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MICROENCAPSULATION OF MICROBIAL CONSORTIUM AND VOLATILE COMPOUNDS OF PALM (*Acrocomia aculeata*) WINE

MICROENCAPSULACIÓN DEL CONSORCIO MICROBIANO Y COMPUESTOS VOLÁTILES DE VINO DE PALMA (*Acrocomia aculeata*)

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Abstract

The objective of this work was to determine the effect of spray drying on the microbial survival of lactic acid bacteria (LAB), acetic acid bacteria (AAB) and yeast (Y), as well as on the volatile compounds retention of the "taberna". Different mixtures of maltodextrin (MD), gum arabic (GA), xanthan gum (XG) and sodium alginate (AL) as coating materials, different proportions coating material:"taberna" (1:1, 1:2 and 1:3 v/v), as well as the effect of the feeding flow (3, 6 and 9 mL min^{-1}) and the inlet air temperature (80, 90 and 100 °C) in the dryer were evaluated on the efficiency of microencapsulation, microbial survival, water activity and the morphology of the microcapsules. Optimal conditions for the encapsulation of "taberna" to maximize microbial survival and microencapsulation efficacy were: feed flow of 3 mL/min, inlet air temperature of 97 °C and a mixture of MD-GA using a coating material:"taberna" ratio of 1:1 (v/v). With these conditions, in addition to maintaining the viability of the three microbial groups, most of the aromatic compounds of the "taberna" were retained.

Keywords: "taberna", orthogonal experimental design, microbial survival, volatile compounds.

Resumen

El objetivo de este trabajo fue determinar el efecto del secado por aspersión sobre la viabilidad de grupos de bacterias ácido lácticas (BAL), bacterias ácido acéticas (BAA) y levaduras, así como la retención de compuestos volátiles de la taberna. Se evaluaron diferentes mezclas de maltodextrina (MD), goma arábiga (GA), goma xantana (GX) y alginato de sodio (AL) como agentes encapsulantes, diferentes proporciones agente encapsulante:"taberna" (1:1, 1:2 y 1:3 v/v), así como el flujo de alimentación (3, 6 y 9 mL min^{-1}) y la temperatura del aire de entrada (80, 90 y 100 °C) sobre la eficiencia de microencapsulación, la supervivencia de los microorganismos, actividad de agua y morfología de las microcápsulas. Las condiciones óptimas para la encapsulación de "taberna" para maximizar la supervivencia de los microorganismos y la eficacia de microencapsulación fueron: flujo de alimentación de 3 mL min^{-1}, temperatura del aire de entrada de 97 °C, usando una mezcla de MD-GA en una relación 1:1 (v/v) con "taberna". Bajo estas condiciones, además de conservar la viabilidad de los tres grupos de microorganismos, se retienen la mayoría de los compuestos aromáticos de la "taberna".

Palabras clave: "taberna", Diseño Experimental Ortogonal, sobrevivencia microbiana, compuestos volátiles.

1 Introduction

In Mexico, "taberna" is a traditional alcoholic drink produced by natural sap fermentation from the *Acrocomia aculeata* (Jacq.) Lodd. ex. Mart. palm. It is a sweet, effervescent drink produced and consumed in the south of Mexico with characteristics similar to palm wine from Africa (Alcántara-Hernández *et al*., 2010). The production of "taberna" is carried out under non-aseptic conditions; for this reason, the microbial consortium involved in the sap fermentation is the one present in the environmental media (Karamoko *et al*., 2012), and then the fermentation process is little controlled. Alcántara-Hernández *et al*. (2010) reported the proliferation of yeast, AAB, LAB and other microorganisms in "taberna".

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The good quality or acceptability of "taberna" is principally in terms of organoleptic properties, which are promoted principally by the microbial consortium in involved during sap fermentation.

Many studies have been done in Mexico, Nigeria and other African countries on the microbial community of palm wine of different species of trees (Alcántara-Hernández *et al*., 2010; Karamoko *et al*., 2012). Thus far, however, very few published studies on the aroma compounds of the palm wine have been reported and none on *Acrocomia aculeata*.

Until now more than 80 volatile compounds have been identified in different palm wine varieties (Lasekan and Otto, 2009). Systematic studies have been performed to indicate the aromatic compounds responsible for the characteristic bouquet of palm wine, but those of "taberna" [*Acrocomia aculeata* (Jacq.) Lodd. ex Mart.] have not been reported. For that reason, novel technologies can be adopted especially to overcome the problem of active compound deterioration and to assure the microbial survival. These technologies are based on the idea of coating the desired active compound. Encapsulation is a process in which small solid particles, liquid components or gaseous materials are coated or entrapped within another inert shell material, which isolates and protects the core material from environmental factors (Zhu *et al*., 2012; Enciso-Sáenz *et al*., 2018; Ocampo-Salinas *et al*., 2017). In addition, this technique helps to mask the unwanted taste and the odor of the ingredient, helps to prevent the evaporation of the volatile components and helps to prevent the contact of the ingredient with oxygen to avoid their oxidation (Turasan *et al*., 2015; Fuentes-Ortega *et al*., 2017).

