



Whey protein supplement as a source of microencapsulated PUFA-rich vegetable oils

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ABSTRACT

Whey protein supplements (WPS) intake has been increasing worldwide as they are mainly used to improve overall athletic performance. Adding other bioactives such as polyunsaturated fatty acids (PUFA) may be an alternative to help fulfill nutritional needs. Microencapsulation is able to protect PUFA-rich oils from oxidation, but important aspects of particle production and their influence on food properties must be evaluated. This study aimed to develop WPS with microencapsulated green coffee and walnut oils using stearic acid as a wall material. Oxidative stability (differential scanning calorimetry) of the oils increased (from 82 ± 4 to 110 ± 10 kJ mol⁻¹ for green coffee oil and from 90 ± 5 to 149 ± 1 kJ mol⁻¹ for walnut oil) after encapsulation and WPS rheological properties were not affected by the microcapsules ($p < 0.05$). Sensory analysis of the supplement containing microencapsulated green coffee oil showed a lower sensory preference than the blank sample, but no difference was found with the blank sample in the case of walnut oil ($p < 0.05$). The encapsulation strategy used to produce an enriched WPS was efficient in protecting the oils from oxidative degradation.

1. Introduction

The combination of nutritional supplements with physical exercise is often seen by fitness enthusiasts and professional athletes as an approach to improve the quality of life and to achieve a healthier lifestyle. The ingestion of supplements has been increasing and whey protein-based products are often used by consumers. Whey protein is the soluble protein fraction of milk serum that is obtained during cheese and casein production and its biological properties have been described (Garrido et al., 2016). Milk proteins and particularly whey are important for human nutrition due to the presence of some natural bioactive substances (Groziak & Miller, 2000). Their enrichment with other bioactive molecules such as polyunsaturated fatty acids (PUFA) might be beneficial and is a potentially important strategy to follow.

Edible vegetable oils with PUFA are important for human nutrition

including concerns related to addressing the imbalanced intake of saturated and unsaturated fatty acids, particularly in western diets. Green coffee (*Coffea arabica*) oil is ~75% triglycerides and contains caffeine, sterols, terpenes and tocopherols. It is high in PUFA and monounsaturated FA (MUFA) and also volatile compounds (Getachew & Chun, 2016). Coffee is one of the most important global commodities (Getachew & Chun, 2016) with an annual production of 8 million tonnes/yr (Bresciani et al., 2014). Walnuts (*Juglans regia*) contain ~60% lipids (Martínez et al., 2011), and ~75% of them are PUFA. The oil is also valuable due to the presence of tocopherols, polyphenols and phytosterols (Calvo et al., 2011).

In comparison to saturated oils, PUFA rich oils are more susceptible to oxidation, which may lead to undesirable sensory characteristics and thus impact on the overall quality (Mexis et al., 2009). Oxidative processes are complex and may be influenced by the presence of free fatty

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acids (FFA), metals and other components such as pigments (Ixtaina et al., 2011). Since oxidative degradation is an exothermic process, differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) techniques can be used to study the thermal behavior of foodstuff, taking into account that the associated oxidative processes are complex and are different upon heating with non-isothermal conditions (Cipriotti et al., 2017).

The stability of various vegetable oils, including canola, soy and corn (Adhvaryu et al., 2000), chia seeds and linseed oils (Grampone et al., 2013) and linoleic acid (Ulkowski et al., 2005) have been evaluated using thermal methods. DSC data can be analyzed using an isoconversional method such as the one of Ozawa, Flynn and Wall (OFW) (Flynn & Wall, 1966; Ozawa, 1970) to determine the activation energy of the oxidation reaction. Vegetable oils have been analyzed using this approach assuming that the oxidation reaction follows pseudo first-order kinetics when oxygen is in excess. A constant conversion rate is assumed for every onset oxidation temperature at different heating rates (Ostrowska-Ligeza et al., 2010).

The protection of edible oils against oxidative degradation can be accomplished through encapsulation techniques (Inácio et al., 2018) and hot homogenization provides a reliable way to incorporate liquid oils into solid lipid matrices. Using this method, solid lipids or waxes form a physical barrier that protects such oils from the atmospheric oxygen and also hinders heat transfer. Hot homogenization has been applied to encapsulate a range of active substances to protect against chemical degradation, and migration to food and volatilization, (Đorđević et al., 2015). It was applied to produce microparticles containing chia seeds (*Salvia hispanica* L.) oil, pumpkin (*Cucurbita maxima*) oil and baru (*Dipteryx alata*) oil (Rojas et al., 2019). DSC thermograms showed oil stability increased by comparing the activation energy of the oxidation reaction.

