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Fermented milk supplemented with pequi oil microcapsules: effects on physicochemical properties, stability, *in vitro* digestion, and bioaccessibility

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Abstract

Pequi oil presents high levels of unsaturated fatty acids and beta-carotene and has been used for nutraceutical purposes. Microcapsules can improve the protection of bioactive compounds in pequi oil and mask the inherent smell and taste for food applications. However, the stability of the microencapsulated oil and its influence on the product characteristics need to be investigated. This work evaluated the impact of pequi oil microcapsules on the physicochemical characteristics of fermented milk and other aspects of stability, digestion, and bioaccessibility via the analysis of beta-carotene present in the oil. During 28 days of storage, the pH values decreased from 4.5 to 4.32 for all fermented milk. Syneresis values decreased (<50%) due to water retention caused by the biopolymers on the microcapsules. Microencapsulation promoted an improvement in oil stability and a gradual release for 120 min, which allowed a better condition for beta-carotene quantification in the micellar phase and resulted in better bioaccessibility. Thus, the microcapsules positively influenced the physicochemical properties of the fermented milk and improved oil bioaccessibility aspects.

Keywords: complex coacervation; *Caryocar coriaceum*; beta-carotene; biopolymers; dairy products.

Practical Application: Microencapsulation positively influenced the fermented milk's physicochemical properties, enhanced beta-carotene stability, and increased beta-carotene bioaccessibility.

1. Introduction

Fermented milk is a product that results from the addition of suitable bacteria to usually heat-treated animal milk, followed by incubation to reduce the pH, with or without coagulation pretreatment. The most famous examples of fermented milk are yogurt, buttermilk, and kefir, although variations of these products are found based on historical practices, geography, and type of milk (Savaiano & Hutkins, 2021).

Because of the high consumer acceptance of fermented milk, it can be excellent to ensure and improve the daily intake of nutrients, thereby causing positive impacts on the health of consumers (Silva et al., 2018). However, fermented milk is usually not considered a rich source of bioactive compounds (Ozturkoglu-Budak et al., 2016), and adding these compounds can be very beneficial in enriching the nutritional value of these products.

Pequi oil has stood out as a natural plant material for the food and pharmaceutical industry due to its excellent qualities associated with high levels of unsaturated fatty acids, predominantly oleic (60.6%) and carotenoids (Pinto et al., 2018). Beta-carotene is found in pequi oil (about 270 μL g^{-1}) and is an essential vitamin A source. It has a crucial role in human health, such as improving immunity, decreasing the risk of heart disease, and increasing gastrointestinal functions (Geng et al., 2022). Pequi has excellent potential to be explored as a source of nutrients (Magalhães et al., 2019), despite its strong aroma and smell. For this reason, using microencapsulation methods can mask the strong flavor of the pequi oil, and recent studies have used the microencapsulation technology to boost the stability and bioaccessibility of beta-carotene (Niu et al., 2020; Šeregelj et al., 2021).

In complex coacervation, the associative electrostatic interactions of two oppositely charged polyelectrolytes, initially soluble in water, undergo reciprocal and reversible complexation upon temperature and/or pH changes, producing a complex of insoluble hydrophilic macromolecules (González-Monje et al., 2021). Animal proteins, plant proteins, and polysaccharides are widely used as polyelectrolytes during the complex coacervation process (Muhoza et al., 2021). Previously, microparticles of pequi oil with cashew gum/gelatin and cashew gum/chitosan were studied separately and showed favorable physical and physicochemical characteristics, e.g., small particle sizes (<10 μm) favor sensory aspects, good retention capacity, high efficiency, and good stability of the wall material against stress conditions (temperature and pH) (Nascimento et al., 2020; Silva et al., 2018; Silva et al., 2022). The composition of wall material used in the formation

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of microcapsules plays a crucial role in the physicochemical, rheological, morphological, microbial, and sensory properties of the final product (Gumus & Gharibzahedi, 2021), as well as the chosen food matrix influences the stability and bioaccessibility of the bioactive compound of interest (Donhowe et al., 2014).

Microencapsulation is a promising technology that promotes the dispersion of hydrophobic compounds, such as carotenoids, in water-rich food matrices, thus improving their bioaccessibility (Campo et al., 2019). Bioaccessibility is considered as the fraction of micronutrients initially present in the food, which is solubilized in the intestinal lumen and consequently, it would be susceptible to be taken up by enterocytes. It can be easily obtained by subjecting the food to a process of gastrointestinal digestion *in vitro*, similar to that in the human gastrointestinal tract (Grenha et al., 2023). According to Timilsena et al. (2017), the physicochemical properties of microcapsules, such as their size, interfacial characteristics, and degree of lipid crystallization, significantly affect the rate and extent of lipolysis in the gastrointestinal tract.

Because of our interest in using microencapsulated pequi oil in food products, in this work, we evaluated the influence of the addition of pequi oil microcapsules on the physicochemical and morphological properties of fermented milk; the stability of the encapsulated and non-encapsulated bioactive compounds in the fermented milk during storage, and the release profile during *in vitro* gastrointestinal digestion and the bioaccessibility of beta-carotene with respect to the microcapsules in the fermented milk product.