Spray drying is a technique used for the microencapsulation of microorganisms (Peighambardoust *et al*., 2011; Broeckx *et al*., 2016) and volatile compounds (Sultana *et al*., 2018; Turasan *et al*., 2015; Lasekan and Otto, 2009; Villalobos-Castillejos *et al*., 2017) with the aim of protecting them during storage. Many works have been performed to protect *Lactobacillus* spp. (Bielecka and Majkowska, 2000; Kumar and Mishra, 2004; Olivarez-Romero *et al*., 2018) and *Saccharomyces* (Chandralekha *et al*., 2016; Chandralekha *et al*., 2017), among others, but little works has been reported about encapsulating microbial consortium (Pedroza-Islas, 2014; De Araújo-Uribe, 2018). "Taberna" is a complex mixture of microorganisms and aromatic compounds that could be stabilized by this technique, the problem is then to find spray drying process conditions to maximize the microbial survival and volatile compounds retention in microcapsules. The aim of this study was to optimize the encapsulation of "taberna" by spray drying using maltodextrin, gum arabic, xanthan gum and sodium alginate in terms of encapsulation efficiency and microbial survival of consortium and to evaluate the retention of volatile compounds in microcapsules.

2 Materials and methods

2.1 Taberna production

For "taberna" production, 10-year-old *Acrocomia aculeata* palms were used. For that, a "taberna" producer of Centro Tabernero from Benito Juarez, Chiapas, Mexico (16° 25' 33.94" N, 93° 19' 16,152" W, located at an altitude of 646 m above sea level) made the incision in apice of the palm (canoa) to drain the sap towards the "canoa". The producer had permission from the Ministry of the Environment and Natural Resources (SEMARNAT) a government agency from Mexico for palm exploitation. For 12 h palm sap accumulated in the "canoa" and fermented in vivo through natural microbiota. Every 12 h, fermented palm sap was then removed from the "canoa". "Taberna" corresponds to the beverage fermented seven days after the incision on the palm is made. The samples were collected each 24 h in sterile bottles and closed with screw caps; they were then transported to the Instituto Tecnológico de Tuxtla Gutiérrez at 20 °C and processed within 2 h after sampling (Ogbulie *et al*., 2007).

2.2 Enumeration of microorganisms

The initial microbial count of lactic acid bacteria, yeast and acetic acid bacteria of "taberna" was determined for serial dilutions with sterile peptone water solution (1 g). To evaluate the presence of lactic acid bacteria, 1 mL of bacterial suspension was inoculated in MRS agar (DIBICO 1267-A, Mexico) containing 10 mg mL^{-1} cycloheximide (Sigma-Aldrich-01810, St. Louis, USA) to suppress the growth of yeasts. The Petri dishes were stored at 37 ºC for 72 h (Amoa-Awua *et al*., 2006) and incubated under anaerobic conditions. To test the yeast, 1 mL of dilution was inoculated in YM agar composed of 3 g L^{-1} yeast extract (DIBICO 3003-E, Mexico) 5 g L^{-1} peptone (BD Bioxon 252,606, Mexico), 3 g L^{-1} malt extract (Fluka 41768

/ 1 42904120, Spain), 10 g L^{-1} dextrose (JT Baker 1916-01, Mexico) and 20 g L^{-1} bacteriological agar (DIBICO 1001-A, Mexico) containing 0.004 g L^{-1} of oxytetracycline in order to suppress the growth of bacteria. The Petri dishes were incubated aerobically at 30 ºC for 72 h. For acetic acid bacteria, CARR agar was used, which was composed of 30 g L^{-1} yeast extract (DIBICO 3003 -E, Mexico), 20 g L^{-1} ethanol, 20 g L^{-1} agar (DIBICO 1001-A, Mexico) and 0.02 g L^{-1} bromocresol green (954 HYCEL, Mexico) containing 36 g L^{-1} penicillin and 0.005% cycloheximide (Sigma-Aldrich-01810, St. Louis USA). All determinations were performed in triplicate and after the incubation period, the colony forming units (CFU mL^{-1}) were enumerated.

2.3 Preparation of microcapsules

2.3.1 Preparation of coating materials

The maltodextrin (MD) (INAMALT, IMSA Guadalajara, Mexico), gum arabic (GA) (HYCEL of Mexico- CAS 9000-01-5, Mexico), sodium alginate (AL) (Cosmopolitan-Mexico) and xanthan gum (XG) (Fufeng FCCIV, Republic of China) were hydrated in distilled water at 40ºC and then kept refrigerated for 24 h. The concentrations of solutions were 300 g L^{-1} for the MD and GA, 30 g L^{-1} for AL and 10 g L^{-1} for XG. The mixtures of coating materials were prepared in the proportion 60:40 v/v (MD-GA, MD-AL and MD-XG) in each case. Solutions were sterilized at 121 °C for 15 min and allowed to stand at room temperature for 24 h. Before spray drying, the sterile coating material was mixed with "taberna" samples in ratios (coating material:taberna) corresponding to 1:1, 1:2 and 1:3 v/v, at 7200 rpm for 2 min using a T 25 ULTRA-TURRAX T25 (IKA, Germany).

2.3.2 Spray drying

The emulsions were dehydrated in a mini spray dryer (B-290 BUCHI, Switzerland). Drying was performed at inlet air temperatures of 80, 90 and 100 ºC and a feed flow of 3, 6 and 9 mL min^{-1}. The dried samples were stored in sealed plastic bags and stored under vacuum in amber glass bottles with silica gel at room temperature.

