Vol. 17, No. 2 (2018) 407-420
Revista Mexicana de Ingeniería Química

LEMONGRASS (*Cymbopogon citratus* (DC) Stapf) ESSENTIAL OIL ENCAPSULATION BY FREEZE-DRYING

ENCAPSULACIÓN DEL ACEITE ESENCIAL DE ZACATE LIMÓN (*Cymbopogon citratus* (DC) Stapf) POR LIOFILIZACIÓN

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Received October 3, 2017; Accepted December 26, 2017

Abstract

The objective of this study was to determine the effect of different encapsulating agents (gum arabic (GA), maltodextrin (MD) and/or xanthan gum (XG) and two different freezing methods (slow and fast)) on the encapsulating efficiency of *Cymbopogon citratus* essential oil and on its release during storage. Retention of $\alpha-$ and $\beta-$citral, $\beta-$myrcene and linalool were determined by Gas Chromatography Mass Spectra (GC MS). Release of the encapsulated compounds was determined at 76% relative humidity and 30°C. The highest retention obtained was 78±9% for $\beta-$citral, 100±16% for $\alpha-$citral, 81.6±5.8% for $\beta-$myrcene and 39.7±2.3% for linalool with the slow tested freezing protocol and a mixture of 50% GA, 40% MD, and 10% XG (volume). Kinetic release during storage was exponential and adequately fitted with Avrami´s model. Lemongrass essential oil can be encapsulated by freeze-drying using a mixture of gum arabic, maltodextrin and xanthan gum with a high retention of volatile compounds by using a slow freezing protocol.

Keywords: *Cymbopogon citratus*, citral, myrcene, linalool, freeze-drying.

Resumen

El objetivo de este trabajo fue determinar el efecto de diferentes mezclas de agentes encapsulantes (goma arábiga (GA), maltodextrina (MD) y/o goma xantana (GX) y dos diferentes métodos de congelamiento (lento y rápido)) sobre la eficiencia de encapsulamiento del aceite esencial de *Cymbopogon citratus* y evaluar su liberación durante el almacenamiento. La retención de $\alpha-$ y $\beta-$citral, $\beta-$mirceno y linalol se determinaron mediante cromatografía de gases con espectrometría de masas (CG EM). La liberación de los compuestos durante el almacenamiento se evaluó a 76% de humedad relativa y 30°C. Las retenciones más altas obtenidas fueron 78±9% para $\beta-$citral, 94±5.6% para $\alpha-$citral, 81.6±5.8% para $\beta-$mirceno y 39.7±2.3% para linalol, utilizando congelación lenta y una mezcla de 50% GA, 40% MD y 10% GX (volumen). La cinética de liberación de compuestos durante el almacenamiento fue exponencial y ajustada con el modelo de Avrami. El aceite esencial de zacate limón puede ser encapsulado mediante liofilización usando una mezcla de goma arábiga, maltodextrina y goma xantana con una retención elevada de compuestos volátiles utilizando una congelación lenta.

Palabras clave: *Cymbopogon citratus*, citral, mirceno, linalol, liofilización.

1 Introduction

Essential oils are frequently obtained from plants by hydrodistillation. They can contain hundreds of components, mainly monoterpenes and sesquiterpenes, hydrocarbons and their oxygen derivatives, as well as a few types of phenylpropanoids (Gautam and Agrawal, 2017); Kujur *et al*., 2017). *Cymbopogon citratus* (DC) Stapf (*Gramineae*) is an herb well known as lemongrass, lemon herb, lemon tea, as well as some other names. The tea extracted from the leaves is very popular and is

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Tel. 96-15-03-80 ext 325
doi: 10.24275/10.24275/uam/izt/dcbi/revmexingquim/2018v17n2/Enciso
issn-e: 2395-8472

used as an analgesic, anti-inflammatory, antipyretic, diuretic and anesthetic, among other uses. Essential oil obtained from the leaves of *C. citratus* contains a large amount of compounds that give it the characteristic smell; citral ($\alpha-$ and $\beta-$citral) is the most abundant compound with percentages that oscillate between 65 and 73% as was reported by Rocha *et al*. (2011) and up to 75.3% according to Gautam and Agrawal (2017). Citral content depends on a great variety of factors such as the place of origin of the plant, the weather, the habitat in which it is cultivated and its agronomic management, as well as others. Other compounds found in concentrations lower than citral are linalool, $\beta-$myrcene, geraniol and geranyl acentate (Gautam and Agrawal, 2017). These authors reported, however, that citral ($\alpha-$ and $\beta-$citral), myrcene, and linalool are the main compounds in *C. citratus* essential oil. Poonpaiboonpipat *et al*. (2013) also showed that citral ($\alpha-$ and $\beta-$citral), myrcene and linalool constitute 91% of total compounds in this essential oil. A common problem that has been reported in essential oils is the loss of aromatic quality and their biological activity during storage and transportation because of oxidation caused by environmental conditions, such as storage temperature and light exposure (Kujur *et al*., 2017). Because of this, a solution to this problem could be the isolation and protection of the compounds, making their useful period longer. Encapsulation is an attractive technology for protecting flavors and aromas of essential oils to avoid unwanted changes like oxidation; encapsulation also may help to control the release of aromatic compounds (Jafari *et al*., 2008; Dima *et al*., 2015), giving it more possible uses. The protecting capsule or encapsulating matrix can be formed by one or more compounds, usually called encapsulating agents, such as gum arabic, gelatin, starch, soy protein, whey protein, and chitosan, among others, which can be used as a blend or alone (Fernandes *et al*., 2014; Martínez *et al*., 2015). The optimal combination of encapsulating agents and their concentrations can lead to a better encapsulation and protection of the compounds during the drying process and storage (Dima *et al*., 2015). Dima *et al*. (2015) reported that microcapsules of coriander essential oil microencapsulated by spray drying using different encapsulating agents such as chitosan, alginate and blends of chitosan/alginate and chitosan/inulin, were resistant to pH and temperature variation. Furthermore, the encapsulating agents can ensure a slow release of essential oil.