The incorporation of PUFA-rich oil-loaded microparticles in whey protein supplement might provide an ingredient that would improve foodstuffs. It is of particular importance to determine the impact on sensory properties of adding the microcapsules to the whey protein product. Therefore, the objective of this study was to microencapsulate green coffee oil and walnut oil using stearic acid as a wall material and to add the microparticles into commercial whey protein supplement. In addition to the microencapsulation process to avoid oil oxidation, the whey protein supplement was characterized using rheology, colorimetry and evaluated using sensory tests.

2. Materials and methods

2.1. Materials

Commercial whey protein supplement was acquired in the local market (Whey Protein 5w Health Time brand, São Paulo, Brazil, which is composed of whey protein concentrate, 80.0% crude proteins, 1.0% total lipids, 5.0% carbohydrates and 3.3 kcal g⁻¹ according to the manufacturer). Green coffee oil and walnut oil (without added antioxidants) were acquired from Veris do Brasil Co. (São Paulo, Brazil) and kept protected from light at -10 °C until use (maximum of 3 wk). Stearic acid (Sigma-Aldrich Co., St. Louis, MO, USA, analytical grade), sodium caseinate (Sigma-Aldrich, analytical grade) and distilled water were used as the wall material, stabilizer and continuous medium, respectively, in the microencapsulation process. Methyl tricosanoate (23:0 Me, Sigma-Aldrich, chromatographic standard, 0.3 mg), 2:1 v/v ether: absolute alcohol (Vetec Co., São Paulo, Brazil, analytical grade) solution, 0.01 mol.L⁻¹ sodium hydroxide solution (Dinâmica Co., São Paulo, Brazil, analytical grade), isooctane (Dinâmica, chromatographic grade), 0.5 mol.L⁻¹ methanolic sodium hydroxide solution, saturated sodium chlorate solution and potassium bromide (Sigma-Aldrich, chromatographic standard) were used. FAME mix (Supelco 37 Component FAME mix, Sigma-Aldrich) was used as the chromatograph standard. Synthetic air (Air Liquid Co., Maringá, Paraná, Brazil, 79% N₂,

21% O₂) and gaseous nitrogen were used in the thermal analyses.

2.2. Microparticles production

The procedure described by Inácio et al. (2018) was used to produce the microparticles. Briefly, sodium caseinate (0.275 g) was dissolved in 250.0 g distilled water and heated to 75 °C with gentle stirring. Meanwhile, 16.75 g stearic acid was melted at 75 °C in a jacketed glass flask connected to a thermostatic bath. Thereafter, the oils (green coffee or walnut, 8.25 g) were added with gentle stirring. After 1 min, the caseinate aqueous solution was added and the system stirred at 8600 rpm for 5 min (Ultraturrax IKA- T25-S25N10G, IKA Werke GmbH & Co. KG, Staufen, Germany). The dispersion was quickly quenched with an ice bath to give solid microparticles. The microparticles were lyophilized (Liotop L101, Liobras Co., Sao Paulo, Brazil) and stored at -10 °C protected from light for a maximum of 10 days. Blank microparticles were obtained following the same procedure but without oil addition.

2.3. Oils and microparticles characterization

Trans-esterification was carried out in triplicate as described by Barros et al. (2013), using methyl tricosanoate as an internal standard at 1.0 mg.L⁻¹ in isooctane. In a screw-cap tube with a screw-cap, 300 µL internal standard were added. After evaporation of the solvent under the flow of nitrogen gas, 20 mg oil was added. Then, 4.0 mL NaOH 0.50 mol L⁻¹ in methanol was added and vigorously agitated for 30 s. The mixture was heated in a bath at 100 °C for 5 min and cooled to 25 °C. 5 mL esterifying reagent were added (mixture of 2.0 g ammonia chloride, 60.0 mL methanol, and 3.0 mL sulfuric acid) followed by saturated NaCl solution (0.5 mL) and isooctane (2 mL). The esterified sample was left at 10 °C for better phase separation. The supernatant containing the methyl fatty esters was collected and kept at -18 °C for a maximum of 1 h for the gas chromatograph analysis. Fatty acids (FA) profile was obtained using gas chromatography coupled to a flame ionization detection (GC 1000, GC-FID, Dani Co., Milan, Italy). FA identification was done by comparing the relative retention time of the FAME standards with the samples. Results were expressed as relative percentage of each FA.

