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ORIGINAL RESEARCH

Optimization of *Pinhão* Extract Encapsulation by Solid Dispersion and Application to Cookies as a Bioactive Ingredient

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Abstract

Pinhão residues have a wide range of bioactive compounds and encapsulation can be one of the alternatives to increase their bioavailability. Thus, this work aimed to apply *pinhão* extract, pure and encapsulated by solid dispersion, in the formulation of cookies as a bioactive ingredient. For that, *pinhão* extract was encapsulated in different biopolymers (sodium caseinate, gelatin, and gum arabic) and with different shear mechanisms (sonication, Ultra-Turrax, and magnetic stirring). The best encapsulation procedure has been defined by a chemometric analysis (hierarchical cluster analysis), considering thermal properties (DSC) of particles and (+)-catechin encapsulation efficiency (HPLC). The optimized conditions were gelatin as encapsulation agent and Ultra-Turrax as shear mechanism (70.1 ± 2.8 °C maximum endothermic peak temperature and $96.0 \pm 2.3\%$ (+)-catechin encapsulation efficiency). The phenolic profile of the encapsulated extract showed the presence of (+)-catechin (0.31 ± 0.01 (mg/$g_{particle}$), protocatechuic acid (0.29 ± 0.00 mg/$g_{particle}$), and (−)-epicatechin (0.11 ± 0.00 mg/$g_{particle}$). Both the pure and encapsulated extracts were incorporated into the cookie formulation, which was characterized in terms of centesimal composition, color parameters, texture, and sensory aspects. It was found that cookies with the pure and the encapsulated extract showed significant differences concerning the centesimal composition, products added with *pinhão* extract and encapsulated extract presented higher values when compared to the control, probably influenced by the mineral content of the *pinhão.* In addition, higher hardness values were detected for cookies formulated with the encapsulated extract, which possibly negatively affected the consumer's sensory perception.

Keywords *Araucaria angustifolia* (Bertol.) Kuntze · Solid dispersion · Encapsulation · Hierarchical cluster analysis

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Introduction

The seeds of *Araucaria angustifolia* (Bertol.) Kuntze are a native product widely consumed in southern Brazil. These seeds, called *pinhão*, are collected by small producers as an alternative for sustainable income (Carvalho et al., 2020). They are rich in starch, proteins, and flavonoids, presenting high nutritional value (Brandão et al., 2019). The use of *pinhão* bio-waste as a sustainable alternative has been proposed by several research groups, which have evaluated different strategies, such as, for example, the use of sterile bracts as a natural absorbent to remove methylene blue from aqueous solutions (Matias et al., 2019) and pinhão seed coats as a source of antimicrobial extract (Trojaike et al., 2019), as well as to produce extracts with other bioactive properties (Branco et al., 2019; da Silva et al., 2014; Koehnlein et al., 2012; Peralta et al., 2016; Souza et al., 2014).

The bioactivities related to *pinhão* extracts are linked to the presence of phenolic compounds (de Souza et al. 2020; Silva et al., 2019). In the case of *pinhão* seed coat extracts, catechins are the main compounds obtained when hydroalcoholic solvent mixtures are used (Santos et al., 2018). Catechins are flavonoid compounds known to have improved antioxidant properties than α-tocopherol, butylated hydroxyanisole, or butylated hydroxytoluene (Ahmad et al., 2019). However, catechins, as other phenolic compounds, present low bioavailability, which has been partly attributed to degradation and metabolism in the gastrointestinal tract, low membrane permeability, and pre-systemic hepatic elimination (Ye & Augustin, 2019).

The encapsulation of bioactive compounds is an effective strategy to improve their stability and improve bioavailability. Other results can be achieved when encapsulation is applied to bioactive extracts or compounds, such as masking an unpleasant taste/aroma and allowing it transformed into a useful physical form, e.g., from a liquid to a powder (Ye & Augustin, 2019). According to Raddatz and Menezes (2021), the encapsulation of active compounds is an effective means of meeting the constant changes of the consumer market, allowing the food industry to create products with a functional and nutritional appeal. The size of the food encapsulation market exceeded USD 34 Billion globally in 2019, being this demand driven by the improvement of end-products shelf life along with reduced nutrient loss (Ahuja & Rawat, 2020).

The encapsulation of *pinhão* extracts has been proposed by a few authors as follows. Dorneles and Noreña (2020) used hydrolyzed pectin/collagen and partially hydrolyzed polydextrose/ guar gum as encapsulating agents. The authors obtained microparticles by spray-drying and also by lyophilization, resulting in improved stability of antioxidant capacity during storage. Fonseca et al. (2020) encapsulated the *pinhão* extract in electrospun starch fibers. Improved thermal stability, protection against harmful external elements, and control of the phenolic compound were the outstanding results.

The solid dispersion encapsulation technique is widely used to increase the solubility and bioavailability of poorly water-soluble compounds. This technique allows the formation of solid amorphous solutions, where the encapsulated compound and the carrier (encapsulating agent) are miscible and soluble. Thus, a homogeneous molecular interaction between the components of the formulation is created (Vasconcelos et al., 2007) as miscibility seems to be essential to maintain the long-term physical stability of the solid dispersion (Newman et al., 2012).

The interaction between the bioactive compounds and encapsulating agents during the encapsulation procedure can be improved by the shear mechanism (Phunpee et al., 2018). Shear forces can be generated using several different approaches, including the application of ultrasound generated by sonicators, the use of high-shear mixers (for example, rotor–stator systems), or passing through high-pressure homogenizers (Chandrapala et al., 2014).

