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Enhancement of Bioactive Components in Mango Kernel Seed Oil Microencapsulated with Cassava Starch, Corn Starch, and Their Composites

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Abstract

Mango Kernel seed oil extract contain high antioxidants activities. The study evaluated the influence of corn cassava starches, their blends, as well as the cell wall materials on the bioactive components of mango kernel seed extract microcapsule. Microencapsulation was prepared by using the cell wall materials together with mango kernel seed extract and water homogenized using a high-speed homogenizer at using high pressure at 15,000 rpm for about 10 min. the homogenate was later freeze dried, packaged and stored for 40 days. The total phenol content of non-freeze-dried homogenate samples ranged between 0.47-0.88 mg/100g, but reduced when stored for 40 days (0.36-0.67 mg/100g). The flavonoid content ranged from 0.16 to 0.26 mg/100g, but increased with storage from 0.30- 0.60 mg/100g. Both total phenol and flavonoid reduced with freeze drying (0.07 -0.69 and 0.03-0.04 mg/100g respectively) but picked during storage (0.36 – 0.67 and 0.30-0.60 mg/100g). Fourier transform infrared spectroscopy analysis indicated freeze dried samples of cassava and corn starch carrier contained more bioactive compounds than freeze dried samples with the blends as cell wall materials. Bioactive compounds functional group identified in the samples using FTIR include sp^3 C-H, aldehyde C-H, ketone, amine, sp^2 C-H, strong alkyl C-O, medium alkyl C-O, weak alkyne, alcohol O-H and Carboxylic acid. Use of cassava starch as a cell wall material preserves the bioactive compound better than using composite starch when freeze dried and in stored.

Keywords: *Antioxidants, Cell wall materials, FTIR, Mango kernel seed oil, Microencapsulation*

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Introduction

Microencapsulation is a method in which tiny particles or droplets are surrounded by a coating wall, or are embedded in a homogeneous or heterogeneous matrix, to form small capsules (Gharsallaoui *et al*., 2007; Castillo *et al*., 2011). Microencapsulation technique is widely studied, and it helps to protect sensitive food ingredients, develop novel food formulation and deliver sensitive bioactive to the consumer without destruction (Awolu *et al*., 2022). Presently, spray drying, freeze drying, spray cooling, extrusion, coacervation, liposome entrapment, co-crystallisation, emulsion (Augustin and Hemar, 2009; Jeyakumari *et al*., 2016) are some of the widely used encapsulation techniques used in the food industries. The use of freeze drying has the advantage of non-application of heat but increase the processing cost (Lopez *et al*., 2012). Basically, freeze drying has application in the encapsulation of heat sensitive volatile compounds and pharmaceuticals (Awolu *et al.*, 2023; Tai *et al*., 2011).

Fruits and vegetables are the major sources of bioactive compounds and also contain various kinds of natural antioxidant compounds. Mango (*Mangifera indica* L.) is one of the most important tropical fruits in the world (Ibarra *et al*., 2015). Based on its chemical composition, it is regarded as the king of fruits, a distinction that makes it the second most traded tropical fruit in the world and fifth in total production (FAOSTAD, 2015). The world production of mango is estimated at 42 million tons per year (FAOSTAD, 2015). In

2014, it was estimated that more than one million tons of mango seeds were annually produced as wastes with no commercial purposes (Leanpolchareanchai *et al.*, 2014).

Mango seed has been reported as a bio-waste with high content of bioactive compounds (phenolic compounds, carotenoids, vitamin C, and dietary fibre) that improve human health (Olorunfemi *et al.*, 2022; Awolu et al., 2018; Jahurul *et al*., 2015). Studies hve also shown that mango seed kernel contain oil ranging from 2.72-25.57% depending on cultivating climate, cultivar, extraction solvent and extraction time (Olagunju, 2013; Amel *et al* ., 2015;Karunanithi *et al*., 2015). Oil extracted from mango seed kernel was reported to be semi solid at room temperature but with a melting point of 32 - 42°C (90 - 108°F). The oil was said to contain total saturated fat of 45% and total unsaturated fat of 55% essential bioactive compounds including antioxidants such as phenols and flavonoids (Awolu and Manohar, 2019; Nzikou *et al*., 2009; Amel *et al*., 2015).

