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Microencapsulation of guava pulp using prebiotic wall material

Microencapsulamento de polpa de goiaba com material de parede prebiótico

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Abstract

Important functional compounds present in fruits are often lost in technological processes and during storage. Microencapsulation technique allows maintaining the compounds of interest and adding value to the product using functional encapsulating materials. This work aimed to produce microencapsulated guava pulp using the spray-drying technique and a functional encapsulant material, i.e., a mix of inulin and maltodextrin. The guava pulp was analyzed for centesimal composition, carotenoid content, and antioxidant activity. The microspheres were analyzed for retention of carotenoids, antioxidant activity over time, and morphology by Scanning Electron Microscopy (SEM) and X-ray diffraction. Two proportions of coating material could maintain the antioxidant activity of guava pulp. The microencapsulation with a higher percentage of inulin is a preferred option due to the good results of retention and stability regarding antioxidant activity over time, relevant retention of the carotenoid content, and a more stable microstructure. In addition, inulin can add value to powders owing to its inherent functional properties. The product obtained in the study is innovative and interesting, as well as may provide a capable use of these materials as encapsulated agents. In fact, it can be considered a potential functional ingredient.

Keywords: Spray-drying; Food ingredients; Antioxidants; Carotenoid; Inulin; Shelf-life.

Resumo

Importantes compostos funcionais presentes nas frutas são frequentemente perdidos nos processos tecnológicos e durante o armazenamento. Com a técnica de microencapsulamento, é possível manter os compostos de interesse e agregar valor ao produto usando materiais de encapsulamento funcionais. Este trabalho tem como objetivo produzir polpa de goiaba microencapsulada através da técnica de secagem por pulverização, utilizando material encapsulante funcional, uma mistura de inulina e maltodextrina. A polpa de goiaba foi analisada quanto à composição centesimal, ao teor de carotenoides e à atividade antioxidante. As microcápsulas foram analisadas quanto a retenção de carotenoides, atividade antioxidante ao longo do tempo e sua morfologia por microscopia eletrônica de varredura e difração de raios-X. Ambas as proporções utilizadas no material de revestimento foram capazes de manter a atividade antioxidante da polpa de goiaba. O microencapsulamento com maior porcentagem

de inulina mostrou-se como a melhor opção, devido aos bons resultados de retenção e estabilidade da atividade antioxidante ao longo do tempo, maior retenção do conteúdo de carotenoides e microestrutura mais estável, além de a inulina poder agregar valor aos pós devido às suas propriedades funcionais inerentes. O produto obtido no estudo representa um uso inovador, interessante e de possível utilização dos materiais como agentes encapsulantes, além de poder ser considerado como um potencial ingrediente funcional.

Palavras-chave: *Spray-drying*; Ingredientes alimentícios; Antioxidantes; Carotenoides; Inulina; Vida de prateleira.

1 Introduction

The consumption of fruits has been growing in the global market due to the recognition of their nutraceutical, functional, and nutritional values (Rufino et al., 2010). Several epidemiological studies have associated consumption of fruits rich in antioxidant compounds with a lower risk of cardiovascular diseases, cancer, and other degenerative diseases. This characteristic contributes to a greater scientific interest on the composition and antioxidant capacity of foods (Genkinger et al., 2004; Wang et al., 2011).

The guava (*Psidium guajava L.*) is a native fruit of tropical America. It is produced throughout the year and consumed mainly as a fresh fruit or as juice (Dembitsky et al., 2011). It presents highly antioxidant compounds such as vitamin C, carotenoids, and phenolic compounds, highlighting the high content of lycopene in its pulp (Alothman et al., 2009; Mercadante et al., 1999; Rodriguez-Amaya et al., 2008; Thaipong et al., 2006). Indeed, guava also has a significant dietary fiber content that can lead to a positive effect on human health by reducing the glucose levels in blood, lowering blood pressure, triglycerides and cholesterol, and acting against rheumatism (Gutiérrez et al., 2008).

