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Micellar Casein and Whey Protein as Encapsulating Agents of Natural Extracts: Antioxidant Capacity and Phenolic Compounds

Caseína Micelar e Proteínas Do Soro Como Agentes Encapsulantes de Extratos Naturais: Capacidade Antioxidante e Compostos Fenólicos

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Dairy proteins can be used as encapsulating agents for natural compounds during spray drying. This enhances the health benefits of both ingredients. This study aimed to determine the best encapsulating agent for three different natural extracts, considering their trolox equivalent antioxidant capacity (TEAC) and phenolic contents. The experimental strategy was based on three different natural extracts (passion fruit, avocado seed, and green coffee) encapsulated in two types of proteins (whey protein [WPC] and micellar casein [MC]). The particle size, morphology, Raman spectroscopy, TEAC, phenolic compounds, moisture, and water activity were measured during the experiment. The results indicated that only the green coffee extract modified the spectra of WPC and MC in the dry powders, which could be attributed to chlorogenic acid. MC promoted greater retention of phenolic compounds (226.30 mg GAE/100 g for passion fruit; 245.20 mg GAE/100 g for avocado seed; 331.05 mg GAE/100 g for green coffee in) and trolox equivalent antioxidant capacity (28.03 µmol TE/g for passion fruit; 27.29 µmol TE/g for avocado seed; 13.31 µmol TE/g for green coffee) compared to those of WPC. Passion fruit and avocado seed extracts encapsulated in MC showed the highest trolox equivalent antioxidant capacity. In conclusion, the encapsulating agent exerted different effects on the encapsulation of natural extracts, depending on the TEAC and phenolic content.

Keywords: Spray drying; milk proteins; natural extracts; particle size; morphology; Raman spectroscopy.

1. Introduction

Currently, the global trend is the consumption of healthier foods, which can improve people's health.[1] In this sense, it is important to develop foods with raw materials containing bioactive compounds, such as antioxidants and polyphenols, which help reduce the indicators of various disorders, such as cardiovascular diseases, cancer, Parkinson's disease, diabetes, Alzheimer's disease,[2] rheumatoid arthritis,[3] and obesity.[4] According to Lobo *et al.*[5], an antioxidant is a molecule that is sufficiently stable to donate an electron to a free radical and neutralize it, thus reducing its capacity for damage. These antioxidants delay or inhibit cellular damage, mainly through their free radical inhibitory properties. In general, antioxidants are found in fruits and vegetables,[6] such as Corozo (*American Oil palm oleifera*), Curuba criolla (*Passiflora mollissima*), Marañon (*Anacardium occidentale*), Arazá (*Eugenia estipitata*), avocado,[7] and green coffee.[8,9]

Although these foods contain antioxidants, many of them are lost because they do not satisfy marketing characteristics such as size, color, and shape, and in other cases, the loss is due to post-harvest damage.[10] This is an environmental problem because food byproducts contaminate the soil and water. In that sense, products such as avocado, curuba, and coffee are important sources of byproducts. Curuba byproducts are 41% peel, 18% seeds, and 41% pulp;[11] whereas for coffee, the largest byproduct is the spent coffee ground; approximately 6 million tons of spent coffee ground are annually generated worldwide[12]. The waste generated by the avocado industry is approximately 45% of the world's production, of which 65% is pulp, 17% seed, and 18% peel.[13] Antioxidant capacity values of 60000 µmol trolox/100 g,[14] between 100.8 µmol trolox/100 g to 468.4 µmol trolox/100 g,[15] and 250 µmol trolox/100 g[16] were reported for curuba pulp, coffee, and avocado seeds, respectively. Given the antioxidant content of these fruits, including their byproducts, it is important to determine valorization strategies, one of which is

encapsulation, which involves the encapsulation of different bioactive compounds, such as polyphenols, antioxidants, and quercetin, using different wall materials, such as inulin, maltodextrins, gum arabic, soy lecithin, and milk proteins.[17]

Dairy proteins are used as encapsulating agents during spray drying, aiming, for example, at the production of dehydrated products with the addition of natural compounds.[18-21] The benefits of the formulation of these products are described in the literature; for instance, delivery of bioactive compounds such as rhodamine B and curcumin,[22,23] enhanced bioavailability of lycopene, anticancer activity,[24] application in food and cosmetic formulations,[25] iron fortification of solid and liquid food products,[26] incorporation of nanoparticles into living cells with enhanced transport properties,[27] encapsulation of lipophilic bioactive ingredients such as fish oil,[28] stability of vitamins C and D3,[29,30] agents, and carriers of natural colorants.[31,32]

Studies that determined the antioxidant capacities and concentrations of phenolic compounds to study encapsulation comparing micellar casein (MC) and whey protein concentrate (WPC) are not found in the literature. Furthermore, it has not yet been reported, in the same study, the comparison of the use of two different milk proteins, as encapsulating agents, of the three different extracts chosen: passion fruit, avocado seed and green coffee. Therefore, a new product with potentially differentiated and functional properties would be a combination of milk proteins with natural extracts, enhancing the nutritional benefits of both and meeting this new market demand.