Bioactive compounds are natural constituents of foods that provide health benefits (Biesalski *et al*., 2009) beyond the basic nutritional value of the product. Bioactive compounds are unstable once they are isolated from their natural source and need to be protected from the environment and undesirable interactions with other component. One of the alternatives used to improve bioactive compounds stability is the microencapsulation technique (Olabiran et al., 2023; Awolu et al., 2022), which entraps a sensitive ingredient inside a coating material. The need for protecting, increase shelf life and stabilizing bioactive compounds brought about the use of coating materials (wall materials). There are a number of commercially approved coating materials available to produce various microencapsulated foods. Coating materials are often used in combination with other coating materials and modifiers such as oxygen scavengers, antioxidants, chelating agents and surfactants in order to meet all the properties needed for protection, stabilization and slow release of food ingredients (Jeyakumari *et al*., 2016).

Carbohydrates such as starch and cyclodextrins have good ability to absorb volatiles from the environment and are good for flavour encapsulation. Gum Arabic is a commonly used capsule material due to its viscosity, solubility and emulsification characteristics but its cost is a major disadvantage. Protein based materials are able to form stable emulsions with volatile flavor components but their solubilities in cold water, potential to react with carbonyls and high cost limit their application. Ethyl cellulose is a good material to encapsulate water soluble vitamins because it is water soluble itself and as the shell thickness increases, the water permeability of the core vitamin is reduced (Jeyakumari *et al*., 2016).

This study will examine the use of starch from corn, cassava and; the composite starch of corn and cassava as shell materials to preserve bioactive compounds as well as the effect of freeze drying techniques on microencapsulation stability of bioactive compounds found in mango seed kernel oil in storage.

Materials and Methods

Materials

Mango fruits were obtained from local market in Akure and authenticated in the laboratory of Federal University of Technology, Akure. Cassava and corn starch were obtained from Pascal Scientific, Akure. All reagents are of analytical grade and obtained from accredited distributor of Sigma-Aldrich chemical company in Nigeria.

Preparation of Mango kernel seed

The mango fruits were washed and manually peeled to remove the kernels. The kernels were sundried for two weeks, de-coated to obtain the seed and the seed further dried for one week. The dried kernel seed was blended into fine powder, packaged in polythene bags and stored in a desiccator for further use.

Extraction of Mango kernel seed oil

One kg of the dried mango kernel seed powder was weighed and placed in a muslin cloth before been placed in the soxhlet extractor. Using N-hexane as solvent, the soxhlet extractor was set to a temperature of 60°c to avoid the extract burning off, after which the oil extract was collected in a glass container and stored prior to use.

Microencapsulation

The extract was encapsulated using different wall materials (corn starch, cassava starch and composite of corn and cassava at 50:50). About 20g of each wall material was prepared with 66.6g of the oil extract and 46.7g of water to achieve a wall to core ratio of 1:1. Sodium phosphate buffer (80% wt) and tween 80 (10% wt) was used as emulsifier. All the samples were stirred using a high-pressure homogenizer to obtain nano-particle. The samples were freeze dried, packaged and stored in a desiccator for further use.

Moisture content analysis

The average moisture content of encapsulated powders was measured gravimetrically by oven drying method using hot air oven according to the method of AOAC (2005).

Total Phenol and Flavonoid Contents Determination

The total phenol content of samples (freeze dried and non-freeze dried encapsulate) was determined by the method of Singleton *et al.,* (1999). Exactly 0.2ml of the sample mixture was mixed with 2.5ml of 10% Folinciocalteau's reagent and 2ml of 7.5% sodium carbonate. The mixture was then subsequently incubated at 45°c for 40mins, and the absorbance was measured at 700nm in the spectrophotometer, and garlic acid was used as standard phenol. Flavonoid was determined using catechin as standard according to the method reported by Castro-Alatorre *et al.* (2021) and absorbance taken at 500 nm.

Fourier-Transform Infrared (FTIR) Spectroscopy

A small quantity of the mango kernel oil was taken separately and potassium bromide (KBr) was added. Pellet was prepared with the help of pellet maker and this pellet was placed in IR chamber and analyzed by Apodization Happ-Genzel Alpha-T Model Fourier Transform Infrared Spectroscope (FTIR). This was used to detect the characteristic peaks corresponding to the functional groups.

Statistical Analysis

Analysis was carried out in triplicates and the results subjected to statistical analysis using the statistical package for social statistics (SPSS version 17). The mean and standard deviation of the triplicate analysis of the samples were calculated. The analysis of variance (ANOVA) was performed to determine significant differences between the means of bioactive properties (total phenol and total flavonoid), while the mean was separated using the Duncan's multiple range test at $p \leq 0.05$.