Chranioti and Tzia (2013) suggest that a blend of starch, maltodextrin, and chitosans leads to a good retention of oleoresin encapsulated by lyophilization. On the other hand, Cano-Higuita *et al*. (2015) show that the retention of curcumin was higher using lyophilization compared to spray drying when a ternary blend of maltodextrin-gum arabic-modified starch was used. Lyophilization is a technology used in order to prolong the useful period of a product that is sensitive to heat, such as microorganisms, enzymes, and aromatic compounds, among others (Tovar-Benítez *et al*., 2016). Lyophilization presents higher yields in the process and percentage of retention of solid matrix compounds when compared to spray drying (Cortes-Rojas *et al*., 2014). For lyophilization, however, it is necessary to freeze the sample, and then when the water becomes ice during the freezing step, the water could be separated from the other components of the formulation. Thereafter, the highly concentrated droplets can start to aggregate and irreversibly fuse, leading to the destabilization of the system (do Vale Morais *et al*., 2017). Therefore, the freezing of the sample is a critical step of the lyophilization process because there are changes in the physical properties caused by ice crystal formation that may cause aggregation of the droplets in the emulsified systems (Tang and Pikal, 2004). Freezing is the first step of the freeze-drying process, in which the sample is exposed to low temperatures so that the sample becomes solid (do Vale Morais *et al*., 2016), then freeze-drying occurs. The freezing rate is a parameter that should be optimized because freezing rates result in the formation of different types of ice crystals. Fast freezing leads to small and numerous ice crystals, whereas slow freezing forms larger and less numerous crystals. Thus, the surface area of these crystals will influence the further freeze-drying steps (Ingvarsson *et al*., 2011). Big crystal formation can provoke the collapse of the emulsion that contains encapsulating agents and the essential oil. Consequently, it will have lower yields of encapsulation. Choi *et al*. (2008) indicated that the flavor losses are attributed to the changes of structure in the capsule´s wall which are associated with freezing before sublimation. On the other hand, small crystal with points can break the capsules, promoting the liberation of their content in a continuous phase and probably lowering the retention percentage of aromatic compounds in the solid matrix. That way, in the field of essential oil encapsulation, it is not evident if the freezing methods promote a better retention percentage from the aromatic compounds.

There are some works on the encapsulation of the essential oil of *C. citratus* by spray drying

(Weisheimer *et al*., 2010; Sosa *et al*., 2014; Moreira Ribeiro *et al*., 2015, Punpee *et al*., 2017), but there is no information about encapsulation using the lyophilization process. Therefore, the objectives of this research were as follows: i) to investigate the effect of several blends of encapsulating agents (arabic and xanthan gums and maltodextrin) and two different methods of freezing (slow and fast) on the encapsulation of *C. citratus* essential oil and ii) to determinate the release kinetics of encapsulated essential oil during storage.

2 Materials and methods

Healthy and green leaves of *C. citratus* were cultivated in a field located in Cintalapa de Figueroa, Chiapas, México (16°51´30.6"N, 0.93°41´27.6"W). The essential oil was extracted by steam extraction. Briefly, leaves were packed in a stainless steel column, which was made to pass into water vapor from a cauldron containing boiling water with 2kg/cm^2 of manometric pressure. Water vapor crosses the packed leaves of *C. citratus*, dragging the essential oil with the help of condensation, which has to be supplied with water at environmental temperature. Finally, the water-essential oil mix was separated by density difference by using a separation funnel. For this process, citral (>95%), β−myrcene (>95%), linalool (>95%) and hexane (grade HPLC) were used; all were acquired in Sigma-Aldrich, St. Louis, MO, USA and used for calibration curves. The gum arabic used was acquired in Hycel (México), xanthan gum in Proquimposa (México) and maltodextrin 10 DE in INAMALT (México).

2.1 Emulsion preparation

The gum arabic (GA), maltodextrin (MD) and xanthan gum (XG) were hydrated each with distilled water for 24 hours at 8°C before utilization. The gum arabic and maltodextrin were prepared to 30% (w/v) and the xanthan gum to 1% (w/v) (Soottitantawat *et al*., 2005a).

To evaluate the effect of several concentrations of encapsulating agents (MD, GA and XG) on the retention percentage of essential oil, the agents were mixed in the proportions shown in Table 1 to obtain the emulsion to be lyophilized. The total solid content for each 100 mL of emulsion varied between 6.8 and 30 g and the wall material:core material relation varied between 1:2.26 and 1:10 (Table 1). These volumes proportions of encapsulating agents (MD-GA-XG) were chosen to be tested in a wide range of encapsulating agents content and wall material:core material relation in the blends. Furthermore, 100 mL of emulsion were obtained using an ULTRA-TURRAX T25 (IKA ULTRA-TURRAX ®T25 Basic, Wilmington, NC, USA) at 9,600 rpm for 5 min. Briefly, the maltodextrin volume was mixed in ULTRA-TURRAX for 1 min. After 3 mL of essential oil were slowly added, and immediately after, the gum arabic and/or xanthan gum volumes were added according to treatment (Table 1). Therefore, the essential oil concentration in the emulsion was 3 mL/100 mL of emulsion. Finally, the emulsion was maintained for 3 min in an ultrasonic bath (Dima *et al*., 2015) (ULTRASONIC Q500, Newtown, USA) at a constant temperature of 22ºC, for 15 min and finally performing the freezing and freeze-drying.