This study hypothesized that dairy proteins are encapsulating agents capable of maintaining the viability of natural extracts and consequently having nutritional benefits. However, by changing the natural extracts and dairy proteins, there may be differences in the trolox equivalent antioxidant capacity and concentrations of phenolic compounds in dairy powders. This study aimed to identify a better encapsulating agent, whey protein concentrate and micellar casein, for three different natural extracts (passion fruit, avocado seed, and green coffee), considering their TEAC and phenolic contents.

2. Material and Methods

The avocado (*Persea Americana L.* var. Hass) seeds were supplied by Terravocado S.A. (Medellín, Colombia), passion fruit (*Passiflora mollissima*) was obtained from a local market in Plaza Minorista de Medellín, Colombia, and green coffee was obtained from a local market in Juiz de Fora, Minas Gerais, Brazil. Antioxidant compounds from fresh samples of passion fruit and avocado seeds were obtained according to the method described by Contreras *et al.* [6] with a few modifications, and for green coffee according to the method proposed by Dawidowicz and Typek[33] with modifications. Approximately 2.0 g of each sample was extracted with a mixture of ethanol:water (50:50) and acetone:water (60:40), centrifuged, and the supernatant was recovered. The extracts were stored at -18 °C in amber flasks and lyophilized. The extraction and measurements were performed in triplicate. The green coffee beans were ground in a micro knife mill (MSSL-030, Solab, São Paulo, Brazil) and sieved (granulation of 840 µm/mesh N. 20). For the extraction, 10 g of green coffee was mixed with 100 mL of water in an ultrasound device called SoniClear2 (Sanders medical, Minas Gerais, Brazil) for 1 h. Subsequently, the extract was filtered under a vacuum pump using a Büchener funnel and kitassato glassware, using 14 µm millex filter paper.

The drying process was conducted in a MiniSpray Dryer B-290 (Buchi, São Paulo, Brazil), and the three different extracts (passion fruit, avocado seed, and green coffee) were encapsulated in two types of proteins (WPC and MC). The experimental steps started by mixing 50 g of reconstituted lyophilized extract in water, 32 g of whey protein powder (34% w/w of protein in dry matter) or 32 g of micellar casein powder (61.5% w/w of protein in dry matter), 148 g of water, and 7.2 g of Span 80 (emulsifier–sorbitanoleate, Sigma-Aldrich®/Merck, São Paulo, Brazil). The final concentration of each extract before spray drying was 13.5% w/v of protein. The drying parameters were: between 40 to 50 $L \cdot min^{-1}$ air flow (total flow of air used in the heating system of the spray dryer), between 0.25 to 0.50 $L \cdot min^{-1}$ of product flow, between 130 to 150 °C inlet temperature (inlet air temperature), and between 60 to 78 °C outlet temperature (outlet air temperature).

The rehydration capacity of the powders was studied by the particle size distribution, according to Francisquini *et al.*[34]. The particle size distribution of the powders during the rehydration process was obtained using a Beckman Coulter LS 13320 laser diffraction analyser with an aqueous liquid module (Beckman Coulter, Brea, CA, USA). Sufficient quantities of samples (powders without rehydration) to generate the turbidity required for the readings were added to the reservoir of the liquid analysis module containing water at room temperature. Data were collected on the region of 0.04 to 2.000 µm every 90 seconds over 5 different times (1.5; 3.0; 4.5; 6.0; and 7.5 minutes). The data are presented as the percentage (%) of the volume occupied by the particles as a function of size. Beckman Coulter software (particle characterisation) version 5.03 was applied to analyse the data. The indicators Dv10 and Dv90 (volume in which 10% and 90%, respectively, of the particles were found) were used to assess the particle size distribution.

The morphology of the powders was characterized using Scanning Electron Microscopy (SEM; Hitachi TM 3000, Hitachi Ltd., Tokyo, Japan, images magnitudes of 2000x).

Raman spectroscopy was conducted according to the methodology proposed by Torres *et al.*[35]. The Raman spectra of all samples represented in this study were obtained using a Bruker FT-Raman RFS 100 spectrometer equipped

with a liquid nitrogen-cooled Ge detector and a Nd:YAG laser. Spectra were collected using a 100 mW laser beam with near-infrared excitation at 1064 nm, and the scattered radiation was collected at 180°. For all spectra, good signal/noise ratios were obtained by performing an average of 512 scans, which were collected with a spectral resolution of 4 cm^{-1} in the region from 3500 cm^{-1} to 50 cm^{-1}. OPUS platform 6.0 was used for the acquisition of Raman spectra.