Thus, guava is a fruit that suffers high post-harvest losses. The high water content in guava makes it highly perishable, requiring the use of preservation processes such as freezing. The exportation *in natura* of guava to some countries is restrict owing to attacks of tropical fruit flies (Osorio et al., 2011; Wang & Langrish, 2009). Another aggravating factor to post-harvest losses is the incidence of post-harvest damages. Indeed, 63% of fruits traded in warehouse suffered post-harvest mechanical injuries, and 5.5% of fruits showed symptoms of diseases related with the incidence of mechanical injuries (Martins et al., 2007).

To overcome these obstacles, products are needed in which the transferring of molecules responsible for sensory characteristics and functional properties of tropical fruits to a solid phase is capable of improving its stability and its release in food matrices (Osorio et al., 2011).

Through microencapsulation of food it is possible to coat small droplets, solid particles, or gas compounds in a nutritional wall material, turning them into microspheres with a few millimeters or microns of diameter and with different properties (Gharsallaoui et al., 2007).

Spray-drying is one of the most used techniques for microencapsulation, seeing that it is a simple and inexpensive technology and the equipment is widely available in the global market (Gharsallaoui et al., 2007). Through this technique currently used for this purpose, it is possible to generate good quality powders with low water activity that facilitates transport and storage. However, their main limitations are associated with the wall materials available for use (Gharsallaoui et al., 2007; Gouin, 2004).

Many biopolymers such as Arabic gum, alginates, carrageenan, milk proteins, and maltodextrins with different dextrose-equivalent properties have been used as wall materials (Kanakdande et al., 2007; Shaikh et al., 2006; Teixeira et al., 2004). Maltodextrin is the most widely used Encapsulation Agent (EA) of foods. It has a low viscosity, a high solid content, and a good water solubility, in addition to low cost. However, it does not have the properties required for an efficient interfacial microencapsulation. Therefore, it is usually combined with other EAs (Gharsallaoui et al., 2007).

Inulin can be used as a potential EA in combination with maltodextrin (Rivas et al., 2020). Inulin is a naturally occurring carbohydrate reserve in thousands of plants. It is composed of units of β-D-fructofuranosyl

linked by β 2-1 bonds attached to a terminal sucrose unit. Indeed, inulin is obtained mainly from the roots of chicory (*Cichorium intybus L.*) and Jerusalem artichoke (*Helianthus tuberosus* L.) (Bemiller & Huber, 2010). It has a prebiotic function in the organism by promoting selective growth and activity of a limited number of endogenous bacteria in the colon, thus inducing beneficial luminal and systemic effects in the host (Gibson & Fuller, 2000; Ooi & Liong, 2010). In addition, it can contribute to the bioavailability of calcium (Lavanda et al., 2011), reduce blood cholesterol (Letexier et al., 2003; Trautwein et al., 1998), and assist in the modulation of the immune system (Seifert & Watzl, 2007).

This study aimed to investigate different ratios of inulin and maltodextrin as an encapsulating agent for microencapsulation of guava pulp using spray-drying and evaluate the antioxidant stability of the powders during storage in 60 days.

2 Materials and methods

2.1 Characterization of guava pulp

Guava fruits were acquired at a local market in Rio de Janeiro. The fruits were washed, selected, and pulped by using a pulping machine (Bonina 0.25dF). To standardize total solids content and facilitate the drying stage, the pulp was centrifuged in a centrifuge (Liqstar RI1784, Walita).

Analyses of moisture, ash, protein, fat, dietary fiber (Association of Official Analytical Chemists, 2010), soluble solids (Brix), pH, and acidity were performed (Association of Official Analytical Chemists, 2000). Carbohydrate was calculated as the difference between 100 and the sum of the proteins, fats, dietary fiber, moisture, and ash contents.

