

Microencapsulation of aroma compounds produced by *Pichia fermentans*

Adriane Bianchi Pedroni Medeiros, Suzan Cristina Rossi, Mario Cesar Jucoski Bier, Walter José Martínez-Burgos & Carlos Ricardo Soccol

Department of Bioprocess Engineering and Biotechnology, Federal University of Paraná Curitiba, Brazil. adrianebpm@ufpr.br; suzancrisrossi@hotmail.com; mariocjb@gmail.com; wjmartinez02@gmail.com; soccol@ufpr.br

Received: July 2nd, 2021. Received in revised form: January 10th, 2022. Accepted: January 26th, 2022

Abstract

Aroma and fragrance compounds have broad applications in the food, cosmetic and pharmaceutical industries. However, these compounds are mainly produced by chemical synthesis. An alternative, sustainable and promising route for the natural production of flavour compounds is microbial transformation processes. In this work, we obtained the production of different aroma compounds in significant concentrations, such as isoamyl acetate, isoamyl alcohol, ethyl acetate and acetaldehyde, among others, from agribusiness by-products (sugarcane molasses) and using *Pichia fermentans*. These were subsequently concentrated by pervaporation and microencapsulation by spray drying using different encapsulating agents. The results showed that when these techniques were applied to two iced beverages, they produced an odour of fruity notes, especially those of banana. In addition, woody and alcoholic notes were identified by the panellists. Finally, the best encapsulating agents were dextrin (73.49 ppm) and the dextrin and maltodextrin mixture (72.58 ppm).

Keywords: *Pichia fermentans*; molasses; microencapsulation; sensory analysis.

Microencapsulación de compuestos aromáticos producidos por *Pichia fermentans*

Resumen

Los compuestos aromáticos tienen amplias aplicaciones en las industrias alimentaria, cosmética y farmacéutica. Sin embargo, estos compuestos se producen principalmente por síntesis química. Una alternativa, sostenible y prometedora para la producción natural de compuestos aromáticos son los procesos fermentativos usando microorganismos. En este trabajo se obtuvo la producción de diferentes compuestos aromáticos en concentraciones significativas, como acetato de isoamilo, alcohol isoamílico, acetato de etilo y acetaldehído, entre otros, a partir de subproductos de la agroindustria (melaza de caña de azúcar), usando *Pichia fermentans* como inóculo. Los compuestos aromáticos se concentraron posteriormente por pervaporación y microencapsulación mediante secado por aspersión utilizando diferentes agentes encapsulantes. Se encontró que cuando estas técnicas se aplicaron a dos bebidas, produjeron un olor de notas afrutadas, especialmente las de plátano. Además, los panelistas identificaron notas amaderadas y alcohólicas. Finalmente, se encontró que los mejores agentes encapsulantes eran la dextrina (73,49 ppm) y la mezcla de dextrina y maltodextrina (72,58 ppm).

Palabras Claves: *Pichia fermentans*; melaza; microencapsulación; análisis sensorial

1. Introduction

The market for aromas currently plays an important role in the world economy. In the year 2016, it reached 26.5 billion, with an annual growth rate of approximately 4.4% [1]. In the year 2010 alone, in the USA, the demand for aroma

compounds was 75 tons [2]. Aroma and flavour compounds are added to foods for the purpose of conferring, defining, enhancing or reconstituting flavour. They are considered food additives because they have no nutritional function; these compounds can be classified as natural, artificial or identical to natural.

How to cite: Medeiros, A.B.P., Rossi, S.C., Bier, M.C.J., Martínez-Burgos, W.J. and Soccol, C.R., Microencapsulation of aroma compounds produced by *Pichia fermentans*. DYNA, 89(220), pp. 163-171, January - March, 2022.

Artificial or natural-like flavours are chemically synthesized, for example, vanillin is generally synthesized from catechol, which is initially methylated to generate guaiacol, which is subsequently condensed to generate the vanillin [3,4]. However, some of the compounds employed for the synthesis of flavourings such as benzene, toluene, and xylene come from petroleum and have been listed as toxic [5]. So, with the increased industrialization of food, demand for more practical and healthy products and depletion of the oil supply, many studies have been carried out to develop processes to produce natural aromas from renewable sources [5,6]. According to Lee and Wendisch [5], Rodriguez et al. [7], aroma compounds in plants, bacteria and fungi are generally derived from the shikimate route. Therefore, the knowledge about this route is considered the key to understanding the metabolism and biological production of the compounds mentioned.

To this end, Zhu et al. [8] produced 15 types of aromatic compounds, including phenylacetic acid, 4-hydroxy-benzoic acid and vanillic acid, using *Bacillus ligninophilus* L1 and alkaline lignins as the carbon source. Bruckner et al. [9] obtained a concentration of 1.2 g/L of muconic acid using *Saccharomyces cerevisiae*. Bluemke and Schrader [10] produced ethyl acetate, propyl acetate, isobutyl acetate, isoamyl acetate, citronellol and geraniol using *Ceratocystis moniliformis*.

The encapsulation of aromas is a technology of great interest in different areas, especially in food, since preserving the aromas of the products themselves is one of the major concerns of manufacturers. Encapsulation describes different processes used to cover an active compound with a protective wall material. It can be used to treat flavours or convey some degree of protection concerning evaporation, reaction or even migration in food Madene et al. [11]. In general, a microencapsulation process, whereby the active agent is trapped by a coating of polymers with the release of these active agents occurring under specific conditions, is used to protect the material from the adverse conditions of the medium, promoting product stability and increased shelf life.

Encapsulation methods can be divided into two groups: (i) mechanical, such as spray drying, freezing/cooling, extrusion and (ii) chemical, such as coacervation, co-crystallization, ionic gelation, interfacial polymerization and molecular inclusion, ionic gelation, polymeric incompatibility and entrapment in liposomes [11,12]. However, one of the most widely used encapsulation methods is spray drying [11–14].

The spray drying method has been used for decades to encapsulate ingredients used in foods such as aroma compounds (oregano, citronella, cardamom oil etc.), lipids, linoleic acid, essential oils, anthocyanin pigments etc. [11,13,15]. The main advantages of this method are that it provides a high encapsulation efficiency of approximately 96%-100%, as well as being an inexpensive method when compared with other processes [16]. However, this requires studies of encapsulating agents as well as optimization of operating conditions [17].