Thus, to improve the viability of *pinhão* extract encapsulation, a systematic study is proposed in the present work, where three low-cost GRAS (Generally Recognized as Safe) encapsulating agents were applied (gelatin, arabic gum, and sodium caseinate), as well as three shear mechanisms (magnetic stirring, ultra-turrax, and ultrasound) to improve the interaction between the compounds of the extract and the encapsulating agents. The technique of encapsulation by solid dispersion was proposed, as an alternative considered easier to apply and less costly than other encapsulation techniques (Leimann et al., 2019). The lyophilized solid dispersions were evaluated concerning their thermal characteristics and encapsulation efficiency in terms of (+)-catechin. A hierarchical cluster analysis (HCA) was applied to verify similarities between the different encapsulated materials obtained and to select an experimental condition to be applied in the formulation of cookies.

Material and Methods

Materials

The seeds of *Araucaria angustifolia* (Bertol.) Kuntze were purchased in the local market of Campo Mourão, Paraná State, Brazil, in April 2018. Gelatin (bloom type, Gelita, Maringá, Paraná-Brazil), sodium caseinate (Sigma-Aldrich, SP, Brazil), arabic gum (P.A., Vetec, São Paulo, SP, Brazil), and Tween 80 (P.A., Dinâmica, Indaiatuba, SP, Brazil) were used for the encapsulation of the extract. For HPLC–DAD

analyses, methanol and acetic acid (PanReac Química, Spain), and purified deionized water (Milli-Q system-Millipore) were used. The (+)-catechin analytical standard (≥ 98% purity) was acquired from Sigma-Aldrich (São Paulo — SP, Brazil). Acetonitrile (Fisher Scientific, HPLC grade) and formic acid (Panreac Química SLU) were used in the HPLC–DAD-ESI/MSn analyses. The phenolic standards were purchased from Extrasynthèse (France) and the purified deionized water was obtained in a Milli-Q water purification system (TGI Pure Water Systems, USA). The ingredients to produce the cookies were purchased at the local market in Campo Mourão, PR State, Brazil: wheat flour, butter, salt, refined sugar, and baking powder (composed of maize starch, sodium bicarbonate, monocalcium phosphate, and calcium carbonate). The following reagents were used in the proximate composition assay: potassium sulfate (P.A., Neon, Suzano, SP, Brazil), copper sulfate II (P.A., Alphatec, São Bernardo do Campo, SP, Brazil), sodium hydroxide (P.A., Isofar, Duque de Caxias, RJ, Brazil), hydrochloric acid (P.A., Vetec, São Paulo, SP, Brazil), sulfuric acid (P.A., Fmaia, Belo Horizonte, MG, Brazil), boric acid (P.A., Neon, Suzano, SP, Brazil), sodium carbonate (P.A., Neon, Suzano, SP, Brazil), and hexane (P.A., Dinâmica, Indaiatuba, SP, Brazil). In the microbiological analyzes peptone water (KASVI, São José do Pinhais, PR, Brazil), Baird Parker agar (Acumedia, Baltimore, MD, USA), lauryl sulfate tryptose broth (Acumedia, Baltimore, MD, USA), green bile lactose broth (Difco, Sparks, MD, USA), *E. coli* broth (EC broth, Acumedia, Baltimore, MD, USA), Hektoen agar (KASVI, São José do Pinhais, PR, Brazil), and Brilliant-green Phenol-red Lactose Sucrose Agar (BPLS agar, Merck, São Paulo, SP, Brazil) were used.

Extraction and Encapsulation of Pinhão Extract

The extract was prepared as described by de Freitas et al. (2018); the pinhão was boiled in water (500 g/L) for 2 h, filtered on qualitative filter paper, and then frozen in an ultra-freezer (Liobrás, Brazil) at − 90 °C. The encapsulating conditions evaluated in the encapsulation procedure are shown in Table 1. The technique applied to encapsulate the *pinhão* extract was the solid dispersion, described by Karavas et al. (2006) with minor modifications. Briefly, the extract was thawed (50 mL) and then Tween 80 (90 mg), used as a surfactant, was added under magnetic stirring. After that, the encapsulating agent (348 mg of gelatin, sodium caseinate, or arabic gum) was added and the mixture was heated at 50 °C for 15 min to allow the encapsulating agents to solubilize. After this step, each mixture was stirred according to the conditions in Table 1. A Fischer Scientific sonicator (Loughborough, UK, 150 W, 1/8′ tip) was used for the shear acoustic cavitation mechanism, with a 30-s pulse on/10 s off for 15 min. A Fisatom (model 5B, Brazil) magnetic stirrer was used for a low-shear mixture. An Ultra-Turrax (Ika, T25, Germany) was used as the third mechanism for high-shear generated by the rotor–stator system. Finally, all the solutions were lyophilized (Liotop, Liobrás, L101, Brazil).

Encapsulated Extract Characterization

Thermal Characterization

The thermal characterization of the encapsulated extracts was performed using a differential scanning calorimeter (DSC, Perkin Elmer, 4000, USA). Samples (approximately

Table 1 Experimental conditions applied for the production of encapsulated *pinhão* extract and results obtained by DSC (T (°C)-maximum endothermic transition peak temperature) and encapsulation efficiency in terms of catechin (Catechin EE (%)). *MS*, magnetic stirring; *US*, ultrasound; *UT*, Ultra-Turrax