Once the emulsions were obtained, these were placed in polyethylene bags of 7 x 15 cm, with an approximate volume of 25 mL, and were immediately frozen following two methods: fast freezing and slow freezing. For fast freezing, polyethylene bags were immersed in liquid nitrogen liquid for 10 min, and for slow freezing, the bags were placed in a conventional freezer for 24 h. Thereafter, the samples were lyophilized using a freeze dryer (Labconco FreeZone 2.5 L, Kansas City, MO, USA), at -50°C for 24 h (Viveros-Contreras *et al*., 2013). Finally, the lyophilized samples were weighed to determine the yield of the freeze-drying and stored in glass amber bottles at -17°C until their use.

2.2 Superficial and encapsulated essential oil determination

Gautam and Agrawal (2017) reported that α− and β−citral, myrcene and linalool are the main compounds in *Cymbopogon citratus* essential oil, which correspond to 91% of the composition of essential oil (Poonpaiboonpipat *et al*., 2013). For that reason, the total retention percentage was determined for these four compounds. Superficial and encapsulated essential oil were determined by gas chromatography (GC) and mass spectrometry (MS) according to method reported by Soottitantawat *et al*. (2005b). Briefly, for encapsulated essential oil, 100 mg of powder were blended with 3 mL of distilled water and 5 mL of hexane. The solution was mixed and sonicated for 10 min at 80% amplitude (90 W) at 22ºC. In addition, 1 μL of the organic phase was

analyzed by GC-MS, Agilent 5975C, Santa Clara, CA, USA) provided by a DB-WAX capillary column of 60 m × 0.25 mm and 0.25 μm of thickness (J & W Scientific, USA). The column temperature used was of 70 to 250°C with increases of 16°C/min up to 150°C and after with increases of 25°C/min up to 250°C. The injector temperature was 250°C. Helium was used with a flux of 1 mL/min. Each sample was injected in duplicate. In addition, a standard curve of $\alpha-$ and $\beta-$ citral, linalool and $\beta-$ myrcene was performed. The method reported by Fuentes-Ortega *et al*. (2017) was used to determine the superficial oil on the encapsulated with some modifications. Briefly 100 mg were added to 5 mL of hexane. The blend was shaken at 120 rpm for 5 s and kept at 25°C for 24 h. Afterwards, the samples were filtrated and evaporated in a rotary evaporator (BUCCHI, Labortechnik, AG). One μL of the organic phase was analyzed with GC-MS to determine the $\alpha-$ and $\beta-$ citral, linalool and $\beta-$myrcene content.

The total retention of essential oil was calculated with the sum of the retention of $\alpha-$ and $\beta-$citral, linalool and $\beta-$myrcene. The retention percentage (RP) of $\alpha-$ and $\beta-$citral, linalool and $\beta-$myrcene each was calculated by using Eq. (1):

$$RP = (EE * ER) * 100 \tag{1}$$

where the encapsulation efficiency (EE) of each component was calculated with the subtraction between total and superficial contents for $\alpha-$ and $\beta-$citral, linalool and $\beta-$myrcene each (Eq. 2) (Fuentes-Ortega *et al*., 2017). The encapsulation retention (ER) was calculated with the relation between $\alpha-$ and $\beta-$citral, linalool and $\beta-$myrcene encapsulated and the $\alpha-$, $\beta-$citral, linalool and $\beta-$myrcene added in the emulsion to be dried (Eq. 3).

$$EE = \text{Xi in lyophilized sample} - \text{Xi superficial compound in lyophilized sample} \tag{2}$$

$$ER = \frac{\text{Xi essential oil encapsulated}}{\text{Xi essential oil in emulsion}} \tag{3}$$

Where Xi can be the $\alpha-$ and $\beta-$citral, linalool or $\beta-$myrcene content.

2.3 Scanning electron microscopy (SEM)

Micrographs were obtained by scanning electron microscopy (TOPCON SM-510 model, Singapore). The sample was fixed on an aluminum plate and coated with a thin layer of gold. The accelerating voltage used was 5 kV (Cano-Higuita *et al*., 2015). The porous structure of the encapsulated was determined on micrographs using Orion 6.7 software.

2.4 Release retention during storage

One g of sample was stored at 30°C and 75.5% of relative humidity (Shiga *et al*., 2001) to evaluate release retention at three different storage times: 2, 4 and 7 days. After each time 0.1 g of the samples was obtained, and the total and superficial essential oils were then determined as described in the Section 2.2. The relative retention (RR) of compounds during storage was calculated as the relation between the retention percentage time and the initial retention percentage of storage.

Avrami´s equation (Soottitantawat *et al*., 2005a; Phunpee *et al*., 2017) (Eq. 4) was used to represent the release of essential oil during storage:

$$RR = \exp[-(kt)^n] \tag{4}$$

where RR is the relative retention of compounds (dimensionless), t is the time of storage (h), n is the exponent of the time and k is a constant which defines the release velocity of the compounds of lyophilized (h^{-1}).

2.5 Experimental design

A completely randomized experimental design was used, and all treatments were done by triplicate. Retention percentages of $\beta-$myrcene, $\alpha-$, and $\beta-$citral and linalool were analyzed by variance analysis ($p \leq 0.05$), and the minimal significant difference was used to compare the treatments by using the Statgraphics Centurion XV program (Statgraphics Centurion XV, 2007).

