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Evaluation of physicochemical properties of honey powder using rice and pea proteins as carriers

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ABSTRACT

Honey is a special product widely appreciated because of its peculiar flavor and aroma as well as its beneficial effects on health due to its constituents. However, the use of honey in its natural form can present several disadvantages to the food industry because of its high viscosity and density. This work aimed to obtain honey powder using rice, pea, or a mixture of both proteins as carriers by spray drying and to characterize physiochemically. Also, the mass balance was performed to evaluate changes in humidity and temperature that occurred by the drying air during the process. The honey showed acceptable physicochemical parameters by the legislation of honey quality control in regard to color (143.43 ± 4.34) mm Pfund, free acidity (46.41 ± 0.53) meq/kg, pH (3.73 ± 0.03), fructose content (46.52 ± 0.56) g/100 g and glucose content (35.88 ± 0.16) g/100 g, which leads to the production of honey powder. Among the carriers tested, the honey powder using rice protein achieved the highest powder recovery yield at (64.88 ± 0.64) %. The physicochemical properties were evaluated and the phenolic compounds were not negatively affected by spray drying conditions, maintaining a value of gallic acid equivalent (GAE) content at (301.31 ± 20.95) mg/kg of honey. Therefore, this work shows honey as an alternative food ingredient in powdered form, including the growing market for using alternative protein.

Keywords: honey; spray drying; microparticles; mass balance; plant protein; phenolic compounds.

1. Introduction

Honey is a natural product that has been considered an important carbohydrate source since the beginning of humanity (Bogdanov et al., 2008; Crane, 1975). Throughout human history, honey has been used as a nutrient and for medical purposes (Bogdanov et al., 2008; Jones, 2009), and it is considered a natural preservative with antimicrobial properties due to its high osmolarity (Molan, 1992), acidity (Yatsunami & Echigo, 1984) and hydrogen peroxide produced by the glucose oxidase of honey (Alvarez-Suarez et al., 2010; Bogdanov, 1997; Samborska, 2019; White, Subers & Schepartz, 1963;).

Honey composition has been shown to act synergistically and may contribute to several health benefits (Samat et al., 2018; Vică et al., 2021; Zainol, Yusoff & Yusof, 2013). Honey is mainly composed of sugar (76 g/100 g), fructose being the major monosaccharide, and water (less than 20 g/100 g) (Afrin et al., 2020; Martinotti & Ranzato, 2018; White, 1979;). Honey also contains minerals, vitamins, proteins, amino acids, and enzymes (White, 1979). The minor constituents are the phenolic acids and flavonoids (Dimitrova, Gevrenova & Anklam, 2007; Martos, Ferreres & Yao, 2000) that have been shown to provide biological effects, such as antimicrobial (Estevinho et al., 2008), antioxidant (Biluca et al., 2020), anti-inflammatory (Sun et al., 2020), and antimutagenic (Wang, Andrae & Engeseth, 2002).

The commercial production of honey in the world is approximately 1.2 million tons per year (Bogdanov et al., 2008), suggesting an average global consumption of 0.64 g/day and the largest consumption per capita is in the Central African Republic, which is 9.62 g/day (FAO, 2019b). However, this is still a low value compared to the global sugar consumption, which is expected to increase to 65.20 g/day in 2027 (FAO, 2019a), exceeding the value of 50 g/day of sugar, which is the amount recommended by the World Health Organization to maintain healthy body weight and reduce the risks of noncommunicable diseases (WHO, 2015; WHO, 2021). Thus, to promote the consumption of nutritional foods, it is important to increase the use of honey as an ingredient. However, developing food products using honey can be a challenge due to its physical properties,

such as high viscosity and density (Cui et al., 2008; Hebbar, Rastogi, & Subramanian, 2008). Additionally, honey is a supersaturated sugar solution, and can crystalize spontaneously during storage, decreasing consumer acceptance (Cui et al., 2008; Hebbar, Rastogi, & Subramanian, 2008; Samborska, 2019). Therefore, to significantly minimize such difficulties, an alternative is to use honey in a powder form.

Several techniques are used for honey drying, such as spray drying (Nurhadi et al., 2012; Samborska, Gajek, & Kamińska-Dwórznicka, 2015; Samborska, Sokołowska & Szulc, 2017; Suhag, Nayik & Nanda, 2016), vacuum drying (Devi et al., 2016; Nurhadi et al., 2012), microwave vacuum drying (Cui et al., 2008), vacuum foam drying (Sramek et al., 2016) and freeze-drying (Sramek et al., 2016). However, spray drying is the usual technique applied to dry honey (Samborska, 2019). It has some advantages because the food industry is used to convert liquid or paste food into powder. Spray drying is characterized as a continuous method with a short processing time, resulting in a product with low water activity. During the drying of the particles, the high mass and heat transfer of the water allow for maintaining a low temperature, thus permitting heat sensitive compounds to dry without excessively affecting their quality (Ré, 1998). The efficiency is also comparable to other types of dryers and the process can be considered low-cost (Filková & Mujumdar, 1995). However, drying honey can be a challenge due to its sugar-rich composition causing it to remain as a syrup and stick to the drying chamber walls (Bhandari & Howes, 1999), decreasing the powder recovery yield or not generating a powder (Samborska, Gajek, & Kamińska-Dwórznicka, 2015). This phenomenon called the *stickiness problem* occurs because of the low glass transition temperature (T_g) of monosaccharides: 31 °C for anhydrous glucose and 5 °C for anhydrous fructose (Bhandari & Howes, 1999; Samborska et al., 2019). To overcome this problem, it is necessary to add high molecular weight drying agents, which can modify the drying process due to their high T_g (Samborska, Gajek, & Kamińska-Dwórznicka, 2015).

