Received: 20 October 2021 | Revised: 3 February 2022 | Accepted: 6 February 2022

DOI: 10.1111/jfpp.16468

Journal of Food Processing and Preservation | Institute of Food Science + Technology ifst | WILEY

ORIGINAL ARTICLE

Microencapsulation of hibiscus bioactives and its application in yogurt

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Abstract

The aim of this study was to evaluate the application of microparticles of anthocyanins from *Hibiscus sabdariffa* L. extract, obtained by ionic gelation, to commercial natural yogurt. Two ionic gelation techniques were used as follows: atomization (Spray Dryer B-290) and dripping (Encapsulator B-390). Microparticles produced were evaluated for size, morphology, color, and encapsulation efficiency (total phenolic compounds, anthocyanins, and antioxidant capacity). Hibiscus extract (1.5%–4.5%) and 20% of hibiscus microparticles, obtained by atomization or dripping, were applied to whole sweetened natural yogurt and fat-free natural yogurt. Samples were evaluated for sensory acceptance and color changes under refrigerated conditions after the application of different levels of hibiscus extract (1.5%–4.5%). The atomization technique generated smaller particles with a greater encapsulation efficiency of bioactive compounds. Application of free hibiscus extract (4.5%) and hibiscus microparticles (20%) in yogurt matrix proved to be technically feasible, providing color and functional appeal to the product, with good sensory preference.

Novelty Impact Statement: The double emulsion followed by ionic gelation has allowed good retention of hibiscus anthocyanins.

The atomization technique generated smaller particles with a greater encapsulation efficiency of bioactive compounds.

Hibiscus extract combined with microencapsulated hibiscus promoted color and functional appeal to yogurt, with good sensory preference.

1 | INTRODUCTION

Hibiscus sabdariffa belongs to the *Malvaceae* family, known to be cultivated in tropical and subtropical climates worldwide (Ifie et al., 2018). Its calyx, which is the commercially important part of *H. sabdariffa*, is mainly exported from these regions and added to jam, juice, jelly, syrup, wine, ice cream, pudding, and cake. It is a product rich in secondary metabolites, mainly anthocyanins, flavonoids, and a large number of organic acids (Da-Costa-Rocha et al., 2014; Ifie et al., 2018). The anthocyanins can inhibit α-glucosidase which, in turn, may have a potential therapeutic effect on blood glucose levels after meals (Huang et al., 2018; Ifie et al., 2018).

The main pigments of hibiscus calyx are anthocyanins, a soluble in water compound that can have a red, purple, or blue color, depending on system pH, which is an important alternative to the replacement of artificial dyes (Ochoa-Velasco et al., 2017). However, anthocyanin is unstable in the presence of oxygen, enzymes, copigments, metal ions, sulfur dioxide, sugar, sugar degradation products, as well as variations in temperature, pH, light, and solute concentration (Ochoa-Velasco et al., 2017; Wallace, 2010). Studies have shown that the hibiscus extract has a high level of antioxidant activity and such effect may be related to the presence of anthocyanins (Da-Costa-Rocha et al., 2014; Maciel et al., 2018; Su et al., 2020).

Microencapsulation is considered an effective technique for protecting sensitive chemical compounds against environmental damages, such as temperature, humidity, radiation, and microorganisms. It is an approach of interest regarding the storage, separation, and packaging of such sensitive materials in a physical system on a microscopic scale (Ochoa-Velasco et al., 2017). Such technique can be an alternative to protect bioactive compounds from hibiscus extract during processing and storage, such as encapsulation with mesquite gum (Ochoa-Velasco et al., 2017), encapsulation using spray drying (Gómez-Aldapa et al., 2019), or using coextrusion technology (Goh et al., 2021), but the use of ionic gelling has not been studied yet.

Ionic gelation is a simple microencapsulation method occurring when a polymer solution containing active material is dripped on a calcium ion solution at appropriate concentrations (Patil et al., 2010). Ionic gelation by dripping-extrusion is a simple, efficient, and low-cost encapsulation technique not requiring specific equipment, high temperature, or organic solvents, which makes it appropriate for hydrophobic or hydrophilic compounds (Arriola et al., 2019). Different polymer solutions such as guar gum, gum Arabic, and low- and high-methoxyl pectins have been studied in order to improve the bioactive compound protection efficiency (Calvo et al., 2019).

Regulations and definitions vary somewhat among countries, but in order to meet the Codex standard, yogurt is defined as a fermented milk product containing two strains of live bacteria, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*. During yogurt preparation (Donovan & Hutkins, 2018), optional nonlactic ingredients are added before, during, or after fermentation up to a maximum of 30% w/w, then the product is classified as yogurt with additives.

The dairy products sector is one of the most innovative in the food industry and it has been following the trend of healthiness and well-being for some years. This industry has strongly invested in products with functional appeal, probiotic and prebiotic products, and natural antioxidants (Francisco et al., 2018; Lucey, 2016). Therefore, the purpose of this project was to use ionic gelation to encapsulate the anthocyanins from hibiscus extract (*Hibiscus sabdariffa* L.) and evaluate the application of microparticles to commercial natural yogurt.

2 | MATERIALS AND METHODS

2.1 | Materials

Hibiscus extract (*Hibiscus sabdariffa* L.)—food grade (30%), supplied by the company *Heide Extratos Vegetais*, Pinhais/PR, Brazil; GENU amidated low methoxyl pectin (*CPKelco*, Limeira/SP, Brazil); calcium chloride food grade (*Dinâmica*, Diadema/SP, Brazil); canola oil—commercial brand Liza (*Cargill Agrícola S.A.*, Mairinque/SP, Brazil); PGPR surfactant (*Danisco*, Pirapozinho/SP, Brazil); analytical grade reagents; low-fat natural yogurt (*Danone*, Poços de Caldas/SP, Brazil); and whole-milk natural yogurt with added sugar (*Danone*, Poços de Caldas/SP, Brazil).

2.2 | Methods

2.2.1 | Extract characterization

Hydroalcoholic extract of hibiscus supplied by company *Heide Extratos Vegetais* was characterized by the following.

Moisture content

Moisture content was gravimetrically determined by drying at 70°C with no vacuum for 24 hr, followed by vacuum drying for an additional 24 hr. The analysis was performed in triplicate with approximately 10 g of the sample (AOAC, 2006).

pH

pH was measured with a potentiometer (Digimed, model DM 20, Brazil) according to the methodology described by *Instituto Adolfo Lutz* (Brasil, 2005). The analysis was performed in triplicate.

Total phenolic compounds

Total phenolic compounds were determined according to the Folin Ciocalteau spectrophotometric method, described in Singleton and Rossi (1965) and used by Turkyilmaz et al. (2013).

Total monomeric anthocyanin concentration

Total anthocyanin concentration was determined by using the differential pH method, as per method no. 37.1.68 of AOAC (2006).