2.3.3 Experimental design

An orthogonal experimental design L_9 was used (Table 1). The factors and levels were as follows: inlet air temperature (80, 90 and 100 ºC), feed flow (3, 6 and 9 mL min^{-1}), mixtures of coating material (MD-GA, MD-XG and MD-AL) and coating material:"taberna" ratio (1:1, 1:2 and 1:3 v/v). All treatments were carried out in triplicate. Results were analyzed by an ANOVA test with a significance of 5% using Statgraphics Centurion XV software (Statgraphics, 2007).

Table 1. Orthogonal L_9 experimental design.

Treatment	Inlet Temperature	Flow	Coating material	Coating material: "taberna" ratio
	(ºC)	mL min^{-1}		(v/v)
1	80	3	MD-GA	1:01
2	80	6	MD-XG	1:02
3	80	9	MD-AL	1:03
4	90	3	MD-XG	1:03
5	90	6	MD-AL	1:01
6	90	9	MD-GA	1:02
7	100	3	MD-AL	1:02
8	100	6	MD-GA	1:03
9	100	9	MD-XG	1:01

MD: maltodextrin; GA: gum Arabic; AL: sodium alginate; XG: xanthan gum. The total volume to the mixture of coating material and "taberna" was 100 mL.

2.4 Analysis of microcapsules

2.4.1 Microencapsulation efficiency

Microencapsulation efficiency (ME) was calculated using Ec. (1) (Su *et al*., 2007):

$$ME(\%) = \frac{Dry\ \ powder\ \ (g)}{Solids\ \ in\ \ encapsulant\ \ solution\ \ (g)} * 100 \quad (1)$$

2.4.2 Microbial survival

The viability of microorganisms after spray drying was determined as follows: the samples were rehydrated in sterile water based on the amount of total solids in the emulsion before spray drying. The colony forming units (CFU g^{-1}) were determined by preparing serial dilutions in peptone water (1 g L^{-1}) and spread plated on MRS agar plates for lactic acid bacteria, YM agar for yeast, and for acetic acid bacteria CARR agar was used.

The microbial survival (MS) in each sample tested was calculated using Ec. (2) (Yu *et al*., 2010):

$$MS\,(\%) = \left[\frac{\log(N)}{\log(N_i)}\right] * 100 \quad (2)$$

where N is the number of microorganisms per gram of dry matter before drying and N_i is the number of microorganisms per gram of dry matter in the microcapsules.

2.4.3 Water activity determination

Water activity (Aw) of microcapsules was measured by "HygroPalm AW" water activity indicator (Rotronic Instruments, USA).

2.4.4 Surface morphology analysis of microcapsules

To analyze the structures and surface of the microcapsules, a scanning electron microscope (TOPCON, SM-510, UK) was used. Samples were coated with the mixture of gold/palladium by a sputter coating device (ANATECH, Union City, CA, USA). The scanning electron microscope was operated at 40 mA in a high vacuum at 10 KV and 5 mm away (Su *et al*., 2007).

2.4.5 Morphology analysis of encapsulated microorganisms

Encapsulated microorganisms were observed by scanning electron microscopy using the methodology described by Nation (1983) with some modifications. A powder sample was dissolved in 1 mL of a 0.1 M sodium cacodylate buffer solution (SCBS) of pH 7.0. After, it was centrifuged at 5000 rpm for 5 min, and the supernatant was removed. Washing with SCBS was repeated twice and after the supernatant was removed. The pellet was re-suspended in 0.5 mL of 40 g L^{-1} of glutaraldehyde in SCBS, allowed to stand for 30 min. Afterward, it was then centrifuged again, and the cell pack was resuspended in SCBS for 5 min. In addition, the supernatant was removed. The pellet was resuspended in 1 mL of SCBS and filtered using a microfilter (0.2 μm), which was then dehydrated through ethanol solutions of 30%, 70%, 90%, and 100% for 10 min in each of the solutions. Hexamethyldisilazane (HMDS) was added and maintained for 5 min. Finally, the HMDS was filtered, and the membrane was left inside a desiccator with silica gel for 24 h. The powder samples dried after treatment were finally coated with gold/palladium before the scanning electron microscopy.

2.5 Optimization of spray drying microencapsulation process

The spray drying microencapsulation process was optimized by response surface methodology, with the aim of finding the optimum conditions for maximizing microencapsulation efficiency and microbial survival. This analysis was performed using the Statgraphics Centurion XV software (Statgraphics, 2007). The optimal treatment in triplicate was carried out in order to know the percentage of error prediction between the model and the experimental, which was calculated according to Ec. (3):

$$Error(\%) = \left[\frac{V_e - V_r}{V_r}\right] * 100 \quad (3)$$

where V_r is the predicted value provided by software and V_e is the experimental value obtained by performing validation (Taylor, 1982).

2.6 Volatile compounds determination

For the extraction of volatile compounds and the analysis by GC-MS followed the methodology reported by Bleve *et al*. (2015) with some modifications. First 5 mL of "taberna" was stored in a 10 mL headspace vial with a crimp type cap and a PTFE/silicone septum to prevent the leakage of volatile compounds.

For the "taberna" encapsulated sample, first 1 g of powder was hydrated in 10 mL of water and mixed with a vortex. Next, the vials with samples were heated at 70 ºC for 30 min with magnetic stirring. The fiber coating to carboxen/polydimethylsiloxane (CAR/PDMS) with 75 μm of film thickness was inserted and exposed in the vial containing the samples for 20 min.