Moisture content was determined according to the AOAC methodology,[36], that is, gravimetric method at 105 °C with successive weighing until constant mass. And water activity (A_w) was obtained using the methodology described by Matinez *et al.*[12], sprint TH-500 water activity system (Novasina, Switzerland) was used.

All analyses were performed in duplicate.

The trolox equivalent antioxidant capacity (TEAC) was performed using a 2,2'-azino-bis-3-ethylbenzthiazolin-6-sulfonic acid (ABTS) assay, and the total polyphenolic content was determined using the Folin-Ciocalteu test. Both tests were based on the methodology proposed by Calderon *et al.*;[37] and the analyses were performed in triplicate. Approximately 1.0 g of each sample was extracted with a mixture of ethanol:water (50:50) and acetone:water (60:40), centrifuged, and the supernatant was recovered. The extracts were stored at -18 °C in amber flasks.

To compare the results, Student's t-test was performed.

3. Results and Discussion

The moisture and water activities (Table 1) of the products encapsulated with WPC were within an adequate range compared to those reported in the literature. However, the moisture content of the products encapsulated in MC was high. Casein is a protein with a high capacity to link water; therefore, MC has a higher moisture content but the same water activity as that of WPC. According to Rocha *et al.*,[20] moisture values from 2.58% to 4.98% and water activity from 0.358 to 0.447 were detected in powders formulated with different extracts (jabuticaba, jussara, and blueberry extracts) and different encapsulating agents (maltodextrin, gum Arabic, and whey protein concentrate). Student's *t*-test was performed, considering the protein source as a variable and the extracts as constants (passion fruit WPC × passion fruit MC, avocado seed WPC × avocado seed MC, and green coffee WPC × green coffee MC). A significant difference ($p \leq 0.05$) was observed in water activity and moisture between WPC and MC for each extracts, except water activity for WPC avocado seed x MC avocado seed (showed no significant difference; $p > 0.05$).

The fresh passion fruit extract exhibited an trolox equivalent antioxidant capacity and total phenolic content similar to those reported in the literature (Table 1). In contrast, the green coffee extract used in this study showed superior results compared to those reported in the literature. The trolox equivalent antioxidant capacity varied between 18.91 and 23.02 µmol Trolox/g for products formulated with WPC, 13.31 and 28.03 µmol Trolox/g for products formulated with MC, and 111.40 and 269.50 µmol Trolox/g for fresh extracts. Total phenolic compounds ranged from 107.40 to 115.70 mg GAE/100g for products formulated with WPC, from 226.30 to 331.50 mg GAE/100g for products formulated with MC, and from 1624.63 to 3154.40 mg GAE/100g for fresh extracts. Compared to that of the fresh extracts of passion fruit, avocado seeds, and green coffee, there were reductions of 8×, 10×, and 8×, respectively, in the TEAC of the dry extracts with micellar casein. When formulated with WPC, there were reductions of 11×, 12×, and 6× in the trolox equivalent antioxidant capacity of the fresh extracts of passion fruit, avocado seed, and green coffee, respectively. Micellar casein promoted a higher TEAC than that of WPC in passion fruits and avocado

Table 1. The parameters obtained at 5 minutes intervals during the drying and analysis of the physicochemical properties (moisture and water activity), trolox equivalent antioxidant capacity (TEAC), and phenolic compounds of the powders.

Attributes	Whey protein			Micellar casein			Fresh extract		
	Passion fruit	Avocado seeds	Green coffee	Passion fruit	Avocado seeds	Green coffee	Passion fruit	Avocado seeds	Green coffee
Inlet air RH[a] (%)	62.47 ± 0.57	64.28 ± 0.39	52.36 ± 2.95	55.03 ± 0.96	57.10 ± 0.08	49.82 ± 0.28	-	-	-
Outlet air RH[a] (%)	23.74 ± 0.44	23.84 ± 0.40	22.36 ± 0.90	25.27 ± 1.44	23.84 ± 0.08	33.54 ± 3.69		-	-
Inlet air AH[b] (g/kg)	10.97 ± 0.17	10.45 ± 0.04	11.38 ± 0.28	10.22 ± 0.15	10.20 ± 0.09	9.90 ± 0.11	-	-	-
Outlet air AH[b] (g/kg)	21.33 ± 0.60	21.33 ± 0.28	22.53 ± 0.12	24.99 ± 2.93	33.12 ± 2.10	30.37 ± 1.55	-	-	-
g evaporated water/kg dry air	10.36 ± 0.52	10.88 ± 0.25	11.15 ± 0.24	14.76 ± 3.03	22.92 ± 2.01	20.47 ± 1.55	-	-	-
Product flow rate (g/h)	488.75 ± 49.89	508.50 ± 21.25	508.75 ± 13.74	631.75 ± 146.96	1117.33 ± 67.10	927.25 ± 92.44	-	-	-
Moisture ($w \cdot w^{-1}$)	3.86 ± 0.01	3.97 ± 0.02	4.13 ± 0.00	7.24 ± 0.00	7.31 ± 0.00	7.74 ± 0.00	-	-	-
A_w[c]	0.28 ± 0.05	0.29 ± 0.06	0.30 ± 0.06	0.42 ± 0.06	0.41 ± 0.06	0.42 ± 0.05	-	-	-
TEAC by ABTS[d] (µMol Trolox/g)	19.24 ± 1.52	23.02 ± 1.13	18.91 ± 2.41	28.03 ± 0.01	27.29 ± 1.53	13.31 ± 3.29	221.30 ± 1.85	269.50 ± 4.30	111.40 ± 27.00
TP[e] (mg AG/100g)	112.90 ± 7.80	115.70 ± 17.10	107.40 ± 1.75	226.30 ± 19.40	245.20 ± 14.40	331.50 ± 39.30	1624.63 ± 111.76	2776.47 ± 269.16	3154.40 ± 310.00