Polysaccharides are commonly used as carriers for dry honey, for example, maltodextrin and gum arabic (Devi et al., 2016; Nurhadi et al., 2012; Samborska, Gajek, & Kamińska-Dwórznicka, 2015; Samborska et al., 2019; Suhag, Nayik, & Nanda, 2016). Plant protein derived from rice and pea has already shown important functional properties strongly necessary to act as a carrier during the honey drying process, such as foaming stability and emulsifying capacity (Nesterenko et al., 2013). In addition, rice belongs to the most important cereal crop in the world, and it can be a potential source of inexpensive high-quality proteins (Hamada, 2000; Saunders, 1990). Thus, plant protein (rice protein and pea protein) was used as a carrier in this work, since it has gained significant interest and reflects the current "green" trends in the food industry (Moser et al., 2020; Nesterenko et al., 2013). Furthermore, the pea protein used in this study was micronized, which consists of a technique applied to reduce the particle size at the micrometer level, resulting in changes in structural, physicochemical, and functional properties. This size reduction is important to promote functional and physicochemical properties, such as water retention capacity, swelling capacity, and solubility (Dhiman & Prabhakar, 2021). In this context, this study aims to develop honey powder by spray drying with isolated rice protein or isolated micronized pea protein, or a mixture of both. To the best of our knowledge, the use of pea protein to obtain honey powder by spray drying has not been explored previously in the literature.

2. Materials and Methods

2.1 Materials

Rowse honey (ETHIOPIAN honey) was purchased from a local market (Leeds, UK). Isolated rice protein (ORYZAPRO) and micronized pea protein, used as carriers, were donated by Healy Group, Leicestershire, UK. The protein content of the rice protein was (80.6 g/100 g) in dry matter. Micronized pea protein was extracted from yellow peas, and the protein content was (84 g/100 g) in dry matter, characterized by low viscosity, and excellent emulsifying properties. It is worth mentioning that the tests were performed with pea protein without micronization. Therefore, it was not possible to obtain a proper dispersion for the atomization. Ultrapure water (Direct Q3® system,

104 Millipore, USA) was used throughout the experiments. All the chemicals used were of analytical
105 grade.

106 **2.2 Methods**

107 **2.2.1 Physicochemical characterization of honey**

108 2.2.1.1 *Color*

109 The color was determined by spectrophotometry (Cecil, CE 3021, 3000 series, Cecil Instruments,
110 England), according to Ferreira et al. (2009). For that purpose, (50 g/100 g) honey solutions with
111 ultrapure water were prepared and the absorbance value was measured at 635 nm. The honey was
112 classified according to the Pfund scale in millimeters after the conversion of the absorbance values,
113 according to White et al. (1984), using Eq. 1:

114 $$mmPfund = -38.70 + 371.39 \times Abs \quad (1)$$

115 According to USDA classification (USDA, 1985), the color mm Pfund scale ranges from 8 or less
116 to over 114, classified as water (lighter in color) to dark (amber in color), respectively.

117 2.2.1.2 *Free acidity and pH*

118 The acidity of honey is caused by organic acids (tartaric, citric, oxalic, acetic, etc.), both from nectar
119 and bee secretions (Yadata, 2014). Free acidity was determined by potentiometric titration
120 according to the International Honey Commission (2009). Briefly, 10 g of honey was dissolved in
121 50 mL of ultrapure water, the electrode of the pH meter (Mettler Toledo, SevenCompact S220,
122 Switzerland) was inserted into the solution and, under magnetic stirring, the solution was titrated
123 with NaOH (0.05 N) up to pH 8.5. The result was expressed by milliequivalents/kg of honey. The
124 pH of honey was measured using a pH meter (Mettler Toledo, SevenCompact S220, Switzerland).

125 2.2.1.3 *Total soluble solid content*

126 The total soluble solid content was determined using an optical handheld refractometer (Bellingham
127 & Stanley Ltd. Tunbridge Wells, UK). Honey was homogenized and submitted to a temperature of
128 50 °C to prevent any sugar crystals. The samples were analyzed after cooling the temperature to 20
129 °C. The results were expressed as grams of total soluble solid content g/100 g of honey.

130 2.2.1.4 *Honey sugar content*

131 The honey sugar content was analyzed by HPLC, with an ELSD evaporative light scattering
132 detector (Shimadzu Prominence, Japan), according to Bogdanov et al. (1999) and the International
133 Honey Commission, (2009), with some modifications. Honey was diluted at 6.25 mg/mL, using
134 methanol at a concentration of 25 mL/100 mL, and filtered in a MILIPPORE membrane of 0.45
135 µm. The separation of sugar was performed in a Grace Davison Prevail Carbohydrate Es column
136 (5 µm, 250 mm × 4.6 mm), using acetonitrile as mobile phase B and ultrapure water (Direct Q3®
137 system, Millipore, USA) as mobile phase A. The mobile phases were delivered at 1 mL/min in a
138 binary gradient mode: (0.01-15 min: from 75 % to 60 % of B; 15.00-15.01 min: from 60 % to 75 %
139 of B; 15.01-20 min: 75 % of B). Measurements were carried out at 25 °C, and the sample injection
140 volume was 10 µL. Sugar content quantifications were achieved in triplicate by the standard curve
141 of glucose ($Y = 2\times10^6 x - 10^6$, $r^2 = 0.9991$, retention time: 6.09 min), fructose ($Y = 2\times10^6 x - 10^6$,
142 $r^2 = 0.9992$, retention time: 6.87 min), and sucrose ($Y = 2\times10^6 x - 691932$, $r^2 = 0.9992$, retention
143 time: 7.84 min). The sugar content was expressed as grams of sugars (fructose, glucose, and
144 sucrose) per 100 g of honey.

145 2.2.1.5 *Total phenolic compounds (TPC) in honey*

146 The TPC was analyzed according to Dżugan et al. (2018) and Piljac-Žegarac, Stipčević & Belščak
147 (2009), with some modifications. Honey solutions at (50, 25, and 12) mg/mL in ultrapure water
148 (Direct Q3® system, Millipore, USA) were prepared. Aliquots of 0.4 mL of the honey solutions
149 were mixed with 2 mL of Folin-Ciocalteu reagent 10 mL/100 mL (Merck, Germany) and 1.6 mL of
150 sodium carbonate 7.5 g/100 mL (Sigma-Aldrich Co., EUA), and incubated at ambient temperature
151 for 2 h, protected from light. After that, the absorbance was measured using a spectrophotometer
152 (Cecil, CE 3021, 3000 series, Cecil Instruments, England) at 760 nm. The blank was prepared
153 following the same procedure with ultrapure water. Gallic acid (Sigma Aldrich Co., USA) ranging
154 from (0.1 to 16) µm/ mL was used to build the standard curve ($Y = 0.0451x + 0.0013$, $r^2 = 1$).
155 Results were expressed as gallic acid equivalent (GAE) content (mg/kg) of honey.