2.2 Preparation of guava microspheres

The encapsulation was carried out according to Rivas et al. (2019) with some modifications. Inulin (Raftiline® ST, BENEO-Orafti, Clariant, Brazil) and maltodextrin (Avebe MD 14P, Avebe, Brazil) were used as EAs, whereas inulin and maltodextrin as encapsulating materials at a 2:1 ratio (w/w)

To evaluate the potential use of inulin associated with maltodextrin as an EA, two formulations were evaluated as following: Formulation 1 (F10), encapsulating materials at a 1:1 ratio (w/w), was prepared with 6% of inulin and 6% of maltodextrin in relation to the pulp volume; and Formulation 2 (F2),), encapsulating materials at a 2:1 ratio (w/w), was prepared with 8% of inulin and 4% of maltodextrin in relation of pulp volume. The atomization process was carried out in a mini spray dryer (Buchi B-190; Flawil, Switzerland) with a 0.3-mm spray nozzle. The air inlet and outlet temperatures were 190 ± 2 °C and 94 ± 2 °C, respectively, for both formulations. The atomization pressure used was 7 bar, with an average drying air flow rate of 700 L/h and a mean flow rate of 23 mL/min.

2.3 Storage

The microencapsulated pulp was stored at room temperature. To evaluate stability in the presence of light, the microencapsulated pulp was stored in transparent Tradpouch 60 M polyethylene packaging (Iperó, Brazil) and to evaluate stability in the in the absence of light, it was stored in laminated Tradpouch 60 M polyethylene packaging (Iperó, Brazil). The analyses of antioxidant activity, carotenoid content were evaluated at times 0, 30, 45, and 60 days.

2.4 Antioxidant activity

The antioxidant activity of the pulp and powders were determined by spectrophotometry (Rufino et al., 2007). The quantification was performed based on the discoloration of the free radical ABTS (2,2'-azino-

bis(3-ethylbenzothiazoline-6-sulfonic acid)) salt with 98% purity (Sigma-Aldrich® Brazil, Sao Paulo) (Re et al., 1999). For the determination of antioxidant capacity, 200 µL of sample extract was reacted with 1.8 mL of the $ABTS^{+}$ ethanolic solution, and the decrease in the absorbance at 734 nm was measured after 15 min of reaction. A calibration curve, using Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) with 97% purity (Sigma -Aldrich®) as a standard antioxidant, was constructed for the quantification. The results were expressed as the Trolox-equivalent antioxidant capacity. The results were expressed as Trolox-equivalent antioxidant capacity (TEAC) in µmol/g of sample.

2.5 Carotenoids

The carotenoids from pulp and powders were extracted (Rodriguez-Amaya, 2001) by macerating the sample with Celite® and acetone until total discoloration. The carotenoid solution obtained was transferred to a separatory funnel containing petroleum ether, and then the solution was washed in distilled water until all acetone solution was completely removed. The carotenoid solution was funnel-filtered over anhydrous sodium sulfate and collected in an amber volumetric flask containing petroleum ether. The total carotenoids in the sample extracts were determined by spectrophotometry at 450 nm. The analyses were performed in triplicate.

The quantification and identification of lycopene and β-carotene were determined in an acetone extract by High Performance Liquid Chromatography (HPLC) (Pacheco et al., 2012). Chromatography was performed on a Waters® Alliance 2695 (Milford, USA) system equipped with a Waters® 2996 photodiode array detector. Carotenoid separation was obtained in a C30 column (S-3 Carotenoid, 4.6 mm × 250 mm, YCM) by a gradient elution of methanol and methyl tert-butyl ether. The flow rate was 0.8 mL min^{-1} and the running time was 28 min. Column temperature was 33 °C and the injection volume of the samples was 15 µL. Carotenoids were identified based on their retention times and Ultraviolet-Visible (UV/Vis) absorption spectra, compared to the retention times of the carotenoid standards. Carotenoid's profile was determined in an acetone extract by HPLC.

2.6 Carotenoid retention

Retention (%) was determined on a dry basis according to Equation 1.

$$\textit{Carotenoid Retention}(\%) = \frac{Ce}{Cp/(TSp + WMm)} x\,100 \quad (1)$$

where *Ce* was the amount of carotenoid encapsulated in µg/100 g, *Cp* was the content of pulp carotenoid in µg/100 g, *TSp* was the total solids pulp (g), and *WMm* was the mass (g) of wall material used to encapsulated the pulp.