Results and Discussion

Proximate composition of the crunchy snacks is presented in Table 1. The data reviewed that the moisture contents of the formulated snack samples were lower than the moisture content (16.66%) of rice snack reported by (Folorunso *et al.*, 2016). The highest level of protein was observed in sample WRKBO1 (9.52%) having white maize flour 70%, red kidney beans 25% and onion flour 5%. The combined consumption of beans and cereals can ensure a balanced protein diet due to the nutritional complementation of essential amino acids (Hayat *et al.*, 2014).

Nutritional Quality and Relative Weight of Rats Fed with Crunchy Snacks

The nutritional quality of crunchy snack produced from corn, red kidney bean and onion composite flour depicting the protein quality and relative weight gain of experimental animals is shown in Table 2.

Total phenol and flavonoid content of non-freeze dried and freeze-dried mango kernel seed oil microcapsules

The total phenol and flavonoid content ranges between 0.47–0.88mg/g and 0.16-0.26mg/g respectively as shown in Table 1. Sample with cassava starch (sample B) as wall material had the highest value while sample with composite of cassava and corn starch (sample C) had least phenol content. Sample A (mango extract with corn starch) had the highest flavonoid content of 0.26 % while sample C (mango oil extract with composite starch) had the lowest flavonoid content of 0.16%. Phenolic and flavonoid contents of the encapsulated samples (corn, cassava and composite starch as wall material) were significantly ($P \leq 0.05$) different from each other. This was an indication that phenol and flavonoid content of encapsulated mango seed oil was dependent on the type and concentration of coating materials (Sahin-Nadeem *et al*., 2013). The values were generally lower than those reported by (Pitchaon, 2011) on encapsulation of extract from mango kernel waste using Maltodextrin, whey protein isolate and Arabic gum as wall material. After freeze drying, total phenol and flavonoid range from 0.07 mg/100g for sample A (corn starch wall) to 0.69 mg/100g for sample B (cassava starch wall) and 0.03 to 0.04 mg/100g respectively.

There was decrease in total phenol and flavonoid content of the three samples with freeze drying. However, decrease observed in sample A after freeze drying for total phenol was very high (about 88%) when compared with other samples (about 21 and 27% for sample B and sample C respectively) and decrease in flavonoid for all the samples was also high (about 75 -87%). Ogrodowska *et al*., (2017) also reported highest bioactive compound losses in freeze dried sample of encapsulated pumpkin seed oil when compared with homogenized and non-homogenized spray dried emulsion. The decrease observed in freeze dried sample may be due to long time aeration of the sample during freeze drying which could have resulted in the degradation of phenols and flavonoid compounds (Ogrodowska *et al*., 2017).

Table 1: Total phenol and flavonoid content of non-freeze dried and freeze dried encapsulate

Sample	Total phenol mgGAE/g		Flavonoid mgCE/g	
	Non-freeze dried	Freeze dried	Non freeze dried	Freeze dried
A	0.60 ± 0.02	0.07 ± 0.01	0.26 ± 0.02	0.03 ± 0.01
B	0.88 ± 0.01	0.69 ± 0.02	0.23 ± 0.01	0.03 ± 0.01
C	0.47 ± 0.01	0.34 ± 0.01	0.16 ± 0.01	0.04 ± 0.01

Values reported are means ± standard deviation of triplicate determinations. A = core material + corn starch; B= core material + cassava starch; C = core material + composite (corn + cassava) starch.

Total Phenol and Flavonoid content of non-freeze dried and freeze-dried mango kernel seed oil microcapsule stored for 40 days

The total phenol content and flavonoid content of the high pressure homogenized emulsion encapsulate stored for 40 days is shown in Table 2. The total phenol content ranged between 0.36-0.45% while the flavonoid content ranged between 0.30-0.60%. Sample SB had the highest total phenol content as well as flavonoid content while sample SC has the lowest total phenol and sample SA had the lowest flavonoid content when stored for 40 days. The value obtained for microcapsule stored for 40 days after freeze drying as presented in Table 2 ranged from 0.25 to 0.78 for total phenol and from 0.12 to 0.53 for flavonoids. Freeze dried sample B had the highest total phenol content while freeze dried sample A had the lowest. Also, freeze dried sample C had the highest flavonoid content while freeze dried sample B had the lowest flavonoid content after storage for 40days.

