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Morphological characterization of whey protein concentrate admixture of microencapsulated curcumin by spray drying

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Abstract

Spray drying was used to develop two products: whey protein concentrate (WPC) and whey protein concentrate admixture of microencapsulated curcumin (TWPC). We aimed to characterize the concentrate's technological attributes to verify whether lactose remains in an amorphous state even after the addition of turmeric extract containing curcumin, a compound with antioxidant and anti-inflammatory properties. Analysis of morphology, particle size distribution, Raman spectroscopy, sorption isotherms and colorimetry were carried out. WPC and TWPC showed spherical, irregular, particulate morphology with agglomeration points, without apparent cracks. Differences in the L^*, a^*, b^* values between WPC and TWPC showed that the addition of curcumin extract, which is a natural orange-yellow dye, has led to a tendency toward yellow coloration. The sorption isotherms indicated no difference in its curved shape. The presence of turmeric extract in TWPC sample modified WPC Raman spectrum. Thus, it was possible to develop TWPC without altering WPC technological characteristics.

Practical applications

Whey protein concentrate (WPC) and whey protein concentrate admixture of microencapsulated curcumin (TWPC) showed spherical, irregular, particulate morphology with agglomeration points, without apparent cracks. The differences in the L^*, a^*, b^* values between WPC and TWPC showed that the addition of curcumin extract, which is a natural orange-yellow dye, has led to a tendency toward yellow coloration. Thus, we developed a TWPC without altering WPC technological characteristics.

1 | INTRODUCTION

Whey protein has been studied and consumed by athletes and physically active individuals due to its content of branched-chain amino acids (leucine, isoleucine, and valine), bioactive peptides, vitamins, and minerals. It is considered a product with physiological and functional properties that intensify hypertrophy (Bergia et al., 2018; Corgneau et al., 2019; Davies et al., 2018; Patel, 2015; Simovic et al., 2019; Souza et al., 2015).

Whey proteins have also been used as biopolymers to increase the shelf life of sensitive molecules and conduct the release of bioactive/nutritional substances into the body. Whey proteins have the ability to form gels and microcapsules under relatively moderate heating conditions and without the need for chemicals, making them a natural vehicle for bioactive (hydrophobic and hydrophilic) components for medical and food applications (Awad et al., 2015; Hu et al., 2019; Jiang et al., 2018; Nishanthi et al., 2018).

Turmeric (*Curcuma longa* L.) belongs to the Zingiberaceae family. It is a medicinal plant native to India and Southeast Asia, popularly

known as turmeric, saffron, safflower, and yellow ginger (Cañamares et al., 2006; Mohajeri et al., 2017). Its main phenolic component is curcumin [1,7-bis(4-hydroxy3-methoxyphenyl)-1,6-heptadiene-3,5-dione], a chemical difluoromethane that consists of two bonded ferulic acid molecules with a methylene bridge forming β-diketone (Cañamares et al., 2006; Mohajeri et al., 2017; Pereira & Stringheta, 1998; Teymouri et al., 2017). Turmeric's rhizome has been used as dye, flavoring agent, and ingredient in the food industry to prepare seasonings, processed foods, and dairy products (Apisariyakul et al., 1995; Govindarajan, 1980; Ricardo, 2014; Silva et al., 2004).

Recently, curcumin has been studied for its benefits to sports medicine because of its antioxidant and anti-inflammatory potential (Zhao et al., 2015). However, because curcumin is sensitive to alkaline conditions, heat treatment, light, metal ions, enzymes, oxygen, and ascorbic acid, it is often encapsulated to improve solubility, stability, and bioavailability (Chin et al., 2009; Dar et al., 2017; Hatcher et al., 2008).

Raman spectroscopy offers analytical possibilities for production and quality control, especially where milk matrices are involved, and can be used for various purposes. Stephani et al. (2017) paired Raman spectroscopy and chemometrics and found that the association could be used as a screening method for routine analysis and production line control. Another author has used the same parameters to quantify lactose amounts in whey powder (Norgaard et al., 2005).