The analysis of the samples was performed using a GC-MS (Agilent Technologies, USA). A DB-WAXter capillary column (60 m x 0.25 mm x 0.25 μm) (Agilent Technologies, USA) was utilized. The GC system was used in splitless mode with the injector maintained at 250 ºC. Helium as the carrier gas was maintained at a constant flow of 1 mL/min. The temperature conditions of the column were as follows: a primary temperature of 40 ºC for 5 min, which after was increased to 230 ºC at 3 ºC/min, and held for 10 min.

The temperature of the ion source was 230 ºC. The MS detection proceeded in electron impact mode, and the ionization energy was 70 eV. Compound identifications were carried out manually by matching the unknown spectra with mass spectral NIST 08 libraries.

3 Results and discussion

3.1 *Microbial and chemical composition of taberna during in vivo fermentation*

The microbiological analysis from the "taberna" samples collected during tapping is presented in Table 2. During the *in vivo* fermentation of *Acrocomia acualeata* sap, three groups of microorganisms were identified principally: yeast, acetic acid bacteria and lactic acid bacteria. In the first tapping, corresponding to 4 hours after making the "canoa", a high load (6.01 $\log_{10}$ CFU mL^{-1}) of LAB was found. This value was increased quickly during 24 h until about 8.5 $\log_{10}$ CFU mL^{-1} remaining constant during all of the sampling. In relation to yeast, the microbial count was 5.74 $\log_{10}$ CFU mL^{-1} in the first three days and after the value increased to about 8 $\log_{10}$ CFU mL^{-1} until the end of fermentation. Acetic acid bacteria were identified at the beginning of fermentation with an initial microbial count of 5.29 $\log_{10}$ CFU mL^{-1}, and an average of 7.9 $\log_{10}$ CFU mL^{-1} were counted after the third day until the end of fermentation. The results showed that the microbial community increased in the first three days of fermentation and after were maintained constant over 13.5 d.

Table 2. Mean values of the populations of LAB, AAB and yeast microorganisms in "taberna" samples during *in vivo* fermentation of the *Acrocomia aculeate* sap.

Time of tapping palm wine (h)	**Microbial count ($\log_{10}$ CFU mL^{-1})**		
	LAB	**AAB**	**Yeast**
4	6.01 j	5.29 g	5.74 g
12	8.76 b	8.08 a	7.72 cde
36	8.84 b	7.88 ab	7.69 cdef
60	8.23 de	7.29 f	8.26 b
108	8.52 c	7.75 bc	7.90 c
132	8.12 e	7.43 def	7.78 cd
156	7.91 f	7.59 cd	7.57def
180	7.40 g	7.32 f	7.48 ef
204	9.26 a	7.57 cde	7.45 fg
228	7.23 h	7.30 f	7.58 def
252	8.36 d	7.37 f	7.69 cdef
276	7.07 i	7.33 ef	7.43 f
300	8.53 c	7.7 cd	8.63 a
324	8.50 c	7.39 def	7.46 ef
LSD	0.1377	0.2346	0.2799

LAB= lactic acid bacteria, AAB= Acetic acid bacteria. Values with different letters are significantly different within the columns ($P < 0.05$). LSD: Low significant difference ($P < 0.05$)

These results are similar to those reported by Amoa-Awua *et al*. (2007) for another palm wine, which reported that the microbial population was constant after 5 days until the end of fermentation. For that, "taberna" samples for spray drying were obtained from the fourth day. Physicochemical analysis in the first 4 h after tapping the palm sap showed a pH of 7.5, soluble solids of 11.9% and a density of 1.04 g mL^{-1}. Other carbohydrates, were identified as glucose (48 g L^{-1}), fructose (51 g L^{-1}) and fructans (4.43 g L^{-1}), which are used also as a carbon source for metabolism of microbial consortium. After 12 h of fermentation, the pH was between 3.7 and 4, and the soluble solid between 4.5 and 5%. At the beginning of fermentation, the sucrose concentration was 110 g L^{-1}; however, this value decreased to 22.92 g L^{-1} after 12 h. After the third day, however, all physicochemical parameters evaluated stayed constant.

Higher values of organic compound in "taberna" were 51 g L^{-1} for ethanol, acetic acid 5.9 g L^{-1} and lactic acid of 3 g L^{-1} during the tapping of the "canoa" around 15 days. These organic compounds are probably the compounds with the highest influence on the aroma of "taberna" as reported by Amoa-Awua *et al*. (2007) for palm wine. According Faparusi and Bassir (1972), palm wine generally becomes unacceptable to consumers when the concentration of acetic acid is higher than 6 g L^{-1}. Palm wine drinkers know that the drink tastes differently at different stages of fermentation as a result of yeast fermentation and the accumulation of organic acids, especially acetic acid from fermentation by AAB as the fermentation progresses each day (Nwaiwu *et al*., 2016).

3.2 Effect of spray drying conditions on microbial encapsulation

The microencapsulation efficiency varied between 9.44 and 40.56% for all treatments. These values are lower than reported by other authors (Arepally and Goswami, 2019; Xavier dos Santos *et al*., 2019).

Table 3. Multifactorial analysis of variance to evaluate the main effects on the microencapsulation efficiency, microbial survival and water activity of powder.