Table 2: Total phenol and flavonoid content of non-freeze dried and freeze dried encapsulate stored for 40 days

Sample	Total phenol mgGAE/g		Flavonoid mgCE/g	
	Non-freeze dried	Freeze dried	Non-freeze dried	Freeze dried
A	0.45 ± 0.03	0.25 ± 0.17	0.30 ± 0.06	0.47 ± 0.03
B	0.67 ± 0.06	0.78 ± 0.06	0.60 ± 0.06	0.12 ± 0.06
C	0.36 ± 0.06	0.51 ± 0.44	0.47 ± 0.03	0.53 ± 0.11

Values reported are means ± standard deviation of triplicate determinations. A = core material + corn starch; B= core material + cassava starch; C = core material + composite (corn + cassava) starch.

Comparing Tables 1 and 2, it was observed that there was decrease in total phenol and increase in flavonoid content with storage for non-freeze-dried samples. However, an increase with storage was observed for total phenol and flavonoid of freeze dried. This indicated that the bioactive components of mango seed oil are more stable in storage when encapsulate is freeze dried. This is reflected in consistent trend observed for freeze dried samples.

FTIR analsis for non-freeze dried and freeze dried sample A (Mango oil encapsulated with corn starch).

The Fourier-Transform Infrared Spectroscopy (FTIR) analysis for sample A (freeze dried emulsion encapsulate) is shown in Figure 1a. The peak value of the bio-active component presents based on wavelength are 2922.2, 2855.1, 1744.4, 1481.1, 995.2, 1151.7, 1100, 2094.8, 3347.1 cm^{-1} which represent sp^3 C-H, aldehyde C-H, ketone, amine, sp^2 C-H, strong alkyl C-O, medium alkyl C-O, weak alkyne, alcohol O-H respectively as shown in Table 3.

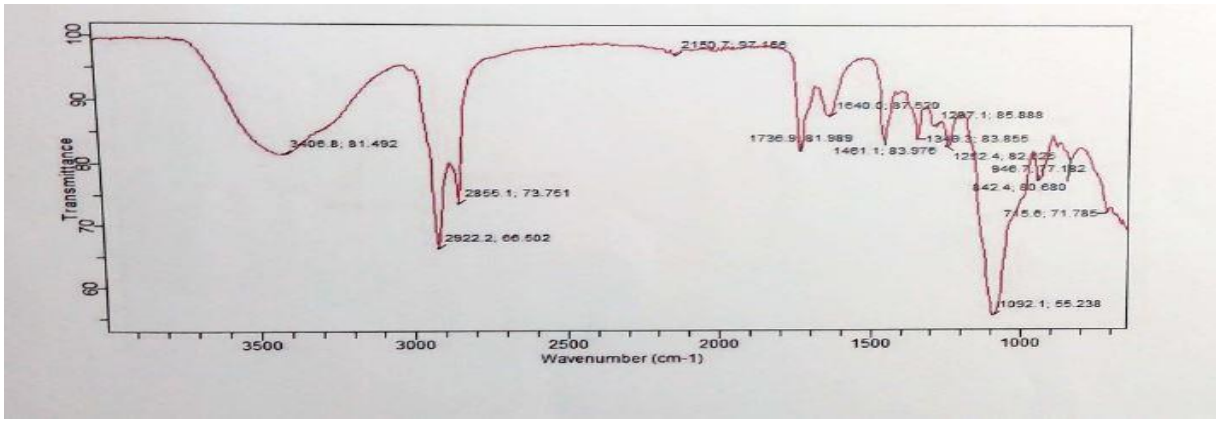

Figure 1a: FTIR analysis of freeze dried sample A (Mango oil encapsulated with corn starch)

Figure 1b showed the Fourier-Transform Infrared Spectroscopy (FTIR) analysis for sample SA (non-freeze dried encapsulate). The peak value of the bio-active component present based on their wave length are 2856, 2918, 1736, 1095, 3421, 1647, 1347, 1461, 9467 cm^{-1} (Figure 1b)which indicate the presence of aldehydes , aldehyde, aldehyde (N.U), sulfoxide (S=O), carboxylic acid, alkene, sp^3 C-H, sp^2 C-H, aromatic respectively (Table 3).

