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Research Article

Microencapsulated Walnut Oil (*Juglans neotropica* Diels) by Spray Drying Technology and Determination of Fatty Acids Composition Stability

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Abstract

Background and Objective: walnut tocte (*Juglans neotropica* Diels) have high content of oil with good proportion of fatty acids. The aim of this study was to evaluate the microencapsulated walnuts (*Juglans neotropica* Diels) oil components using the Fourier-transform infrared spectroscopy (FTIR) technique and to determine its fatty acids composition before and after microencapsulation using the gas chromatography (GC) technique. **Materials and Methodology:** Walnut tocte oil was microencapsulated with temperatures at entrance and exit of 180 and 95°C, respectively, at a speed of 15 kg h^{-1}. The fatty acid profile was evaluated before and after microencapsulation using gas chromatography with a mass spectrum (GC-MS). Walnut tocte oil was characterized using the FTIR technique. Morphology structure of powder microencapsulated of walnut tocte oil was analyzed using scanning an electronic microscope (SEM). Microencapsulation yield (MEY), microencapsulation efficiency (MEE), water content and free oil was calculated. **Results:** Walnut tocte oil MEY was 79.19% and walnut tocte oil MEE was 62.21%. Walnut tocte oil fatty acids profile was analyzed with the gas chromatography technique, presenting no statistical differences in fatty acids total content. Linoleic acid was the most abundant fatty acid with a value of 65.30%, before microencapsulation and 67.57% after microencapsulation. Walnut tocte oil presented a high content of unsuturated fatty acids with 87.61% and a saturated fatty acids content of 18.05%. **Conclusion:** Walnut tocte oil, due to its composition, can be used for different purposes in the food industry and can be conserved using the spry drying technique.

Key words: Tocte oil, *Juglans neotropica* diels, microencapsulation, walnut, fatty acids, spray drying

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

It is scientifically accepted that eating habits are related to the prevention of certain metabolic diseases. For example, soy consumption is related to the prevention of certain types of cancer including colon cancer[1]. Nut consumption is indicated to prevent cardiovascular risk. Omega 3 monounsaturated acids (oleic acid) is recommended to prevent cardiovascular risk and cholesterol control[2]. The USA Functional Food Center defines functional food as "Natural or processed foods that contain known or unknown biologically-active compounds, the foods, in defined, effective and in non-toxic amounts, provided a clinically proven and documented health benefits for the prevention, management, or treatment of chronic diseases"[3-8]. Thus, nuts may possess functional characteristics due to their high amount of nutritive oils. Nuts, such as walnuts, peanuts, almonds, hazelnuts, among others, have a good nutritional composition. Those walnuts can contribute with their unsaturated fatty acids, when included in the daily diet. In these fruits, oleic and linoleic acids make 75% of the total fat content while the amounts of saturated fatty acids do not exceed 7%[9,10]. Nuts have a suitable fatty acids profile in the fat composition as well as other functional chemicals with antioxidant capacity[11,12], such as vitamin E and phenolic compounds[13-15].

Walnut tocte (*Juglans neotropical* Diels) is obtained of a native tree of South America in Colombia, Venezuela, Peru and Ecuador. Tocte oil is extracted of a walnut kernel with cold and heat press processes. Tocte oil has high quality bio-compounds such as protein, lipid, fiber, antioxidants and vitamins. Microencapsulation is a technique used for maintaining and, or protecting sensitive (to physical or chemical environments) materials or chemicals such as bioactive compounds[16], essential oils[17], seeds oils[18-20], nuts oils flavorings[21,22], hazelnut oil[23], walnut oil[24], palm oil[25], fish oil[26], and flaxseed oil[27]. In general, microencapsulates consist of the material "Of interest" to be protected and the carrier material that in general does not react with the material to be encapsulated and can protect of lipid oxidation of lipids microencapsulated[28].

Microparticles such as bio-compounds (fatty acids, omega 3, 6 and 9) can be added to different food products to modify flavor and aroma as well as changing texture and color. In addition, they can be added as antimicrobials and antioxidants. However, only a few studies have addressed their application in the food industry. To this extent, studies have been conducted on flaxseed oil microencapsulation in bread[29] and the application of lycopene microcapsules in cakes[30]. The aim of this study was to evaluate fatty acids content and morphological (electron microscopy) characteristics of microencapsulated oils of tocte walnuts (*Juglans neotropica* Diels) cultivated in Ecuador.