2.7 Scanning Electronic Microscopy (SEM)

The morphology of the microspheres obtained were studied by Scanning Electron Microscopy (SEM). First, the samples were prepared and placed directly on a carbon double-faced adhesive tape in a metal cylinder of 10.0 mm in diameter and 10.0 mm in height. After this procedure, they were then coated with gold under high vacuum using pulverization equipment (LEICA SPRAY IN ACE600; Wetzlar, Germany), and then analyzed using SEM (JEOL, JSM 5460LV; Tokyo, Japan) at a magnification of 2300× (Sheu & Rosenberg, 1998).

2.8 X-ray diffraction

The measurements of degree of crystallinity of powders were performed using the methodology proposed by the manufacturer high-resolution diffractometer (Carl Zeiss®, HGZ-4, Germany) with X-ray generator

(Seifert®, ID3000, Germany). For this analysis, the microspheres samples were kept in a double-sided tape that covered the aluminum trays and then exposed to CuKx radiation (λ = 15418 Å) at diffraction angles of 2θ, from 10 to 100°C (step size 0.05, time per step 1.0 s).

2.9 Statistical analysis

All analyses were performed in triplicate. The results were expressed as the mean of three determinations plus standard deviation. In the statistical analysis of characterization of guava pulp microencapsulated, the Student's t test was used ($p < 0.05$). The Analysis of Variance (ANOVA) and Tukey's test at 5% significance were used to evaluate the effects of storage on antioxidant activity. In both analyses, the statistical software XLSTAT 7.5 was used.

3 Results and discussion

3.1 Characterization of guava pulp

Table 1 shows the results of the characterization of guava pulp. The guava pulp presented a high moisture, fiber content, and acidity; on the other hand, low protein, fat, carbohydrate, and pH values. Similar values for moisture (87.00 g/100 g), ashes (0.5 g/100 g), proteins (0.8 g/100 g), and pH (4.25) were reported in a study with guava (Osorio et al., 2011). Such differences can be attributed to different varieties and cultivars, climate, geographical location of the crop, agronomic techniques, fruit ripening at harvest, post-harvest handling, and storage (Rodriguez-Amaya, 2001).

An important bioactive compound present in the guava is carotenoid. Seeing that carotenoids are characterized as an important precursor of vitamin A, they also contribute to the antioxidant activity in the human body. The total content of carotenoids found in guava pulp was 7,832 μg/100 g, 156 μg/100 g of β-carotene, and 5,876 μg/100 g of lycopene (Table 1). In a study on carotenoid quantification and identification in guava, there were 420 μg/100 g of β-carotene and 4,700 μg/100g of lycopene (Wilberg & Rodriguez-Amaya, 1995). Another study determined the carotenoid content of 18 Brazilian fruits and found values ranging from 300 to 4,200 μg/100 g in guava (Rufino et al., 2010).

A study on the extract of lycopene obtained from red guava showed a decrease in the viability of human breast adenocarcinoma cells and suggested potential applications of lycopene from red guava in the development of economically viable and sustainable anticancer products (Santos et al., 2018). Fruits rich in carotenoids have been used for the development of food products aiming to offer consumers more healthy food options (Rivas et al., 2019).

The guava pulp had 18.65 μmol Trolox/g, which was related to a high antioxidant activity value for fruits. It could be possible to observe differences in antioxidant activity in several studies with guavas. In a characterization of antioxidant activity in 62 fruits, values of 15.18 μmol Trolox/g of antioxidant activity for guava were obtained (Fu et al., 2011). On the other hand, a study with three red guava genotypes reported values of antioxidant activity between 22.3 and 34.4 μmol Trolox/g (Thaipong et al., 2006). The guava fruit presented a higher antioxidant activity than that of several other exotic Brazilian tropical fruits considered excellent sources of antioxidants, such as *murici* (15.73 μmol Trolox/g), *mangaba* (10.84 μmol Trolox/g) (Almeida et al., 2011), *cajá* (7.8 μmol Trolox/g), cashew (11.2 μmol Trolox/g), and *umbu* (6.3 μmol Trolox/g) (Rufino et al., 2010).

