Heliyon 7 (2021) e07627

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Encapsulation of bioactive compounds from byproducts of two species of passionflowers: evaluation of the physicochemical properties and controlled release in a gastrointestinal model

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ARTICLE INFO

Keywords:
Gulupa
Cholupa
Microencapsulation
Gastrointestinal digestion
Phenolic compounds
Antioxidant activity
ACE inhibitory activity

ABSTRACT

This study aimed to evaluate the release of active components with antioxidant and antihypertensive capacity from encapsulated extracts of the peel and seeds of Gulupa (*Passiflora edulis* f. *edulis*) and Cholupa (*Passiflora maliformis*) in an *in vitro* gastrointestinal digestion model. Microencapsulated extracts were prepared with enzymatically modified rice starch as the encapsulating material and ethanol extracts of seeds and peel of *P. edulis* f. *edulis* and *P. maliformis* as encapsulated material. Microcapsule characterization was performed by scanning electron microscopy with values of 4.54–5.13 μm and ξ potential values of -6.34 mV and -6.66 mV. Dynamic light scattering (DLS) analysis was conducted with polydispersion values from 1.33 to 1.51, and dispersion stability analysis was also conducted. The total phenol content and antioxidant activities (ABTS, DPPH, and FRAP) and ACE inhibitory activity (*in vitro* antihypertensive activity) were evaluated after each stage of digestion, with values greater than 80% of activity before gastrointestinal transit and with values greater than 55% activity after the end of gastrointestinal transit. Gastrointestinal evaluation of the encapsulated extracts was performed with an *ex vivo* model using pig intestines and simulating the conditions of digestion in three phases: the gastric (pH 2.0 with 1.0 M HCl +0.5 g/L pepsin), enteric (pH 8.0 with Krebs solution +1.0 mL/L bile) and final enteric (pH 7.5 Krebs solution only) phases. The microencapsulation of passionflower extracts showed good behavior against changes in pH and enzymatic activities throughout digestion, thus promoting a controlled release and targeted delivery of bioactive compounds, undergoing a paracellular mechanism through the intestinal barrier to preserve the antioxidant activity and ACE inhibitory that was shown by the extracts before encapsulation of the material.

1. Introduction

Colombia is a country with an abundant agro-industrial occupation, which annually generates a large amount of easy-to-buy and low-cost waste, among which the fruit sector stands out, which can reach 712,000 tons per year [1]. These waste materials negatively affect the environment due to their high concentration of organic matter and their inadequate final disposal. Among the most important organic waste of fruit and vegetable processing are the peel, seeds, and pulp and individual fruits that do not meet quality standards, among others [2]. It is important to note that after agroindustrial processing, approximately 70–80% of the weight of the fruits belongs to the passiflora varieties is not used by the industry, generating a significant volume of residual biomass [3].

The Passifloraceae family has approximately 700 species [4]. In America, there are five genera within this family; however, most of the family's diversity corresponds to the genus Passiflora, of which 95% are American species [5]. The main species of passionflower in terms of marketing and consumption are Maracuyá (*Passiflora edulis* f. *flavicarpa*), Granadilla (*Passiflora ligularis*), Curuba (*Passiflora tripartite*), Badea (*Passiflora quadrangularis*), Gulupa (*Passiflora edulis* f. *edulis*and*)* and Cholupa (*Passiflora. maliformis*) [6]. In Colombia, it was estimated that in 2018, more than 241,000 tons of passionflower fruits were produced among all the aforementioned species [7].

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https://doi.org/10.1016/j.heliyon.2021.e07627
Received 21 December 2020; Received in revised form 31 March 2021; Accepted 15 July 2021

The *P. edulis* f. *edulis* is between 1100 and 2750 m above sea level (m.a.s.l.) [8]. This fruit is promising and pleasant with a characteristic flavor, slightly acidic and sweet. [9], and its organoleptic and nutritional properties have allowed it to be widely used in the food industry for the production of sweets, jellies, jams, pulps and juices, among other products [10]. *P. maliformis* has been used in the food industry due to its contribution of carbohydrates and proteins, among other things because they attribute a high nutritional value to it. [11]. However, other uses have been explored, including the use of vines for skin rejuvenation and blood pressure control, and the use of the fruit as an aphrodisiac has also been reported [12].

In general, passionflowers have great potential as bioactive components with sedative, antispasmodic, antibacterial [13], antioxidant, antiproliferative and insecticidal [14] properties. A significant number of these species have been used in traditional medicine in many countries as a remedy to treat anxiety, insomnia, hysteria, epilepsy, spasms, and pain [15]. In addition, the Passifloraceae family is distinguished by its great use in the fruit sector due to the contribution of minerals, vitamins, and fibers from the fruit and their attractiveness related to texture, aroma and flavor [20].

The byproducts of passionflowers, such as the peel and seeds, are rich in secondary metabolites, such as 4-hydroxybenzoic acid, chlorogenic acid, vanillic acid, caffeic acid, p-coumaric acid, ferulic acid, rutin, quercetin, and trans-cinnamic acid, which have antioxidant and gastroprotective properties [16]. Other compounds also found in seeds and peels are syringic acid, gallic acid, resveratrol and piceatanol, with similar bioactive properties [17]. On the other hand, phenolic compounds such as cyanidin anthocyanins have been found, and this type of compound has been reported to have antioxidant, antiallergic, anti-inflammatory and antihypertensive properties and is useful in the treatment of arthritis, diabetes and cancer [8]. Other studies have reported apigenin and catechin in peels and seeds with analgesic, antipyretic, antioxidant and anti-inflammatory properties [18, 19].

Passionflower peels and seeds are an important source of several compounds that can have different types of bioactivities. These byproducts, which are mostly discarded in the agroindustrial production chain, have the potential to be transformed and give added value to the production chain, thus generating a process of circular economy friendly to the environment and improving the sustainability of the passionflower processing chain [47].

Microencapsulation has various applications in the food industry to improve the properties and characteristics of food ingredients or the addition of antimicrobial agents [21]. There are several processes to produce microcapsules, including spray drying, cooling spray, coacervation/phase separation, gelation, solvent evaporation, supercritical fluid expansion, interfacial polymerization and extrusion [22].