The encapsulation process through spray drying is generally carried out in three stages: emulsion preparation, homogenization and atomization. The principle of the process is to atomize the material while it is in the liquid state, and when the small drops come into contact with the hot gas, the solvent evaporates, forming a thin layer of the coating material [17]. In this method, the compound to be encapsulated is covered by a matrix generally made of starch, modified starches, some gums, maltodextrins and carboxymethylcellulose, among others [18]. Because of its relatively low cost, starch is one of the most widely used encapsulating agents. The objective of this work was to microencapsulate aromas produced by the yeast *Pichia fermentans* in sugarcane molasses and apply them to two iced beverages.

2. Methodology

2.1 Microorganism and inoculum preparation

The aroma-producing yeast strain used in this study, *P. fermentans* ITD 00165, was provided by Microbial Biotechnology Lab's Culture Collection at Durango Institute of Technology (Mexico). For inoculum preparation, the cells were transferred with a wire loop to an Erlenmeyer flask containing 50 mL of YM broth (Himedia). The flask was incubated at 30 °C and 120rpm for 24 h.

2.2 Aroma production

Fermentation occurred in a 4.5 L bioreactor filled with 1.5 L of the medium as described by Rossi et al. [19]. Sugarcane molasses was used as the carbon source in fermentations. The culture medium was prepared with 50 g/L molasses, 1 g/L leucine and 5 g/L magnesium sulphate ($MgSO_4 \cdot 7H_2O$). Leucine and $MgSO_4$ were added to the medium to improve isoamyl acetate production. After cooling, the bioreactor was inoculated with 10^6 cells/ml of aroma-producing yeast and incubated under the following conditions: 30°C and 120 rpm. The profile of volatile compound production was monitored by gas chromatography (GC) over 28 h of fermentation. The fermented broth was concentrated by using a pervaporation module.

2.3 Pervaporation process

The process of recovery and purification of the aromas in the fermented product occurred by pervaporation. The pervaporation process used was previously described according to [19]. The module consisted of a thermostatic bath (Quimis), diaphragm pump (Bomax, Brazil), 4 L feed tank, permeation cell, support for the 1.5 L 'cold trap' (Corning Pyrex) containing liquid nitrogen, a vacuum pump (Boc Edwards, model RV12) and a digital vacuum pressure gage (Instrutherm VDR-920 model). A commercial polymeric organophilic membrane with a PDMS separation film, PERVAP™ 4060 (Sulzer Chemtech AG, Allschwil, Switzerland) was placed in the permeation cell.

The membrane has a diameter of 8cm, and its effective area was 50 cm². The permeation cell consisted of a metallic mesh as a solid support to provide tangential flow to the membrane surface. In the process, two different fractions of

pervaporate were obtained. Fraction one consisted of the early pervaporate obtained in the first 6 hours. Fraction two consisted of the late pervaporate obtained from 7 hours to the end of the pervaporation process (13 hours).

2.4 SPME/headspace gas chromatography (HS-GC)

The volatiles produced (samples of fermentation broth and fraction recovered by pervaporation) were quantified by gas chromatography (GC) from the analysis of the headspace by solid-phase microextraction (SPME) as already performed by Rossi et al. [19]. The fibre used was carboxen/polydimethylsiloxane of thickness 75 µm (SPME CAR/PDMS), coupled to a manual holder (Supelco, Bellefonte, PA, USA). A 5-mL sample was placed in a 20-mL vial. The septum vials were kept at 30°C under stirring with a magnetic bar. The microfiber was exposed to the sample headspace for 30 min. Desorption was carried out by manual injection into a gas chromatograph equipped with a Shimadzu GC17A, DB-5 column (30m×0.32mm), and a flame ionization detector. The fibre was thermally desorbed by direct exposure to the injector port for 2 min. The injector and detector temperatures were maintained at 250°C. The oven temperature was initially programmed to 40 °C for 5 min and increased at a rate of 20 °C/min to a final temperature of 150 °C, which was maintained for 4 min. Nitrogen was used as the carrier gas, at a flow rate of 1.5mL/min. The split ratio was 1:5. The concentration of volatiles was calculated from a standard curve of isoamyl acetate. Compounds were identified by chromatography by comparing the retention time of the compounds present in the headspace with the retention times of pure standards (Sigma Aldrich) analysed under the same conditions.

2.5 Analysis of aroma compounds by GC-MS

In order to confirm their identification, aroma compounds present in the feed and in the permeate were also analysed in a GC Varian 450 coupled to a Varian 320-MS TQ mass detector with an HP-1MS column (30m×0.25mm) and 1µm film (Agilent J & W). The run time was set to 21 min with manual injection of 400µL of headspace using a gastight syringe.

The initial oven temperature was set to 40 °C and raised by 10°C/min to 200 °C. The injector temperature was 250 °C, and the split ratio was 1:2. The mass spectrometer ionization chamber was operated at 250 °C and the transfer line at 280°C. The scan range was 10–300 m/z. Identification was accomplished by comparison with the NIST MS Library 98 (VARIAN) in addition to specific literature [20].

2.6 Microencapsulation

The encapsulating agents tested were dextrin (modified starch ‘snowflake 6131’ provided by Corn Products Brasil), maltodextrin Mor Rex 1920 (Corn Products Brasil) and pure gum Arabic (Synth, Brasil). The drying and microencapsulation of the aroma were carried out in a spray dryer Labplant model ST05. The drying conditions were air inlet temperature of 150 °C, air flow 57 m³/h and feed rate 390 mL/h. The atomizer nozzle used had a diameter of 0.5 mm.

The formulations initially proposed to obtain the powdered aroma can be found in Table 1. Formulations with ethyl acetate standard (>99.5%, Vetec) were tested in order to define the most suitable encapsulating agents. The formulations were prepared with the addition 15 mL of ethyl acetate in 100 mL of the emulsion prepared with addition of the encapsulating agent in water under magnetic stirring. Following identification of the best encapsulating agent, tests were performed with natural flavour (fermented and concentrated/permeated aroma) in an emulsion with the encapsulating agent according to Table 1. The samples were resuspended after drying (0.1 g of the dry powder in 5 mL of 1% NaCl solution) and analysed by GC. The natural aroma produced by fermentation and concentrated by pervaporation was microencapsulated after selection of the best encapsulating agent, according to the encapsulation efficiency and characteristics of the microcapsules (size and formation of grooves).