3 Results and discussions

3.1 Retention of essential oil

The yield of lyophilization was higher than 98% for all treatments (data not shown). The total retention of *C. citratus* essential oil in the lyophilized samples varied between 5 and 72% (Table 1). The retention of $\beta-$citral varied from 2.3 to 78% and the $\alpha-$citral between 4.0 and 96%. The retention of $\beta-$myrcene was of 1.0 to 81.6%, while that of linalool was between 2.7 and 39.7% (Table 1).

Table 1. Total retention of *C. citratus* essential oil and β-myrcene, linalool, α- and β- citral retention for all treatments

Treatment	Volume of solutions in the emulsion (%)			Freeze Method	Relation core material: wall material mL essential oil:g total solids encapsulating agents	Total solids for each 100 mL of emulsion	Total retention of essential oil (%)	Retention for component (%)			
	MD	GA	XG	FM		(g/100 mL emulsion)		β-citral	α-citral	β-myrcene	linalool
1	40	60	0	2[a]	1:10	30	20.10±3.9	31.9±5.2	51.2±7.2	22.3±3.9	14.0±2.9
2	40	60	0	1[b]	1:10	30	32.44±4.0	44.6±3.9	66.0±5.2	41.6±3.8	24.6±2.0
3	40	50	10	2	1:09	27.1	25.85±4.1	34.9±8.0	53.7±6.0	12.3±2.3	12.8±2.9
4	40	50	10	1	1:09	27.1	72.1±13.8	78.06±8.7	94.0±5.6	81.6±5.8	39.7±2.3
5	40	40	20	2	1:08	24.2	33.74±1.2	38.7±1.0	61.7±2.6	15.3±1.9	15.7±2.8
6	40	40	20	1	1:08	24.2	51.27±0.9	61.3±0.1	90.7±0.2	24.2±1.7	32.2±1.9
7	40	30	30	2	01:07	21.3	17.64±1.9	14.0±2.4	21.6±2.9	8.0±1.1	10.9±1.4
8	40	30	30	1	01:07	21.3	59.99±3.6	49.2±4.8	75.2±5.4	24.4±2.5	27.1±2.3
9	40	20	40	2	01:06	18.4	12.49±0.9	8.5±1.0	13.4±1.8	4.2±0.6	7.7±1.6
10	40	20	40	1	01:06	18.4	44.15±3.1	30.0±3.7	44.4±4.9	9.9±1.9	16.2±2.2
11	40	10	50	2	01:05	15.5	8.17±0.9	4.4±0.7	7.6±0.8	2.4±0.6	5.3±0.6
12	40	10	50	1	01:05	15.5	36.29±4.1	31.0±4.0	25.4±4.0	6.9±1.8	20.3±3.3
13	40	0	60	2	01:04	12.6	5.01±1.0	2.3±0.7	4.0±1.5	1.0±0.4	4.5±1.2
14	40	0	60	1	01:04	12.6	44.92±0.9	29.2±0.4	43.2±0.4	7.9±1.2	18.0±1.2
15	20	80	0	2	1:10	30	30.64±4.2	34.4±5.4	44.6±7.5	8.9±2.3	20.3±1.8
16	20	80	0	1	1:10	30	56.37±5.0	68.3±4.5	85.5±8.5	22.2±2.4	43.2±2.9
17	20	60	20	2	1:08	24.2	51.13±3.0	36.8±5.6	31.9±3.9	61.9±2.2	9.4±2.3
18	20	60	20	1	1:08	24.2	49.04±3.0	50.7±3.0	45.7±1.8	16.7±5.2	23.0±3.0
19	20	40	40	2	01:06	18.4	9.80±1.5	7.1±1.8	11.4±0.6	3.8±1.2	4.1±1.0
20	20	40	40	1	01:06	18.4	44.97±1.8	41.9±0.7	54.7±1.4	14.2±3.6	16.5±3.9
21	20	20	60	2	01:04	12.6	6.90±1.2	2.5±0.2	4.5±2.1	2.2±0.9	2.7±0.9
22	20	20	60	1	01:04	12.6	43.50±1.9	22.5±2.0	32.5±1.6	5.0±0.6	11.0±1.2
23	20	0	80	2	01:02	6.8	15.91±2.2	6.72±2.2	8.5±2.6	1.7±0.9	1.7±0.5
24	20	0	80	1	01:02	6.8	53.71±0.6	16.60±0.3	21.4±0.4	5.5±0.6	7.9±0.7

[a] samples freeze using nitrogen immersion during 10 min.
[b] samples freeze dried at -17°C during 24 h.
Retention is the average of 3 replicates ± standard deviation
MD maltodextrin; GA, gum arabic; XG, Xanthan gum; FM freezing method

The maltodextrin is frequently used as wall material because of its low cost, in addition to being a cryoprotective agent during lyophilization.

Gum arabic and xanthan gum have been used principally for emulsifying and thickening properties, respectively, as well as for their low cost. Reddy *et al*. (2009) showed that lactose, skimmed milk, and maltodextrin can be used as cryopreservative media for the freeze-drying of three probiotic lactic acid bacteria. For maltodextrin and gum arabic in our work, we used a volume of 40% and 50%, respectively (Table 1), resulting in the higher retention percentage for four compounds. In relation to the xanthan gum, a volume of 10% is indicated as the better treatment for maximizing the retention for four compounds. In this treatment, the gum arabic allowed us to reach a good emulsion of essential oil in the aqueous phase, the maltodextrin probably formed the wall of the matrix, the gum arabic was the emulsifying agent and the xanthan gum increased the viscosity of the emulsion and stable maintenance of the emulsion during freeze-drying.