MATERIALS AND METHODS

Oil extraction: Shelled nuts (1 kg) were ground in the heat press (PITEBA oil expeller). To remove particles, oils were centrifuged at 4,000 rpm for 40 min at room temperature. Clarified oils were placed in amber vials with nitrogen atmosphere and stored at 4°C to continue with the analysis.

Emulsions preparation: Emulsions were prepared according to the Calvo *et al*.[31] methodology with some modifications. Table 1 shows the ingredients used to prepare emulsions. Gum Arabic (Roig Farma, Spain), maltodextrin DE 16 (Roig Farma, Spain) were dissolved in water at 50°C using a magnetic stirrer until complete dissolution. The tocte oil was added slowly and mixed with an international GLH-3005 OMNI homogenizer (GA, USA) at 10,000 rpm for 10 min.

Spray drying: Emulsions were spray dried using a LPG-5 High Speed (China) of 15 kg h^{-1}. Temperatures at the entrance and exit of the system were 150±2 and 95±2°C, respectively. After cooling at room temperature, the powder was placed in plastic bags and stored until analysis.

Microencapsulation yield (MEY): MEY was calculated using equation (A) as reported by Zhong *et al*.[32]:

Table 1: Analysis of fatty acids using GS-MS of tocte oil micro encapsulated and no micro encapsulated

	Before spry drying		Spry drying	
FAMEs	Percentage	SD	Percentage	SD
C14:0	0.40	0.10	0.06	0.02
C15:0	0.06	0.04	0.06	0.00
C16:0	5.20	0.00	5.23	0.00
C16:1	0.17	0.06	0.23	0.03
C17:0	0.08	0.03	0.06	0.01
C18:0	2.20	0.00	2.12	0.06
C18:1	18.07	0.15	16.49	0.06
C18:2	65.30	0.82	67.57	0.38
C18:3	3.70	0.10	3.49	0.00
C20:0	0.10	0.00	0.08	0.01
C20:1	0.10	0.00	0.06	0.00
C21:0	0.53	0.12	0.30	0.00
C22:1	0.27	0.06	0.06	0.01
Σ saturated	18.51			0.11
Σ unsaturated	87.61			0.48
Total FAMEs	96.10		96.00	

Results represent the average of three determinations±SD (n = 3)

$$\text{MEY}(\%) = \frac{\text{Mass of collected product}}{\text{Non-solvent mass in the feed}} \times 100$$

Microencapsulation efficiency (MEE): MEE was calculated according to the Davidov-Pardo *et al.*[33] methodology. It was calculated as the amount of oil (O) in the total amount of powder (P) obtained as follows:

$$\text{E}(\%) = \frac{\text{O}}{\text{P}} \times 100$$

FTIR analysis: The FTIR analysis of samples was performed with an FTIR spectrophotometer (FT/IR4100 Schimadzu, Japan). The range was from 4000-600 cm^{-1} while the resolution was 4 cm^{-1}. For each spectrum 30 interferograms were co-added before Fourier transformation and zero-filled to give a data point spacing of 1.9 cm^{-1}[34].

Gas chromatography analysis: Free fatty acids were extracted from tocte oil. One milliliter of freshly prepared transesterification reagent (methanol/acetyl chloride, 20:1 v/v) was added to each tube. The tubes were heated at 100°C for 1 h for the transmethylation, being shaken every 10-15 min. The mixture was cooled to room temperature and 1 mL each of water and hexane were added. The tubes were then shaken and centrifuged. Two phases were formed: The upper one (hexane) was transferred to another tube. This operation was repeated twice, to optimize sample lipid extraction. Hexanic phase (about 3 mL) was dried under N2 atmosphere and FAMEs were resuspended in 0.5 mL of hexane and injected into the gas chromatograph. The fatty acid composition of oil extracted from tocte walnut was analyzed by injecting FAMEs into an Agilent Technologies 7980 A system gas chromatography (Agilent, Santa Clara, CA) equipped with a MSD 5977A GC/MSD, an auto-sampler 7693, column (60 m×250×0.25 µm, Agilent 122-7062). The oven temperature was programmed as follows: From 80°C, ramp 1: to 100°C at 20°C/min for 1 min, ramp 2: at 200°C at 25°C/min for 10 min, ramp 3: at 250°C at 2°C/min. The injector and detector temperatures were set at 250°C. Helium was used as carrier gas at a linear flow velocity of 1.4 mL min^{-1}[35].