*Data are presented as mean ± standard deviation. where: [a]RH (relative humidity); [b]AH (absolute humidity); [c]Aw (water activity);[d]ABTS); [e]TP (total phenolic compounds)

seeds. Green coffee extract maintained a higher trolox equivalent antioxidant capacity in WPC compared to MC. The results of the phenolic compounds verified that they were reduced by the use of the encapsulating agents when compared to those of the fresh extract. When MC was used, the phenolic compounds from the fresh extracts of passion fruit, avocado seed, and green coffee were reduced by 7×, 11×, and 10×, respectively. An even greater reduction was observed with the use of WPC (14×, 23×, and 29× in the extracts of passion fruit, avocado seed, and green coffee, respectively).

The antioxidant capacity of passion fruit species varies from 50 to 2240 μmol Trolox/g and the total phenolics from 659 to 4605 mg GAE/100g.[37,38] Segovia *et al.* [39] reported antioxidant capacity values ranging between 58.45 and 725 μmol (TE)/g and total phenolic compounds between 615 and 29200 mg GAE/100 g in avocado seed extract. Skowron et al.[40] reported antioxidant capacity between 27.3 and 67.6 μmol TE/g and total phenolics between 35.4 and 170 mg GAE/100g in green coffee. The same findings of this study (reduced antioxidant capacity and phenolic compounds) were previously reported, where hibiscus extract was encapsulated with yogurt and later dried via a spray dryer.[18] In addition, Nunes *et al.* (2016)[41] reported a reduction in phenolic compounds and antioxidant capacity in fresh guava using different drying methods. The reduction in antioxidant compounds was attributed to the dilution of the extracts in the encapsulating material.

In a study by Caleja *et al.*,[42] chamomile and fennel extracts were added to yogurt. Herein, differences in the antioxidant capacity were observed for the different types of extracts used. Similarly, Vasco *et al.,*[43] reported different values of antioxidant capacity and phenolic compounds in different Equatorian fruit extracts. Therefore, both studies corroborated the changes observed with the different extracts used in this study. Talón *et al.* [21] reported a reduction in the antioxidant capacity when compared to that of the pure extract with the addition of isolated whey protein and soy lecithin. In the same study, modification of the encapsulating agent (addition of chitosan) was responsible for the difference in the final antioxidant capacity. The same result was reported in a study by Sarabandi *et al*.[44] In this work, the authors modified the type of encapsulating agent (maltodextrin, gum arabic, and maltodextrin + gum arabic) and observed a substantial difference in the antioxidant capacity and total phenolic content. Therefore, the work formulated here is in agreement with both cited studies.

Comparing only the products formulated with MC, passion fruit extract retained the best trolox equivalent antioxidant capacity, whereas green coffee extract retained the lowest. In contrast, comparing the products formulated with WPC, it can be inferred that the avocado seed extract retained the best TEAC, whereas the green coffee extract retained the lowest trolox equivalent antioxidant capacity. The ability of MC to maintain approximately 2× more phenolic compounds than those with WPC was observed for all the extracts used. This indicates a difference in the maintenance of phenolic compounds by the different encapsulating agents used (better encapsulation properties). Student's *t*-test was performed for the total phenolic parameters and trolox equivalent antioxidant capacity, comparing different milk protein sources for each extract (passion fruit WPC × passion fruit MC, avocado seed WPC × avocado seed MC, and green coffee WPC × green coffee MC). A significant difference ($p<0.05$) was observed, confirming that the protein source did not affect the phenolic parameters or antioxidant capacity of the powders.