Considering the aforementioned information, the objective of the present study was to obtain whey protein concentrate admixture of microencapsulated curcumin (TWPC) via spray drying and to characterize the concentrate's technological attributes to verify whether lactose remains in an amorphous state.

2 | MATERIALS AND METHODS

2.1 | Turmeric extract preparation

Turmeric was purchased from rural producers in the city of Viçosa, Minas Gerais, Brazil. Samples were collected from the rhizome of different plants to obtain a representative sample.

The analytical method used for obtaining curcumin was based on NBR 13624 which used dilution and spectrophotometric reading to carry out turmeric extraction as described below (Lara, 1984).

Sanitized, peeled turmeric root was weighed, placed in a saucepan, covered with water, and boiled for 20 min. The contents of the pan were then transferred to a container and cooled to room temperature.

The cooked turmeric was subsequently ground in a blender (Black & Decker® SB40), while 70% ethanol was added gradually (just enough to grind the sample). The ground mixture was covered with aluminum foil and let rest for 4 hr.

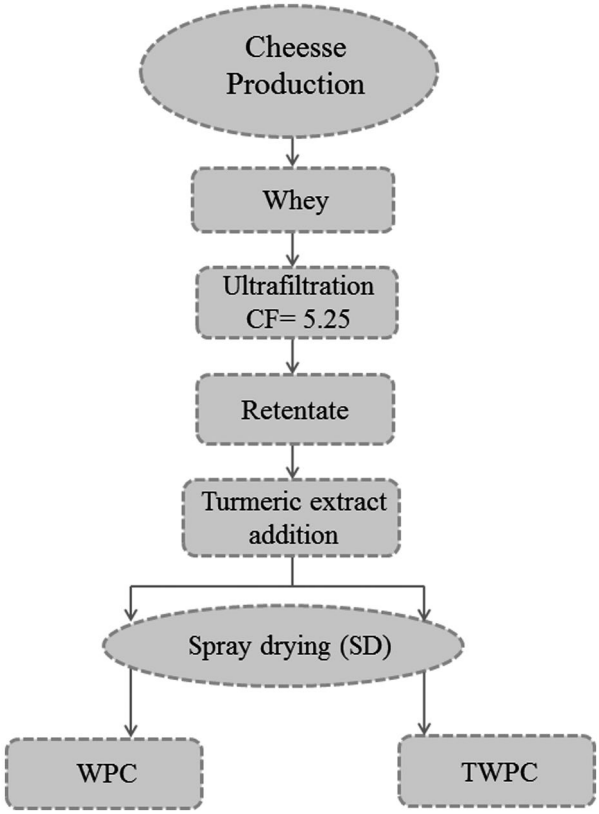

FIGURE 1 Sample Production Flowchart. Where Cf, concentration factor; TWPC, whey protein concentrate admixture of microencapsulated curcumin; WPC, whey protein concentrate

Next, the mixture (turmeric, water, and alcohol) was centrifuged (Multi-purpose® centrifuge, NF 1200/1200R) at 6,000 rpm for 10 min. The supernatant was vacuum filtered using a Büchner funnel with filter paper. Ethanol was evaporated from the mixture at 70°C ± 1°C for 20 min using a rotary evaporator (QUIMIS® model Q344.1) coupled with the vacuum pump (Tecnal® model TE058). The extract was stored in an amber bottle at −18°C ± 1°C until it was ready to use in the TWPC and in the nutritional and technological analyses.

2.2 | Whey protein concentrate admixture of microencapsulated curcumin

Two products were developed: WPC and TWPC (Figure 1). The amount of turmeric extract added to WPC was calculated according to the daily acceptable intake of curcumin of 0–3 mg/kg body weight/day as recommended by the Food and Agriculture Organization/World Health Organization (FAO/WHO, 2004).