Microencapsulates storage: Powders were collected in glass vials, protected from light with aluminum foil and stored at room temperature (25±1°C). Microencapsulates were analyzed at 2, 8 and 15 days in oil content water activity and free oil. Micrographs were also taken from powders.

Micrographs of tocte oil microencapsulated: A Scanning Electron Microscope (SEM) JEOL JSM-6610 LV (Akyshama, Japan) was used to obtain micrographs using the back scattered electrons technique with an acceleration voltage of 10 KV[32].

Statistical analysis: Results are presented as means±standard deviation from three replicates of each experiment. Differences between mean values were determined by the analysis of one- way ANOVA. The *post-hoc* analysis was performed by the Tukey test. All tests were considered significant at $p<0.05$. Statistical analysis was performed using the software package Prism 4 for Windows, version 4.3 (GraphPad Software Inc., www.graphpad.com).

RESULTS AND DISCUSSION

Microencapsulation yield (MEY), microencapsulation efficiency (MEE) and free oil content: Figure 1a shows the tocte walnut seeds (*Juglans neotropica* Diels) cultivated in the Andean region in Ecuador. Seeds are of brown color with vertical dark lines. The skin was retired and kernels were ground to obtain oil with a cold press. Walnut oil presented no particles in suspension and a homogenous yellow color (Fig 1b). One kilogram of tocte walnut was used to obtain tocte oil. Yield was 77.0% of oil. The formulation of emulsions has 50% of water, 18.75% of Arabic gum, 12.5% of maltodextrin and 18.75% of tocte oil. This formulation was used to generate the microcapsules, the powder microencapsulated has a homogenous white color (Fig 1c). Tocte oil walnut MEY was of 79.19%. Tocte oil MEE was 62.21%. Different studies have reported MEE with a value of 35.3% for peanut oil (*Arachis hypogaea*), 38.5% for pecan oil (*Carya illinoinensis*), 44.1% for walnut oil (*Juglans regia*), 74.70% for halzenut oil (*Corylus avellana*), 70% for chia oil (*Salvia hispanica*) and from 73.70-93.90% for sacha inchi oil (*Plukenetia volubilis*). These results depend on the conditions of work, the emulsions of the different formulations and the initial concentrations of solids used in the assays[18,36,37]. Free oil of microencapsulated oil was calculated with a value of 13.0% of free oil. This result is compared to the three oils of reference with values of 20.82% for sacha inchi oil, 28.0% for linseed oil and 25.07% for omega 3 oil. Luna-Guevara *et al.*[37] have reported a content of free oil for walnut (*Juglans regia*) with a value of 5.50%, peanut (*Arachis hypogaea*) with a value of 5.18% of free oil and pecan oil (*Carya illinoinensis*) with a value of 4.48% of free oil[37].

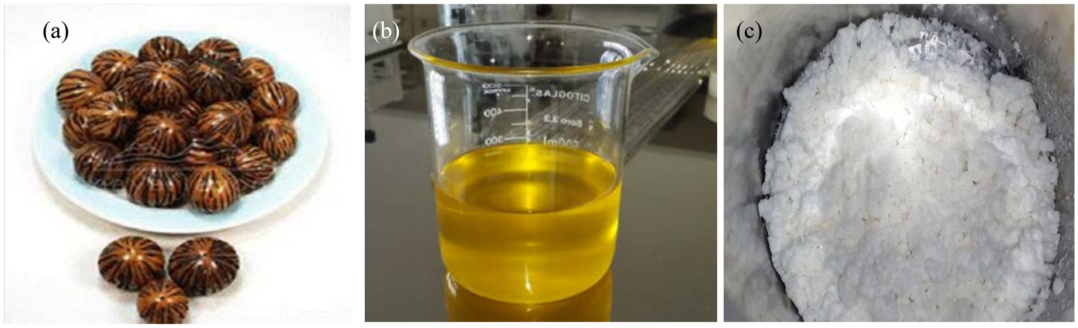

Fig. 1(a-c): (a) Walnuts of tocte (Juglans neotropica: Diels) seeds cultivated from Ecuador, (b) Walnut tocte oil extracted using PITEBA oil expeller and (c) Walnut tocte oil microencapsulated

