

RESEARCH ARTICLE

Effect of whey and cassava starch on the production of honey powder: Spectroscopic and morphological analysis

Efeito do soro de leite e do amido de mandioca na produção de mel em pó: Análise espectroscópica e morfológica

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Abstract

Honey is considered a nutritious food, produced primarily by bees (*Apis mellifera* L.) from flower nectar. However, its high viscosity and handling difficulties have driven the development of honey powder production. The use of adjuvants such as whey and cassava starch, due to their versatility and availability, is a viable option for honey powder production. Therefore, mixtures of honey, whey, and cassava starch were evaluated. The mixtures were dried in a forced air oven at 60 °C for 24 h. The resulting powders were physicochemically characterized and analyzed by FT-IR, DLS, and SEM. The mixture with the highest whey content showed a tendency to slightly decrease the absorption peaks in the spectral range of 1400 to 700 cm⁻¹ in FT-IR spectral analysis. The electrostatic stability showed values of -34.7907 mV, a peak distribution of -35.3467 mV with a conductivity of 0.0091 mS/cm and an electrophoretic mobility of -2.7116 μm²/Vs. The morphology of the resulting powder consisted of agglomerated particles with starch granules ranging in size from approximately 45.7 to 90.2 μm. Whey and cassava starch are used as additives and stabilizing agents to produce powdered honey.

Keywords: Honey. Whey. Cassava starch. FT-IR. DLS. SEM.

Resumo

O mel é considerado um alimento nutritivo, produzido principalmente pelas abelhas (*Apis mellifera* L.) a partir do néctar das flores. No entanto, sua alta viscosidade e as dificuldades de manuseio impulsionaram o desenvolvimento da produção de mel em pó. O uso de adjuvantes como soro de leite e amido de mandioca, devido à sua versatilidade e disponibilidade, é uma opção viável para a produção de mel em pó. Portanto, misturas de mel, soro de leite e amido de mandioca foram avaliadas. As misturas foram secas em estufa de ar forçado a 60 °C por 24 h. Os pós resultantes foram caracterizados físico-quimicamente e analisados por FT-IR, DLS e MEV. A mistura com o maior teor de soro de leite apresentou uma tendência a diminuir ligeiramente os picos de absorção na faixa espectral de 1400 a 700 cm⁻¹ na análise espectral de FT-IR. A estabilidade eletrostática apresentou valores de -34,7907 mV, um pico de distribuição de -35,3467 mV com condutividade de 0,0091 mS/cm e mobilidade eletroforética de -2,7116 μm²/Vs. A morfologia do pó resultante consistiu em partículas aglomeradas com grânulos de amido variando em tamanho de aproximadamente 45,7 a 90,2 μm. O soro do leite e o amido de mandioca são utilizados como adjuvantes e agentes estabilizantes na produção de mel em pó.

Palavras-chave: Mel. Soro de leite. Amido de mandioca. FT-IR. DLS. MEV.

Graphical Abstract



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1. Introduction

Honey is a viscous substance produced from the enzymatic conversion of nectar from different floral sources and secreted by the honeybee *Apis mellifera* L. Honey is mainly composed of 80% sugars, 15% water, and smaller amounts of amino acids, enzymes, vitamins such as B complex and ascorbic acid, minerals such as K, Na, Ca, Fe, Mg, P, Cu, S, and polyphenols, most of which are flavonoids such as apigenin, hesperidin, kaempferol, luteolin, myricetin, naringenin, pinocembrin, quercetin, and rutin; phenolic acids; phenolic acid derivatives (gallic acid, chlorogenic acid, vanillic acid, caffeic acid, syringic acid, ferulic acid, and ellagic acid); and flavones, compounds of great interest due to their diverse biological activities, such as neuroprotective properties, antimicrobial, chemoprotective, anticancer, antifungal, antioxidant, anti-inflammatory, and immunomodulatory (Baglio, 2018; Filomeni et al., 2012; Santos et al., 2021).

The quantity of compounds in each honey sample varies depending on the climate, pollen type, altitude, and other environmental conditions of the production area (Tenore et al., 2012). However, due to its high sugar content, honey is difficult to handle because of its high viscosity and hygroscopicity. Therefore, it is necessary to add additives with high molecular weight molecules derived from native or modified starch, whey, vegetable protein, and other components capable of maintaining an emulsifying equilibrium. This increases the glass transition temperature of the mixture, reduces viscosity by creating a semicrystalline system, and improves drying efficiency and desirable physicochemical properties (Samborska et al., 2025; Toniazzo et al., 2023; Vosoghi et al., 2025; Yadav et al., 2026).