2. Materials and methods

2.1. Material

Cashew gum polysaccharides were obtained from the exudate of *Anacardium occidentale* L. and collected from cashew trees in the Pacajus experimental field of Embrapa Agroindústria Tropical. Polysaccharide isolation was performed using the methodology previously described by Silva et al. (2018). The pequi oil (*Caryocar coriaceum* Wittm.) was extracted from the fruit pulp, according to Lima et al. (2019). Chitosan, of low molecular weight and 75–85% deacetylated, was purchased from Sigma-Aldrich (St. Louis, MO, USA), and bovine gelatin 225H type B (isoelectric point 5.2) was provided by Rousselot® (The Netherlands). The microorganism culture to produce fermented milk was purchased commercially from Bio Rich® (*Lactobacillus acidophilus* LA-5 1×10^6 UFC/g, *Bifidobacterium* BB-12 1×10^6 UFC/g, and *Streptococcus thermophilus*). The beta-carotene (beta-carotene; ≥93%, UV), porcine gastric mucosa pepsin (P7000), porcine bile extracts (B8631), and porcine pancreatin (P7545, 8 × USP specifications) were purchased from Sigma-Aldrich.

2.2. Microcapsules formation

The cashew gum/chitosan microcapsules with the pequi oil (CCP) and the cashew gum/gelatin microcapsules with the pequi oil (CGP) were prepared according to the methodology described by Nascimento et al. (2020) and Silva et al. (2018). The authors optimized ratios of pequi oil and polymers to produce microcapsules. An ultra turrax (IKA T-25 digital) was used for 3 min at 10,000 rpm for homogenization. The CCP was prepared from 100 mL of chitosan (0.5% w/v) with 2 g of pequi oil, which was added to 100 mL of a solution of cashew gum (11% w/v). For the CGP, 100 mL of gelatin (2% w/v) and 2 g of pequi oil were added to 100 mL of cashew gum (4% w/v). Finally, 400 mL of deionized water was added to both emulsions. After preparing the CCP and CGP emulsions, the pH was adjusted to 4.5, cooled (5 °C) overnight, and freeze-dried (Liotop LP 820) at -23 °C and pressure of 0.07 mbar.

2.3. Fermented milk preparation

UHT liquid whole milk was used to produce fermented milk according to the methodology applied by Yu et al. (2021), with modification. The milk was heated to 43 °C and added to the microorganism culture at a 0.4 g L^{-1} (w/v). The mixture was kept in an oven (43 °C) until clot formation at pH 4.5 (approximately 4 h). After coagulation, the fermented milk was cooled (4 °C) overnight to add pequi oil microcapsules and pure oil later. The fermented milk formation process (fermentation stage) was performed in a single batch. The fermented milk produced was partitioned, and pequi oil (encapsulated and non-encapsulated) was added in triplicate. Four fermented milk were obtained, where FM-C was the control-fermented milk without additions; FM-PO was the fermented milk added with 0.8% (w/w) pure pequi oil; FM-CCP was the fermented milk added with 2.4% (w/w) of CCP microcapsules; and FM-CGP was the fermented milk added with 1.7% CGP microcapsules. The calculations for additions of microcapsules and pure oil to fermented milk were done in accordance with the suggestions of the National Health Surveillance Agency (ANVISA), which recommends a daily intake of vitamin A of 600 µg for adults and 375–500 µg for children (Brasil, 2005), where 1 µg of beta-carotene is equivalent to 0.167 µg of retinol (vitamin A). Fermented milk samples were stored in a refrigerator (4 °C) and analyzed at 1, 7, 14, 21, and 28 days of storage.

2.4. Characterization of fermented milk

2.4.1. pH and syneresis

The pH of the samples was analyzed using a pH meter (mPA210, MS Tecnopon®) and a viscous liquid electrode (Analyser® 2A09E). An amount of 50 g of fermented milk samples were weighed and analyzed during the storage period in a beaker.

The amount of syneresis was measured according to the method described by Akgün et al. (2019), with modification. An amount of 20 g of fermented milk was centrifuged for 10 min at 4,000 rpm at 20 °C. The supernatant was removed and weighed. Syneresis (%) was expressed as the percentage weight of the supernatant over the initial weight of the fermented milk sample.

2.4.2. Colorimetry

For colorimetric analysis, all fermented milk during storage was monitored. For color measurement, each fermented milk sample (20 g) was placed in a petri dish, and the values of L*

(light), a* (red/green), and b* (yellow/blue) were obtained using a colorimeter ChromaMeter CR-400 (Konica Minolta, Sensing, INC, Japan). The total color difference (ΔE) at 28 days of storage was obtained by Equation 1.

$$\Delta E = [(\Delta L^*)^2 + (a^*)^2 + (b^*)^2]^{1/2} \quad (1)$$

2.4.3. Rheology

The viscosity of fermented milk was analyzed using a HAAKE MARS III rheometer (Thermo Scientific). The fermented milk sample was loaded onto the rheometer with the cone-and-plate geometry (C35/2° Ti L) and 0.105 mm gap with the temperature maintained at 4 °C. The viscosity flow curve was determined by logarithmically increasing the frequency from 0.1 to 100 s^{-1} and descending from 100 to 0.1 s^{-1} as controlled by the Haake Rheowin Job Manager software. The samples were conditioned for 5 min at 4 °C to recover the fermented milk structure, and the readings were carried out at 5 min intervals. Apparent viscosity was measured at a shear rate of 1 s^{-1}. The pseudo-plasticity of the fermented milk samples was determined using the following power-law model (Ostwald-de Waele) (Equation 2):

$$\tau = k(\gamma)^n \quad (2)$$

2.4.4. Morphology

Optical micrographs were obtained on a Zeiss optical microscope coupled with a digital image acquisition system using a CCD camera. Images were also obtained by transmission electron microscopy (TEM) (Model Vega 3, Tescan). An aliquot of fermented milk was placed on a carbon-coated copper grid and allowed to stand for 3 min. Then, the sample was stained with phosphotungstic acid (0.4% w/v) for 3 min, and the excess was removed with filter paper. The TEM analysis was performed using a tungsten source at an accelerating voltage of 30 kV.