Factor	Levels	Microencapsulation efficiency (%)	Microbial Survival (%)			Water activity
			LAB	AAB	Yeast	
	(mL min^{-1})					
	3	33.50 a	99.84 a	99.66 a	99.1 a	0.18 a
Flow rate	6	23.68 b	92.76 b	91.98 a	88.3 a	0.21 a
	9	9.44 c	95.8 ab	89.58 a	99.0 a	0.13 b
	(ºC)					
Inlet Air	80	18.86 b	99.84 a	99.69 a	99.2 a	0.11 b
Temperature	90	12.26 c	96.2 ab	96.86 a	90.7 a	0.11 b
	100	35.50 a	92.32 b	84.67 a	96.6 a	0.30 a
	MD-GA	30.28 a	96.38 a	94.81 a	96.8 a	0.20 a
Coating material	MD-AL	26.89 a	95.79 a	89.58 a	99.0 a	0.13 b
	MD-XG	9.44 b	96.21 a	96.83 a	90.6 a	0.19 a
	(v/v)					
Coating material:	1:01	40.56 a	91.87 b	86.13 a	88.9 a	0.34 a
taberna ratio	1:02	14.63 b	99.99 a	99.97 a	99.9 a	0.08 b
	1:03	11.42 b	96.5 ab	95.12 a	97.6 a	0.10 b
LSD		5.62	6.96	16.55	13.59	0.048

Values with different letters are significantly different within the columns ($P < 0.05$). LSD: Low significant difference ($P < 0.05$)

According to Yousefi *et al*. (2010), the low yields during spray drying could be attributed to the low glass transition temperatures of the samples. The glass transition is the passage of a metastable thermodynamic state, characterized by high molecular order and aggregation, to another thermodynamic state, known as rubbery state, where there is more molecular mobility and thus lower stability (Gutiérrez *et al*., 2014). "Taberna" contains around 23 g L^{-1} of sugars and these compounds have a low glass transition temperature (Tg), around of 62 °C for sucrose, 5 °C for fructose and glucose with 32 °C (Patist and Zoerb, 2004; Yousefi *et al*., 2010). The use of polysaccharides such as maltodextrin, gum Arabic, sodium alginate and xanthan gum during the microencapsulation, considerably enhance the viscosity of the mixture and increase the Tg in the food solutions thanks to its high molecular weight (Richards *et al*., 2002). Nevertheless, possibly during the encapsulation of the "taberna", the temperature of the wet bulb in the drying chamber was higher than the Tg of the mixtures, causing problems of stickiness on the dryer chamber during drying, leading to low yield and operational problems (Yousefi *et al*., 2010).

Multifactorial analysis showed that the inlet air temperature, feed flow, coating material and the core to coating ratio had a statistical effect on microencapsulation efficiency ($P < 0.05$) (Table 3). By using a feed flow at 3 mL min^{-1} and an inlet air temperature at 100 ºC, greater encapsulation efficiency was obtained. These results are similar to those reported by Tonon *et al*. (2008), who concluded that higher feed flow rates reduce the contact time between droplets and drying air, producing a less efficient heat transfer. This results in less water evaporation and therefore higher moisture content and, consequently, reducing yield. León-Martínez *et al*. (2010) suggest an inverse relationship between feed flow and microencapsulation efficiency caused by lower heat and mass transfer in the drying chamber.

The effect of the coating material on the encapsulation efficiency depends on the possible interactions between the active compounds and carrier materials, as well as the viscosity of the emulsion. The MD-GA mixture had the highest encapsulation efficiency. Toluna *et al*. (2016) reported that compounds with electron donating groups in their molecular structure can form hydrogen bonds with the surface of hydroxyl-containing compounds, such as gum Arabic and maltodextrin. Gum Arabic is recognized too for its properties of high solubility and low viscosity in a solution.

Rajabi *et al*. (2015) mentioned that increasing the coating viscosity has a negative effect on the retention of the active compounds.

The microencapsulation efficiency was significantly higher when the coating material: "taberna" ratio was 1:1 ($P < 0.05$) (Table 3). This is attributed to the lower concentration of "taberna" in the emulsion so that a better coating is achieved during the encapsulation. The "taberna" is rich in carbohydrates so that an augmentation of the coating material:"taberna" ratio (i.e. 1:3) increases the mono- and disaccharides in the solution to be dried, which could have very low glass transition temperatures causing a stickiness of the sample in the drying chamber during spray drying (Garofulić *et al*., 2016) decreasing then the drying efficiency.

The microbial survival of AAB and yeast after encapsulation did not have a significant statistical effect caused by drying conditions, the type of encapsulating material or the coating material:"taberna" ratio ($P > 0.05$) (Table 3). Furthermore, AAB presented survival in the range of 86.13 to 99.97%, while for yeasts it was 88.93 to 99.93%. LAB had a survival in the range of 91.87 to 99.99% for all treatments; the survival percentage was not influenced by any factors. The microbial survival depends on their own defense mechanisms induced in conditions of thermal and osmotic stress, as well as possible interactions between the microorganism and the encapsulating agent (Huang *et al*., 2017). Moreover, Yu *et al*. (2010) reported that at a higher feed flow rate it is necessary to supply more thermal energy to ensure the evaporation of moisture content because at a higher flow, the residence time in the drying chamber is very short. Several authors attribute the survival of the microorganisms to the short residence time during spray drying (Edelson-Mammel and Buchanan 2004, Arku *et al*., 2008).