2.2.1.6 *Total protein content*

The standard orthophthaldialdehyde (OPA) spectrophotometric assay (Church et al., 1983) was applied to quantify the total protein content of honey. The OPA reagent was prepared by dissolving 3.81 g of sodium tetraborate in approximately 80 mL Milli-Q water stirring at 50 °C. Then 0.088 g of dithiothreitol and 0.1 g of sodium dodecyl sulfate (SDS) were added after cooling to ambient temperature. Finally, 0.080 g of OPA dissolved in 2 mL of absolute ethanol was added to the solution and completed to 100 mL with Milli-Q water. In microtiter plates, 20 µl of standard/sample were loaded into each well and mixed with 200 µl of OPA reagent, allowing the reaction to proceed for 15 min at ambient temperature. The absorbance was then measured at 340 nm using a microplate photometer (Multiskan FC, ThermoFisher Scientific, USA). The standard curve was obtained using L-leucine ranging from (0.16 to 4) mM of the standard solution made in 10 mM phosphate buffer solution (Sigma Aldrich Co., USA). The standard curve obtained was: $r^2 = 0.9986$. The results were expressed as L-leucine equivalent (L-leuE) content (g/100 g) of honey. Each measurement was conducted in triplicate.

2.2.2 Production of powdered honey by spray drying

The initial honey content in the dispersions was chosen due to the first optimization study (Toniazzo et al., 2023). The honey powders were produced according to Toniazzo et al. (2023), with some modifications. Firstly, (16 g/100 g) of honey were mixed with ultrapure water (Direct Q3® system, Millipore, USA), until complete dissolution. After that, (14 g/100 g) of isolated rice protein or isolated micronized pea protein, or a mixture of both proteins ratio (50:50) were added, resulting in dispersions with a total solid concentration of (30 g/100 g) in dry matter. Dispersions were kept under magnetic stirring at ambient temperature (25 °C) during the drying process to prevent separation between liquid and solid phases. Finally, the dispersions were atomized in a laboratory scale spray dryer (Büchi, B290, Switzerland) coupled with a 0.7 mm diameter nozzle, under the following conditions: drying air flow rate at 35 m^3/h (corresponding to 100 % of its capacity), average inlet/outlet air temperature at (130.11 ± 0.61, 75.58 ± 2.55) °C, respectively, and

feed flow rate at 10 mL/min. Honey powder was collected at the bottom of the cyclone and stored in the absence of light in an aluminum bag until analyses. It is worth mentioning that the analyzes were performed only with freshly produced honey powder.

2.2.3 Mass balance

Spray drying mass balance can be performed from the dried product and the evaporated water. However, it is useful to determine the conditions under which the food product will be dried. In this situation, it is possible to evaluate the changes in humidity and temperature that occurred by the drying air during the process. A mass balance to the component water was described, according to Eq. 2:

$$\dot{m}_{ms}\,\overline{X}_{w1} + \dot{m}_{air}\,\overline{Y}_{w1} = \dot{m}_{ms}\,\overline{X}_{w2} + \dot{m}_{air}\,\overline{Y}_{w2} \quad (2)$$

wherein $\dot{m}_{ms}$ is the dry matter mass flow rate contained in the current of the material entered to be dried [$g \cdot s^{-1}$], $\overline{X}_{w1}$ is the initial moisture of the dispersion entered to be dried [$g_{water} \cdot g_{dry\ matter}^{-1}$], $\dot{m}_{air}$ is dry air mass flow rate [$g \cdot s^{-1}$], $\overline{Y}_{w1}$ is the initial air absolute humidity [$g_{water} \cdot g_{dry\ air}^{-1}$], $\overline{X}_{w2}$ is the moisture of the honey powder [$g_{water} \cdot g_{dry\ matter}^{-1}$], and $\overline{Y}_{w2}$ is the final air absolute humidity [$g_{water} \cdot g_{dry\ air}^{-1}$].

Ambient temperature (°C) and relative humidity (%) were measured using a digital temperature probe and a Thermometer/Humidity Monitor (Traceable® 4040, USA). Also, the air proprieties, such as absolute humidity, wet-bulb temperature, and relative humidity were found with Psychrometric Chart (Toledo, 1991) and psychrometric calculator auxiliary. The psychrometric calculator was based on the formulations of thermodynamic properties of moist air, according to Hyland & Wexter (1983a,b). The experimental wet-bulb temperature was measured according to Beck (2021), using a thermometer with the wet bulb.

2.2.4 Global and thermal efficiency of spray dryer

The global and thermal efficiency of the spray dryer (Masterd, 1972) were estimated, according to Equations 3 and 4, respectively:

$$\eta_{global} = 100\left(\frac{T_{air0} - T_{airf}}{T_{air0} - T_{amb}}\right) \quad (3)$$

$$\eta_{thermal} = 100\left(\frac{T_{air0} - T_{airf}}{T_{air0} - T_{bu}}\right) \quad (4)$$

wherein: T_{air0} is the inlet air temperature, T_{airf} the outlet air temperature, T_{bu} the wet-bulb temperature, and T_{amb} is the ambient air temperature.

2.2.5 Physicochemical characterization of honey powder

2.2.5.1 *Determination of moisture content and water activity (a_w)*

The moisture content was determined according to AOAC (1996), using an oven (Memmert, UL 40, Germany) at 105 °C, until the samples achieved a constant weight. The results were expressed as grams of water content per 100 g of dry matter. Water activity was measured using a HygroLabC1 water activity meter (Rotronic, Switzerland).