There are numerous reports on the encapsulation of plant extracts by different methods using various biopolymers as encapsulating materials [21], but no study was found on the encapsulation of *P. edulis* f. *edulis* and *P. maliformis* extracts. Therefore, the present study aimed to explore and utilize some of the bioactive properties of *P. edulis* f. *edulis* and *P. maliformis*, such as the antioxidant potential and antihypertensive activity, through a mechanism of inhibition of the angiotensin converting enzyme - ACE. This study was conducted to promote the development of new products with nutraceutical or functional characteristics as well as the development of models, methodologies and techniques applicable to the integral use of byproducts such as the peels and seeds of passionflowers, which are generated as an agroindustrial waste.

2. Materials and methods

2.1. Chemicals

All the reagents used in the present study were: 3-ethyl benzothiazoline-6 sulfonic acid (ABTS), 2,2-diphenyl-1-pyrrole hydrazyl (DPPH), diphenyl bromide 3- (4,5-dimethylthiazol-2 -yl) -2,5-tetrazolium (MTT), the substrate HHL (hippyl-histidyl-leucine) and the enzyme ACE (EC 3.4.15.1, 5.1 U/mg), both for in vitro and ex vivo tests, were purchased from SIGMA-ALDRICH® (St. Louis, MO, USA). Chromatographic solvents were acetonitrile LC-MS grade from J.T. Baker (Deventer, The Netherlands) and purified water from a Milli-Q Millipore system (Bedford, MA, USA). The other chromatographic chemicals used were HPLC grade and supplied by J.T. Beaker. Other chemicals used were analytical grade (Merck, USA).

2.2. Materials

Collection of plant material. Ripe fruits of *P. edulis* f. *edulis* and *P. maliformis* were obtained in the local markets of the cities of Ibagué and Neiva, respectively. The fruits of the first species were collected in the municipality of Anzoátegui, located north of the department of Tolima, with an average altitude of 2010 m above sea level, and an average temperature of 17 °C. For the second species, the fruits were purchased at the Central Mayorista Surabastos, Industrial Zone of the city of Neiva. The plant material was harvested in the municipality of Rivera, located 20 km north of the city of Neiva, with an average altitude of 700 m above sea level and an average temperature of 25 °C.

2.3. Preparation of plant material

The peel and seeds were manually separated from the pulp and then dried (45 ± 2 °C, 24 h) in an oven with forced air circulation (Thermolab, DiEs, Antioquia, Colombia). The material was ground and subjected to a degreasing process using the Soxhlet technique. The sample was dried at room temperature and macerated with ethanol (in a ratio of 1:20 w: v), the solvent was renewed every 2 h until exhaustion of the sample (24 h). The extracts obtained from all extraction times were pooled. The ethanolic extract of peel (EEP) and seeds (EES) obtained were concentrated under vacuo using a rotary evaporator at 45 ± 2 °C (R-114, Büchi, Flawil, Sweden) and stored at – 85 °C (freezer Kaltis 390) until analysis.

2.4. Chemical composition

The moisture, ash, lipids, protein, and raw fiber content of samples were analyzed according to the methods of the AOAC [23]. The mineral content (K, Na, Mg, Ca, Fe, Mn, Cu, and Zn) was determined using an atomic absorption spectrophotometer (Thermo Scientific ice 3000 Series AA). The content of phosphorus (P), boron (B) and sulfur (S) were determined using an ultraviolet (UV)-vis spectrophotometry (Evolution 260 BIO) [47].

2.5. Phytochemical analysis of extracts

The extracts were phytochemically (qualitative screening) evaluated to determine the presence of phenols, tannins, flavonoids, terpenes, anthraquinones and reducing sugars according to standard methods [24, 25]. In addition, a quantification of phenolic compounds was carried out using the method describe by Singleton et al., [26]. Total flavonoids and tannins were quantified by the methods described by Palomino et al. [27] and Makkar & Harinder [28], respectively.

2.6. Chromatographic analysis

The identification and quantification of the main phenolic compounds in the EEP and EES samples were done using a Waters Atlantis dC18 column (5 μm, 2.1 mm × 150 mm) in a Waters ACQUITY UPLC® System with diode array detector (DAD) (Waters, Milford, MA). For the separation of the compounds, a mobile phase composed of H_2O (solvent A, 0.1% v/v formic acid) and 100% methanol (solvent B, acetic acid 0.1% v/v) was used. The linear gradient elution was performed according to Delpino-Rius et al. [29]. Eluates were monitored at 280 and 320 nm. Samples were assessed in triplicate and the compounds were identified

using chromatographic behavior and UV-vis absorption spectra together with published information on the main phenolic compounds in the samples [17].

2.7. Antiradical and antioxidant capacity

Antioxidant capacity of EEP and EES samples was evaluated according to DPPH· [30], $ABTS^{\cdot+}$ [31], and ferric reducing power (FRAP) [31] assays and using a microplate UV-vis reader (Multiskan® GO Thermo Scientific). For DPPH and ABTS, results were expresses as Trolox equivalent per 100 g of sample, and for FRAP assay, results were expressed in g equivalents of ascorbic acid per 100 g of sample (g equivalents of ascorbic acid/100 g).

2.8. ACE inhibition

The ACE inhibitory activity of samples was measured by the method described by Cushman and Cheung (1971) with some modifications, as described in a previous publication [48]. 40 μL of the samples were mixed with 100 μL of substrate (HHL prepared in sodium borate buffer), and with 2 mU of ACE (EC 3.4.15.1, 5.1 U/mg) dissolved in 50% glycerol. The mixture was incubated for 30 min at 37 °C. Then, 150 μL of HCl (1 N) was added to stop the reaction. 1 mL of ethyl acetate was added to the mixture with the aim to extract the hippuric acid formed in the reaction. The mixture was stirring and subsequent centrifuge at 4000 x g for 10 min at room temperature. The organic phase (supernatant) was collected and evaporated by heating using a water bath (95 °C). The hippuric acid residue was dissolved in 800 μl of distilled water and the absorbance was read at 228 nm.

The ACE inhibitory activity (ACEI) of samples were assessed in triplicate, and calculated as the sample concentration necessary to inhibit 50% of the enzyme (IC_{50}) by the following Eq. (1):

$$\%\ ACEI = \frac{ABScontrol - ABS\ sample}{ABS\ contol - ABS\ blank} * 100 \quad (1)$$

where:

ABS control: absorption of hippuric acid formed after the action of ACE without inhibitor;

ABS blank: absorption of HHL that has not reacted and that has been extracted with ethyl acetate; and

ABS Sample: absorption of hippuric acid formed after the action of ACE in the presence of inhibitory substances.