The water activity of microcapsules varied between 0.08 and 0.34 for treatments (Table 3). Multifactorial analysis showed that inlet air temperature, feed flow, mixtures of encapsulating agents and coating material:"taberna" ratio had a statistical effect on Aw ($P < 0.05$). These values are similar to those reported by Poddar *et al*. (2014) who found *Lb. paracasei* CRL 431 encapsulated by spray drying that survive better when the Aw was lower than 0.33.

Another study performed on *Lb. rhamnosus* GG also showed that the viability of bacteria in dried crushed flaxseed dropped rapidly, with an Aw at 0.43 and 0.22 during long-term storage, while a loss of viability of only 0.29 $\log_{10}$ units was found with an Aw at 0.11 (Vesterlund *et al*., 2012).

3.3 Surface morphology of microcapsules

The surface morphology of the microcapsules is observed in Figure 1. Microcapsules presented a spherical shape, with the particle size from 1 to 10 μm and a smooth surface. In the powder, it is observed that the encapsulating material is porous, which is due probably to the volatilization of the water (principally) during drying (Turasan *et al*., 2015). Su *et al*. (2007) attribute the smooth shape of the microcapsules to the high degree of dehydration and low moisture content of the microcapsules. It can be seen that both samples had smooth surfaces free of cracks and dents. This lack of surface deformation could be explained by the high content of wall matrices and by the high rate of dehydration at the moment of being sprayed (Turasan *et al*., 2015).

The microcapsules obtained were treated in order to show the interior of the microcapsules. Figure 2 shows the presence of microorganisms from the encapsulated "taberna". The encapsulated cellular diversity includes short and long bacilli that are grouped as *diplobacillus, streptobacillus* and *coccobacillus*, as well as cocci, diplococci and yeast. These results are similar to the morphological characteristics of the main microbial groups identified during the *in vivo* sampling of the "taberna".

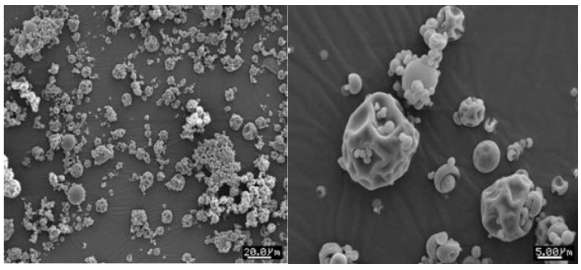

Fig. 1. Scanning electron microscopy images of microcapsules of "taberna" microencapsulated using a mixture of maltodextrin-gum Arabic with a coating material: "taberna" ratio of 1:1 (v/v) which were dried with a feed flow of 3 mL min^{-1} and an inlet air temperature of 97 ºC by spray drying.

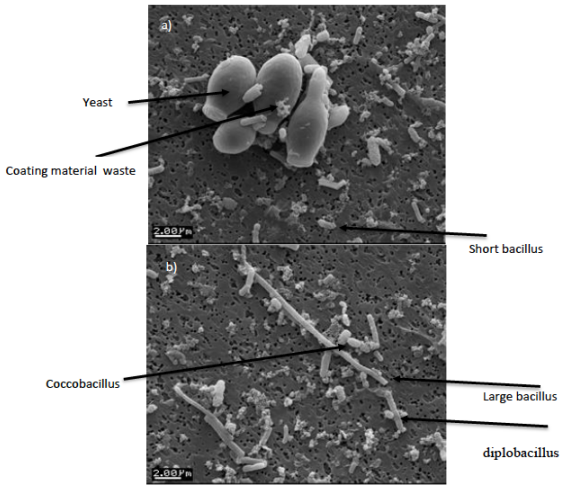

Fig. 2. Microphotographs of the microbial diversity in microcapsules of "taberna" obtained by optimal condition (mixture of maltodextrin-gum Arabic with a coating material: "taberna" ratio of 1:1 v/v which were dried with a feed flow of 3 mL min^{-1} and an inlet air temperature of 97ºC) of spray drying. a) Yeast and short bacillus encapsulated. b) Large *bacillus*, *diplobacillus* and *coccobacillus*.

3.4 Optimization of the "taberna" encapsulation sample

Drying conditions were optimized to maximize encapsulation efficiency and microbial survival in the encapsulated samples. A feed flow of 3 mL min^{-1}, an inlet air temperature 97 ºC, and a mixture of MD-GA in a coating material:"taberna" ratios of 1:1 are the optimum values for "taberna" encapsulation. Microbial survival after spray drying for optimal conditions were 89% for LAB, 84% for AAB and 80% for yeast. This optimal treatment was validated in triplicate, and the error of prediction obtained for encapsulation efficiency was ±1.89, while for LAB, AAB and yeast survival it was ±9.94%, ±15.39% and ±18.70%, respectively.