2.2.5.2 *Hygroscopy*

This procedure was conducted according to Cai & Corke (2000), with some modifications. Amounts of 1.2 g of honey powder were stored for 1 week in a desiccator containing a NaCl-saturated solution, with a relative humidity of (75.3) % in an incubator (SciQuip, Incu-80s, UK) at 25 °C. The mass of water adsorbed by the samples was expressed as grams of adsorbed water per 100 g of dry matter.

2.2.5.3 *Sugar content and total phenolics compounds (TPC)*

The sugar content was determined following the same procedure detailed in session 2.2.1.4. The determination of the TPC followed the same procedure previously described with one modification: the honey powder solutions were prepared at 0.40 mg/mL with ultrapure water (Direct Q3® system, Millipore, USA).

228 2.2.5.4 *Total protein by the Kjeldahl method*

229 Protein was analyzed using the Kjeldahl method according to AOAC (1996). The conversion factors
230 by Mariotti, Tomé & Mirand (2008) were 5.95 and 5.24 for honey powder with rice protein and pea
231 protein, respectively. The total protein content was expressed as grams per 100 g of dry matter.

232 2.2.5.5 *Morphology by Scanning Electron Microscopy (SEM)*

233 Morphology was visualized using scanning electron microscopy (Carl Zeiss EVO MA15,
234 Germany). The samples were first coated with 20 nm of Iridium (Ir) to act as an electricity
235 conductor using the secondary electron detector. The images were obtained at 10 keV.

236 2.2.5.6 *Particle size distribution and mean particle diameter*

237 Particle size distribution and mean particle diameter were determined using low-angle laser light
238 scattering (Mastersizer 2000, Malvern Panalytical, UK). The sample was dispersed in water and
239 remained under agitation during the procedure at 2500 rpm, including 1 min of ultrasonic agitation.
240 Volume-weighted mean diameter ($d_{4,3}$) was obtained using Eq. (5):

241 $$D[4,3] = \sum \frac{n_i d_i^4}{n_i d_i^3} \quad (5)$$

242 wherein n_i is the number of particles with diameter d_i. Each measurement was carried out in
243 triplicate.

244 **2.3 Statistical analyses**

245 All experiments were carried out in triplicate, and the data are presented as average plus standard
246 deviations. Tukey tests were performed to compare the treatment means. The significance level for
247 all tests was 5 %, which was calculated using SAS version 9.4.

248 **3. Results and discussion**

249 **3.1 Physicochemical characterization of honey**

250 The color value found at (143.43 ± 4.34) mm Pfund can be considered dark amber, according to the
251 mm Pfund scale (USDA, 1985). The color of the honey is largely influenced by the chemical
252 composition of nectar, associated with its botanical origin (Nordin et al., 2018; Scholz et al., 2020;

253 Solayman et al., 2016). Additionally, it can be influenced by the mineral content of honey, closely
254 linked with the soil characteristics and, therefore, geographic regions (Alvarez-Suarez et al., 2010;
255 Bobis et al., 2020;). Minor constituents of honey, such as flavonoids and carotenoids, can also
256 influence its color. Alvarez-Suarez et al. (2010), Bobis et al. (2020) and García-Tenesaca et al.
257 (2017) found high concentrations of these constituents in darker honey compared to lighter ones.
258 For that reason, dark honey was chosen to develop this work.
259 The measured pH of honey (3.73 ± 0.03) is low enough to avoid the growth of undesirable
260 microorganisms, maintaining its stability (Terrab et al., 2004). Free acidity is related to the presence
261 of organic acids in equilibrium with their lactones, or internal ester, and some inorganic ions, such
262 as phosphate or sulfate (Terrab et al., 2004; White, 1979). The free acidity value found in this work
263 of (46.41 ± 0.53) meq/kg is an acceptable value according to the legislation on honey quality
264 control (Brasil, 2000; Codex Alimentarius, 2001; International Honey Commission, 2009). The
265 maximum acceptable value is 50 meq/kg of honey; values above this limit can indicate the presence
266 of undesirable fermentation (Habib et al., 2014).
267 The total soluble solid content was (82 ± 0.2) g/100 g; therefore, the water content, which is the
268 second major component in honey, was considered (18 ± 0.2) g/100 g. This value is acceptable,
269 according to Brasil (2000) and Codex Alimentarius (2001); both legislations limit the maximum
270 value of water content to 20 g/100 g. The water content of honey can naturally range from (13.6 to
271 23) g/100 g and is influenced by different factors, such as the source, nectar geographical origin,
272 climatic conditions, harvest season, and the manipulation by the beekeepers (Bogdanov & Martin,
273 2002; De-Melo et al., 2018;). However, according to Bogdanov & Martin (2002), fermentation
274 issues can only be avoided if honey contains less than 18 g/100 g of water; otherwise, it will be a
275 suitable medium for yeast proliferation, decreasing its quality. Fermentation is caused by
276 osmophilic yeasts present in honey, which are responsible for forming ethyl alcohol and carbon
277 dioxide. Alcohol, in the presence of oxygen, breaks down into acetic acid and water, promoting a
278 sour taste in honey. The main yeast genus reported to be responsible for honey fermentation is