2.5. Beta-carotene stability

The stability of beta-carotene in the FM-PO, FM-CCP, and FM-CGP was analyzed during the 28-day storage period. For 50 g of each fermented milk, 100 mL of 4M HCl was added to break the microcapsules (for 4 h). After that, 10 mL of hexane was added and centrifuged (15,303×*g* for 10 min at 25 °C) to capture the supernatant oil. The collected organic phase was dried under a stream of nitrogen, where an aliquot of 50 mg of the oil was redissolved in 5 mL of hexane to quantify the beta-carotene. The stability of beta-carotene was expressed as a percentage (%) by taking the ratio of the beta-carotene amount calculated over the storage time over the initial beta-carotene amount.

All analyses of beta-carotene quantification were performed with independent samples. Beta-carotene quantification was evaluated in a spectrophotometer (Varian Cary 50) at 450 nm. A standard curve was obtained as $y = 0.1536x - 0.0351$, with R^2 of 0.99 from 1 mg of beta-carotene in 100 mL of hexane (10 μg mL^{-1}). Results were expressed as μg beta-carotene g^{-1} oil sample.

2.6. Beta-carotene release profile via simulated in vitro gastrointestinal digestion

The digestion behavior of beta-carotene present in pequi oil (encapsulated and non-encapsulated) as formulated in fermented milk was evaluated using the two-stage *in vitro* static digestion model by Chen et al. (2020) and Rutz et al. (2017) with modification. In 10 g of fermented milk was added 10 mL (1:1 w/v) simulated gastric fluid (SGF) (pH 2.0, NaCl 0.15 M) containing 3.2 mg mL^{-1} of pepsin (≥250 U USP/mg) and kept in a shaker incubator (Solab SL222) at 150 rpm, 37 °C for 1 h. After SGF incubation, the mixture was immediately adjusted to pH 7.0 using 2.5 M NaOH and 20 mL (1:1 v/v) of simulated intestinal fluid (SIF) (pH 7.0, $CaCl_2$ 5 mM) containing 8 mg mL^{-1} bile salts and 1 mg mL^{-1} pancreatin (8 U USP/mg). The mixture was incubated and shaken at 150 rpm for 2 h at 37 °C.

In the SIF step, beta-carotene release was analyzed at five different time intervals (0, 30, 60, 90, and 120 min). Hexane (5 mL) was added, and the mixture was vortexed for 1 min to extract the beta-carotene. Subsequently, the samples were centrifuged at 1,057×*g* for 3 min. The procedure was performed in three cycles for complete beta-carotene extraction. The hexane-containing phase was collected and dried (the volume of hexane was adjusted to 5 mL) under a stream of nitrogen. Beta-carotene released during SIF digestion was expressed as a percentage (%), corresponding to the ratio of the beta-carotene amount calculated over time to the initial beta-carotene amount.

2.7. Bioaccessibility of beta-carotene after digestion

Bioaccessibility, the micellization rate of beta-carotene after digestion, was calculated according to the method described by Chen et al. (2020) with modifications. The material submitted for complete digestion in two stages was collected and centrifuged at 15,303×*g* for 30 min at 4 °C. It was separated into three phases after centrifugation, namely, the oil phase, the aqueous phase (micellar phase), and the sediment phase. The aqueous phase was collected and filtered. The beta-carotene in the micellar phase was extracted with methanol and hexane in a 2:3 (v/v) ratio, vortexed for 30 s, and centrifuged (15,303×*g* for 4 min). The upper yellow organic phase was removed and transferred to a glass tube. The extraction process was repeated three times to ensure the complete extraction of beta-carotene from the aqueous phase. The collected organic phase was dried under a stream of nitrogen and redissolved in 5 mL of hexane. Bioaccessibility was calculated according to Equation 3:

$$\text{Bioaccessibility (BC)}\% = \frac{\text{BC concentration in micelles}}{\text{BC concentration in digesta}} \times 100 \quad (3)$$

where:

BC concentration in micelles: the beta-carotene quantified in the micellar phase;

BC concentration in digesta: the total beta-carotene present after digestion (oil phase + micellar phase).

2.8. Statistical analysis

The results were expressed as mean±standard deviation (SD). The analyzed samples were submitted to a two-way ANOVA with a significance level of p=0.05, using Tukey's test for comparison. Statistical analyses were performed using the STATISTICA software, version 13.0.

3. Results and discussion

3.1. pH and syneresis

The effect of incorporating CCP and CGP microcapsules into fermented milk during storage was evaluated by pH, total solids, and syneresis analysis (Table 1).

The pH values ranged from 4.5 to 4.32 during the 28-day storage period. In all fermented milk studied, the pH values decreased significantly (p<0.05) in the first 14 days of storage, and maintained their values for up to 28 days. According to Estrada et al. (2011), the decrease in pH may be related to the persistent metabolic activity of microorganisms, also called post-acidification. Post-acidification or post-fermentation acidification is an unwanted process in fermented dairy products that refers to continuous acidification beyond its ideal range due to the persistent metabolic activity of the microbiota in the development during its shelf life (Deshwal et al., 2021). Baba et al. (2018) also observed a decrease in pH, especially during the first 15 days of storage, of yogurt fortified with walnut oil emulsions and linseed oil with guar gum.

