Research Article

Effect of Formulation Variables on the Microencapsulation of Cassava Seed Oil and Evaluation of the Antimicrobial Properties of its Cream Formulations

*Odeniyi M.A.[1], Adegbolagun T.I.[1], Odeniyi O.A.[2], Adebayo-Tayo B.C.[2]

[1]*Department of Pharmaceutics & Industrial Pharmacy, University of Ibadan, Ibadan, Nigeria*
[2]*Department of Microbiology, University of Ibadan, Ibadan, Nigeria*

ABSTRACT

Cassava seed oil obtained from Manihot esculenta Crantz possesses antimicrobial and anti-oxidant properties and can be incorporated in topical pharmaceutical formulations for treatment of wounds, skin infections and irritations. This work determined the effects of formulation variables on the properties of cassava seed oil microcapsules and the antimicrobial properties of its cream formulations. Cassava seed oil was formulated into alginate microcapsules using calcium chloride and aluminum sulphate as cross-linkers at varying oil: alginate ratios, cross-linker concentrations, and curing times. The surface morphologies, particle sizes, encapsulation efficiencies (EE) and FT-IR spectra of the microcapsules were determined. Selected microcapsules were formulated into creams and the antimicrobial activities of the cream formulations were determined against Acinetobacter baumannii NCTC 7363, Serratia marcescens ATCC 8155, Staphylococcus aureus ATCC 29213, Citrobacter freundii ATCC 8090, Escherichia coli ATCC 25925, Salmonella typhimurium 14028, Pseudomonas aeruginosa 27853 and Staphylococcus aureus ATCC 6571 by agar diffusion. Microcapsules crosslinked with calcium chloride were smaller with smoother surfaces, while those with aluminum sulphate were large, irregularly shaped with rough surfaces. The EE of microcapsules crosslinked with aluminum sulphate were higher than those with calcium chloride. The EE (23.6 – 66.7%) increased with increase in alginate:oil ratio, concentration of cross-linkers and curing times. The oily cream retained its antimicrobial property against many of the microorganisms, showing highest inhibitory activities against Pseudomonas aeruginosa 27853 and Acinetobacter baumannii NCTC 7363. Creams incorporating Cassava seed oil microcapsules with antimicrobial and antioxidant properties were produced using a simple microencapsulation process with biodegradable and renewable materials.

Keywords: *Cassava seed oil, Microencapsulation, Microcapsules, Antimicrobial activity*

INTRODUCTION

Antimicrobial resistance to available antibiotics is one of the current major issues facing global health. This danger has been on the increase and highlights the need for novel antibiotics with enhanced antibacterial activity. No antibiotic meant for systemic use should ideally be applied topically, due to possible risks of promoting resistance. This resistance in microorganisms is on the increase and has been escalated by widespread topical use of antibiotics. Hence, the need to investigate novel antibacterial agents from natural sources and improved topical formulations of the same.

Cassava seed oil exhibits a broad spectrum of antibiotic and antimycotic activity (Okeke *et al*., 2001; Pawar and Thaker, 2006). As a result, it is rapidly gaining the attention of cosmetic microbiologists and dermatologists because it is

effective, cheap, safe, eco-friendly and most importantly readily available within the community (Popoola *et al*., 2007). Cassava seed oil is liquid at room temperature (25°C), pale yellow, odorless and tasteless with specific gravity of 0.94 at room temperature. It has a lipid content of 25.2%, Iodine value of 90 Wijs. The oil is found to be low in free fatty acid, peroxide and cyanide., Seven fatty acids have been identified upon analysis of the lipid by colorimetric method. These include linolenic, Lauric, arachidonic acids, stearic, oleic, linoleic, and palmitic. The major fatty acids are Palmitic (31.26%) Linoleic (35.16%) and Oleic (13.09%) (Popoola and Yangomodou, 2006). However, gas chromatography analysis, by Jayaprakas *et al*. (2013), on the lipid profile of cassava oil indicates the presence of 13 fatty acids with dominance of C18 component (oleic). Other major components of the oil were

stearic acid (19.61%) and myristic acid (10.73%). Cassava seed oil is highly unsaturated (Jayaprakas *et al*., 2013).

Due to their unsaturated nature, fixed oils with high content of omega fatty acids (e.g. cassava seed oil) are chemically unstable and prone to oxidative degradation which produces free radicals and unpleasant tastes and adversely affect the organoleptic characteristics shelf-life, stability and overall acceptability of food products (Velasco *et al*., 2003). Microencapsulation is a feasible method of maintaining and improving the characteristics, biological and functional, of fixed oils like cassava seed oil. Microencapsulation is applied in oils to protect against oxidation, to control or sustain release and conceal unpleasant taste (Drusch and Mannino, 2009) and also improve the skin permeability and targeting of topical natural antioxidants (Aljuffali *et al*., 2015).

Alginate is relatively cheap, natural, safe, flexible, biocompatible and biodegradable (Reis *et al*., 2007; Boateng *et al*., 2008; Damgé *et al*., 2008; Lee and Mooney 2012). Also, alginate has been experimentally studied and proven to have appreciable stability against Ultra-violet rays (Rijo *et al*., 2014; Flores-Céspedes *et al*., 2015).

In cosmetics, cassava seed oil may be used as a natural active in topical and body care products because of its inherent antimicrobial and anti-oxidant properties. This work was thus aimed at developing new formulations of cassava oil as microcapsules embedded in cream formulations in order to improve its stability and ease of application.