Owing to the high antioxidant potential of red guava, a study reported that a lycopene-rich extract of red guava had a beneficial effect on acute inflammation. It offers protection against consequences of oxidative stress by downregulating inflammatory mediators and inhibiting gene expression involved in inflammation (Vasconcelos et al., 2017).

Table 1. Guava pulp characterization used in microencapsulation.

Analysis	Mean
Moisture content (g/100 g)	89.78 ± 0.02
Ashes (g/100 g)	0.62 ± 0.11
Protein (g/100 g)	0.69 ± 0.04
Fat (g/100 g)	0.39 ± 0.03
Fiber (g/100 g)	3.17
Carbohydrates (g/100 g)	7.11
Calorific value (kcal/100 g)	34.71
Total acidity (g citric acid/100 g)	*0.62*
pH	3.70
°Brix	11
TEAC (µmol Trolox/g)	18.65 ± 1.61
Total Carotenes (µg /100 g)	7,832 ± 89.0
β-carotene (µg /100 g)	156 ± 18.3
Lycopene (µg /100 g)	5,876 ± 330.9

3.2 Characterization of microencapsulated guava pulp

3.2.1 Carotenoids

Table 2 shows the results of total carotenoid, β-carotene and lycopene contents in the spray-dried material. The microspheres of Formulation 2 showed a greater retention of total carotenoids, β-carotene, and lycopene than the microspheres of Formulation 1. The β-carotene was the carotenoid with the highest retention for the Formulations 1 and 2 i.e., 52.86% and 70.57%, respectively.

Carrier agents play the role of a physical barrier against degrading agents in bioactive compounds. The highest content of inulin could be responsible for the higher retention of β-carotene.

In the microencapsulation of fruits rich in carotenoids, the use of inulin combined with maltodextrin showed values of encapsulation efficiency of 72% to 55% (Rivas et al., 2020). Factors of degradation, such as storage with access to light and air, did not cause significant degradation of bioactive compounds in capsules made of inulin and guar gum (Pieczykolan & Kurek, 2019). A better coating of microspheres could be reached with low-molecular weight sugar, which could act as a plasticizer and avoid irregular shrinkage of the microparticle surface during drying, thus improving the process (Silva Carvalho et al., 2016). This effect can be created by using inulin as wall material. It improves the efficiency of powders. The use of inulin is also an interesting option to enrich these powders due to their beneficial effects to human health and its functional properties.

Inulin is considered a functional food in several countries such as France, Holland, Japan, South Korea, Singapore, Brazil, Chile, and Colombia. It has allowed a health claim for products containing inulin in its composition (Rivas, et al., 2019). The recommended intake values of inulin range among countries from 1.25 to 20 g/day (Brownawell et al., 2012). In the development of cakes using microencapsulated fruits with inulin and maltodextrin, the consumption of such cakes may strongly contribute to a proper daily recommended intake of inulin (Rivas, et al., 2019).

Table 2. Total Retention of Carotenoids.

Samples	Total carotenoid (µg/g)	Retention (%)	β carotene (µg/g)	Retention (%)	Lycopene (µg/g)	Retention (%)
Pulp	193.10 ± 2.19	-	3.84 ± 0.45	-	144.87 ± 8.15	-
F1	69.38 ± 0.37	35.79	2.08 ± 0.70	52.86	63.80 ± 0.50	44.46
F2	82.50 ± 0.51	42.73	2.64 ± 0.51	70.57	72.51 ± 49.46	49.46

Data are presented as mean ± Standard Deviation in three replicates.

