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Microencapsulated Hovenia dulcis: application in yogurt and evaluation of gastrointestinal digestion *in vitro*

Microencapsulado de Hovenia dulcis: aplicación en yogur y evaluación de la digestión gastrointestinal *in vitro*

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Abstract

This study aimed to determine if adding different forms of *Hovenia dulcis* (HD) to yogurt enhances bioactive compound concentrations and their effects on gastrointestinal digestion *(in vitro*). Freeze-dried (Y-HD) and microencapsulated (YEn-HD) forms were added to various yogurt formulations. The yogurts were analyzed on days 1 and 21 post-processing revealing no changes in the fermentation kinetics, proximate composition, water-holding capacity, and hardness of the different formulations. Lower Hunter Whiteness ($p>0.05$) was observed in yogurts with free and encapsulated (Y-HD) compared to the control (without HD). Although YEn-HD yogurt provided higher values of total phenolic compounds, there was no difference before and after the digestion process. An opposite result was observed for ABTS, which able to be determined only after passing through gastrointestinal digestion, with an 83% higher value in YEn-HD compared to Y-HD. This increase may be due to the conditions of the digestive environment, which can cause changes in molecular structure and form new compounds with bioactive effects. This study shows that HD microcapsules can be prepared using the freeze-drying method, becoming a natural and promising additive to increase bioactivity in dairy products such as yogurt, particularly after the digestion process.

Keywords: Japanese grape; Bioactive compounds; Phenolic compounds; Antioxidant activity; Freeze-drying.

Resumen

Este estudio ha determinado si la adición de *Hovenia dulcis* (HD) al yogur mejora las concentraciones de compuestos bioactivos y sus efectos en la digestión gastrointestinal (*in vitro*) (DG). Se agregaron formas liofilizadas (Y-HD) y microencapsuladas (YEn-HD) a formulaciones de yogur. Los yogures fueron analizados en los días 1 y 21 después del procesamiento, revelando que no hubo cambios en la cinética de fermentación, la composición proximal, la capacidad de retención de agua y la dureza de las diferentes formulaciones. Se observó una menor blancura de Hunter ($p>0.05$) en los yogures con HD libre y encapsulado (Y-HD) en comparación con el control (sin HD). Aunque el yogur YEn-HD proporcionó valores más altos de compuestos fenólicos totales, no hubo diferencias antes y después del proceso de digestión. Se observó un resultado opuesto para ABTS, que solo pudo ser determinado después de pasar por la digestión gastrointestinal, con un valor 83% más alto en YEn-HD en comparación con Y-HD. Este aumento puede deberse a las condiciones del ambiente digestivo, que pueden causar cambios en la estructura molecular y formar nuevos compuestos con efectos bioactivos. Este estudio muestra que las microcápsulas de HD pueden prepararse utilizando la liofilización, convirtiéndose en un aditivo natural y prometedor para aumentar la bioactividad en productos lácteos como el yogur, especialmente después del proceso de digestión.

Palabras clave: Uva japonesa; Compuestos bioactivos; Compuestos Fenólicos; Actividad antioxidante; Liofilización.

Introduction

Yogurt is among the most consumed dairy products worldwide due to its nutritional and therapeutic value (1). It contains nutrients such as calcium and vitamins, however, it has a low content of bioactive compounds (2,3). The development of novel dairy products using plants as a source of bioactive compounds is an effective approach to improving the healthiness of these products (3,4). However, although the intake of bioactive compounds from plants can confer health benefits, there are some limitations to their use in food products. Problems such as off-flavors, bitterness, discoloration of the product (1), and instability of the compounds during processing and storage have been reported (5). In addition, gastrointestinal digestion can influence the bioavailability and bioaccessibility of bioactive constituents in yogurt (6).

Microencapsulation is a "packing" technique in which an active ingredient is coated by an encapsulating material, promoting its protection. It is defined as a process of coating small particles of solid, liquid, or gaseous components with a protective coating material. The typical wall materials include proteins (sodium caseinate, whey protein, soy protein, and gelatin) and hydrocolloids (modified starch and gum Arabic) (7,8). Thus, this technique can be used to improve the delivery of bioactive compounds and the characteristics of the final product.