As the syneresis values of the fermented milk were compared, the fermented milk samples added with microcapsules presented lower values than the FM-C and FM-PO, showing the effect of the addition of biopolymers in the fermented milk matrix. Syneresis values were not changed during the storage time after each treatment of fermented milk. The presence of biopolymers causes a stabilization of the yogurt's three-dimensional network, reducing the diffusion or separation rate of whey in yogurts (Gharibzahedi & Chronakis, 2018). Akgün et al. (2019) and Yu et al. (2021) obtained similar results and reported that the lower syneresis results were due to water retention caused by the biopolymers.

3.2. Colorimetry

The color parameters L*, a*, and b* obtained can vary from white (100) to black (0), from green (-) to red (+), and from blue (-) to yellow (+), respectively, according to the data in Table 2. A comparison of the results of L* of the FM-C and FM-PO

Table 1. Effect of the incorporation of pequi oil and pequi oil microcapsules on the pH and syneresis of fermented milk, where FM-C is the control-fermented milk, FM-PO is the fermented milk added with pequi oil, FM-CCP is the fermented milk added with pequi oil microcapsules using cashew/chitosan gum, and FM-CGP is the fermented milk added with pequi oil microcapsules using cashew gum/gelatin.

Properties	Fermented milk	Day 1	Day 7	Day 14	Day 21	Day 28
pH	FM-C	4.50±0.01^{aA}	4.40±0.01^{aB}	4.34±0.01^{abC}	4.34±0.01^{aC}	4.34±0.01^{aC}
	FM-PO	4.44±0.02^{bA}	4.36±0.01^{bB}	4.36±0.01^{bB}	4.31±0.03^{aC}	4.33±0.01^{aBC}
	FM-CCP	4.47±0.01^{abA}	4.39±0.01^{aB}	4.34±0.01^{abCD}	4.31±0.02^{aC}	4.36±0.01^{bD}
	FM-CGP	4.46±0.01^{bA}	4.36±0.01^{bB}	4.32±0.01^{aC}	4.30±0.01^{aC}	4.32±0.01^{aC}
Syneresis (%)	FM-C	59.33±0.08^{aA}	55.93±0.23^{aB}	56.25±0.41^{aB}	56.52±1.20^{aB}	57.53±1.91^{aAB}
	FM-PO	57.63±0.06^{bA}	56.22±0.03^{aA}	57.38±0.12^{aA}	57.63±0.12^{aA}	57.83±1.49^{aA}
	FM-CCP	44.28±0.43^{cA}	44.67±0.48^{bA}	46.90±0.61^{bB}	47.63±1.44^{bB}	47.73±0.76^{bB}
	FM-CGP	45.30±0.43^{dA}	44.47±1.85^{bA}	44.35±1.73^{cA}	44.33±0.66^{cA}	45.30±2.08^{bA}

Different lowercase letters show a significant difference (p<0.05) between the columns of each property. Different capital letters show a significant difference (p<0.05) between lines for each property.

Table 2. Color parameters of the fermented milk with and without microcapsules.

Color	Fermented milk	Day 1	Day 7	Day 14	Day 21	Day 28
L*	FM-C	92.19±0.77^{aA}	89.5±0.94^{aB}	96.8±0.60^{aC}	88.63±0.66^{abB}	88.66±0.36^{aB}
	FM-PO	91.37±0.59^{abA}	100.34±0.25^{bB}	95.96±0.66^{aC}	91.96±0.81^{cA}	88.57±0.44^{aD}
	FM-CCP	90.47±0.23^{bA}	98.86±0.59^{cB}	93.99±0.96^{bC}	89.4±0.50^{bA}	90.33±0.43^{bA}
	FM-CGP	90.42±0.31^{bA}	96.75±0.62^{dB}	95.25±0.98^{abB}	87.85±0.82^{aC}	90.32±0.79^{bA}
a*	FM-C	-1.24±0.05^{aA}	-1.39±0.17^{aA}	-1.36±0.43^{abA}	-1.12±0.08^{aA}	-1.03±0.32^{aA}
	FM-PO	-2.2±0.14^{bA}	-1.96±0.25^{bA}	-1.71±0.36^{aAB}	-1.45±0.17^{aB}	-1.22±0.14^{abB}
	FM-CCP	-0.95±0.15^{acAB}	-0.71±0.13^{cA}	-0.81±0.18^{bAB}	-1.17±0.3^{aB}	-1.68±0.1^{bC}
	FM-CGP	-0.95±0.18^{cAB}	-0.59±0.3^{cA}	-0.94±0.27^{bAB}	-1.24±0.34^{aB}	-1.48±0.25^{abB}
b*	FM-C	13.3±0.52^{aA}	14.61±0.2^{aB}	12.47±0.40^{aA}	15.12±0.07^{aB}	15.45±0.52^{abB}
	FM-PO	15.8±0.94^{bAB}	15.59±0.99^{aAB}	14.55±0.69^{bA}	15.27±0.36^{aAB}	16.7±0.67^{aB}
	FM-CCP	14.34±0.47^{acA}	15.29±0.33^{aA}	15.04±0.28^{bA}	14.45±0.75^{aA}	12.08±0.63^{cB}
	FM-CGP	14.73±0.29^{bcA}	15.56±0.85^{aA}	14.39±0.94^{bA}	12.46±0.66^{bB}	14.23±0.98^{bA}

Different lowercase letters show a significant difference (p<0.05) between the columns of each property; Different capital letters show a significant difference (p<0.05) between lines for each property; FM-C: the control-fermented milk; FM-PO: the fermented milk added with pequi oil; FM-CCP: the fermented milk added with pequi oil microcapsules using cashew gum/chitosan; FM-CGP: the fermented milk added with pequi oil microcapsules using cashew gum/gelatin.

showed a significant decrease ($p<0.05$) during the storage time, which differed from the fermented milk with microcapsules with better stability of the L* values. No relationship was apparent between the presence and/or absence of microcapsules and non-encapsulated bioactive compounds. Similar brightness values were found in this work, and a similar result was obtained by Comunian et al. (2017).