3.2.2 Antioxidant activity

The antioxidant activity in microencapsulated guava pulp achieved 27.58 µmol Trolox/g and 24.79 µmol Trolox/g for the Formulations 1 and 2, respectively. There was no significant difference between formulations. In a study on shelf-life, the microspheres of Formulation 1 remained stable over time in storage in the absence of light. However, in the presence of light, the microspheres had a significant loss at 60 days: 18.80 µmol Trolox/g (Figure 1). There was no significant loss of antioxidant activity over time in both storage conditions for Formulation 2 (Figure 2).

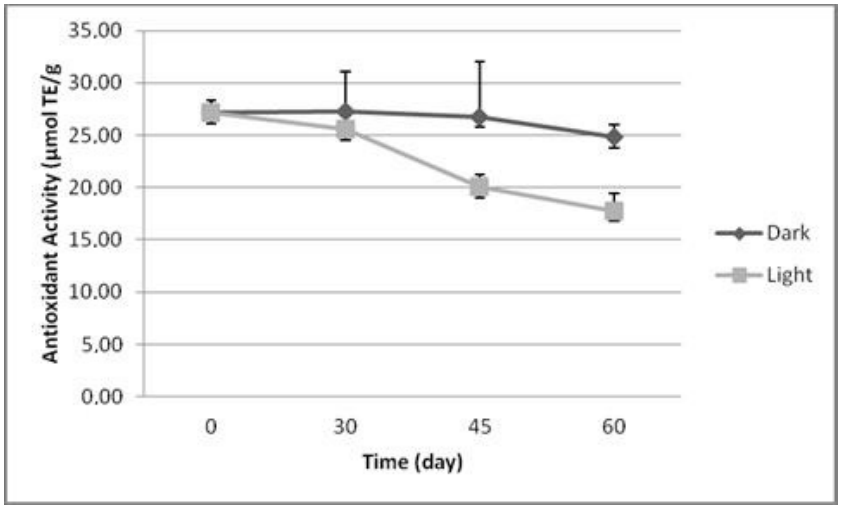

Figure 1. Storage stability of antioxidant activity regarding guava pulp encapsulated (Formulation 1) in absence and presence of light.

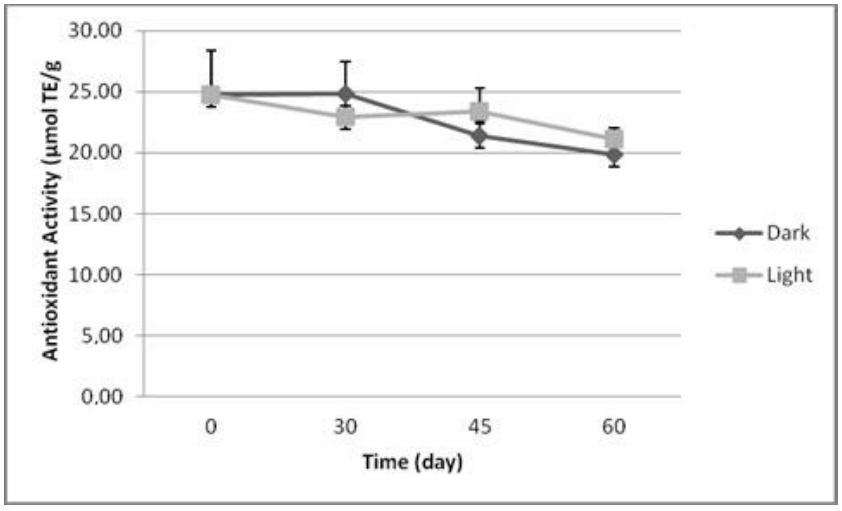

Figure 2. Storage stability of antioxidant activity regarding guava pulp encapsulated (Formulation 2) in absence and presence of light.