Hovenia dulcis Thunb, popularly known as Japanese grape, is a globose pseudo fruit, which when mature becomes brown in color, fleshy, and sweet (9). During *H. dulcis* ripening, important changes occur in color, hardness, and sweetness, as well as in the content of bioactive compounds, which provide health benefits such as liver protection, anti-fatigue, anti-steatosis, anti-diabetic, and anti-inflammatory activities (10,11). The powder form of *H. dulcis* can be a potential raw material for the development of new functional dairy foods, providing compounds with bioactive properties (12).

Several studies have been performed on the use of this pseudo fruit in meat products (13), fermented vinegar (14), and cookies (15). However, the addition of *H. dulcis* in encapsulated form to dairy products, such as yogurt, has not been evaluated. This study aimed to evaluate the effect of the addition of *H. dulcis* in the form of powder and microencapsulated in yogurts, concerning the increase in the concentration of bioactive compounds and the positive effects of these compounds on gastrointestinal digestion (*in vitro*).

Materials and methods

Obtaining the powder of mature H. dulcis pseudofruit

H. dulcis pseudofruits were collected at maturity stage (soluble solids to acidity ratio of 18.29), in the municipality of Pinhalzinho, Santa Catarina, Brazil (Latitude 22° 46' 46'' S, Longitude 45° 35' 26'' W). The material was separated from the seeds and stems and cleaned in running water. The excess water was removed, and the sample was ground using a domestic processor (CK1026, Clinck, BRA) to particle sizes of 2-5 mm, packaged (1 cm layer) in 250 mL plastic bags, and frozen in an ultra-freezer at -80 ºC (IULT 335D, Indrel, BRA). The freeze-drying process was carried out in a tray dryer at a temperature of -50 ºC and a pressure of 0.05 mTorr, for 24 h (TDF 5503, IIShin, KOR).

The freeze-dried sample was ground in a blender (Diamante 800, Britânia, BRA) and the particle size was standardized on a 32 Tyler mesh size (<500 mm). Then, the *H. dulcis* (HD) powder was stored in an ultra-freezer at -80 ºC protected from light (IULT 335D, Indrel, BRA) until use.

Obtaining the encapsulated H. dulcis

The microencapsulated *H. dulcis* (En-HD) was produced according to the methodologies of Yadav *et al.* (1) and Yadav *et al.* (16), with modifications. Whey protein concentrate (WPC) and gum Arabic (GA) (Êxodo Científica, BRA) were used as coating materials in a protein-to-gum ratio of 3:2. The HD and the coating materials (1:5) were dissolved in water (0.6 g/mL) and agitated in a magnetic stirrer (CE-1540 / A-18, Cienlab, BRA) at 1800 rpm for 2 h. Then, the mixture was placed in an ultrasound bath (SSBuc, SolidSteel, BRA) at 160W and 40 kHz for 30 min, at 25 ºC, and immediately arranged in layers of 2 and 3 mm, in Zip Lock bags, for freezing in an ultra-freezer at -80 ºC (IULT 335D, Indrel, BRA) and subsequent freeze-drying at -50 ºC, at a pressure of 0.05 mTorr for 24 h (TDF 5503, IIShin, KOR). The particles were stored protected from light, in an ultra-freezer at -80 ºC (IULT 335D, Indrel, BRA) until use.

Manufacture of yogurt supplemented with H. dulcis

Pasteurized milk (HTST, 72 °C for 10 s), with 2.7 % fat and a density of 1032.5 g/L, was kindly provided by a local dairy. Yogurt was produced in duplicate batches, as described by Yadav *et al.* (1), with adaptations, with the addition of skim milk powder to milk until reaching 16% total solids. Three yogurt formulations were made as follows: (I) YC, control yogurt; (II) Y-HD, yogurt with the addition of 1% HD powder (w/w); and (III) YEn-HD, yogurt with the addition of 1% encapsulated powder (w/w).

The formulations were homogenized at 42 ºC for 20 min in Thermomix™ (Thermomix® 5, Vorwerk, FRA), and then 3% (w/v) starter culture was inoculated. The started culture inoculated was *Streptococcus thermophilus* + *Lactobacillus delbrueckii* subsp. *Bulgaricus* (CHR-HANSEN, BRA).

The mixture was placed in 50 mL sterile plastic

containers and incubated at 42 ºC (BOD Luca 161/01, Lucadema, BRA). The pH of the formulations was monitored until reaching pH ~ 4.8. Then, the fermentation was interrupted, and the samples were cooled at 4±2 ºC in a chamber (BOD Luca 161/01, Lucadema, BRA) until analysis (1 and 21 days after manufacture).