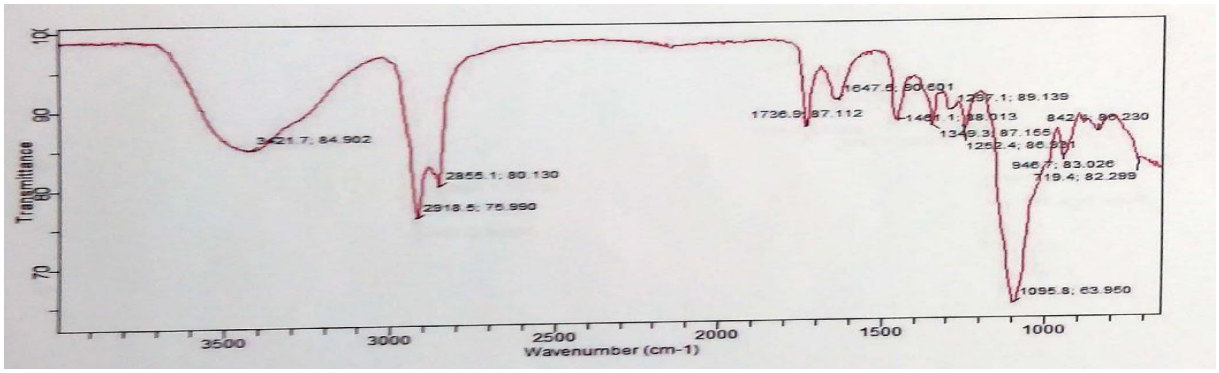

Figure 1b: FTIR analysis of non-freeze dried sample A (Mango oil encapsulated with corn starch)

Table 3: Summary of the Bioactive compounds in the microcapsules samples using FTIR

Sample A (corn starch)		**Sample B (cassava starch)**		**Sample C (composite starch)**	
Freeze dried	**Non-freeze dried**	**Freeze dried**	**Non-freeze dried**	**Freeze dried**	**Non-freeze dried**
Aldehyde C-H	Aldehyde	Aldehyde C-H	Aldehyde	Aldehyde	Aldehyde
Alkyl carboxyl (ketone)	Sulfoxide	Alkyl carboxyl (ketone)	Sulfoxide	Alkyne	Sulfoxide
Amine	Carboxylic acid	Aromatic	Carboxylic acid	Sulphones	Carboxylic acid
Strong Alkyl C-O	Aromatic	Alkyl C-O	Aromatic		Aromatic
Alcohol O-H		Alkyne			Alkyne
Medium Alkyl C-O		Unsaturated acid			Alcohol
					Amide
					Ethers

The result indicated that sample A freeze dried (corn starch wall) cotains more active compounds as against sample A which is not freeze dried. The results obtained can related to those gotten by (Durga *et al*., 2014) on the bioactive compounds of *Clitoria ternatea* leaf and flower extract.

FTIR analsis for non-freeze dried and freeze dried sample B (Mango oil encapsulated with cassava starch)

The Fourier-Transform Infrared Spectroscopy (FTIR) analysis for sample B, freeze dried microcapsule is shown in Figure 2a. The peak value of the bio-active component present based on their wave length are 3258.9, 2922.2, 2855.1, 955.2, 1148, 1640, 2079, 1744 cm^{-1} which indicated the presence of sp C-H, aromatic sp^2 C-H, sp^3 acldehyde C-H, sp^2 C-H, alkyl C-O, sp^2 C=O ketone, alkyne, unsaturated acid respectively.

