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ENCAPSULATED BIOACTIVE COMPOUNDS-RICH POWDERS: PREPARATION OF A NON-ALCOHOLIC BEVERAGE FROM AMAZON SPECIES

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Dissertation submitted in fulfillment of the requirements for the Degree of Master in Materials Science and Engineering.

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ABSTRACT

Interest in the discovery of new antioxidants through unconventional food plants (UFP) has been growing mainly to prevent food deterioration and minimize harm to human health. The Amazon region is known for its rich biodiversity, with several fruit species presenting high amounts of bioactive compounds. In the Chapter 3, the effects of different drying methods (freeze- or spray- drying) and carriers (gum Arabic or inulin) on the powder characterization and stability of the bioactive compounds of *Hibiscus acetosella* extract (HAE) during storage were evaluated. The ^{1}H NMR spectrum of the HAE revealed characteristic signals of the caffeoyl hydroxycitric acid. The encapsulated extract powder presented different morphology according to the drying processes. Powders produced by the freeze-drying process presented higher retention of bioactive compounds due to the low temperature process. The mixture of gum Arabic and inulin as carrier resulted in the absorption of larger amount of water. The half-life time of the encapsulated extract powders ranged from 31 – 36 h (DPPH) and from 34 – 71 h (phenolic compounds). In general, the powders constituted of gum Arabic and produced by the freeze- drying process presented better protection and retention of the bioactive compounds from the HAE. The Chapter 4 reports the encapsulation of bioactive compounds from *Clidemia japurensis* and *Clidemia hirta* juices in maltodextrin of different dextrose equivalents (DE). Microparticles containing the encapsulated juices were obtained by freeze-drying process. The stability of the encapsulated bioactive compounds was evaluated under different relative humidity (22 and 77%) at 25 °C by DPPH and ABTS methods. Twelve bioactive compounds were identified by UFLC-Q-TOF- MS/MS and classified as organic acids, flavonoids and anthocyanins. The juices presented good antioxidant properties [DPPH value of 943 ± 15 μM TE (*C. japurensis*) and 994 ± 14 μM TE (*C. hirta*); ABTS value of 1119 ± 24 μM TE (*C. japurensis*) and 1273 ± 18 μM TE (*C. hirta*)]. Encapsulation Efficiency (EE) ranged from 97.0 to 99.8% (DPPH) and from 87.8 to 99.1% (ABTS). The encapsulated juices did not present Water Activity (WA) values that could favor microbial growth. According to the ABTS results, the bioactive compounds of *C. hirta* and *C. japurensis* encapsulated in MD10 wall material and stored at RH = 22% presented half-life time around 45 and 37 days, respectively. These results represent an interesting possibility of application in food industry.

Keywords: unconventional food plants; encapsulation; *Hibiscus acetosella*; *Clidemia japurensis*; *Clidemia hirta.*

RESUMO

O interesse na descoberta de novos antioxidantes em plantas alimentícias não convencionais (PANC) vem crescendo devido ao interesse na preservação desses constituintes nos alimentos, bem como melhorar sua contribuição para a saúde humana. A região amazônica é conhecida por sua rica biodiversidade, com diversas espécies de frutas apresentando altas quantidades de compostos bioativos. No capítulo 3, foram avaliados os efeitos de diferentes métodos de secagem (liofilização ou *spray drying*) e carreadores (goma arábica ou inulina) na caracterização do pó e na estabilidade dos compostos bioativos do extrato de *Hibiscus acetosella* (HAE) durante estocagem. O espectro de ^{1}H RMN do HAE revelou sinais característicos do ácido hidroxicítrico. O extrato encapsulado em pó apresentou morfologia diferente de acordo com os processos de secagem. Os pós produzidos pelo processo de liofilização apresentaram maior retenção de compostos bioativos devido à baixa temperatura do processo. A mistura de goma arábica e inulina como carreadores resultou na absorção de maior quantidade de água. O tempo de meia-vida dos extratos encapsulados variou de 31 a 36 h (DPPH) e de 34 a 71 h (compostos fenólicos). Em geral, os pós constituídos por goma arábica e produzidos pelo processo de liofilização apresentaram melhor proteção e retenção dos compostos bioativos do HAE. O capítulo 4 relata o encapsulamento de compostos bioativos dos sucos de *Clidemia japurensis* e *Clidemia hirta* em maltodextrina com diferentes concentrações de dextrose (DE). As micropartículas contendo os sucos encapsulados foram obtidas pelo processo de liofilização. A estabilidade dos compostos bioativos encapsulados foi avaliada sob diferentes umidades relativas (22 e 77 %) a 25 °C pelos métodos DPPH e ABTS. Doze compostos bioativos foram identificados por UFLC-Q-TOF-MS/MS e classificados como ácidos orgânicos, flavonóides e antocianinas. Os sucos apresentaram boas propriedades antioxidantes [valor de DPPH de 943 ± 15 μM TE (*C. japurensis*) e 994 ± 14 μM TE (*C. hirta*); valor de ABTS de 1119 ± 24 μM de TE (*C. japurensis*) e 1273 ± 18 μM de TE (*C. hirta*)]. A eficiência de encapsulamento (EE) variou de 97,0 a 99,8 % (DPPH) e de 87,8 a 99,1 % (ABTS). Os sucos encapsulados não apresentaram valores de atividade de água que pudessem favorecer o crescimento microbiano. De acordo com os resultados do ABTS, os compostos bioativos de *C. hirta* e *C. japurensis* encapsulados em carreadores MD10 e armazenados em UR = 22% apresentaram tempo de meia-vida em torno de 45 e 37 dias, respectivamente. Esses resultados representam a possibilidade interessante de aplicação desses produtos na indústria de alimentos.

Palavras-chave: plantas alimentícias não convencionais (PANC); encapsulamento; *Hibiscus acetosella*; *Clidemia japurensis*; *Clidemia hirta*.

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MD10 – maltodexitrina Mor-Rex 1910;

MD20 – maltodexitrina Mor-Rex 1920;

MD30 – maltodexitrina Mor-Rex 1930;

MD – maltodextrina;

C. hirta – Clidemia hirta;

C. japurensis – Clidemia japurensis;

H. acetosella – Hibiscus acetosella;

DE – dextrose;

HAE – *Hibiscus acetosella* extract;

SEM – Scanning Electron Microscopy;

MC – moisture content;

WA – Water Activity;

TGA – thermogravimetric analysis;

G – gum Arabic;

I – inulin;

GI – gum Arabic + inulin;

FD – freeze-drying technique;

SD – spray-drying technique.

TABLE OF CONTENTS

1. INTRODUCTION

Interest in the discovery of new antioxidants from unconventional food plants (UFP) has been growing in recent years, mainly due to the interest in preserving these constituents in food as well as improving their contribution to human health (Kinupp, 2014).

The Amazon region is known for its rich biodiversity, with several fruit species containing high amounts of bioactive compounds (Uekane et al., 2017). Consumption of tropical fruits has been increasing in domestic and international markets due to the growing recognition of their importance to human health (Contreras-Calderón, Calderón-Jaimes, Guerra- Hernández and García-Villanova, 2011). The antioxidant properties of fruits depend on their content of vitamin C, E, carotenoids, flavonoids and other polyphenols. The main dietary sources of phenolic compounds are fruits and beverages (Saura-Calixto & Goñi, 2006).

The search for a healthy lifestyle has been conducting scientific researches on new antioxidants, mainly of natural origin, which can be used in food, pharmaceutical, cosmetic and other industries (Melo, 1989). As a consequence, much attention has been directed to the research of natural antioxidants and their mechanisms of action. As a result, several plant extracts or their secondary metabolites have been demonstrated to show significant antioxidant activity, as well as protection against oxidant-induced damage (Collins, 2005).

Encapsulation of active substances is a technique that has been used to solve problems related to volatility and stability of bioactive compounds (Campelo et al, 2018). This technique allows to protect an active agent within a protective matrix using different types of polymeric materials as carriers.

The freeze-dried powdered food production process has been widely used by the food industry, mainly due to the low process temperatures, since most foods have thermolabile bioactive compounds, losing their nutritional and functional quality during the process (Murali, et Al, 2015). Several studies confirmed the efficiency of the lyophilization process for food encapsulation (Murali et al., 2015; Silva et al., 2018).

The production of powdered fruit juices and teas represents an application of industrial interest because they are highly demanded products in the world market, besides their production in the powder form has been strongly targeted. However, the high hygroscopicity and thermoplastic properties of these powders have been causing problems such as adhesion to the dryer walls and agglomeration.

In this context, the aim of this dissertation was to study the microencapsulation process of bioactive compounds from Amazon unconventional food plants (UFP) aiming the preparation of non- alcoholic beverages.

In the first and second chapters, a review of some Amazon UFP (*Hibiscus acetosella*, *Clidemia japurensis*, *Clidemia hirta*), as well as a brief report concerning the encapsulation technique and some of the most important carrier agents (gum Arabic, maltodextrin and inulin) were reported. In the third chapter, the effects of different carriers (gum arabic and inulin) and drying methods (freeze- and spray drying) were evaluated in relation to the physical and chemical properties of the encapsulated *Hibiscus acetosella* extract (HAE). In the fourth chapter, the *Clidemia japurensis* and *Clidemia hirta* juices were encapsulated in maltodextrin of different dextrose equivalents (DE). The bioactive compounds and stability were evaluated according to color analysis, retention of bioactive compounds and half-life time evaluation. The conclusions were reported on the fifth chapter.

2. LITERATURE REVIEW

2.1. Unconventional Food Plants (UFP) as antioxidant source

Nowadays people have experienced the so-called nutritional transition period, with an increase in the intake of fatty, refined and high sugar foods, as well as a low consumption of fruits and vegetables, especially the unconventional ones (Rocha et al., 2008).

Humanity has been suffering from recurrent hunger on a local scale and widespread hunger. As UFP has been underused, they have the potential to increase and diversify the sources of family incomes, agroecological and gastronomic tourism (Kinupp & Lorenzi, 2017). Consumption of tropical fruits has increased due to the growing recognition of their importance for human health (Contreras-Calderón, Calderón-Jaimes, Guerra-Hernández and García-Villanova, 2011). The antioxidant properties of tropical fruits depend on the concentration of vitamins C and E, carotenoids, flavonoids and other polyphenols. The Amazon region is known for its rich biodiversity of fruit species with high amounts of bioactive compounds (Bataglion, Da Silva, Eberlin and Koolen, 2015; Uekane et al., 2017).

Antioxidants are substances that, when present in low concentrations in relation to the oxidizable substrate, significantly retard or inhibit its oxidation (Halliwell & Gutteridge, 1989). Natural antioxidants are linked directly to aging processes by decreasing or inhibiting degenerative processes such as oxidative stress, cancer and atherosclerosis. Thus, several antioxidant nutrients, especially phenolic compounds, contribute to the antioxidant activity of membranes, and other cellular compartments of the human body. In addition, a combination of antioxidants with different sites of action may provide more effective inhibition than an antioxidant used alone, being a much more effective inhibitor of chronic degenerative diseases (Raven & Witzum, 1995; Owen, et al., 1997).

Food deterioration is inevitable over time. During production, processing, distribution and storage, food present several modes of deterioration that include biological changes caused by microorganisms normally found in the environment, as well as intrinsic chemical and biochemical changes. The latter are represented by enzymatic and non-enzymatic oxidation of lipids and phenolic substances, which may cause undesirable changes in flavour, taste, appearance, physical structure, nutritional value and toxicity (Namiki, 1990).

Tropical and subtropical countries have the largest diversity of plant species. However, the number of proportionally used fruit trees is significantly small. Brazil is the third largest

fruit producer (Andrigueto et al., 2010), but Amazon fruits represent less than 0.2% (Romero, 2009). The traditional agriculture of the Amazon region is basically composed of vegetables, native roots, medicinal plants and exotic fruits (Clay et al., 1999), which are used both for fresh consumption and for processing products (Andrigueto et al., 2010; Souza et al., 2001).

The demand for fruit worldwide has increased due to the results obtained in various epidemiological and nutritional studies, which have been shown apparent relationship between increased fruit and vegetable intake and decreased radical disease (Terry, et al., 2001).

2.1.1. *Hibiscus acetosella* Welw. Ex Hiern

Hibiscus, one of the largest genera of Malvaceae, is distributed in tropical and subtropical regions. Among the diversity of plants, the genus *Hibiscus* stands out in the ornamental area due to its diversity of colorful flowers. However, in recent years, it has gained space in the food area due to its edible flowers and natural dyes. Most flowers of this species present vitamins A and E, quercetin and anthocyanins (Bovini et al., 2001). The genus *Hibiscus* contains 220 species distributed around the world. It is an interesting source of potential bioactive molecules, such as phenolic compounds, triterpene derivatives, phytosteroids, besides antioxidant, cardioprotective, antihypertensive and antiproliferative activities (Maganha, 2010).

Hibiscus acetosella Welw. Ex Hiern (**Figure 1**) is originally from Africa and popularly known as vinegar, gooseberry or purple okra. Its botanical features consist of a shrubby plant, semi-woody stem, dark burgundy leaves with webbed ribs, and purplish pink solitary flowers (Lorenzi et al., 1999).

Figure 1: *H. acetosella* leaves.

Anthocyanins have been found in its flowers. On the other hand, tannins, flavonoids, coumarins, cardiotonic heterosides and alkaloids were identified in its leaves. Furthermore, antibacterial action of the leaves hydroalcoholic extract was previously reported (Março, 2011).

The main phenolic acid found in the *H. acetosella* extract was different from those from *H. cannabinus* and *H. sabdariffa*. Studies reported the presence of flavonoids, besides caffeoyl-hydroxycitric acid as the major phenolic acid of the *H. acetosella*. However, the neochlorogenic acid is reported as the main phenolic acid in *H. cannabinus* and *H. sabdariffa* (Kapepula, 2017).

2.1.2. Clidemia hirta and *Clidemia japurensis*

The Melastomataceae family has approximately 4,570 species distributed throughout the tropical and subtropical regions. About 175 species belong to the genus *Clidemia* D. Don, which occurs from southern Mexico to Paraguay and southern Brazil, and was introduced to Africa. Like all species of Melastomataceae, the genus *Clidemia* prefers a warm, tropical climate (Raffauf, 1996).

Reports on ethyl acetate extracts from *Clidemia hirta* roots described tannins derived from ellagic and gallic acid. In addition, they present antibacterial activity without cytotoxicity (Abdellaoui et al, 2014).

Clidemia rubra is a shrub belonging to the Melastomataceae family. The bluish or black berries are oval with 4 to 5 mm in diameter. The growing area extends from Central America (Oaxaca, Mexico) to Panama (Raffauf, 1996). In South America it is spread mainly in Colombia, Ecuador, Venezuela, French Guiana, while different varieties can also be found in Bolivia and southern Brazil. The edible and succulent fruits of *Clidemia rubra* are collected from wild plants or grown in greenhouses before being offered in local markets (Ternes, Täufel, Tunger & Zobel, 2005).