Within the same protein source, no difference was detected in the distribution of particle size among the different extracts analyzed, demonstrating that passion fruit, avocado seed, and green coffee did not influence the size of the powder particles. The particle size distribution (Figure 1, a–f) during rehydration did not change, indicating that milk proteins maintained their main properties after the addition of the natural extracts. This means that the solubility of dairy protein powders was preserved, even in the presence of natural extracts. Both WPC and MC showed an improvement in rehydration over the analysis time (1.5-7.5 min) in this study. However, the particle size distribution was different among the different protein sources. The whey protein showed a bimodal particle distribution with particles in the submicrometric region (<1 μm). Micellar casein exhibited an unimodal structure with no particles in the region below 1 μm. When the particles are in this submicrometric region and have a bimodal distribution, as observed in whey protein, the efficiency of the powder rehydration process is increased in water, which can be beneficial from the consumer's point of view because reconstitution of the powder is easier to perform.

No or little differences were found when the extract used was modified (passion fruit; avocado seeds; green coffee). The greatest differences in particle size were observed with the change of encapsulating agent. For products formulated with whey protein, the average value of all extracts used, was 1.20 μm for Dv10 and 41.92 μm for Dv90. While for products formulated with micellar casein, the average value also for all extracts used, was 7.49 μm for Dv10 and 55.29 μm for Dv90. The value of Dv10 and Dv90 corresponds to 10% and 90%, respectively, of the particles below the value found. Therefore, it appears that products formulated with WPC exhibited a higher population of small particle when compared to products formulated with MC. It is known that the smaller the number of particles below the region smaller than 1 μm, the better the rehydration capacity of the powder, which is directly related to the quality of the powder in terms of consumer perception.[45,46]

The use of different extracts did not influence the morphological (Figure 2a-2f) characteristics of the powders. This is because the images showed only small differences according to the protein source. In other words, the whey protein presented a morphology different from that of micellar casein. However, when using the same protein, it

was not possible to observe changes in the powder images of different extracts. Despite the small difference between whey protein and micellar casein, both presented the typical morphological structure of spray-dried products (spherical particulate particles with agglomeration points). These results are in line with those reported in the literature[45] and corroborate the results of the particle size distribution with respect to the efficient encapsulation of extracts by milk proteins.

In work carried out by Gomes *et al.,*[45] the manufacture of whey protein added with curcumin was carried out, the powders were analyzed by scanning electron microscopy and they remained spherical in shape (irregular), particulate morphology with agglomeration points (interconnected) and without apparent cracks. Furthermore, in this work the particle size of the curcumin extract was 8.41 % volume of particles below 1 µm, Dv10 of 1.14 µm, Dv90 of 97.52 µm, while the powders encapsulated with WPC had a value of 2.51 % volume of particles below 1µm, Dv10 of 1.51 µm, Dv90 of 43.01 µm.

Barbosa *et al.,*[46] carried out characterization of coffee silverskin extract microencapsulated with instant skimmed milk powder and concentrated whey protein. The Dv90 values were: 31.57 µm pure extract; 196.17 µm extract with instant skimmed milk powder; 18.61 µm extract with concentrated whey protein. And the amount of particles below 1 µm corresponded to: 9.50% pure extract; 6.92% extract with instant skimmed milk powder; 9.07% extract with concentrated whey protein. The morphology of the pure extract was completely different from the encapsulated products due to the different types of drying (lyophilization and spray drying). However, the morphologies of products encapsulated with skimmed milk powder and WPC were quite similar (spray drying).

Raman spectroscopy (Figure 3) did not reveal bands attributable to chemical interactions between MC or WPC and the extracts. Caseins have higher glass transition temperatures than those of whey proteins[47,48] enabling better encapsulation stability[R1]; therefore, MC powders present higher levels of total phenolic compounds and antioxidant capacity.

The Raman spectra are shown in Figure 3a and 3b, indicating that all the powders showed a peak in the region between 2750 and 3000 cm^{-1}, which is characteristic of C–H stretching vibrations. In addition, in this region, the absence of a crystalline profile in the products formulated with WPC was verified. All samples exhibited peaks in the region close to 1600 cm^{-1}, which is related to the presence of aromatic rings. The spectra of the samples formulated with passion fruit and avocado seeds were similar, with small differences resulting from the different compositions of the different milk proteins used. By analyzing the spectra of the powders, it can be concluded that they showed a difference in relation to the other analyzed powders. The influence of the green coffee extract on both WPC and MC is different from that observed for passion fruit and avocado seed extracts in the bands 1633 and 1606 cm^{-1}. These bands are attributed to chlorogenic acid, a phenolic acid comprising an ester from caffeic acid and quinic acid, and are related to stretching vibrations of the phenyl ring (1633 cm^{-1}) and the