Antioxidant capacity—DPPH

The antioxidant capacity by the DPPH method was measured according to the Brand-Williams et al. (1995) methodology and used by Arend et al. (2017).

Antioxidant capacity—ABTS

The antioxidant capacity by the ABTS method was measured according to the Arts et al. (2001) and used by Turkyilmaz et al. (2013).

Analysis of color

Colorimetric determinations were conducted by using Chromameter CR-400 (Konica-Minolta Sensing Inc., Osaka, Japan), programmed in the CIELAB system, where L^* (0 = black and 100 = white), a^*($+a^*$ = red and $-a^*$ = green), and b^* ($+b^*$ = yellow and $-b^*$ = blue) are determined. The colorimeter was calibrated with illuminant D65.

To obtain the readings, the samples were put in a test tube. Equipment was previously calibrated with a white calibration plate. The following values were calculated:

$$\text{Chroma or Saturation} = C^* = \sqrt{(a*^2 + b*^2)}, \quad (1)$$

$$\text{Hue or Tone} = H* = \arctan\left(\frac{b*}{a*}\right). \quad (2)$$

Analysis of extract color stability

In order to evaluate extract stability in different media, a color change test was performed at a pH range of 3–8 and at a temperature of 25°C.

2.2.2 | Production of microparticles

Aiming to enable hydrosoluble extract retention in a gel matrix, a simple emulsion (W/O) was initially elaborated with canola oil and surfactant PGPR (4 g/100 g) at a ratio of hibiscus extract: canola oil + surfactant (35:65) (w/w) in an ultra-turrax dispersing machine IKA T18 (15,000 rpm/30 min) and controlling the temperature with a thermostatic bath at 25°C. Then, double emulsion (W/O/W) was produced with pectin solution (2 g/100 g) at 15,000 rpm/5 min, at a ratio of simple emulsion: pectin solution (20:80) (w/w). Microparticles were formed by atomization using the mini-Spray Dryer B-290 atomizer (brand Büchi) under the following conditions: nozzle 0.7 mm, 0.15 bar, and 1.30 ml/min and by dripping using the Encapsulator (B-390, brand Büchi) under the following conditions: nozzle 300 μm, 200 mbar (5.5–7 ml/min), 100 Hz, and 400 V. Crosslinking solution used was $CaCl_2$ (3 g/100 g) acidified at pH 3.0, positioned at a distance of 18 cm from the nozzle and under agitation (for 15 min). A detailed description of the methodology can be found in Moura et al. (2018).

2.2.3 | Characterization of microparticles

Mean diameter and size distribution

The sample mean diameters and size distribution were determined in Laser Light Scattering Analyzer LA-950 V2 (Horiba Instruments Inc., Japan) by light scattering using the liquid dispersion module and filtered water as a dispersing agent. Analysis was performed in sextuplicate. Mean diameter was determined based on the mean diameter of a sphere of the same volume (De Brouckere diameter—D[4,3]), calculated by Equation 3 as follows:

$$D_{[3,2]} = \frac{\sum n_i d_i^3}{\sum n_i d_i^2}, \quad (3)$$

where d_i is the diameter of particles and n_i is the number of particles.

The polydispersity index (PDI) was calculated according to Jafari et al. (2007) using Equation 4 as follows:

$$\text{PDI} = \frac{d_{90} - d_{10}}{d_{50}}, \quad (4)$$

where d_{10}, d_{50}, and d_{90} are the diameters at 10%, 50% and 90% of accumulated volume, respectively. That is, d_{90}–d_{10} is the data interval and d_{50} is the mean diameter.

Analysis of color

Colorimetric determinations were performed according to Section 2.2.1.7. Parameters L^*, a^*, b^*, C^*, and H^* of particles were evaluated. The color reading of the particles was performed after the samples had been filtered on filter paper (no vacuum) and placed in Petri dishes. The readings were taken in quadruplicate with the cannon of the device positioned at a small distance from the Petri dish with the particles. The equipment was previously calibrated with a white calibration plate.

Microstructure

Particle microstructure was analyzed according to methodology adapted from Moura et al. (2018) by using an optical microscope (model BX41, brand Olympus, Scientific Equipment Products Group, Center Valley, Pennsylvania, USA) for visualization at 40x magnification.

Moisture content

According to Section 2.2.1.1, the analysis was performed in triplicate.

Total phenolic compounds

For particle dissolution, EDTA (0.2 M) was used under stirring (30 min) at 10 g of moist particles. Then, extraction was performed in a homogenizer/crusher Turratec (brand Tecnal model TE102, Piracicaba, Brazil) at 11,000 rpm for 5 min, four times, of 2 x 20 ml with ethanol 70% and 2 x 20 ml with acetone 70%, alternatingly. After each extraction, the sample was filtered into a grinding-mouth Erlenmeyer flask covered with aluminum foil and, in the last wash, the bioactive compound was transferred to a 100-ml volumetric flask completing the volume with ethanol 70%, covering it with aluminum foil and protecting from light, then it was taken to quantification. Microparticles without the active compound were considered as a control to disregard the effect of interfering substances on quantifications. Quantification of the recovered bioactive compound was conducted according to Section 2.2.1.3. The analysis was performed in triplicate.

Total monomeric anthocyanins

According to Section 2.2.1.4. The analysis was performed in triplicate.

Antioxidant capacity—DPPH

According to Section 2.2.1.5, the analysis was performed in triplicate.

Encapsulation efficiency (EE)

It indicates the amount of active ingredient is effectively retained in the microparticle structure after processing. This is important because it defines the number of microparticles to be used (Kim et al., 2009) in subsequent applications. The encapsulation efficiency was calculated on a wet basis as follows:

$$\text{EE}\ (\%) = \frac{\frac{\text{mg of acive in microparticle}}{100\ \text{g of microparticle}}}{\frac{\text{mg of active added to double emulsion}}{100\ \text{g of double emulsion}}} \times 100, \quad (5)$$

where the double emulsion is composed of simple emulsion (oil + hibiscus extract) + pectin solution.

2.2.4 | Application of microparticles in yogurt

In the first step, three whole natural yogurt formulations were elaborated: yogurt with 1.5% (w/w) of extract, yogurt with 20% (w/w) of particles obtained by atomization, and yogurt with 20% (w/w) of particles obtained by dripping. The amount of anthocyanins in 1.5% of extract corresponds to 1.5 mg of anthocyanins per 100 g of yogurt. The amount of anthocyanins in 20% of the microparticles also corresponds to 1.5 mg of anthocyanins per 100 g of yogurt. The particles were applied 24 hr after production. This time was constant for all samples. These samples were considered in order to evaluate sensorial characteristics based on ABNT (2014).

In the second step, eight formulations were elaborated for each type of natural yogurt (whole and fat-free). Then the samples were stored at 5 ± 1°C.