MATERIALS AND METHODS

Preparation of plant material and extraction: Dry cassava seeds were obtained from the Cassava Breeding Unit, International Institute for Tropical Agriculture, Ibadan, Nigeria. The fixed oil was extracted with hexane using a Soxhlet extractor.

Antioxidant Activity

DPPH-free radical scavenging: The 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability of the seed oil was investigated using different test concentrations (200–1 µg/mL) of the oil, employing spectrophotometric method according to the method of Ali *et al*., (2017) and described by Odeniyi *et al.,* (2020). The percent scavenging activity of DPPH was calculated using the following formula:

$$\% \, Scavenging = [(1 - ABS/ABC)] \times 100$$

Where "ABS" and "ABC" correspond to absorbance of sample and absorbance of control, respectively.

Formulation of microcapsules: Cassava seed oil alginate microcapsules were prepared by extrusion/external gelation method (Urbano, 2004) through ionotropic gelation. 30mL oil-alginate emulsions (in alginate: oil ratios 2:1 and 5:1) were prepared by mixing 20mL of 2% sodium alginate with 10mL of cassava seed oil (2:1) and 25mL of 2% sodium alginate with 5mL of cassava seed oil (5:1). Each oil-alginate emulsion was mixed with a magnetic stirrer (PC-420, Coring, United States of America) at about 500 rpm for 10 minutes.

Each emulsion obtained were extruded through a fine syringe (21-gauge, diameter 0.8mm) (Henriques *et al*., 2017) into 50mL of 10% $CaCl_2$, 20% $CaCl_2$, 10% $Al_2(SO_4)_3$ and 20% $Al_2(SO_4)_3$ under magnetic stirring for 15 minutes and 30 minutes in each cross-linker solution. The resulting solutions were filtered and washed with cold water to obtain gelled hydrated beads (Henriques *et al*., 2017). The hydrated beads were then dried in the hot air Oven (OVB-305, Gallenkamp, Germany) at 40°C for 24 hours after which they were stored in a desiccator. The formulation components of the cassava seed oil- alginate microbeads samples are presented in Table 1.

Table 1.
Formulation compositions of cassava seed oil microcapsules

Formulations	Alginate Conc. (%)	Alginate: Oil ratio	Cross-linker Concentration (%)	Stirring time (minutes)	Encapsulation Efficiency (%)	Mean Particle Size (mm)
A-1	2	2:1	10% $CaCl_2$	15	25.45 ± 0.07	2.42±0.39
A-2	2	2:1	10% $CaCl_2$	30	23.63 ± 0.06	2.29±0.42
A-3	2	2:1	20% $CaCl_2$	15	47.45 ± 0.07	2.47±0.43
A-4	2	2:1	20% $CaCl_2$	30	36.86 ± 0.23	2.39±0.65
A-5	2	2:1	10% $Al_2(SO4)_3$	15	26.30 ± 0.14	3.13±1.34
A-6	2	2:1	10% $Al_2(SO4)_3$	30	30.85 ± 0.07	3.18±1.38
A-7	2	2:1	20% $Al_2(SO4)_3$	15	67.66 ± 0.05	3.51±0.59
A-8	2	2:1	20% $Al_2(SO4)_3$	30	45.85 ± 0.11	3.25±0.83
B-1	2	5:1	10% $CaCl_2$	15	52.25 ± 0.07	2.16±0.65
B-2	2	5:1	10% $CaCl_2$	30	59.21 ± 0.10	2.37±0.45
B-3	2	5:1	20% $CaCl_2$	15	35.73 ± 0.13	2.28±0.74
B-4	2	5:1	20% $CaCl_2$	30	36.75 ± 0.11	2.13±0.43
B-5	2	5:1	10% $Al_2(SO4)_3$	15	24.25 ± 0.06	2.34±0.34
B-6	2	5:1	10% $Al_2(SO4)_3$	30	28.50 ± 0.07	2.28±0.55
B-7	2	5:1	20% $Al_2(SO4)_3$	15	23.82 ± 0.01	3.49±1.03
B-8	2	5:1	20% $Al_2(SO4)_3$	30	36.09 ± 0.13	2.29±0.50

Size Analysis of Formulated Microcapsules: Particle size and size distribution of each batch of formulated microbeads were determined using a light microscope (Gallenkamp MNH550-R, Germany). Samples of each batch of formulated microbeads were loaded onto a microscope slide and the mean particle size was calculated by measuring the diameter of 20 microbeads at 40x magnification with the help of a pre-calibrated ocular micrometer and the mean diameter and standard deviation for each formulation were calculated and recorded.

Particle Shape and Morphology: The shape and surface topography of $CaCl_2$ and $Al_2(SO4)_3$ crosslinked microcapsule formulations were studied using a Scanning Electron Microscope (Vega 3, Tescan) at an accelerating voltage of 20kV and at magnifications 70x, 250x and 500x (Ayorinde *et al.*, 2017). Samples were coated with carbon to make the samples electrically conductive. Particle sizes of samples were also measured at 28x magnification. The surface morphologies of all formulated cassava seed oil microcapsules were also studied using a stereomicroscope (Vickers Instrument VP/34, England).

Encapsulation efficiency

Determination of Maximum Absorbance Wavelength : The maximum wavelength of UV absorption was determined using an Ultraviolet Scanning Spectrophotometer (Lambda 25/35/45 UV/Vis spectrophotometer, United Kingdom). Standard dilutions (0.01%, 0.1% and 1%) of cassava seed oil in n-hexane were prepared and scanned at ultraviolet wavelength from 200nm-800nm. The UV spectra obtained were recorded. The concentration which gave the most suitable spectra was noted, and the maximum wavelength with absorbance value less than 0.6 was used for further analysis.