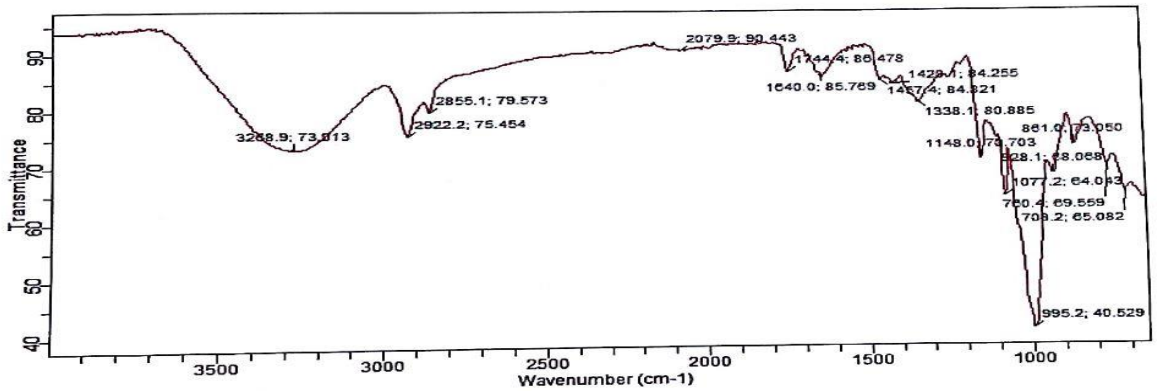

Figure 2a: FTIR analysis of freeze dried sample B (Mango oil encapsulated with cassava starch).

The peak value of the Fourier-Transform Infrared Spectroscopy (FTIR) analysis of non-feeze dried sample B are 3295, 2922, 1640, 1461, 1080, 946, 1740 cm^{-1} (Figure 2b). The peak value is an indication of the presence of carboxylic acid, alkane, amide C=O, sp^2 C-H, sulphate, aromatic, esther respectively (Table 3). The result showed that freeze dried mango seed kernel oil coated with cassava starch (sample B) contained more active compounds than non freeze dried sample B.

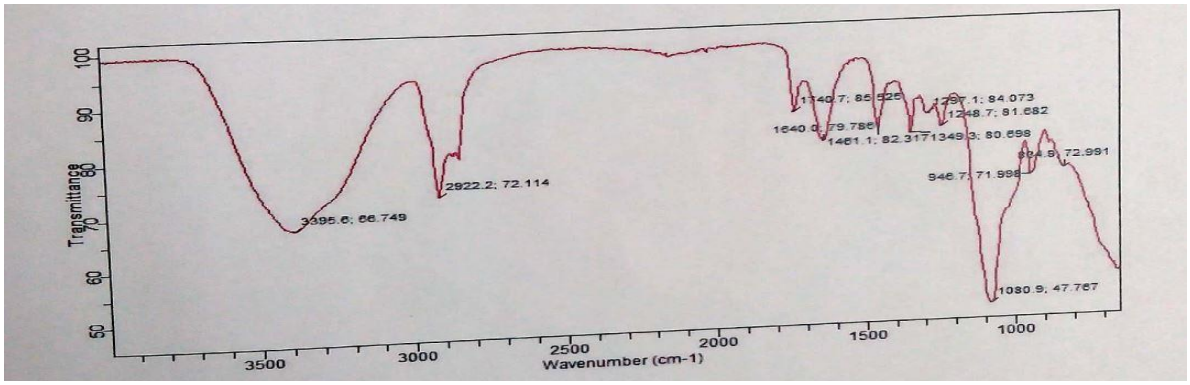

Figure 2b: FTIR analysis of non-freeze dried sample B (Mango oil encapsulated with cassava starch).

FTIR analsis for non-freeze dried and freeze dried sample C (Mango oil encapsulated with composite cassava and corn starch) before and after freeze drying.

The peak values of the compounds present in freeze dried sample C are 2922, 2856, 3004.2, 2160, 1744, 1162, 1462, 1237, 1114 cm^{-1} (Figure 3a) which indicated the presence of the following compounds alkane, aldehyde, alkane, alkyne, aldehyde, sulphones, sp^3 C-H, sulphtate, sulponyl respectively (Table 3).