The a* parameter had negative results, showing a slight tendency to a greenish color. Still, the b* parameter had positive results, showing a yellowish hue, with larger values for the fermented milk with the non-encapsulated oil. The results suggested that adding microcapsules was favorable in maintaining the original color appearance of the fermented milk, as was also shown by Tan et al. (2018).

With regard to the total color difference (ΔE), values of 4.14, 3.10, 2.38, and 0.74 were obtained for the fermented milk FM-C, FM-PO, FM-CCP, and FM-CGP, respectively. The highest values obtained may be related to higher whey separation in the fermented milk without microcapsules (FM-C and FM-PO).

3.3. Morphological characterization

Micrographs of fermented milk samples were obtained by optical microscopy (Figures 1A, 1B, and 1C) and transmission electron microscopy (TEM) (Figures 1D, 1E, and 1F).

A dense structure was visualized in Figures 1A and 1E (control-fermented milk FM-C). These fermented milk samples were not submitted to mechanical stress for homogenization of the added materials. The dark areas corresponded to the protein structure in the fermented milk. The small structures observed in the fermented milk samples were related to the fat globules, characteristic of dairy products such as yogurt (Campo et al., 2019).

In Figure 1B, it is possible to observe the presence of oil in the FM-PO. The denser and more compact microstructures in the fermented milk that contained microcapsules, FM-CCP (Figure 1C) and FM-CGP (Figure 1D), proved that there was dispersion and no agglomeration of the microcapsules, as was also observed in the articles by Comunian et al. (2017) and Yu et al. (2021).

In the micrographs obtained by TEM was not possible to observe oil particles (Figure 1F) and microcapsules of FM-CCP (Figure 1G) and FM-CGP (Figure 1H). All these structures can be confused with the structures of protein and fat globules of natural fermented milk. The mechanical stress has not influenced the structure of the fermented milk, with no adverse effect on the food matrix.

3.4. Rheology aspects

Fermented milk is commonly classified as pseudo-plastic liquids ($n<1$), and one of the mathematical models that describe this type of liquid (non-Newtonian) is the Ostwald-de Waele model. As shown in Table 3, the R^2 values were above 0.9, thus indicating the applicability of this model. Regardless of the storage day, all fermented milk had fluid behavior index values (n) lower than 1 and could therefore be classified as non-Newtonian pseudo-plastic liquids. When subjected to a specific shear rate, the rheological analysis can evaluate the deformation and flow of matter. A fermented milk's apparent viscosity decreases with increasing deformation rate during shear. This result agrees