Berries of *Clidemia rubra* seemed to be a good source of dietary fiber and some minerals (Ca, Mn and Zn). In contrast, titratable acid and ascorbic acid contents were found in low concentration. Notable amounts of cyanidin 3-O-rutinoside (39.43 ± 1.66 mg/100g fresh weight), delphinidin 3-O-rutinoside (23.74 ± 1.18 mg/100 g), cyanidin 3-O-glycoside (11.68 ± 0.56 mg/100 g) and delfinidine-3-*O*-glucoside (6.08 ± 0.35 mg/100 g) were reported. Non-

anthocyanin phenolic constituents are represented by the gallic acids, protocatechuic, *p*-hydroxy-benzoic, vanillic and caffeic. Epigallocatechin, epigallocatechin gallate and epicatechin gallate, besides 11 different myricetin and quercetin derivatives such as quercetin-3-*O*-arabinoside (5.26 ± 0.16 mg / 100 g) and quercetin-3-*O*-ramnoside (5.06 ± 0.08 mg / 100 g) were also reported. Anthocyanins and ascorbic acid were mainly responsible for the antioxidant capacity of *Clidemia rubra* berries (Gordon, Schadow, Quijano, & Marx, 2011).

(a)  **(b)**

Figure 2: (a) *Clidemia hirta* and (b) *Clidemia japurensis*.

2.2. Microencapsulation technique

Several technologies have been associated with the release of active substances present in drugs and pesticides, colorants, flavorings etc. Among them, polymeric matrix systems have been widely applied in the form of microparticles. The microcapsule concept is related to the idealization of the cellular model: the membrane that surrounds and protects the cytoplasm and other components simultaneously performs other functions, such as controlling the entrance and exit of different compounds. Similarly, the microcapsule consists of a layer (or encapsulating agent - carrier), usually constituted of a polymeric material, that acts as a protective film, isolating the active substance and avoiding its exposure. This membrane responds to specific stimulus, releasing the substance at the ideal place or time.

The main objective of the microencapsulation process is to protect and delay unwanted reactions in the core material, as they partially or completely isolate the encapsulated material

from the external environment (Fernandes, 2013). Other reasons such as reducing evaporation of volatile compounds, reducing water adsorption and improving handling are relevant for food industry and can be achieved by the microencapsulation technique (Ray; Raychaudhuri; Chakraborty, 2015).

2.2.1. Carrier Agents

Natural polymers (polysaccharides and/or oligosaccharides) represent a class of materials of high interest and prominence as carriers due to their properties of low toxicity, biodegradability, filmogenic characteristics, ease of derivatization, availability and low cost for pharmaceutical purposes (Vandamme et al., 2002).

The encapsulation technique has been employed in different areas in order to improve the stability of bioactive compounds. Moreover, this technique has also been extensively applied for food encapsulation by the lyophilization method (Pedro H. Campelo, Sanches, Barros, Botrel and Borges, 2018; Murali, Kar, Mohapatra and Kalia, 2015; Silva et al., 2018). As most foods present thermolabile bioactive compounds, the lyophilization method represents a suitable alternative for the industrial production of powdered foods (Murali et al., 2015).

Fruits and beverages can be considered the main dietary sources of phenolic compounds (Saura-Calixto & Gon, 2006). The development and production of powdered fruit juices are of industrial interest because they are highly demanded products in the world market. However, its high hygroscopic and thermoplastic properties have resulted in adhesion to freezer and agglomeration in the dryer wall materials. These problems can be solved by using the encapsulation technique.

Encapsulation of bioactive compounds from fruit juices can be performed using different wall materials. In this context, maltodextrins (MD) are recommended for fruit juice encapsulation due to their low hygroscopicity (Carmo et al., 2018; Moser et al., 2017). In addition, several studies have been conducted to evaluate the influence of dextrose equivalents (DE) on the physicochemical properties of food encapsulation (Pedro H. Campelo et al., 2017; Ghani et al., 2017; Matsuura et al., 2015).

2.2.1.1. Gum Arabic

Given the possibility of obtaining new materials, special attention should be given to one of the main classes of natural polymers, which are called gums. Gums are exudated hydrocolloids from plant, as their defense mechanism response (Bagal-kestwal et al., 2014).

Gum Arabica is a product obtained by spontaneously desiccating the exudate of the trunks and branches of *Acacia senegal* (Linne). Its application as pharmaceutical excipients finds recognition and wide application in the preparation of emulsions, suspensions, as well as a binder in the manufacture of conventional tablets (Hovgaard, Brondsted, 1996). Gum Arabic is a polysaccharide widely used as a vehicle for encapsulating bioactive compounds due to its stabilizing, emulsifying and film-forming properties.

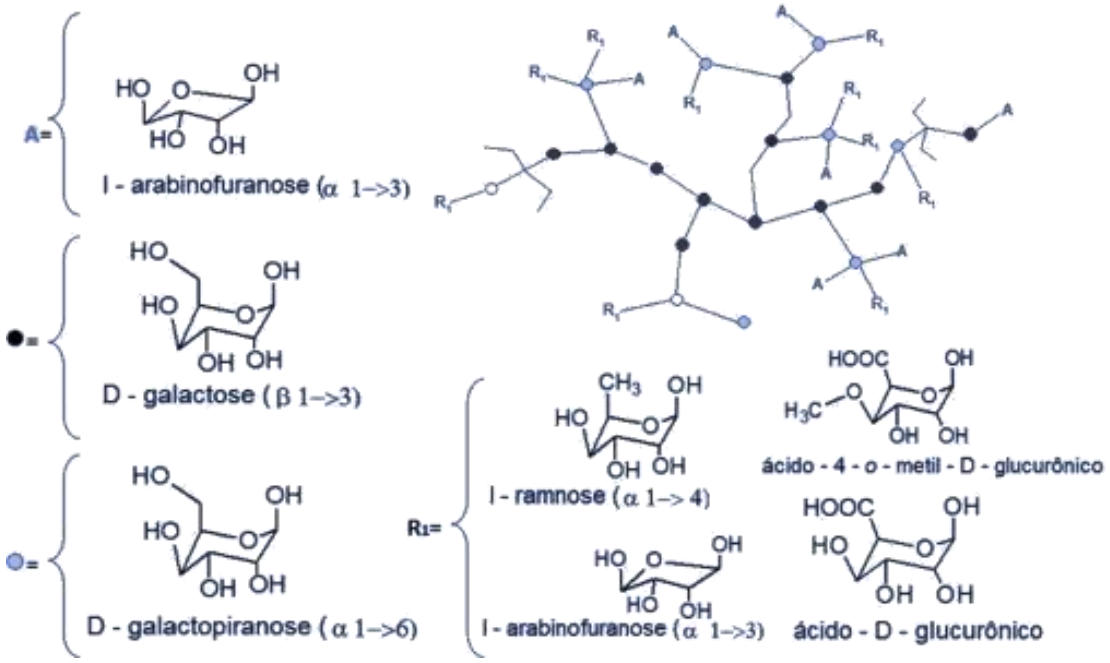

Figure 3: Gum Arabic molecular structure.

Recent studies have shown other interesting applications for gum Arabic: incorporating conducting polymers such as polyaniline into flexible natural polymer matrices may result in good solubility, while maintaining conductive properties. Gum Arabic has also been used as a stabilizer for polyaniline, preventing precipitation (Tiwari, A. 2008).

2.2.1.2. Maltodextrin

Maltodextrins are recommended for the production of powdered fruit juice, mainly due to its low hygroscopicity (Carmo et al., 2018; Moser et al., 2017). Several studies have evaluated the influence of dextrose equivalents on the physicochemical properties of encapsulated foods (Abd et al., 2017; Campelo et al., 2017; Matsuura et al., 2015).

Maltodextrins do not have good retention properties of volatile compounds. It has usually been used in combination with another emulsifying compounds to improve retention in microencapsulation processes (Fernandes et al., 2014b; Matiolo; Rodriguez- Amaya, 2002).

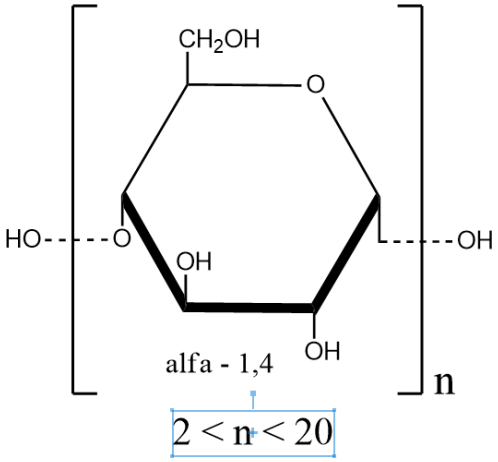

Figure 4: Maltodextrin molecular structure.

Dextrose Equivalent (DE) is a measure related to the total α-D-glucose unit. Its degree of polymerization is described as the monomeric unit number in a polymer molecule (DE = 100/SD). Unhydrolyzed starch has a value of ED = 0 and glucose has a value of ED = 100. To be called maltodextrins, the value of DE must be less than 20 (Tonon, 2009). Maltodextrins of different DE present different physicochemical properties, altering the stability of powder food microparticles, as observed in other food microencapsulation studies (Abd Ghani et al., 2017; Matsuura et al., 2015; Mulcahy; Mulvihill; O'Mahony, 2016; Takeiti; Kieckbusch; Collares- Queiroz, 2010).

2.2.1.3. Inulin

Inulin, an undigestible fructoligosaccharide commonly extracted from chicory root, can be used as a fat substitute in dairy products due to its ability to form microcrystals that result in a creamy texture, causing a fat-like sensory sensation (Debon, 2009). It is considered a prebiotic because it is selectively metabolized in the intestine colon by one or more probiotics (Mattilasandholm et al., 2002).

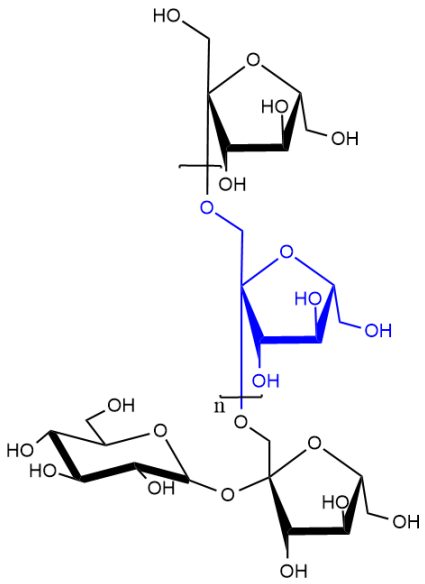

Figure 5: Inulin molecular structure.

Due to its gelling characteristics, inulin allows the development of low-fat products without compromising its taste and texture. Thus, it is used in milk, yogurt, creams, ice cream and sorbets, as well as meat, gravy and soup products (Franck, 2002).

Inulins are dietary fibers that resist digestion in the upper gastrointestinal tract and are fermented in the colon. Its energy value is lower than that of nutrients absorbed in the small intestine. Its fermentation generates physiological effects such as acceleration of intestinal transit time, reducing the risk of cardiovascular disease and colorectal cancer (Cherbut, 2002; Roberfroid, 2007).

On the other hand, inulin is a water-soluble prebiotic carbohydrate that has also been used for encapsulation purposes to ensure the functionality of powdered foods, as well as improving consumer health. In addition, inulin improves intestinal health with microflora stimulation, reduces the inflammatory state of the body and has beneficial effects on glucose

and lipid metabolism. However, inulin does not exhibit good surface properties, impairing encapsulation efficiency. For this reason, carrier mixtures (gum Arabic and inulin) represent an alternative for preserving bioactive compounds, improving the nutritional and functional properties of powdered foods.

2.3. References

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3. BIOACTIVE COMPOUNDS-RICH POWDERS: INFLUENCE OF DIFFERENT CARRIERS AND DRYING TECHNIQUES ON THE CHEMICAL STABILITY OF THE *Hibiscus acetosella* EXTRACT

3.1. Abstract

The effects of different drying methods (freeze- and spray-drying) and carriers (gum Arabic and inulin) on the powder characterization and stability of the bioactive compounds of *Hibiscus acetosella* extract (HAE) during storage were evaluated. The ^{1}H NMR spectrum of the HAE revealed characteristic signals of the caffeoyl hydroxycitric acid. The encapsulated extract powder presented different morphology according to the drying processes. The powders produced by the freeze-drying process presented higher retention of bioactive compounds due to the low temperature process. The mixture of gum Arabic and inulin as carrier resulted in the absorption of larger amount of water. The half-life time of the encapsulated extract powders ranged from 31 – 36 h (DPPH) and from 34 – 71 h (phenolic compounds). In general, the powders constituted of gum Arabic and as carrier obtained by the freeze-drying process presented better protection and retention of the bioactive compounds from the HAE.

3.2. Introduction

Natural products extracted from plants of the Malvaceae family have been used in the treatment of many diseases worldwide. *Hibiscus spp.* is one of the important genus of this family, representing over than 220 species distributed in tropical and subtropical regions (Tsuda, Horio, & Osawa, 2000), which have been extensively used in traditional medicine due to their interesting bioactive properties (Donsì, Sessa, Mediouni, Mgaidi, & Ferrari, 2011). Several antioxidant constituents have been found in the calyx and flower petals of *Hibiscus spp.*, such as anthocyanins, quercetin, ascorbic acid, steroid glycosides (including *β*-sitosteroid glycoside), protocatechuic acid (Freitas, Santos, & Moreira, 2013), polyphenolic acids and flavonoids (Chang, Huang, Hsu, Yang, & Wang, 2005).

The development of functional foods based on bioactive compounds holds many technological challenges: most bioactive compounds are susceptible to temperature, humidity, oxygen and light. For this reason, the use of carriers to protect and release the

bioactive compounds of functional foods has become evident (da Silva Carvalho et al., 2016; Gonçalves, Estevinho, & Rocha, 2017). The encapsulation technique is considered a physical process in which a wall material is used to protect solids, liquids and gases. The freeze (FD) and spray-drying (SD) processes are the most used techniques for encapsulation of food bioactive compounds (Di Battista, Constenla, Ramírez-Rigo, & Piña, 2015).

Different carriers have been used in the microencapsulation of food, aiming to improve the encapsulation efficiency, stability and controlled release. Gum Arabic is a polysaccharide extensively used as carrier to encapsulate bioactive compounds due to its stabilizing, emulsifying and film forming properties (Turchiuli, Jimenez Munguia, Hernandez Sanchez, Cortes Ferre, & Dumoulin, 2014). On the other hand, inulin is a water-soluble prebiotic carbohydrate that has also been used for encapsulation purposes to guarantee functionality to powdered foods, besides improving consumer health. Furthermore, inulin improves intestinal health upon stimulatory modulation of microflora, reduces inflammatory state of the body, as well as present beneficial effects on glucose and lipid metabolism (Guimarães et al., 2018). However, inulin does not exhibit good surface properties, impairing the encapsulation efficiency (Pedro Henrique Campelo et al., 2017; E. K. Silva & Meireles, 2015; Turchiuli et al., 2014). For this reason, the mixtures of carriers (gum Arabic and inulin) represent an alternative to preserve bioactive compounds, improving the nutritional and functional properties of the powdered foods.