Evaluation of yogurts with H. dulcis powder and encapsulated H. dulcis powder

The yogurts were evaluated, in triplicate, on day 1 for proximate composition, and on days 1 and 21 for lactic acid bacteria count, titratable acidity, water retention capacity, instrumental color, texture profile analysis, total phenolic compounds, and antioxidant activity by ABTS and DPPH• assays.

Physicochemical characterization

Titlable acidity and pH were monitored throughout the fermentation time to assess the fermentation kinetics of the yogurts. The pH was evaluated in a potentiometer (mPA210, Tecnopon, BRA), and titratable acidity was determined according to method 942.15b of AOAC (17). The determinations of ash content (method 920.153), total solids (method 990.20), protein (method 928.08), and moisture (947.05) were performed according to AOAC (17).

Lactic acid bacteria counts, titratable acidity and water-holding capacity

Total lactic acid bacteria counts were performed by plating on MRS agar at pH 6.5, under anaerobic conditions, and incubation at 37±1 °C for 72 h (18). The titratable acidity of the samples was determined according to the methodology 942.15b of AOAC (17).

The water-holding capacity (WHC) of the yogurt formulations was evaluated as described by Yadav *et al.* (1), with modifications. For that, 15 g of yogurt (Y) was centrifuged for 30 min at 1255 g (SL-700, Solab, BRA) and 25 °C. The whey released (WE) was quantified, and the WHC was calculated according to Eq. 1.

$$\text{WHC}\ (\%) = 100\frac{(Y - WE)}{Y} \quad \text{(Eq. 1)}$$

Instrumental color measurements

Instrumental color was evaluated for the parameters L^*, a^*, and b^*, using a colorimeter (Konica Minolta, Chroma Meter CR400, USA) with direct reading of the samples. The international CIELAB system was used, in which L^* represents brightness, ranging from dark (0) to white (100), a^* ranging from green (-60) to red (+60), and b^* ranging from blue (-60) to yellow (+60) (13). The Hunter Whiteness (WH) was determined when compared to the control, according to Wang *et al.* (18).

Texture profile analysis

Texture profile analysis of the yogurt formulations was performed as described by Wang *et al.* (19) with modifications, using a texture analyzer (CT-3 50K, Brookfield, USA) at a penetration distance of 30 mm, using a cylindrical acrylic probe 25.10 mm in diameter and 20 mm height, and trigger force of 0.04 N. The data acquisition rate was 50 points per sec in two cycles, at a speed of 1 mm/s. The sample (50 mm diameter, 35 mm height, 50 g) was analyzed in quintuplicate for the parameter's hardness, adhesiveness, cohesiveness, elasticity, and gumminess.

Total phenolic compounds and antioxidant activity

The determination of Total phenolic compounds (TPC) and antioxidant activity was performed according to the methodology proposed by Yadav *et al.* (1). For that, 10 g of yogurt was centrifuged at 4025 g (SL-700, Solab, BRA) for 10 min and the supernatant was collected to make up the sample extract.

The TPC was determined as described by Roesler *et al.* (20), with adaptations. For that, 0.5 mL of the extract was mixed with 2.5 mL of 0.1 N Folin-Ciocalteu reagent and kept in reaction for 5 min. Then, 2 mL of 7.5% sodium carbonate was added and kept at rest protected from light at 25 °C for 2 h. The absorbance of the mixture was measured at 760 nm (80 AS, Cirrus, BRA). The blank was prepared by replacing the extract with ultrapure water. The TPC was calculated using a standard curve of Gallic acid (GAE) with concentrations ranging from 10 to 80 mgGAE/L ($R^2 = 0.97$). The results were expressed as mgGAE/100 g sample.

The antioxidant activity measured by the ABTS assay was determined as described by Helal and Tagliazuchi (21), using Trolox as a standard. For that, 40 µL of the extract was mixed with 1960 µL of the ABTS radical. The mixture was incubated at 37 °C for 10 min and the decrease in absorbance was measured at 734 nm (80 AS, Cirrus, BRA). The concentration was calculated using the standard curve of Trolox (R^2= 0.99) ranging from 100 to 2000 mgTrolox/L. The results were expressed as mgTrolox/g of HD.