The developed sample contained 3.6 mg of curcumin (for 30 g whey protein). Because turmeric has a distinctive and pungent flavor, artificial pineapple flavoring (composition: sugar, cornstarch, citric acid, artificial flavoring, artificial coloring: yellow tartrazine and dusky yellow) was added to the sample to improve acceptance

levels. One gram of artificial pineapple flavoring was added for each 3 g of TWPC according to the product label instructions.

Whey was ultrafiltered (ultrafiltrator, GEA®). The retentate obtained was spray-dried (GEA®, model Minor Production) using a 180°C ± 1°C inlet air temperature and an 85°C ± 2°C outlet air temperature. Prior to drying, the retentate was divided into two aliquots. The first one (WPC) went directly into the spray dryer for drying. Turmeric extract was added to the second aliquot, which was spray-dried to obtain TWPC.

2.3 | Scanning electron microscopy

The morphology of *in natura* lyophilized turmeric and turmeric extract, as well as the two samples (WPC, TWPC), was studied to determine their specific characterizations using Scanning Electron Microscopy technology (Hitachi®, TM3000, Hitachi Ltd., Tokyo, Japan).

2.4 | Particle size distribution in the rehydrated WPC by laser diffraction

Particle size distribution was examined using a Beckman Coulter LS 13 320 laser diffraction analyzer (Beckman Coulter®, Miami, FL, USA) coupled with a liquid analysis module (Beckman Coulter®, Miami FL, USA). Samples were added in liquid form (turmeric extract) and powder form (WPC and TWPC) without prior rehydration. Sufficient amounts of the powders were added slowly to a vessel containing room temperature water until an opaque mixture was obtained. Five series of data were collected in the region of 0.375 μm to 2,000 μm at 90-s intervals (1.5, 3.0, 4.5, 6.0, and 7.5 min). The results were obtained using the Fraunhofer approximation method to determine the total solubility. The data were represented by the percentage of the volume occupied by the particles as a function of their size. Beckman Coulter (Particle Characterization) software (version 5.03) was used for the statistical analysis of the obtained data.

2.5 | Colorimetric analysis

Colored powder was measured using a spectrophotometer (CM-5, Konica Minolta®, Osaka, Japan) operated according to the CIELa * b * scale. The CIELa * b * color system uses three ordinates, where L^* corresponds to brightness, a^* to the chroma measurement on a red-green axis, and b^* to the chroma measurement on the yellow-blue axis. In layman's terms, the L^* coordinate represents how light or dark the sample is, with values ranging from 0 (totally black) to 100 (totally white). The a^* coordinate values range from −80 (green) to +100 (red); and the chromaticity coordinate b^* values range from −50 (blue) to +70 (yellow). Planar diffraction grating using an SCI (specular component included) determined the spectral separation device model with a reflectance and wavelength range from 360 to 740 nm (Gadonski et al., 2018; Minolta, 2019).

2.6 | Isotherm sorption

Isotherm analysis was performed at 25°C for 21 days until mass transfer equilibrium was established between the samples and the different saturated salt solutions in desiccators. The salts used in the saturated solutions and the respective water activities were: LiCl (0,112); $KC_2H_3O_2$ (0,226); K_2CO_3 (0,438); $Mg(NO_3)_2$ (0,544); NH_4NO_3 (0,620); NaCl (0,753).

2.7 | Raman spectroscopy

The Raman spectra for turmeric extract (liquid), WPC, and TWPC were obtained using an FT-Raman spectrometer (Bruker®, model RFS100) equipped with cooled liquid nitrogen Ge detector and Nd: YAG laser with an excitation line at 1,064 nm. A 90 mW beam was trained on the samples. A good signal-to-noise ratio was obtained for all spectra by averaging a total of 512 scans, collected at a spectral resolution of 4 cm^{-1}. Raman spectra acquisition of Raman spectra was carried out with OPUS 6.0 software (Almeida et al., 2010).