The aim of this paper was to evaluate the influence of different drying methods, as well as the mixture of carriers on the chemical stability of *Hibiscus acetosella* extract (HAE). The bioactive compounds were identified by NMR and DI-HRMS techniques; microparticle characterization was carried out using Scanning Electron Microscopy (SEM), moisture content (MC), Water Activity (WA), wettability, hygroscopicity and thermogravimetric analysis (TGA). Then, these results were correlated with the accelerated stability test of the encapsulated extract.

3.3. Materials and methods

3.3.1. Materials

H. acetosella, known as 'vinagreira' in Brazil (SISGEN authorization A26CD5E), was collected in the mature state in Manaus, Brazil (3°6'26"S/60°1'34" W) in 2018. Gum Arabic and

inulin Frutalose DP 2-10 were purchased, respectively, from Alland & Roberts-France) and Sensus Ingredients-Netherland).

3.3.2. Extract encapsulation

H. acetosella extract was prepared using 1.6 g of dried leaves and 200 mL of potable water at 95 °C (Nunes et al., 2017). Three different combinations of carriers were evaluated: gum Arabic (G), inulin (I) and gum Arabic + inulin (1:1) (GI). Extract and G, I or GI carriers (80:20 w/w) were homogenized using magnetic stirrers for 1 h and storage at 4 °C for 12 h allowing the biopolymers hydration. Then, the dispersions were homogenized using a mechanical stirrer (10.000 rpm, 5 min) at 25 °C, and submitted to the drying processes.

3.3.3. NMR and DI-HRMS analyses

An aliquot of 500 µL of extract was prepared using 40 µL of D_2O containing 6.0 mM TMSP. NMR analysis was performed in a Bruker Advance IIIHD 500 MHz spectrometer. The standard used to calibrate ERETIC2 (Electronic Referencing to Access In Vivo Concentrations) was 5.0 mM quinine. The values of P1 and D1 (D1 = 5 x T1) were calibrated for all samples, standardized and acquired under quantitative conditions. The HSQC and HMBC experiments were performed to confirm the molecular structure of the quantified constituent.

The DI-HRMS analysis was performed in an ESI-MicroTOF-Q II hybrid quadrupole time-of-flight mass spectrometer (Bruker Daltonics®, Fremont, CA, USA). Sample (1 mg) was diluted in methanol/water (1:1, v/v) with 0.1 % formic acid and 3 mM of ammonium formate. The mass spectrometer parameters were as follow: capillary voltage (-3.5 kV to negative, and 4.5 kV to positive ion modes); nebulizer gas (nitrogen, 2.0 bar); dry gas (nitrogen, 6.0 L/min) and mass range (m/z = 100 – 800 Da). The instrument was calibrated with sodium formate. Data acquisition and processing were carried out using the Bruker® Compass Data Analysis 4.1 software.

3.3.4. Determination of metals

Dried leaves of *H. acetosella* (0.5g) were added in HNO_3 (10 mL) and digested for 15 min in a microwave oven (MarXpress, CEM, USA). The determination of metals (Al, Cd, Ca, Cr, Cu, Fe, K, Ni, Na, Mg, Pb, Co, Se, P and Zn) was carried out in an inductively coupled plasma optical emission spectrometer (ICP/OES) with axial configuration (model iCAP 6000 series, Thermo

Scientific, UK). Argon was used as main and auxiliary gas with flow rate of 0.5 L/min. The analytical curves considered the concentrations of 0.10 to 12,000 μg L^{-1} of the evaluated metals.

3.3.5. Freeze and spray-drying processes

After homogenization, all dispersions were rapidly frozen and subjected to freeze-drying (FD) method (Lobov, USA). Then, the microparticles were powdered manually until reach granulometry less than 30 mesh. All dispersions were also dried using a spray-dryer (SD) (model MSD 1.0, Labmaq, Brazil) equipped with two-fluid nozzle atomizer. The operational conditions were as follow: inlet temperature of 170 ± 2 °C, outlet temperature of 101 ± 2 °C, flow air of 1.5 m^3 min^{-1} and feed rate of 8 mL min^{-1}. Powders were stored at 4 °C until further analysis.

3.3.6. SEM analysis

The morphology of the encapsulated extract powders was obtained in a NA3, TECSCAN - Czech Republic microscope using 20 keV at 25 °C. Powders were placed on carbon tape and coated with a thin gold film before analysis.

3.3.7. Powder recovery, moisture content (MC) and Water Activity (WA)

The powder recovery (%) was calculated after spray-drying method according to Eq. 1 (Bhusari, Muzaffar, & Kumar, 2014):

$$Powder\ recovery\ (\%) = \frac{total\ weight\ of\ resulting\ powder}{total\ solid\ content\ in\ feed} \qquad \textbf{Eq. 1}$$

The moisture content (MC) of the encapsulated extract powder was gravimetrically determined using an infrared thermobalance (MOC-120H, Shimadzu, Japan) at 105 °C. The Water Activity (WA) was measured by a water activity meter device (Labtouch, Tecnal, Brazil) at 25 °C.

3.3.8. Apparent density, tapped density, flowability and compressibility index

Apparent density, tapped density, flowability (Eq. 2) and compressibility index (Eq. 3) were determined according to previous work (Bhusari et al., 2014). The apparent density is defined by the mass of powder (g) occupying 1 cm^3. The tapped density is the mass of powder (g) occupied in 1 cm^3 after 50 beats on a flat surface:

$$HR = \frac{tapped\ density}{aparrent\ density} \qquad \textbf{Eq. 2}$$

$$CI = \frac{tapped\ density - apparent\ density}{tapped\ density} \qquad \textbf{Eq. 3}$$

where, *HR* is the Hausner ratio and *CI* is the compressibility index.

3.3.9. Particle size distribution

Particle diameter (D_{32}; **Eq. 4**) and size distribution of powders were determined by light scattering using laser diffraction (Mastersizer 2000 Malvern Instruments Ltd., Malvern, UK) (da Silva Carvalho et al., 2016). Samples were analysed on a wet basis, with dispersion in ethanol (99.5%). Polydisperse index was calculated according **Eq. 4**.

$$D_{32} = \frac{\sum n_i d_i^3}{\sum n_i d_i^2} \qquad \textbf{Eq. 4}$$

$$Span = \frac{d_{90} - d_{10}}{d_{50}} \qquad \textbf{Eq. 5}$$

where d_i is the mean diameter of the droplets; n_i is the number of droplets; and d_{90}, d_{50} and d_{10} are the volume diameters at 90 %, 50 % and 10 % of the cumulative volume, respectively.

3.3.10. Wettability and hygroscopicity evaluation

For wettability, 1 g of the encapsulated extract powder was sprinkled, without stirring, on a surface containing 100 mL of distilled. The time required for the powder particles to sediment, sink, submerge, and disappear from the surface was recorded and used to compare

the extent of wettability of the samples (Campelo-Felix et al., 2017). Encapsulated extract powders (0.5 g) were packed in containers with saturated NaCl solution at controlled relative humidity (RH = 75%) until reach constant weight. The hygroscopicity was calculated as a percentage relative to the dry base of the microparticles (R. S. Silva et al., 2018). Analyzes were performed in triplicate at 25°C.

3.3.11. Thermogravimetric analysis

The thermal stability of the extract powders was evaluated through thermogravimetric analysis (TGA) on a TG-DTA H Shimadzu 60 under nitrogen atmosphere (10 mL/min), from 25 to 550 °C at a rate of 10 °C min^{-1}.

3.3.12. Bioactive compounds evaluation

Antioxidant activity and phenolic compounds were evaluated in the *in natura* extract, as well as in the encapsulated extract powders. The values obtained for the extract before the encapsulation process were used as control to evaluate the bioactive compounds retention. Antioxidant activity was evaluated by the DPPH radical (Molyneux, 2004). Trolox was used as positive control (2000 -125 mM). The absorbances were measured at 515 nm. Total phenolic contents were determined using a modified Folin-Ciocalteu colorimetric method (Singleton, V. L. and Rossi, 1965). Microparticles (~100 µg) were added to phenol reagent (150 µL) and sodium bicarbonate (150 µL, 10%). Total phenolic concentration was calculated from a calibration curve. The absorbances were measured at 730 nm. Results were expressed as mg of gallic acid equivalents for 100 mg of microparticles. The bioactive compounds retention (R) was calculated according to Eq. 6:

$$R(\%) = 100 \times \frac{BC_{encap}}{BC_{extract}} \qquad \textbf{Eq. 6}$$

where BC_{encap} represents the bioactive compounds of the encapsulated and $BC_{extract}$ represents the bioactive compounds of the *in natura* extract before encapsulation.

3.3.13. Accelerated storage stability

The powder samples (1 g) obtained in each treatment (FD or SD processes) were maintained at 60 °C and 85 % RH. Samples were characterized according to the antioxidant activity (DPPH) and phenolic compounds every day, for 5 days. The half-life of the bioactive compounds of the encapsulated extract powders was calculated according to Eq. 7 and 8:

$$kt = -\ln\left(\frac{C}{C_0}\right) \quad \textbf{Eq.7}$$

$$t_{1/2} = \frac{ln2}{k} \quad \textbf{Eq.8}$$

where C_0 corresponds to the initial DPPH and phenolic compounds, C is the bioactive compound concentration at time t (h), and k (h^{-1}) is the first-order reaction rate constant.

3.3.14. Statistical analysis

R software (version 3.5.2) was used for analysis of variance (ANOVA) to evaluate the effects of different carrier agents and drying processes on the stability of the extract powders. Differences between the mean values obtained for each treatment were evaluated at a 5% significance level (p-value ≤ 0.05) using the Tukey´s test.

3.4. Results and discussion

3.4.1. Identification of bioactive compounds

Previous phytochemical analysis of flower and seed extracts from *Hibiscus* spp. have reported the abundance of several phenolic compounds. The chemical composition has been related to the different varieties, genetics, ecology, harvest conditions and types of extracts (Fernández-Arroyo et al., 2011).

The ^{1}H NMR spectrum of the HAE revealed signals in the region of aromatic compounds, which were attributed to a caffeic acid derivative. The doublet in δ_H 7.23 (J = 1.9 Hz, 1H) and the double doublet in δ_H 7.16 (J = 8.3 and 2.2 Hz, 1H), both with coupling constants of hydrogen atoms coupled in meta position, in addition to the doublet in δ_H 6.95 (J = 8.3 Hz, 1H) with coupling between hydrogen atoms in the *ortho* position, suggests the presence of 1,2,4-

trisubstituted benzene nucleus. The signals in δ_H 7.73 (*d*) and 6.49 (*d*) with coupling constant of 16.0 Hz were assigned to the *trans*-olefin system hydrogen atoms of aromatic derivatives. The signals in 4.46 (s), 3.06 (d, 16 Hz) and 2.96 (d, 16 Hz) are characteristic of the hydroxycitric acid unit. This compound was identified as caffeoyl hydroxycitric acid and was quantified by ERETIC2 in about 0.40 ± 0.01 mM (N/S≥100). The presence of caffeoyl hydroxycitric acid corroborates to the antioxidant activity of the HAE. The identified compounds are shown in **Table 1**.

The main phenolic acid detected in the HAE was different from those found in *H. cannabinus* and *H. sabdariffa*. Studies report the presence of flavonoids, and the caffeoyl-hydroxycitric acid as the major phenolic acid of the *H. acetosella*. However, the neochlorogenic acid is reported as the main phenolic acid in *H. cannabinus* and *H. sabdariffa* (Kapepula et al., 2017). Our results obtained by DI-HRMS confirm the presence of the caffeoyl hydroxycitric acid by the ion *m/z* 369.0491 $[M-H]^-$.

Table 1: Compounds identified by DI-HRMS in the Hibiscus acetosella extract.

	Mode	Observed ion	Molecular Formula	Compounds
1	M-H$^-$	127.0025	$C_5H_4O_4$	Mesaconate
2	M-H$^-$	189.0061	$C_6H_6O_7$	Oxalosuccinic acid
3	M-H$^-$	207.0146	$C_6H_8O_8$	Hydroxycitric acid
4	M-H$^-$	351.0535	$C_{19}H_{12}O_7$	Daphnoretin
5	M-H$^-$	369.0491	$C_{15}H_{14}O_{11}$	Caffeoyl hydroxycitric acid
6	M-H$^-$	379.0153	$C_{12}H_{12}O_{14}$	2-(3-carboxy-2-oxalooxypropanoyl)oxypropane-1,2,3-tricarboxylic acid
7	M-H$^-$	397.0243	$C_{12}H_{14}O_{15}$	2-(3,4-dicarboxy-2,3-dihydroxybutanoyl)oxy-1-hydroxypropane-1,2,3-tricarboxylic acid
8	M+H$^+$	307.0323	$C_{10}H_{10}O_{11}$	2-(2,3,4,5-tetrahydroxypyran-2-yl)oxypyran-2,3,4,5-tetrol
9	M+H$^+$	393.0452	$C_{17}H_{12}O_{11}$	tri-*O*-methoxyellagic acid
10	M+H$^+$	597.1394	$C_{33}H_{24}O_{11}$	Tetrahydroxy-trimethoxy-flavone-chalcone derivative
11	M+H$^+$	635.4088	$C_{43}H_{54}O_4$	Flexirubin
12	M+H$^+$	679.4329	$C_{45}H_{58}O_5$	Difarnesyl-genistein derivative

3.4.2. Concentration of metals

The concentration of metals in the HAE are shown in the Supplementary material, Figure S2. The highest concentration was found for K (73 ± 1 µg mL^{-1}), followed by Ca (30 ± 1 µg mL^{-1}) and Mg (11 ± 2 µg mL^{-1}). Potassium can change the physical, chemical and anatomical characteristics of plants. Furthermore, it influences on the level of total soluble sugars of leaves. On the other hand, Pb, Cu and Cd were not detected in the HAE, which can be useful for the food industry since these minerals, in high amounts, are toxic to humans. The *H. sabdariffa* L. species

are described containing high contents of iron in stems (11.91 mg L^{-1}) and leaves (30.04 mg L^{-1}), which are above the average concentration found in vegetables (Freitas et al., 2013).

3.4.3. Morphological evaluation

Powder morphology is an important parameter for the evaluation of encapsulates since cracked surfaces can decrease the stability bioactive compounds (Pedro H. Campelo et al., 2017). The encapsulated extract powder (**Figure 6**) presented different morphology according to the drying processes: spherical and smooth surface were observed for the encapsulates produced by the SD process, whereas lamellar morphology was observed in those powders obtained by the FD process. Furthermore, porous are clearly observed in the microparticles containing gum Arabic (G) and (GI) and obtained by the FD process.

Rough surfaces present in microparticles produced by SD process may be associated to shrinkage of the carrier in the early stages of the drying process. Also, less rough surface can be observed in microparticles produced using carriers of good viscoelastic properties and favorable drying rates (D. A. Botrel, de Barros Fernandes, Borges, & Yoshida, 2014). The morphology of powdered foods produced by the FD process is a result of the breaking down of the spongy structure formed after the removal of ice crystals through sublimation (Chen, Zhong, Wen, McGillivray, & Quek, 2013). Reports of encapsulated grape polyphenols (Tolun, Altintas, & Artik, 2016) showed that more irregularities and cracking are observed when the concentration of gum Arabic is increased.