2.9. Preparation of microcapsules

The encapsulates were prepared used enzymatically modified rice starch (AAM) as the wall material according to the methodology of Pranee [32]. Initially, wall material were solubilized in acetone (0.005% w/v) over a period of 4 h at room temperature (~25 °C) and 100 rpm. Then, the AAM solubilized was filtrated using a vacuum pump system. At the same time, extracts were solubilized in acetone (0.002% w/v) while stirring for 10 min at 100 rpm. The solubilized extract was incorporated into the AAM filtrate for 15 min by stirring at 500 rpm. The resulting solution was dispersed on glass plates using a nebulizer coupled to a negative pressure pump. The plates were dried at room temperature for 10 min. AAM-EP microcapsules retained on the plates were collected and stored. The encapsulated extracts, as well as the wall material, were coded as follows: wall material (M1), microencapsulated of *P. edulis* f *edulis* Sims seed (M2), and microencapsulated *P. maliformis* seed (M3). For the microencapsulation process, the extract with the highest content of phenolic compounds of each of the species was selected, taking into account its relevance for the pharmaceutical and food industry.

The encapsulation efficiency was determined taking into account Eq. (2), measuring the total phenolic content (method described in section 2.5) of the sample before and after the encapsulation process. The encapsulated samples (50 mg) were extracted by shaking using distilled water (5 ml) as a solvent. Then the material was centrifuged at 6000 rpm for 10 min.

$$\%\ EE = \frac{TP_O - TP_S}{TP_O} * 100 \quad (2)$$

2.10. Hygroscopicity (H)

The hygroscopicity of the encapsulates was established according to the methodology described by Cai and Corke [33], with certain modifications. Samples, approximately 0.5 g of the encapsulated material, were placed in a desiccator containing a saturated solution of NaCl (RH 75.3%). The results were expressed as the mass of water absorbed per 100 g of the sample after 7 days of storage.

2.11. Solubility (S)

The solubility of the encapsulates was determined according to the methodology reported by Eastman and Moore [34], with some modifications. The sample (0.5g) was added to containers containing 50 mL of distilled water, stirred at 110 rpm for 30 min using a TE-424 brand Tecnal shaker and then centrifuged at 4000 rpm for 5 min. An aliquot (25 mL) was removed from each supernatant, transferred to porcelain plates and dried to constant weight in an oven at 105 °C. Solubility was expressed as a percentage (%).

2.12. Morphology and size of microencapsulated particles

The morphological characteristics of the microcapsulates and acetylated starch were examined by scanning electron microscopy (Phillips XL30). The samples were placed on stubs using doublesided carbon tape and covered with a gold layer using a rotary-pump coating system (Q150R, Quorum, Lewes, UK). Images were obtained at an accelerating voltage of 12.5 kV and at varying degrees of magnification.

2.13. Stability of the encapsulates

To determine the stability of the encapsulates, the determination of the zeta potential (ξ) and dynamic light scattering (DLS) analysis were carried out using a Zetasizer nano-ZS ZEN3600 kit (Malvern Instruments, Herrenberg, Germany).

2.14. Color parameters

The color parameters were evaluated using a reflectance spectrophotometer (CM-5, Konica Minolta). The modified starch was evaluated as a color standard and the microencapsulates of the passiflowers as evaluation samples. The results were expressed according to the CIELAB color system (L * = lightness, a * = redness and b * = greenness).

2.15. Gastrointestinal evaluation

For the evaluation of the encapsulates through an *ex vivo* gastrointestinal model, a pig intestine was used, which was supplied by the Ibagué city slaughter plant. The model consisted of simulating the digestion conditions, dividing it into three phases: the gastric, enteric and final enteric phases [35, 36]. In the first phase, it was carried out in a 400 mL beaker with constant stirring of 500 r.p.m. The medium used was NaCl at 5 g/L to obtain an isotonic medium and the pH of the gastric fluid was adjusted with 1.0 M HCl. To this fluid, pepsin was added at 0.5 g/L, with a container volume 300 mL, and the residence time of the encapsulates in the gastric part was 90 min. For the second enteric phase, it was used in a 400 mL beaker with constant stirring at 400 r.p.m. and a Krebs solution with the following composition (mM) was used: NaCl, 118.7; KCl, 4.7; CaCl2, 2.5; NaHCO3, 25.0; MgSO4.7H2O, 1.2; glucose, 11.0;

and ascorbic acid, 0.1. Bile (1 ml/L) was added, this solution was adjusted to pH 7.0 to 7.5 with 1.0 M NaOH. The residence time in this phase was 90 min [37]. For the third phase, the procedure was the same as the previous phase, the same composition as the enteric phase was used, but the pH of the bile juices was adjusted to 8.0 and the residence time was 90 min. (Figure 1).

The small intestine was obtained from the slaughtered pigs, 5 min after the slaughter of the animal. The tissue was kept on ice during the procedure. Some anatomical regions of the small intestine, the duodenum (starting at 10 cm from the pylorus, total length ±0.5 m), proximal, middle and distal part of the jejunum (1.5, 5 and 10 m from the pylorus, proportionally) were collected, proximal, middle and distal part of the ileum (1.5 1 and 0.5 m proximal to the ileal cecal valve) and finally the colon. The intestine was stored in an ice-cold Krebs solution and fed with a carbogen gas mixture (95% O2/5% CO2) to avoid ischemia, later it was quickly transported to the laboratory. Upon arrival, intestinal tissue was carefully rinsed and rinsed with an ice-cold Krebs solution to remove luminal debris and placed in a Krebs beaker on ice. In the gastric phase, only tissue was not used, only the gastric mixture was stirred for 90 min and a 500 μL sample was taken to determine the phenol content qualitatively. For the final enteric and enteric phase, the everted portions of intestine were placed in 400 mL beakers with krebs solution and the microcapsules on the outside, and 5.0 mL of the krebs solution were placed inside the intestine, where they were collected. The bioactive compounds that managed to cross the intestinal barrier, these compounds dissolved in the krebs solution were taken through a capillary adapted to a syringe in order to qualitatively determine the presence of phenolic compounds and quantitatively their antioxidant and antihypertensive activity. *in vitro*.