279 *Saccharomyces* spp. (Snowdon & Cliver, 1996). Note that drying honey could prevent spoilage by
280 yeast besides facilitating and increasing the application of the honey as an ingredient in the food
281 industry.
282 The fructose content was found at (46.52 ± 0.56) g/100 g and the glucose content at (35.88 ± 0.16)
283 g/100 g, while sucrose was not found in honey. Honey is mainly composed of sugar, and fructose is
284 normally the major monosaccharide (Afrin et al., 2020; Martinotti & Ranzato, 2018; White, 1979).
285 Sugars are directly related to honey crystallization, and the time over which this phenomenon
286 occurs depends mostly on the ratio of fructose to glucose (F/G), considering that glucose is less
287 soluble in water than fructose (Escuredo et al., 2014; Gleiter, Horn, & Isengard, 2006; Laos et al.,
288 2011; Nascimento et al., 2018). Honey with an F/G ratio of >1.33 does not crystallize for a long
289 time; in turn, if the ratio is <1.11 the honey crystallizes very fast (Escuredo et al., 2014; Smanalieva
290 & Senge, 2009;). In this work, the F/G ratio of the samples was at (1.30 ± 0.02), which suggests a
291 slow natural crystallization of honey.
292 In this work, the total phenolic compounds (TPC) found was GAE content at (301.31 ± 20.95) mg
293 /kg of honey, and this value is consistent with those reported by several other authors. Nascimento
294 et al. (2018) found similar values in honey collected from the south of Brazil, with a GAE content
295 ranging from (260 to 1000) mg/kg of honey. Furthermore, Dżugan et al. (2018) analyzed TPC in
296 Polish honey and the GAE content values ranged from (254.52 to 1353.66) mg/kg. Kavanagh et al.
297 (2019) found values of GAE content ranging from (25.9 to 811) mg/kg of honey in Irish multifloral
298 honey.
299 The total protein content found in this work was the L-leuE content at (0.0956 ± 0.0066) g/100 g of
300 honey. According to De-Melo et al. (2018), total honey protein can range from (0.1 to 0.5) g/100 g
301 of honey. Azeredo et al. (2003) evaluated total protein in honey of different floral origins and found
302 values ranging from (0.0199 to 0.2236) g/100 g, within the average value found in the present work.

3.1.1 Production of honey powder by spray drying

Tables 1 and 2 show the parameters and the water mass balance obtained from the production of honey powder by spray drying. The spray drying conditions, such as ambient temperature, relative humidity, inlet, and outlet air temperature indicated global efficiency of (48.33 ± 1.53, (48.67 ± 2.08 and 54.00 ± 1.00) % for the honey powder produced with rice protein, pea protein, or a mixture of both proteins, respectively, which corresponds to the total heat fraction provided by the equipment used to dry. Spray drying has the advantage of completing the drying process within a few seconds, maintaining the very low temperature of the droplets, and consequently drying heat-sensitive products without excessively affecting their quality (Ré, 1998; Tan, Zhong, & Langrish, 2020). Therefore, at a determined point of droplet drying, its temperature is the wet-bulb temperature of the drying air (Bhandari, Datta & Howes, 1997; Ré, 1998), which was (38.00 ± 1.00, 36.67 ± 0.58 and 37.33 ± 2.08) ºC for the honey powder produced with rice protein, pea protein, or a mixture of both proteins, respectively. Only at the end of the drying process did the particles reach the temperature close to the outlet air temperature of (77.75 ± 4.19, 77.00 ± 2.16 and 72.00 ± 1.82) ºC for the honey powder produced with rice protein, pea protein, or a mixture of both proteins, respectively. Under these conditions, the inlet air temperature was not high enough to affect the possible heat-sensitive components probably existing in the honey, for example, the phenolics compounds.

For example, for the honey powder produced with rice protein, changes in air humidity during the process were due to the water lost in the initial dispersion moisture at 2.356 ± 0.001 $g_{water} \cdot g_{dry\ matter}^{-1}$, leading the air absolute humidity to increase from approximately 0.0051 ± 0.0001 to 0.0200 ± 0.0001 $g_{water} \cdot g_{dry\ air}^{-1}$, providing a thermal efficiency around 56.67 ± 2.08 %, which indicates the approximation to the drying air saturation degree.

The powder recovery yield for the honey powder using rice protein, pea protein, or a mixture of both proteins as carriers was (64.88 ± 0.64, 45.32 ± 1.20 and 52.34 ± 4.59) %, respectively.

328 According to Bhandari et al. (1997), to consider a successful drying operation, the powder recovery
329 yield for sugar-rich products should be above 50 %. As can be seen, using pea protein as a carrier,
330 the value of the powder recovery yield was lower compared to that produced with rice protein. In
331 addition, it was observed that in the honey powder produced with pea protein as a carrier, the
332 particles easily stuck to the internal wall of the drying chamber, resulting in a low powder recovery
333 yield. However, with the honey powder produced with the mixture of proteins, the value found can
334 still be considered satisfactory. Therefore, when the objective is to obtain a high powder recovery
335 yield, with the drying parameters used in this study, it is suggested that for the formulation of honey
336 powder produced with the mixture of both proteins, the proportion of pea protein as a carrier should
337 not exceed the (50:50) ratio.

338 **3.2 Physicochemical characterization of honey powder**

339 Table 3 presents the physicochemical characterization of the honey powder produced with rice
340 protein, pea protein, or a mixture of both proteins. According to Labuza (1980), it is very important
341 to control water activity (a_w) to guarantee food stability, avoiding microbial growth and chemical
342 deterioration. The measured a_w values were similar and between (0.25 ± 0.07 and 0.427 ± 0.002) for
343 all samples, and the honey powder produced with rice and pea protein did not show significant
344 differences during storage time. The honey powder produced with the mixture of both proteins
345 showed a significant difference on day 21. However, all samples remained below 0.6, which is
346 considered to indicate stability to microbial deterioration (Labuza, 1980).
347 Regarding moisture, the use of pea protein as a carrier resulted in higher moisture of the honey
348 powder in comparison to rice protein, and the mixture of both proteins. According to Goula &
349 Adamopoulos (2005), the powder moisture is influenced by the particle size, and when the powder
350 has a smaller particle size, drying is facilitated. This is possible for two main reasons: (i) smaller
351 particles have a larger surface area per unit mass; therefore, more surfaces are in contact with the
352 heating air and, consequently, permit the moisture to escape; (ii) the heat capacity of the particles is
353 reduced for smaller particles; the distance is also reduced for moisture to go from the center of the

particle to the surface and escape. As can be seen in Figures 1A and 2, the honey powder produced with rice protein as a carrier has a higher quantity of smaller particles, when compared with the honey powder produced with pea protein as a carrier (Fig. 1B) and with the mixture of both proteins (Fig. 1C).