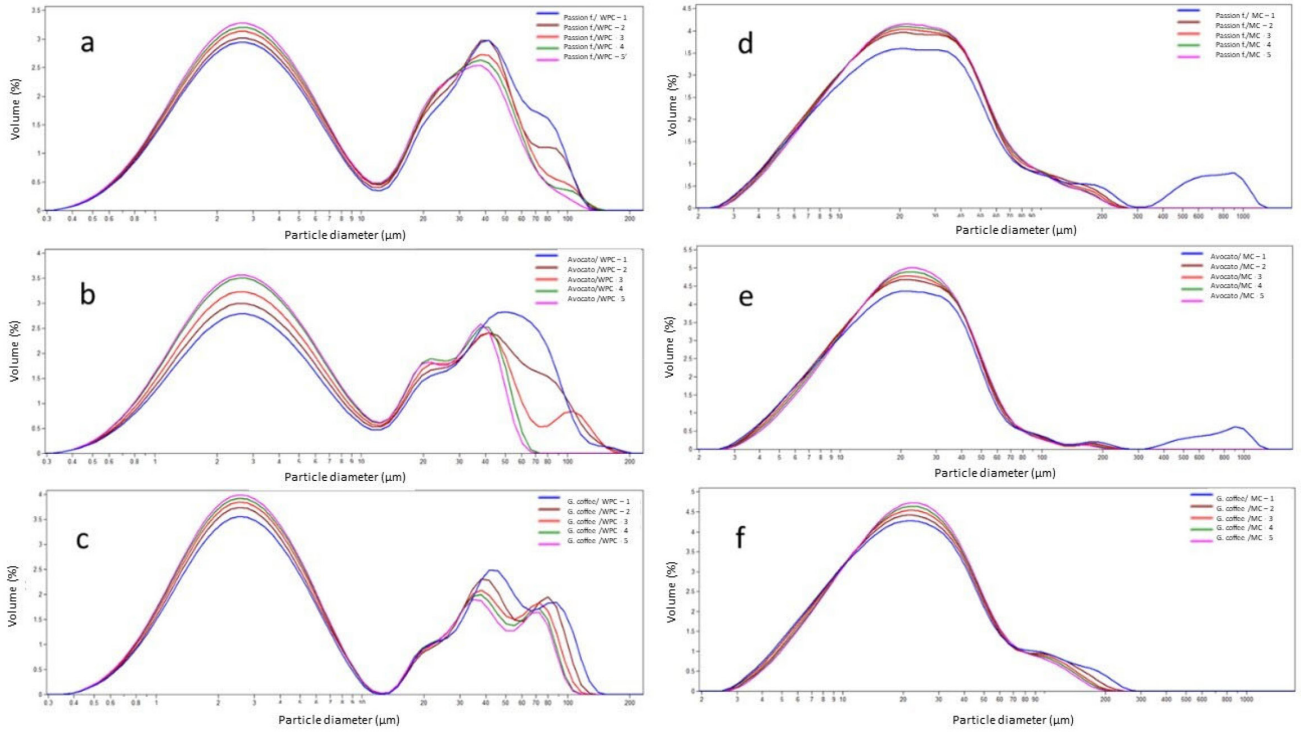

Figure 1. Curves of particle size analyses of the powders elaborated in this work. a, b, c: passion fruit, avocado seed, and green coffee extract, respectively, with whey protein. d, e, f: passion fruit extract, avocado seed, and green coffee, respectively, with micellar casein