Powdered honey is a product derived from liquid honey, which has gained popularity in the food industry due to its versatility, ease of handling, stability, and storage, as well as the preservation of its attributes. Powdered honey can be obtained through dehydration processes, transforming liquid honey into a dry powder while preserving its nutritional and organoleptic properties, such as its natural flavor and sweetness (Samborska, 2019).

The use of natural additives such as milk protein and starch to produce honey powder is a sustainable, economical, and readily available alternative. Whey is a byproduct of the dairy industry derived from cheese production and is typically discarded. However, recent studies have demonstrated the nutritional value of whey, which contains essential branched-chain amino acids that influence the insulin response in humans. Studies have shown that incorporating whey protein into foods can improve insulin release and lower postprandial blood glucose levels. Furthermore, various chronic conditions, such as diarrhea, biliary diseases, skin problems, urinary tract infections, hypertension, and cardiovascular disorders, can be addressed through the use of biological components of whey. The diverse therapeutic benefits of whey have also been demonstrated through different administration methods, including supplements, functional foods, and topical applications (Solanki et al., 2023; Verma et al., 2024). Some food products, such as bread and confectionery, have been made with whey powder, obtaining physicochemical and organoleptic properties desirable to the consumer (Britchenko & Mickiewicz, 2022; Chaven, 2023). Another important additive in the food industry is starch.

Starch is a ubiquitous polysaccharide composed of numerous glucose units linked by glycosidic bonds. It is a biomolecule produced in plants as an energy reserve. Various starches from different botanical sources, such as corn, rice, potato, wheat, and cassava, among others, exhibit distinct

physicochemical properties for use in diverse applications. Cassava starch has a granule size of 5 to 35 μm , a silky texture, high viscosity, and is an excellent binder, furthermore, it exhibits a degree of crystallinity of 25 to 35%, evidenced by a Type C (intermediate) X-ray diffraction pattern, standing out above potato and corn starch with Type B (open structure) and Type A (compact structure) crystallinity degrees respectively, related to the amylopectin content, being a physicochemical property with an area of opportunity to study its behavior when mixed with other organic components (Wang et al., 2022; Zhu, 2015). Cassava starch is used in the formulation of various food products, primarily in snacks (gelled cassava pearls) for soft drinks, as well as in the pharmaceutical, cosmetic, and textile industries (Breuninger et al., 2009).

The development of powdered honey responds to the need for a practical alternative to liquid honey, especially in industrial and culinary applications. Its powdered form facilitates storage, transport, and dosage, reducing problems such as dripping, crystallization, and sticking (Samborska, 2019). Furthermore, it mixes easily with other ingredients, enhancing its use in products such as baked goods, confectionery, instant beverages, and functional foods (Sathivel, 2013).

The production process for powdered honey typically includes techniques such as spray drying, freeze-drying, fluidized bed drying, and air-flow oven drying. These techniques are frequently combined with carrier agents such as maltodextrin or gum arabic to prevent clumping and improve the stability of the final product (Samborska, 2019; Suhag & Nanda, 2016). In this context, powdered honey has become an innovative and cost-effective option for developing and innovating new products with this natural additive, as well as opening up new applications and marketing possibilities globally (Samborska, 2019; Tomczyk et al., 2021). However, no studies have yet been reported on spectroscopic analysis using instrumental techniques to verify its composition when combined with modified starch and whey. Therefore, the objective of this study was to analyze the composition of a honey powder from FT-IR, DSL, and SEM analysis, in order to obtain an efficient, economical, easy-to-handle, and functional powder.

2. Materials and Methods

2.1. Sample

The honey sample was acquired from artisanal producers in the Mexicali Valley region. The whey powder was purchased commercially (Perley, Vieley, Aguascalientes, MX). The starch used was commercial food-grade cassava starch (Ingredient, Incorporated Food and Beverage Services, Westchester, Illinois, USA), 12% moisture by weight, with an amylose to amylopectin ratio of 18:82. The reagents used were analytical grade from J.T. Baker.