Encapsulating agent	Experiment code	Stirring mechanisms	Stirring conditions	T (°C)	Catechin EE (%)
Gelatin	G1	Magnetic stirring	200 rpm for 16 h	$82.1^{a} \pm 2.1$	$94.9^{a} \pm 3.2$
	G2	Ultrasound	30-s pulse on/10 s off, for 15 min with 100% amplitude	$67.7^{b,c} \pm 3.3$	$97.4^{a} \pm 2.9$
	G3	Ultra-Turrax	12,000 rpm for 15 min	$70.1^{c} \pm 2.8$	$96.0^{a} \pm 2.3$
Sodium caseinate	C1	Magnetic stirring	200 rpm for 16 h	$77.7^{a} \pm 2.5$	$94.3^{a} \pm 1.9$
	C2	Ultrasound	30-s pulse on /10 s off, for 15 min with 100% amplitude	$73.7^{a,b,c,d} \pm 1.9$	$95.3^{a} \pm 2.3$
	C3	Ultra-Turrax	12,000 rpm for 15 min	$77.4^{a} \pm 2.7$	$94.8^{a} \pm 2.4$
Arabic gum	GA1	Magnetic stirring	200 rpm for 16 h	$82.1^{a,e} \pm 2.7$	$91.5^{a} \pm 3.9$
	GA2	Ultrasound	30-s pulse on /10 s off, for 15 min with 100% amplitude	$77.4^{a} \pm 2.1$	$90.1^{a} \pm 2.5$
	GA3	Ultra-Turrax	12,000 rpm for 15 min	$77.4^{a} \pm 1.3$	$91.4^{a} \pm 3.7$

[a,b]Means in a column followed by different letters are significantly different ($p < 0.05$) by Tukey's test

5 mg) were placed in closed aluminum holding pans and analyzed in a temperature range of 0 to 200 °C under a nitrogen flow rate of 20 mL/min and a heating rate of 20 °C/min (Almeida et al., 2018). The analysis was done in duplicate for each sample.

Encapsulation Efficiency

To determine the encapsulation efficiency of the extract concerning (+)-catechin (CEE, %), particles (400 mg) were initially extracted with 50 mL of ethanol using Fischer Scientific sonicator (Loughborough, UK, 150 W, 1/8′ tip), with 30-s pulse on/10 s off for 5 min. After the extraction, the solution was filtered (0.45-μm hydrophilic PTFE filter) and the resulting solution was dried at a low temperature (≤ 40 °C) under reduced pressure in a rotary evaporator (TECNAL, TE – 211, Brazil). The dry extracts were solubilized in 300 μL of ethanol, filtered through a 0.45-μm hydrophilic PTFE filter, and subjected to HPLC–DAD chromatographic analysis in a Dionex UltiMate 3000 chromatography (Germering, Germany), equipped with a quaternary analytical pump (LPG-3400SD), autosampler (WPS-3000TSL), column compartment (TCC-3000SD), and diode array detector. The chromatographic separations were carried out using a C_{18} reverse-phase column (Acclaim 120, Germany), with 250 × 4.6 mm, 5 μm (particle), and 120 Å (porosity), coupled to a C_{18} pre-column (Phenomenex), 4 × 3.0 mm ID, at a flow rate of 1 mL/min. The autosampler was kept at 10 °C, the column compartment at 40 °C, and the detection wavelength used was 279 nm. Samples were eluted using a gradient of solvent A (acidified water_ acetic acid 1%, v/v), and B (methanol). The gradient of solvent B used was as follows: 5 − 10% (0 − 2 min), 10 − 12% (2 − 3 min), 12 − 16% (3 − 5 min), 16 − 23% (5 − 10 min), and 23 − 28% (10 − 15 min). After each separation, the column was cleaned with 100% B and re-equilibrated with the initial conditions. The calibration curve used for the quantification of (+)-catechin calibration was $y = 83.8300\ x - 0.9468$, $R^2 = 0.9993$, with a LOD = 34.9400 μg/mL and LOQ = 105.8700 μg/mL.

In the case of the aqueous *pinhão* extract, quantification and identification of the phenolic compounds were done by HPLC–DAD-ESI/MSn (Dionex Ultimate 3000 UPLC, Thermo Scientific, San Jose, CA, USA), as previously described by Bessada et al. (2016). To submit the extract to the same conditions under which the particles were treated before being analyzed in the HPLC–DAD, the extract was initially dried, after that was diluted in ethanol (400 mg in 50 mL), filtered (0.45-μm hydrophilic PTFE filter), evaporated (Tecnal, E-211, Brazil) and dried, and finally suspended in methanol:water (20:80 *v/v*, 1 mL). Separation was achieved using a Waters Spherisorb S3 ODS-2 C18 (3 μm, 4.6 × 150 mm, Waters, Milford, MA, USA) column thermostatted at 35 °C, using a gradient solvent system with (A) 0.1% formic acid in water and (B) acetonitrile. Detection was performed using a DAD (280, 330, and 370 nm as preferred wavelengths) and a mass spectrometer (MS). MS detection with electrospray ionization was performed in negative mode (Linear Ion Trap LTQ XL, Thermo Finnigan, San Jose, CA, USA). The identification of phenolic compounds was achieved by comparing retention times, UV–Vis, and mass spectra with available standard compounds. Otherwise, available data reported in the literature were applied to tentatively identify the compounds. The quantification was performed using a manual integration using a baseline to valley integration mode with baseline projection performed (Xcalibur® program, Thermo Finnigan, USA). The following calibration curves were used for quantification: (+)-catechin ($y = 84950x - 23{,}200$, $R^2 = 1$; LOD = 0.17 μg/mL; LOQ = 0.68 μg/mL), (−)-epicatechin ($y = 10314x + 147{,}331$, $R^2 = 0.9994$; LOD = 0.15 μg/mL; LOQ = 0.78 μg/mL), and protocatechuic acid ($y = 214168x + 27{,}102$, $R^2 = 0.9999$; LOD = 0.14 μg/mL; LOQ = 0.52 μg/mL). Results were used for the encapsulation efficiency determination in terms of (+)-catechin.

Finally, the results were used to determine (+)-catechin encapsulation efficiency (CEE) according to Eq. (1), where C_T is the total concentration of (+)-catechin present in the extract used to prepare the particles according to HPLC–DAD-ESI/MSn analysis ($mg/g_{extract}$), and C_F is the concentration of free (+)-catechin extracted from particles determined by HPLC–DAD ($mg/g_{extract}$) (Pillai et al., 2012). Samples were analyzed in triplicate.

$$CEE(\%) = 100 - \frac{(100 \times C_F)}{C_T} \tag{1}$$

Enrichment of Cookies with Encapsulated Pinhão Extract

Cookie Production

The optimized particle formulation was morphologically characterized by transmission electron microscopy (TEM; JEOL model JEM 2100, 200 kV, USA). Samples diluted with ethanol were dripped onto 300 mesh copper grids coated with carbon and submitted to analysis.