Tocte oil fatty acids profile: Tocte oil (*Juglans neotropica* Diels) fatty acids profile was analyzed with a gas chromatography mass spectrometry (GC-MS) before and after microencapsulation. Fatty acids total content was 96.10 % of fatty acids before microencapsulation and 96.00% of fatty acids after microencapsulation. No statistical differences were detected. Linoleic acid was the most abundant fatty acid with a value of 65.30% before microencapsulation and presented an increase to 67.57% content after microencapsulation at statistical difference $p<0.05$ and a decrease of linolenic acid content with a value of 3.70% before microencpasulation and 3.49% of linolenic acid after microencpasulation. Barroso *et al.*[38], reported changes in fatty acids flexseed oil after the micro-encapsulation process. They reported an incresase of linoleic acid with a value of 13.30% before microencapsulation and 15.46% after microencapsulation with a decrease of linolenic acid with a value of 58.40% before microencapsulation and 54.41% after microencapsulation. They indicate that the increase of omega 6 can be due to the oxidation of omega 3 (linolenic acid)[38]. In this study, we observed the same situation with an increase of omega 6 and a decrease of omega 3 in walnut tocte oil. The next fatty acid in proportion was the oleic acid with a value of 18.07% before microencapsulation and 16.49% after microencapsulation. Statistical difference was $p<0.05$. Palmitic acid presented a value of 5.20% before microencapsulation and 5.23% after microencapsulation. Statistical difference, $p<0.05$. Tocte oil fatty acids content in this study is in accordance with the contents reported by Vilcacundo *et al.*[39] palmitic acid (5.05%), oleic acid (19.50%), linoleic acid (65.81%) and linolenic acid (2.79%) in tocte oil cultivated in Ecuador[39]. Tocte oil presented a good proportion of unsaturated fatty acids with a value of 87.61% of unsaturated fatty acids total content and 18.51% of saturated fatty acids total content. When tocte oil is compared to other vegetable oils cultivated in Ecuador, tocte oil has a good proportion of omega 9 (18.07%), omega 6 (65.30%) and omega 3 (3.70%). Sacha inchi oil (*Plukenetia volubilis*) present a high content of omega 6 and 3 (34.98 and 47.04%, respectively)[40], Sambo oil (*Cucurbita ficifolia* L) with a value of omega 9 and 6 (41.36 and 33.98%, respectively)[41], chia oil (*Salvia hispanica* L) with a high content of omega 3 (54.08%) and omega 6 (18.69%)[42]. Corn oil (*Zea mays* L) has a high content of omega 6 (52.68%) and omega 9 (29.70%)[43]. Kahai oil (*Caryodendron orinocense* Karst)[44] presents a high content of omega 6 (68.04%) and omega 9 (18.59%), both oils present high content of omega 6 as in tocte oil. Only macadamia oil (*Macadamia intergrifolia*)[35] and ungurahua oil (*Oenocarpus batua*) present similar or higher oleic acid content with values of 63.36 and 82.03% of oleic acid[45], with low contents of omega 6 and omega 3.

The water content in the microencapsulated tocte oil was determined for a 15 days storage period with a value of $2.56\pm0.07\%$, respectively. Three oils were used as oil of reference (Sacha inchi oil, Linseed oil and omega 3 oil) obtaining values of 5.18 ± 0.05, 3.73 ± 0.09 and $4.56\pm0.04\%$ of water content. The value of water content in tocte oil was the lowest compared to the other oils assayed.

Morphology of particle: Figure 2 shows the SEM micrographs of the microparticles obtained of walnut tocte oil. The power microencapsulated present spherical particles and collapsed particles (Fig. 2a). This can be related to temperature entrance and exit used in this study in the spray dry (150-95°C). Figure 2b shows the surface of spherical particles, the surface is uniform and regular. Luna-Guevara *et al.*[37] have reported the same situation to walnut, pecan and peanut microencapsulated oils. They used temperature at entrance