2.2. Preparation of blends

Three treatments (T1, T2, and T3) were performed, varying the amount of whey (0.5, 1, and 2 g). The amounts of cassava starch and honey were 10 g and 6.5 g, respectively. For each treatment, the honey was first dissolved in 10 mL of distilled water, and then the starch and whey, previously mixed dry, were gradually added. Finally, the mixture was homogenized using a digital laboratory mixer (MXBAOHENG Instrument Co., Guangdong, China) for 30 min to 2500 rpm. After homogenization, the mixtures for each treatment were dried in an airflow oven (LabLine, Ambi-Low Chamber, Livonia, Michigan, USA) at 60 °C for 24 h. These conditions were established based on preliminary

trials (results not shown). Finally, the mixtures were ground using a mortar and sieved with a pore size of 250 μm and stored in airtight, vacuum-sealed Ziploc bags at a temperature of 8 $^{\circ}\text{C}$ for further analysis.

2.3. Physicochemical characterization

T1, T2 and T3 were characterized by moisture content, total soluble solids, and pH, following the AOAC (2023) methodology. The pH was measured using an Ohaus ST5000-F pH meter (Columbus, Ohio, USA).

Water-soluble solids (WSS) were quantified by pressing 10 g of sample and dissolving it in 30 mL of distilled water until a homogeneous mixture was obtained and deposited in porcelain crucibles previously at constant weight and placed in a drying oven at 110 $^{\circ}\text{C}$ for 60 min, finally they were placed in a desiccator and the crucibles were weighed again until the sample was obtained in powder and the water-soluble solids content was calculated using Eq. 1.

$$\%WSS = [(P_i - P_f) / P_i] * 100 \quad \text{Eq. 1}$$

Where: P_i = (weight of constant crucible + weight of sample); P_f = (weight of constant crucible + weight of dry sample)

For the determination of moisture, first, porcelain crucibles were pre-weighed to a constant weight. Five grams of the flour sample were placed in triplicate in each crucible and heated in an air-cooled oven (LabLine, Ambi-Low Chamber, Livonia, Michigan, USA) at 130 $^{\circ}\text{C}$ for 60 minutes. After heating, the crucibles were removed and placed in a desiccator to constant weight at room temperature (25 $^{\circ}\text{C}$). Finally, they were weighed, and the moisture content was calculated according to Eq. 2. The mixture with the lowest moisture content, as well as the cassava starch and whey, were characterized by spectroscopic and morphological analysis.

$$\% \text{ Moisture} = \frac{(P_i - P_f)}{P_i} \times 100 \quad \text{Eq. 2}$$

Where: P_i = Initial weight (Crucible + sample); P_f = Final weight (Crucible + sample / drying).

2.4. Fourier transform infrared spectroscopy (FT-IR)

The cassava starch, whey, and sample were placed on the surface of the ATR crystal. FTIR spectra were obtained between 4000 and 600 cm^{-1} using an Agilent 4300 FT-IR (Frontier, PerkinElmer, Waltham, Massachusetts, USA), with 40 scans and resolutions of 4 cm^{-1} .

2.5. Dynamic light scattering (DLS)

The cassava starch, whey, and sample were prepared beforehand. 100 ppm solutions were made with distilled water and vortexed at 3000 rpm for 30 seconds. The electrostatic stability of the sample's particle distribution was determined by static light scattering laser diffraction (DLS) using a Microtrac Nanotrack Wave II, Microtrack, Haan/Duesseldorf, Germany.

2.6. Scanning electron microscope (SEM)

The sample was observed by scanning electron microscopy using a low-vacuum SEM-EDS (JEOL JSM-6010LA SEM-EDS, JEOL, Tokyo, Japan), operated at 10 kV and 500X magnification to obtain the micrographs.

2.7. Statistical analysis

All experiments were performed in triplicate. The results were analyzed using a one-way analysis of variance (ANOVA) with Origin 8.0 software (Origin Inc., MA, USA). Mean differences were analyzed using Tukey's test ($p \leq 0.05$).

3. Results and Discussion

Table 1 shows the results obtained from the physicochemical characterization of the mixtures. The mixture with 2 g of whey protein had a moisture content of $11.39 \pm 0.18\%$, showing significant differences compared to the other samples. A lower moisture content does not promote agglomeration between particles; therefore, the opposite charges on the powder surfaces, caused by friction and attrition, lead to ionic bonding between the particles (Hazlett et al., 2021).