The DPPH• radical scavenging activity was determined according to Ardabilchi *et al.* (22), with modifications. For that, 250 µL of the extract was homogenized with 3 mL of DPPH• solution (60 µM in ethanol) and the mixture was kept in the dark at 25 °C until stabilization (approximately 1 h). The control sample was obtained by replacing the extract with ultrapure water. The absorbance was measured at 515 nm (80 AS, Cirrus, BRA) and the percentage of inhibition was calculated according to Eq. 2.

$$DPPH^{\bullet}\text{inhibition (\%)} = \frac{(A_{control} - A_{sample})}{A_{control}}.100 \quad \text{(Eq. 2)}$$

Gastrointestinal digestion in vitro

The gastrointestinal digestion *in vitro* of the yogurts was evaluated according to methodologies adapted from Tang *et al.* (23) and Verruck *et al.* (24). The following steps were performed for the simulated digestion: (1) esophagus-stomach stage, at 37 °C for 2 h: 10 g of yogurt was mixed with 5 mL of 35 mmol.L^{-1} sodium chloride, and the pH was adjusted to 2.0 with 1 mol.L^{-1} HCl. Then, 0.05 mL of pepsin solution (*25* mg.mL^{-1} in 0.1 mol.L^{-1} HCl) per g of sample was added; (2) duodenum stage: the pH of the mixture was adjusted to 7.0 with 1 mol.L^{-1} NaOH. Then, 0.25 mL of bile salts and pancreatin solution (2.0 g.L^{-1} pancreatin and 12 g.L^{-1} bovine bile salts in 0.1 mol.L^{-1} NaOH) per gram of sample was added, homogenized, and kept at 37 °C for 2 h. The hydrolysate was kept at 50 °C for 5 min for enzyme inactivation and then centrifuged at 3260 g for 10 min at 4 °C (Megafuge 8R, Thermo Scientific, USA). The supernatant extract was characterized for total phenolic compounds, and antioxidant activities by ABTS and DPPH assays, according to methodologies previously described.

Statistical analysis

The formulations were made in duplicate, and the analyses were performed in triplicate. The significant differences were analyzed by analysis of variance (ANOVA) and Tukey's test for comparison of means, with 95 % confidence level ($p<0.05$) using the software STATISTICA 14 Trial (Statsoft). The results were expressed as mean ± standard deviation.

Results and discussion

Physicochemical characterization of the yogurts

Figure 1 shows the fermentation kinetics of the formulations YC, Y-HD, and YEn-HD. The pH decreased with the increase in lactic acid levels, for the three formulations studied, indicating that the addition of HD and En-HD to the yogurt formulations did not affect the fermentation time, thus with no effect on the development of starter cultures.

All formulations (Table 1) showed an increase in titratable acidity and lactic acid bacteria counts ($p<0.05$) during the storage. This behavior is due to the metabolic activity of the bacteria, which can grow even after cooling the product, as well as the degradation of macromolecular substances in the yogurt (18). No differences were observed between formulations ($p<0.05$), indicating that the supplementation did not affect the bacteria growth, as also observed during fermentation of yogurt. Similar results were found by De Moura *et al.* (5), who studied the addition of encapsulated hibiscus extract to yogurt and observed an increase of 1 log cycle after 30 days of storage, showing a positive effect of the bioactive compounds in maintaining the bacteria viability.

Figure 1: Fermentation kinetics of the control yogurt (YC), yogurt with the addition of *H. dulcis* powder (Y-HD), and yogurt with the addition of encapsulated *H. dulcis* (YEn-HD).

Table 1: Stability of control yogurt (YC), yogurt with addition of *H. dulcis* powder (Y-HD), and yogurt with addition of encapsulated *H. dulcis* (YEn-HD) at days 1 and 21 of storage for the parameters lactic acid bacteria counts, titratable acidity, and water-holding capacity (WHC).

	Lactic acid bacteria (log CFU/mL)		Titratable acidity (g lactic acid/100 g sample)		WHC (%)	
	Storage time (Days)					
Formulation	1	21	1	21	1	21
YC	6.6±<0.1^{aA}	9.7±<0.1^{aB}	0.8±<0.1^{aA}	1.1±<0.1^{aB}	46.2±0.7^{aA}	46.9±2.0^{aA}
Y-HD	7.2±<0.1^{aA}	9.9±<0.1^{aB}	0.9±<0.1^{aA}	1.1±<0.1^{aB}	45.1±2.6^{aA}	46.4±0.9^{aA}
YEn-HD	7.1±0.1^{aA}	9.7±<0.1^{aB}	0.9±<0.1^{aA}	1.2±<0.1^{aB}	47.6±2.1^{aA}	48.7±0.9^{aA}

Mean followed by equal lowercase letters in the column, for each storage time, and uppercase letters in the row, for the same formulation, do not differ significantly among the formulations by Tukey's test ($p<0.05$).