The use of inulin as EA has a greater protective effect over time to bioactive compounds and antioxidant activity. A study with microencapsulation of mango and passion fruit pulp mixed with inulin and maltodextrin reported stability during 90 days with no losses of phenolic compounds, vitamin C, total carotenoids, and antioxidant activity (Rivas et al., 2019). The encapsulation of extract of cactus pear provided a higher rate of degradation of bioactive compounds in microcapsules made of maltodextrin rather than made of inulin (Saenz et al., 2009). In the microencapsulation of black currant, inulin has created significantly more stable black currant microcapsules than maltodextrin (Bakowska-Barczak & Kolodziejczyk, 2011).

3.2.3 Particles morphology by Scanning Electron Microscopy (SEM)

Figure 3 shows the SEM of guava pulp microspheres. It could be observed, for both formulations, a perfect spherical microsphere with a smooth surface, as expected from obtaining microspheres using spray-drying process.

Study with combined use of inulin and maltodextrin in microencapsulation showed microspheres with continuous walls without fissures or cracks. It had the same characteristics during storage of 90 days and presented uniformity between the sizes of microspheres, with smooth surfaces and some small depressions (Rivas et al., 2020). This was also observed when guava was microencapsulated with maltodextrin. However, when combining maltodextrin with Arabic gum, the authors observed a greater presence of regular spherical particles (Osorio et al., 2011).

The combination of polymers tends to result in better effects on the formation of microspheres than the use of a single polymer does. The interaction between polymers generates a greater formation of microspheres of smooth surfaces and spherical forms. In a study with encapsulation of betalain with maltodextrin and other variations such as gum arabic, pectin and xanthan gum showed an increased stability of betalains and higher pigment retention with EAs combination when compared to maltodextrin alone (Ravichandran et al., 2014). Furthermore, the absence of fissures or cracks in microspheres tend to preserve the aroma of fruits in the microspheres. When they are used as ingredient in food products, such as cakes, they have a better sensorial evaluation (Rivas et al., 2019).

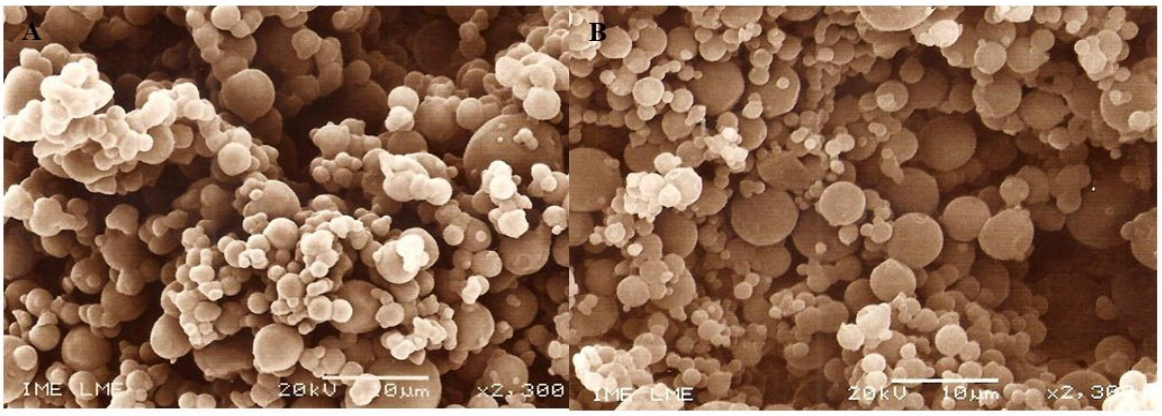

Figure 3. Scanning electronic microscopy of guava fruit juice encapsulated using Formulation 1 (A) and Formulation 2 (B) at a magnification of 2300×.

3.2.4 X-ray diffraction (XRD)

Figure 4 shows the XRD patterns of the encapsulating material, inulin and maltodextrin, and the microcapsules of Formulations 1 and 2. Inulin and maltodextrin had a completely amorphous surface, as could be shown by the presence of broad peaks and large amounts of noise. The microspheres of Formulation 1 showed an amorphous profile with no crystallinity. The microspheres of Formulation 2 showed some small peaks not well defined and many noises, at 10º to 25°, characterizing a partially crystalline structure. It could be attributed to the interaction of lycopene from guava, a crystalline structure, with the carrier material. The presence of a partially crystalline structure is favorable.