Generation of Calibration Curve: Standard dilutions (0.05%, 0.06%, 0.07%, 0.08%, 0.09% and 0.10%) of cassava seed oil in n-hexane were prepared. Duplicate readings of the absorbance values of the dilutions at 235nm were taken using an Ultraviolet Spectrophotometer (Jenway 6305, Bibby Scientific Ltd. United Kingdom). A calibration curve of average absorbance against concentration was plotted.

Determination of Encapsulation Efficiency: The amounts of cassava seed oil loaded within the microcapsules were determined by modifying the methods developed by Parris *et al.* (2005) and Soliman *et al.* (2013). A 0.5g quantity of each batch of formulated Cassava seed oil microcapsules was weighed. The oil in the microcapsules was extracted by mixing the microcapsules with 5mL 10% Tri-sodium citrate and 5mL n-hexane (Soliman *et al.,* 2013). The extraction system was tightly covered and left to stand for 24 hours in a dark cupboard to ensure complete dissolution of the microcapsules and release of encapsulated oil. After 24 hours, the n-hexane layer was diluted by a 100 fold (250µL was diluted to 25mL with n-hexane) and the absorbance of the dilution at 235nm wavelength was measured using an Ultraviolet Spectrophotometer (Jenway 6305, Bibby Scientific Ltd., United Kingdom). Empty microcapsules were subjected to the same extraction and dilution processes and the n-hexane layer obtained was used as the blank. Absorbance readings were taken twice. The actual concentration of oil was determined from the plots generated from cassava seed oil calibration curve.

Encapsulation efficiency was calculated using the following formula:

$$Encapsulation\ efficiency\ (\%) = \frac{Actual\ Oil\ content\ (mL)}{Theoretical\ Oil\ content\ (mL)} \times 100 \qquad (2)$$

Fourier- Transform Infrared Spectroscopy (FT-IR)
Fourier- transform Infrared spectroscopy of selected batches of formulated cassava seed oil microcapsules, free cassava seed oil and empty beads were recorded with an FT-IR spectrophotometer (Spectrum BX, PerkinElmer, United states of America). All samples were compressed into potassium bromide pellets and scanned from 4000 to 600 cm^{-1}. The infrared spectra obtained for each sample were recorded.

Preparation of oily cream base and incorporation of alginate microcapsules

Preparation of wool alcohol ointment: Hard paraffin (4.8g) was melted in a crucible dish over a water bath (Gallenkamp, England) at 50ºC. Afterwards, 2g of soft para, 1.2g of Lanolin and 12mL of liquid paraffin was added and mixed. The resulting molten mixture removed from the water bath and stirred till it was cool.

Formulation of Oily Creams and Incorporation of Microcapsules: Freshly prepared Wool Alcohol Ointment (3g) was melted on a water bath (Gallenkamp, England) at 50ºC. 5mL of water was heated on the water bath to the same temperature and gently added to the melted Wool Alcohol Ointment with constant stirring. The resulting mixture was removed from the water bath heater and stirred until cool and transferred into a clean container.
Selected cassava seed oil microcapsules containing to 2mL of oil were dispersed in each of the cooled oily cream bases and levigated using a spatula till homogenous creams were formed. The finished creams were kept in a clean container at room temperature away from light (Ang *et al*., 2019).

Evaluation of the antimicrobial activity of formulated creams containing cassava seed oil microcapsules: The eight bacterial strains used for the study were *Acinetobacter baumannii* NCTC 7363, *Serratia marcescens* ATCC 8155, *Staphylococcus aureus* ATCC 29213, *Citrobacter freundii* ATCC 8090, *Escherichia coli* ATCC 25925, *Salmonella typhimurium* 14028, *Pseudomonas aeruginosa* 27853 and *Staphylococcus aureus* ATCC 6571. Antimicrobial activity was determined by agar diffusion method.

Agar medium preparation towards antimicrobial agar diffusion tests: Approximately 22 mL quantities of freshly prepared sterile molten nutrient agar were poured into 16 sterile 90 mm Petri dishes. After cooling and setting, the agar plates were used, in duplicates, to determine the antimicrobial

activities of the formulated cassava seed oil microcapsules oily creams.

Inoculum preparation and antimicrobial agar diffusion test to determine the antimicrobial activities of formulated creams containing cassava seed oil microcapsules: The turbidity of the test bacteria to be used in the screening of the antimicrobial activities of the formulated cassava seed oil microcapsules oily creams was adjusted to a 0.5 McFarland standard (comparable to a bacterial suspension of 1.5 x 10^8 CFU/mL) and their suspensions were swabbed on the surface of the nutrient agar plates using sterile swab sticks. Each pair of agar plates were bored to create 7 mm diameter agar wells. Dilutions (2:1) of the oily creams incorporating the seed oil capsules and the oily cream base were prepared by diluting 2 g of the creams with 1 mL of liquid paraffin. The diluted microcapsules oily creams (0.1g), the oily creams base (0.1g) were introduced into different wells in duplicate plates in each group of labelled nutrient agar plates. Streptomycin, (130 µg/mL), was used as the positive control and the oily cream base was used as a negative control. 30 µL of the cassava seed oil were also introduced into different wells. The plates were incubated at 37ºC for 48 hours after which the zones of bacterial inhibition were measured in millimeters.