No significant differences were observed for WHC ($p<0.05$) between formulation (Table 1). Studies have shown that the increase in lactic acid concentration can cause the weakening of the protein network, decreasing the water-holding capacity and leading to whey syneresis, which accumulates on the product surface and can affect consumer acceptance (2). However, the present results showed that the yogurt supplementation had no impact on WHC, with small variations in the volume of whey released. Also, the changes in titratable acidity were not enough to cause significant changes ($p<0.05$) in the water-holding capacity.

The addition of HD and En-HD to the yogurt formulations did not affect ($p<0.05$) the parameters moisture, total solids (TS), ash, and protein (Table 2). No significant difference was observed for the yogurt with the addition of 1% (w/w) HD and HD-En ($p<0.05$) when compared to the control (YC), which can also be correlated with the maintenance of water-holding capacity (WHC) throughout the period studied.

Table 2: Moisture contents, total solids (TS), ash and protein contents of control yogurt (YC), yogurt with addition of *H. dulcis* powder (Y-HD), and yogurt with addition of encapsulated *H. dulcis* (YEn-HD)

Formulation	Moisture (%)	TS (%)	Ash (%)	Protein (%)
YC	88.7±0.4^{a}	11.3±0.4^{a}	0.9±0.3^{a}	4.5±0.1^{a}
Y-HD	88.1±0.3^{a}	11.9±0.3^{a}	0.8±0.2^{a}	4.4±<0.1^{a}
YEn-HD	88.3±0.1^{a}	11.7±0.1^{a}	0.8±0.2^{a}	4.4±<0.1^{a}

Means followed by the same lowercase letters in the column, for each parameter, do not differ significantly between formulations by Tukey's test ($p<0.05$).

Color profile

Color is one of the major attributes that affects the consumer perception of quality, playing a decisive role in the purchase intention (4). The addition of *H. dulcis* led to the formation of darker color in the yogurts (lower *L** values), and lower whiteness indices (HW). However, no significant differences ($p>0.05$) were observed for the same samples throughout the 21 days of storage (Table 3). The sample YEn-HD presented a lower *L** value even protected by encapsulation (only after 21 d of storage), when compared to the non-encapsulated sample. Gum Arabic and whey protein concentrate may have interacted with casein micelles, which together with fat globules, are responsible for the diffusion of incident light (25). On the other hand, the reduction of hunter whiteness with *H. dulcis* in free and encapsulated form may inhibit the diffusion of incident light in yogurt during storage, impacting the whiteness index of the product (18).

Texture profile

The texture profile analysis of the yogurts is shown in Figure 2. The texture attributes are important characteristics for the acceptance of yogurts by consumers. According to Sah *et al.* (26), the texture of yogurts is formed by casein aggregation due to pH reduction and disulfide bond formation between κ-caseins and denatured whey proteins, and further pH reduction during the storage. Hardness is the main parameter used to evaluate the texture of yogurts (27). The addition of encapsulated *H. dulcis* (HD-En) to the yogurt formulations also incorporates whey proteins and starch, which, in appropriate amounts, can increase hardness and reduce syneresis due to the formation of complexes between the denatured whey proteins and casein micelles (28). However, the present results showed that the addition of En-HD (1% w/w) did not lead to significant changes ($p<0.05$) in hardness when compared to the other formulations and storage times, possibly due to the low concentration used. This result corroborates the findings obtained for protein contents, moisture, and WHC, which are parameters that can affect the hardness.