2.16. Statistical analysis

All experiments were performed in triplicate. Analysis of variance (ANOVA) and Tukey's multiple range tests were applied to determine significant differences among samples using Statgraphics Centurion XVI software (Statistical Graphics Corporation, USA).

3. Results and discussion

3.1. Phytochemical composition of extracts

Chemical composition and mineral analysis of the two species analyzed can be seen in Table 1. The results for phytochemical screening and the quantification of the main secondary metabolites analysis of the peel and seeds of *P. edulis* f. *edulis* and *P. maliformis* can be seen in Tables 2 and 3. The phytochemical screening revealed the presence of reducing carbohydrates in the peel and seed extracts of both passionflowers. Terpenes and phenolic compounds of tannic nature, in addition to flavonoids, were also observed. Results obtained from *P. edulis* f. *edulis* corroborate the results reported previously by different authors [38, 46, 47]. On the other hand, the secondary metabolites of *P. maliformis* have not been studied in the peel and seeds, but they have been observed in the pulp [39].

The components of pharmaceutical and nutritional interest found in the peel and seeds of both passionflower species were phenols, flavonoids, tannins and terpenes, compounds that were not evidenced in the same proportion in the ethanolic extracts of the peel of *P. maliformis*; however, the phytochemical reports are similar to those obtained in other phytochemical studies for the peel of these two species, where the presence of a higher content of phenols in the seeds than in the peel was observed [40].

In order to validate the qualitative analysis carried out on the extracts, the metabolites of interest for the pharmaceutical and food industries were quantified, such as total phenols, tannins and flavonoids. The results obtained show a higher concentration of the compounds evaluated in the seed extracts obtained from both species (Table 3). Regarding the content of total phenols, it was observed that the results obtained in this study agree with those obtained for *P. ligularis* and *P. edulis* f. *flavicarpa* [41]. However, a lower flavonoid content was observed compared to that reported for *P. cincinnata* Mast [42]. Differences between the content of evaluated compounds may be due to agroecological differences, species type, geographic location and method of extraction. The presence of this type of compounds has been related to multiple biological activities such as antioxidant, antihypertensive and antidiabetic [43, 44].

3.2. Chromatographic analysis

Figure 2 shows the chromatograms obtained for the evaluation of the peel and agroindustrial seed residues of *P. edulis* f. *edulis* and *P. maliformis*. The main compounds identified and quantified in the *P. edulis* f. *edulis* and *P. maliformis* peels were chlorogenic acid (27.34 and 25.28 mg/100 g), rutin (20.43 and 21.26 mg/100 g, respectively), quercitrin (11.22 and 12.53 mg/100 g), epicatechin (9.64 and 8.78 mg/100 g) and isoquercetin (9.37 and 8.58 mg/100 g) (Figure 2). While, for the seeds of the samples, a lower number of compounds were identified. The main compounds found in the *P. edulis* f. *edulis* and *P. maliformis* seeds were quercitrin (10.67 and 11.23 mg/100 g, respectively), rutin (6.25 and 7.34 mg/100 g), isoquercetin (2.78 and 3.05 mg/100 g) and p-coumaric acid (0.96 and 0.88 mg/10 g).

In the peel, very similar results were observed between the two passionflowers, with *P. edulis* f. *edulis* presenting higher contents chlorogenic acid, epicatechin and isoquercetin, and *P. maliformis* presenting higher

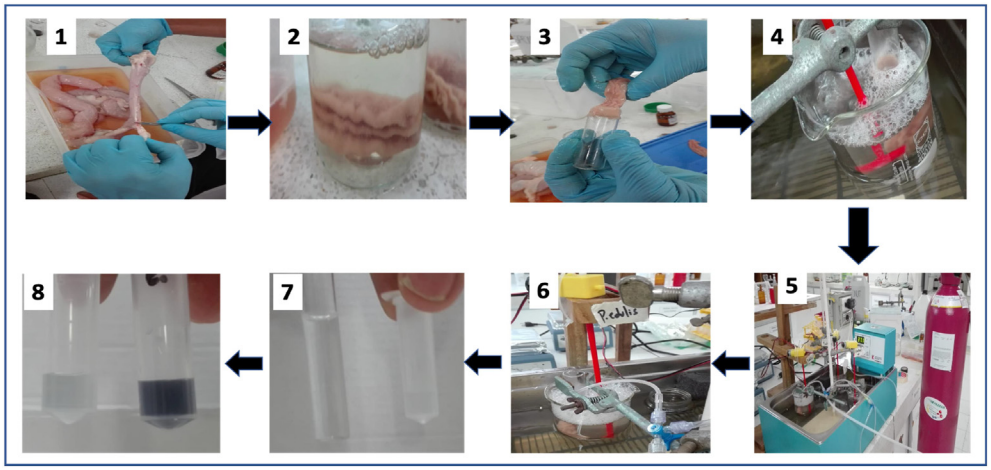

Figure 1. Gastrointestinal model with a pig intestine. 1. Cleaning the small intestine, 2. everted small intestine for testing, 3. placement of the intestine in collection tube, 4. mounting of the intestine in the gastrointestinal solution with agitation, 5 and 6. complete assembly of the encapsulates of each passionflower and positive control (captopril), 7. qualitative testing of phenols in gastric samples at pH 2,0 and 8. qualitative testing of phenols in a gastrointestinal sample at pH 8,5. The intestines used in this experiment were taken from pigs slaughtered for human consumption and donated by the municipal slaughter plant.

Table 1. Chemical composition and mineral analysis.

Determination	*P. edulis* var *edulis* Sims	*P. maliformis*
Dry material (%)	91.6	93.4
Ethereal extract (%)	13.5	29.4
Crude protein (%)	11.8	16.4
Potassium (mg/Kg)	857.5	812.6
Sodium (mg/Kg)	448.3	363.1
Iron (mg/Kg)	263.7	185.9
Cooper (mg/Kg)	19.1	25
Zinc (mg/Kg)	17.3	19.8
Boron (mg/Kg)	34.6	37.5
Manganese (mg/kg)	10.3	12.2

Table 2. Phytochemical analysis.