In the whole yogurt, 20% of particles obtained by atomization were added plus a range of 1.5%, 2.5%, 3.5%, and 4.5% of the extract, producing four formulations. The same was done with 20% of particles obtained by dripping, resulting in the other four formulations (1.5%, 2.5%, 3.5%, and 4.5% of extract).

Similarly, in fat-free yogurt, 20% of particles obtained by atomization were added plus a range of 1.5%, 2.5%, 3.5%, and 4.5% of the extract, producing four formulations. The same was done with 20% of particles obtained by dripping, resulting in the other four formulations (1.5%, 2.5%, 3.5%, and 4.5% of extract).

For these samples, the following analyses were performed:

Analysis of color

Colorimetric determinations were performed according to Section 2.2.1.7. Parameters L^*, a^*, b^*, C^*, and H^* of yogurt samples were evaluated. Color reading was made in quadruplicate after the samples had been put in Petri dishes. Equipment was previously calibrated with a white calibration plate.

Sensory evaluation

For the samples obtained in the first step (Section 2.2.4), a descriptive method with a consensus profile was applied (ABNT, 2014). Sensory characteristics were evaluated by consensus by a highly trained panel of seven evaluators selected for sensory acuity. Attributes such as appearance, odor, sensation in the mouth, and taste were described, giving scores from 0 to 10.

For the samples obtained in the second step (Section 2.2.4), an ADQ descriptive method combined with the test of preference as per Meilgaard et al. (2006) was used. For this evaluation, 32 yogurt consumers were recruited and trained, with no restrictions with respect to sex and age, of social classes A/B/C according to Brazilian Criteria of Economic Classification 2015 (ABEP, 2016). After evaluating the samples, the evaluator was requested to put the samples in order of preference. The samples were evaluated in a single, sequential monadic test, following a balanced complete block design, presented with three random number codes. Each sample was put in clear acrylic square glasses and covered with plastic film. The samples were evaluated for pink color (light to dark) and characteristic appearance of yogurt (unsatisfactory to excellent) through a 9-point structured scale. Then the evaluators were requested to put the samples in order of preference (Meilgaard et al., 2006). The sensory data were collected through paper ballots. Structured scale data were submitted to analysis of variance and Tukey's test for mean value comparison. Data related to tests for order of preference were submitted to Friedman and Fisher's tests.

2.2.5 | Statistical analysis

The study results were statistically evaluated by analysis of variance (ANOVA) and Tukey's test at a level of significance of 5%, using program Statistica, version 12 (StatSoft Inc., Tulsa, USA).

3 | RESULTS AND DISCUSSIONS

3.1 | Content of total anthocyanins and total phenolic compounds, and antioxidant capacity of hibiscus extract

The hibiscus extract has been studied due to the possibility of extracting total phenolic compounds from different parts of the plant. The mean results obtained in the present study for an extract from hibiscus calyx in wet base (total phenolic compounds 288.84 ± 0.33 mg/100 g, total anthocyanins 78.20 ± 0.31 mg/100 g, antioxidant capacity—DPPH 17.00 ± 0.50 μmol/g, and antioxidant capacity—ABTS 30.10 ± 0.37 μmol/g) were consistent with values observed in the literature (Ifie et al., 2018; Sinela et al., 2017). The study conducted by Mohd-Esa et al. (2010) showed the plant calyx is rich in polyphenols (291 mg/100 g) and the leaves had a polyphenol content (220 mg/100 g) 24% lower than the calyx. In compliance with this result, Zhen et al. (2016) found out the total phenolic content in *Hibiscus sabdariffa* leaves ranged from 189.8 ± 27 to 299.0 ± 50 mg/100 g on a wet basis. Total phenolic compounds (3,604 mg/100 g) and anthocyanin (1,967 mg/100 g) values on a dry basis found by Ifie et al. (2018) are somewhat higher than the values found in the present study. The anthocyanin value observed (1.13 g/100 g of dry extract) is slightly above the value found by Sinela et al. (2017). These authors performed the identification and quantification by HPLC-MS at 360 nm of anthocyanin present in *Hibiscus sabdariffa* L. extract, observing main anthocyanin contents of 0.46 and 0.30 g/100 g of dry extract. According to Sinela et al. (2017), total anthocyanin content can be affected by variety, production year, ground, and climate conditions. Anthocyanin content had values within the range shown by Juliani et al. (2009) for *Hibiscus sabdariffa* varieties of different origins and periods of production (1 to 1.5 g/100 g of dry extract). With respect to polyphenol content, the value is also within the range (2 a 3 g/100 g of dry extract), as shown by Juliani et al. (2009). Yang et al. (2012) conducted a study of the antioxidant capacity of hibiscus calyx extract, which was stated to be rich in antioxidants with values of 289.01 ± 16.68 μg/ml using

DPPH methodology and 423.25 ± 31.38 μg/ml using ABTS methodology. The methodology used by these authors was changed and the results cannot be directly compared, but the values of this study are compliant with those, concluding the antioxidant capacity by ABTS was superior to the antioxidant capacity by DPPH.

3.2 | Hibiscus extract behavior based on pH changes

Aiming to evaluate hibiscus extract behavior based on pH changes, changes in color at a pH range from 3 to 8 were evaluated. The original pH of the extract was 2.76 ± 0.01.

The pH values of the extract were increased, and its color was evaluated according to CIELAB parameters. Hibiscus extract had a significant reduction in parameter a^* (1.44–0.74) and parameter C^* (1.46–0.75). On the other hand, parameters L^*, b^*, and H^* did not show a clear trend of behavior. These results comply with a study conducted by Cabrita et al. (2000), who showed that anthocyanins have lower stability at high pH, with such instability rapidly increasing at pH 5–6. However, for such authors, the stability of some anthocyanins was higher at alkaline pH, with maximum stability at pH 8–9. During the analysis of hibiscus extract, there was a noticeable change in color at a pH range of pH 5–6, also noted by the increased deviation in readings, mainly L^* and b^*. When pH 8 was reached, the increase in NaOH volume (1 M) virtually did not change the extract pH.

Figure 1 shows the color change based on pH increase in hibiscus extract diluted in distilled water at a ratio of 1:5 ($g_{extract}$:g_{water}). The extract loses its original red color as the pH is increased. Anthocyanin stability at low pH is widely attributed to a higher concentration of flavylium cation (Gradinaru et al., 2003). According to these authors, a higher ratio of anthocyanins in the hydrated form and pH above 3.6 may lead to a higher rate of anthocyanin degradation. The application of hibiscus extract (pH 2.76 ± 0.01) directly to yogurt (pH 4.4 to 4.8) can irreversibly affect the stability of anthocyanin. Therefore, the microencapsulation of the extract can contribute to the protection of this dye and yogurt color stability.