Table 1 Physicochemical characterization of honey powder produced with whey and cassava starch

Treatments	Whey (g)	Moisture (%)	Water-soluble solids (%)
T1	0.5	18.41 \pm 0.15 ^a	12.46 \pm 0.02 ^a
T2	1	14.05 \pm 0.11 ^b	15.16 \pm 0.08 ^b
T3	2	11.39 \pm 0.18 ^c	18.62 \pm 0.06 ^c

Letters with different letters in each column show significant differences, $p > 0.05$.

The quantity of water-soluble solids in the sample with 2 g of whey was $18.62 \pm 0.06\%$, the highest value compared to the other treatments. This behavior is attributed to whey proteins, which have high natural solubility across a wide pH range. When added to the mixture, they directly contribute to the total soluble solids content. Furthermore, cassava starch can interact with whey proteins, forming covalent complexes between starch amylose and soluble whey proteins (Allen et al., 2007; Wu & Guo, 2025; J. Zhang et al., 2025).

The pH increased with the addition of more whey, reaching values from 5.9 to 6.5. This behavior is attributed to the fact that pH directly influences the protonation state of whey proteins. When the pH rises above the isoelectric point of the proteins, they develop a more negative net charge, which can lead to altered or enhanced electrostatic interactions with the hydroxyl groups of starch. These interactions, especially during thermal processing, can involve the binding of hydrogen ions (H^+) by certain molecular groups, reducing their free concentration in the solution and, consequently, increasing the pH (Bravo-Núñez et al., 2019; Chandrapala et al., 2015; Pelegrine & Gasparetto, 2005; Zhen et al., 2022).

Depending on the type of drying and the adjuncts, there will be variability in the physicochemical properties of the powdered honey, presenting moisture values of 2 to 18%, pH between 2.6 and 6.8, and water-soluble solids of 10 to 72% (Masyithoh et al., 2025; Saraugi et al., 2026; Yadav et al., 2026).

Honey, when homogenized with distilled water at room temperature, along with cassava starch and whey, ensures uniform dispersion. However, further studies are needed on the molecular

stability of the blend during storage (time/temperature). To obtain a powdered product, such as honey, the dehydration process is used. This involves mixing the honey with adjuncts such as starch, maltodextrin, gums, syrups, whey, and others, and then removing moisture through various methods such as spray drying, vacuum drying, microwave vacuum drying, foamed material vacuum drying, internal surface expansion, and low-temperature vacuum drying. The specific temperatures used are key to creating a fine powder and maintaining the physicochemical and organoleptic properties upon rehydration (Samborska, 2019; Yadav et al., 2026).

Once the results obtained from the three treatments were analyzed, T3 with cassava starch and whey was compared, because it presented the lowest amount of moisture compared to the other treatments, and consequently avoids particle agglomeration. Cassava starch, whey, and honey mixture samples were analyzed by FT-IR (Fig. 1). The cassava starch sample (Fig. 1a) showed a spectrum with characteristic peaks in the 3500–3200 cm^{-1} range, attributed to the amount of water present due to the stretching vibrations of OH groups. The peaks observed in the 1200–700 cm^{-1} region of the FTIR spectra have been correlated with changes in the double helix structure, specifically associated with crystalline and amorphous starch regions, respectively (Soler et al., 2026).

The whey sample (Fig. 1b) exhibited vibrations of single bonds (O–H, C–H, and N–H) at 4000–2500 cm^{-1} . Furthermore, a complex vibration pattern in the low wavenumber range of 1800 to 700 cm^{-1} is characteristic of the presence of proteins in amide groups at 1750–1650 cm^{-1} , C=O at 1750–1700 cm^{-1} , Amide II at 1550–1500 cm^{-1} , N–H at 3500–3300 cm^{-1} , and C–N at 1300–1000 cm^{-1} (He et al., 2025; Nabgan et al., 2025; Saji et al., 2024).

In the sample of the mixture of cassava starch, whey, and honey components (Fig. 1c), a tendency to slightly decrease the absorption peaks in the spectrum described above was observed. This behavior is attributed to interference with the ability of the starch microstructure to retain the honey and whey components, along with the bound water. The study conducted by Yamul & Lupano (2005) analyzed the structural and functional properties of whey protein concentrate gels with varying amounts of honey and wheat flour. They demonstrated that wheat flour, due to its physicochemical and functional properties, interacted with whey and honey, highlighting the importance of incorporating higher molecular weight adjuncts to achieve synergies between components with different molecular structures. Another study by Torley et al. (2004) showed that honey acts as a plasticizing agent due to its sugar content (fructose and glucose), improving flexibility and reducing brittleness in starch-based films.