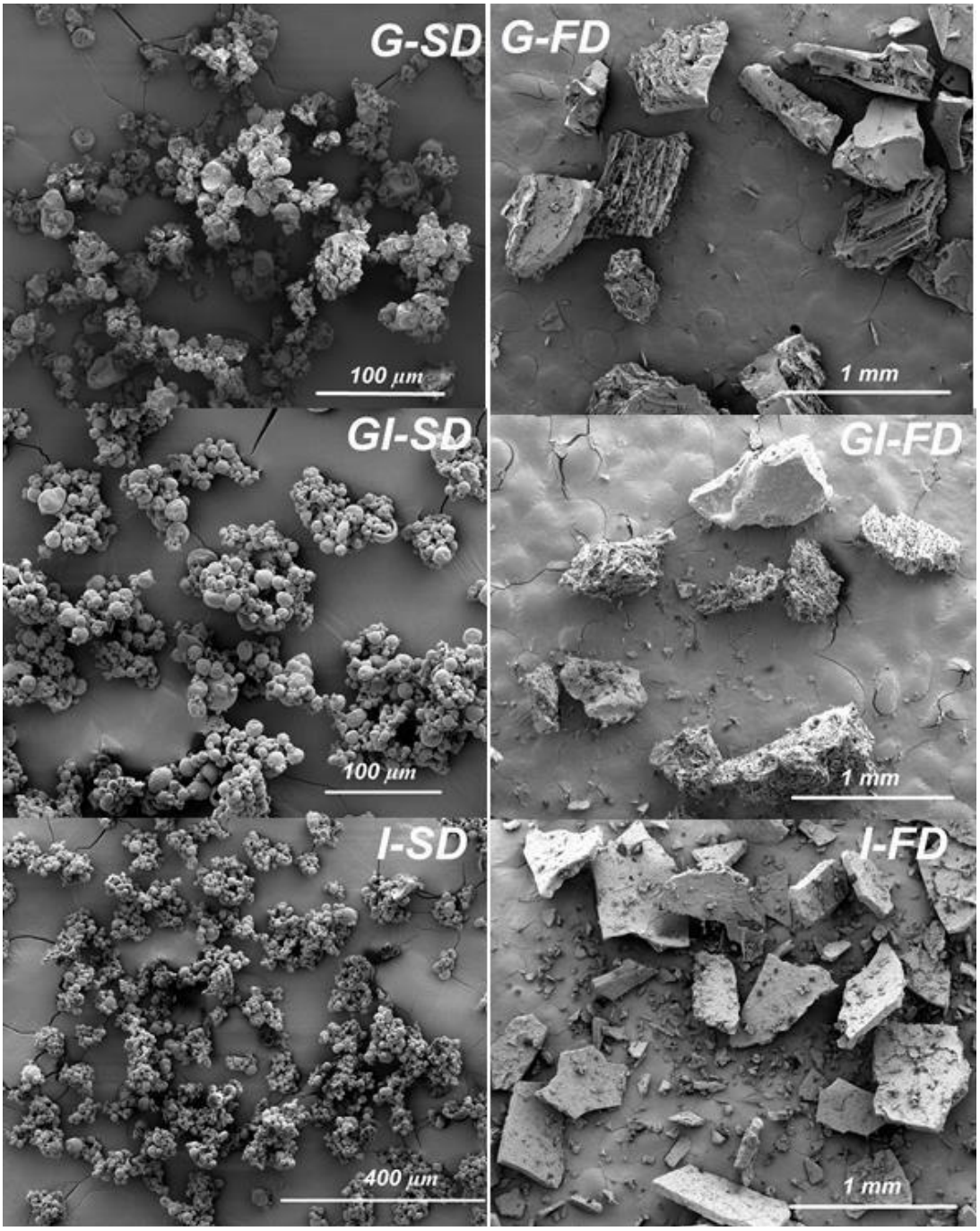

Figure 6: SEM images of the encapsulated HAE powder. G: Gum Arabic; I: Inulin; SD: Spray-drying process; FD: Freeze-drying process.

3.4.4. Physicochemical properties of the extract powders

The recovered powder yield obtained by the SD process presented values in the range of 23 – 43% w/w, with a significant difference (p-value <0.05) according to the type of carrier. The microparticles containing inulin (I and GI) presented lower recovered powder yield. The

encapsulation of astaxanthin oleoresin in different carriers (Bustos et al., 2013) revealed that the partial substitution of gum Arabic by inulin also reduced the power recovery. The lack of interfacial activity of inulin, with low emulsifying and stabilizing properties, can reduce the efficiency of the microencapsulation process. The yield of the FD powders recovered in this present work was not measured because there was practically no loss of solids during the drying process.

Table 2: Physicochemical properties of the encapsulated *H. acetosella* extract powder.

Parameters	G-SD	G-FD	I-SD	I-FD	GI-SD	GI-FD
Powder recovery (%)	43 ± 2^{a}	-	23 ± 2^{c}	-	35 ± 3^{b}	-
Water Activity	0.07 ± 0 ^{e}	0.24 ± 0 ^{c}	0.28 ± 0 ^{b}	0.38 ± 0 ^{a}	0.15 ± 0 ^{e}	0.38 ± 0 ^{a}
Moisture content (g. water.100g^{-1} dry powder)	1.3 ± 0.1 ^{d}	0.93 ± 0.06 ^{e}	5.0 ± 0.1 ^{a}	1.81 ± 0.07 ^{c}	3.5 ± 0.2 ^{b}	1.65 ± 0.09 ^{c}
Wettability (s)	257 ± 2 ^{a}	20 ± 1 ^{c}	9 ± 1 ^{c}	13 ± 1 ^{c}	216 ± 16 ^{b}	15.5 ± 0.7 ^{c}
Particle size (µm)	11 ± 1^{d}	510 ± 12^{b}	13 ± 1^{c}	610 ± 21^{a}	15 ± 2^{c}	540 ± 12^{b}
Span	1.2 ± 0.2^{d}	2.6 ± 0.7^{b}	1.7 ± 0.1^{c}	3.1 ± 0.6^{a}	1.5 ± 0.2^{c}	3.0 ± 0.4^{a}
Apparent density	0.6 ± 0.2^{a}	0.4 ± 0.2^{c}	0.5 ± 0.3^{a}	0.4 ± 0.2^{c}	0.5 ± 0.1^{b}	0.4± 0.2^{c}
Tapped density	0.8 ± 0.1^{a}	0.5 ± 0.3^{b}	0.7 ± 0.1^{a}	0.5 ± 0.1^{b}	0.7 ± 0.1^{a}	0.5 ± 0.1^{b}
Hausner ratio	1.4 ± 0.2^{b}	1.4 ± 0.2^{b}	1.3 ± 0.1^{c}	1.3 ± 0.1^{c}	1.4 ± 0.1^{a}	1.4 ± 0.1^{b}
Carr´s compressibility index	0.20 ± 0.02^{e}	0.30 ± 0.01^{c}	0.20 ± 0.01^{c}	0.20 ± 0.02^{d}	0.30 ± 0.02^{b}	0.40 ± 0.02^{a}
DPPH (Retention, %)	72 ± 4^{c}	67 ± 4^{c}	63 ± 5^{c}	90 ± 5^{a}	87 ± 2^{a}	78 ± 4^{b}
Phenolic compounds (Retention, %)	35 ± 4^{c}	27 ± 4^{cd}	25 ± 5^{d}	41 ± 5^{a}	31 ± 2^{ab}	28 ± 4^{b}

Values are expressed mean ± standard deviation. $^{(a-e)}$ Different letters in the group are significantly different (p-value < 0.05). G: Gum Arabic; I: Inulin; SD: Spray drying process; FD: Freeze-drying process.

Moisture content (MC) and Water Activity (WA) of the encapsulated extract powders are shown in **Table 2**. For both parameters, the different treatments presented a significant difference (p-value <0.05). The limit of the AW values for non-microbial growth is 0.3. That is, the particles with inulin (GI and I) produced by FD process presented values above this limit, which can decrease the stability of these encapsulates. Inulin has the ability to control the AW levels due to its hydroxyl groups (E. K. Silva & Meireles, 2015). Comparing the different treatments, the AW values were highest for the freeze-dried microparticles, although these particles presented lower MC. Similar results were observed in studies reporting the encapsulation of pulp and acerola residue using FD and SD processes (Rezende, Nogueira, & Narain, 2018). The MC values were higher for the SD process when inulin was used as carrier. Particles produced by SD process tend to present higher MC values due to its high surface area, favoring the adsorption of water molecules. In the drying processes, inulin can agglomerate

around the encapsulated food more quickly due to the high temperature. This fact prevents the diffusion of water through the pores and increases the MC of the powders (Castel, Rubiolo, & Carrara, 2018).

Table 2 also shows the wettability values of the encapsulated extract powders, ranging from 9 to 257 s. The highest dissolution times were observed for those powders containing gum Arabic (G and GI), and obtained by the SD process. Foods produced by this process have low particle size, resulting in increased wettability (Ferrari, Germer, Alvim, Vissotto, & de Aguirre, 2012). For fish essential oil encapsulated in different carriers (D. A. Botrel et al., 2014), the addition of inulin significantly reduced the wettability time, as observed in this present work. Powders produced using inulin as carrier tend to agglomerate, improving the wettability of powdered foods.

The powder diameter and particle size distribution are also shown in **Table 2**. A significant difference (p-value <0.05) may be due the manually maceration of the recovered powder after the FD process, taking into account that powder cracks can lead to the degradation of the bioactive compounds during storage. Considering the type of carrier, the different treatments were significant (p <0.05), with smaller particle sizes observed for those powders containing gum Arabic (G and GI). Due to the good stabilizing and emulsifying properties of gum Arabic (Thevenet, 2006), the particle size tends to become smaller, resulting in no agglomeration. The span values were found higher for the powders obtained by the FD process as a consequence of the manually maceration.

Physical parameters such as bulk density, tapped density and compressibility affect the powder's flowability and storage stability (Goyal et al., 2015). The different types of carrier and drying processes resulted in a significant difference (p-value<0.05) in the apparent and tapped density values. Microparticles containing inulin as carrier presented lower density. Larger powders tend to present lower apparent and tapped density due to the voids presented between particles. Furthermore, the particles produced by the FD process presented lower values of apparent and tapped density when compared to those produced by SD process. During the FD process, the frozen water rapidly boils, resulting in porous powder structure (E. K. Silva & Meireles, 2015). This fact increases the volume of air in the structure and reduces the density. On the other hand, the water is rapidly evaporated in the powders produced by the SD process, resulting in more compact structures (R. V. de B. Fernandes et al., 2016). As a result, the powder density is increased. Produced blueberry powders (Darniadi, Ho, & Murray, 2018) presented

higher density values for spray-dried powders when compared to those obtained by the FD process.

According to the Hausner's ratio (HR) and Carr's compressibility index (CI) (Bhusari et al., 2014), all recovered powders presented low fluidity and high compactability. There was a significant difference between treatments (p-value < 0.05), with higher HR values for the GI sample. The rough and porous morphology observed in the powder surfaces confirm the low powder flowability due to the friction between particles, hindering the flow (Ghodki & Goswami, 2016).

3.4.5. Bioactive compounds retention

Table 2 shows the bioactive compounds retention of the extract after the encapsulation process, ranging from 25% to 90%. For both bioactive compounds [antioxidant (DPPH) and phenolics], the different drying process and carrier resulted in a significant difference (*p*-value < 0.05). The drying process showed to be important to the bioactive properties' maintenance of the encapsulated extract. The powders produced by the freeze-drying process presented higher retention of the bioactive compounds due to the low temperature process, preserving those bioactive compounds that are sensitive to high temperature. During the SD process, the water steam within the powders may favor the bioactive compound degradation, reducing their retention (Rezende et al., 2018). Even at low temperatures, the FD process can reduce the concentration of the encapsulated bioactive compounds mainly by carrying them together with the sublimated water (E. K. Silva, Zabot, A. Meireles, & Meireles, 2015). For acerola pulp (Rezende et al., 2018) and encapsulated coffee bioactive compounds (Ballesteros, Ramirez, Orrego, Teixeira, & Mussatto, 2017), high retention was observed in the powders produced by the FD process. The emulsifying and film forming properties of carrier are related to their volatile compound retention capacity (Goubet, Le Quere, & Voilley, 1998). Gum Arabic presented higher retention, with values between 80 and 40% for antioxidant compounds (DPPH) and phenolics, respectively. Gum Arabic is a highly branched sugar heteropolymer containing a small amount of protein covalently attached to the carbohydrate chain. For this reason, it is considered an excellent film forming property agent that better capture the bioactive molecules (Daniel Daza, Fujita, Granato, Silvia Fávaro-Trindade, & Inés Genovese, 2017). The powders containing gum Arabic and inulin presented better retention values when compared to those constituted only of inulin. Presenting low surface activity, inulin is not able

to form films around the microparticles (E. K. Silva & Meireles, 2015). For this reason, the addition of gum Arabic can enhance its protective property. Lower retention of bioactive compounds of cajita juice encapsulated in inulin carrier was observed in comparison to encapsulates produced using gum Arabic (Daniel Daza et al., 2017). Also, lower rosemary oil retention was observed when gum Arabic was partially replaced by inulin (R. V. D. B. Fernandes, Borges, & Botrel, 2014).

3.4.6. Hygroscopicity evaluation

Foods with high water absorption capacity may have higher rates of bioactive compounds degradation during storage (R. V. D. B. Fernandes et al., 2014). In general, all powders recovered by the SD process presented higher hygroscopicity when compared to those obtained by the FD process, as shown in **Figure 7**.