3.3 | Characterization of particles

3.3.1 | Particle mean diameter and size distribution

Table 1 shows the size results for particles generated by atomization and by dripping. It was observed that the particles obtained by atomization are more than four times smaller than the particles obtained by dripping. The polydispersion index of particles obtained by atomization (1.38) is significantly higher than the polydispersion index of particles obtained by dripping (0.59). This fact can be explained by the characteristics when obtaining the particles through different microencapsulation processes. For the atomization process, the particles are sheared, resulting in a large difference in the size of the particles. In the dripping process, the particles are generated individually, thus not resulting in a large difference in particle size (Ferreira & Nicoletti, 2020; Kim et al., 2011).

As shown in Figure 2, the distribution of microparticles obtained by atomization was characterized by monomodal behavior, with the curve forming a peak represented by higher volume, from 9% to 11% for particles (0.15 bar/1.30 ml/min), close to D_{50} 246.6 μm.

The D_{50} values achieved were lower than those found by Yamdech et al. (2012), who studied the stability of anthocyanins from blackberry extract in microparticles obtained by ionic gelation by atomization and at different alginate concentrations (1.0%–2.5%). The value presented by these authors for mean microparticle size was 400 μm.

The size of the microcapsule may be important for anthocyanin stability and microencapsulation efficiency (Zhao et al., 2008). According to these authors, larger microcapsules generally give better protection than smaller microcapsules, but their dispersion

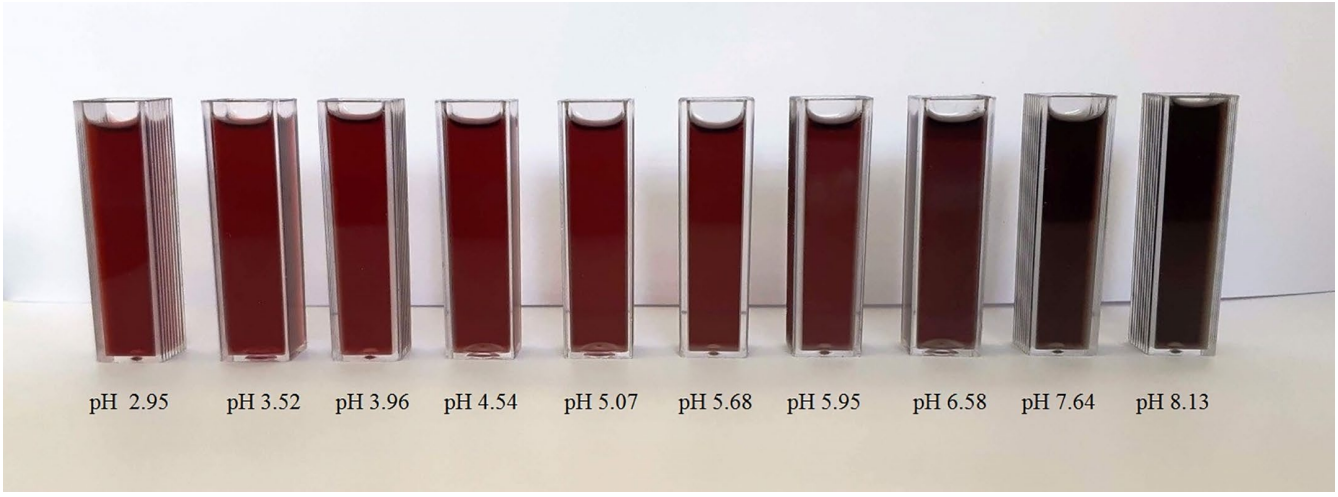

FIGURE 1 Picture of hibiscus extract (diluted in distilled water at a ratio of 1:5) at different pH values and adjusted with NaOH (1 M) according to the pH value intended

is not good in food matrices. Therefore, an optimal particle diameter should be established, not only to have a good protection of the active compound and achieve maximum shelf-life stability but also to have a good microparticle distribution in food applications.

As per Dorati et al. (2013), the dripping technique enables the formation of controlled-size droplets with the application of low flow rates of liquid and simultaneous application of a sinusoidal vibration. In this case, the droplet size depends on the nozzle diameter and the sinusoidal vibration frequency since this technology is based on the subdivision of a laminar flow into droplets by applying superimposed vibrations. Another parameter involved in this process is the voltage of the electrode, whose objective is to stabilize the droplet flow, dispersing the drops and avoiding droplet coalescence. The higher the voltage of the electrode, the smaller and more separated the droplets. The liquid jet speed (V), which is controlled by the air pressure applied, the nozzle diameter (D), and the vibration frequency are the most critical parameters to be set up in order to obtain batches of homogeneous size microparticles at a high reproducibility level. Microparticle diameter (d) can be calculated by the base flow rate (V) and the vibration frequency (f), as the following equation:

$$d = \sqrt{\frac{6V}{\pi f}} \quad (6)$$

According to Equation (6), low rates of liquid jet flow (V), that is, low air pressure values, together with high vibration frequencies result in smaller microparticles (Dorati et al., 2013). The results achieved in this study follow such authors when lower pressure values have led to smaller particles. Therefore, it was evidenced the better working option would be a pressure of 200 mbar.

The results shown in Table 1 are also in agreement with Aizpurua-Olaizola et al. (2016), who studied the variation of voltage, frequency, and air pressure variation in Encapsulator Büchi B-390 when obtaining particles rich in polyphenols from wine production residue. The results achieved by these authors showed the better process conditions when using a 300 μm nozzle were high frequency (1150 Hz) and voltage (2,000 V) values as well as low air pressure (77 mbar), resulting in particles of 600 ± 90 μm.

As shown in Figure 2, the mean distribution of microparticles by dripping was characterized by a monomodal behavior, forming a peak represented by higher volume, between 25 and 30% for particles close to 1100 μm. Similar results of D_{50} (1009.09 ± 38.53 μm) were observed by Cutrim et al. (2019) in microencapsulation of green tea extract using ionic gelation.

The D_{50} values achieved (788–1119 μm) were higher than those observed by Cho et al. (2014) on resveratrol microencapsulation by ionic gelation using chitosan as core material. These authors prepared different concentrations of chitosan solution (0.5% and 1% m/v) by dissolving it in lactic acid solutions at 1% (ml/100 ml) and used an Encapsulator B-390 Pro (Büchi). The mean diameter of particles obtained by these authors ranged from 160.58 to 206.52 μm. The viscosity of the emulsion used in this study implied reducing the equipment flow by increasing the air pressure used, which led to an increase in particle size. On the other hand, the D_{50} values found in this study were lower than those observed by Belscak-Cvitanovic et al. (2016) when encapsulating polyphenols from the dandelion extract. These authors used sodium alginate and pectin as core material and obtained microparticles with D_{50} values ranging from 2150 to 2250 μm.

TABLE 1 Mean diameter and size distribution of microparticles (μm) generated by atomization and by dripping

	$D_{4.3}$ (μm)	D_{50} (μm)	SD (sample)	PDI
By atomization	268.16[b]	246.59[b]	140.3[b]	1.38[a]
By dripping	1169.22[a]	1118.62[a]	311.3[a]	0.59[b]

Note: Different letters in the same column show a significant difference ($p < .05$).