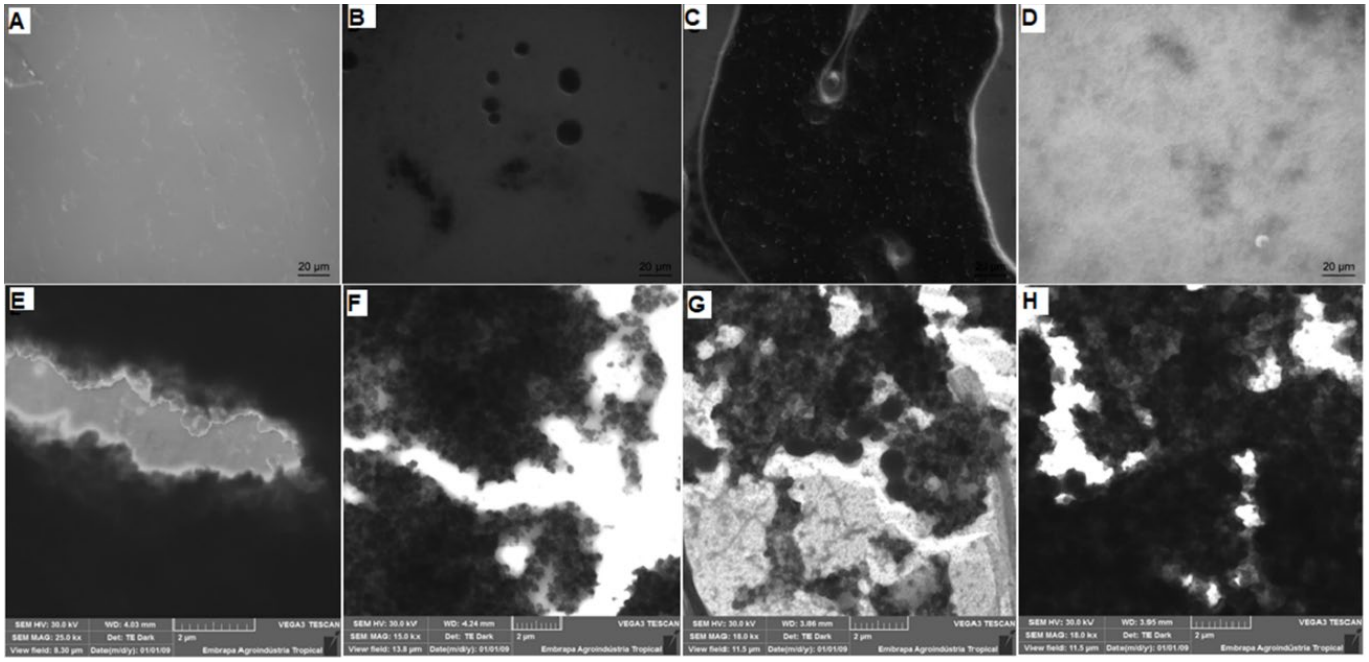

Figure 1. Optical micrographs of fermented milk, where: (A) the control-fermented milk (FM-C); (B) the fermented milk added with pequi oil (FM-PO); (C) the fermented milk added with pequi oil microcapsules using cashew gum/chitosan (FM-CCP); (D) the fermented milk added to pequi oil microcapsules using cashew gum/gelatin (FM-CGP). Transmission electron micrographs (TEM) of fermented milk, where E is FM-C, F is FM-PO, G is FM-CCP, and H is FM-CGP.

Table 3. Parameters of the Ostwald-de Waele model (*K*, *n*, and R^2) and apparent viscosity (η) calculated for a shear rate of 1 s^{-1} during storage time at 4 °C.

Parameters	Fermented milk	Day 1	Day 7	Day 14	Day 21	Day 28
K	FM-C	2.84	2.86	4.87	3.91	3.24
	FM-PO	2.45	1.19	1.16	1.47	1.26
	FM-CCP	1.26	1.74	1.51	2.21	1.8
	FM-CGP	43.97	1.27	5.09	6.14	5.22
n	FM-C	0.12	0.23	0.22	0.2	0.15
	FM-PO	0.37	0.37	0.31	0.37	0.3
	FM-CCP	0.3	0.4	0.34	0.36	0.42
	FM-CGP	0.52	0.53	0.26	0.28	0.2
R^2	FM-C	0.98	0.99	0.99	0.97	0.99
	FM-PO	0.85	0.96	0.95	0.95	0.99
	FM-CCP	0.99	0.96	0.95	0.98	0.92
	FM-CGP	0.96	0.83	0.98	0.77	0.98
η (mPa.s)	FM-C	3268	2710	5237	3820	3355
	FM-PO	7512	881,8	1240	1559	1252
	FM-CCP	1252	1970	1620	2336	2051
	FM-CGP	4380	1834	5387	7166	9613

FM-C: the control-fermented milk; FM-PO: the fermented milk added with pequi oil; FM-CCP: the fermented milk added with pequi oil microcapsule using cashew gum/chitosan gum; FM-CGP: the fermented milk added with pequi oil microcapsule using cashew gum/gelatin.

with earlier publications (Comunian et al., 2017; Tan et al., 2018) (Figure 2).

In the case of the FM-C, the apparent viscosity remained stable throughout storage at a shear rate of 1 s^{-1}. However, there was a more significant difference between the FM-PO on the first day of storage and the samples stored for 28 days, showing a negative influence of the fermented milk containing the non-encapsulated pequi oil. The FM-CCP and FM-CGP showed higher values when the results on the 1st and 28th day of storage were compared due to the addition of microcapsules, which increased the solids content of the final product. In contrast to the microcapsule added to fermented milk, the gel structure of the pequi oil-added fermented milk was weak. This eventually reduced the viscosity, increasing the fermented milk's tendency to flow, shrinking and expelling the whey (Dai et al., 2016).

3.5. Beta-carotene stability

The stability analysis of beta-carotene in pequi oil that was incorporated, with or without encapsulation, into fermented milk was performed to verify the protection of the oil during the 28 days of storage; the data are shown in Figure 3A, where all the analyzed effects obtained statistical difference ($p<0.05$). In all fermented milk, the decrease in beta-carotene concentration was more accentuated in the first 7 days of storage, followed by a more gradual reduction up to 28 days. This beta-carotene degradation in the first days of storage occurred during the decrease in pH in the fermented milk matrix, where the environment was favorable to beta-carotene oxidation. From day 1 to day 7, beta-carotene values ranged from 77.04 to 22.49, 82.94 to 38.33, and 83.66 to 33.50 for the FM-PO, FM-CCP, and FM-CGP, respectively. Šeregelj et al. (2021) suggested that the carotenoids' apolar and highly unsaturated structure made them susceptible to oxidation and isomerization during processing or storage. In this context, encapsulation technology to improve their oxidative stability is desirable for their application in foods.

The influence of microencapsulation on the protection of beta-carotene can be proven by the higher beta-carotene concentrations at all storage times when comparing FM-CCP and FM-CGP with FM-PO. At 28-day storage, beta-carotene retention was 13.26, 28.03, and 20.16 for the FM-PO, FM-CCP, and FM-CGP, respectively. Campo et al. (2019) evaluated the stability of zeaxanthin nanoparticles applied in yogurts. They observed a decrease in retention during storage time, in the range of 16–22%, thus corroborating the results obtained herein. Šeregelj et al. (2021) observed that beta-carotene content in fortified yogurt was slightly changed during the storage period, showing decreasing trends for the tested fermented milk.