Three cookie formulations were prepared as described by de Almeida et al. (2018), with modifications. A control formulation (C, without adding encapsulated or pure extract) was prepared, one with the addition of 10% of encapsulated extract concerning the total weight of the wheat flour (EE) and the other with the incorporation of the equivalent of pure extract to the weight of extract present in the added particles (PE). To produce the cookies, flour (111 g), salt (1.05 g),

sugar (50 g), and baking soda (2.50 g) were initially mixed manually. Then, the particles were added with encapsulated extract (11.1 g), or the pure extract (1.49 g), being mixed for 3 min. After that, butter (33.75 g) was added and mixed for another 3 min. In the sequence, water was incorporated (20 mL for control and 25 mL for EE and PE due to the increase in the solids content in these formulations), and the mixture was homogenized for 3 min. The dough was spread with the aid of a roller, with a standardized thickness (5 mm) and then cut into discs of 50 mm in diameter. Finally, the cookies were baked in an oven (Tedesco, FTT 240E, Caxias do Sul, RS, Brazil) at 170 °C for 15 min. The cookies were cooled to room temperature, then packed in polyethylene bags (20 units per package), and stored at 25 °C.

Weight Loss, Texture Measurements, and Color Determination

Weight loss (WL) of the cookies was calculated using Eq. (2), where W_{dough} is the weight (g) of the cookie dough before baking, and W_{cookie} is the weight (g) of the cookies after baking (Rodríguez-García et al., 2014).

$$WL(\%) = \left(\frac{W_{dough} - W_{cookie}}{W_{dough}} \right) \times 100 \quad (2)$$

Mechanical testing was performed first in the cookie dough (Texture Profile Analysis test, TPA) and also in baked cookies (hardness test), both using a Stable Micro Systems texturometer (TA-XT Express model, Godalming, Surrey, UK). To assess the cookie dough, TPA was applied to dough cylinders (50 mm in diameter × 5 mm in height) equipped with a 10-kg load cell and using a 34-mm cylindrical probe (P/34), following the method described by Venturini et al. (2018), with minor modifications. Ten samples of cookie dough were analyzed for each treatment (C, EE, and PE), with a compression of 25% of samples height, a speed test of 5 mm/s, and an interval of 5 s between compressions. Parameters evaluated were hardness (N), adhesiveness (N.s), springiness (mm), cohesiveness (dimensionless), and resilience (dimensionless).

The hardness test was applied to evaluate baked cookies also as described by Venturini et al. (2018). Initially, the thickness of baked cookies was measured on a digital caliper (Ford, Brazil), then the cookies (10 samples for each treatment and storage time: 0, 15, and 30 days) were compressed to 50% of their height using a 2-mm cylindrical probe (P2) with 10 (mm/s) of compression speed. The puncture force is the highest force (N) detected in the test.

For color analysis, ten samples of each treatment were evaluated concerning the parameters L^* (luminosity), C^* (Chroma), and $h°$ (Hue angle) with the Delta Vista 450G colorimeter (Delta Color, Brazil) with standard illuminant D65 and standard observer of 2°. The samples were evaluated at time intervals of 0, 15, and 30 days.

Proximate Composition of the Cookies

Moisture was determined by the gravimetric method, at 105 °C (air circulation oven, Cienlab, CE220/1152, Brazil) until a constant weight was reached. In the analysis of the ashes, the cookies were incinerated in a muffle at a temperature of 550 °C (muffle, Nova Ética NT380, Brazil). The Microkjeldahl method (TE-0364, Tecnal, Brazil) was used to obtain the protein content, and the lipid determination was done by the Soxhlet method (Marconi MA 044/5/50, Brazil) (Lutz, 2008).

Microbiological Quality and Sensory Analysis

The cookies were submitted to microbiological analysis of coliforms at 45 °C, using *Salmonella* sp. and coagulase-positive *Staphylococcus aureus* as required by the ANVISA resolution (Brazil, 2001).

The sensory tests were performed at the Sensory Analysis Laboratory of the Federal Technological University of Parana (UTFPR), under the approval of the Research Ethics Committee of the same university, under protocol number 13799119.9.0000.5547. The cookie samples were sensorially evaluated by the ranking preference test (ISO, 2006) by 63 untrained assessors (29 male and 34 female), aged between 17 and 49 years old, involving students and employees of the institution. Three samples (C, EE, and PE) were randomly presented, and the assessors were asked to order them in decreasing order of overall preference. Cookies were served on white plates coded with three random digits, in random order. The assessors were asked to drink water before testing the samples. In the analysis of sensory data, a rating of 1 was assigned to the most preferred sample and a rating of 3 to the least preferred cookie.

Statistical Analysis

Hierarchical cluster analysis (HCA) was performed using MATLAB R2008b (MathWorks Inc., Natick, MA). The objective was to identify similarities between the results of the experimental conditions tested. The results obtained with the thermal characterization (maximum endothermic transition peak temperature) and encapsulation efficiency were placed in columns and the experimental runs in rows. Ward's method based on Euclidean distance was used for cluster formation and sample pattern recognition (Granato et al., 2018; Šoronja Simović et al., 2017). The results obtained for extract composition (HPLC–DAD-ESI/MSn), cookie texture, color, and proximate composition were evaluated using analysis of variance (ANOVA), and the averages of the

results were compared using Tukey's test at a significance level of 5% ($p < 0.05$) using the software Statistica 7.0 (Statsoft, USA). In the sensory tests, a Friedman test ($p \leq 0.05$) was used to estimate whether there were significant differences, and tables of the maximum distance between the samples (Christensen et al., 2006) were used to compare these rank sums.