$D_{4.3}$ and D_{50} mean diameter.

Abbreviations: PDI, polydispersity index; SD, the standard deviation of distribution curve.

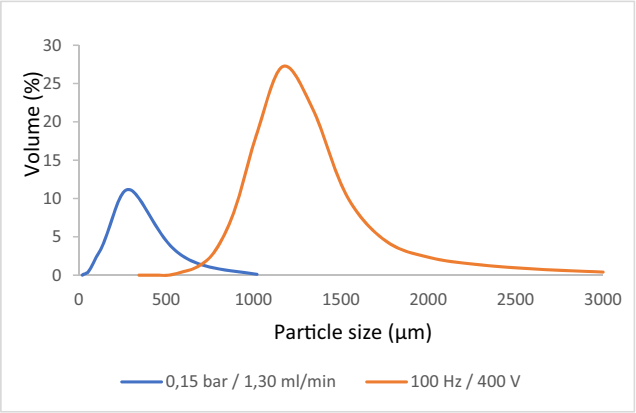

FIGURE 2 Mean distribution of microparticles produced by atomization at the conditions: 0.15 (bar)/1.30 (ml/min) and mean diameter and size distribution of microparticles produced by dripping at conditions: 100 Hz/400 V

TABLE 2 Parameters of the color of microparticles generated by atomization and dripping

	L^*	a^*	b^*	C^*	H^*
By atomization	53.20[a]	25.62[a]	3.15[b]	25.81[a]	0.12[b]
By dripping	53.16[a]	24.46[b]	5.78[a]	25.14[b]	0.23[a]

Note: Different letters in the same column show a significant difference ($p < .05$).

3.3.2 | Analysis of color

The results of microparticle color (Table 2) were similar for particles generated by atomization and dripping. The particles obtained by atomization had higher Chroma or Saturation and a* values, in addition to lower b* and Hue or Tone values than the particles generated by

dripping. This fact was related to the smaller size of the particles, which provides the greater color. Particles obtained by dripping have greater Hue or Tone value, although Chroma or Saturation value was lower.

3.3.3 | Microstructure

Figure 3 shows the microstructure of particles (40x magnification) with optical microscope illumination (under the sample) and lighting by incident light (over the sample), using an external light source of optical fiber (Olympus). Then it was possible to observe several emulsion droplets of different sizes, covered by the core material.

Figure 4 shows optical microscopy of particles generated at 100 Hz/400 V and 40x magnification. We can verify the particle surface is irregular, however, it is not possible to see the inner part of the particle. For better viewing, lighting by incident light (over the sample) was applied using an external light source of optical fiber (Olympus). Then it is possible to observe several emulsion droplets close to one another and covered by the core material.

Microparticles obtained in this study had an irregularity in their spherical form. Tzatsi and Goula (2021), using alginate at ionic gelation of chokeberries extract, produced particles with spherical morphology of the smooth and continuous surface. These results differ from Belscak-Cvitanovic et al. (2016) who, in their work about microencapsulation of polyphenols and β-carotene of dandelion, found that particles generated by external ionic gelation using pectin as core material had lower regularity in their spherical form than particles having alginate as core material.

In the dripping method, each particle is formed individually so that the results of microscopic observation and light scattering coincide. However, when using the atomization method, particles are formed in a disruptive way due to the shear caused by the air on the liquid and can be adsorbed again, forming agglomerates, which can be seen in Figures 3 and 4. This difference can affect not only morphology but also encapsulation properties.

3.3.4 | Total phenolic compounds and total anthocyanin contents and antioxidant capacity

Particles have 20% simple emulsion and 80% pectin solution; in simple emulsion, 35% correspond to extract and 65% correspond to oil. Therefore, if encapsulation efficiency was 100%, 35% of the 20% of active compounds present in hibiscus extract were expected to be present in particles.

Table 3 shows the extract analysis results, the initial value (which would be present in microparticles generated by atomization if there were no losses during the process), and the value found in microparticles.

Final values for total phenolic compounds, anthocyanins, and antioxidant capacity were lower than the initial values. Once again, losses may have occurred during extraction of the active compound to conduct the analyses due to contact with oxygen and exposure to light, characterized by lower stability of extract under these conditions (Lopes et al., 2007; Moura et al., 2018).

By converting the anthocyanin results found in particles to dry basis, the following values are observed: particle (moisture = 77.33%) = 14.34 mg of cyanidin-3-glucoside equivalent/100 g dry sample. When these results are compared with those shown by Yamdech et al. (2012), we verify the hibiscus microparticles had anthocyanin contents, similar to those observed by the authors for blackberry microparticles encapsulated by ionic gelation and using alginate as a core material (13–17 mg/100 g of dry sample).

The observed EE values were the following: polyphenols: EE 80.0%, anthocyanins: EE 84.3%, and antioxidant capacity maintained at 92.1%, which is considered high. Smaller microcapsules as those obtained by atomization generally have better EE (%) than larger microcapsules, since there is a greater relationship between the particle surface and the crosslinking solution (Moura et al., 2018).

These results comply with those observed by Santos et al. (2013), who evaluated the stability of black currant peel extract encapsulated using supercritical CO_2 as a solvent. The authors compared this method with the ionic gelation (Ca-alginate) by dripping and concluded both systems have increased the anthocyanin stability, with different characteristics. Extract encapsulation efficiency for anthocyanin using supercritical CO_2 as a solvent achieved 80%.

The encapsulation efficiency values were quite similar to those values observed in the work developed by Gomes et al. (2016), who showed high efficiency for encapsulation in W/O emulsion with 4% of PGPR, 86.75 ± 0.33%.

This noted encapsulation efficiency was also similar to EE for polyphenols (80–82%) shown by Belscak-Cvitanovic et al. (2016) at the microencapsulation of dandelion by ionotropic gelation. Lower

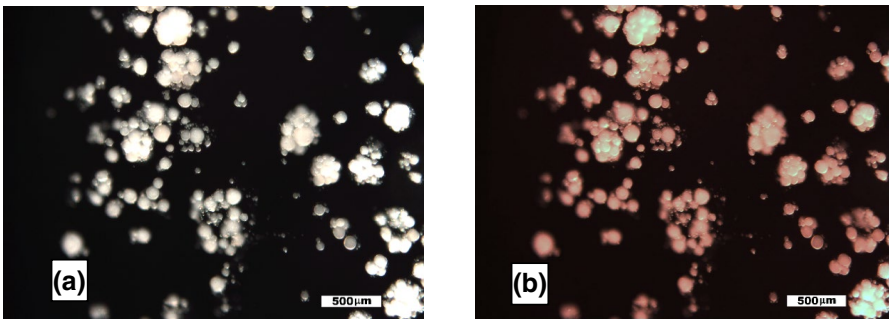

FIGURE 3 Microstructure of particles generated by atomization at 0.15 bar/1.30 ml/min and 40x magnification: (a) with optical microscope illumination (under the sample) and (b) by incident light (over the sample), using an external light source of optical fiber (Olympus)