Statistical analysis
Statistical analysis was carried out using the Students' t-test and ANOVA at $p<0.05$ limit of significance (Graphpad Prism version 6.00 for Windows, GraphPad Software, La Jolla California, USA, www.graphpad.com).

RESULTS AND DISCUSSION

Antioxidant activity (DPPH scavenging assay): The DPPH scavenging activity of the Cassava seed oil and a standard antioxidant (ascorbic acid) is shown in Table 2. Activity ranged from 10.8 to 31.1% at a concentration of 100–1000 µg/mL. The scavenging activity increased in a dose dependent manner. The scavenging potential of the seed oil was lesser to that of the standard ascorbic acid. The antioxidant activity of the oil was observed to increase with increasing concentration and this may be attributed to the unsaturated nature of the oil due to the presence of several fatty acids in the oil. At 100 µg/mL, the percentage scavenging activity was 10.8 and this increased to 31.1 at 1000 µg/mL.

Particle shape and morphology of cassava seed oil microcapsules: Morphologies of the hydrated microcapsules formulated with calcium chloride were found to be discrete and generally spherical (Plate 1) in shape (Das and Senapati, 2007). While hydrated microcapsules crosslinked with aluminum sulphate were observed to be oblate and existed as aggregates. There was a color change from opaque cream to translucent yellow on drying. The mixture of sodium alginate and cassava seed oil were extruded as cream emulsions. After gelling and drying, small cassava seed oil globules entrapped within the matrix aggregated to form larger globules within the microcapsules. Moreover, ionotropic gelation of alginate precipitates it from the emulsion-like mixture and reduces its

emulsifying capacity causing the entrapped oil to aggregate within the microcapsules.

Batch A formulations had more intense yellow color than formulation in batch B because the alginate:oil ratio of batch B (5:1) was higher than batch A (2:1)., since batch A microcapsules contained more cassava seed oil than the batch B.

Table 2:
DPPH scavenging Activity of Cassava seed oil

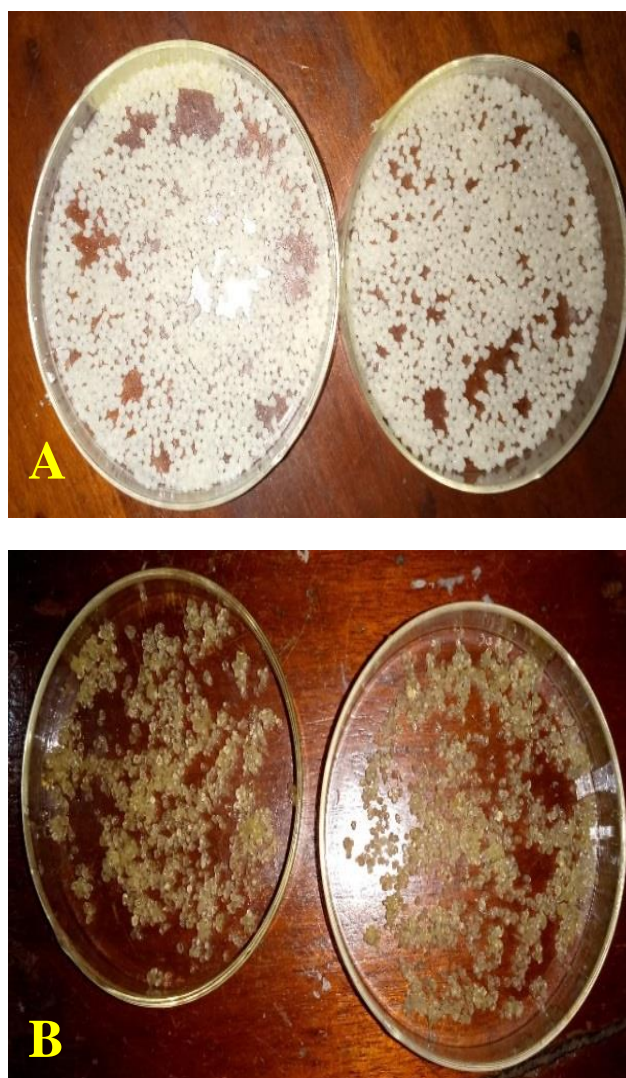| Concentration (µg/ mL)	Scavenging activity (%)	
	Cassava seed oil	Ascorbic acid
100	10.8	95.5
200	24.6	95.6
400	28.6	95.7
600	28.6	95.7
800	30.9	95.8
1000	31.1	96.9