The amorphous states tend to be hygroscopic, make the sample sticky, and form agglomerates during storage time. This occurs because amorphous sugars are transformed into crystalline sugars to reach an energetically more stable state, implicating in weight gain, microstructure collapses, and potential microbiological instability (Borrmann et al., 2011; Rivas et al., 2020).

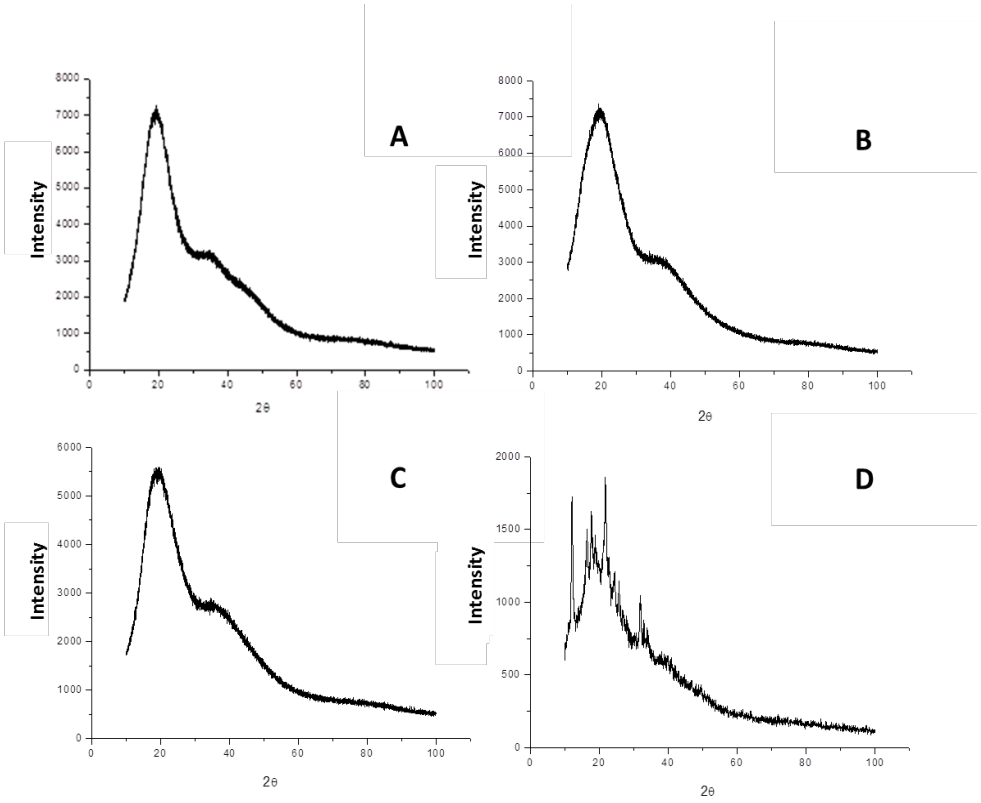

Figure 4. X-ray diffraction of maltodextrin (A) and inulin (B) in microcapsules using Formulation 1(C) and microcapsules using Formulation 2 (D).

4 Conclusion

The microspheres obtained by using inulin and maltodextrin as encapsulating materials at a 2:1 ratio (w/w) (Formulation 2) showed desirable characteristics in the final product considering the variables studied in this work. The powders presented a good quality in terms of morphology. They did not present cracks or fissures, thus reflecting a good protection of the encapsulated material. They presented a higher encapsulation efficiency and showed no loss of antioxidant activity during 60 days in storage in the presence or absence of light. According to the results, the mixture of inulin with maltodextrin was a good alternative for the encapsulation process of fruits due to the presence of technological characteristics of relevant interest and to a significant functional potential. It could also be used as a replacement of food additives, such as flavoring and coloring materials.

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