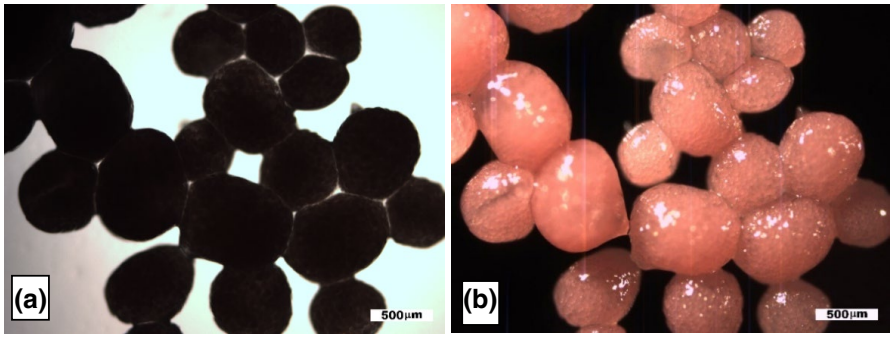

FIGURE 4 Microstructure of particles generated by dripping at 100 Hz/400 V and 40x magnification: (a) with optical microscope illumination (under the sample) and (b) by incident light (over the sample), using an external light source of optical fiber (Olympus)

TABLE 3 Characterization of bioactive compounds in hibiscus extract and microparticles generated by atomization and by dripping

Analyses	Hibiscus extract	Particle initial value (w.b.)	Particle final value (w.b.)	EE[a] (%)
By atomization				
Total phenolic compounds[b]	298.54 ± 0.42	20.90	16.63 ± 0.79	80.0
Total anthocyanins[c]	55.40 ± 0.28	3.88	3.25 ± 0.06	84.3
Antioxidant capacity—DPPH[d]	22.36 ± 0.40	1.56	1.44 ± 0.02	92.1
By dripping				
Total phenolic compounds[b]	279.14 ± 0.25	19.54	14.46 ± 0.15	74.4
Total anthocyanins[c]	101.03 ± 0.10	7.07	4.78 ± 0.06	67.9
Antioxidant capacity—DPPH[d]	11.64 ± 0.60	1.36	0.75 ± 0.05	55.2

Abbreviation: w.b., wet basis.

[a]EE: encapsulation efficiency or retention (%).

[b]mg of gallic acid equivalent/100 g of sample.

[c]mg of cyanidin-3-glucoside equivalent/100 g of sample.

[d]µmol of Trolox equivalent/g of sample.

TABLE 4 Color parameters of whole-milk yogurt with the addition of particles by atomization and dripping varying the free extract percentage

	1.5% of extract	2.5% of extract	3.5% of extract	4.5% of extract
20% of particles by atomization				
*L**	63.46 ± 0.25[a]	59.06 ± 0.32[b]	58.8 ± 0.25[b]	56.75 ± 0.25[c]
*a**	8.89 ± 0.18[a]	9.33 ± 0.08[b]	10.91 ± 0.17[c]	11.94 ± 0.1[d]
*b**	3.64 ± 0.1[a]	3.7 ± 0.18[a]	3.77 ± 0.08[a]	3.98 ± 0.07[b]
*C**	9.09 ± 0.18[a]	9.52 ± 0.09[b]	11.08 ± 0.17[c]	12.1 ± 0.1[d]
*H**	0.39 ± 0.01[a]	0.38 ± 0.01[a]	0.33 ± 0.002[b]	0.32 ± 0.003[b]
20% of particles by dripping				
*L**	64.17 ± 0.79[a]	60.06 ± 0.59[b]	59.63 ± 0.52[b]	56.2 ± 0.6[c]
*a**	7.12 ± 0.33[a]	8.79 ± 0.42[b]	9.85 ± 0.32[c]	10.17 ± 0.29[c]
*b**	3.22 ± 0.1[a]	3.28 ± 0.11[a]	3.3 ± 0.1[a]	3.26 ± 0.07[a]
*C**	7.34 ± 0.32[a]	8.97 ± 0.42[b]	10.02 ± 0.31[c]	10.33 ± 0.29[c]
*H**	0.43 ± 0.02[a]	0.36 ± 002[b]	0.320.01[c]	0.31 ± 0.01[c]

Note: Different letters in the same line show a significant difference ($p < .05$).

EE values (72.6%) were found by Cutrim et al. (2019) at the microencapsulation of green tea extract polyphenols by ionic gelation using Encapsulator B390.

Table 3 also shows the results of extract analysis, the initial value that would be present in microparticles generated by dripping if there were no losses during the process, and the final value found in microparticles.

When the results of total phenolic compounds found in particles were converted into dry basis (Table 4), the following values were observed: particle (100 Hz/400 V, moisture = 83.07%) = 85.41 mg of gallic acid equivalent/100 g of dry sample. By comparing these results with values shown by Aizpurua-Olaizola et al. (2016), we verified the hibiscus microparticles had higher amounts of total phenolic compounds than those observed by the authors at the encapsulation

of wine production residues. The authors used ionic gelation with alginate and $CaCl_2$ and the total polyphenol values found were 67 mg/100 g of dry sample.

Encapsulation efficiency (EE) values obtained were the following: polyphenols 74.4%, anthocyanins 67.9%, and antioxidant capacity maintained at 55.2%.

These encapsulation efficiency values were lower than those presented by Cho et al. (2014) from 94% to 99%, for resveratrol encapsulation by ionic gelation using Encapsulator (B-390 Pro, Büchi) and values shown by Tzatsi and Goula (2021) of 94.2% for chokeberry extract encapsulation at sodium alginate solution 2.0% w/w, calcium chloride solution concentration 2.5%, and extract concentration 20.0% w/w.

The values observed in this work are higher than encapsulation efficiency values for polyphenols and anthocyanins found by Hosseini et al. (2013) when they studied a multiple emulsion method (O/W/O) to produce alginate microparticles carried with essential oil of *Satureja hortensis* (SEO). The encapsulation efficiency values and mean diameter of particles were 52–66% and 47–117 μm, respectively. The authors suggest that for ionic gelation by dripping, there was a saturation of the polymer to the essential oil being encapsulated, leading to a decrease in encapsulation efficiency. The same may have occurred with the encapsulation of anthocyanin from the hibiscus used in the present study. In ionic gelation by dripping, as the droplets are larger, the wall that protects the double emulsion has greater difficulty in retaining the active compounds, leading to lower encapsulation efficiency. It is almost like the bonds between the pectin and the calcium solution have become saturated or the polymer solution has become saturated.

3.3.5 | Sensory characteristics of yogurt samples

During the sensory characteristic analysis of yogurt samples with the presence of extract only (1.5%), those having 20% of particles by atomization and those having 20% of particles obtained by dripping, the following results were observed.