Tocopherols were determined following a procedure described by Barros et al. (2013) using a HPLC system (Smartline system 1000, Knauer Co., Berlin, Germany) coupled to a fluorescence detector (excitation at 290 nm and emission at 330 nm; FP-2020; Jasco, Easton, WA, USA). BHT solution in hexane (Fisher Scientific, Lisbon, Portugal, 10 mg mL⁻¹, 100 µL) and the internal standard solution in hexane (racemic tocol; 50 µg mL⁻¹, 400 µL) were added to the sample prior to the extraction procedure. The samples (~500 mg) were homogenized with methanol (4 mL) for 1 min and hexane (4 mL) was added. Saturated NaCl aqueous solution (2 mL) was added, the mixture was homogenized in the Ultraturrax (1 min), centrifuged (5 min, 4000×g) and the clear upper layer was transferred to a vial. The sample was re-extracted twice with hexane. The combined extracts were taken to dryness under a nitrogen stream, redissolved in 2 mL of n-hexane, dehydrated with anhydrous sodium sulphate, filtered through 0.2 µm nylon filters (Whatman, Maidstone, UK), transferred into a dark injection vial and analyzed using the HPLC system described above. The chromatographic separation was achieved with a normal-phase column (Polyamide II 250 mm × 4.6 mm i.d., YMC, Waters) at 30 °C. The mobile phase used was a mixture of n-hexane and ethyl acetate (HPLC grade, Fisher Scientific, Lisbon, Portugal, 70:30 v/v) at 1 mL min⁻¹ and the injection volume was 20 µL. Quantification was done using commercial standards of tocopherols (α-, β-, γ-, and δ-isoforms, Matreya, Pleasant Gap, PA, USA), by applying the internal standard methodology. The results were expressed in mg/100 g oil.

Fourier transform infrared spectroscopy (FTIR, IR Affinity-1, Shimadzu Inc., Tokyo, Japan) analysis was carried out at 2 cm⁻¹ resolution by combining 32 scans in the spectral range of 4000 to 400 cm⁻¹ at 0.2

cm s⁻¹. Sample (2 g) was mixed with 200 mg of potassium bromide and pelletized under pressure at 10 kg_f cm⁻².

DSC (DSC-4000, PerkinElmer Inc., Waltham, MA, USA) with a N₂ flow rate of 50 mL min⁻¹ N₂ and heating rate of 20 °C min⁻¹ from 0 to 150 °C was used to evaluate microparticles thermal behavior. An amount of ~10 mg of each sample was put in a closed aluminum sample holder.

The parameters D₁₀, D₅₀ and D₉₀, corresponding to the size of 10, 50 and 90% of the microparticles, respectively, were calculated from the volumetric cumulative sizes distribution (Mastersizer 3000, Malvern Inc., Malvern, Worcestershire, UK) using water as the dispersant. The span was calculated using Equation (1).

$$\text{Span} (-) = \frac{D_{90} - D_{10}}{D_{50}} \quad (1)$$

Scanning electron microscopy (SEM) images of gold coated (Quorum Q150R, Quorum Technologies Inc., Laughton, UK) microparticles were obtained using a EVO MA 15 SEM (Carl Zeiss Inc., Oberkochen, Germany) coupled with a backscattering detector, acceleration voltage of 15 kV and 500× magnification.

Encapsulation efficiency was determined using the methodology described by Inácio et al. (2018). Lyophilized microparticles (20–30 mg) were esterified as described earlier to determine the total amount of FA ([FA]_{total}) using gas chromatography. An aliquot of the microparticles dispersion was washed with 100% ethanol and filtered (paper filter with 3 μm porosity, Dinâmica) to remove the free oil, and then also esterified to determine the amount of FA entrapped inside the microparticles ([FA]_{entrapped}). Esterified samples were analyzed using gas chromatography as described above. Encapsulation efficiency was calculated using Equation (2) for linoleic acid and α-linolenic acid because they were the most abundant PUFA in the oils.

$$EE (\%) = \frac{[FA]_{\text{entrapped}}}{[FA]_{\text{total}}} \cdot 100 \quad (2)$$

TGA was carried out using a thermobalance (TGA-50, Shimadzu Inc.). Samples (~4 mg) were put inside aluminum sample holders and heated from 30 to 650 °C at 10 °C min⁻¹ under a nitrogen atmosphere (150 mL min⁻¹) to determine transition temperatures and enthalpy.