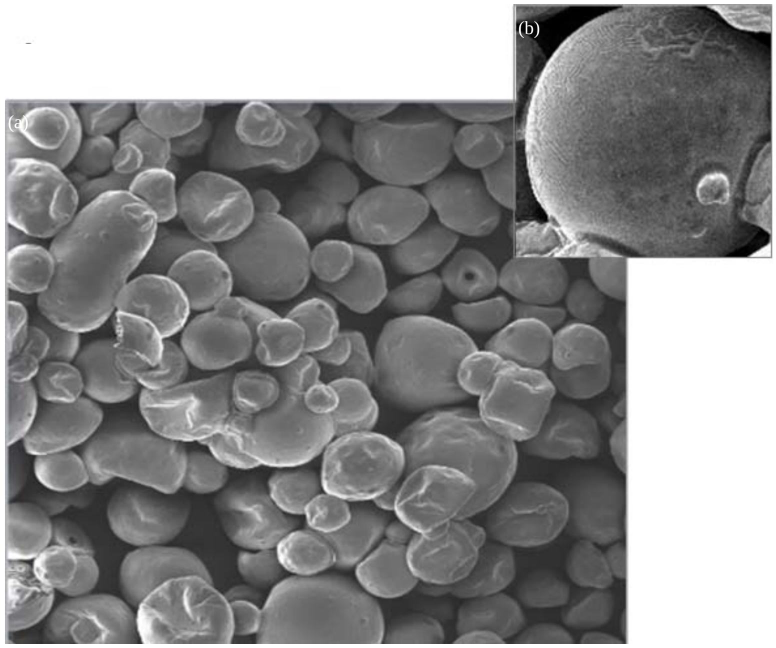

Fig. 2(a-b): SEM micrographs of the microparticles of microencapsulated tocte oil. Morphology of microcapsules of tocte oil powders augmented in (a) 510x and (b) 10,000x

and exit of 180 and 80°C[37]. Independently of the conditions of spray dryer temperatures and formulations used, the particles were spherical and collapsed particles distributed in the powder obtained. The particle size distribution was performed with a laser light diffractometry using a Horiba LB 550 particle analyzer. The particle size obtained for the microencapsulated particle was uniform with a size of 2.49±0.14 µm, indicating uniformity in the microencapsulated powder.

Microencapsulated tocte oil FTIR analysis: The spectra obtained presented great similitudes with the FTIR spectra of different edible oils reported in the literature. Guillen and Cabo[46] have reported the profile of lard, extra virgin olive and sunflower oil analyzed with the FTIR method. They identified 25 peaks corresponding to functional groups present in the samples. In this study, the FTIR was used to analyze a sample of tocte oil without microencapsulation (Figure 3a). In this spectrum, we identified eight functional groups of fatty acids from vegetal oil:

- 3007.44 cm^{-1} corresponding to = C-H (Cis) stretching vibration of the trans and cis olefinic double bands
- 2919.7 cm^{-1}-C-H-(CH2) methylene asymmetrical stretching band
- 2851.24 cm^{-1}-C-H-(CH2) methylene symmetrical stretching band
- 2361.41 cm^{-1} band was not identified
- 1741.41 cm^{-1}-C = O (ester) of triglycerides shows a stretching vibration band assigned to free fatty acid, this peak was intensive
- 1460.81 cm^{-1}-C-H (CH2-CH3) scissoring of the bending vibration of the methylene group
- 1158.04 cm^{-1} band is generally appreciably weaker than bands resulting of methylene scissoring
- 1095.37 cm^{-1}-C-O stretching group ester
- 715.46 cm^{-1}-HC = CH-(cis) band result of the overlapping of the methylene rocking vibration and the out-of-plane bending vibration of the *cis*-disubstituted olefins

Our results are in accordance with results reported by Guillen and Cabo[47]. Figure 3b shows peaks of tocte oil microencapsulated with the following identified bands:

- 2924.52 cm^{-1}-C-H (CH2) stretching asymmetric
- 2857.02 cm^{-1}-C-H (CH2) stretching symmetric
- 1741.41 cm^{-1} both corresponding to the -C = O (ester) of stretching vibration band assigned to free fatty acids. These bands confirm the presence of oil in power microencapsulated in this study. Figure 3c shows a mix of polymers used in the formulation maltodextrin and Arabic gum. In this spectrum, we do not see peaks of characteristic oil