Our retention percentages of $\alpha-$ and $\beta-$citral, $\beta-$myrcene and linalool were accurate according to Bertolini *et al*. (2001), who reported values of 44 to 64% for linalool, from 47 to 86% for citral and from 74 to 88% for $\beta-$myrcene encapsulated by spray drying using gum Arabic. They demonstrated that the retention of volatile monoterpenes in gum arabic capsules depends on their chemical functionality (solubility and diffusion through the forming matrix) and steric factors of the volatile compounds. Kaushik and Roos (2007) encapsulated limonene by lyophilization using a mix

of gum arabic, saccharose and gelatin. They found that a relation 0.66:0.17:0.17 for the blend gelatin-saccharose-gum arabic allowed the higher retention of limonene (71%); this retention was similar to the citral retention percentage in this work. On the other hand, Chranioti and Tzia (2014) show that the encapsulation efficiency of an oleoresin was between 48 and 85% by lyophilization, but the higher value was obtained using modified starch and chitosan.

The higher retention percentage was obtained with a blend of 40% maltodextrin, 50% of gum arabic and 10% xanthan gum volumes using a freezing slow method, where a retention for $\alpha-$citral and $\beta-$citral was of 96% and 78% respectively. While a retention for $\beta-$myrcene and linalool was of 81.6 and 39.7% respectively (Table 1). The fact that compounds $\alpha-$ and $\beta-$citral showed a bigger percentage of retention in comparison with $\beta-$myrcene and linalool could be because the affinity of the encapsulating agent and the active substance that has to be protected. Bertoloni *et al*. (2001) indicate that compounds that show donator groups of electrons in their molecular structure (such as citral) can form links hydrogen bonds with the surface of compounds that contain hydroxyl groups, as in the case of gum arabic and maltodextrin. The retention of $\beta-$ myrcene could be due to the union of partial electrostatic interactions between double links of the molecule and the hydrogen available in the contained agent. Thus, these compounds could be associated with the wall material and therefore present greater retentions than the hydrocarbons. The retention of linalool is shown to be probably caused by a synergistic effect through the interaction of encapsulating agents with the electron´s donation groups and for linalool has its higher solubility in water. The linalool has a solubility in water of 1.45 g/L, while citral ($\alpha-$ and $\beta-$) and $\beta-$myrcene show a water solubility of 0.42 g/L and 0.006 g/ L, respectively (Merck, 2010). The α-citral presents a higher retention percentage than $\beta-$citral. This is probably due to their molecular configuration (Merck, 2010). The aldehyde functional group in $\beta-$citral (Z-isomer) generates probably the interaction by a hydrogen bond in a position of steric hindrance, decreasing the possibility of interaction with our encapsulating agents. This case is contrary to the $\alpha-$citral which is an $E-$isomer. Ruktanonchai *et al*. (2011) observed different affinities of cyclodextrins ($\alpha-$cyclodextrin, $\beta-$cyclodextrin, and HP-$\beta-$cyclodextrin) to bind with the $E-$citral and $Z-$citral using molecular modelling and physicochemical characterization.

Table 2. Total solids effect in emulsion on total retention of essential oil in lyophilized microcapsules.

Total solids for each 100 mL of emulsion	Total retention of essential oil
(g/100 mL emusion)	(%)
27.1	49 a
24.2	42 ab
21.3	39 ab
30	38 ab
18.4	33 bc
12.6	27 c
6.8	22 c
15.5	22 c
LSD	9.3

Values with different letter in a column are different significantly.
LSD = least minimum difference to $p \leq 0.05$.

They reported that inclusion complex between $E-$citral and the cyclodextrins was significantly more favourable than $Z-$citral.

Table 2 shows the results of the effect of total solids in the emulsion on the total retention of essential oil in lyophilized microcapsules. The total solids in emulsion had a significant effect ($p \leq 0.05$) on the total retention of essential oil. It was observed that a total solid higher than 27.1 g by 100 mL of emulsion gave the higher total retention of essential oil. These results are according to El Asbahani *et al*. (2015), who showed that maltodextrin-gum arabic-xanthan gum blend is a good encapsulating material for essential oil; however, the content of these in the blend should be optimized.

The statistical effect ($p \leq 0.05$) of factors studied in this work are showed in Table 3. The higher retention percentage values for $\beta-$myrcene, linalool, $\alpha-$ and $\beta-$citral were obtained by using a slow freezing (Table 3). The slow freezing for 24 h at -17oC allowed obtaining higher retentions than immersion in nitrogen. Ghio *et al*. (2000) reported that when a slow freezing is used, larger crystals are formed, while a faster freezing promotes the building of small crystals with sharpened forms that can break the emulsion. Choi *et al*. (2004) reported that the freezing process can break the particles and provoke the liberation of compound of the lyophilized samples. At a slow rate of freezing, the cryoprotectant (maltodextrin) can migrate to the concentrated phase liquid and prevent droplet aggregation. This mechanism cannot occur during fast freezing, when the cryoprotectants do not have enough time to diffuse completely, resulting in poor dispersion. The addition of a suitable amount of

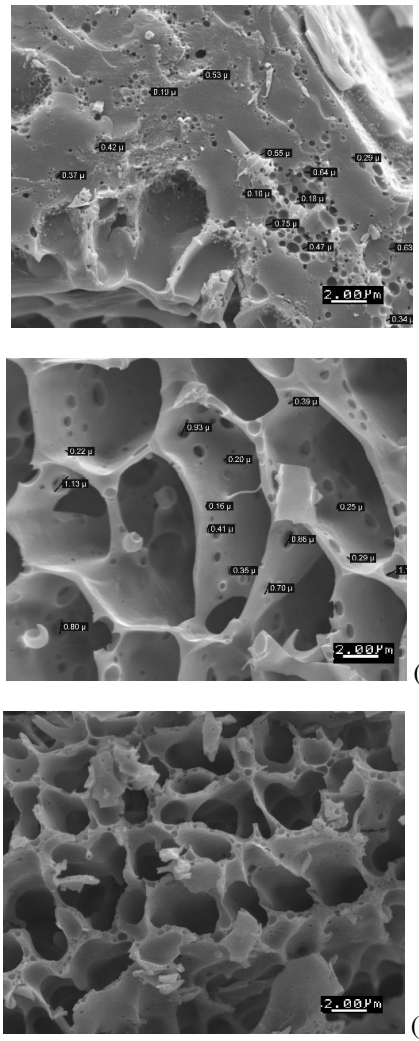
cryoprotectant at a high freezing rate, however, can improve such problems (Lee *et al*., 2009).