Oxidative stability was determined using DSC as described elsewhere (Rojas et al., 2019) using zinc (429.75 K and 107 J g⁻¹) and indium standards (692.68 K and 28.5 J g⁻¹) for calibration. Briefly, oil or lyophilized microparticles were put in open aluminum holders under a synthetic air flux at 100 mL min⁻¹ from 0 to 440 °C using different heating rates (1, 5, 10 and 20 °C min⁻¹). The activation energy (E_a, kJ mol⁻¹) involved in the lipid oxidation reaction was calculated using Equations (3) and (4) (Flynn & Wall, 1966; Inácio et al., 2018; Ostrowska-Ligeza et al., 2010; Ozawa, 1970) using MATLAB R2008b software (The MathWorks, Inc., CA, USA).

$$\log \beta = aT^{-1} + b \quad (3)$$

$$E_a = -2,19R \frac{d \log \beta}{dT^{-1}} \quad (4)$$

In these equations, β is the heating rate (°C min⁻¹), “a” and “b” are the slope and intercept of the adjusted curve, respectively, and R is the universal gas constant (8.3143 J mol⁻¹K⁻¹). The oxidation onset temperature (T_o, °C) was found by extrapolation considering the tangent line (leading edge) and the thermogram baseline (Inácio et al., 2018; Ostrowska-Ligeza et al., 2010). The experimental procedure was carried out in duplicate for each heating rate.

2.4. Formulation of the whey protein supplement with the oil-loaded microparticles

Oil-loaded microparticles were added to the commercial whey protein supplement at 2.5, 5.0 and 7.5% w/w concentration. Then, 25 g of

the whey supplement were added to 125 mL water and mixed for 5 min. A colorimeter (CR-400, Konica Minolta Inc., Tokyo, Japan) was used to determine the parameters L* (luminosity), a* (from green to red) and b* (from blue to yellow) at the surface of the solution (Altunkaya et al., 2013). Saturation (chroma, C*) and tonality angle (hue angle, h°) were calculated using Equations (5) and (6), respectively (Lancaster & Lister, 1997; Lemes et al., 2017). Color was analyzed in duplicate with three measurements for each experimental condition.

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (5)$$

$$h^\circ = \tan^{-1} \left(\frac{b^*}{a^*} \right) \quad (6)$$

Rheological measurements (DV-III and spindle SC431, Brookfield Inc, Middleboro, MA, USA) were carried out in duplicate at a spindle speed varying from zero to 100 rpm at 25 and 37 °C (approximate room temperature and the average human body temperature, respectively). The parameters of the Bingham, Casson and Herschel-Bulkley models were evaluated using Equations (7)–(9), respectively, where σ is the shear stress (Pa), σ₀ the yield stress (Pa), η_a the limiting viscosity (Pa.s), γ̇ the shear rate (s⁻¹), k_c the viscosity at infinite shear rate (Pa.s), k the consistency index (Pa.sⁿ), and n the flow behavior index (dimensionless) (Chivero et al., 2016; Tabilo-Munizaga & Barbosa-Cánovas, 2005).

$$\sigma = \sigma_0 + \eta_a \cdot \dot{\gamma} \quad (7)$$

$$\sigma^{0.5} = \sigma_0^{0.5} + k_c \cdot \dot{\gamma}^{0.5} \quad (8)$$

$$\sigma = \sigma_0 + k \cdot \dot{\gamma}^n \quad (9)$$

Instituto Adolfo Lutz (2008) methodologies were applied to determine crude protein, ash, total lipids, moisture and energy content of the whey protein supplement (carbohydrates were calculated by difference). The moisture content was determined gravimetrically by heating the sample in porcelain crucibles in an oven (105 °C) until constant weight was achieved. The ash content was determined with the dried material, using a muffle furnace at 600 °C, where the crucibles were heated until constant weight was achieved and the ash content determined gravimetrically. Total lipids were extracted with a mixture of chloroform:methanol; water at 2:2:1.8 v/v/v and the total lipids content was determined gravimetrically with Soxhlet equipment. Crude protein contents were determined using the semi-micro Kjeldahl method (TE 0363, Tecnal Inc., Piracicaba, São Paulo, Brazil) in which total nitrogen was determined and the factor 6.25 was used for the conversion to total crude protein.