Metabolite tests	*P. maliformis*		*P. edulis f. edulis*	
	Peel	Seeds	Peel	Seeds
Phenols	+	+++	++	+++
Tannins	+	++	+	++
Flavonoids	++	+++	++	+++
Terpenes	++	++	++	++
Reducing sugars	++	+++	++	+++
Anthraquinones	ND	+	ND	+

Positive (+), Very positive (++), Highly positive (+++), Not detected (ND).

contents of rutin and quercitrin. The results of the seed extracts showed that *P. maliformis* has a high content of all compounds than *P. edulis* f. *edulis* except for the content of p-coumaric acid. The compounds identified in the samples are widely recognized for their antioxidant, antiplatelet, vasodilator, antiarthritic, antibacterial, anti-inflammatory and oncoprotective activities [45], which demonstrates the nutraceutical potential of the analyzed samples. In addition, the results obtained in the identification and quantification of phenolic compounds corroborate the presence of these compounds in the passionflower species [46].

3.3. Antiradical and antioxidant capacity

The antiradical or antioxidant capacity, as reported in some studies for Passiflora species such as *P. quadrangularis*, *P. edulis* f. *edulis* and *P. maliformis*, presented a high value in the DPPH and ABTS test and a relatively low value in the FRAP tests. Table 4 shows the values obtained in this study, where a high antiradical content of the peel and seed byproducts of *P. edulis* f. *edulis* and *P. maliformis* is shown; additionally, the table also shows the values of FRAP. It should be noted that the values of antiradical capacity were higher in the peel than in the seeds for both passionflowers. In addition, *P. edulis* f. *edulis* presents a higher activity in both the peel and seeds than those of *P. maliformis* with an equivalent Trolox value µmol/100 g sample of 3256.15 compared to 1111.16 in the seeds for the DPPH test, while for the peels, the values in Trolox equivalents µmol/100 g sample were 1529.98 and 1187.39 for *P. edulis* f. *edulis* and *P. maliformis*, respectively. This same behavior was presented in the ABTS assay, where *P. edulis* f. *edulis* showed greater activity in both the peel and seeds compared to that of *P. maliformis*, with values in Trolox equivalents µmol/100 g sample of 6575.29 compared to 2398.25 for the seeds and 3345.34 compared to 2321.21 for the peel. The data reported for FRAP showed greater activity by *P. edulis* f. *edulis* than by *P. maliformis* for both the peel and seeds. The values reported in this study for the two passionflowers species are higher than those reported in previous studies [45] and similar to those reported by Gonzalez et al. [47].

An explanation for the antioxidant capacity of these byproducts of the two passionflowers species can be attributed to the chemical nature, diversity and concentration of the secondary metabolites present in the extracts. Phytochemical analysis revealed the relative abundance of metabolites of phenolic nature: tannins and flavonoids pigments. Terpenes and anthraquinones were also abundantly detected. Other investigations have also associated bioactivity responses similar to those obtained in this test with the content of phenolic compounds [45, 48].

3.4. ACE inhibitory activity

The IC_{50} of the inhibitory activity of angiotensin converting enzyme is shown in Figure 3. The peel and seed extracts of *P. edulis* f. *edulis* and *P. maliformis* showed good ACE inhibitory activity, with values between 53.4 and 86.6 mg/L in the *Passiflora edulis* f. *edulis* peel. For seeds, values of 58.4–90.4 mg/L were obtained. The *P. maliformis* peel presented values in mg/L 43.1 to 73.5, and the values obtained in *P. maliformis* seeds were between 50.6 mg/L and 86.9 mg/L. At the lowest concentrations evaluated of 100 mg/L, in *P. edulis* f. *edulis*, more than 50% inhibition could be obtained for both the peel and seed extracts, while in *P. maliformis*, the values at the same concentration of 100 mg/L in the peel presented values below 50%, but in seeds, an inhibition greater than 50% was obtained.

The data reported in this work are slightly lower than those previously reported for *P. edulis* f. *edulis* [47], where a maximum value of 96.26 mg/L for the peel and 95.21 mg/L for seeds was reported; however, the results in this work show better results than those reported for *Passiflora incarnata*, with moderate inhibition of the ACE enzyme (72.9% and 66.1%) [49], and those reported for badea and *Passiflora quadrangularis* [50]. It should be noted that for *P. maliformis*, there are no reports of ACE inhibitory activity thus far, and these are the first reports of antihypertensive activity *in vitro*.

3.5. Microencapsulated extract characterization

The production of agro-industrial residues or by-products in the different levels of the production processes is today a global problem because in many cases these residues are not managed or disposed of properly, which contributes to the process of environmental deterioration. Agro-industrial residues such as the peel or the seeds of many fruits are not used for the most part and have a high potential to be managed in different processes that include the elaboration of new products, thus

Table 3. Total phenolic content of passionflower seed and peel extracts.

Phenolic compounds				
Quantified Metabolite	*P. maliformis*		*P. edulis f. edulis*	
	Peel	Seeds	Peel	Seeds
Flavonoids mgEQ	7.64 ± 0.01	92.59 ± 0.33	12.34 ± 0.01	99,43 ± 2.64
Tannins	0.36 ± 0.01	1.16 ± 0.01	0.57 ± 0.01	2.86 ± 0.01
Total Phenols mgEAG	0.11 ± 0.01	4.17 ± 0.04	0.19 ± 0.01	9.55 ± 0.06

mgEQ: milligrams quercetin equivalent mgEAG: gallic acid equivalent milligrams.