3 | RESULTS AND DISCUSSION

3.1 | Scanning electron microscopy and colorimetric analysis

Turmeric and turmeric extract showed similar morphological properties (Figure 2-A,-B), with non-spherical and agglomerated particles. The results were compatible with established curcumin morphology (Thorat & Dalvi, 2014). WPC and TWPC showed spherical (irregular), particulate morphology with agglomeration points (interconnected), without apparent cracks (Figure 2-C,-D). These characteristics are similar to samples obtained using a single-stage spray dryer which facilitates encapsulation (Beran et al., 2018; Sierra et al., 2013; Xu et al., 2018). By encapsulating extracts of pequi carotenoids using the same drying method at three different drying temperatures (150, 170, and 190°C), Alves et al. (2017) found the same morphological similarities. Irregular surfaces on the spherical particles of the spray-dried samples can be attributed to their shrinkage during the drying process (Janiszewska & Witrowa-Rajchert, 2009). The absence of cracks and breaks in the microspheres plays a fundamental role in guaranteeing greater protection and retention of the microencapsulated extract (Mendes, 2002). Another important feature is the formation of agglomerates, that is, the occurrence of small particles located on the surface of larger particles.

This feature yields a microencapsulated compound with enhanced stability, since the external particles protect the internal compound and, consequently, its pigments (Mendes, 2002). The