Plate 1:
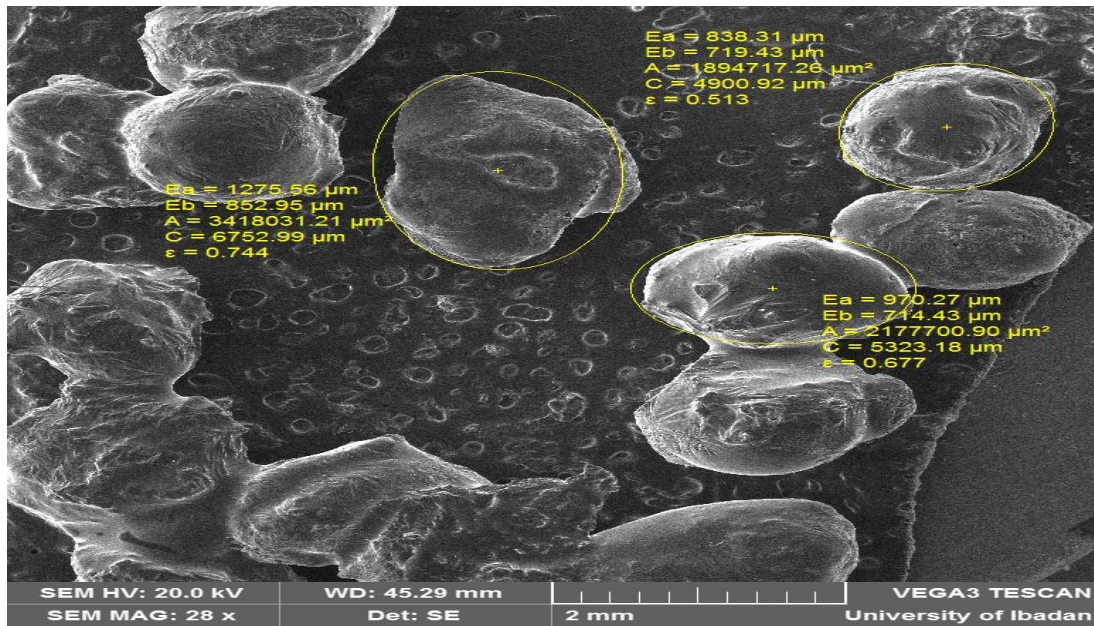Formulation **A-4** (a) Hydrated (b) Dried microbeads of cassava seed oil

Plate 2:
Scanning electron micrograph of formulation B-2 incorporating CaCl$_2$ as crosslinker
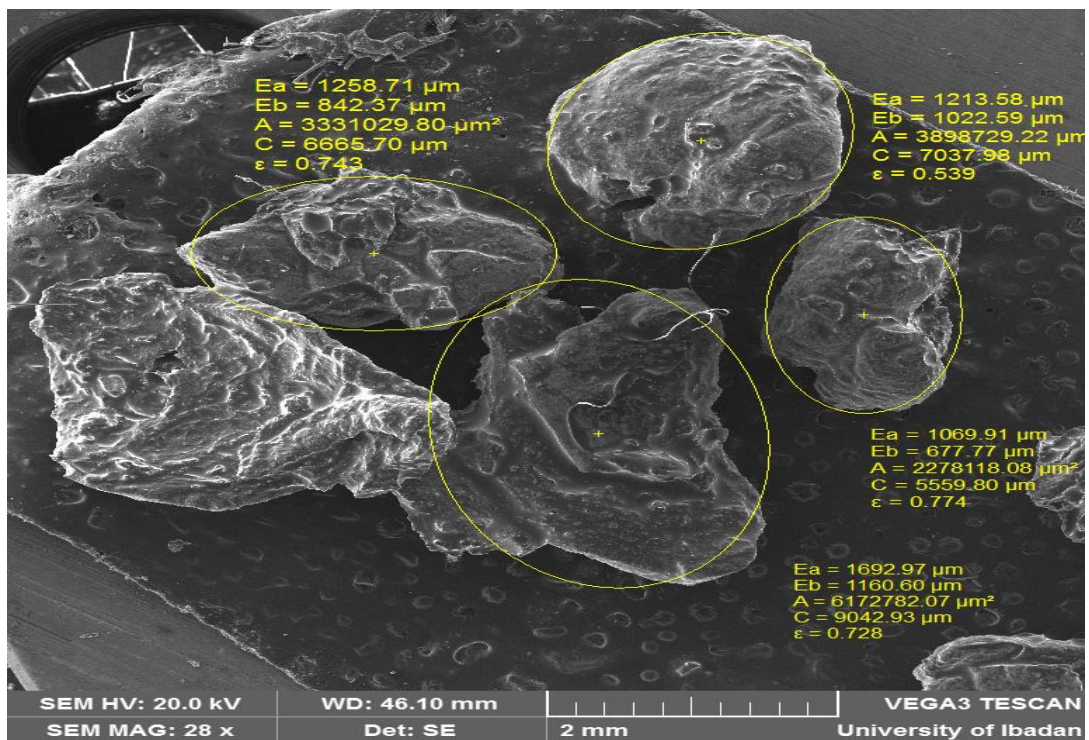

Plate 3:
Scanning Electron Micrograph of formulation A-7 with Al$_2$(SO4)$_3$ as crosslinker

Stereomicroscopic analysis of the topological features and shapes of the dried alginate microcapsules showed that microcapsules crosslinked with calcium chloride were relatively small, roughly spherical with relatively smooth surfaces, while those crosslinked with aluminum sulphate were relatively larger, irregularly shaped with rough surfaces. This was enhanced with the SEM photomicrographs of the dried alginate microcapsules that had the highest encapsulation efficiencies (Plates 2 & 3).

However, at higher SEM magnifications, the surfaces of the alginate microcapsules were observed to be rough; they exhibited crimpy surfaces with the appearance of noticeable lumps. This is due to the high concentration of dispersed oil which is deposited at the molecular level onto the inner or outer surface of the external layer of the microcapsules. The presence of oil components on the alginate surface causes the surface to plasticize and results in the formation of lumps (Soliman *et al.,* 2013).

At higher magnifications, surface of formulation cross-linked with $Al_2(SO4)_3$ (A-7) had pores and cracks, while the surface of B-2 (cross-linked with $CaCl_2$) had pores but no cracks. The microcapsules sizes ranged from 2.13 ± 0.43 to 3.51 ± 0.59 mm and the particle diameters were affected by alginate:oil ratio, curing time, polymer concentration and cross-linking agent used. The particle size distribution of each formulation was within a narrow (Table 1).