3.6. Beta-carotene release profile via in vitro digestion

The beta-carotene release profile in a fluid that simulates gastrointestinal conditions is shown in Figure 3B, where all the analyzed effects obtained statistical differences ($p<0.05$). As observed, there was a considerable amount of beta-carotene in the gastric phase, but this does not compromise the bioaccessibility values, since the material was mostly transported to simulated intestinal phase. Searches of reticulation or chemical modification of the materials could be an option for future studies, to obtain better stability of the wall against gastric conditions.

The fermented milk with microcapsules had a more gradual release, different from the fermented milk with only pequi oil. The fermented milk with CGP microcapsules had lower levels of release up to 90 min in contact with SIF. However, at 120 minutes, beta-carotene release reached values similar to those of CCP microcapsules. The non-full release was due to the fraction of beta-carotene that might have been absorbed in the digesta, which will be considered in the bioaccessibility step below.

The wall materials provided different behavior of beta-carotene release of the particles. CCP microcapsules have a higher wall density because due to the surface charge of the chitosan more cashew gum is used to form the complex (Nascimento et al., 2020; Silva et al., 2022). In this case, the lower wall density of the CGP released beta-carotene faster, facilitating its digestion.

Keršienė et al. (2020) verified the release kinetics of bioactive molecules during the *in vitro* digestion of the product and compared it to the incorporation of the bioactive (loaded in double emulsion or added directly to the product). At the end of the simulated intestinal condition, a complete release (approximately 100%) of the vitamins was recorded, independent of the incorporation method. The fact that the FM-PO did not show full release during the first contact with SIF could be explained by the influence of the fermented milk matrix, which made digestion difficult. During the digestion time, the matrix was destabilized and there was an increase in oil release. Rutz et al. (2017) observed that after application in food, the release of microencapsulated carotenoids from palm oil was lower and the

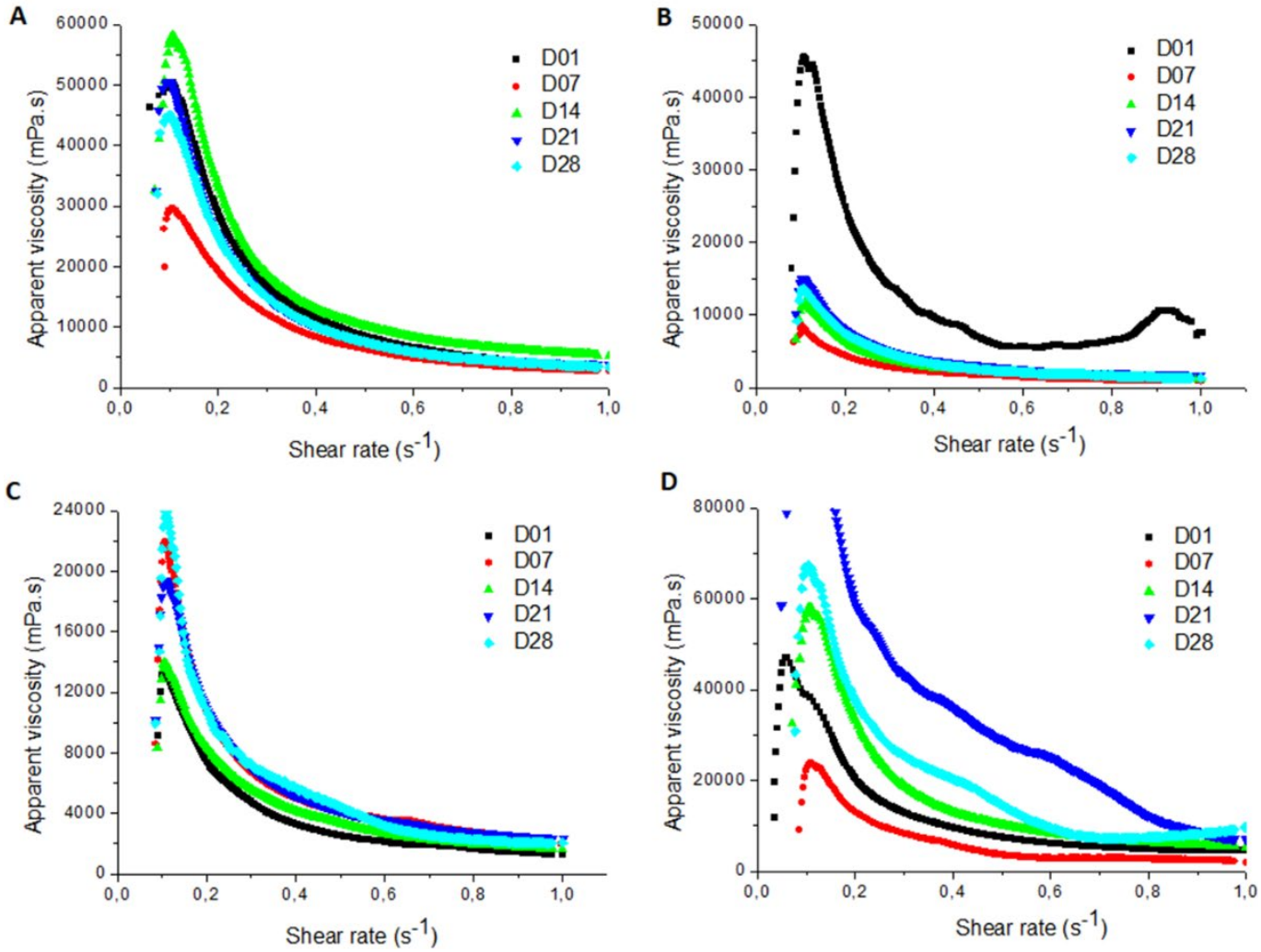

Figure 2. Apparent viscosity of: (A) the control-fermented milk, (B) the fermented milk added with pequi oil, (C) the fermented milk added with pequi oil microcapsules, using cashew gum/chitosan, (D) the fermented milk added with pequi oil microcapsules using cashew gum/gelatin, as a function of the shear rate at 4 °C.