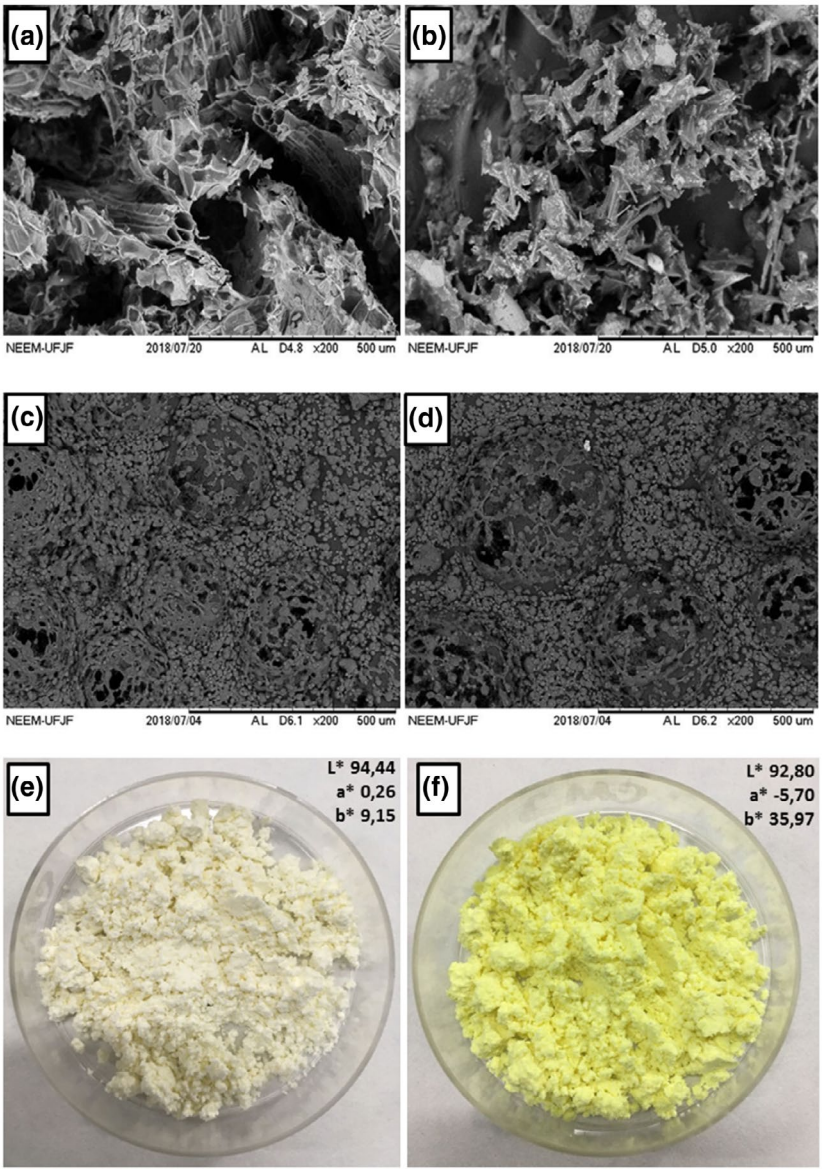

FIGURE 2 Scanning Electron Microscopy (x200): (a) Turmeric in natura (lyophilized); (b) Turmeric extract (lyophilized); (c) Whey protein concentrate; (d) whey protein concentrate admixture of microencapsulated curcumin. Colorimetric Analysis Results: (L^* = Brightness, a^* = Red/green agitation, b^* = yellow/blue color): (e) Whey protein concentrate; (f) Whey protein concentrate admixture of microencapsulated curcumin

addition of turmeric extract did not alter the morphology of the powders, indicating that there was no change in adhesion properties among the particles.

Furthermore, the turmeric extract addition did not cause a significant change in the tendency toward moisture absorption, a factor that can cause modifications in the morphology of powders obtained using spray drying (Juarez-Enriquez, 2019; Torres et al., 2017; Zafar et al., 2017).

Figure 2 shows macroscopic images of the WPC (Figure 2-E) and TWPC (Figure 2-F) powders as well as their respective colorimetric analysis results. WPC showed higher brightness (L^*) compared to TWPC (94.44 and 92.80, respectively). For ordinate analysis (a^*) on the red-green axis, WPC demonstrated a positive result (0.26), inferring a greater red-color tendency. Conversely, TWPC demonstrated a negative result (−5.70), which demonstrates a greater green color tendency. Finally, in the ordinate analysis (b^*) on the yellow-blue axis, both WPC (9.15) and TWPC (35.97) showed positive results, suggesting yellow targeting. However, it should be noted that TWPC showed a higher value for b^* (35.97), thus showing a greater target for the yellow region. The differences in the L^*, a^*, b^* values between WPC and TWPC are a result of the addition of curcumin extract, which is a natural orange-yellow dye (Custódio, 2014; Gadonski et al., 2018.

In view of these results, we confirmed that the addition of the extract did not alter the characteristics of WPC, which allows us to conclude that curcumin was indeed encapsulated. Hence, the process allows for the reduction of particle core interactions with outside environmental factors, thus delaying any changes that may result in the loss of bioactive compounds from the turmeric extract added to WPC.

3.2 | Particle size distribution and sorption of WPC

Particle size distribution of particles in WPC and TWPC indicates that adding turmeric extract to the retentate did not change the

TABLE 1 Granulometric extract analysis results for WPC and TWPC samples obtained by spray drying

	Turmeric extract	WPC	TWPC
Means (μm)	38.61	13.99	12.92
Standard deviation (μm)	36.62	26.31	21.83
d_{10} (μm)	1.14	1.44	1.58
d_{90} (μm)	97.52	45.77	40.25
<1 μm (% volume)	8.41	2.23	2.78

Abbreviations: *d*, particle diameter; TWPC, whey protein concentrate admixture of microencapsulated curcumin; WPC, whey protein concentrate.

powder structure. The average particle sizes, d_{10} and d_{90} (Table 1) showed no difference between the powders tested with or without the addition of turmeric. It is worth noting that the volume for particles smaller than 1 μm (Table 1) and the mean values for d_{10} and d_{90} indicate that no difference in these was found between the rehydration processes of WPC and TWPC when the particle volume determination had been performed in a liquid modulus.

