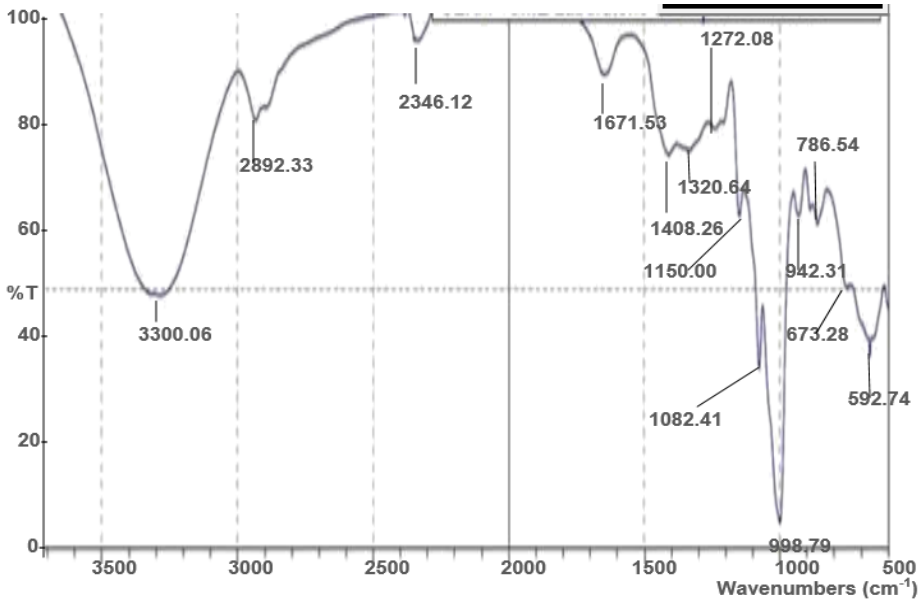

Fig. 14. Fourier Transform Infrared Spectroscopy (FTIR) using Corn Starch Wall material

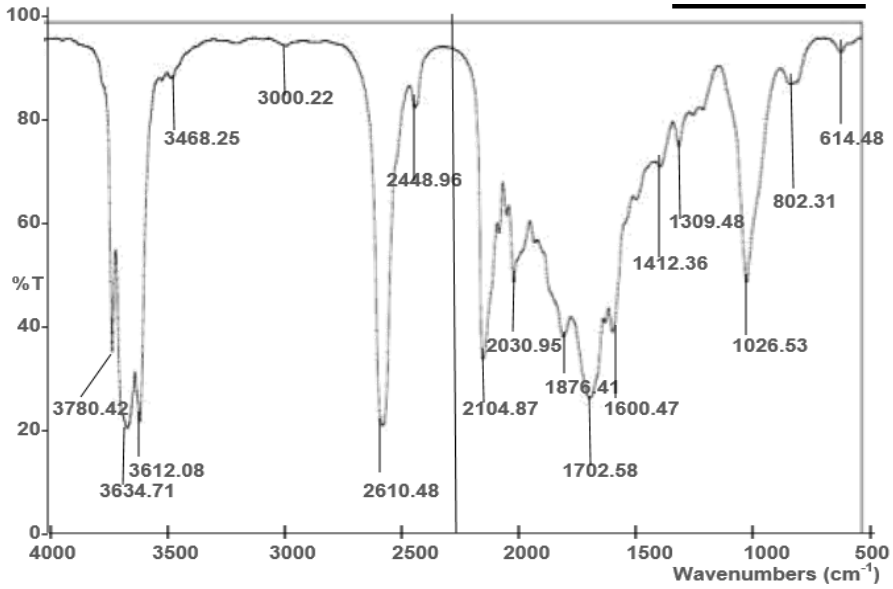

Fig. 15. Fourier Transform Infrared Spectroscopy (FTIR) using Cassava Starch Wall material.