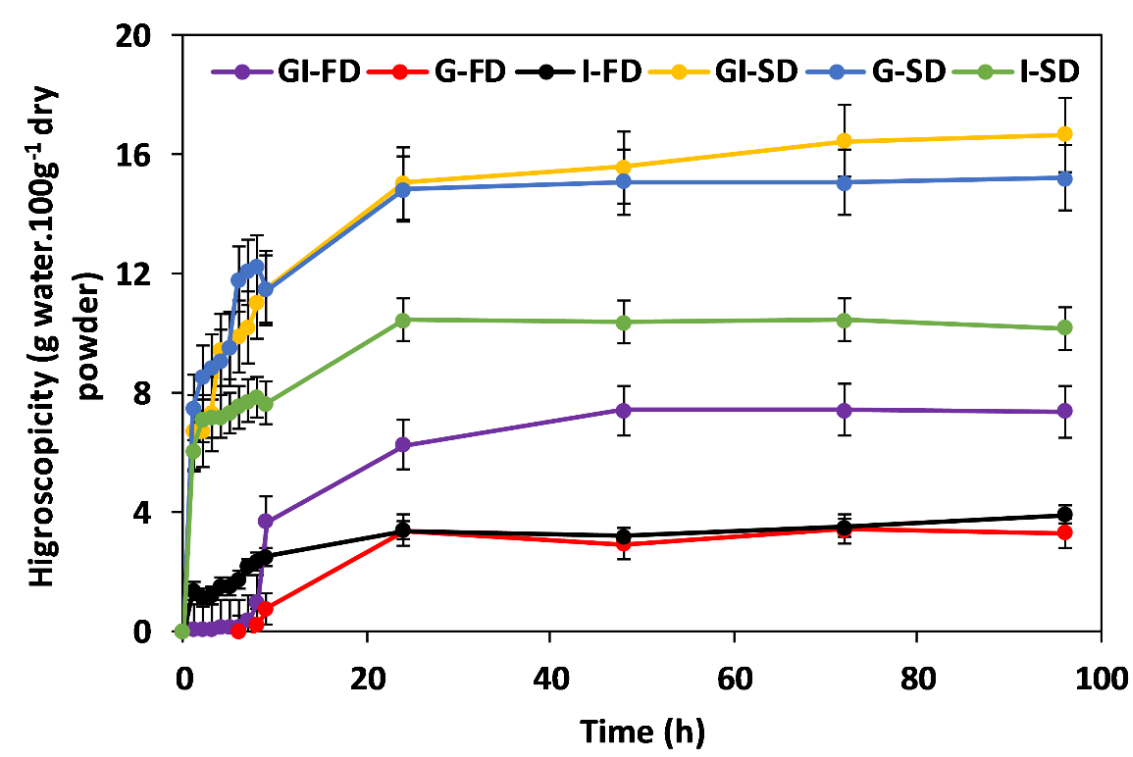

Figure 7: Hygroscopicity curves of the encapsulated HAE powder at 75% RH and 25 °C. G: Gum Arabic; I: Inulin; SD: Spray-drying process; FD: Freeze-drying process.

The powders produced by the SD process presented rapid water absorption up to 8 h, with hygroscopicity increasing slowly up to 20 h, with values stabilizing along the analysis time. This rapid adsorption of water in the first hours is related to the large quantity of active sites in

the first layer of the adsorbed molecules (Campelo-Felix et al., 2017). For the freeze-dried powders, the hygroscopicity presented values under 8 g water $100g^{-1}$ dry powder. The hygroscopicity of acerola pulp encapsulated by the FD process was also lower when compared to the SD (Rezende et al., 2018). In FD processes, the microparticles tend to be larger, reducing the surface area and, consequently, the water adsorption (Kuck & Noreña, 2016).

The mixture of gum Arabic and inulin (GI, 1:1) as carrier resulted in the absorption of larger amount of water when compared to the G and I powder. Furthermore, inulin presented lower hygroscopicity than gum Arabic. Inulin is an interesting carrier since its lower hygroscopicity may increase the stability of encapsulated bioactive compounds (R. V. de B. Fernandes et al., 2016). The dissolution of the carbohydrates occurs at high relative humidity values, exposing the active sites to connections with water (R. V. D. B. Fernandes et al., 2014). It was also observed that the addition of inulin as carrier reduced the hygroscopicity of *Annona crassiflora* pulp (Diego Alvarenga Botrel, Rodrigues, de Souza, & Fernandes, 2016).

3.4.7. Thermogravimetric analysis

The thermal stability represents an important parameter to predict the behavior of powdered foods during processes in the food industry. It is common for powdered foods to have three stages of mass loss: the first stage is due to the loss of water; the second one is related to the degradation of the biopolymers, and the third stage is attributed to the degradation of carbonaceous residues. For all treatments (**Figure 8**), the type of carrier influenced on the thermal characteristics of the recovered powders. The microparticles containing gum Arabic presented thermal degradation starting at 260 °C. For those powders containing inulin, the degradation started at 200 °C. This result shows that the addition of inulin reduced the thermal stability of the recovered powders. Gum Arabic is reported to presented degradation at 252°C (Mothé & Rao, 2000) and inulin at 214 °C (Leone, Colman, Schnitzler, Ellendersen, & Masson, 2014). The second stage of thermal degradation may be related to the depolymerization of the carrier (Ballesteros et al., 2017).

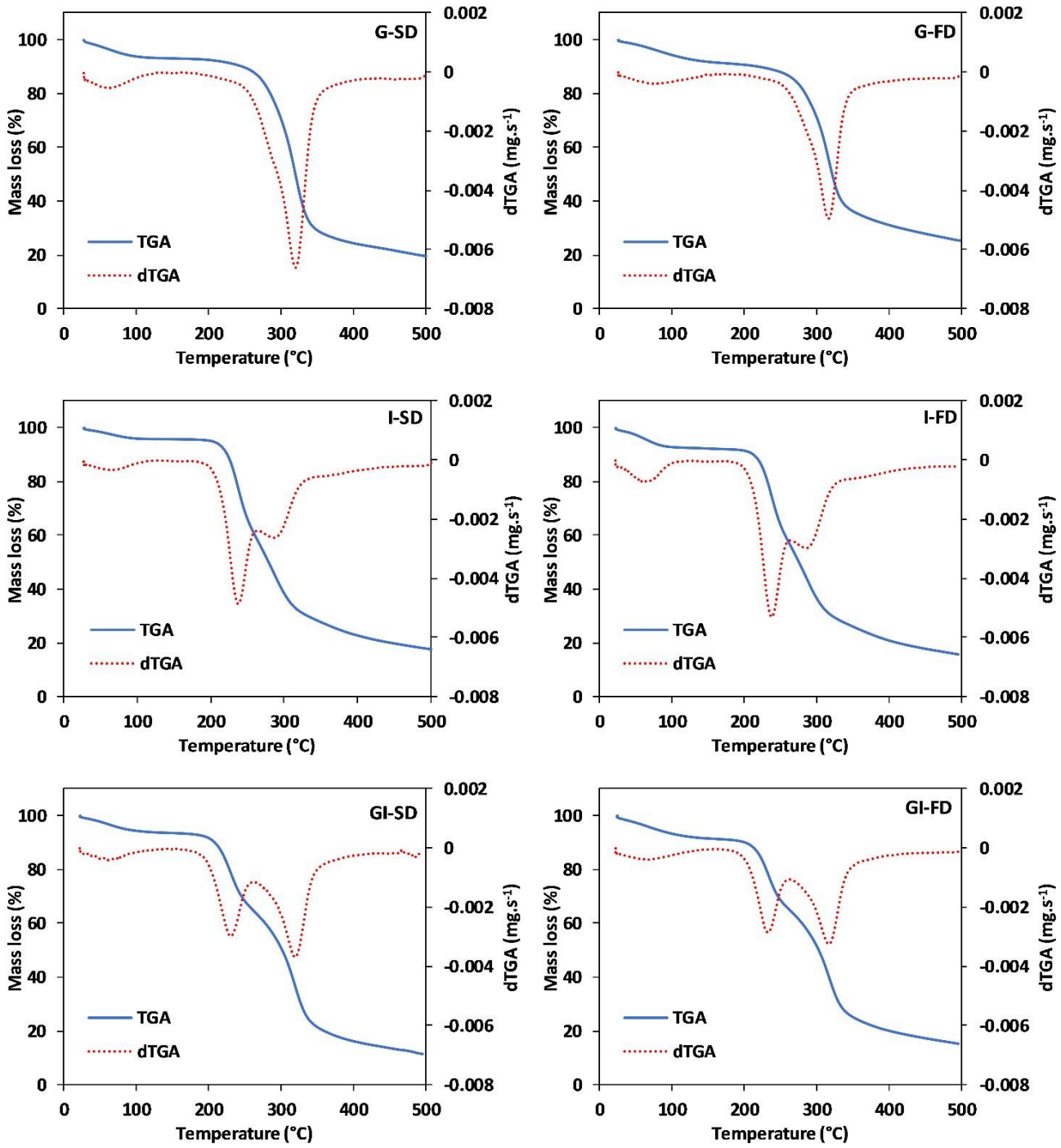

Figure 8: Thermogravimetric curves of the encapsulated HAE powder. G: Gum Arabic; I: Inulin; SD: Spray-drying process; FD: Freeze-drying process.

3.4.8. Accelerated storage stability

The thermal degradation curves of the bioactive compounds (DPPH and phenolic compounds) of the encapsulated extract powders stored at 60 °C are shown in **Figure 9**. The

kinetic parameters of degradation resulted from the linear regression of the degradation curves are presented in **Table 3**. The half-life time values ranged from 31 h to 36 h (DPPH) and from 34 h to 71 h (phenolic compounds). For microparticles of beet extract with different biopolymers (Carmo et al., 2018), inulin powders presented high bioactive compounds retention after 15 weeks. The short stability time found in the present work may be related to the high air humidity in the Amazon region (annual average about 83%).

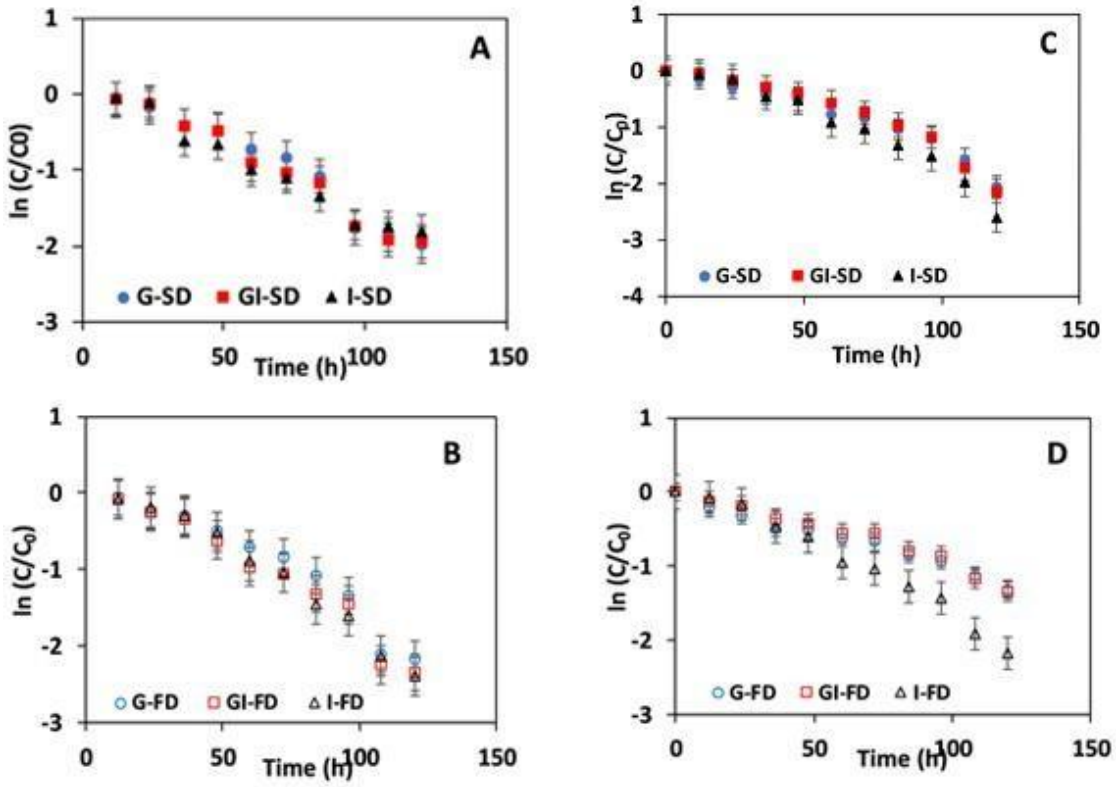

Figure 9: Bioactive compounds stability at 35 °C based on the DPPH (A and B) and phenolic compounds assays (C and D). G: Gum Arabic; I: Inulin; SD: Spray-drying process; FD: Freeze-drying process.

For DPPH and phenolic compounds, the microparticles containing inulin presented higher degradation constant considering both FD and SD processes. The low surface activity of inulin can reduce their film forming capacity during the homogenization and drying processes, reducing the protection of the encapsulated bioactive compounds. By adding any biopolymer with high emulsifying capacity, this ability to retain and protect thermosensitive compounds can be improved, as observed in the GI powders.

Table 3: Kinetic parameters of degradation based on the DPPH and phenolic compounds evaluation.

Powder	DPPH			phenolic compounds		
	$k\ (10^3\ h^{-1})$	$t_{1/2}\ (h)$	R^2	$k\ (10^3\ h^{-1})$	$t_{1/2}\ (h)$	R^2
G-SD	19 ± 1^{b}	36 ± 3^{a}	0.95	15 ± 4^{b}	45 ± 2^{c}	0.94
GI-SD	20 ± 2^{a}	36 ± 2^{a}	0.97	17 ± 1^{ab}	42 ± 1^{c}	0.91
I-SD	22 ± 1^{a}	32 ± 1^{b}	0.97	20 ± 1^{a}	34 ± 1^{d}	0.94
G-FD	20 ± 2^{ab}	35 ± 2^{a}	0.93	10 ± 1^{c}	71 ± 2^{a}	0.98
GI-FD	21 ± 1^{a}	32 ± 2^{b}	0.96	10 ± 2^{c}	65 ± 1^{b}	0.97
I-FD	22 ± 1^{a}	31 ± 2^{b}	0.97	18 ± 1^{a}	38 ± 2^{c}	0.98

k: kinetic constant; $t_{1/2}$: half-life time; R^2: correlation coefficient; G: Gum Arabic; I: Inulin; SD: Spray-drying process; FD: Freeze-drying process.

For encapsulation of oregano extracts (Zabot, Silva, Azevedo, & Meireles, 2016), partial replacement of inulin with modified starch improved the thymol retention and encapsulation efficiency. When comparing the drying processes, the phenolic compounds degraded faster for the recovered powders produced by the SD process. For the FD process, the lamellar morphology created a smaller surface area/volume ratio when compared to the microspheres produced by the SD process. Due to the larger surface area for the same amount of materials, the degradation of the surface phenolic compounds (Ballesteros, 2017).

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4. ENCAPSULATION OF AMAZONIAN BLUEBERRY EXTRACTS: EVALUATION OF BIOACTIVE COMPOUNDS AND STABILITY

4.1. Abstract

Bioactive compounds of *Clidemia japurensis* and *Clidemia hirta* juices were encapsulated in maltodextrin of different dextrose equivalents (DE). Microparticles containing the encapsulated juices were obtained by freeze-drying process. The stability of the encapsulated bioactive compounds was evaluated under different relative humidity (22 and 77%) at 25 °C by DPPH and ABTS methods. Twelve bioactive compounds were identified by UFLC-Q-TOF-MS/MS and classified as organic acids, flavonoids and anthocyanins. The juices presented good antioxidant properties [DPPH value of 943 ± 15 μM TE (*C. japurensis*) and 994 ± 14 μM TE (*C. hirta*); ABTS value of 1119 ± 24 μM TE (*C. japurensis*) and 1273 ± 18 μM TE (*C. hirta*)]. Encapsulation Efficiency (EE) ranged from 97.0 to 99.8% (DPPH) and from 87.8 to 99.1% (ABTS). The encapsulated juices did not present Water Activity (WA) values that could favor microbial growth. The encapsulated juices within MD10 wall material presented lower retention of the bioactive compounds due to the water sublimation occurred along the drying process. According to the ABTS results, the bioactive compounds of *C. hirta* and *C. japurensis* encapsulated in MD10 wall material and stored at RH = 22% presented half-life time around 45 and 37 days, respectively. These results represent an interesting possibility of application in food industry.