Curcumin is a lipophilic agent and its own hydrophobic nature makes it insoluble in water (Chin et al., 2009; Shehzad et al., 2017). Thus, its biological activity in some aqueous medium is limited due to its low solubility, stability, and bioavailability (Awad et al., 2015; Liang et al., 2009).

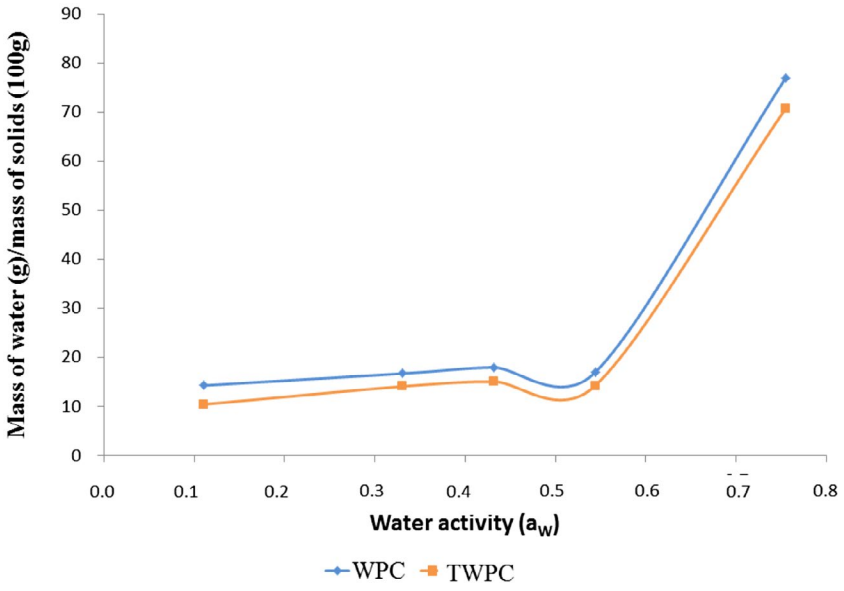

FIGURE 3 Sorption isotherm at 25°C of whey protein concentrate (WPC) and whey protein concentrate admixture of microencapsulated curcumin (TWPC) by spray drying

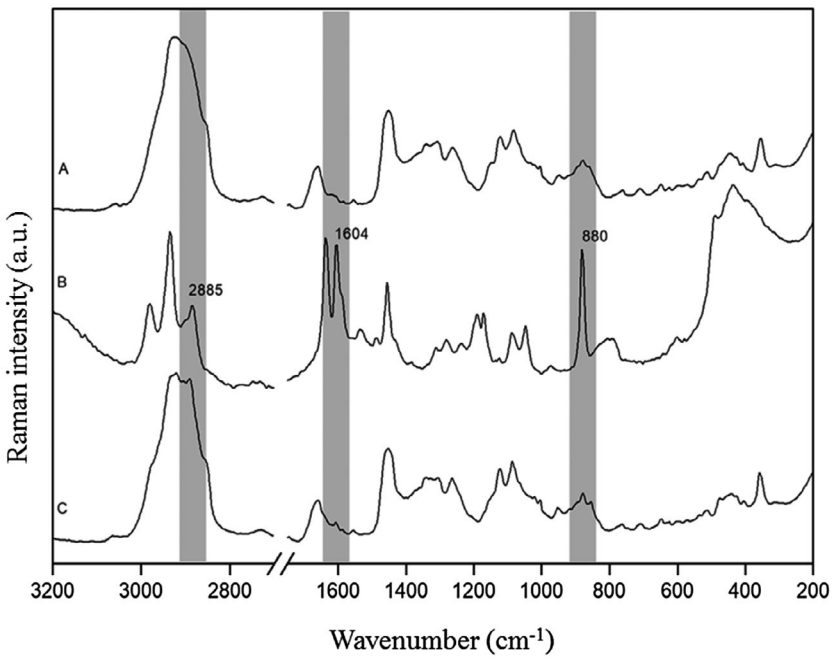

FIGURE 4 Raman spectroscopy: (a) Whey protein concentrate; (b) Turmeric extract; (c) whey protein concentrate admixture of microencapsulated curcumin

β-lactoglobulin (a major milk protein) has an affinity for curcumin which allows it to form a complex during encapsulation that is capable of increasing its solubility and stability through hydrophilic interactions (Awad et al., 2015; Sneharani et al., 2010).