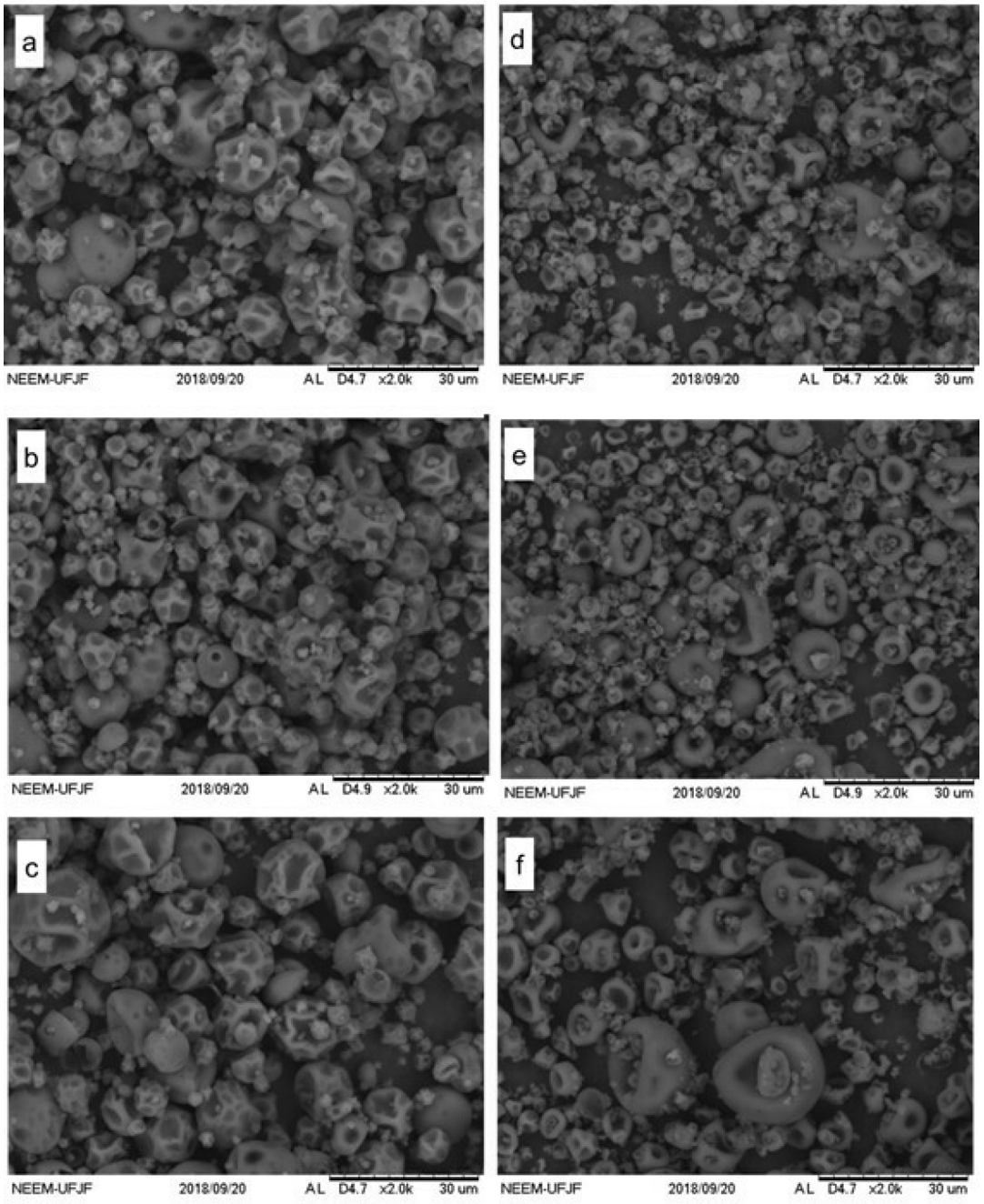

Figure 2. Scanning electron microscopic analysis of the samples. a, b, c: passion fruit, avocado seed, and green coffee extract, respectively, with concentrated whey protein. d, e, f: passion fruit, avocado seed, and green coffee, respectively, with micellar casein. Scale bar = 30 **μm**

C=C stretching vibration of the aromatic group (1606 cm^{-1}).

According to Almeida *et al.*,[49] the Raman spectroscopy peak is in the region between 2750 and 3000 cm^{-1} with characteristic C–H stretching vibrations. Stephani *et al.*,[50] reported that the effects of different treatments and storage conditions on the crystallization of lactose were identified by spectral changes near 2900 cm^{-1} and between 1200 and 800 cm^{-1}, which were attributed to the phase change of amorphous lactose to crystalline lactose. Mangolim *et al.*[51] related the peaks in the region close to 1600 cm^{-1} with the presence of aromatic rings. Abreu *et al.*[52] reported the same peaks of this study in samples of green coffee (1632 and 1606 cm^{-1}).

Whey protein has a higher proportion of branched-chain amino acids, leucine, isoleucine, and valine, whereas micellar casein contains a higher proportion of histidine, methionine, phenylalanine, and valine.[50] For these samples, it appears that the spectra were maintained according to the literature, showing little or no interference in the structure of the milk protein by the addition of the passion fruit extract or avocado seed.[50] The change in the spectra of products formulated with green coffee extract is due to the