Results and Discussion

Characterization of Extract and Microencapsulated Extracts

The phenolic profile determined for the *pinhão* extract is shown in Table 2. Three compounds were identified by comparing the retention time, maximum absorption wavelengths in the visible region, and mass spectral data with the available standard compounds. (−)-Epicatechin was the major compound found in the extract. The total phenolic content of the extract was equal to 2.43 mg/mL, lower than those reported by de Oliveira (2021) (4.50 ± 0.06 mg/$g_{extract}$), de Souza (2020) (6.42 ± 0.01 mg/$g_{extract}$), and da Silva et al. (2019) (8.25 ± 0.05 mg/$g_{extract}$). With the statistical analysis, it was found that there was a statistical difference between the concentrations of protocatechuic acid and (−)-Epicatechin ($p < 0.05$). On the other hand, the concentration of (+)-Catechin was equivalent to the other compounds ($p > 0.05$).

The thermograms of *pinhão* extract microencapsulated in gelatin, sodium caseinate, and arabic gum are shown in Fig. S1(a, b, and c), respectively. The maximum endothermic peak temperatures are presented in Table 1.

In the DSC thermograms, the maximum temperature of the endothermic peak was recorded since the melting temperature of the encapsulating agents and the evaporation of water are overlapped in this region. Lower values of maximum endothermic transition temperatures were obtained for all the particles in which the applied shear mechanism was the sonicator and the particles produced with gum Arabic as encapsulating material presented a higher transition temperature, differing significantly from the other matrices (gelatin and sodium caseinate, Table 1). On the other hand, higher temperature results were determined for all samples submitted to magnetic stirring, and there is no significant difference between the encapsulating agents used (Table 1). This result may be associated with the cavitation-induced cleavage of macromolecular chains (Leimann et al., 2013). The sound waves generated during sonication pulses interact with gas bubbles present in the liquid, leading to their coalescence and collapse. This cavitation process results in the generation of high temperatures within these bubbles (Ashokkumar et al., 2007). Samples processed by Ultra-Turrax presented similar transition temperatures to sonicated samples. This may also be attributed to the high shear physical mechanism, which can generate shear force orders of magnitude higher than overhead stirring (Chandrapala et al., 2014).

Encapsulation efficiency results are also shown in Table 1. The results revealed that for all the encapsulating agents, as well as the shear mechanisms used, the encapsulation efficiencies were at least 91.4% and did not differ statistically ($p < 0.05$) about the encapsulating matrix and agitation mechanism used in the production of particles. Jain et al. (2015) when developing microcapsules using Whey Protein Isolate and acacia gum by the complex coacervation method to encapsulate beta-carotene, achieved an encapsulation efficiency close to 77.3%, a lower value than that achieved by the solid dispersion technique used in the present study.

Selection of Encapsulation Conditions

Figure 1 presents the similarities between the experimental conditions tested found by the hierarchical cluster analysis (HCA). It is possible to observe that four groups were formed considering a dissimilarity equal to 0.3. All experiments with sodium caseinate as the encapsulating agent were grouped (C1, C2, and C3). Furthermore, among these samples, the experiments submitted to magnetic stirring and Ultra-Turrax were the most similar among all samples (C1 and C2). This is an indication that sodium caseinate was the least encapsulating agent influenced by the shear conditions in encapsulation efficiency and thermal properties.

Table 2 Retention time (Rt), maximum absorption wavelengths in the visible region (λmax), mass spectral data, tentative identification, quantification (concentration $mg_{compound}/g_{extract}$) of the phenolic compounds, and total phenolic content (TPC) present in *pinhão* extract

Peak	Rt (min)	λmax (nm)	$[M-H]^-$ (*m/z*)	MS^2 (*m/z*)	Tentative identification	C ($mg/g_{extract}$)
1	5.61	293	153	109 (100)	Protocatechuic acid	$0.74^{a} \pm 0.17$
2	7.16	280	289	245 (100), 203 (6), 137 (5)	(+)-Catechin	$0.78^{a,b} \pm 0.01$
3	9.67	280	289	245 (100), 203 (10), 137 (5)	(−)-Epicatechin	$0.92^{b} \pm 0.31$
					TPC	2.43 ± 0.47

[a,b]Means in a column with different letters are significantly different ($p < 0.05$) by Tukey's test