4.2. Introduction

The consumption of tropical fruits has been increased due to the growing recognition of its importance to human health (Contreras-calderón, Calderón-jaimes, Guerra-hernández, & García-villanova, 2011). The antioxidant properties of tropical fruits depend on the concentration of vitamins C and E, carotenoids, flavonoids and other polyphenols. The Amazon region is known for its rich biodiversity of fruit species with high amounts of bioactive compounds (Bataglion, Da Silva, Eberlin, & Koolen, 2015; Uekane et al., 2017).

The encapsulation technique has been employed in different areas aiming to improve the stability of bioactive compounds. Furthermore, this technique has also been extensively applied for food encapsulation using the freeze-drying method (Pedro H. Campelo, Sanches, de Barros Fernandes, Botrel, & Borges, 2018; Murali, Kar, Mohapatra, & Kalia, 2015; Silva et al.,

2018). Since most of foods present thermolabile bioactive compounds, the freeze-drying method represents a suitable alternative for industrially food powder production (Murali et al., 2015).

Fruits and vegetables can be considered the main dietary sources of phenolic compounds (Saura-Calixto & Gon, 2006). The development and production of powdered fruit juices represent an area of industrial interest since they are high demanded products on the world market. However, their high hygroscopicity and thermoplastic properties have resulted in adhesion to the freeze-dryer wall materials and agglomeration. These problems can be overcome by the use of the encapsulation technique.

The encapsulation of bioactive compounds from fruit juices can be performed using different wall materials. In this context, maltodextrins (MD) are recommended for the encapsulation of fruit juice due to their low hygroscopicity (Carmo et al., 2018; Moser et al., 2017). Furthermore, several studies have been conducted to evaluate the influence of dextrose equivalents (DE) on the physicochemical properties of food encapsulation (Pedro H. Campelo et al., 2017; Ghani et al., 2017; Matsuura et al., 2015).

Clidemia hirta (L.) D. Don and *Clidemia japurensis* DC. (popularly known as buxixu in Brazil) are shrubs from the Melastomataceae family with oval berries of 4–5 mm (Kinupp & Lorenzi, 2017). Few studies have reported the encapsulation of bioactive compounds from Amazonian species. For this reason, the present paper aims the encapsulation of the *C. hirta* and *C. japurensis* juices using maltodextrins of different DE (MD10, MD20 and MD30) as wall materials. SEM analysis was performed to evaluate the juice powder morphology. The chemical evaluation of the bioactive compounds was performed using ultra-fast liquid chromatography quadrupole-time-of-flight tandem mass spectrometry technique (UFLC-Q- TOFMS/MS). Furthermore, the stability of the encapsulated powder juices was verified by DPPH and ABTS methods under different relative humidity (RH) according to their moisture content (MC) and Water Activity (WA).

4.3. Materials and Methods

4.3.1. Materials

C. hirta and *C. japurensis* fruits were purchased in Manaus (3° 6 '26 "S, 60 ° 1' 34" W; SISGEN A26CD5E) in January 2018. Maltodextrin (Ingredion™, Campinas, Brazil) of different

DE (10, 20 and 30), labeled as MD10, MD20 and MD30, were used as wall materials for the encapsulation of the fruit juices.

4.3.2. Preparation of extracts

C. hirta and *C. japurensis* fruits were washed using distilled water and sodium hypochlorite solution (1%). Fruits were kept under -18 °C for 24 h, freeze-dried for 5 days in a Terroni Enterprise I freeze-dryer and then powdered using a mortar. Juices (50gmL^{-1}) were prepared using potable water.

4.3.3. UFLC-Q-TOF-MS/MS analysis

About 2 mg of the freeze-dried juices were subjected to ultrasonic extraction using acetonitrile/water (1:2, v/v) for 5 min. The supernatant was membrane-filtered and subjected to the UFLC-Q-TOF-MS/MS analysis. The LC-MS/MS system consisted of a UFLC (Shimadzu) coupled to the MicroTOF-QII mass spectrometer (Bruker Daltonik GmbH). A Shimadzu Prominence UFLC equipment consisting of a binary pump (LC-20AD) was used as separation system, with automatic injector (SIL-20A HT), furnace for column (CTO-20AC) and DAD detector (SPD-M20A). A Kinetex C18 analytical column (100 mm x 2.1 mm, 2.6 µm particle, 30 °C) was used. Water (eluent A) and acetonitrile (eluent B) were used as mobile phases, both with 0.1% formic acid. The programmed gradient consisted of 2 min in 3% of eluent B, increasing linearly to 100% in 28 min and kept constant for 3 min. Then, the column was reconditioned in 3% of eluent B for 3 min. The injection volume was 6 µL and the flow rate was 0.2 mL min^{-1}. The MS/MS system consisted of a source of ESI ionization in the positive and negative modes operating in a mass range of 100-1000 *m/z*. The following acquisition parameters were used: nebulizer at 4.0 bar, dry heat of 200 °C, capillary voltage of 2.6 kV for ESI$^-$ and 4.5 kV for ESI$^+$, and gas flow rate of 9.0 L min^{-1}. Data were acquired in AUTOMSMS mode, selecting a maximum of 4 precursors per cycle using a total cycle of 3.0 s. A concentration of 10 mM sodium formiate was used for internal calibration. The OTOF Control 3.4 and Data Analysis 4.1 programs were employed to acquire analyzes and processing, respectively.

4.3.4. Encapsulation process

Maltodextrins (MD) of different DE (MD10, MD20 or MD30) were mixed with the *C. hirta* and *C. japurensis* juices in the ratio of 85:15 (juice:MD). Solutions were stirred (Gehaka Ultra

DU-15) at 10,000 rpm for 5 min at 25 °C, maintained at -18 °C for 24 h and subjected to the freeze-dryer equipment (Terroni Enterprise I) for 5 days. Then, the microparticles were manually powdered until reach granulometry less than 100 mesh.

4.3.4. DDPH/ABTS antioxidant assays

The antioxidant activity was determined by the DPPH (Molyneux, 2004) and ABTS (Re et al., 1999) methods with slight modifications. The encapsulated juices were analyzed to evaluate the influence of the wall materials (MD10, MD20 and MD30) on the stability of the bioactive compounds, according to specific methods (Moser et al., 2017).

4.3.5. Color analysis

The juice color analysis was performed by the evaluation of the coordinates L*, a* and b* in a DeltaVista (450G, DeltaColor, Brasil) spectrophotometer. The L*, a* and b* parameters indicate, respectively, the black/white, red/green, and yellow/blue color variations. The CIELab color scale was used to measure the L*, a* and b* parameters (Negrão-Murakami et al., 2017).

The color difference (ΔE*) was obtained according to the Eq. 1. The color parameters variation (Δ) was calculated as the difference between the initial values (0 days) and those obtained during 28 days.

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \qquad \textbf{Eq. 1}$$

4.3.6. Scanning Electron Microscopy (SEM) analysis

The powder morphology analysis of the encapsulated juices was performed in a NA3, TECSCAN-Czech Republic, 20 keV. Powders were placed on carbon tapes coated with a gold thin film. Measurements were performed at 25 °C.

4.3.7. Moisture Content (MC) and Water Activity (WA)

Moisture content (MC) of the encapsulated juices was gravimetrically evaluated in a thermobalance (i-Thermo, Bel-Italy). Water Activity (WA) was measured by an AquaLab Water Activity Meter device (Series 4TE, Decagon, Pullman, USA) at 25 °C.

4.3.8. Solubility evaluation

The encapsulated nonalcoholic drinks were weighed (1 g) and stirred in 25 mL of distilled water for 5 min. The solution was centrifuged at 3300 rpm for 15 min. An aliquot of 10 mL of the supernatant was transferred to a pre-weighed Petri dish and dried at 105 °C until reach constant weight. Solubility ($g.100g^{-1}$ of water) was calculated as the percentage of the dried supernatant to the amount of the powder originally weighted (1g) (Campelo, Sanches, Fernandes, Botrel, & Borges, 2018).

4.3.9. Retention of bioactive compounds

The retention of bioactive compounds [(R%), Eq.2] were measured before and immediately after the freeze-drying process by the DPPH and ABTS methods. BC_{encap} represents the encapsulated bioactive compounds (µM) and $BC_{in\ natura}$ represents the bioactive compounds presented in the *in natura* juices (µM).

$$R(\%) = 100 \times \frac{BC_{encap}}{BC_{in\ natura}} \qquad \textbf{Eq. 02}$$

4.3.10. Stability of the encapsulated bioactive compounds

The encapsulated juices powder (0.1 g) were stored in opened vials under two different relative humidity (22 and 77%) in a temperature-controlled chamber (25 °C) for 28 days. Samples were submitted to the DPPH/ABTS methods and color analysis every 7 days.

4.3.11. Half-life time analysis

The degradation of the bioactive compounds was considered as a first-order kinetics (Moser et al., 2017). The half-life time of the encapsulated juices were determined by DPPH/ABTS methods according to the Eq. 3 and 4.

$$kt = -\ln\left(\frac{C}{C_0}\right) \qquad \textbf{Eq. 3}$$

$$t_{1/2} = \frac{ln2}{k} \qquad \textbf{Eq. 4}$$

where C_0 corresponds to the initial anthocyanins and ascorbic acid concentrations (mg.100 g^{-1} of powder), C is the bioactive compound concentration at the time t (h), and k (h^{-1}) is the first-order reaction rate constant.

4.3.12. Statistical analysis

The analysis of variance (ANOVA) was performed using the R software (version 3.5.1) to evaluate the effects of different wall materials on the properties of the encapsulated non-alcoholic drinks. Differences between the mean values obtained for each treatment were evaluated at 5% of significance level ($p \leq 0.05$) using the Tukey´s test.

4.4. Results and discussions

4.4.1. Chemical identification of the bioactive compounds

Twelve compounds were identified in the *C. japurensis* and *C. hirta* juices, including organic acids, sugars, flavanol, flavan-3-ols and anthocyanins derivatives (**Table 1)**. Chemical compounds of *C. japurensis* and *C. hirta* juices have not been reported. However, gallic acid [**4**], flavan-3-ols epigallocatequin [**5**], epigallocatequin gallate [**7**] and flavonol quercetin-3-*O*-rhamnoside [**9**] were previously identified in berries of *C. rubra* (Gordon, Schadow, Quijano, & Marx, 2011). The presence of gallic acid and quercetin-3-*O*-rhamnoside in *C. hirta* is related to its remarkable antioxidant activity. Some of the ions shown in **Table 4**, such as tannin derivatives, have already been reported, besides its antioxidant activity (Abdellaoui et al., 2014).

The compounds **6** and **8** presented characteristic fragmentation pattern of hydrolysable tannins with hexahydroxyldiphenoyl (HHDP) units, which is common in the Melastomataceae family (Serna & Martínez, 2015). The molecular deprotonated ion $[M]^-$ at 783.0618 and fragments at *m/z* 481.0653 and *m/z* 300.9977 indicated the structure of bis-(HHDP)-glucose. The fragmentation pattern of the O $[M]^-$ ion at 935.0720, *m/z* 633.0725 and *m/z* 300.9971 was assigned as galloyl-*bis*-HHDP-glucose.

Table 4: Chemical compounds of *C. japurensis* and *C. hirta* juices.

	T_r (min)	Compounds	*C. japurensis*		*C. hirta*	
			$[M-H]^-$ *m/z*	**MS/MS *m/z***	**$[M-H]^-$ *m/z***	**MS/MS *m/z***
1	1.3	hexosyl hexose	341	**225**, 161, 101	**-**	**-**
2	1.4	quinic acid	-	-	191	128, **111**
3	1.5	citric acid	191	114, **111**	191	114, **111**
4	1.6	gallic acid	-	-	169	**128**, 125, 108
5	6.4	epigallocatequin	305	215, **125**	305	215, **125**
6	7.9	bis-(hexahydroxydiphenoyl)-glucose	-	-	783	481, **301**
7	9.7	epigallocatequin gallate	457	305, 269, **169**, 125	457	305, 269, **169**, 125
8	10.3	galloyl bis-(hexahydroxydiphenoyl)-glucose	935	633, **301**	935	633, **301**
9	10.8	quercetin-3-*O*-rhamnoside		-	447	**306**, 300
			$[M]+$ m/z	**MS/MS m/z**	**$[M]+$ m/z**	**MS/MS m/z**
10	10.1	Delphinidin-3-*O*-rutinoside-5-glucoside	773	611, 465, **303**	773	611, 465, **303**
11	10.7	Delphinidin-3-*O*-rutinoside-5-pentoside	-	-	743	611, 370, **303**
12	10.8	cyanidin-3-*O*-rutinoside-5-glucoside	-	-	757	714, 629, **287**

The compound **10,** with molecular cation at *m/z* 773.1917 $[M+H]^+$ and ions at *m/z* 611.1395, *m/z* 465.1094 and *m/z* 303.0493, showed loss of *m/z* 161 due to the hexose split off at C5, followed by loss of *m/z* 146 by rhamnose (*m/z* 465.0953) and loss of *m/z* 161 by hexose, respectively. The positive ion product was at *m/z* 303.0493, indicating an aglycone fragment of delphinidin, which was identified as delphinidin-3-*O*-rutinoside-5-glucoside. The substance **11** with *m/z* 743.1768 $[M+H]^+$ showed a fragmentation pattern similar to the substance **10**, showing loss of *m/z* 132, indicating the cleavage of a pentoside (*m/z* 611.1395), as well as a radical aglycone fragment in *m/z* 303.0493 which is assigned to delphinidin-3-*O*-rutinoside-5-pentoside. The compound **12** with *m/z* 757.1981 $[M+H]^+$ showed *m/z* 303.0493, indicating an aglycone fragment of cyanidin. By comparing the fragmentation pattern, it can be assigned as cyanidin-3-*O*-rutinoside-5-glucoside.

The mono substituted delphinidin-3-*O*-rutinoside and cyanidin-3-*O*-rutinoside were previously identified in berries of *C. rubra* (Gordon et al., 2011). The anthocyanins and anthocyanidins isolated from Melastomataceae family are delphinidin, cyanidin, pelargonidin, peonidin and malvidin glycosides, whereas the fruits have shown mainly the presence of delphinidin and pelargonidin glycosides (Serna & Martínez, 2015).

4.4.2. SEM analysis

Figure 10 shows the SEM images of the encapsulated non-alcoholic drinks. The irregular and cracked surface morphology may be resulted from the powder grinding process. However, this behavior is also typical of microparticles produced by the freeze-drying process (Caparino et al., 2012). Moreover, the sample MD30 presented microparticles with smoother surfaces, which can be related to the smaller molecular chain size of dextrose. On the other hand, the sample MD30 looked like smooth spheres, presenting less resistance to the applied vacuum during experiment (Ersus & Yurdagel, 2007).