The encapsulation efficiency (EE), the concentration of aroma present in the microcapsules compared with the concentration of those initially used for drying, was calculated (Eq. 1) according to Rocha et al. [21] as follows:

$$EE\% = \frac{\text{Microencapsulate aroma (ppm)}}{\text{Aroma infeed (ppm)}} * 100 \quad (1)$$

2.7 Scanning electron microscopy

The encapsulated aromas were analysed by scanning electron microscopy to verify the size and surface of the microcapsules in order to relate these results to the volatile retention capacity and to evaluate the efficiency of the spray drying process. The microencapsulated aromas were fixed on a stub support with double carbon faceted coated with gold under an argon atmosphere in an SCD 30 Balzers metallizer. Afterwards, they were analysed by using a Tescan Vega 3 LMU micro-scanning microscope. The microcapsules were checked for uniformity of size and the formation of grooves on their surfaces.

Table 1. Formulations of aroma (ethyl acetate) and natural aroma compounds with different encapsulating agents

Formulation	1	2	3	4	5	6
Encapsulating Agent	20% dextrin	20% Arabic gum	30% maltodextrin	15% maltodextrin and 15% dextrin	20% dextrin	15% maltodextrin and 15% dextrin
Feed 15% ethyl acetate					35% permeated *	35% permeated *

* Natural concentrated aroma after pervaporation.

Source: The authors.

2.8 Application of the natural aroma in beverages

The natural aroma obtained was applied to sugar-based soda and in a cocktail ('Ice' type mixed sparkling drink), both developed by Duas Rodas Industrial Ltda (Jaraguá do Sul, SC). In both products the dosage was 1.0%.

2.9 Sensorial profile

These samples were kept frozen until application of the product in the drinks. Sensory testing was carried out by using a panel of seven trained evaluators. The sensory profiles evaluated were appearance, odour and taste. Analyses began by looking at the appearance of the products, then noticing the odour and finally the taste. Each rater received approximately 25 mL of each sample at 25 °C ± 2 °C in 50 mL clear plastic cups identified with a combination of three random numbers. The sensorial profile was established by discussion and consensus of the group of trained evaluators.

3. Results and discussion

3.1 Volatile profile of the natural aroma

The volatile compound found in the highest concentration in natural aroma by HS-SPME/GC analysis was isoamyl acetate (banana fruity odour) in both samples 1 and 2 (20.02 ppm and 11.89 ppm), comprising 66.66% and 49.54% of the total composition, respectively.

Sample 1 contained significant concentrations of propyl acetate (ethereal fruity odour of banana, 1.475 ppm) and isoamyl alcohol (1.315 ppm), while aroma sample 2 presented 2.791 ppm isoamyl alcohol (Table 2). The compound 2-heptanone, which has a sweet, fruity coconut odour, was found in both samples and showed higher concentrations in aroma sample 2 (1.33 ppm).

This result is important, given that isoamyl acetate and 2-heptanone are products with high added value and demand in the food, pharmaceutical and perfume industries [22,23]. Isoamyl acetate and 2-heptanone have been widely used in food processing, mainly in the manufacture of jams, fruit punches, honey, caramels, artificial coffee, etc. [24]. Other works carried out on *P. fermentans* also reported on the production of the aroma compounds isoamyl acetate and 2-heptanone [25-27].

On the other hand, other researchers have used enzymes such as lipases for the production of natural isoamyl acetate; however, high-purity reagents are required (ethyl acetate >99 %, isoamyl alcohol >99 %, acetic anhydride >99 %) [1,24]. The fact that isoamyl acetate can be obtained in a natural way and at low cost from a renewable substrate is an advantage for its commercialization because, at present, the trend is toward the consumption of naturally synthesised products [1,24]. According to Zare et al. [24], Yuso et al. [28], isoamyl acetate is traditionally produced by chemical synthesis whereby additional steps are required to separate the solvents from the catalysts employed. Furthermore, chemical syntheses in general have a negative impact on the environment, with low yields and high production costs [2].

Isoamyl acetate and isoamyl alcohol were identified in both fractions at significant concentrations. The latter compound has also been reported by Kong et al. [29], who used *Pichia fermentans* and *Saccharomyces cerevisiae* to change the aroma of wine. Table 2 shows the presence of compounds such as acetaldehyde, 2-heptanone, propyl acetate etc. and others that were not identified. This indicates that in the fermentative process, different metabolic routes predominate. Some aroma compounds were detected and identified by GC/MS and did not appear in the CG/FID analysis.

Table 2.
Volatile profile of the natural aroma analysed by HS-SPME-GC

*R.I. (min)	Compound	C.A.S.	Concentration (ppm) Fraction (1)	Concentration (ppm) Fraction (2)
1.829	Acetaldehyde	75-07-0	0.16	0.53
1.974	Ethanol	64-17-5	3.29	4.06
2.238	N.I.	-	0.01	0.04
2.297	N.I.	-	0.02	-
2.623	Ethyl acetate	141-78-6	2.26	2.76
2.941	N.I.	-	0.06	0.08
3.854	N.I.	-	0.01	0.01
3.902	N.I.	-	0.01	0.02
4.291	Propyl Acetate	109-60-40	1.47	0.01
4.357	Isoamyl alcohol	123-51-3	1.31	2.79
5.114	Ethyl Isobutyrate	97-62-1	0.04	0.16
5.738	2-hexanona	591-78-6	0.01	0.01
7.426	1-hexanol	111-27-3	0.02	0.06
7.683	Isoamyl Acetate	123-92-2	20.02	11.89
7.826	2-heptanone	110-43-0	0.99	1.33
8.240	N.I.	-	0.01	-
8.956	N.I.	-	0.09	0.11
Volatile total		-	30.03	24

R.I: retention index; NI: Not identified.

Source: The authors

Table 3.