The honey powder produced with pea protein as a carrier presented a smooth surface morphology, with less porosity than the honey powder produced with rice protein and the mixture of both proteins, suggesting that this characteristic hindered the evaporation of water during drying. For all samples, the particles are observed to be linked by bridges; this morphology is typical for sugar-rich product powder (Samborska et al., 2019).

In dried honey, high values for hygroscopy are expected due to the high sugar content; however, the powders obtained in this study did not show significant differences, and the hygroscopy values were similar to those of other authors that also used spray drying, as can be seen in the review from Samborska (2019). Tonon, Brabet & Hubinger (2008) dried açai (*Euterpe oleraceae* Mart.) by spray drying and found hygroscopy values ranging from (12.48 ± 0.10 to 15.79 ± 0.29) g/100 g. The honey powder produced with rice protein as a carrier showed a lower moisture value. It has been suggested that the lower moisture increases the capacity to absorb moisture from the environment due to the greater water concentration gradient between the powder and the surrounding air (Tonon, Brabet & Hubinger, 2008). In addition, the morphology of the powder may influence its hygroscopy. The honey powder produced with pea protein presented a smooth surface, with less porosity than the one produced with the mixture of both proteins. Thus, the porosity of the honey powder produced with the mixture of both proteins may influence the hygroscopy value, mainly because rice protein increased the surface area of the particles and exposure to the moisture of the environment.

The sugar content values of the honey powder produced with rice protein, pea protein or a mixture of both proteins corroborate the initial spray-drying feed formulations. For example, in the feed formulation for the honey powder produced with rice protein, the honey content was (53.37 ± 0.04)

g/100 g, with the protein content of (37.59 ± 0.03) g/100 g, both in dry matter. At the end of the process, the honey powder produced with rice protein had a sugar content of (55 ± 0.25) g/100 g, and a protein content of (37.38 ± 1.26) g/100 g, both in dry matter, indicating that these macromolecules did not decrease during the spray drying process.

In the TPC assay for the honey powder, the proteins were verified to interfere with the results, increasing the TPC values. According to Ikawa et al. (2003), the use of the Folin-Ciocalteu phenol reagent can also detect certain nitrogen compounds. To overcome this challenge, powders were produced by spray drying only with rice protein, pea protein, or a mixture of both (without honey in the composition), and the TPC assay was performed. Finally, the absorbances measured from the powders only with proteins were subtracted from the corresponding TPC results of the honey powder. The values found after spray drying (Table 3) were similar to those found in the GAE content of (301.31 ± 20.95) mg/kg of honey, suggesting that the phenolic compounds were not negatively affected by the spray drying conditions and that the microencapsulation method was effective in protecting these bioactive compounds.

The particle size distributions of the honey powder shown in Figure 2 support the images in Figure 1 and show that the honey powder produced with rice protein has smaller particles compared to the honey powder produced with the pea protein and with the mixture of both proteins. The honey powder showed a mean particle diameter of (12.154, 26.835, 17.905) µm for the honey powder produced with rice protein, pea protein, or a mixture of both, respectively. This can be considered an appropriate size when the aim is to add the particles to a food matrix. According to Hansen et al. (2002), the particle size should be less than 100 µm to avoid a negative influence on the food texture.

4. Conclusions

In this work, the development of honey powder by spray drying using plant proteins as a carrier was successively achieved. The honey used as raw material showed acceptable physicochemical parameters by the legislation of honey quality control, leading to the production of honey powder.

406 The isolated rice protein provided a higher powder recovery yield compared to pea protein or a
407 mixture of both. The proportion of pea protein as the carrier should not exceed the (50:50) ratio to
408 obtain a high powder recovery yield. Total phenolic compounds were not affected by spray drying
409 conditions, since at a determined point of the droplets drying, their temperature is the wet-bulb
410 temperature of the drying air. High values for hygroscopy are expected as a result of the high sugar
411 content; however, the honey powders showed values similar to those reported by other authors that
412 also used the spray drying process. The honey powders produced with two different plant proteins
413 and a mixture of both can be an option for food ingredients, even for individual consumption as a
414 sports supplement or for new product development (protein bars and cookies). Thus, this study
415 suggests that honey powder is a suitable ingredient to be applied to a real food matrix that can be
416 commercialized.

Declaration of Competing Interest

None declared

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Authors' contributions

Taíse Toniazzo: Conceptualization, Data curation, Formal analysis, Writing - original draft, Review & Editing, Methodology, Investigation. **Mar Collado-González**: Investigation, Methodology, Review **Carmen Cecília Tadini:** Supervision, Conceptualization, Writing - review & editing, Funding acquisition. **Alan Mackie:** Supervision, Writing - review & editing.

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661 **Figure Captions**

662 **Fig. 1.** Morphology by scanning electron microscopy of the honey powder produced with isolated
663 rice protein (**A**), isolated micronized pea protein (**B**), or a mixture of both proteins (**C**) as carriers
664 during atomization. Magnification: 1,000 X.

665 **Fig. 2.** Distribution of the particle size of honey powder produced with isolated rice protein, isolated
666 micronized pea protein, or a mixture of both proteins as carriers during atomization.

667 **Table 1.**

668 Parameters from the spray drying process to obtain a honey powder with isolated rice protein, isolated
669 micronized pea protein, or a mixture of both proteins as carriers

Sample	Honey powder Rice protein	Honey powder Pea protein	Honey powder Rice+Pea proteins
Inlet air temperature (ºC)	130.83^{a} ± 0.75	130.17^{a} ± 0.41	129.33^{a} ±1.63
Outlet air temperature (ºC)	77.75^{a} ± 4.19	77.00^{a} ± 2.16	72.00^{a} ± 1.82
Ambient temperature (ºC)	22.10^{a} ± 0.17	21.03^{b} ± 0.40	21.57^{ab} ± 0.12
Relative humidity (%)	31.00^{a} ± 1.00	33.33^{ab} ± 1.15	33.67^{b} ± 0.58
Experimental wet-bulb temperature (ºC)	38.00^{a} ± 1.00	36.67^{a} ± 0.58	37.33^{a} ± 2.08
Global efficiency (%)	48.33^{a} ± 1.53	48.67^{a} ± 2.08	54.00^{b} ± 1.00
Thermal efficiency (%)	56.67^{a} ± 2.08	57.67^{a} ± 2.89	62.00^{a} ± 3.00
Powder recovery yield (%)	64.88^{a} ± 0.64	45.32^{b} ± 1.20	52.34^{c}± 4.59

670 Means followed by the same lowercase letter in the same line were not significantly different (p>0.05) by Tukey's test.