Effect of alginate concentration: The diameter of the microcapsules was observed to decrease with increase in the alginate:oil ratio from 2:1 to 5:1, implying that there was a decrease in the particle size with increasing amounts of the sodium alginate in the formulations (Table 1). Das and Senapati (2007) showed a progressive increase in encapsulation efficiency with increased sodium alginate concentration. This pattern was also observed in the results obtained; on average, that samples in batch B (Alginate: oil ratio 5:1) had higher encapsulation efficiency than samples in batch A (Alginate: oil ratio 2:1). This is explained by the increased availability of active cation-binding sites in the polymeric chains of alginate polymers following increased quantity of sodium alginate (El-Kamal *et al.*, 2003).

Effects of type of Cross-linker: Batch A microcapsules crosslinked with aluminum sulphate had markedly higher particle size than those crosslinked with calcium chloride. But in batch B formulations, only B-7 had a significantly higher particle size than others. This is explained by the tight crosslinking between alginate and calcium ions which brings adjacent polymer chain closer so that the microcapsule size is smaller (Das and Senapati, 2007).

With an increase in the concentration of cross-linkers used from 10% to 20% some microcapsule formulations showed an increase in particle size; this increases in particles sizes were not significant. However, an increase in concentrations of cross-linkers results in more cross-linker cations which improves cross-linking between the alginate chains but gelation proceeds inward to the center, internal crosslinking shrinks the core space and consequently the particle size reduces (Babu *et al*., 2010).

The increase in encapsulation efficiency may be because of increased cross-linking density by Al^{3+} cations that reduce diffusion of the drug out of the beads during in the curing solution and formation of porous beads by Ca^{2+} cations which results in diffusion of the drug out of the bead at the time of curing (Das and Senapati, 2007). In addition, Ca^{2+} cations are more hydrophilic than Al^{3+} cations and they produce pore on the surface of the bead through which the core material may escape during the curing process (Sankalia *et al.,* 2005). Moreover, because alginate polymer chains have higher affinity for calcium ions than aluminum ions, they produce microcapsules of smaller diameter; smaller free volume of core space to entrap cassava seed oil compared to Al^{3+} cations that produce larger microcapsules (Soliman *et al.,* 2013).

Effect of Concentration of Cross-linker; Previous studies on the microencapsulation of essential oils into alginate microcapsules by Soliman *et al.,* (2013), showed an increase encapsulation efficiency following an increase in concentration from 0.125 to 0.5% beyond which the encapsulation efficacy decreased (Soliman *et al.,* 2013). Similarly, Babu *et al.*, (2010), noted that increased concentration of calcium chloride cross-linker caused a progressive decrease in the encapsulation efficiency of gellan gum microbeads loaded with amoxicillin. Gelation proceeds radially from the surface of the bead to its center (Babu *et al*., 2010). Initially, an increase in concentrations of cross-linkers will consequently lead to higher levels of cross-linker cations improving cross-linking between the alginate chains and formation a dense network which improves the ability to load and retain encapsulated oil (Soliman *et al.,* 2013). However, as gelation proceeds inward to the center, internal crosslinking shrinks the core space and the encapsulated material is expelled along with water (Babu *et al*., 2010). However, the results obtained did not follow this pattern as there were increases in the encapsulation efficiencies with increase in concentrations of crosslinking agents from 10 - 20%.

Effect of Curing time/Stirring time; In the study done by Soliman *et al.,* 2013, initial increase in curing time increased encapsulation efficiency, however beyond 20 minutes the encapsulation efficiency decreased (Soliman *et al.,* 2013). Babu *et al.*, (2010), and Sharma *et al.*, (2009), also reported decreases in encapsulation efficiencies with increased curing time. However, only batches A-2, A-4 and A-8 followed this trend where as other batches showed increased encapsulation efficiency with curing time.

Encapsulation efficiency; The encapsulation values were between the ranges of 23.63 ± 0.06 to $6.66 \pm 0.05\%$ (Table 1). Most of the encapsulation efficiencies obtained were below 50%, this may have been due to the concentration of alginate used (2%) employed in the emulsification of the cassava seed oil with distilled water. Moreover, the FT-IR analysis indicated presence of chemical interactions between calcium chloride and the encapsulated cassava seed oil which may have affected the UV absorbing capacity at the wavelength (235nm) employed in the determination (Figure 3). A fifteen-minute curing time was optimal for encapsulation of alginate:oil 2:1, compared to the 5:1 formulations. Higher curing time was required for the higher alginate concentration formulations. At alginate:oil ratio of 2:1, $Al_2(SO4)_3$ exhibited higher encapsulation efficiency compared to 5:1 alginate:oil, where $CaCl_2$ showed better efficiency. This highlights the need for careful choice of crosslinker depending on the type and proportion of formulation components