Table 3. Effect of the freezing method, gum arabic volume, xanthan gum volume and maltodextrin volume agents on individual retentions for β-myrcene, linalool, α- and β- citral.

Factor	Retention (%)			
	β-myrcene	Linalool	β-citral	α-citral
Freezing method				
Slow[1]	15.0 a[n]	22.7[a]	42.3[a]	59.5[a]
Fast[m]	10.1 a	8.9 b	15.7 b	22.3 b
LSD (0.05)[q]	4.9	3.0	8.6	12.3
Gum Arabic				
0	4.0 d	7.0 d	11.6 d	17.0 b
10	4.6 cd	10.4 cd	13.3 d	20.9 b
20	5.3 cd	9.4 cd	17.8 cd	22.9 b
30	16.2 b	19.0 b	31.6 bc	48.4 a
40	14.4 b	17.0 bc	33.9 bc	57.6 a
50	23.0 ab	28.4 a	63.7 a	74.7 a
60	29.4 a	17.9 b	41.0 ab	55.9 a
80	14.2 bc	29.5 a	47.9 a	65.9 a
LSD (0.05)	7.3	6.4	12.6	19.5
Maltodextrin (%)				
20	10.8 a	12.15 b	26.4 a	38.3 a
40	13.4 a	17.6 a	30.7 a	42.1 a
LSD (0.05)	5.7	4.8	11.0	15.7
Gum Xanthan (%)				
0	18.9 b	23.2 ab	43.6 ab	51.0 bc
10	23.0 ab	28.4 a	63.7 a	74.7 ab
20	30.3 a	20.2 b	46.9 ab	78.3 a
30	16.2 bc	19.0 bc	31.6 bc	48.4 cd
40	8.0 cd	10.5 d	19.9 cd	30.2 de
50	4.6 d	10.3 cd	13.3 d	20.9 e
60	4.0 d	7.8 d	13.5 d	16.5 e
80	3.6 d	5.4 d	12.0 d	17.1 e
LSD (0.05)	7.4	6.4	13.3	19.9

[1] frozen samples at -17°C for 24 h.
[m] frozen samples by nitrogen immersion during 10 min.
[n] Values with different letter for each in a column are different significantly.
[q] LSD = least minimum difference to $p \leq 0.05$.

Figure 1 shows the microphotographs of solid matrix obtained by lyophilization using different conditions, where the matrix structure and porous of the lyophilized matrix can also be observed. The porous structure and irregular particles that resemble flakes with sharp and broken glass-like surfaces were obtained that because grinding. Similar results were reported by Cano-Higuita *et al*. (2015), who lyophilized curcumin using gum arabic, a binary blend of maltodextrin and modified starch, or a ternary mixture of gum arabic, maltodextrin and modified starch. Figure 1a corresponds to a mix between 50% gum arabic, 40% maltodextrin and 10% xanthan gum by using a slow freezing process, in which a higher retention of $\alpha-$ and $\beta-$citral was observed. In Figure 1a, we can observe the diameter of the pore in the structure matrix varied between 0.16 and 0.64 m, while for lower retention of and $\beta-$citral (0% gum arabic, 40% maltodextrin and 60% xanthan gum using fast freezing) the porous diameter varied between 0.16 and 1.14 m (Figure 1b). These pores probably correspond to spaces occupied by essential oil content



a)

(b)

(c)

Fig. 1. Microphotography of solid matrix after lyophilization using a) 50% gum arabic, 40% maltodextrin and 10% xanthan gum (with higher retention of α and β citral) using a slow freezing, b) 0% gum arabic, 40% maltodextrin y 60% xanthan gum (with lower retention of α and β citral) using a fast freezing, and c) 50% gum arabic, 40% maltodextrin and 10% xanthan gum using a fast freezing.

in the structure matrix solid lyophilized. In the microphotographs, one observes that cavernous structure in the structure matrix solid lyophilized increases when the faster freezing method is used (Figure 1c).

Consequently, the essential oil in the inner surface of the solid matrix probably evaporated more easily than in the slow freezing process (Figure 1a), where samples showed a higher cavernous structure. Jafari *et al*. (2006, 2008) reported that for a good retention of the essential oil drops of essential oil should have a diameter lower than 1 μm, as in the case of Figure 1a. When the diameter of the drop is higher, however, the emulsion could to collapse making the drops of essential oil can to increase of size, reducing the retention percentage (Figure 1b). Soottintantawat *et al*. (2005a) show that when the diameter of the drop grows, the retention percentage of d-limonene decreases. Topography, form, and size of the pore found are also shown in the microphotographs. In addition, in the structure matrix solid lyophilized a glassy - amorph microstructure of the sample is observed in them, without apparent crevices. This solid, flat and crackless microstructure could be allow the entrapment of aromatic compounds and protect them when they are submitted to heat and oxygen as reported by Kaushik and Roos (2007).