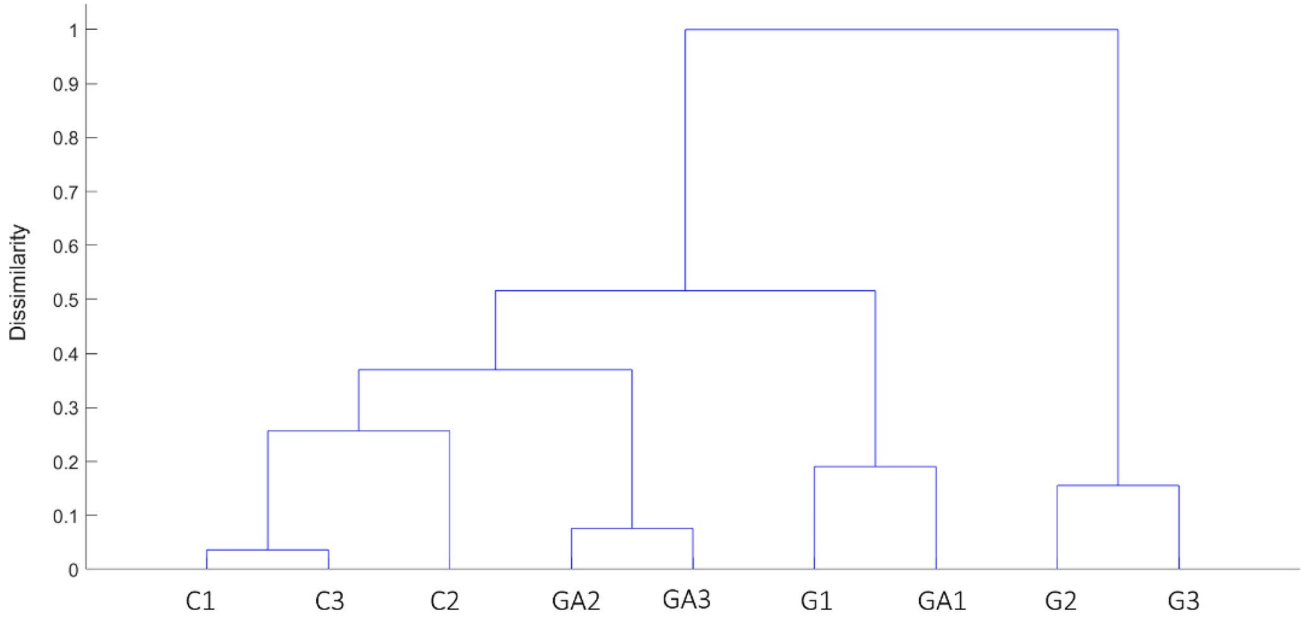

Fig. 1 HCA dendrogram of experimental data of thermal transition temperature and encapsulation efficiency

On the other hand, for gelatin and arabic gum, the dissimilarities were evidenced by the clustering of samples prepared with magnetic stirring in one group (G1 and GA1), and two groups related to each encapsulating agent: G2 and G3; GA2 and GA3. This result evidence that for these encapsulating agents, only the shear mechanism by magnetic stirring affected the evaluated responses.

Based on the hierarchical cluster analysis, the experimental condition with gelatin as encapsulation applying Ultra-Turrax as shear mechanism (G3 sample) was chosen to be applied in the formulation of cookies.

Application of Microencapsulated Pinhão Extract in the Formulation of Cookies

The gelatin particles containing *pinhão* extract, produced with Ultra-Turrax (G3), were characterized in relation to the phenolic profile of the encapsulated extract. The results showed the following profile: protocatechuic acid = 0.29 ± 0.00 ($mg/g_{particle}$), (+)-catechin = 0.31 ± 0.01 ($mg/g_{particle}$), (−)-epicatechin = 0.11 ± 0.00 ($mg/g_{particle}$), and total phenolic compounds = 0.71 ± 0.00 ($mg/g_{particle}$).

The produced particles were also characterized in terms of their morphology and size by transmission electron microscopy (TEM) and the images are presented in Fig. 2A. It is possible to observe a needle-shaped structure with a size in the range of 50–150 nm. Encapsulated formulations can be presented either with spherical geometry (single-particle structure), with irregular geometry (aggregated structure) (Jain et al., 2016), or even more complex, depending on the encapsulation technique, encapsulating material, shear forces, pH, etc. which are applied to the encapsulating system (Gharieh et al., 2019; Silva et al., 2017).

The gelatin particles containing *pinhão* extract as well as the crude extract were applied to cookie formulations. The results of the texture profile analysis (TPA) applied to cookie dough are shown in Table 3.

It is possible to observe that the hardness parameter showed a significant difference ($p < 0.05$) among all treatments. The hardness result of cookies added with the encapsulated extract was approximately twofold higher than the result obtained for the control formulation. For cookies added with unencapsulated *pinhão* crude extract, this effect was less evidenced. Wang et al. (2007) found that the addition of green tea extract to bread formulations increased hardness and adhesiveness. The interactions between phenolics with proteins and starch affect the physical and rheological properties of wheat doughs (Xu et al., 2019). In the present study, there was no significant difference in adhesiveness for the PE sample when compared to the control, and cohesiveness also increased. For the dough added with encapsulated *pinhão* extract, there was a decrease in adhesiveness and an increase in springiness and cohesiveness. Yu et al. (2019) added pigskin gelatin to bread formulations and noted that gelatin induced the dough to be less stiff and softer. In addition, the authors found that an increase in the level of gelatin gave rise to an increase in dough water absorption, due to more hydrogen bonding interactions between water and hydroxyl groups in the gelatin structure.