3.5 Volatile compounds in microencapsulated "taberna" samples

The profile of main aromatic volatile compounds present in natural and microencapsulated "taberna" is shown in Table 4. The presence of higher alcohols, acetic acid and esters is observed in both samples. The secondary aroma of drink could be attributed to the compounds generated by the metabolism of

yeasts and the esters make up the group of compounds that significantly affect the sensory quality of the final product. These compounds can be divided into two main groups: acetate esters, such as ethyl acetate, isoamyl acetate and phenylethyl acetate, and ethyl esters, such as ethyl hexanoate and ethyl octanoate. Both groups together are desirable in most fermented alcoholic beverages because they provide floral/fruit aromas and flavors and contribute to their characteristic complexity of aroma and flavor (Loviso and Libkind, 2018). These esters are mostly found in concentrations close to the threshold level, which implies that small changes in their concentration could affect the aroma and flavor of the drink (Loviso and Libkind, 2018). Ester formation occurs during primary fermentation and is highly associated with the metabolism of lipids and the growth of yeasts. These compounds are synthesized in the cytoplasm from reactions catalyzed by enzymes acyl transferases (or ester synthases), where acyl-CoA is required as a co-substrate (Saerens *et al*., 2008). In the absence of oxygen, the reaction between acetyl-CoA and alcohol (ethanol or higher alcohols) allows the formation of acetate esters, such as ethyl acetate, isoamyl acetate, isobutyl acetate and phenylethyl acetate, while the combination between the long chains of acyl-CoA and ethanol produces ethyl esters (Pires *et al*., 2014). Among the latter, ethyl hexanoate and ethyl octanoate are of great importance for obtaining drinks balanced in terms of aromatic profiles.

The ethyl acetate is reported as the majority ester in wines and is attributed to undesirable aromatic connotations (Lambrechts and Pretorius, 2000). Other esters of higher alcohols, such as pentyl acetate and short-chain fatty acids such as ethyl butanoate, ethyl hexanoate (ethyl caproate) and ethyl octanoate (ethyl caprylate), known as fruity esters that could be responsible for the fruity and floral aroma of wines (Ferreira *et al*., 1995).

Table 4. Profile of aromatic volatile compounds of the natural and encapsulated "taberna".

Compounds	**Relative abundance (%)**	
	Natural	**Encapsulated**
3-methyl-1-Butanol	0.170 a	0.345 a
3-methyl-2-Butanol	0.015 a	0.140 a
Ethyl Acetate	39.32 a	32.19 b
Propanoic acid, 2-methyl-ethylester	0.040 b	0.615 a
Acetic acid, 2-methylpropylester	0.375 b	6.985 a
Butanoic acid, ethylester	18.890 a	4.140 b
Acetic acid, butylester	0.735 a	0.275 b
Acetic acid, 3-methylbutyl ester	5.535 a	0.385 b
Butyric acid, isobutylester	0.160 b	0.335 a
Butanoic acid, butylester	0.160 a	0 b
Hexanoic acid, ethylester	7.625 a	0.175 b
Butanoic acid, 3-methylbutylester	0.855 a	0.075 a
Octanoic acid, ethylester	14.475 a	5.175 b
Decanoic acid, ethylester	5.33 b	20.350 a
Butanedioic acid, diethylester	0.455 a	0.250 b
9-Decenoic acid, ethyl ester	0.490 a	0.625 a
Acetic acid, 2-phenylethylester	0.250 b	0.410 a
Dodecanoic acid, ethylester	0.575 b	4.415 a
Benzenepropanoic acid, ethyl ester	0.080 b	0.330 a
Acetic acid	1.445 b	19.470 a
D-Limonene	2.330 a	2.520 a
Styrene	0.190 a	0.015 b
O-Cymene	0.360 a	0.270 b
Decanal	0.070 a	0.210 a
Benzaldehyde	0.075 b	0.925 a

Values with different letters in the rows are significantly different ($P < 0.05$)

The presence of acetic acid, 3-methylbutyl ester is considered a positive quality that inspires fruity (banana oil) fragrances and therefore enhances the quality of the product (Lasekan *et al*., 2007).

Other compounds synthesized by different species of lactic acid bacteria during the malolactic fermentation process also contribute to a lesser degree to the sensory quality of the final product (Lambrechts and Pretorius, 2000). The profile of alcohols produced in the "taberna" is similar to that reported by Lambrechts and Pretorius (2000), who found in wine that the main alcohols from yeast metabolism are ethanol and glycerol, followed by diols and higher aliphatic alcohols (1-propanol, 2-methyl-1-propanol, 2-methyl-1-butanol and 3-methyl-1-butanol) and aromatic alcohols (2-phenylethanol). Lasekan *et al*. (2007) evaluated the compounds with aromatic potential present in palm wine (*Elaeis guineensis*) and reported that the presence of 3-methyl-1-butanol is associated with malt aroma.

Lasekan *et al*. (2007) reported the presence of 41 aromatic compounds in samples of palm (*Elaeis guineensis*) wine produced in Nigeria, using solvent extraction, Headspace-SPME and olfatometry coupled with gas chromatography with FID and MS detectors. The compounds that match both samples of palm wine (*Elaeis guineensis* and *Acrocomia aculeata*) are 3-methylbutanol, hexanoic acid, ethyl ester, ethanol, acetic acid, and butanoic acid. Lasekan and Otto (2009) used the proton-mass spectrometry (PTR-MS) transfer reaction technique and reported that the aromatic profile of palm wine in Nigeria is composed mainly of acetic acid, butanoic acid, methyl-butanoic acid, 3-methylbutanoic acid, pentanoic acid, ethanol, acetoin, 2-phenylethanol, 3-methylbutanal, 2,3-butanedione, ethyl butanoate, ethyl 2-methylbutanoate and ethyl acetate compounds, which were also identified in both of the "taberna" samples (Figure 3). Generally, most compounds exhibited fruity, flowery and sweet impressions. It is also interesting to note that the fruity smelling esters, methyl butanoate, ethyl pentanoate and ethyl hexanoate, as well as acetoin, produced the most intense odor qualities (Lasekan *et al*., 2007).