A significant difference in adhesiveness was observed for the treatment made with YEn-HD ($p<0.05$) when compared to YC and Y-HD, indicating a certain stickiness of the yogurt (29), probably due to the coating materials used for encapsulation. Concerning the parameter elasticity, a significant difference was observed for YC ($p < 0.05$) when compared to Y-HD and YEn-HD, showing a greater ability to return to the initial condition after the shear forces were eliminated (27), which may be due to the non-addition of *H. dulcis* and the absence of bonds between the yogurt molecules, causing changes in the adhesiveness and elasticity. Regarding the parameter's cohesiveness and gumminess, no significant differences ($p<0.05$) were observed between the samples and the storage times. Thus, the supplemented formulations showed no changes in internal bond strength (cohesiveness) when compared to the control, as also observed for the parameter viscosity (gumminess) of the formulation made with 1% supplementation (29).

Table 3: Color characterization of control yogurt (YC), yogurt with addition of *H. dulcis* powder (Y-HD), and yogurt addition of encapsulated *H. dulcis* (YEn-HD) on days 1 and 21 of storage

		Storage time (days)	
Determination	**Formulation**	**1**	**21**
L*	YC	78.2 ± 2.2^{aA}	77.4 ± 0.1^{aA}
	Y-HD	71.0 ± 0.9^{aB}	70.5 ± 0.7^{aB}
	YEn-HD	70.1 ± 0.5^{aB}	68.5 ± 1.0^{aC}
a*	YC	-7.5 ± 0.3^{aA}	-7.4 ± 0.1^{aA}
	Y-HD	-7.4 ± 0.3^{aA}	-7.2 ± 0.1^{aA}
	YEn-HD	-7.0 ± 0.1^{aA}	-7.1 ± 0.2^{aA}
b*	YC	13.0 ± 0.6^{aA}	12.7 ± 0.1^{aB}
	Y-HD	13.5 ± 0.4^{aA}	13.5 ± 0.3^{aA}
	YEn-HD	$12.3\pm<0.1^{aB}$	$12.7\pm0.5a^{AB}$
HW	YC	73.5 ± 1.5^{aA}	73.0 ± 0.1^{aA}
	Y-HD	67.2 ± 0.6^{aB}	66.8±0.5aB
	YEn-HD	66.9 ± 0.5^{aB}	65.3 ± 1.2^{aB}

Mean ± standard deviation. HW: Hunter Whiteness. Means followed by equal lowercase letters in the row and uppercase letters in the column for the same analysis do not show significant difference between the samples ($p<0.05$).

Total phenolic compounds and antioxidant activity before and after the gastrointestinal digestion process

No phenolic compounds were detected in the sample YC before and after gastrointestinal digestion (Table 4). The TPC was ~85% higher in the encapsulated samples (YEn-HD) when compared to the yogurt made with the pseudofruit in free form (Y-HD), with similar behavior before and after gastrointestinal digestion. It is worth noting that Y-HD contained higher *H. dulcis* concentrations when compared to YEn-HD. Although both formulations received 1% *H. dulcis*, the encapsulated yogurt contained the pseudofruit plus the wall materials (gum Arabic and whey) and these were able to prevent lactic acid bacteria from degrading phenolic compounds during the fermentation process and also during storage and gastrointestinal digestion (30).

According to Lima *et al.* (4), phenolic compounds can provide functionality for dairy products. Such functionality is based on the ability of phenolic compounds to interact with milk proteins and form soluble complexes through multiple weak interactions between amino acid side chains and aromatic polyphenol rings. This association is a surface phenomenon influenced by factors such as the nature of the protein and polyphenol residues, temperature, and the presence of other components (e.g., sugars).