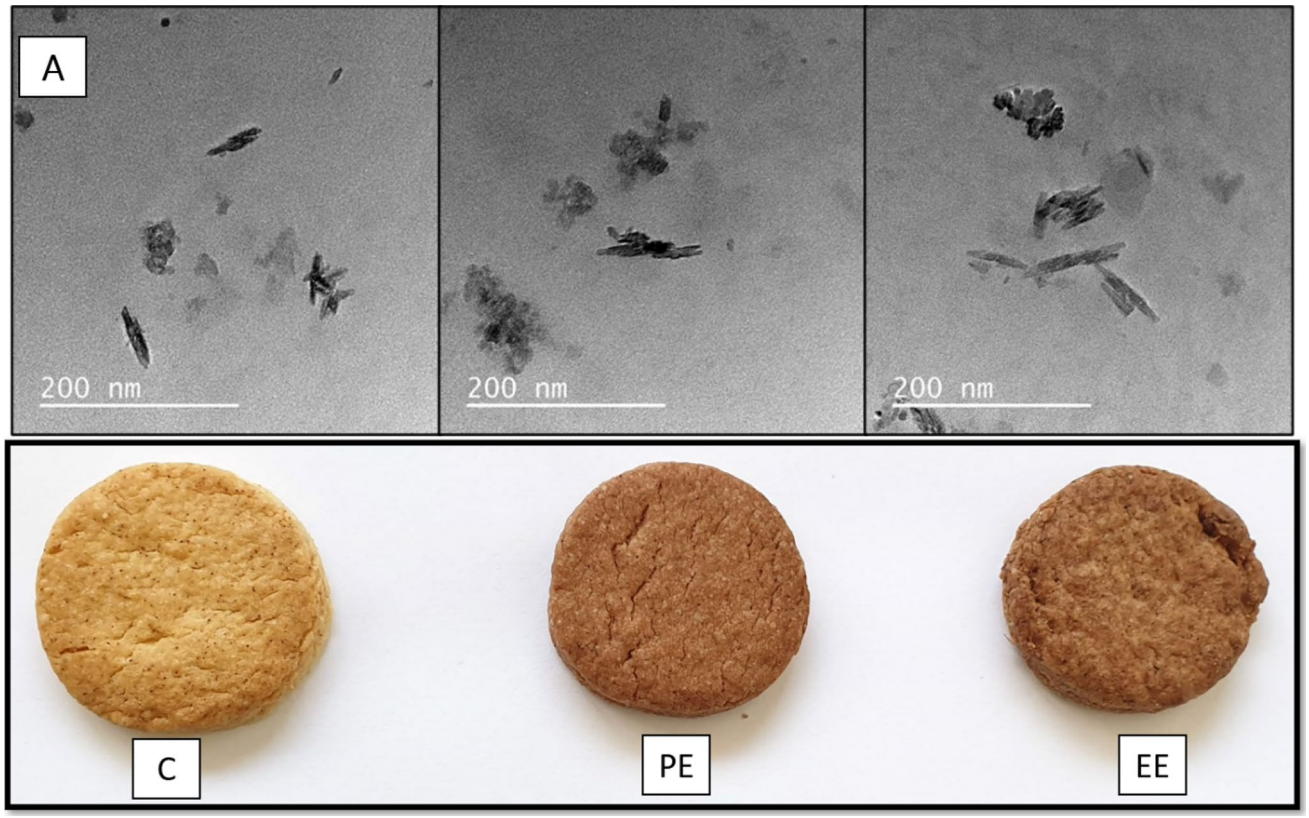

Fig. 2 **A** Transmission electron microscopy images of gelatin particles produced with encapsulated *pinhão* extract (experiment G3); Images of the cookies produced: C — control formulation; EE — addition of encapsulated *pinhão* extract; PE — addition of crude *pinhão* extract

In the present work, a larger amount of water had to be added to the EE cookie formulation, due to the presence of gelatin.

The only significant differences ($p < 0.05$) were found among the proximate composition parameters for ash and moisture content. Cookies added with crude *pinhão* extract and encapsulated extract presented higher values for both parameters when compared to the control. This behavior may be related to the presence of mineral content in the *pinhão* extract (Biel et al., 2020), as well as to a greater water retention capacity of both, crude and encapsulated extract (due to gelatin). This was confirmed by the weight loss since the results were statistically equal for all treatments ($p > 0.05$), and the moisture content was higher for cookies added with crude *pinhão* extract and encapsulated *pinhão* extract compared to control, suggesting a higher load of water.

The results of puncture force showed that the formulation prepared with the addition of encapsulated *pinhão* extract (EE) resulted in harder cookies. In addition, during the first 15 days of storage, there was a significant increase ($p < 0.05$) in the puncture force of 1.6-fold for cookies added with crude *pinhão* extract (PE) and encapsulated *pinhão* extract (EE). This result is probably related to moisture loss during storage (Piga et al., 2005).

Barros et al. (2020) when analyzing cookies formulated with cocoa shells, also noticed an increase in hardness. The authors stated that water-absorbent compounds, such as fibers and proteins, can contribute to the sticky nature of the dough, reducing its extensibility and increasing hardness, especially after baking. Rocha Parra et al. (2019) realized that the product showed greater hardness by partially replacing wheat flour with apple pomace in the formulation of cookies.

Table 3 Texture profile analysis (TPA) applied to cookies dough (*C*, control formulation; *EE*, addition of encapsulated *pinhão* extract; *PE*, addition of crude *pinhão* extract)

TPA parameter	Formulation		
	C	PE	EE
Hardness (N)	$24.68^{a} \pm 4.08$	$32.32^{b} \pm 1.99$	$49.28^{c} \pm 5.65$
Adhesiveness (N.s)	$-0.71^{a} \pm 0.06$	$-0.65^{a} \pm 0.14$	$-0.11^{b} \pm 0.02$
Springiness (mm)	$0.87^{a} \pm 0.05$	$0.85^{a} \pm 0.02$	$0.97^{b} \pm 0.01$
Cohesiveness (−)	$0.61^{a} \pm 0.01$	$0.68^{b} \pm 0.03$	$0.76^{c} \pm 0.02$
Resilience (−)	$0.72^{a} \pm 0.02$	$0.72^{a} \pm 0.05$	$0.77^{a} \pm 0.04$

[a,b]Means in a row with different letters are significantly different ($p < 0.05$) by Tukey's test

The process of encapsulating the *pinhão* extract was not able to reduce the effect of the color change on the cookies, as can be seen in the images presented in Fig. 2 and results from Table 4. Regarding the luminosity (L^*), it was possible to note that cookie samples added with crude *pinhão* extract (PE) and encapsulated *pinhão* extract (EE) are darker than control cookies ($p < 0.05$). Also, there was a slight decrease in luminosity throughout storage time and the luminosity was constant over time for PE and EE cookies. The *pinhão* extract has a dark brown color, but even with the encapsulation, which could mask the color of the extract, there was a visible effect on the final color of the cookies.

Chroma results also showed that the control samples have more saturated color than cookies added with crude and encapsulated *pinhão* extracts (Wrolstad & Smith, 2017). The hue angle ($h°$) represents the attribute of visual perception according to which the color of the object appears to be similar to red, yellow, green, or blue, or also to a combination of adjacent pairs of these colors considered in the L^*C^*h color space (Minolta, 2020; Zhang et al., 2019). The red color is represented by a hue angle of 0° and yellow of 90°; thus, it can be seen that the control cookies present a tonality tending to light yellow, in contrast to cookies containing crude extract and encapsulated *pinhão* extract, which showed lower hue angle values, as well as lower luminosity results, tending to a dark red tonality.