Microencapsulated aroma compounds produced by submerged fermentation by *Pichia fermentans*

Compound	Fraction		Aroma powder (dextrin)	Aroma powder fermented	Aroma powder (dextrin and maltodextrin)
	1	2			
Acetaldehyde	+	+	-	-	-
Ethanol	+	+	-	-	-
Isobutyraldehyde	+	-	-	-	-
1 propanol	+	+	-	-	-
Ethyl acetate	+	+	+	+	-
Isobutyl alcohol	+	+	-	-	-
3 methyl butanal	+	+	-	-	-
Butyraldehyde	+	-	-	-	-
Isoamyl alcohol	+	+	+	+	+
3 methyl butanoic acid	+	+	+	-	-
Isoamyl acetate	+	+	+	+	+
1 butanol, 3 methyl Butanoate	+	+	+	-	-
2 ethyl 1 hexanol	+	+	-	-	-
Acetophenone	-	-	+	-	-
N.I.	-	-	-	+	-

* (+): Component detected in aroma, (-): Component not detected in the aroma, N.I.: compound not identified by CG FID and CG/MS.

Source: The authors.

On the other hand, the encapsulated aroma contained fewer components than the concentrated form, which may have been due to the degradation of components such as acetaldehyde, ethanol and 1-propanol, among others (Table 3) by the process of spray drying. According to Favaro-trindade et al. [16], the efficiency of the process of encapsulation by spray drying exceeds 90%, that is, some compounds undergo degradation; however, it is still one of the most efficient encapsulation methods. Aroma compounds concentrated by pervaporation have a higher content of isoamyl alcohol than isoamyl acetate, according to quantification by GC/FID.

3.2 Sensorial Profile of the natural aroma

Sensorial analysis characterized the sugar-based soda containing the natural aroma (fraction 1) as an intense, yellow, turbid liquid. It was described as having an odour with fermented and cooked fruit notes, referring to the banana. It also had soft, fleshy and woody notes reminiscent of sugarcane juice. The taste was sweet, with alcoholic and fermented fleshy notes of fruit. It presented a mild residual taste that was both bitter and phenolic.

The flavoured sugar-based soda (fraction 2) also appeared as a bright yellow turbid liquid. It had an odour with soft, alcoholic notes of fermented and cooked fruit, fused with sweet notes. The taste was alcoholic, sweet and fruity, with fermented features resembling those of cider.

The cocktail/mixed sparkling flavoured drink (fraction 1) was a yellow, turbid liquid with an alcoholic odour and fermented, fruity and oxidized features. It had an acholic, acidic and mildly bitter taste. It presented soft fermented and citrus notes, resembling albedo. The cocktail/mixed sparkling flavoured drink (fraction 2) was described as a yellow, turbid liquid with an intense odour. It had fermented notes with citric features. The flavour was alcoholic, with an intense, bitter and mildly acidic taste. It had a mild, oxidized residual taste. This can be explained by the fact that the aroma compounds were not pure but present in a mixture (Table 2). Thus it was observed in all organoleptic analyses that the fruity notes of banana odour were always more intense, since isoamyl acetate, responsible for banana odour [24], was present in a higher concentration. In addition, the fermented product contained significant amounts of acetaldehyde and 2-

heptanone, which also give off a fruity odour [30], and ethyl acetate. According to [31,32], the main constituents of banana flavouring are ethyl acetate, ethanol, propyl acetate, isobutyl acetate, ethyl butyrate, isobutyl butyrate, 3-methyl butyl acetate (isoamyl acetate or isoamyl acetate), 2-pentanol, 3- methyl-1-butanol (isoamyl alcohol), 2-methylpropyl butanoate etc. On the other hand, it was observed that the panellists also noticed some alcoholic notes. This could be attributed to the presence of compounds such as ethanol and 1-hexanol.

The aromas presented variations in the sensorial profile according to the application. In soda, fruit notes were described, which referred to banana and fusible notes characteristic of sugarcane liquor. In the cocktail application, citrus features were detected, which were reminiscent of albedo and cider. The sensorial profile detected the presence of fermented notes and residual flavour, suggesting the need to improve these aspects for application in cocktails or mixed sparkling drinks. Aroma (fraction 2) was the product that presented the highest acceptability when applied to beverages by a trained aroma panellist. In order to preserve the original characteristics of the aroma and avoid oxidation, the product was dried and microencapsulated in a spray dryer. The efficiency of microencapsulation of the compounds (ethyl acetate and natural flavour) was determined according to the concentration of the volatile components measured by SPME-CG. The concentrations of powdered compounds (ethyl acetate and natural flavour) in the different formulations were compared in the spray drier feed. The results can be seen in Fig. 1. According to the highest final concentration in the resuspended powder, the best encapsulating agents were dextrin (modified starch, 73.49 ppm) and the mixture of dextrin and maltodextrin (72.58 ppm), which were formulations 1 and 4, respectively.

From the results obtained in tests using ethyl acetate, the encapsulating agents dextrin and maltodextrin were the most efficient at retaining aroma compounds. These agents were used to encapsulate the natural aroma obtained by fermentation. In microencapsulation, the aroma produced by fermentation without any downstream process was called 'fermented', and the aroma recovered and concentrated by pervaporation was called 'permeated'.

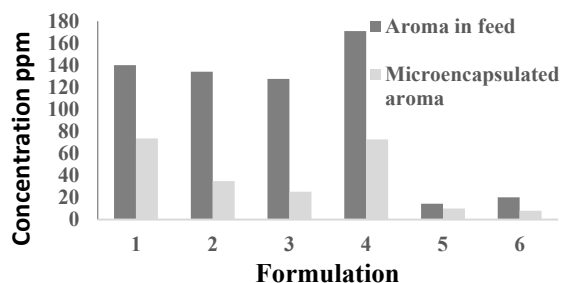


Figure 1. Concentrations of aroma compounds (ethyl acetate and isoamyl acetate) according to the formulations studied for drying. 1) ethyl acetate/dextrin, 2) ethyl acetate/gum Arabic, 3) ethyl acetate/maltodextrin, 4) ethyl acetate/dextrin and maltodextrin, 5) natural flavour/dextrin and 6) natural flavour/dextrin and maltodextrin.

Source: The authors.