The retention percentage of $\beta-$myrcene, linalool, $\beta-$citral and $\alpha-$citral ($p \leq 0.05$) was influenced statistically by the gum arabic (Table 3). The higher values of these compounds were obtained with an emulsion containing 50% of gum arabic. When the gum arabic content was lower than 50%, the retention percentage decreased for all compounds. The maltodextrin had a statistical effect on the retention of linalool but not on $\beta-$myrcene, $\beta-$citral and $\alpha-$citral (Table 3), while xanthan gum had an effect on the retention of $\beta-$myrcene, linalool, $\beta-$citral and $\alpha-$citral ($p \leq 0.05$). The higher values of retention percentage were obtained using 10% or 20% of xanthan gum.

3.2 Release kinetic of compounds during storage

The kinetic of release controlled of $\alpha-$ $\beta-$citral, $\beta-$myrcene and linalool is shown in Figure 2. The four compounds show an exponential decrease in the liberation during storage, which was faster in the first 48 h. Relative retention (*RR*) of $\alpha-$ and $\beta-$citral decreases faster than $\beta-$myrcene and linalool. The same behavior was obtained for all treatments during storage. After 96 h, the relative retention of compounds had a variation between 0.2 and 0.4 for all compounds. In the case of the release controlled of encapsulated compounds by spray drying, an exponential liberation has been reported, but little information is known about the liberation in lyophilized samples. Soottitantawat *et al*. (2005b) analyzed the liberation of l-menthol obtained by spray drying at 43ºC with different relative humidity by using gum Arabic and modified starch (CAPSU and

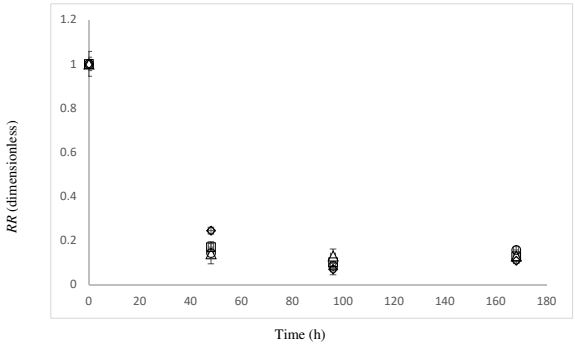


Fig. 2. Release kinetics of volatile compounds of *Cymbopogon citratus* essential oils microencapsulated by lyophilization using a blend (v:v:v) of maltodextrin (40%), gum arabic (50%) and xanthan gum (10%) and a slow freezing method (◊-β-myrcene, Δ- linalool, o -β-citral and □-α citral) during storage.

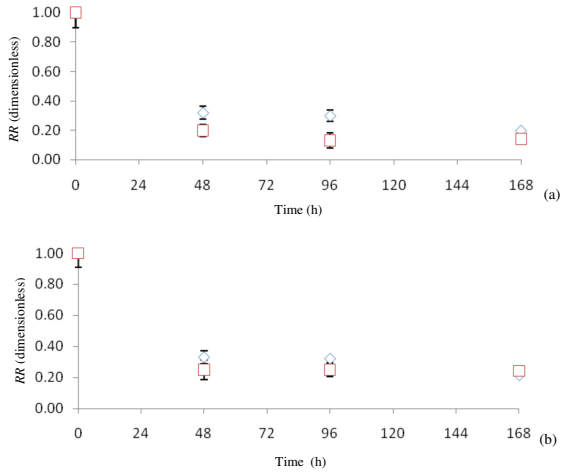


Fig. 3. Release kinetics of α and β-citral compounds of *Cymbopogon citratus* essential oil microencapsulated by lyophilization using a) a blend of maltodextrin (40%) - gum arabic (50%) and xanthan gum (10%), and b) a blend of maltodextrin (20%) - gum Arabic (40%) - xanthan gum (20%); using □ fast freezing and ◊ slow freezing.

Hi-CAP-100) as encapsulating agents. They found release kinetics similar to our results for others treatments. Sigha *et al*. (2001) analyze the liberation of limonene and n-ethyl hexanoate encapsulated by inclusion and spray drying in β−cyclodextrin using gum arabic as an emulsifier. Liberation of n-ethyl hexanoate was between 0.2 and 0.6 after 30 days and between 0.4 and 0.9 for limonene. The difference with our results could be caused by the type of encapsulating agent and encapsulation method used which they were different. Relative liberation of α−, β−citral, β−myrcene and linalool of the compounds of *C. citratus* was adjusted to Avrami´s equation. The parameters of Avrami´s equation for all treatments are shown in Table 4. The parameter "n" varied between 0 and 0.61. These values are similar to Phunpee *et al*. (2017), who reported values between 0.52 and 0.65 for citral encapsulation by spray drying. Values of $n < 1$ indicated that release corresponds to the diffusion-limited kinetic model. The mechanism of molecular diffusion can be explained by the low presence of water in the powder, which interacts with the polar surface of the encapsulating agents, then during storage, compounds of essential oil could be replaced by water. In microspheres/microcapsules, the values of n indicate the following release mechanisms: for $n \leq 0.43$, the dominant release mechanism is the Fickian diffusion (case I transport); $0.43 \leq n < 0.85$ indicates the diffusion and the swelling release mechanism (non-Fickian or anomalous transport); and $n \geq 0.85$ corresponds to zero order release kinetics (case II transport) (Maderuelo *et al*., 2011; Siepmann and Peppas, 2001). Figure 3 shows the effect of the freezing method in the release of α− and β−citral (addition of both retentions) during storage in two different matrices. In addition, in this figure we can see that faster freezing allowed a faster release of α− and β−citral. This speed of freezing, however, is constant until the end of 198 h of treatment. In the first hours at beginning of storage, the release compounds could depend only of composition of encapsulating agent and after, release compound could also to depend of freezing method when the moisture gradient to core of structure matrix increase.