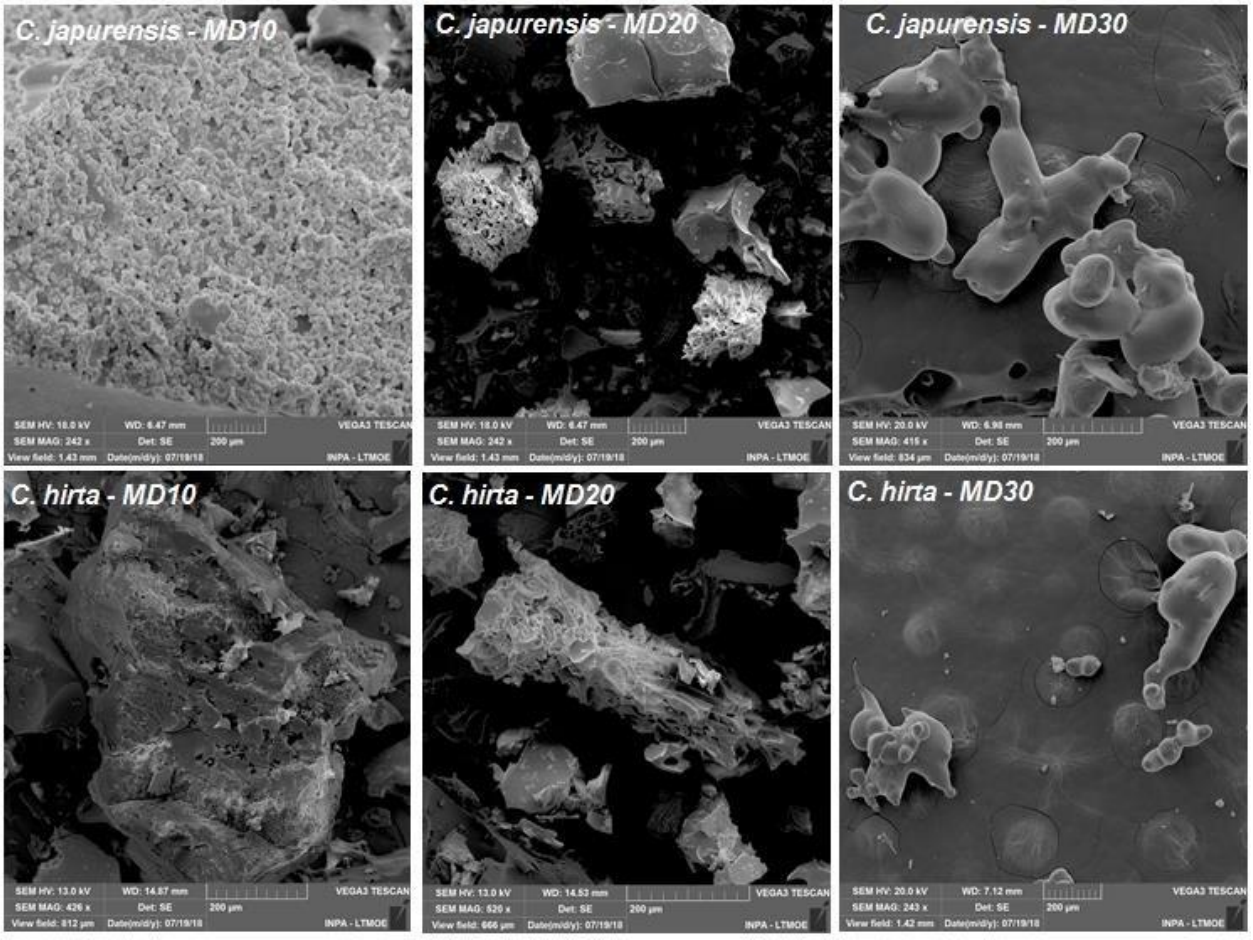

Figure 10: SEM images of the encapsulated juices.

4.4.3. Moisture Content (MC) and Water Activity (WA)

Table 5 shows the MC values of the encapsulated juices. The increase of the DE values reduced significantly the MC of the microparticles ($p < 0.05$). For black currant polyphenol (Bakowska-Barczak & Kolodziejczyk, 2011) and fish oil encapsulates (Ghani et al., 2017), a decrease of MC values with the increase of DE was also observed. High MC values observed in

microparticles of lower DE can be explained by the larger surface vacuoles, which increases the water adsorption due to the surface area (Ghani et al., 2017).

Table 5: Moisture Content (MC), Water Activity (WA) and water solubility values of the encapsulated juices.

Parameter	*C. japurensis*			*C. hirta*		
	M10	**M20**	**M30**	**M10**	**M20**	**M30**
Moisture Content (MC) (g water.100g^{-1} of powder)	5.50 ± 0.1^{a}	3.51 ± 0.2^{b}	1.07 ± 0.1^{c}	6.03 ± 0.5^{a}	4.32 ± 0.5^{b}	2.27 ± 0.2^{c}
Water Activity (WA)	0.31 ± 0.2^{b}	0.51 ± 0.1^{a}	0.55 ± 0.2^{a}	0.39 ± 0.3^{b}	0.51 ± 0.4^{a}	0.53 ± 0.3^{a}
Water Solubility	76 ± 3^{c}	88.1 ± 0.4^{b}	94 ± 1^{a}	75 ± 3^{c}	86.4 ± 0.4^{b}	93 ± 1^{a}

^{a,b,c} Same letter represents no significant difference ($p < 0.05$).
Results are presented as the mean ± standard deviation.
MD10, MD20 and MD30 = maltodextrins of different dextrose equivalent (DE).

The AW is an important parameter related to the microbiological quality of foods. The low hygroscopicity of the maltodextrins allows the reduction of the surface water. For this reason, the AW accounts for the "free" water present in the wall material. Low AW is found in dry atmospheres where the water is strongly attracted by the polar sites of the wall materials, so they are not available for chemical reactions (Pitalua, Jimenez, Vernon-Carter, & Beristain, 2010). Since fungi grow in AW > 0.62 and bacteria in AW > 0.86 (Silva et al., 2018), none of the treatments presented in this paper favor the microbial growth. The increase of DE values resulted in a significant increase of the AW (p-value < 0.05), since larger chains can be able to bind more water molecules.

Powdered foods with high solubility in water present great technological potential to be used in the food industry. All treatments presented solubility above 70 g.100g^{-1} of water, with a significant effect ($p < 0.05$) for those samples with maltodextrins of higher DE. The more hydrolyzed the maltodextrin polymer chain, the more water binding regions will be exposed, leading to increased solubility (Kearsley & Dziedzic, 1995). Studies reported encapsulates containing essential oil of *Citrus aurantifolia* (Campelo et al., 2018), concentrated extract of *Ilex paraguariesis* (Negrão-Murakami et al., 2017) and black mulberry juice (Fazaeli, Emam-Djomeh, Kalbasi Ashtari, & Omid, 2012) using different maltodextrins. The authors also observed better solubility of the microparticles in water when DE was increased.

4.4.4. Retention of bioactive compounds

The encapsulated juices presented good antioxidant activities, with DPPH value of 943 ± 15 µM TE (*C. japurensis*) and 994 ± 14 µM TE (*C. hirta*). The ABTS values were found around 1119 ± 24 µM TE (*C. japurensis*) and 1273 ± 18 µM TE (*C. hirta*). Among the hydrobenzoic acids, gallic acid is the most effective for the inactivation of the radicals ABTS and DPPH (Soares et al., 2008). The effects of the quercetin-3-*O*rhamnoside have been associated with antioxidant-rich plant extracts (Huang et al., 2010). Quercetin glucosides are widely distributed in plants and have attracted attention because of their multiple chemical and biological effects, such as antioxidation (Yamazaki, Inagaki, Kurita, & Inoue, 2007).

The retention of bioactive compounds within microparticles is an important parameter because it is related to the encapsulation process efficiency. **Figure 11** and **12** shows the retention of the bioactive compounds of the encapsulated juices based on the DPPH and ABTS methods, respectively. In general, the freeze-drying process presents high retention of bioactive compounds. The retention of the *C. hirta* and *C. japurensis* juices within the microparticles ranged from 97.04 to 99.79% (DPPH) and from 87.80 to 99.11% (ABTS). This observed retention efficiency may be related to the low temperatures of the drying process, which can reduce the degradation of the bioactive compounds (Silva et al., 2018). High retention of bioactive compounds was also observed when black carrot (Murali et al., 2015) and tucumã juices were encapsulated by freeze-drying process using maltodextrin as wall materials (Silva et al., 2018).

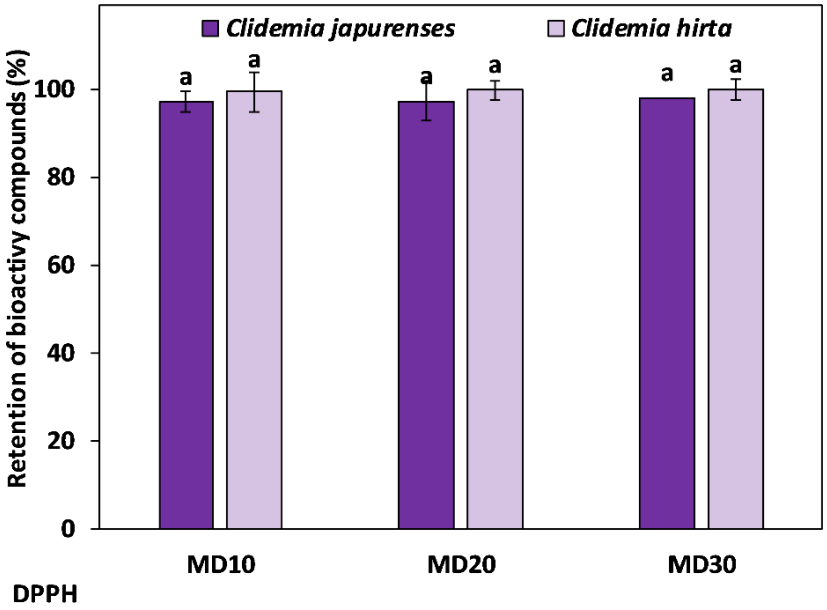

Figure 11: Retention of the bioactive compounds (%) of the encapsulated non-alcoholic drinks based on the DPPH method.

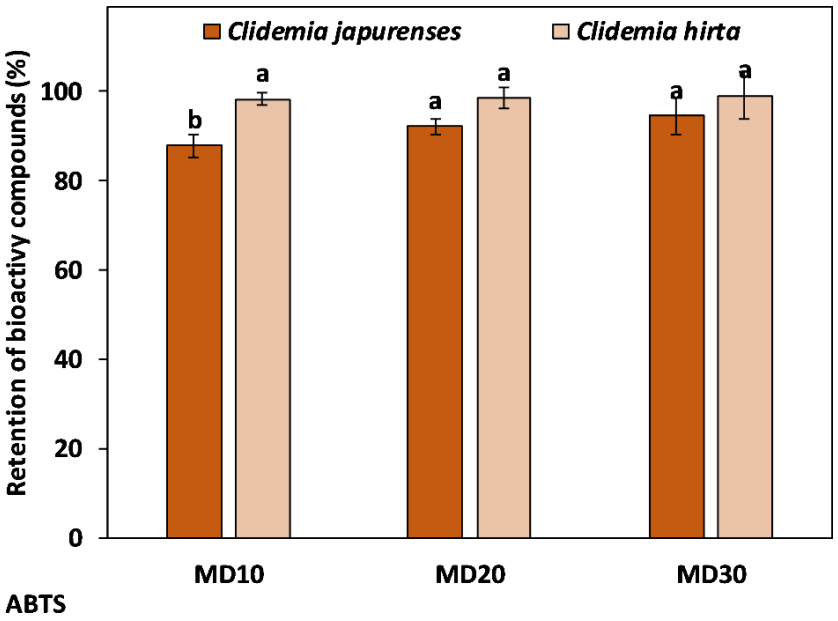

Figure 12: Retention of the bioactive compounds (%) of the encapsulated non-alcoholic drinks based on the ABTS method.

For ABTS analysis, the different treatments presented significant difference (*p*-value<0.05). The MD10 sample revealed lower retention of the bioactive compounds, which can be explained by the water sublimation along the drying process. In this process, the bioactive compounds may be linked to the water molecules through the pores formed by the water crystals (Rezende, Nogueira, & Narain, 2018).

4.4.5. Storage stability of the encapsulated non-alcoholic beverages

Food stability in the storage process is an important tool to determine the time in which the concentration of the bioactive compounds is guaranteed (Moser et al., 2017). The protective effect of maltodextrin of different DE over the *C. hirta* and *C. japurensis* juices is shown in **Figure 13** (DPPH) and **Figure 14** (ABTS).

The encapsulated juices stored at higher humidity presented higher degradation rate of the bioactive compounds. In addition, high water contents observed after harvest may favor the development of microorganisms in the plant matrix, compromising the activity of their bioactive compounds (Barbosa, Demuner, Clemente, Paula, & Ismail, 2007). Powdered food stored at high relative humidity tends to degrade further due to the increase of mobility of the bioactive compounds (Kuck, Wesolowski, Pelayo, & Noreña, 2017). Different relative humidity conditions were evaluated during the storage of the encapsulated bioactive compounds from

Opuntia ficus-indica (Carriazo, Iturriaga, Otálora, Nazareno, & Osorio, 2016). The authors observed that the degradation of the encapsulated bioactive compounds increased with the relative humidity. Also, the treatments with maltodextrin of higher DE presented higher degradation rate of the bioactive compounds. Since more hydrolyzed maltodextrins present higher hydroxyl groups, the mobility of biopolymers, loss of their molecular structure and exposure of the encapsulated bioactive compounds tend to increase.