In Figure 3, a heat map and the clustering of volatile compounds for natural and encapsulated "taberna" were constructed. In the hierarchical cluster analysis, each of the chemical variables was normalized. Moreover, a heat map was produced to display how the most aromatic volatile compounds present in the sample were retained during encapsulation by spray drying, using Euclidean distance as a similarity measure. All samples were grouped into two clusters (natural and encapsulated "taberna"), via cluster analysis. The clustering of volatile compounds demonstrated that both samples grouped closely with high contents of esters.

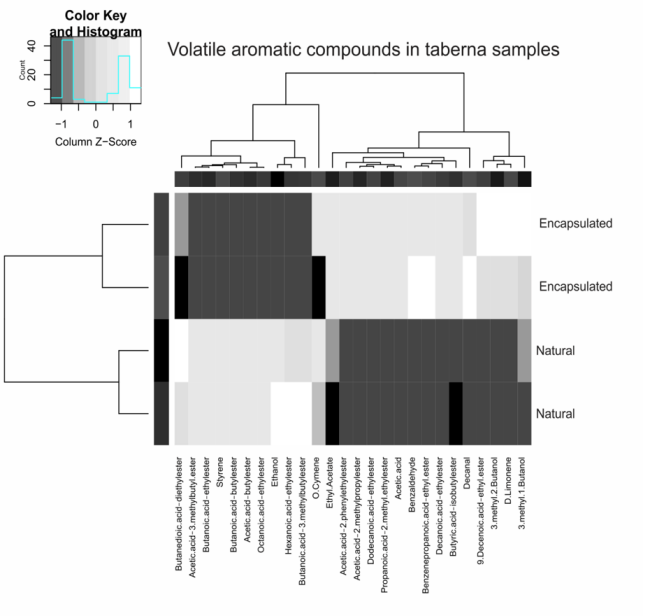

Fig. 3. Heat map and clustering of the total data set for natural and encapsulated "taberna". Values were normalized between the maximum and minimum values for each variable.

Heat map (Figure 3) analysis shows that esters are the principal groups of compounds which were identified in natural and encapsulated "taberna". Results shown that three cluster can be identified principally. Compounds in cluster 1 (as butanoic acid- ethylester, acetic acid- butylester, acetic acid- 3-methylbutyl ester, butanoic acid- butylester, hexanoic acid- ethylester, butanoic acid- 3-methylbutylester, octanoic acid- ethylester and styrene) showed a consecutive decrease in encapsulated "taberna".

However, compounds of cluster 2 (as propanoic acid- 2-methyl-ethylester, acetic acid- 2-methylpropylester, butyric acid- isobutylester, decanoic acid- ethylester, acetic acid- 2-phenylethylester, dodecanoic acid- ethylester, benzenepropanoic acid- ethyl ester, acetic acid, decanal and benzaldehyde) and cluster 3 (D-limonene, 9-decenoic acid- ethyl ester, 3-methyl-1-butanol, 3-methyl-2-butanol) increase with encapsulation. This could mainly be due to the loss of ethanol by evaporation during spray drying, which decreases significantly during encapsulation.

Table 4 shows also the retention of aromatic compounds of "taberna". This retention could be attributed to the intermolecular interactions that are formed with the encapsulating agents, mainly hydrogen bonding, as was previously explained for microorganisms. In addition, it is observed that the compounds retained in the encapsulated matrix are mainly the higher esters and higher alcohols that are characterized by being less polar and insoluble in water compared to the ethanol and low molecular weight esters.

Avellone *et al*. (2018) evaluated the correlation between the microencapsulation technology applied to wines and the resulting quality of the wine itself in terms of volatile composition and phenolic profile. Their results demonstrated a reduction of active odor compounds in microencapsulated wines, after resolubilization in water/ethanol, when considering the total amount of volatiles. They conclude, however, that the encapsulation of wine by spray drying could be an alternative technique for the worldwide marketing of wine powder, with a considerable cost reduction because of the elimination of the liquid volume. Prior to selling/consumption, the wine powder can be safely reconstituted as normal wine through the addition of a hydroalcoholic solution. The final product might have a slightly poorer aroma, but it would certainly remain a wine of acceptable quality.

Conclusions

The optimal condition for "taberna" (wine) encapsulation of *Acrocomia aculeate* palm by spray drying, which maximizes encapsulation efficiency and microbial survival was a feed flow of 3 mL min^{-1}, an inlet air temperature of 97 ºC, with coating material MD-GA and coating material:"taberna" ratio of 1:1. Analyses with SEM showed that the powder of microencapsulated "taberna" had a classical morphology, and it was shown that microorganisms were microencapsulated with high levels of survival (>80%) for LAB, AAB and yeast. Lastly, Headspace-SPME with GC-MS analyses showed that the encapsulation allows retaining the aromatic volatile compounds of "taberna", however, other techniques such as the addition of internal standard could be used to determine the aromatic compounds concentration in microcapsules.

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Nomenclature

Aw	Water activity
ME	Microencapsulation efficiency (%)
MS	Microbial survival (%)
N	Number of microorganisms per gram of dry matter before drying (CFU/g)
N_i	Number of microorganisms per gram of dry matter in the microcapsules (CFU/g)
V_r	Predicted value provided by the software
V_e	Experimental value obtained by performing validation
Relative abundance	Is the percent composition of a particular compound relative to the total area of the volatile compounds in the sample (%)
Tg	Glass transition temperature (°C)

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