Fourier- transform Infrared Spectroscopy (FT-IR)
The FT-IR spectra suggests the presence of chemical interactions between cassava seed oil and the alginate polymers. This supports the values obtained for the encapsulation efficiencies and the result of the antimicrobial assay (Figure 1). Some prominent peaks observed on the FT-IR spectra of free cassava seed oil include: Symmetric C-H stretching of CH_2 at 2860 cm^{-1}, asymmetric C-H stretching at CH_2 at 2927 cm^{-1}, Ester C=O stretching at 1743 cm^{-1} and C-O stretching at 1164 cm^{-1}. The spectra of encapsulated cassava seed oils from the microcapsules showed the same peaks at similar wavenumbers to that of the free cassava seed oil. The peaks indicate the presence of aromatic fatty acids and esters.
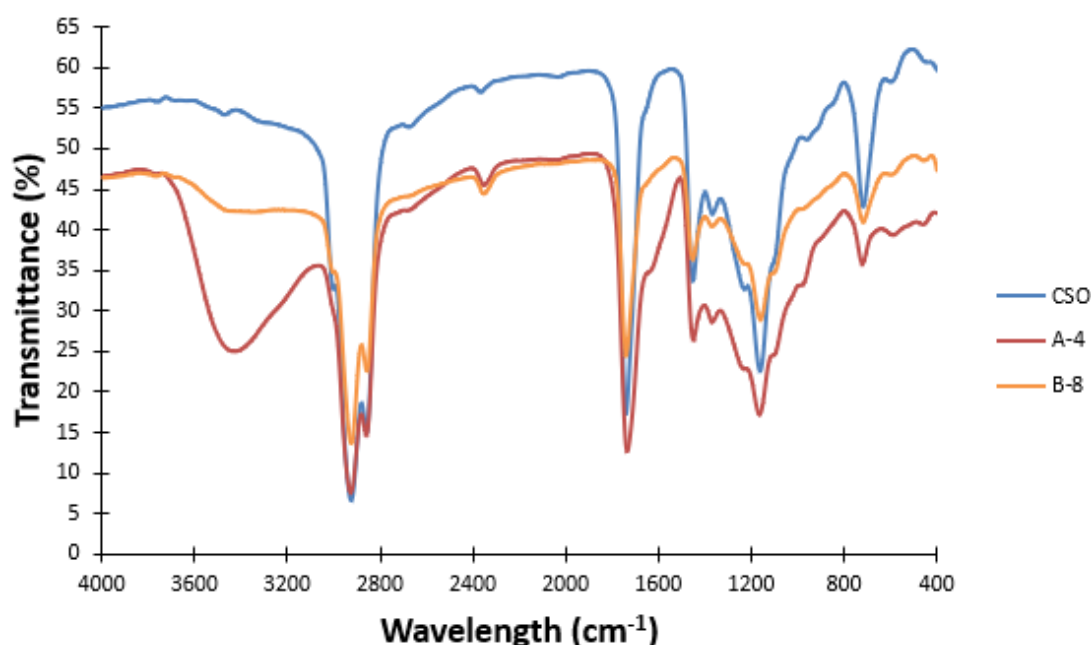
Figure 1:
Combined FT-IR spectra of Empty Cassava seed oil (CSO), A-4 microcapsules and B-8 Microcapsules

Table 3:
Inhibitory zones of Cassava seed oil microcapsule creams against test bacteria

Test	Zone of Inhibition (mm)							
	Acinetobacter baumannii NCTC 7363	*Serratia marcescens* ATCC 8155	*Staphylococcus aureus* ATCC 29213	*Citrobacter freundii* ATCC 8090	*Escherichia coli* ATCC 25925	*Salmonella typhimurium* 14028	*Pseudomonas aeruginosa* 27853	*Staphylococcus aureus* ATCC 6571
A-4 cream	24	12.5	10.5	10	9	18	18	20
B-4 cream	25	18	14	20	13	23	28	22
A-8 cream	11.5	22	13	10.5	11	-	32	11
B-8 cream	12.5	18.5	11	14.5	15	14.5	15	-
Oily Cream	11.5	-	-	-	-	-	-	-
Streptomycin	24	20	18.5	11	25	15	12	22
Cassava seed oil	19.5	26	16	29.5	26.5	27.5	38.5	30

Similar to a research done by Henriques *et al.*, 2017, in the formulation of alginate microcapsules of olive oil, there were differences in the FT-IR spectra of the free cassava seed oil and encapsulated oils (Henriques *et al.*, 2017). For instance, the spectra of cassava seed oil in A-4 microcapsules recorded the appearance of a carboxylic acid O-H stretching at 3428 cm^{-1} which was absent in the spectra of the free cassava seed oil. B-8 spectra also recorded a less prominent carboxylic acid O-H stretching at 3341cm^{-1}. This implies that during the process of microencapsulation and storage of microcapsules, esters in cassava seed oil were hydrolyzed into carboxylic acid. The extent of this reaction was greater in microcapsules crosslinked with calcium alginate.

Also, the IR spectra showed that the cassava seed oil from A-4 and B-8 had lower band intensity than free cassava seed oil. The oil from A-4 had the lowest intensity especially at the fingerprint region. Alginate microcapsules crosslinked with calcium chloride showed greater chemical interaction with cassava seed oil than alginate microcapsule crosslinked with aluminum sulphate.