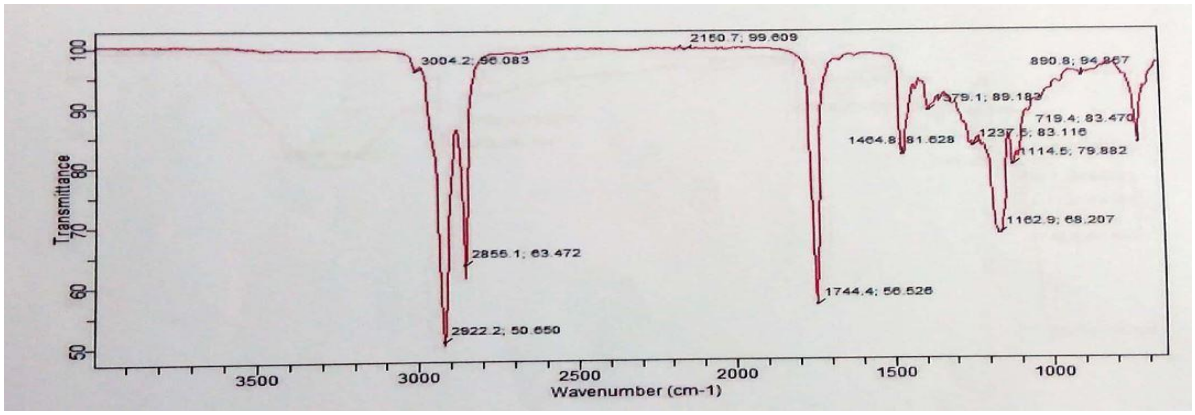

Figure 3a: FTIR analysis of freeze dried sample C (Mango oil encapsulated with composite of corn starch and cassava starch).

The bioactive compounds present in non-freeze dried sample C (high pressure homogenized emulsion) have peak values of 3406, 2855, 1736, 1461, 2150, 2922, 648, 1252, 1640, 1092 cm^{-1} (Figure 3b). This indicated the presence of carboxylic acid, alkane, aldehyde, sp^2 C-H, alkyne, alkane, alkene, alcohol, amide, ethers respectively (Table 3). It can be deduced that sample C (not freeze dried) has more active compound than freeze dried sample C.

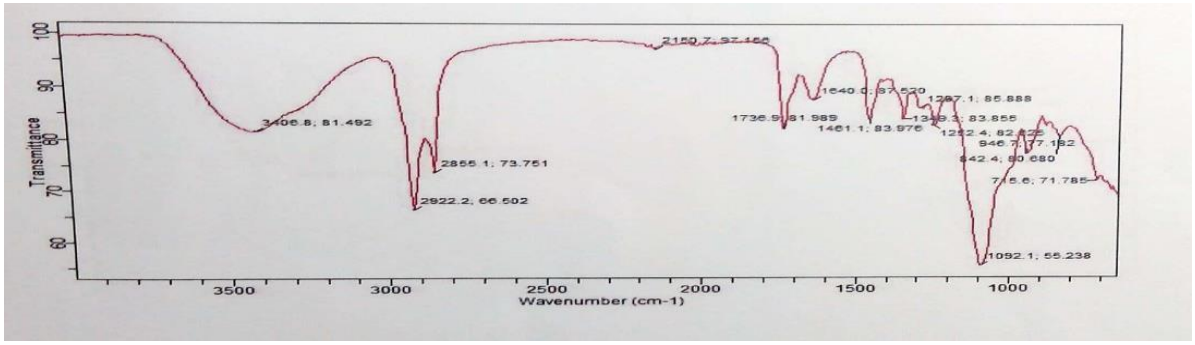

Figure 3b: FTIR analysis of non-freeze dried sample C (Mango oil encapsulated with composite of corn starch and cassava starch).

Moisture content analysis

The average percentage moisture content of freeze dried, sample A (corn starch), sample B (Cassava starch) and sample C (cassava+corn starch) encapsulates were 2.87%, 3.43% and 4.76%respectively. The results indicated that all the three encapsulated powders have lower moisture content (below 10%) due to low temperature (-40 to 30°C) application in freeze drying technique. The higher temperature difference between the drying medium and particles is required to increase the rate of heat transfer into particles, which provides the driving force for moisture removal (Goula and Adamopoulos, 2010).

Moreover, sublimation of ice crystal during freeze-drying leaves a porous structure (Anandharamakrishnan *et al*., 2010). The lower freezing temperature (-40°C) results in smaller pore size in the freeze-dried systems due to higher cooling rate and increased nucleation (Harnkarnsujarita *et al*., 2012). The small pores should effectively resist mass transfer and act as a barrier against sublimation (Pikal *et al*., 2002) Comparatively, freeze dried composite encapsulates had higher moisture content due to their combine ability to bind a great number of water molecules through hydrogen bonds.

Conclusion

The encapsulation of bioactive compounds of the mango kernel seed oil is dependent on the interaction between the oil extract and the wall materials - corn starch, cassava starch and composite corn and cassava starch. The result indicated the use of cassava starch as the encapsulating material whether freeze dried or in emulsion form would retain more total phenol and flavonoid as compared to corn starch or a composite of corn and cassava starch. Also the FTIR analysis indicated functional groups in the samples.

Declarations

Competing Interest

The authors declare no competing interest.

Authors' Contributions

The authors contributed equally to the research process, literature writing, review and editing of the article.

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