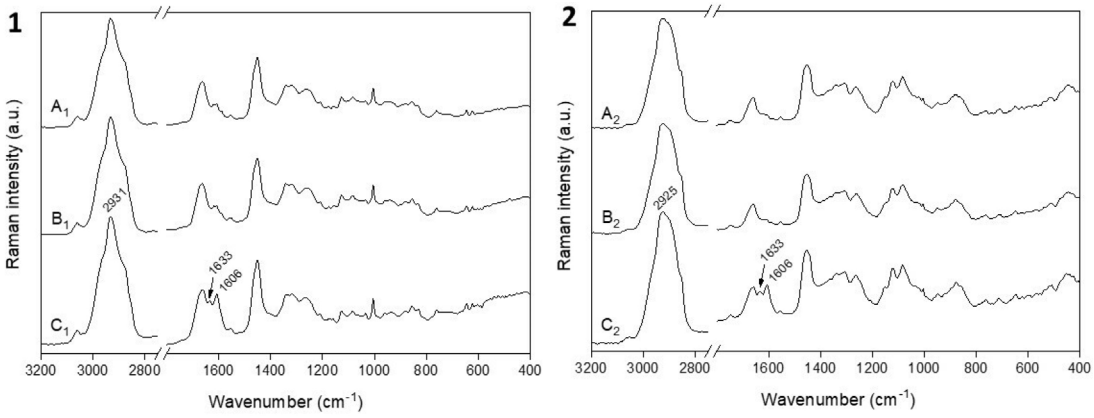

Figure 3. Raman spectroscopic analysis. (1): micellar casein with passion fruit extract (A1), avocado seed (B1), and green coffee (C1). (2): concentrated whey protein with passion fruit extract (A2), avocado seed (B2), and green coffee (C2)

composition of this extract, which has a substantial amount of polyphenolic compounds, in addition to presenting heterogeneity of chlorogenic acids and lipids, differing from the other extracts analyzed here. [52, 53]

To facilitate comparison with the data found here, Table 2 summarizes the main results from the literature.

4. Conclusion

The formulated powders showed differences between them, mainly due to the encapsulating agent used, with few changes due to the type of extract used. An example of this were the results of moisture and water activity. On the other hand, analyzing the results of particle size and morphology, the main differences were due to the comparison of the pure extract with the encapsulated extracts. There is little difference due to the encapsulating agent used (WPC and MC). In addition, the phenolic compounds and trolox equivalent antioxidant capacity decreased with the addition of milk proteins to fresh extracts, and MC promoted higher TEAC retention for passion fruits and avocado seeds than that of WPC. Micellar casein maintains approximately two times more phenolic compounds than those of WPC

Table 2. Results found in the literature for all parameters analyzed in the present wo

Reference	Analyzed product	Analyzed parameter	Value found
Rocha *et al.*,[20]	Powders formulated with different extracts (jabuticaba, jussara, and blueberry extracts) and different encapsulating agents (maltodextrin, gum Arabic, and whey protein concentrate)	Moisture	2.58% to 4.98%
		Water activity	0.358 to 0.447
Pineli *et al.*,[37]	Passiflora extract	Antioxidant capacity	50 to 2240 µmol Trolox/g
		Total phenolics	659 to 4605 mg GAE/100g
Segovia *et al.*,[39]	Avocado seed extract	Antioxidant capacity	58.45 to 725 µmol (TE)/g
		Total phenolic compounds	615 to 29200 mg GAE/100 g
Skowron *et al.*,[40]	Green coffee	Antioxidant capacity	27.3 to 67.6 µmol TE/g
		Total phenolics	35.4 to 170 mg GAE/100g
Barbosa *et al.*,[46]	Coffee silverskin extract microencapsulated with instant skimmed milk powder and concentrated whey protein	Particle size distribution	Dv90 values: 31.57 µm pure extract; 196.17 µm extract with instant skimmed milk powder; 18.61 µm extract with concentrated whey protein Particles below 1 µm: 9.50% pure extract; 6.92% extract with instant skimmed milk powder; 9.07% extract with concentrated whey protein
		Morphology	Pure extract: similar regardless of the encapsulating agent used
Almeida *et al.*,[49]	Milk powder	Raman spectroscopy peak	2750 and 3000 cm^{-1}: C–H stretching vibrations
Stephani *et al.*,[50]	Whey protein concentrate		2900 cm^{-1} and between 1200 and 800 cm^{-1}: amorphous lactose to crystalline lactose
Mangolim *et al.*,[51]	Curcumin–β-cyclodextrin complex		1600 cm^{-1}: aromatic rings
Abreu *et al.*,[52]	Green coffee		1632 and 1606 cm^{-1}: chlorogenic acid

in all the extracts used (better encapsulation properties for MC).

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Authors' Contribution

J.D. Francisquini e participated in conception or design of the work, data collection, data analysis and interpretation, performing the analysis, drafting the article.

E. R. Gomes participated in data collection, data analysis and interpretation, performing the analysis.

O. A. Vega-Castro participated in conception or design of the work, data collection, data analysis and interpretation, performing the analysis, drafting the article, critical revision, final approval of the version to be published.

J. Contreras-Calderon participated in drafting the article, critical revision, final approval of the version to be published.

E. A. Arcila participated in drafting the article, critical revision, final approval of the version to be published.

F. F. Costa participated in conception or design of the work, participated in drafting the article, critical revision, final approval of the version to be published.

M. P. Rodarte participated in conception or design of the work, participated in drafting the article, critical revision, final approval of the version to be published.

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L. F. C. De Oliveira participated in drafting the article, critical revision, final approval of the version to be published.

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