The concentration and powder yield of isoamyl acetate were 9.86 ppm and 18.56%, respectively, for formulation 5 (dextrin) and 7.90 ppm and 26%, respectively, for formulation 6 (dextrin and maltodextrin). In relation to the concentration of isoamyl acetate in the powder, the best encapsulating agent for the permeate (concentrated natural flavour) was dextrin. The formulation fermented with dextrin presented the lowest concentration of isoamyl acetate, 3.2 ppm (Fig. 1). The best encapsulating agents according to their efficiency (%) were dextrin (52.46%), maltodextrin with dextrin (42.46%), gum Arabic (25.92%) and maltodextrin (19.68%) (Table 4). Among the samples with concentrated natural flavour, the highest efficiency was for dextrin, with 68.95%, followed by the mixture of maltodextrin and dextrin encapsulating agents, whose efficiency was 39.33%.

Previous work has shown that gum Arabic, dextrans, maltodextrins etc. or their mixtures are good encapsulating agents [33,34]. Aburto et al. [35] identified modified starch (dextrin), with volatile retention greater than 93%, gum Arabic (85%) and maltodextrin (80%) as the best encapsulating agents for orange essential oil. Balasubramani et al. [36] reported a maximum encapsulation efficiency of 82% for a garlic oleoresin using 60% maltodextrin. In addition, Ascheri et al. [37] microencapsulated orange essential oil and obtained the best result with 36% maltodextrin and 10% modified starch (capsule), in which the

retention efficiencies of aromatic compounds were 83%, 84% and 99% for α -pinene, β -pinene and limonene, respectively.

In this research, the mixture of 15% modified starch and 15% maltodextrin resulted in an encapsulation efficiency of 33.9%. For the natural aroma (permeated samples), the best encapsulating agent was snowflake dextrin modified starch, with a retention rate of 68.95% for isoamyl acetate.

According to the results shown in Table 4, maltodextrin was the encapsulating agent with the lowest encapsulation efficiency (19.68%). This probably occurred because the concentration tested (30%) was 2 times lower than the one used by Balasubramani et al. [36], and the air inlet temperature (150 °C) was also lower than the temperature used this work (200 °C).

In the abovementioned studies, results varied regarding the encapsulation efficiency of the agents tested. This was most likely due to other parameters that influence on this method of drying. Such parameters are the different characteristics and quantities of the aroma compounds added to emulsion, ratio of the nucleus to the wall material and inlet and outlet air temperature in the spray dryer during drying Janiszewska et al. [38].

3.3 Scanning electron microscopy

Microcapsules of natural aroma usually have particles with spherical, smooth and flat characteristics. These are desirable characteristics because they allow greater flavour retention (less surface area and relative proportion/volume ratio), higher mass densities (better powder packaging) and better flow to be reached. As seen in Fig. 2, it is generally observed that the microcapsules have irregular surfaces with folds. According to Ascheri et al. [37], this probably happens due to the internal development of vacuoles, tooth depressions and external cracks. Furthermore, surface imperfections occur when there is a slow formation of crust on the surface of the particles as the pulverized droplets dry. The presence of ‘teeth’ has an adverse effect on the flow properties of the product.

On the other hand, flat spheres were also observed in Figs. 2-2, 2-4 and 2-5. According to Osorio et al. [39], flat spheres are desirable for the encapsulated ingredients stability and controlled release. Balasubramani et al. [36] obtained good retention of active compounds of garlic (81.9%).

Table 4. Characteristics of ethyl acetate spray drier emulsion, microcapsules and natural aroma

Formulation	1	2	3	4	5	6
Encapsulating Agent	Dextrin	Arabic gum	Maltodextrin	Maltodextrin + dextrin	Dextrin	Maltodextrin + dextrin
Compound	Ethyl acetate			Permeated*		
Emulsion solids content (%)	20	20	30	30	20	30
Encapsulation Efficiency (%)	52.5	25.9	19.7	42.5	69	39.4
Average particle size (diameter μ m)	7.0	8.66	10.66	10.33	9.16	10.5

* Permeated: pervaporation concentrated natural liquid aroma

Source: The authors.