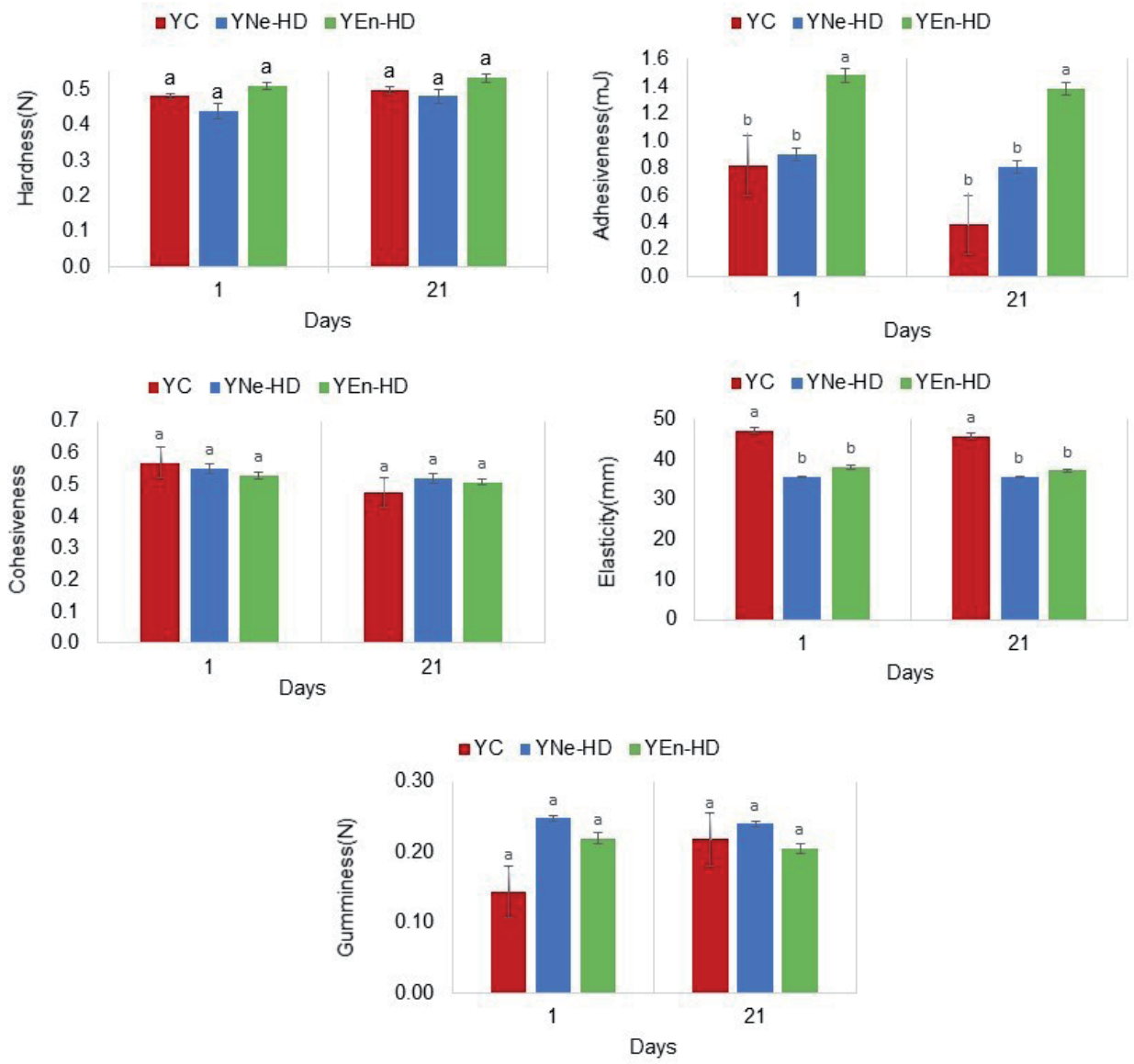

Figure 2: Texture profile analysis of the control yogurt (YC), yogurt with the addition of *H. dulcis* powder (Y-HD), and yogurt with the addition of encapsulated *H. dulcis* (YEn-HD). Cohesiveness is a dimensionless parameter. Means ± standard deviation. Values followed by the same lowercase letter in each column show no significant differences at the 95% confidence level ($p<0.05$) between storage times for each parameter.

No antioxidant activity by the ABTS assay not able to be determined in the yogurts before gastrointestinal digestion. After the process, antioxidant activity was detected in all samples, which were 83% higher ($p<0.05$) for Yen-HD when compared to Y-HD, and lower in the control sample (YC). However, during the 21-day storage, no differences were observed between the yogurt formulations after digestion ($p>0.05$). The interaction between the hydroxyl groups of the bioactive compounds from *H. dulcis* and whey protein (present in large quantities in the encapsulated sample, as it was used as the wall material) may have formed complexes, leading to structural, functional, and nutritional changes during gastrointestinal digestion, thereby improving the bioavailability of the bioactive compounds (31, 32).

The higher antioxidant activities in Y-HD and Yen-HD may be due to the higher content of phenolic compounds in these formulations, and also to the high content of polysaccharides from the pseudo fruits (ratio of 18.29), which also have the capacity to scavenge superoxide anion radicals (33). When subjected to gastrointestinal digestion *in vitro*, TPC and polysaccharides may have a positive effect on antioxidant activity due to the chemical reactions occurring during digestion that are affected by the conditions of the medium, such as types of enzymes and pH, leading to changes in molecular structure and generating new compounds with synergistic effects, thus affecting the free radical release (34).