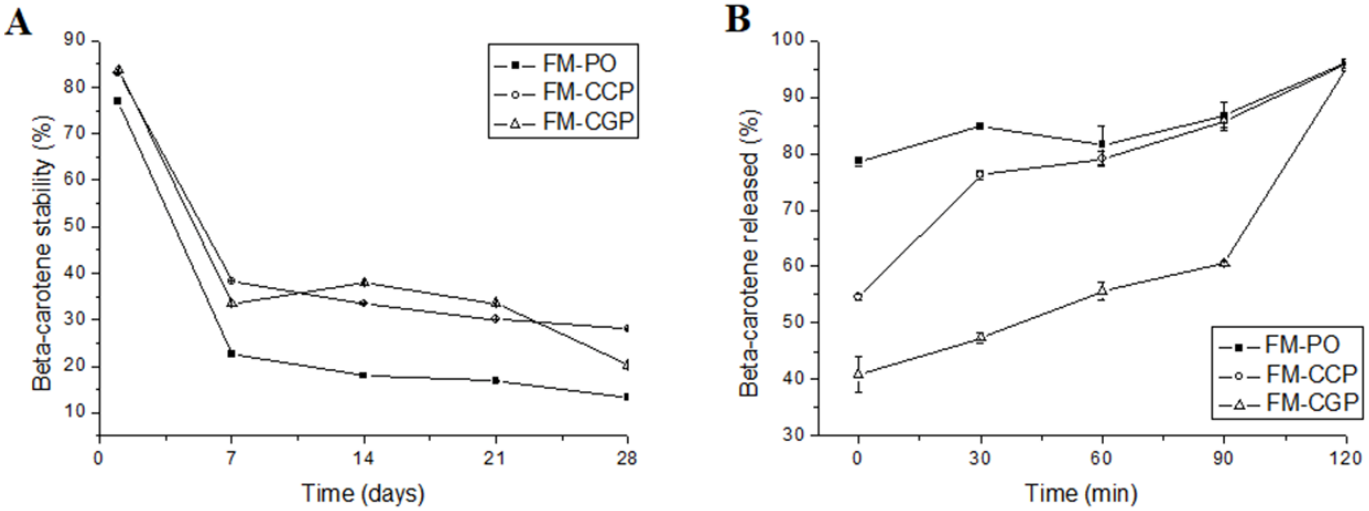

Figure 3. The concentration of beta-carotene (%) of FM-PO (fermented milk added with pequi oil), FM-CCP (fermented milk added with microcapsules of pequi oil, using cashew/chitosan gum), and F-CGP (fermented milk added of pequi oil microcapsules, using cashew gum/gelatin). (A) the stability of beta-carotene over the 28 days of storage, (B) the release profile of beta-carotene during intestinal digestion.

released compounds were not degraded, indicating that food matrices can interact with bioactive compounds in different forms and protect them from oxidation, even after the release of the microcapsules.

3.7. Beta-carotene bioaccessibility

Bioaccessibility was based on beta-carotene concentrations solubilized in the micellar fraction. The values obtained were 4.05±0.03, 4.18±0.04, and 5.08±0.01 for the FM-PO, FM-CCP, and FM-CGP, respectively. In all fermented milk studied, the bioaccessibility values obtained a significant difference ($p<0.05$). The low bioaccessibility values can be explained by the fact that the oil could barely be digested in the intestinal phase due to the large droplet size and the highly acidic environment, which can cause beta-carotene isomerization and/or oxidation (Guo et al., 2022). The gradual release of the oil favors the formation of a lower droplet and facilitates digestion in the intestinal phase, as was verified in the FM-CGP.

Several factors can influence the effectiveness of micellization of carotenoids in the intestinal phase, including physicochemical properties, chemical composition, and interaction with other compounds in the food matrix, such as proteins, lipids, and fibers (Xavier et al., 2018), which can affect digestive behavior, and reduce the release of carotenoids, and consequently their bioaccessibility (Campo et al., 2019). Donhowe et al. (2014) evaluated the bioaccessibility of microencapsulated and free beta-carotene in fermented milk and pudding. They reported that the incorporation of microencapsulated and free beta-carotene in micelles, available after digestion, was lower in fermented milk (0.8 and 5.5%) than in pudding (13.1 and 17%), which implies that the food matrix significantly influenced the decrease of the carotenoids content in the micellar phase.

4. Conclusion

The CCP and CGP microcapsules influenced the fermented milk's physicochemical properties (pH, syneresis, color, and rheology). In this study, the wall material and the oil were shown to interact with the product's other ingredients. Sometimes, this interaction brought desirable characteristics to the product, such as less syneresis. The stability of beta-carotene was considerably higher in all fermented milk with microcapsules during the fermented milk storage, proving the benefit of using microencapsulation technology to protect bioactive compounds. In the release profile via *in vitro* digestion, the oil was gradually released as observed in the FM-CGP, facilitating the absorption of beta-carotene verified by its more excellent bioaccessibility value. Thus, the microencapsulation technique employed in this work has enhanced the utility of pequi oil as a food ingredient in a dairy product like fermented milk.

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