- **Yogurt with free hibiscus extract**

Appearance: light purple viscous fluid (score 6), with the presence of small white lumps typical of yogurt. Odor: typical of natural yogurt (score 7), herbal (score 4). Free from unusual odor. Sensation in the mouth: viscous (score 6), plain, astringent (score 4). Taste: typical of natural unsweetened yogurt (score 8), herbal (score 5), and acid taste (score 4). Free from the unusual taste.

- **Yogurt with particles obtained by atomization**

Appearance: white viscous fluid (score 5), with the presence of tiny well-distributed pink particles and some white lumps, typical of yogurt. Odor: typical of natural yogurt (score 7), herbal (score 4). Free from unusual odor. Sensation in the mouth: viscous (score 5), not plain, with the easy perception of tiny particles, astringent (score 4). Taste: typical of natural unsweetened yogurt (score 8), herbal (score 6), and acid taste (score 4). Free from the unusual taste.

- **Yogurt with particles obtained by dripping**

Appearance: white viscous fluid (score 5), with the presence of spherical, well-distributed pink particles of approximately 1 mm in diameter, and the presence of some white lumps, typical of yogurt. Odor: typical of natural yogurt (score 7), herbal (score 4). Free from unusual odor. Sensation in the mouth: viscous (score 5), not plain, with the presence of smooth and jelly-like particles, astringent (score 4). Taste: typical of natural unsweetened yogurt (score 8), herbal (score 5–6), and acid taste (score 4). Free from the unusual taste.

3.4 | Study of different hibiscus extract use contents

After evaluating the comments from sensory analysis tasters about the lack of pink color in yogurt, we decided to study the influence of using the free extract combined with particles on color and acceptability improvement. Thus, there would be a combination of color improvement by the presence of free hibiscus extract with the functionality increase due to the presence of encapsulated extract. Therefore, tests were conducted with commercial whole-milk yogurt and low-fat yogurt (brand Danone) samples having 1.5, 2.5, 3.5, and 4.5% of hibiscus extract and the addition of 20% of particles both by dripping and atomization methods.

3.4.1 | Analysis of color

Whole-milk yogurt samples, both having dripping and atomization particles, experienced a significant reduction of L^* and H^* and a substantial increase of a^* and C^*, not showing a clear trend of behavior for b^* as the hibiscus extract concentration was increased (Table 4). Figure 5 shows a picture of yogurt samples in different hibiscus extract applications. It is possible to observe the pink color is less homogenous in yogurt samples with particles obtained by the dripping method.

Low-fat yogurt, both with dripping and atomization particles, also showed a significant reduction of L^* and H^* and a substantial increase of a^* and C^*, not showing a clear trend of behavior for b^* as the hibiscus extract concentration was increased (Table 5). It is possible to note the low-fat yogurt samples (Figure 6) have lower brightness than whole-milk yogurt samples (Figure 6). Once more, we can notice the pink color is less homogenous in yogurt samples with particles by the dripping method.

Anthocyanins are sensitive to the acidity level of yogurt, which can influence the final color of the product. According to Scibisz et al. (2019), anthocyanin content in fruit (strawberry, sour cherry, and blueberry) yogurts showed a significant decrease during

storage, especially for the first 2 weeks. The half-life of the pigments in stirred yogurt with the preparation of these fruits was 5.5, 6.7, and 19.0 weeks, respectively.

The addition of 4.5% hibiscus extract significantly improved the pink color of the yogurt, driven by the increase in a^* and C^* values. A loss of anthocyanin color during yogurt storage is expected. The loss of color may be less noticeable to the consumer if the amount of extract added in yogurt preparation is greater.

3.4.2 | Sensory evaluation

Table 6 shows sensory evaluation results for particles generated by atomization and by dripping, for whole-milk yogurt and low-fat milk yogurt.

With respect to pink color acceptability for the whole-milk yogurt, samples having 4.5% of extract both for yogurt with particles obtained by dripping (score 6.9) and those obtained by atomization (score 6.8) were the most accepted ones ($p \leq .05$), with a mean value between "moderate" and "intense."

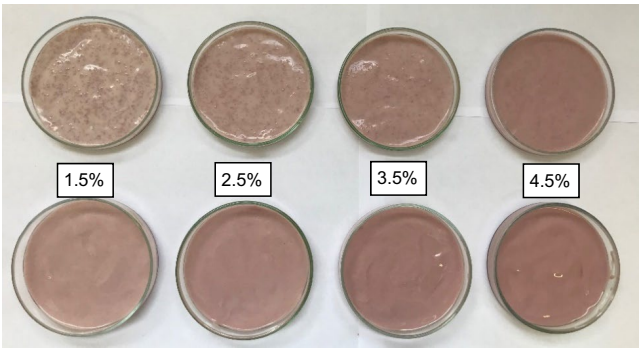

FIGURE 5 Whole-milk yogurt with the application of free extract 1.5% to 4.5%, with 20% of particles obtained by atomization (bottom) and dripping (top)

For the appearance acceptability of whole-milk yogurt, both samples with particles obtained by dripping and those obtained by atomization had similar results, with a mean value close to "good" (score 6.1–6.6). Only the sample with 1.5% of the extract was different from the others, with a mean value between "regular" and "good" (scores 4.2–5.1). Regarding the order of preference, both in samples with particles by atomization and those with particles by dripping, the samples having 4.5% of extract were the preferred ones ($p \leq .05$).

The results are correlated with the study conducted by Moura et al. (2019), where a yogurt matrix with the addition of particles produced by the ionic gelation method by dripping showed a less homogenous distribution of color, resulting in lower scores for sensory acceptance.

For pink color acceptability for the low-fat milk yogurt, samples having 4.5% of the extract, both of yogurt with particles obtained by dripping (score 6.9) and those obtained by atomization (score 6.9), were the most accepted ones ($p \leq .05$), with a mean value between "moderate" and "intense."

Regarding the appearance acceptability of low-fat milk yogurt, samples of yogurt with particles obtained by dripping and those obtained by atomization had similar results, with a mean value close to "good" (scores 5.7–6.1), with only the sample with 1.5% of the extract being different from the others (scores 4.6 to 5.2). With respect to the order of preference, both in samples with particles by atomization and those with particles by dripping, the sample having 4.5% of the extract was the preferred one ($p \leq .05$), as previously observed with whole-milk yogurt samples.

When the same formulations (same type of yogurt and same amount of extract) with different particles (atomization or dripping) were compared, it was possible to perceive that for yogurt consumers, there is no significant statistical difference when different particles are applied.