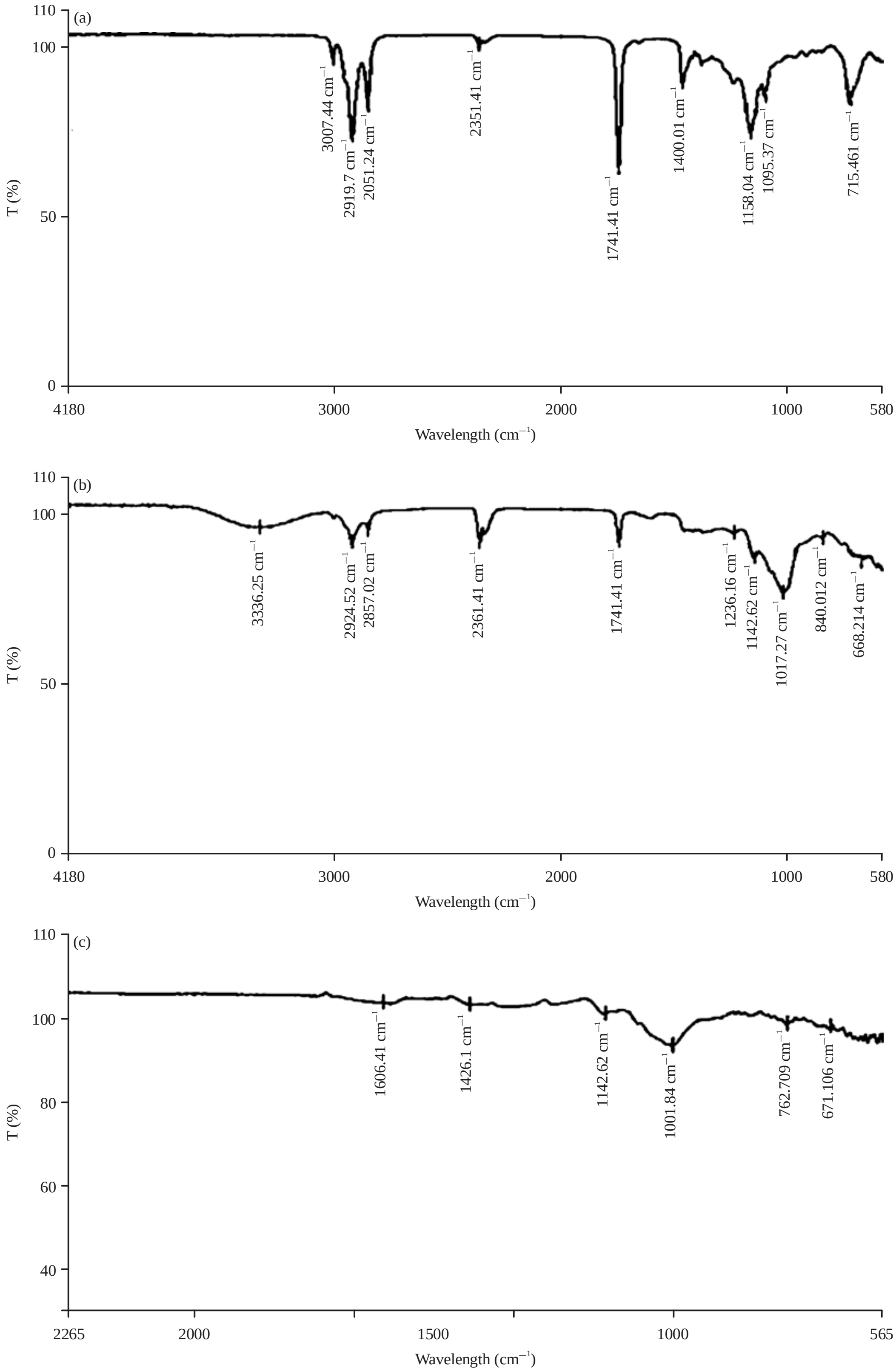

Fig. 3(a-c): FTIR analysis (a) Tocte oil without microencapsulation (b) Tocte oil microencapsulated and (c) mix of polymers: Maltodextrin and gum Arabic

CONCLUSION

Tocte walnut (*Juglans neotropica* Diels) cultivated in Ecuador have a good fatty acids composition. Tocte oil can be microencapsulated using the spray dry technology without affecting the fatty acids total content. The combination of Arabic gum and maltodextrin maintain the fatty acids composition. The tocte microencapsulated water content was low during the storage time. This can prolong the tocte oil expected life. Microspheres present a normal form with adequate small size particles in the microencapsulated powders. Tocte walnut powders can be offered to consumers in different manners, be included in their daily prepared foods at home or be added to more complex processed foods such as functional foods and foodstuffs to increase their nutritional quality.

SIGNIFICANCE STATEMENTS

The present study evaluated the synergetic effect of microencapsulation in the fatty acid composition of walnut tocte oil (*Juglans neotropica* Diels) and its content before and after micro encapsulation and found that there is an increase in unsaturated compounds. This finding has not been evaluated by previous studies. The result of this study helps the researchers to uncover the phenomenon related to the increase in unsaturated fatty acid while using the spry drying method. Morphological analysis of powder microencapsulated presented homogenous particles with normal size described for oil microencapsulated. The microencapsulation method can be used to preserve walnut oil and to be used in the food industry for different purposes.

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