671 **Table 2.**

672 Water mass balance from the spray drying process to obtain honey powder with isolated rice protein, isolated
673 micronized pea protein, or a mixture of both proteins as carriers

Sample	**Honey powder Rice protein**	**Honey powder Pea protein**	**Honey powder Rice+Pea proteins**
Dispersion mass flow rate ($g_{dry\ mass} \cdot s^{-1}$)	0.0553 ± 0.0006	0.0554 ± 0.0002	0.0553 ± 0.0001
Initial dispersion moisture ($g_{water} \cdot g_{dry\ matter}^{-1}$)	2.356 ± 0.001	2.336 ± 0.003	2.336 ± 0.003
Air mass flow rate ($g_{dry\ air} \cdot s^{-1}$)	8.50 ± 0.01	8.50 ± 0.01	8.486 ± 0.002
Initial air absolute humidity ($g_{water} \cdot g_{dry\ air}^{-1}$)	0.0051 ± 0.0001	0.0051 ± 0.0002	0.0053 ± 0.0002
Final powder moisture ($g_{water} \cdot g_{dry\ matter}^{-1}$)	0.04 ± 0.01	0.08 ± 0.01	0.065 ± 0.005
Final air absolute humidity ($g_{water} \cdot g_{dry\ air}^{-1}$)	0.0200 ± 0.0001	0.0198 ± 0.0002	0.0201 ± 0.0001
Wet-bulb temperature (ºC)	37.1 ± 0.5	36.9 ± 0.6	38.4 ± 0.5

674

675 **Table 3.**

676 Physicochemical characterization of honey powder produced with isolated rice protein, isolated micronized
677 pea protein, or a mixture of both proteins as carriers

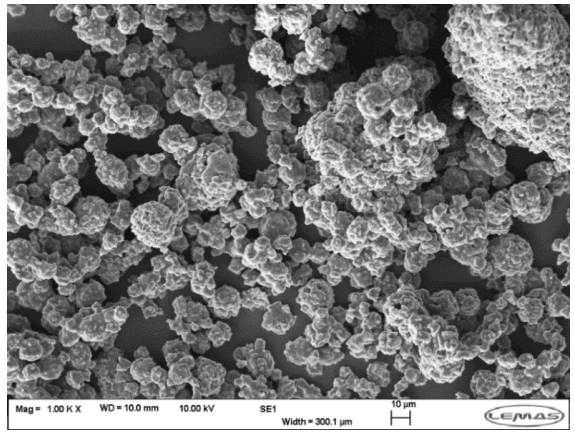
		Honey powder Rice protein	Honey powder Pea protein	Honey powder Rice+Pea proteins
a_w				
	Day 0	$0.28^{aA} \pm 0.10$	$0.32^{aA} \pm 0.06$	$0.25^{aA} \pm 0.07$
	Day 21	$0.39^{aA} \pm 0.02$	$0.398^{abA} \pm 0.007$	$0.427^{bB} \pm 0.002$
	Day 56	$0.31^{aA} \pm 0.01$	$0.327^{aA} \pm 0.002$	$0.324^{aA} \pm 0.003$
Moisture (g/100 g)		$3.36^{a} \pm 0.20$	$7.92^{b} \pm 0.04$	$6.82^{c} \pm 0.01$
Hygroscopy (%)		$21.18^{a} \pm 0.24$	$20.27^{a} \pm 0.02$	$21.26^{a} \pm 0.71$
Fructose content (g/100 g) of honey		$31.56^{a} \pm 0.21$	$31.39^{a} \pm 0.10$	$35.45^{b} \pm 0.43$
Glucose content (g/100 g) of honey		$23.43^{a} \pm 0.13$	$23.04^{a} \pm 0.34$	$27.66^{b} \pm 0.66$
Total protein content (g/100 g)		$37.38^{a} \pm 1.26$	$38.95^{a} \pm 1.77$	—
GAE content (mg/kg) of honey		$353.78^{a} \pm 30.48$	$318.58^{a} \pm 52.80$	$336.18^{a} \pm 52.80$

678 Means followed by the same lowercase letter in the same line were not significantly different (p>0.05) by Tukey's test.
679 Means followed by the same uppercase letter in the same column were not significantly different (p>0.05) by Tukey's test.

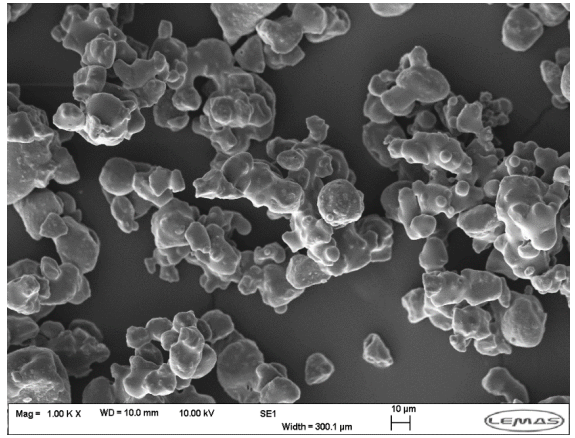
Figure 1

Fig. 1.