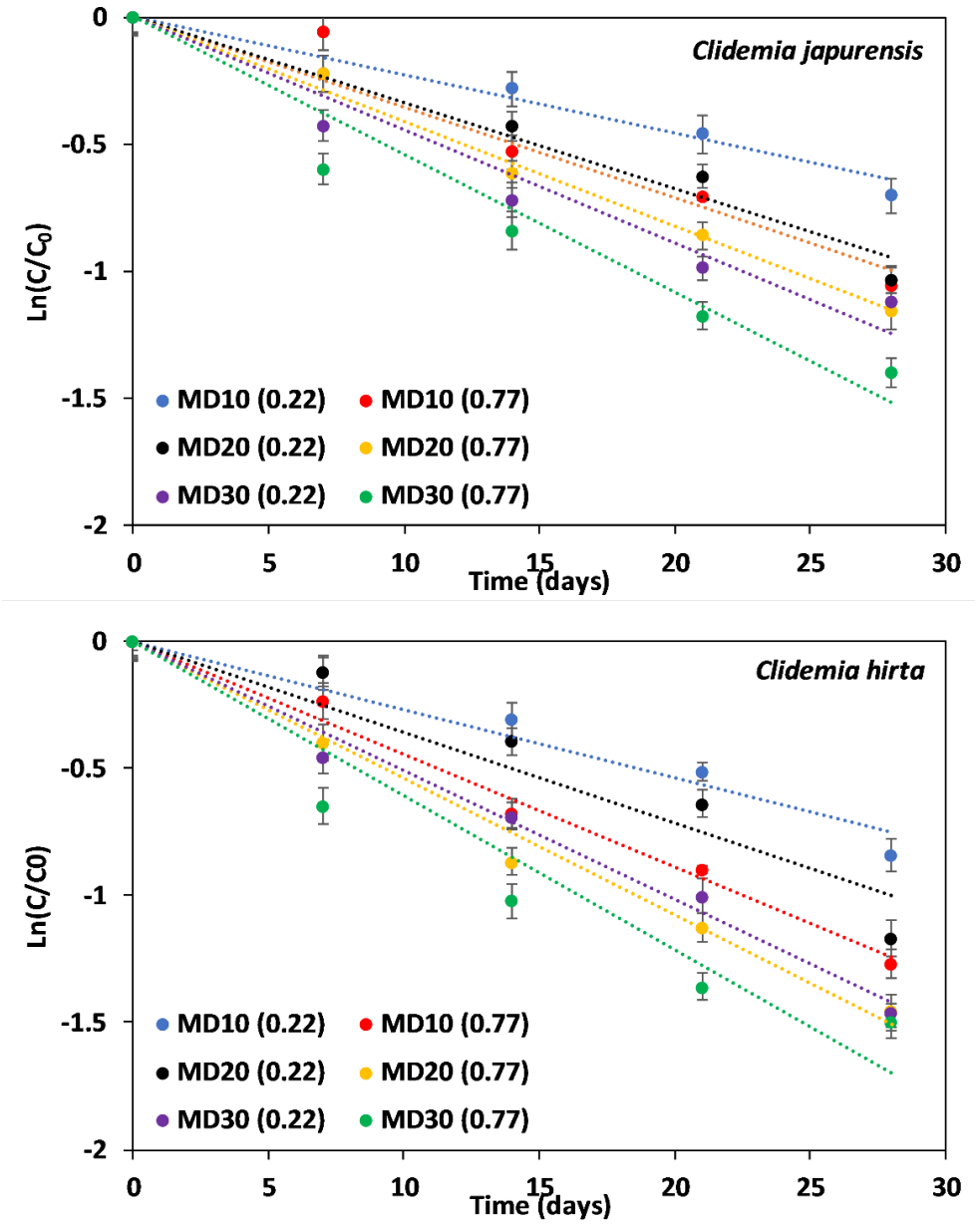

Figure 13: Stability of the antioxidant compounds (DPPH method) of the encapsulated non-alcoholic beverages stored at 25 °C under different relative humidity [CH3COOK (0.22) and NaCl (0.77)].

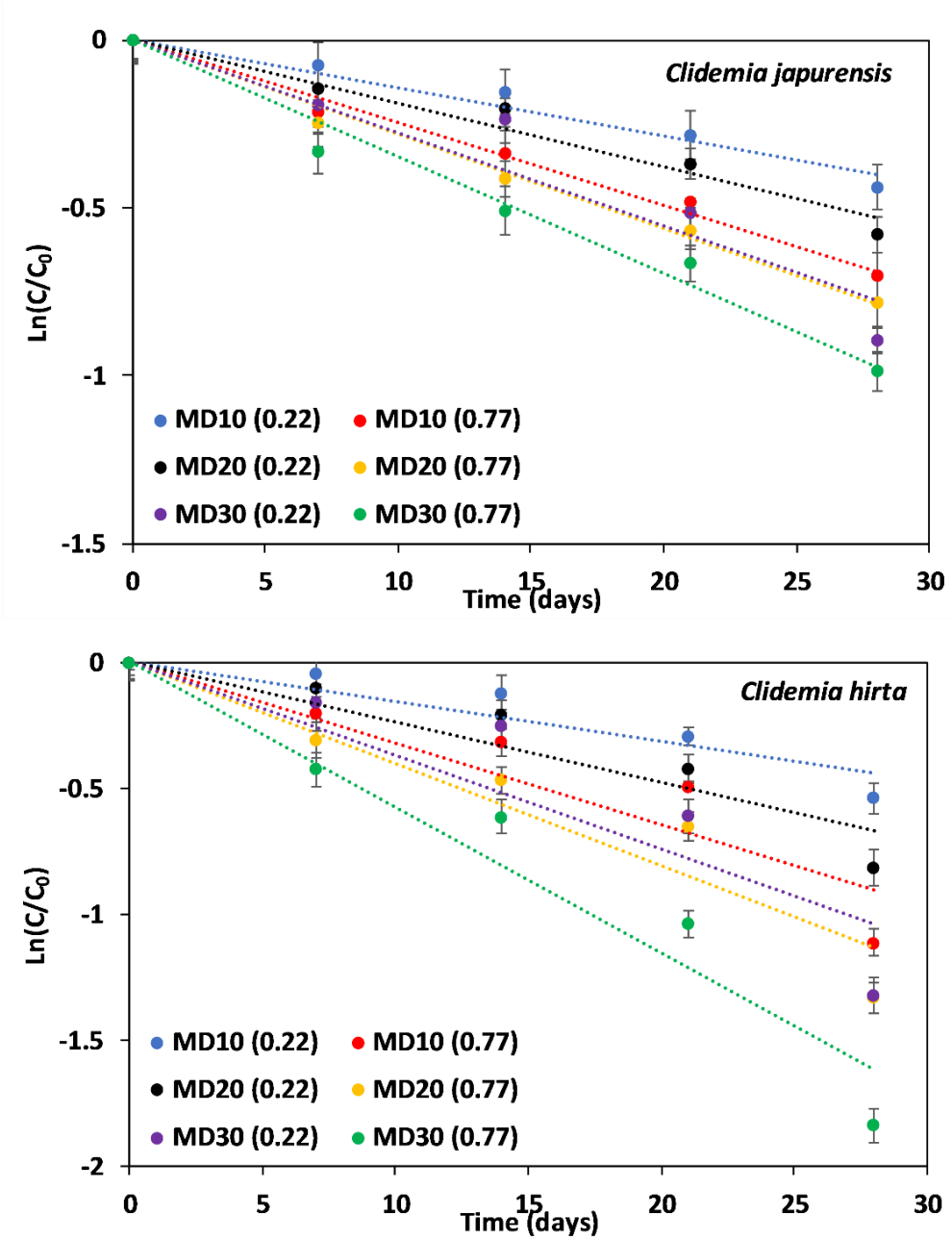

Figure 14: Stability of the antioxidant compounds (ABTS method) of the encapsulated non-alcoholic beverages stored at 25 °C under different relative humidity [CH3COOK (0.22) and NaCl (0.77)].

Therefore, larger amounts of water adsorbed on the microparticle surfaces favors the molecules mobility and increases the degradation of the bioactive compounds (Tonon, Brabet, & Hubinger, 2010). For the encapsulated black currant polyphenols (Bakowska-Barczak & Kolodziejczyk, 2011) and concentrated mate tea (Negrão-Murakami et al., 2017), greater stability of the bioactive compounds encapsulated in maltodextrin of low DE was observed. Microparticles based on maltodextrin presented good stability of phenolic compounds of *Ilex*

paraguariensis (Nunes et al., 2015). Similar behavior was also observed in *Opuntia ficus-indica* (Maria et al., 2010) and *Ribes nigrum L.* (Bakowska-Barczak & Kolodziejczyk, 2011) encapsulates.

Table 6 shows the color variation of the encapsulated juices stored at 25 °C in different RH (0.22 and 0.77). By the values of L* (positive), a* (negative) and b* (positive and negative), the samples presented color tending to light purple. There was a significant difference ($p < 0.05$) related to the wall material and storage humidity. The samples MD30 presented greater color variation during the storage. More hydrolyzed maltodextrins (higher DE) are more hygroscopic due to their number of functional groups which are able to bind with water molecules (da Silva Carvalho et al., 2016). Greater amount of water bounded to the polymeric structure of the microparticles can contribute to the degradation of active compounds in encapsulated systems, modifying their physicochemical characteristics, such as their color. This observation may also be related to the greater variation of color of the microparticles stored in higher RH.

Table 6: Color variation of the encapsulated juices stored at 25 °C in different relative humidity (RH). shows the kinetic degradation parameters of the encapsulated juices.

Time (days)	RH = 0.22				RH = 0.77			
	C. japurensis	**MD10**	**MD20**	**MD30**	***C. japurensis***	**MD10**	**MD20**	**MD30**
0	0	0	0	0	0	0	0	0
7	0.73 ± 0.01^{b}	0.66 ± 0.03^{c}	0.33 ± 0.03^{c}	0.35 ± 0.02^{d}	0.32 ± 0.02^{b}	2.10 ± 0.05^{d}	2.97 ± 0.02^{c}	2.53 ± 0.02^{c}
14	0.90 ± 0.02^{b}	0.91 ± 0.02^{b}	0.67 ± 0.03^{c}	0.70 ± 0.04^{c}	0.59 ± 0.02^{b}	3.88 ± 0.04^{c}	5.12 ± 0.02^{b}	4.35 ± 0.01^{b}
21	1.17 ± 0.01^{a}	1.19 ± 0.04^{b}	1.12 ± 0.02^{b}	1.17 ± 0.05^{b}	1.00 ± 0.02^{a}	5.01 ± 0.04^{b}	6.75 ± 0.04^{b}	6.18 ± 0.02^{b}
28	1.25 ± 0.02^{a}	1.60 ± 0.04^{a}	1.40 ± 0.02^{a}	1.46 ± 0.01^{a}	1.12 ± 0.06^{a}	7.74 ± 0.01^{a}	7.80 ± 0.05^{a}	7.93 ± 0.01^{a}
Time (days)	**RH = 0.22**				**RH = 0.77**			
	C. hirta	**MD10**	**MD20**	**MD30**	***C. hirta***	**MD10**	**MD20**	**MD30**
0	0	0	0	0	0	0	0	0
7	0.22 ± 0.01^{c}	0.41 ± 0.02^{d}	0.34 ± 0.02^{c}	0.35 ± 0.02^{d}	0.16 ± 0.01^{d}	2.17 ± 0.05^{c}	2.44 ± 0.02^{c}	4.18 ± 0.01^{d}
14	0.42 ± 0.01^{bc}	1.08 ± 0.02^{c}	0.68 ± 0.01^{b}	0.71 ± 0.03^{c}	0.34 ± 0.06^{c}	3.56 ± 0.05^{b}	4.76 ± 0.02^{c}	8.3 ± 0.2^{c}
21	0.72 ± 0.03^{b}	1.5 ± 0.1^{b}	1.14 ± 0.01^{ab}	1.19 ± 0.05^{b}	0.55 ± 0.04^{b}	4.52 ± 0.01^{b}	6.99 ± 0.02^{b}	10.29 ± 0.02^{b}
28	1.00 ± 0.06^{a}	2.11 ± 0.02^{a}	1.43 ± 0.01^{a}	1.49 ± 0.06^{a}	0.61 ± 0.04^{a}	5.46 ± 0.02^{a}	9.45 ± 0.03^{a}	12.3 ± 0.1^{a}

The increase of both DE and storage humidity influenced the degradation rate. More hydrolyzed maltodextrins tend to present more water content on their surfaces. For this reason, high water concentrations in powdered foods accelerates the degradation processes (Kuck et al., 2017). According to the ABTS results, the bioactive compounds of *C. hirta* and *C.*

japurensis encapsulated in MD10 wall material and stored at RH = 22% presented half-life time around 45 and 37 days, respectively.

This half-life time may be related to the sugar concentration in the juices, resulting in higher moisture absorption. Indeed, the microparticles appeared to have considerable moisture and fluidity (Villacrez, Carriazo, & Osorio, 2014). Beetroot bioactive compounds encapsulated in maltodextrin and stored at 60 °C and RH = 30% (Carmo et al., 2018) presented half-life time higher than 15 weeks. A half-life time of 373 days was also observed for blackberry encapsulated in maltodextrin and stored at 25 °C (Ferrari, Marconi Germer, Alvim & de Aguirre, 2013).

Table 7: Kinetic parameters of encapsulated non-alcoholic beverages stored under different conditions.

Assay	Species	kinetic parameters	MD10		MD20		MD30	
			RH=0.22	RH=0.77	RH=0.22	RH=0.77	RH=0.22	RH=0.77
DPPH	*C. japurensis*	k (10^3 h^{-1})	25.75	39.51	35.31	42.12	40.11	48.19
		t1/2 (day)	26.90	17.54	19.62	16.45	17.27	14.38
		R^2	0.95	0.93	0.92	0.94	0.91	0.91
	C. hirta	k (10^3 h^{-1})	29.56	45.69	40.73	51.87	49.51	52.86
		t1/2 (day)	23.44	15.16	17.01	13.36	13.99	12.44
		R^2	0.95	0.92	0.94	0.94	0.91	0.90
ABTS	*C. japurensis*	k (10^3 h^{-1})	15.51	23.97	19.72	26.98	30.16	32.94
		t1/2 (day)	44.66	28.92	35.13	25.68	22.98	21.03
		R^2	0.92	0.93	0.95	0.98	0.91	0.89
	C. hirta	k (10^3 h^{-1})	18.93	35.86	27.73	42.91	44.13	61.23
		t1/2 (day)	36.61	19.32	24.99	16.15	15.70	11.32
		R^2	0.92	0.95	0.96	0.98	0.91	0.89

The encapsulated juices of *C. hirta* and *C. japurensis* juices would be in exposed to air moisture only at the moment of the juice preparation. Taking into account that this preparation occurs in few minutes, the found half-life time for both juices is more than sufficient for the encapsulated juices be used for this purpose.

4.5. References

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5. CONCLUSIONS

The powders produced by the FD process presented higher MC and AW values. The drying methods were more significant for hygroscopicity, with higher water adsorption observed for those powders produced by the SD process, reducing the stability of the compounds during the storage. The retention values of bioactive compounds underwent little variation with respect to the different carrier, since their compositions are very close. For the different drying process, the powders recovered by the FD method presented higher retention values of bioactive compounds. In general, the powders constituted of gum Arabic and recovered by the FD process presented better protection and retention of the bioactive compounds from the HAE.

C. hirta and *C. japurensis* juices presented important bioactive compounds such as organic acids, flavonoids and anthocyanins. These compounds may be of great interest to the food industry and be used primarily as an ingredient for a high human health benefit. The encapsulation technique using the freeze-drying method presented efficient protection of these bioactive compounds. The powdered juices prepared with maltodextrin of lower DE as wall materials presented better retention and stability. This fact may be related to the low water adsorption, which can be confirmed by the results of WC and AW. The powdered juices prepared using maltodextrin as wall materials presented satisfactory thermal stability and storage: the sample MD10 achieved higher stability and antioxidant potential. In addition, these materials may offer interesting possibilities for innovation in the food industry by application in food matrices to create synergy between the functional properties of different products.

For *H. acetosella* tea, the powders produced by the lyophilization process presented higher moisture content and water activity values. Drying methods were more significant for hygroscopicity, with higher water adsorption observed for the powders produced by the spray drying process, reducing the stability of the compounds during storage. The retention values of bioactive compounds have little variation in relation to the different wall materials, since their compositions are very close. For the different drying processes, the powders recovered by the lyophilization method presented higher retention values of bioactive compounds. In general, the powders composed of gum arabic and recovered by the lyophilization process presented better protection and retention of bioactive compounds of *H. acetosella* tea.