Microbiological and Sensory Analysis

The microbiological evaluation of the produced cookies was performed to assure the safety of panelists' consumption. Results (Table S1) demonstrate that all prepared cookies are below the tolerable limits for *Staphylococcus aureus* and coliforms at 45 °C, as well as present the absence of *Salmonella* sp, as described in RDC N° 12 of January 2, 2001, and are therefore safe for human consumption.

The results of total rank sums obtained from the ranking preference test were subjected to Friedman's test ($p \leq 0.05$) and the rank of sums was compared to the tables of maximum distance between samples (Christensen et al., 2006). By this method, all possible pairs of tested samples can be compared. Statistical analysis of data from the preference ranking test revealed that all cookie samples differ significantly ($p \leq 0.05$) from each other in terms of preference. The control sample (C) was the most preferred and the EE sample was the least preferred cookie. Cookie PE had an intermediate preference. Preference was possibly influenced by the addition of particles because it significantly affected

Table 4 Proximate composition, weight loss, puncture force, color, and sensory analysis determined for cookie samples (*C*, control formulation; *PE*, addition of crude *pinhão* extract; *EE*, addition of encapsulated *pinhão* extract)

		C	PE	EE
Proximate composition				
Ash (%)		$1.32^{a} \pm 0.01$	$1.56^{b} \pm 0.01$	$1.56^{b} \pm 0.01$
Protein (%)		$0.90^{a} \pm 0.14$	$0.81^{a} \pm 0.05$	$1.15^{a} \pm 0.04$
Moisture (%)		$4.33^{a} \pm 0.02$	$4.84^{b} \pm 0.02$	$4.77^{b} \pm 0.88$
Lipids (%)		$12.99^{a} \pm 0.93$	$13.07^{a} \pm 1.02$	$12.56^{a} \pm 0.10$
Carbohydrate (%)		$80.45^{a} \pm 0.16$	$79.71^{a} \pm 0.99$	$79.95^{a} \pm 0.05$
WL (%)		$14.01^{a} \pm 0.86$	$13.06^{a} \pm 0.74$	$12.82^{a} \pm 0.58$
Puncture force (N)				
0 day of storage		$26.08^{ab,A} \pm 6.31$	$20.37^{a,A} \pm 5.43$	$33.19^{b,A} \pm 7.74$
15 days of storage		$28.04^{a,A} \pm 3.67$	$32.56^{a,B} \pm 3.59$	$50.09^{b,B} \pm 13.81$
30 days of storage		$29.07^{a,A} \pm 2.02$	$31.81^{a,B} \pm 5.20$	$49.48^{b,B} \pm 5.90$
Cookie color				
L*	0 day of storage	$70.46^{a,A} \pm 1.59$	$50.27^{b,A} \pm 1.36$	$50.25^{b,A} \pm 1.33$
	15 days of storage	$66.75^{a,B} \pm 1.93$	$49.65^{b,A} \pm 1.22$	$47.89^{b,A} \pm 1.88$
	30 days of storage	$67.66^{a,B} \pm 1.44$	$52.41^{b,A} \pm 5.83$	$50.77^{b,A} \pm 1.84$
C*	0 day of storage	$35.22^{a,A} \pm 1.27$	$26.38^{b,A} \pm 0.69$	$27.39^{b,A} \pm 1.03$
	15 days of storage	$36.84^{a,B} \pm 0.82$	$27.14^{b,AB} \pm 0.50$	$28.11^{b,AB} \pm 1.11$
	30 days of storage	$37.67^{a,B} \pm 0.63$	$29.08^{b,B} \pm 2.97$	$28.99^{b,B} \pm 1.29$
$h°$	0 day of storage	$73.74^{a,A} \pm 1.78$	$59.21^{b,A} \pm 0.69$	$60.62^{b,A} \pm 2.09$
	15 days of storage	$70.93^{a,B} \pm 0.99$	$58.99^{b,A} \pm 0.81$	$59.76^{b,A} \pm 1.12$
	30 days of storage	$69.93^{a,B} \pm 1.39$	$58.81^{b,A} \pm 3.82$	$57.87^{b,B} \pm 1.41$
Sensory analysis				
Total rank sums (Σ orders)		91^{a}	120^{b}	166^{c}

[a,b]Means in a row with different letters are significantly different ($p < 0.05$) by Tukey's test

[A,B]Means in a column with different letters are significantly different ($p < 0.05$) by Tukey's test

the textural aspects of the product, and the EE presented a higher hardness compared to PE (Table 4). Indeed, the addition of fruit and vegetable by-products may reduce the sensory acceptability of bakery products (Rocha Parra et al., 2019). Ghoshal and Kaushik (2020) when evaluating the partial substitution of wheat flour with some flour in cookies noticed a decrease in sensory acceptability, being attributed to the darker coloration that products developed, a fact that was also observed in the present study.

Conclusions

The *pinhão* extract encapsulated in gelatin and obtained with the Ultra-Turrax shearing mechanism was selected with the hierarchical cluster analysis among the evaluated formulations. The particles were incorporated into cookies, as well as the pure *pinhão* extract. These cookies presented higher values for ash and moisture content when compared to control samples, probably due to the higher mineral content of *pinhão* extract. Also, concerning the texture parameters, cookies containing the particles showed higher hardness values, possibly influencing the sensory perception of the assessors. The encapsulation of extracts from residues of *pinhão* consumption may contribute to the development of food products with high added value and bioactive properties that can bring benefits to the health of consumers. Thus, it becomes important to optimize food formulations procedures aiming to improve sensorial perception.

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Data Availability The authors declare that all data supporting the findings of this study are available within the article and its supplementary information file.

Declarations

Conflict of Interest The authors declare no competing interests.

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