Antimicrobial activity of formulated oily creams of cassava seed oil microcapsules: The highest inhibition by the A4 cream (123.1%) was recorded upon *Acinetobacter baumannii* NCTC 7363, followed by *Staphylococcus aureus* ATCC 6571 (Table 3). However, while the B4 cream also exerted its highest inhibition on *Acinetobacter baumannii* NCTC 7363, an 87.5% inhibition was recorded against *Staphylococcus aureus* ATCC 29213. This B4 cream also exerted a 72.7% and 73.3% inhibition on *Pseudomonas aeruginosa* 27853 and *Staphylococcus aureus* ATCC 29213, respectively. Compared to the B8 cream, the B4 recorded better bacterial inhibition. The A8 cream recorded high inhibition against *Staphylococcus aureus* ATCC 29213 and *Pseudomonas aeruginosa* 27853, while the B8 cream's bacterial inhibitory potentials were generally lower than the other cream formulations, except for *Serratia marcescens* ATCC 8155 (71.1%). Creams A-4 and B-4 both recorded high inhibitory activities (24 and 25 mm, respectively) against *Acinetobacter baumannii* NCTC 7363, which was similar to that exerted by the positive control, streptomycin, while cream

formulations A-8 and B-8 both recorded lower inhibitory activities (11.5 and 12.5 mm, respectively) against it, compared to A4 and B4. Considering the positive streptomycin control, it was instructive that the inhibitory activities exerted by the cassava oil on the eight test bacteria was higher (except for *Acinetobacter baumannii* NCTC 7363 and *Staphylococcus aureus* ATCC 29213, where the positive control recorded a higher inhibition). Out of all the 8 bacteria used *Acinetobacter baumannii* NCTC 7363 recorded the best susceptibility to creams A4 and B4.

Antimicrobial activity of cassava seed oil microcapsules oily creams: When the microcapsules which had the longest contact times (30 minutes) with the highest concentration (20%) of both crosslinking agents (CaCl$_2$ and Al$_2$(SO4)$_3$) from both alginate: oil ratios (2:1 and 5:1) were incorporated into oily cream bases and their antimicrobial activities determined by agar diffusion method, the cassava seed oil in microcapsule creams retained antimicrobial activity and recorded inhibitory activities against the test bacteria (Tadele *et al*., 2008).

The microcapsule creams showed lesser antimicrobial activities compared to free cassava seed oil except formulation A4 on *Acinetobacter baumannii* NCTC 7363 and *Staphylococcus aureus* ATCC 29213. This may be attributed to the partial permeability of the alginate microcapsules walls resulting in the slow release rate of cassava seed oil from the microcapsules and through the oily cream base delaying the onset of antimicrobial action (Sumiga *et. al.,* 2019; Cai *et. al.,* 2019). Moreover, since the presence of chemical interactions between the chemical entities of the microcapsules was confirmed from FT-IR analysis, these chemical interactions may be responsible for the reduced antimicrobial activities when compared to that of the free cassava seed oil.

Cassava seed oil showed significant activity against the selected bacteria except *Staphylococcus aureus* ATCC 29213, a bacterium which also was not very susceptible to the positive streptomycin control. Thus, the antimicrobial activity of cassava seed oil appeared to be broad spectrum based as it showed inhibitory activities against both gram-negative and gram-positive organisms, and significant inhibitory activity against *Staphylococcus aureus* ATCC 6571 and *Eschericia coli* ATCC 25925 that are implicated in a wide variety of skin infections (Popoola *et. al.,* 2007). Cassava seed oil is rich in fatty acids linoleic acid, oleic acid and palmitic acid being the major fatty acids (Popoola and Yangomodou, 2006). Cassava seed oil showed greater activity against *Staphylococcus aureus* ATCC 6571 than other gram-negative bacteria except *Pseudomonas aeruginosa* 27853. Studies have shown that gram negative bacteria are generally more resistant to inactivation by fatty acids than Gram-positive bacteria because of the impermeability of the outer membrane of gram-negative bacteria to hydrophobic substances (Agoramoorthy, *et al*., 2007).

The cream base recorded no inhibitory activity against most of the bacteria but showed low inhibitory activity against *Acinetobacter baumannii* NCTC 7363. Oily cream consists of hard paraffin, soft paraffin, liquid paraffin and lanolin. The paraffin components possess very insignificant antimicrobial activity (Sengupta and Behera, 2014). Main components of refined pharmaceutical lanolin are free fatty acids, fatty alcohols and wax esters; fatty acids and fatty alcohols have been shown to inhibit the growth bacteria by disrupting their cell membrane. As demonstrated by Eder *et al.* (2017), *Acinetobacter baumannii* possesses the ability to assimilate polyunsaturated fatty acids into their phospholipid membrane. Incorporation of these fatty acids causes a remodeling that adversely affects the permeability of the membrane and renders the bacteria more susceptible to other hydrophobic antimicrobial constituents of lanolin compared to other bacteria (Eder *et al.,* 2017).

In conclusion, cassava seed oil-alginate microcapsules were formulated using at 2:1 and 5:1 alginate; oil ratios, 10% and 20% calcium chloride and aluminum sulphate as cross-linkers and stirring times of 15minutes and 20 minutes. The microcapsules crosslinked with calcium chloride were roughly spherical and have relatively smooth surfaces while microcapsules crosslinked with aluminum sulphate are irregularly shaped and have rough surfaces. Encapsulation efficiency of cassava seed oil alginate microcapsules generally increased with increase in alginate:oil ratio, increase in concentration of cross-linkers, increase in curing time. The oily cream of cassava seed oil microcapsules retained its antimicrobial property against some of the microorganisms having highest inhibitory activities against *Pseudomonas aeruginosa* 27853 and the least inhibitory activities against *Escherichia coli* ATCC 2592. Oily creams of microcapsules formulations crosslinked with calcium chloride had better antimicrobial activity than microcapsules crosslinked with aluminum sulphate. Cassava seed oil microcapsule creams with antimicrobial and antioxidant properties were produced using a simple process with biodegradable and renewable materials.

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