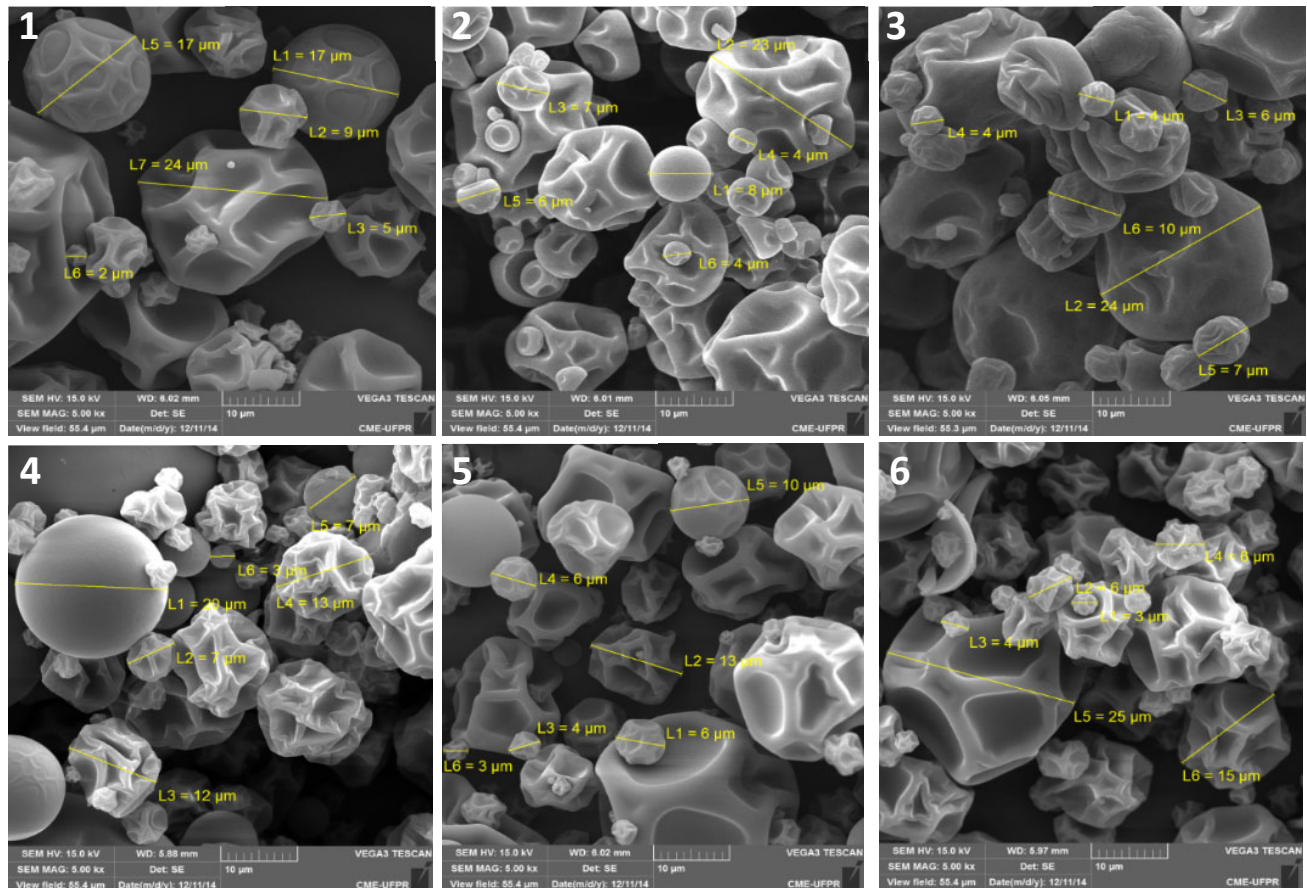


Figure 2. Natural-flavoured ethyl acetate microcapsules obtained by fermentation using different encapsulating agents. 1) ethyl acetate/gum Arabic, 3) ethyl acetate/maltodextrin, 4) ethyl acetate/dextrin and maltodextrin, 5) natural flavour/dextrin, 6) natural flavour/dextrin and maltodextrin.

Source: The authors.

Table 5. Stability test regarding the concentration of encapsulated isoamyl acetate

Microencapsulated aroma with	Initial concentration Isoamyl acetate (ppm)	Concentration Isoamyl acetate after 3 months (ppm)
Dextrin	9.86	8.56
Dextrin and maltodextrin	8.40	7.89
Fermented with dextrin	3.20	2.76

Source: The authors.

The compounds were encapsulated with maltodextrin, and microcapsules presented spherical structures with smooth surfaces and no grooves when analysed by scanning electron microscopy. The only samples which were composed by a few spherical and smooth particles were formulation 2(ethyl acetate/ gum Arabic), 4 (a sample of ethyl acetate with 15% dextrin and 15% maltodextrin) and 5 (natural aroma /dextrin). In these formulations, there was retention of volatile compounds of 25.92%, 42.46% and 69%, respectively. According to the figures below, all samples had particles ranging from 2 to 25 µm and grooves on the surface.

3.4 Microencapsulated flavour stability

After 3 months of storage, the samples were resuspended and analysed in GC by HS-SPME. All the samples were stable (Table 5) with regard to the concentration of microencapsulated isoamyl acetate. There was a decrease of 13.18% in the microencapsulated aroma with dextrin, 6.07% in the mixture of dextrin and maltodextrin and 13.75% for the fermented microencapsulated aroma with dextrin.

According to Pham-hoang et al. [40], aroma compounds are very unstable molecules. When these volatile compounds are obtained by a powder microencapsulation technique, they become more stable and functional due to their controlled release. Water activity and storage temperature are the most important factors in the control of microcapsule stability. In this research, the stability study showed that in 3 months the volatile compounds present in the initial powder remained stable.

4. Conclusion

Spray drying is a promising drying technique for obtaining microcapsules of flavour compounds naturally obtained by

submerged fermentation. Among the tested agents, the highest encapsulation efficiency was obtained using dextrin, while the lowest efficiency was with maltodextrin. The aromas produced were compatible with the characteristic aromas of banana and other fruit notes. Also, the sensory test showed high acceptability by the evaluators. However, improvement in some respects was suggested, as they identified some fermented notes and other residual aromas.