The results of our granulometric analysis found both WPC samples to be in the range of diameters commonly produced by spray drying processes. These vary from 10 to 250 μm (Thomas et al., 2004). Values below 10 μm have negative effects on powder wettability (Ilari & Laila, 2005).

The sorption isotherms of the WPC and TWPC samples exhibited no difference in curve shape and both absorb the same amount of water when exposed to different relative air humidity levels (Figure 3). From this behavior, we can extrapolate that the addition of turmeric extract to WPC does not affect the powder hygroscopicity and therefore TWPC has the same shelf life as WPC. According to Koç et al. (2010), food moisture sorption isotherms provide critical information that can be used to predict shelf life.

Because hydrophobic curcumin has low solubility levels, unencapsulated turmeric would have affected the wettability of the TWPC sample. Thus, it is possible to conclude that the addition of turmeric extract did not modify the powder structure and did not affect the wettability of the WPC sample. It is then possible to state that the curcumin was encapsulated.

3.3 | Raman spectroscopy

The turmeric extract spectrum demonstrated a Raman spectroscopy peak near 1,600 cm^{-1} (Figure 4). This corroborates the findings of Mangolim et al. (2014) who evaluated curcumin encapsulation by means of an added oligosaccharide (β-cyclodextrin) using Raman spectroscopy. According to Kolev et al. (2005), the bands between 880 and 710 cm^{-1} can usually be attributed to different aromatic and skeletal movements outside the COH plane (which may explain the 880 cm^{-1} peak in the TWPC spectrum).

The Raman spectral profile between 2,935 cm^{-1} and 2,888 cm^{-1} corroborates with the profiles found in the literature and clearly demonstrates certain distinctive Raman characteristics, including scattered bands around the CH-stretch vibrations. This profile detail is the characteristic of a WPC sample that demonstrates an amorphous lactose state. Figure 4-A (Almeida et al., 2010; Stephani et al., 2017).

Changes in lactose structure are the primary physical modification found in dairy powders and can lead to other changes during storage. Lactose is the most abundant component of whey. Both during and after water removal by spray drying, lactose remains in a highly hygroscopic amorphous state (Stephani et al., 2017). Therefore, determining the maintenance of lactose in an amorphous state in the developed product was necessary to guarantee the quality of the powder produced.

The presence of curcumin in TWPC modified WPC Raman spectrum (Figure 4-C). The slight modification can be explained by the fact that CH groups were observed (Figure 4-B) in this spectral region of the turmeric extract. Therefore, no alteration of the lactose's amorphous state occurred in TWPC which suggests the powder remained encapsulated (Almeida et al., 2010; Mangolim et al., 2014; Mohan et al., 2012; Stephani et al., 2017).

4 | CONCLUSION

The addition of turmeric extract to whey protein did not alter the morphology of the powder sample obtained. This indicates that there was no change in adhesion properties among the particles. Moreover, the addition of turmeric extract did not cause a significant change in moisture absorption tendencies. Therefore, it has been concluded that curcumin, a hydrophobic compound, was encapsulated in the process, since the addition of the turmeric extract to WPC did not affect powder's hygroscopicity.

The colorimetric analysis showed yellow coloration tendencies for TWPC. The presence of curcumin in TWPC caused a slight modification in the Raman spectrum without altering the amorphous state of lactose. Our results indicate an excellent opportunity to launch potential whey protein products with added antioxidants.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Research data are not shared.

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