In general, the results correspond to those found by Francisco et al. (2019) as they can be understood as offering new possibilities to the dairy products market by using yogurt as an efficient vehicle

TABLE 5 Color parameters of low-fat yogurt with the addition of particles by atomization and dripping varying the free extract percentage

	1.5% of extract	2.5% of extract	3.5% of extract	4.5% of extract
20% of particles by atomization				
L^*	60.89 ± 0.29^a	57.56 ± 0.19^b	56.74 ± 0.58^c	55.87 ± 0.24^d
a^*	8.32 ± 0.22^a	9.65 ± 0.22^b	10.05 ± 0.3^c	11.82 ± 0.09^d
b^*	3.63 ± 0.15^a	4.18 ± 0.12^b	3.63 ± 0.14^a	3.94 ± 0.06^c
C^*	8.53 ± 0.21^a	9.87 ± 0.22^b	10.23 ± 0.3^c	11.98 ± 0.09^d
H^*	0.41 ± 0.02^a	0.41 ± 0.01^a	0.35 ± 0.01^b	0.32 ± 0.004^c
20% of particles by dripping				
L^*	63.13 ± 0.85^a	60.75 ± 0.4^b	59.71 ± 0.55^c	57.18 ± 0.34^d
a^*	6.09 ± 0.51^a	7.99 ± 0.28^b	9.21 ± 0.33^c	10.65 ± 0.23^d
b^*	2.8 ± 0.12^a	2.98 ± 0.12^b	3.22 ± 0.17^c	3.53 ± 0.06^d
C^*	6.31 ± 0.49^a	8.17 ± 0.27^b	9.38 ± 0.32^c	10.82 ± 0.23^d
H^*	0.43 ± 0.04^a	0.36 ± 0.02^b	0.34 ± 0.02^b	0.32 ± 0.01^b

Note: Different letters in the same line show a significant difference ($p < .05$).

for bioactive ingredients. However, expansion into new consumer markets of these developed types of yogurt also requires the standardization of their characteristics in order to achieve a satisfactory acceptance of the consumer.

4 | CONCLUSION

Ionic gelation method both by dripping and atomization techniques has enabled us to obtain microparticles with high retention of anthocyanins and polyphenols, without causing an excessive degradation of bioactive compounds. The atomization technique generated 80% smaller size particles and had better efficiency to encapsulate the bioactive compounds (16% higher). The use of free hibiscus extract (4.5%) combined with microencapsulated hibiscus extract has favored the color and preference of both whole-milk and low-fat yogurts. Additional research using prototypes produced in the pilot plant and scale-up is required, in order to confirm the sensory acceptance with larger groups of consumers.

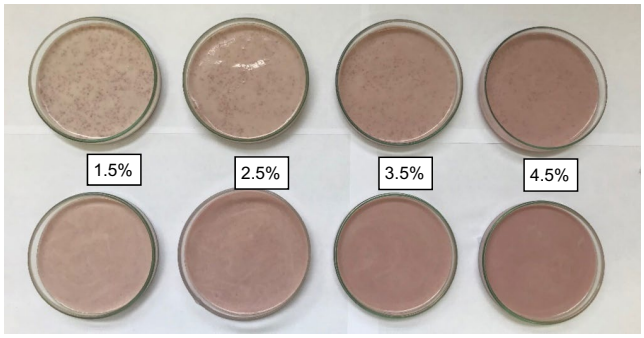

FIGURE 6 Low-fat yogurt with the application of free extract 1.5% to 4.5%, with 20% of particles obtained by atomization (bottom) and dripping (top)

ACKNOWLEDGMENTS

The authors gratefully acknowledge the support of CNPq by granting the fellowship, as well as the company *Heide Extratos Vegetais*, which donated the samples.

CONFLICT OF INTEREST

The authors declare no competing financial interest.

AUTHOR CONTRIBUTIONS

Silvia Cristina Sobottka Rolim de Moura: Conceptualization; data curation; formal analysis; project administration; resources; writing – original draft; writing – review and editing. **Gabriela Nicoluci Schettini:** Formal analysis; resources. **Darlila Aparecida Gallina:** Investigation; methodology. **Izabela Dutra Alvim:** Formal analysis; methodology; **Miriam Dupas Hubinger:** Conceptualization.

TABLE 6 Sensory results of whole-milk and low-fat yogurt with the addition of particles by atomization and dripping, varying the free extract percentage

Whole-milk yogurt—particles by atomization	**1.5% of extract**	**2.5% of extract**	**3.5% of extract**	**4.5% of extract**
Pink color acceptability	$3.5 \pm 1.59^{a,A}$	$5.31 \pm 1.19^{b,A}$	$5.72 \pm 1.25^{b,A}$	$6.84 \pm 1.31^{c,A}$
Appearance acceptability	$5.13 \pm 1.57^{a,B}$	$6.22 \pm 1.36^{a,B}$	$6.31 \pm 1.21^{a,B}$	$6.66 \pm 1.22^{a,B}$
Preference	107^{a}	60^{b}	61^{b}	62^{b}
Whole-milk yogurt—particles by dripping				
Pink color acceptability	$2.41 \pm 1.48^{a,A}$	$4.19 \pm 1.64^{b,A}$	$5.34 \pm 1.41^{c,A}$	$6.97 \pm 1.29^{d,A}$
Appearance acceptability	$4.22 \pm 1.81^{a,B}$	$4.88 \pm 1.89^{a,B}$	$5.59 \pm 1.57^{a,B}$	$6.16 \pm 1.43^{a,B}$
Preference	99^{a}	76^{b}	58^{b}	67^{b}
Low-fat milk yogurt—particles by atomization				
Pink color acceptability	$3.25 \pm 1.45^{a,A}$	$4.75 \pm 1.11^{b,A}$	$6.00 \pm 0.94^{c,A}$	$6.91 \pm 1.19^{c,A}$
Appearance acceptability	$5.25 \pm 1.78^{a,B}$	$6.09 \pm 1.6^{a,B}$	$6.28 \pm 1.24^{a,B}$	$6.06 \pm 1.38^{a,B}$
Preference	102^{a}	70^{b}	59^{b}	53^{b}
Low-fat milk yogurt—particles by dripping				
Pink color acceptability	$2.94 \pm 1.5^{a,A}$	$3.94 \pm 1.13^{a,A}$	$5.53 \pm 1.28^{b,A}$	$6.88 \pm 1.19^{c,A}$
Appearance acceptability	$4.56 \pm 1.75^{a,B}$	$5.41 \pm 1.76^{a,B}$	$5.47 \pm 1.44^{a,B}$	$5.69 \pm 1.79^{a,B}$
Preference	106^{a}	65^{b}	65^{b}	58^{b}

Note: Different lowercase letters in the same line show a significant difference ($p < .05$).
Different uppercase letters in the same column show a significant difference ($p < .05$).

DATA AVAILABILITY STATEMENT

Data available on request due to privacy/ethical restrictions

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How to cite this article: de Moura, S. C., Schettini, G. N., Gallina, D. A., Dutra Alvim, I., & Hubinger, M. D. (2022). Microencapsulation of hibiscus bioactives and its application in yogurt. *Journal of Food Processing and Preservation*, 46, e16468. https://doi.org/10.1111/jfpp.16468