References

- [1] Santos, P, Meireles, M.A.A. and Martínez, J., Production of isoamyl acetate by enzymatic reactions in batch and packed bed reactors with supercritical CO₂, *J. Supercrit. Fluids.*, 127(1), pp 71-80, 2017. DOI: <https://doi.org/10.1016/j.supflu.2017.03.019>.
- [2] Sánchez-Castañeda, A.K., Athès, V., Moussa, M., López-Miranda, J., Jesús, P-L., Soto-Cruz, N. et al., Modeling of isoamyl acetate production by fermentation with *Pichia fermentans* in an aerated system coupled to in situ extraction, *Process Biochem.*, 65(1), pp. 11-20, 2018. DOI: <https://doi.org/10.1016/j.procbio.2017.10.010>.
- [3] Li, K. and Frost, J.W., Synthesis of vanillin from glucose, *JACS.*, 120(1), pp. 10545-10546, 1998. DOI: <https://doi.org/10.1021/ja9817747>.
- [4] Scozzafava, A., Passaponti, M., Supuran, C.T. and Gülçin, İ., Carbonic anhydrase inhibitors: guaiaicol and catechol derivatives effectively inhibit certain human carbonic anhydrase isoenzymes (hCA I, II, IX and XII), *J. Enzyme Inhib. Med. Chem.*, 30(1), pp. 586-591, 2015. DOI: <https://doi.org/10.3109/14756366.2014.956310>
- [5] Lee, J. and Wendisch, V.F., Biotechnological production of aromatic compounds of the extended shikimate pathway from renewable biomass, *J. Biotechnol.*, 257(1), pp. 211-221, 2017. DOI: <https://doi.org/10.1016/j.jbiotec.2016.11.016>.
- [6] Lubbers, R.J.M., Dilokpimol, A., Visser, J., Mäkelä, M.R., Hildén, K.S. and Vries, R.P. De., A comparison between the homocyclic aromatic metabolic pathways from plant-derived compounds by bacteria and fungi, *Biotechnol. Adv.*, 37(1), art. 107396, 2019. DOI: <https://doi.org/10.1016/j.biotechadv.2019.05.002>.
- [7] Rodríguez, A., Martínez, J.A., Flores, N., Escalante, A., Gosset, G. and Bolívar, F., Engineering *Escherichia coli* to overproduce aromatic amino acids and derived compounds, *Microb. Cell Fact.*, 13(1), pp. 1-15, 2014. DOI: <https://doi.org/10.1186/s12934-014-0126-z>
- [8] Zhu, D., Zhang, P., Xie, C., Zhang, W., Sun, J., Qian, W.J., et al., Biotechnology for biofuels biodegradation of alkaline lignin by *Bacillus ligniniphilus* L1, *Biotechnol. Biofuels.*, 10(1), pp. 1-14, 2017. DOI: <https://doi.org/10.1186/s13068-017-0735-y>.
- [9] Bruckner, C., Oreb, M., Kunze, G., Boles, E. and Tripp, J., An expanded enzyme toolbox for production of cis, cis -muconic acid and other shikimate pathway derivatives in *Saccharomyces cerevisiae*, *FEMS Yeast Res.*, 18(1), pp. 1-12, 2018. DOI: <https://doi.org/10.1093/femsyr/foy017>.
- [10] Bluemke, W. and Schrader, J., Integrated bioprocess for enhanced production of natural flavors and fragrances by *Ceratocystis moniliformis*, *Biomol. Eng.*, 17(1), pp. 137-142, 2001. DOI: [https://doi.org/10.1016/S1389-0344\(01\)00072-7](https://doi.org/10.1016/S1389-0344(01)00072-7)
- [11] Madene, A., Jacquot, M., Scher, J. and Desobry, S., Flavour encapsulation and controlled release - a review, *Int. J. Food Sci.*, 41(1), pp. 1-21, 2006. DOI: <https://doi.org/10.1111/j.1365-2621.2005.00980.x>.
- [12] Huertas, R.P., Revisión: microencapsulación de alimentos, *RevFacNalAgrMedellín.*, 63(1), pp. 5669-5684, 2011.
- [13] Sanchez-Reinoso, Z., Osorio, C. and Herrera, A., Effect of microencapsulation by spray drying on cocoa aroma compounds and physicochemical characterisation of microencapsulates, *Powder Technol.*, 318(1), pp. 110-119, 2017. DOI: <https://doi.org/10.1016/j.powtec.2017.05.040>.
- [14] Saifullah, M., Mohammad, S., Rayhana, F., Rahman, R. and Vuong, Q., Micro and nano encapsulation, retention and controlled release of flavor and aroma compounds: a critical review, *Trends Food Sci Technol.*, 86(1), pp. 230-251, 2019. DOI: <https://doi.org/10.1016/j.tifs.2019.02.030>.
- [15] Ribeiro, A.M., Estevinho, B.N. and Rocha, F., Microencapsulation of polyphenols - The specific case of the microencapsulation of *Sambucus Nigra* L. extracts - A review, *Trends Food Sci Technol.*, 1(1), pp. 1-14, 2019. DOI: <https://doi.org/10.1016/j.tifs.2019.03.011>.
- [16] Favaro-Trindade, C.S., Santana, A.S., Monterrey-Quintero, E.S., Trindade, M.A. and Netto, F.M., The use of spray drying technology to reduce bitter taste of casein hydrolysate, *Food Hydrocoll.*, 24(1), pp. 336-340, 2009. DOI: <https://doi.org/10.1016/j.foodhyd.2009.10.012>.
- [17] Gharsallaoui, A. and Chambin, O., Applications of spray-drying in microencapsulation of food ingredients: an overview, *Int. Food Res. J.*, 40(1), pp. 1107-1121, 2007. DOI: <https://doi.org/10.1016/j.foodres.2007.07.004>.
- [18] Ozkan, G., Franco, P., Marco, I. De, Xiao, J. and Capanoglu, E., A review of microencapsulation methods for food antioxidants: Principles, advantages, drawbacks and applications, *Food Chem.*, 272(1), pp. 494-506, 2019. DOI: <https://doi.org/10.1016/j.foodchem.2018.07.205>.
- [19] Rossi, S., Medeiros, A., Weschenfelder, T., Scheer, A. and Soccol, C., Use of pervaporation process for the recovery of aroma compounds produced by *P. fermentans* in sugarcane molasses, *Bioprocess Biosyst Eng.*, 40(1), pp. 959-967, 2017. DOI: <https://doi.org/10.1007/s00449-017-1759-1>.
- [20] Varian. NIST 98 MS Library Database, ver. 1.7.USA 1998. NIST Standard Reference Database 1A n.d.
- [21] Rocha, G., Favaro-Trindade, C. and Grosso, C., Microencapsulation of lycopene by spray drying: Characterization, stability and application of microcapsules, *Food Bioprod. Process.*, 90(1), pp. 37-42, 2012. DOI: <https://doi.org/10.1016/j.fbp.2011.01.001>.
- [22] Rodríguez, Y., López-Bello, N., Martínez, A. and Ley-Chong, N., Design stage of purification for obtaining isoamyl acetate from oil fusel, *Tecnología Química*, 37(1), pp. 405-416, 2017.
- [23] Salcedo, S., Arturo, C., Álvarez, M. y Crespo, C., Producción de ésteres volátiles a través de tecnología de fermentaciones utilizando residuos cítricos, *Revista Con-Ciencia*, 4(1), pp. 83-91, 2016.
- [24] Zare, M., Golmakani, M. and Niakousari, M., LWT - Food science and technology lipase synthesis of isoamyl acetate using different acyl donors: comparison of novel esterification techniques, *LWT - Food Sci Technol.*, 101(1), pp. 214-219, 2019. DOI: <https://doi.org/10.1016/j.lwt.2018.10.098>.
- [25] Rodríguez-Alonso, P., Fern, I.C., Garabal, J.I. and Centeno, J.A., Characterization of yeasts isolated from artisanal short-ripened cows' cheeses produced in Galicia (NW Spain), *Food Microbiol* 53(1), pp. 172-181, 2016. DOI: <https://doi.org/10.1016/j.fm.2015.09.012>.
- [26] Pereira, G., Neto, E., Thomaz, V., Bianchi, A., Lorenci, A. and Soccol, C., Conducting starter culture-controlled fermentations of coffee beans during on-farm wet processing: Growth, metabolic analyses and sensorial effects, *Int. Food Res. J.*, 75(1), pp. 348-356, 2015. DOI: <https://doi.org/10.1016/j.foodres.2015.06.027>.
- [27] Freitas, I. De, Pinon, N., Maubois, J., Lortal, S. and Thierry, A., The addition of a cocktail of yeast species to Cantalet cheese changes bacterial survival and enhances aroma compound formation, *International Journal of Food Microbiology*, 129(1), pp. 37-42, 2009. DOI: <https://doi.org/10.1016/j.ijfoodmicro.2008.10.026>.
- [28] Yuso, N., Sangaran, S. and Rizal, S., Non-enzymatic synthesis route for production of isoamyl acetate in a solvent-free system using miniaturized intensifier reactor, *J. Environ. Chem. Eng.*, 103(1), pp. 103186, 2019. DOI: <https://doi.org/10.1016/j.jece.2019.103186>.
- [29] Kong, C., Li, A., Su, J., Wang, X., Chen, C. and Tao, Y., Flavor modification of dry red wine from Chinese spine grape by mixed fermentation with *Pichia fermentans* and *S. cerevisiae*, *LWT - Food Sci Technol.*, 109(1), pp. 83-92, 2019. DOI: <https://doi.org/10.1016/j.lwt.2019.03.101>.
- [30] Jomori, M., Berno, N. and Kluge, R., Ethylene application after cold storage improves skin color of 'valencia' oranges, *Rev Bras Frutic.*, 38(1), pp. 1-9, 2016. DOI: <https://doi.org/10.1590/0100-29452016636>.
- [31] Virginia, F., Garruti, D., Dias, C., Cordenunsi, B. and Lajolo, M., Influence of different banana cultivars on volatile compounds during ripening in cold storage, *Int. Food Res. J.*, 49(1), pp. 626-633, 2012. DOI: <https://doi.org/10.1016/j.foodres.2012.08.013>.
- [32] Tressl, R. and Jennings, W., Production of volatile compounds in the Ripening Banana, *J. Agric. Food Chem.*, 20(1), pp. 189-192, 1972.