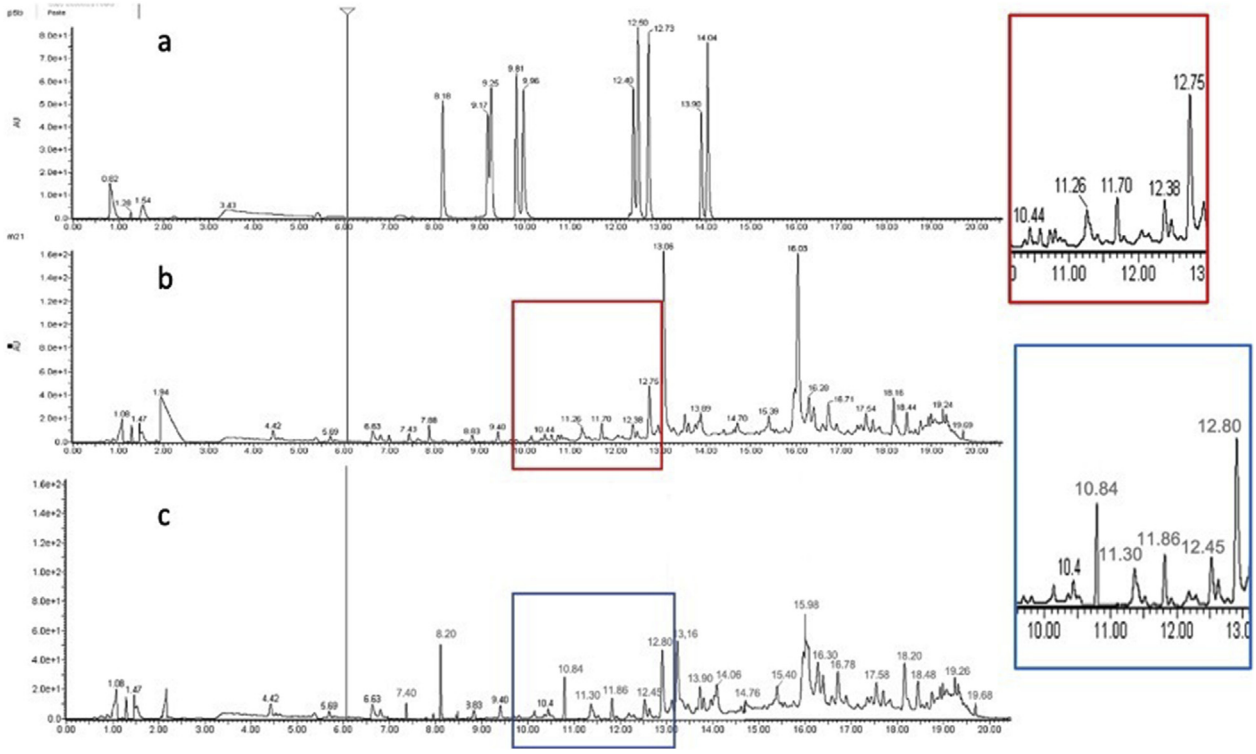

Figure 2. Chromatographic analysis by HPLC. The figure shows (a) the chromatographic profile of the phenolic compound standards, and figures (b) and (c) show the compounds found according to the comparison of the standard with the extracts of passionflower *P edulis* f. edulis and *P. maliformis* respectively.

Table 4. Antioxidant activity of passionflower seed and peel extracts.

Passionflower Extract	DPPH (TE/100 g dw)	ABTS (TE/100 g dw)	FRAP (TE/100 g dw)
P. maliformis seed	1111.16 ± 44.4	2398.25 ± 119.9	13.573 ± 0.5
P. edulis f. edulis seed	3256.15 ± 195.4	6575.29 ± 460.3	24.625 ± 1.2
P. maliformis Peel	1187.39 ± 59.4	2321.21 ± 116.1	34.5 ± 2.1
P. edulis f. edulis Peel	1529.98 ± 76.5	3345.34 ± 250.9	48.6 ± 3.4

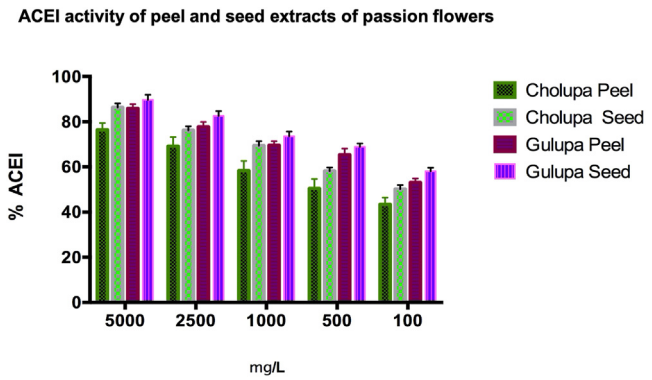

Figure 3. The ACE inhibitory activity of passionflower seed and peel extracts.

incorporating a greater value to the original products and the improvement of affected environmental conditions. Thus, by encapsulating the bioactive extracts obtained from the seeds of *P. edulis f. edulis* and *P. maliformis*, these by-products are expected to achieve added value and contribute to the passionflower processing chain, thus generating new lines of business promoting a green economy. The Table 5 presents the size distribution, polydispersity, and the average size of the encapsulates as observed by scanning electron microscopy (SEM) and the encapsulation efficiency of the encapsulates obtained with modified rice starch and the seed extracts of *Passiflora edulis* f. *edulis* or *Passiflora maliformis*. The results showed that the encapsulates obtained for M2 and M3 had higher size distribution values than that obtained for M1. The incorporation of the seed extracts of both passionflower species led to an increase in the size distribution of the encapsulates. This increase can be explained by the presence of phenolic compounds in the encapsulated extracts. During the formation of the encapsulates by reversed-phase evaporation, the phenolic compounds were incorporated into the aqueous phase of the encapsulates, which led to an increase in the size distribution [38].

The SEM images of rice starch are similar to those described by other authors [51] with small sizes (2–7 μm) and polyhedral and irregular shapes (Figure 4). However, there was some loss in sharpness, and some granules showed slightly rough surfaces, which consistent with the report by Song et al. [52] that the surface appeared deformed with concavities and dents without the presence of breakage or fractures. This description fits with the characteristics of encapsulates produced in this work. The relatively small size and appearance of the encapsulates can be attributed to the incidence of shrinkage because diffusion of water takes longer, allowing for structures to contract and deform to some extent.

Table 5. Size distribution (SD), polydispersity, ξ potential, average microcapsule size (SEM) and encapsulation efficiency (E.E.) of the seed extracts of *P. edulis f. edulis Sims* and *P. maliformis*.

SAMPLE	Polydispersion	SD (nm)	ξ Potential (mV)	SEM Average size (μm)	E. E. (%)
M1	1.62 ± 0,045	86,4 ± 7,9	-7,12 ± 0,52	4,64 ± 1,26	–
M2	1.33 ± 0,03	99,6 ± 2,1	-6,34 ± 0,35	5,13 ± 1,55	78,3 ± 2,5
M3	1.5 ± 0,027	103 ± 3,6	-6,6 6 ± 0,47	4,56 ± 1,59	72,9 ± 3.1

The reported data are the average of three determinations ± SD. M1: acetylated starch, M2: seed encapsulation of *P. edulis f. edulis Sims*, M3: seed encapsulation of *P. maliformis*.