(A)



(B)



(C)

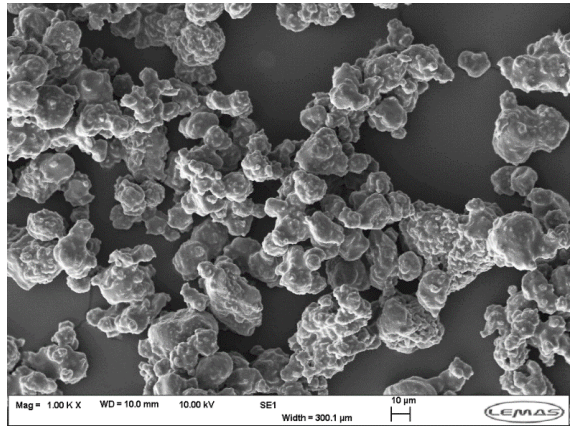
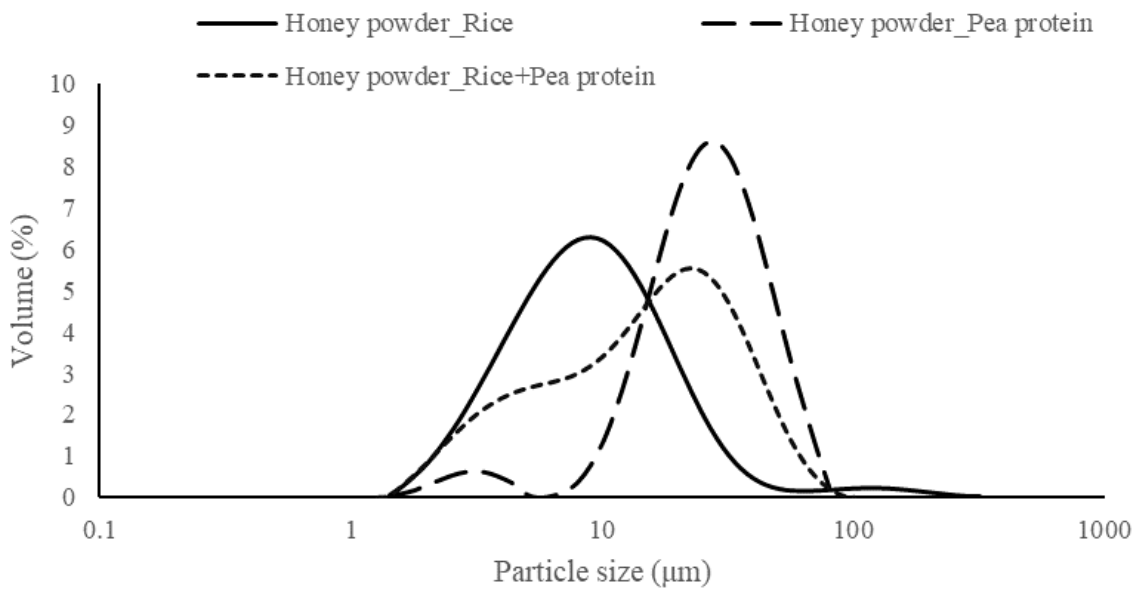
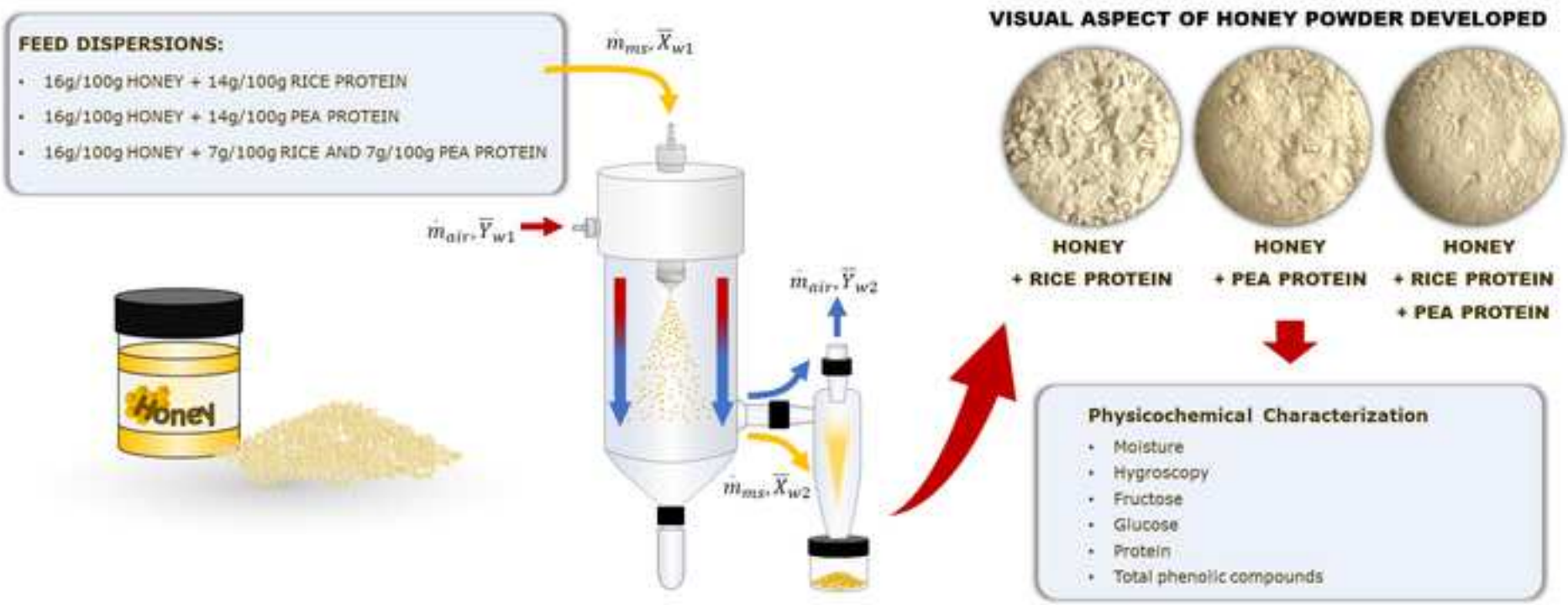


Fig. 2



Graphical Abstract



Credit Author Statement

Taíse Toniazzo: Conceptualization, Data curation, Formal analysis, Writing - original draft, Review & Editing, Methodology, Investigation.

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Declaration of Competing Interest

None declared