The lyophilized samples tended to an equilibrium and absorbed water with the environment, firstly in external layers when it was in contact with a water of environment which was of 75% of moisture. The release of bioactives from encapsulating agents involves three stages: (a) surface release, (b) diffusion through swollen matrix, and finally (c) erosion of the matrix (Peng *et al*., 2010). Therefore, one can assume that when lyophilized samples are hydrated in their first layers, the release of essential oil begins because a high water concentration gradient is created between the surface and the structure matrix solid lyophilized center. This high gradient then decreases with time because of the water diffusion to the interior of the matrix solid lyophilized causing stability loss in the system and the release of the compounds of the essential oil.

Table 4. k and n values of Avrami´s equation for the retention relative percentage of *Cymbopogon citratus* essential oil encapsulated by lyophilization for all treatments.

	Volume of solutions in the emulsion (%)				Constants	
Treatment	Maltodextrin	Gum arabic	Xanthan gum	Freezing method	k (h^{-1})	n (dimensionless)
1	40	60	0	2[a]	0.212	0.1631
2	40	60	0	1[b]	0.0098	0.6089
3	40	50	10	2	2.80×10^{-2}	0.2663
4	40	50	10	1	3.7×10^{-1}	0.1753
5	40	40	20	2	1.3×10^{5}	0.0483
6	40	40	20	1	3.44×10^{-2}	0.4272
7	40	30	30	2	1.26×10^{-1}	0.1877
8	40	30	30	1	1.4367	0.0840
9	40	20	40	2	4.5×10^{7}	0.0133
10	40	20	40	1	2.0×10^{6}	0.0242
11	40	10	50	2	1.8×10^{5}	0.0045
12	40	10	50	1	1.96×10^{-2}	0.2349
13	40	0	60	2	1.96×10^{-2}	0.2335
14	40	0	60	1	8.0×10^{5}	0.0154
15	20	80	0	2	6.2×10^{5}	0.0217
16	20	80	0	1	9.3×10^{-2}	0.2393
17	20	60	20	2	1.9×10^{5}	0.0566
18	20	60	20	1	3.1×10^{4}	0.0385
19	20	40	40	2	2.4×10^{-2}	0.2682
20	20	40	40	1	2.0×10^{3}	0.0273
21	20	20	60	2	1.1×10^{-1}	0.0000
22	20	20	60	1	1.2×10^{6}	0.0389
23	20	0	80	2	2.81×10^{-2}	0.4151
24	20	0	80	1	1.08×10^{-2}	0.5191

[a] frozen samples by nitrogen immersion during 10 min.
[b] frozen samples at -17°C for 24 h.

The liberation constant (k) of Avrami´s equations varied between 0.0098 and 4.7×10^{7} h^{-1} (Table 4) in our treatments. Phunpee *et al*. (2015) encapsulated $\alpha-$ and $\beta-$ citral by spray drying using $\alpha-$, $\beta-$ and $\gamma-$cyclodextrins (CD).

They showed that during storage the $\beta-$CD complexes exhibited slower release rates than the $\gamma-$CD, and $\alpha-$CD complexes; citral release was consistent with the limited diffusion kinetic model. Shiga *et al*. (2001) determined the release of d-limonene and n-ethyl-hexanoate with different agents (βCD-GA-MD). They obtained release constants from 1.86×10^{-14}/h to 1.20×10^{3}/h for d-limonene and between 1.23×10^{-3}/h and 5.62×10^{-3}/h for n-ethyl hexanoate. The differences between constants could be due to the drying technique used (spray drying versus lyophilization), to the agent type (cyclodextrin versus gum arabic, xanthan gum and maltodextrin), and the temperature and relative humidity in equilibrium (water activity) during storage. Baranauskiene *et al*. (2007) found that the capacity of union between aromatic compounds with a different modified starch matrix for encapsulating drops of essential oil of *Mentha piperita* is dependent on water activity, and the release of aromatic contained in dust during storage increases with the water activity.

Conclusions

In this study it was possible to encapsulate the essential oil of *C. citratus* by lyophilization using slow freezing and different volumes of encapsulating agent blends. The blend of 50% of gum arabic, 40% of maltodextrin and 10% of xanthan gum volumes allowed us to obtain the maximum percentage of retention of $\alpha-$ citral, $\beta-$citral, $\beta-$myrcene and linalool (94%, 78%, 81% and 40%, respectively).

These results allow us to suggest that the quality of the essential oil can be maintained in good condition after encapsulation by lyophilization. The release of those volatile compounds during storage was exponential, and the data could be adjusted to Avrami's model. Finally, the technology used in this study gives important knowledge for future research with a view to producing on an industrial scale.

Acknowledgements

This research was financed by the Tecnológico Nacional de México. Borras-Enriquez thanks the Consejo Nacional de Ciencia y Tecnología (CONACyT, México) for the scholarship granted to him. The authors also thank to Guadalupe NIETO, ECOSUR-Tapachula (México) for the support with the scanning electron microscopy technique.

Nomenclature

EE	Encapsulation efficiency, %
ER	Encapsulation retention, %
GA	Gum arabic
GC	Gas chromatography
K	Constant, h^{-1}
MD	Maltodextrin 10DE
MS	Mass spectrometry
n	Exponent of time, dimensionless
R	Retention, %
RR	Retention relative, dimensionless
t	Time of storage , h
XG	Xanthan gum

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