Fig. 2 shows the DLS spectra for ζ potential, a parameter that quantifies the charge developed on the particle surface due to the ionization of chemical groups or ion adsorption (Montes et al., 2019). This parameter is influenced by the particle surface charge characteristics and the composition of the solution in which they are suspended, with electrophoretic mobility being the speed of the particle per unit of electric field and conductivity being the ability of the liquid/medium to conduct current (X. Zhang et al., 2023). The ζ potential obtained for cassava starch (Fig. 2a) was -29.9879 mV, with a peak distribution of -30.5291 mV, a conductivity of 0.0032 mS/cm, and an electrophoretic mobility of -2.3373 $\mu\text{m}^2/\text{Vs}$. Regarding whey (Fig. 2b), the values were -34.4894 mV, with a peak distribution of -35.4995 mV, a conductivity of 0.0155 mS/cm, and an electrophoretic mobility of -2.6881 $\mu\text{m}^2/\text{Vs}$. The sample of the blend of all components and honey (Fig. 2c) presented zeta potential values of -34.7907 mV, a peak distribution of -35.3467 mV, a conductivity of 0.0091 mS/cm, and an electrophoretic mobility of -2.7116 $\mu\text{m}^2/\text{Vs}$. The

theoretical value of the zeta potential, which allows for the evaluation of the stability of a structure, that is, differentiating a surface with low charge from one with high charge, is ± 30 mV. Particles with values above and below this range will exhibit excellent stability, thus preventing aggregation or clumping between particles. However, particles with zeta potential values within the range of $-30 < \zeta < +30$ mV will be more unstable, because the attractive force between particles is greater than the repulsive force, leading to disruption of the dispersion and, subsequently, flocculation (Simunkova et al., 2009). The blend presented ζ -potential values around -35 mV, suggesting good electrostatic stability according to commonly accepted empirical criteria ($|\zeta| > 30$ mV). However, stability in complex food systems may also be influenced by ionic strength, steric interactions, and viscosity effects, which should be further evaluated during storage.

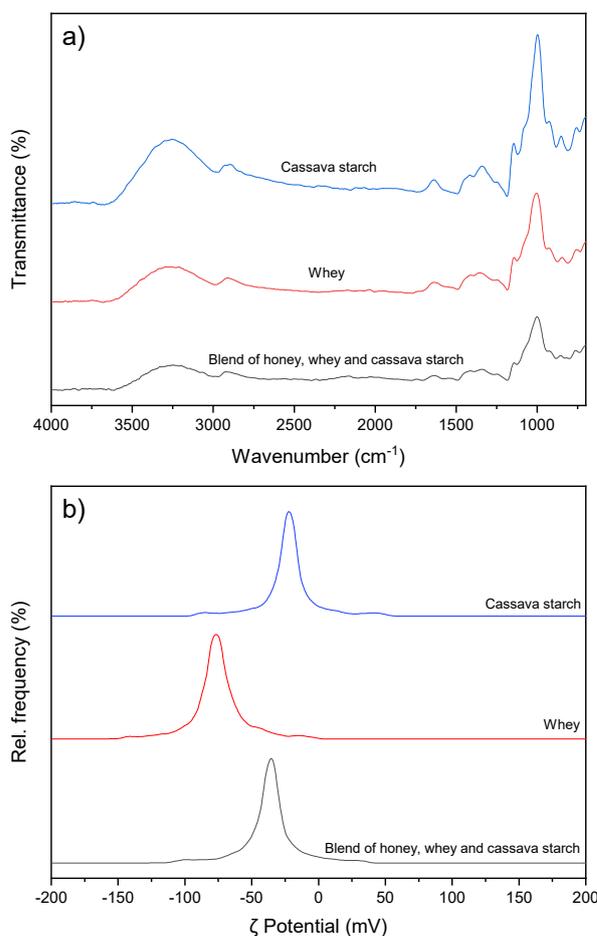


Fig. 1. (a) Fourier transform infrared (FT-IR) spectra and (b) ζ -potential of cassava starch, whey, and a blend of honey, whey, and cassava starch (Formulation T3: 10 g cassava starch, 6.5 g honey and 2 g whey).