- [33] Martins, I.M., Barreiro, M.F., Coelho, M. and Rodrigues, A.E., Microencapsulation of essential oils with biodegradable polymeric carriers for cosmetic applications, *Chem Eng J.*, 245(1), pp. 191-200, 2014. DOI: <https://doi.org/10.1016/j.cej.2014.02.024>.
- [34] Costa, S.S., Machado B., Martin, A., Bagnara, F., Regadalli, S. and Alves, A., Drying by spray drying in the food industry: Microencapsulation, process parameters and main carriers used, *Afr. J. Food Sci.*, 9(1), pp. 462-470, 2016. DOI: <https://doi.org/10.5897/AJFS2015.1279>.
- [35] Aburto, L., De Queiroz, D. and Martucci, E., Microencapsulacão de óleo essencial de laranja, *CiencTecnol Alime.*, 18(1), pp. 45-48, 1998.
- [36] Balasubramani, P., Palaniswamy, P.T., Visvanathan, R., Thirupathi, V., Subbarayan, A. and Maran, J., Microencapsulation of garlic oleoresin using maltodextrin as wall material by spray drying technology, *Int. J. Biol. Macromol.*, 72(1), pp. 210-217, 2015. DOI: <https://doi.org/10.1016/j.ijbiomac.2014.08.011>.
- [37] Ascheri, D.P.R., Marquez, M. and Martucci, E.T., Microencapsulação de óleo essencial de laranja: seleção de material de parede I. *Ciênc Tecnol Aliment.*, 23(1), pp. 1-6, 2003.
- [38] Janiszewska, E., Jedli, A. and Witrowa-Rajchert, D., Effect of homogenization parameters on selected physical properties of lemon aroma powder. *Food Bioprod. Process.*, 4(1), pp. 405-413, 2014. DOI: <https://doi.org/10.1016/j.fbp.2014.05.006>.
- [39] Osorio, C., Forero, D.P. and Carriazo, J.G., Characterisation and performance assessment of guava (*Psidium guajava* L.) microencapsulates obtained by spray-drying, *Int. Food Res. J.*, 44(1), pp. 1174-1181, 2011. DOI: <https://doi.org/10.1016/j.foodres.2010.09.007>.
- [40] Pham-Hoang, B.N., Romero-Guido, C., Phan-Thi, H., Waché, Y., Encapsulation in a natural, preformed, multi-component and complex capsule: yeast cells, *Appl Microbiol Biotechnol.*, 97(1), pp. 6635-6645, 2013. DOI: <https://doi.org/10.1007/s00253-013-5044-1>.
- A.B.P. Medeiros**, is associate professor in the Federal University of Paraná, Brazil, Sp. in volatile fermentation products production and analysis, has experience and interest research on bioH₂, bioaromas, valorization of agroindustrial residues.
ORCID: 0000-0002-8804-0407.
- S.C. Rossi**, is PhD in Bioprocess Engineering and Biotechnology. Experience on enzyme characterization, solid state fermentation, production of natural flavors and immunobiologicals.
ORCID: 0000-0001-8884-0992.
- M.C.J. Bier**, is PhD in Bioprocess Engineering and Biotechnology, research experience in the areas of Biotechnology, involving biotechnological and fermentation processes, as well as applied microbiology.
ORCID: 0000-0002-9390-9955.
- W.J. Martinez-Burgos**, is a BSc. Eng. in Food Engineering, completed his PhD in 2019 from the Federal University of Paraná (UFPR), Brazil. At present he is a postdoctoral researcher at Universidad UFPR, in Curitiba, Brazil. He is currently working on the valorization of agro-industrial residues for the production biolipids and biohydrogen.
ORCID: 0000-0003-4377-6530.
- C.R. Soccol**, is a BSc. Eng. in Chemical Engineer and a PhD in Genie Enzymatique, Microbiologie et Bioconversion, with 30 years of experience green technology, biofuels, applied microbiology, and fermentation technology, especially in industrial bioprocesses.
ORCID: 0000-0001-7630-6864