Table 4: Total phenolic compounds (TPC), antioxidant activity by the ABTS and DPPH• assays for control yogurt (YC), yogurt with addition of *H. dulcis* powder (Y-HD), and yogurt with addition of encapsulated *H. dulcis* (YEn-HD) at day 1 and 21 of storage, before and after the gastrointestinal digestion *in vitro*.

		YC		Y-HD		YEn-HD	
		Storage time (days)					
Analysis	Digestion	1	21	1	21	1	21
TPC	Before	ND	ND	$0.5\pm<0.1^{bA}$	$0.5\pm<0.1^{bA}$	3.3 ± 0.3^{aA}	3.4 ± 0.4^{aA}
	After	ND	ND	$0.5\pm<0.1^{bA}$	$0.5\pm<0.1^{bA}$	3.6 ± 0.2^{aA}	3.5 ± 0.4^{aA}
ABTS	Before	ND	ND	ND	ND	ND	ND
	After	$2.1\pm<0.01^{c}$	$2.0\pm<0.01^{c}$	209.2 ± 2.8^{b}	209.9 ± 2.9^{b}	1227.8 ± 13.6^{a}	1238.8 ± 39.8^{a}
DPPH•	Before	36.7 ± 1.3^{bA}	27.0 ± 0.3^{cA}	54.1 ± 4.2^{aA}	50.5 ± 4.4^{aA}	49.8 ± 2.1^{aA}	48.8 ± 1.2^{aA}
	After	25.0 ± 1.9^{cB}	24.7 ± 2.0^{cA}	44.7 ± 0.7^{aB}	41.5 ± 0.9^{aB}	34.6 ± 2.3^{bB}	35.2 ± 2.0^{bB}

Mean ± standard deviation. Means followed by equal lowercase letters in the row and uppercase letters in the column for the same analysis do not show significant difference between samples ($p<0.05$). ND: not able to be determined. TPC (mgGAE/g); ABTS (mgTrolox/g); DPPH (%inhibition).

Another important factor for the higher antioxidant activity by the ABTS radical in Yen-HD is the presence of milk protein concentrate as a wall material since the degradation of milk proteins leads to the release of peptide fragments that have a number of biological effects, including antimicrobial, angiotensin-converting enzyme (ACE) inhibition, dipeptidyl peptidase IV (DPP-IV) inhibition, opioid agonist and antagonist activities, immunomodulation, mineral binding, and antioxidative functions (35).

An opposite result from the ABTS assay was observed for the DPPH• analysis, where a higher radical inhibition was observed in the DPPH• assay for the Y-HD and YEn-HD formulations when compared to YC before *in vitro* gastrointestinal digestion. The digestion process led to changes in the DPPH• radical constituents, with the highest inhibition observed for Y-HD, followed by YEn-HD and YC, with significant differences ($p<0.05$) among the samples. It is important to note that a reduction in DPPH• radical inhibition capacity after gastrointestinal digestion in the samples containing *H. dulcis* was observed (Y-HD and YEn-HD). The differences detected in the DPPH• and ABTS methods can be attributed to the solubility degree of the radicals. It is known that DPPH• has higher solubility in organic solvents such as ethanol and methanol, and in this study, the extract obtained through yogurt centrifugation was used, which may have resulted in a false positive DPPH• value (36).

Conclusion

The addition of *H. dulcis* in free (Y-HD) and encapsulated (YEn-HD) forms to the yogurts led to no changes in the fermentation kinetics, proximate composition, water-holding capacity, and hardness of the different formulations. Although total lactic acid bacteria did not affect the yogurts, a significant difference in bacteria counts was observed after 21 days of storage, due to the metabolic action of these bacteria that continue to act on the product during refrigerated storage. The yogurts with the addition of Y-HD and YEn-HD showed a decrease in yellowness index and increase in bioactivity, mainly after *in vitro* GI digestion, with a significant increase in the antioxidant activity determined by the ABTS assay when compared to the control, and the more pronounced bioactivity was observed for YEn-HD. This study showed an effective strategy for the production of supplemented yogurt using a simple microencapsulation technology. However sensory studies of the supplemented yogurts are necessary to confirm the consumers' acceptance of the products.

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