Consequently, these surface anomalies could be desirable to improve the rehydration of powders [53].

The images shown in Figure 4 show that the most common geometric shape that can be observed in the entire set of images achieved in this study is an irregular three-dimensional formation of the granules. The polyhedral character described in the literature was observed in samples M1, M2 and M3, in accordance with microencapsulation studies with modified rice starch [54, 55], where it was observed that the encapsulates have a high degree of particle agglomeration, swelling and the appearance of an irregular and heterogeneous solid. These changes in morphology can be explained by the influence of hydrothermal processes on the physicochemical characteristics of starches.

The average size of the granules was 4.64μm ± 1.26 for M1, 5.13μm ± 1.55 for M2 and 4.66μm ± 1.59 for M3, and a similar average size was observed between the three samples. The size of the microcapsules was smaller than that of other encapsulates obtained with modified rice starch with average values of 8.12 ± 3.92 μm [56, 57].

According to Rafiee [58], phenolic molecules increase the charge on the particle surface. The encapsulated passionflower extracts presented a negatively charged zeta potential, considering their contents of phenolic compounds [59]. The zeta potentials of the modified starch dispersions were similar to those observed by Miao et al. [60]. Regarding the optimized formulation (a mixture of extract and wall material), a polysaccharide at a sufficient concentration could trap particle [61] and maintain the dominant zeta potential of the modified starch, as seen in this study.

The retention of bioactive compounds or those with specific properties such as flavors depends on the physicochemical properties and the molecular weight of the encapsulating agent [62]. The relatively high E.E. values obtained for the *P. edulis* f. *edulis* and *P. maliformis* encapsulates, can be inferred from the fact that they present a wall material comprising high concentrations of native and modified rice starch. Materials such as amylose and amylopectin have molecular weights of <0.5 million Daltons to 500 million Daltons respectively, which reduces the diffusivity of the solute during the microencapsulation process, thus increasing a layer dries on the surface of the microcapsule and this increases the efficiency of encapsulation [65]. In addition, there are reports of encapsulation efficiency of 72.7% and 85.7% with protein isolates and whey protein isolates by spray drying [65]. Based on the above results, it can be said that the use of starch as a wall material is a viable alternative for the encapsulation of bioactive compounds due to its high encapsulation efficiency, as was observed in this study.

3.6. Hygroscopicity and solubility

Table 6 shows the hygroscopicity of the modified rice starch and the microencapsulates of the two passion flowers. The hygroscopicity of the samples is low, which may be related to a high storage stability. The reported values are lower than those reported by Volnei Brito de Souza et al. [64], for microencapsulates from byproducts of the Bordo grape and similar to those reported by Marquez-Gómez et al. [65], for microencapsulates with rice starch. Compared to the solubility of the kernels, a moderate solubility can be seen, this is due to the insolubility characteristics of the starch, however they present an appreciable solubility [66].

Solubility is a very important measure to consider according to the purpose that will be given to the microcapsule [65]. The microcapsules could be incorporated into a food matrix according to the results obtained, in the same way, it is important to consider their solubility when establishing the formulation [65]. The powders obtained in this work dissolved completely after 15 min of stirring in water at room temperature.

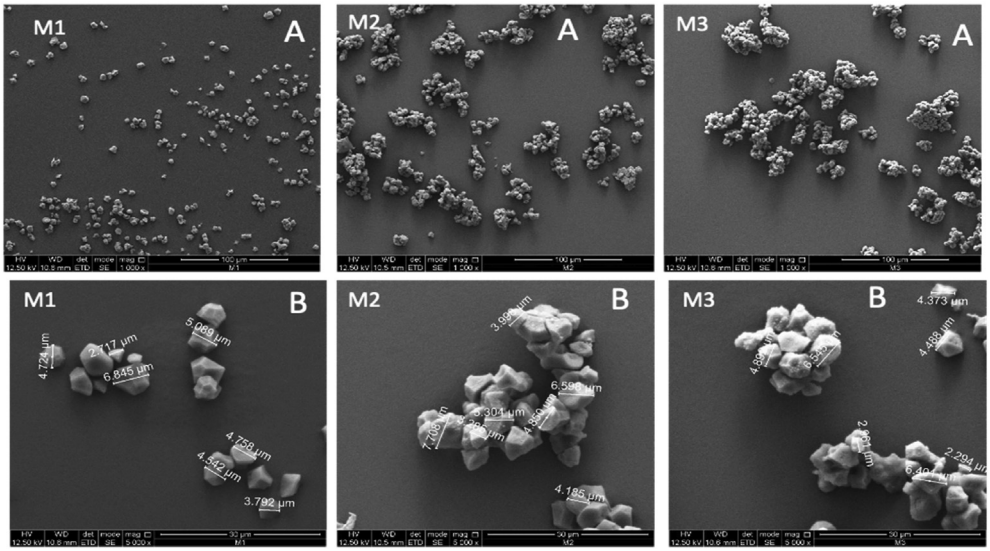

Figure 4. SEM of microcapsules from seed extracts of *Passiflora edulis* var. edulis and *Passiflora maliformis*. M1: acetylated starch; M2 seed encapsulation of *P. edulis* f. edulis Sims, M3: seed encapsulation of *P. maliformis*. The letters A indicate a magnification of 1000x and the letters B indicate a magnification of 5000x, in the magnification of 5000x, sizes of the microcapsules are observed that are between 2.9 and 7.7 μm with an average of 4.64 μm M1, 5.13μm M2 and 4.16μm M3.

Table 6. Hygroscopicity and solubility of microencapsulates.

Sample	*P. maliformis*		*P. edulis f. edulis*	
	H (g of water absorb/g of sample)	S (%)	H (g of water absorb/g of sample)	S (%)
M1	1.286 ± 0.07	73.218 ± 0,23	1.306 ± 0.05	79.408 ± 0,23
M2	1.222 ± 0.07	81.316 ± 0,17	1.278 ± 0.07	81.725 ± 0,17
M3	1,303 ± 0.08	80.431 ± 0.19	1,309 ± 0.08	80.602 ± 0.19

The reported data are the average of three determinations ± SD.