Scanning electron microscopy (SEM) images are shown in Figure 3, revealing the morphology of cassava starch, whey, and the mixture of these components with honey. SEM images of cassava starch revealed its original morphology with oval shapes and smooth surfaces (Fig. 2a), with granule sizes ranging from 12.2 to 19.4 μm . Fig. 2b shows whey powder, which exhibited irregularly shaped, corrugated, flake-like particles ranging in size from 20.5 to 80.9 μm . The mixture of all components and honey (Fig. 2c) showed agglomerated particles with starch granules approximately 45.7 to 90.2 μm in size, indicated by red arrows in Fig. 2c. Additionally, agglomerated cassava granules were

observed. These particle agglomerations can be promoted by whey and honey to agglomerate a specific amount of starch granules, being directly related to the surface charge of each particle. In the food industry, particle size (granulometry) is the critical factor that determines everything from the solubility to the creamy feel of a product. The particle size range is between 15 and 250 μm to obtain a technological effect such as optimal flow, water absorption, smooth texture, and avoiding a gritty feel, among others (Dodds, 2024).

A study by Chandrakar et al. (2024) confirmed the interaction between proteins and polysaccharides, improving thermal stability, functional properties, and physicochemical

properties using a mixture of honey, whey, and maltodextrin. The study demonstrated honey powder with low agglomeration and cohesion, yielding a protein-polysaccharide conjugate suitable for incorporation into the development of various products in the food industry. Regarding morphology, mixtures with a higher concentration of whey exhibited better properties due to the protein-polysaccharide complex, displaying morphologies with holes and cracks resulting from moisture removal, as well as smooth surfaces. According to Ke & Li (2023), the Maillard reaction, induced by temperature during drying of the mixture, promotes a protein-polysaccharide interaction, altering the protein structure and improving the physicochemical properties of the resulting powder (Setiowati et al., 2020).

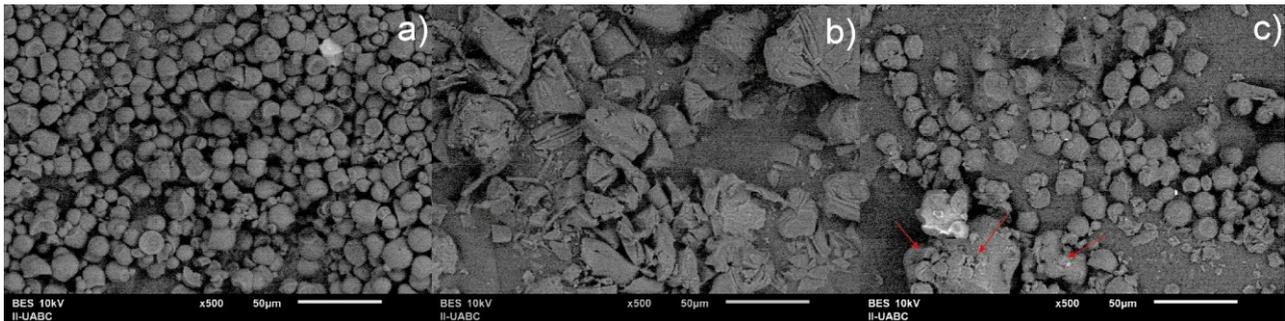


Fig. 2. Scanning electron microscopy images of cassava starch (a), whey (b), and a blend of honey, whey, and cassava starch (c) (Formulation T3: 10 g cassava starch, 6.5 g honey and 2 g whey).

4. Conclusion

Whey protein promotes a molecular synergy with cassava starch to produce powdered honey, offering a new way to utilize this dairy byproduct. Spectroscopic and microscopic instrumental techniques are efficient for revealing the composition and molecular changes, electrostatic charge, and morphological structure of the honey powder, respectively. Therefore, the combination of whey protein and cassava starch creates a stabilizing agent, making it an alternative for obtaining powdered honey for potential use in the food industry for the development of instant beverages, bakery premixes, nutraceutical formulations, and other applications.

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Authors' Contributions

L.G.S.: Conceptualization; J.G.J.D.: methodology; D.D.H., and D.G.M.: validation; B.V.S., and E.B.P.: formal analysis; U.A.B.C.: investigation; U.A.B.C.: data curation, writing-original draft preparation; L.G.S.; writing-review and editing; A.A.S.N.: supervision.

Availability of data and materials

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Conflicts of Interests

The authors declare that they have no competing interests.

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