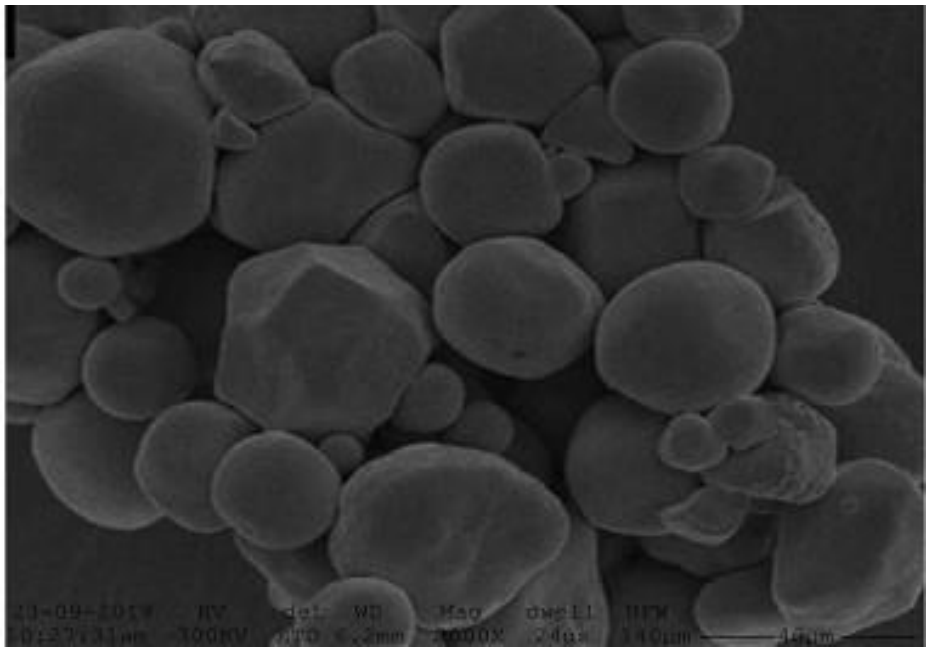

Fig. 16. SEM of sample A3
A3: Corn Starch (48 g): Gum Arabic (2 g): Tween 80 (0.5 ml): peel extract (4.8 ml)

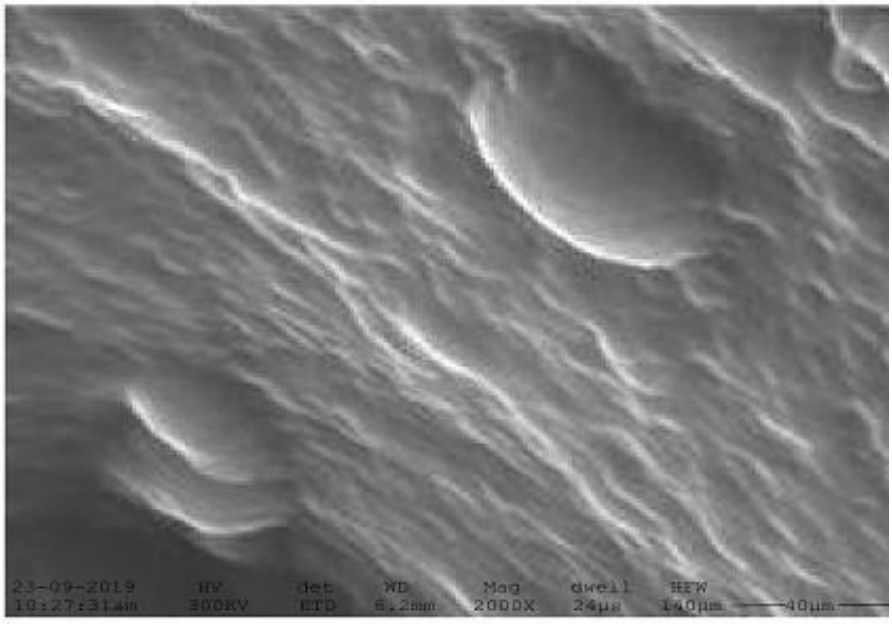

Fig. 17. SEM of sample B3
B3: Cassava Starch (48 g): Gum Arabic (2 g): Tween 80 (0.5 ml): peel extract (4.8 ml)

3.11 Scanning Electron Micrographs (SEM) of Samples A3 and B3

The SEMs of samples A3 and B3 are shown in Figs. 16 and 17 respectively. The micro encapsulate with corn starch cell wall material (sample A3) had unique characteristic shapes (polygonal and spherical). The particle size ranged from 4 – 40 nm. However, sample B3 (with cassava starch cell wall material) with particle size ranging from 2 to 40 nm had no unique shape, and had a smooth uninterrupted but wrinkled surface which might have been formed as a result of the loss of water content during freeze-drying process [70].

4. CONCLUSION

The study established that the avocado peel oil extracted using methanol is a promising source of bioactive compounds, with acceptable antinutrient contents. Both corn and cassava starches show potential as promising cell wall materials for encapsulation of the bioactive compounds in the avocado peel oil methanolic extracts; being able to retain about 90% of the bioactive compounds after 30 days storage at a temperature range of 28^0C – 30^0C.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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