Table 7. Color analysis of microencapsulates.

Analysis	Modified starch	Encapsulated *P. maliformis*	Encapsulated *P. edulis f. edulis*
L*	90,21 ± 0,531	66,63 ± 0,015	66,63 ± 0,015
a*	0,52 ± 0,017	5,59 ± 0,032	5,75 ± 0,037
b*	6,03 ± 0,016	20,43 ± 0,057	20,71 ± 0,017

The reported data are the average of three determinations ± SD.

3.7. Color measurement

The perception and acceptability, by consumers, of the food or pharmaceutical ingredients such as encapsulated materials are related to the sensory or physical characteristics such as color. Encapsulated materials and wall material (standard) were assessed regard to the color characteristic in the CIELab color system (Table 7). a* values showed a slight tendency towards reddish coloration. However, the wall material showed no tendency towards reddish, which means that the reddish tendency of the encapsulates is related to the presence of the seed extracts. As well as for the a* parameter, the b* values of the encapsulates tend to positives values, which means a tendency towards yellow coloration. This behavior can be related to the fact that the extracts obtained showed a tendency to yellow improved by the coloration of the material for encapsulation (standard), which showed a positive value of b* parameter. The luminosity of the encapsulates was high, indicating that the use of the wall material had a great influence on this property due to its white coloration (L ≈ 100).

3.8. Gastrointestinal evaluation

The values obtained for the antioxidant and antihypertensive activity are shown in Figure 5, it should be clarified that it was used as a positive control of the test from the point of view of the commercial antihypertensive activity captopril (pastes of 25 mg of active ingredient) The data obtained on the antihypertensive activity of the encapsulates of the two passiflowers compared to the positive control used captopril, are important in comparison with those of captopril since they present values for *P. edulis* f. *edulis* and *P. maliformis*: 53.5% and 57.2% inhibition of ACE, respectively. These data show that the release of the encapsulated material in the intestine, guaranteed the activity after crossing the gastrointestinal barrier; that is, the active components would reach the bloodstream with an inhibition percentage greater than 50%. To observe the absorption mechanisms of the active ingredient encapsulated and released in the final enteric and enteric phases of the gastrointestinal tract, it is important to highlight that there are different mechanisms that mediate the passage of molecules through the transcellular pathway and that these can be determined by the physicochemical characteristics of the molecule, which in this case are the metabolites contained in the seed extracts of *P. edulis* f. *edulis* and *P. maliformis*.

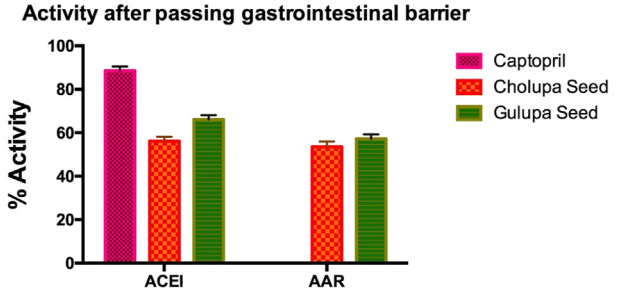

Figure 5. ACEI and ARC Activity after passing gastrointestinal barrier. The positive control used was commercially available captopril 25 mg tablets.

The data obtained on antihypertensive activity are promising compared to that of captopril since there was guaranteed activity after passing the gastrointestinal barrier; that is, the active components would reach the bloodstream with an inhibition percentage greater than 50%.

To look at the absorption mechanisms of the active ingredient encapsulated and released in the final enteric and enteric phases of the gastrointestinal tract, it is important to highlight that there are different mechanisms that mediate the passage of molecules through the transcellular pathway and the use of one or the other is determined by the physicochemical characteristics of the molecule, which in this case were the metabolites of the *P. edulis* f. *edulis* and *P. maliformis* seed extracts.

The data on antihypertensive activity in this study are similar to those reported *in vitro* by González et al [17]. but regarding *P. maliformis*, there are no publications on the activity of its seed extract. This is the first microcapsule gastrointestinal evaluation report for *P. edulis* f. *edulis* and *P, maliformis*.

Regarding the antioxidant activity, the data show that the release of the encapsulated material in the intestine, guaranteed the passage of the intestinal membranes, showing good antioxidant biactivity of the two extracts of the passion flowers with antioxidant activity values of 62.7% for *P. edulis* f. *edulis* and 58.6% for *P. maliformis*. These data are slightly lower than the data reported in *in vitro* tests before encapsulation, the decrease in the value of biactivity may be due to the fact that not 100% of the metabolites contained in the encapsulates manage to pass the intestinal barrier, However, the data shows in the same way that encapsulated extracts can be an alternative for use in food or pharmaceutical products.

4. Conclusions

The absorption mechanisms of the encapsulated active principle released in the enteric and final enteric phases of the gastrointestinal tract may be related to different mechanisms that mediate the passage of molecules through the transcellular pathway, which may be associate with the physicochemical characteristics of the metabolites with biological activity contained in the seed extracts of *P. edulis* f. *edulis* and *P. maliformis*. In the tests carried out, it is evidenced that the ethanolic extracts of *P. edulis* f. *edulis* seeds show better results in the different biological activities compared to the ethanolic extracts of *P. maliformis*

seeds. It is very likely that the antiradical and antihypertensive activity *in vitro* is related to terpenic-type compounds, isoquercetin-type flavonoids, rutin and flavones detected by HPLC, of which there is evidence of their antihypertensive activity *in vivo* studies. The microcapsules showed great stability, which means that they have great potential to be used in the pharmaceutical and food industries. This work demonstrates the great potential of the cultivation of these passion flowers, generating added value to it.

Declarations

Author contribution statement

Jorge Andrés Victoria Taborda: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Walter Murillo Arango: Conceived and designed the experiments; Analyzed and interpreted the data.

Jonh Jairo Méndez Arteaga: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Carlos Martín Guerra Almonacid: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

This work was supported by the Research and Scientific Development Office of Tolima University.

Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

The authors acknowledged Natural Products Research Group GIPRONUT, Research Group in Pharmacy and Occupational Health GIRYSOUT and LASEREX Laboratories of Universidad of Tolima.

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