Sensory evaluation was carried out in accordance with the Brazilian regulations with the protocol number 2.341.697 (approved by the Ethics Committee for Research of the Federal University of Technology – Paraná UTFPR on October 10, 2017). The test was applied at the university's Sensory Analysis Laboratory in individual cubicles without the possibility of communication between the assessors. Tests were carried out under white fluorescent lighting (6 W, Taschibra, São Paulo, Brazil) at a controlled ambient temperature of 23 °C.

Two ranking preference tests (ABNT, 2015) were done to evaluate the impact of the addition of green coffee and walnut oil microparticles on whey protein supplement. Samples without addition of particles were served together with the enriched samples to have their preference evaluated by 60 untrained assessors (56% women and 44% men), aged between 19 and 48 years old, students and university employees, habitual consumers of protein supplements. Thus, in each test the assessors received three coded samples, served in randomized and balanced order, along with a glass of mineral water and an evaluation form. The assessors were asked to rinse with water both before they began testing and between samples, taste the samples from left to right and order them, from the less preferred to the most preferred sample (Fuchs et al., 2013).

Table 1

Composition of FA (%w/w) and of tocopherols (mg/100 g oil) of green coffee and walnut oils.

FA	Green Coffee	Walnut
	Content in %w/w	
16:0 (Palmitic acid)	31.5 ± 0.3	5.66 ± 0.03
16:1 (Palmitoleic acid)	0.24 ± 0.01	0.07 ± 0.01
17:0 (Heptadecanoic acid)	0.24 ± 0.01	0.05 ± 0.01
18:0 (Stearic acid)	7.31 ± 0.04	2.36 ± 0.01
18:1n-9 (Oleic acid)	8.4 ± 0.1	12.3 ± 0.1
18:2n-6 (Linoleic acid)	46.0 ± 0.1	59.5 ± 0.1
18:3n-3 (α-Linolenic acid)	2.17 ± 0.01	19.6 ± 0.1
20:0 (Arachidic acid)	2.65 ± 0.03	0.09 ± 0.01
20:1 (Eicosenoic acid)	0.35 ± 0.01	0.15 ± 0.01
SFA (%)	41.7 ± 0.2	8.16 ± 0.02
MUFA (%)	9.0 ± 0.1	12.9 ± 0.01
PUFA (%)	48.2 ± 0.1	79.1 ± 0.1
PUFA:SFA	1.16	9.58
n-6:n-3	21.2	3.03
Tocopherols	Concentration in mg/100 g oil	
α-Tocopherol	0.75 ± 0.05	nd
β-Tocopherol	12.6 ± 0.1	nd
γ-Tocopherol	nd	3.4 ± 0.1
δ-Tocopherol	nd	2.4 ± 0.1
Total Tocopherols	13.4 ± 0.1	5.8 ± 0.2

SFA: Saturated FA; MUFA: Monounsaturated FA; PUFA: Polyunsaturated FA.

2.5. Statistical analysis

Color parameters for each formulation were compared using Tukey test with 5% significance using Statistica 13.3 software (Tibco Software Inc., Palo Alto, CA, USA). For the sensory analysis, results were analyzed using the Friedman Test ($p < 0.05$). The curves that showed similar rheological parameters indicating the same rheological behavior had their viscosity compared using the Tukey test with 5% significance, seeking to verify that each rheological curve did not show any significant difference with each other separately.

3. Results and discussion

3.1. Oil characterization

FA composition of green coffee oil and walnut oil are shown in Table 1 (retention times and identification are shown in Fig. S1, Supplementary Material).

Linoleic acid and α-linolenic acid were the most abundant FA in walnut oil while the predominant FA in green coffee oil were linoleic and palmitic acids. Values are consistent with those reported in the literature (Bertrand et al., 2008; Budryn et al., 2012). For walnut oil, values of 11.9, 63.2 and 14.6% (Calvo et al., 2011) and 17.6, 54.1 and 15.0% (Christopoulos & Tsantili, 2015) were reported for linolenic, linoleic and oleic acids, respectively. PUFA content was considerably higher in both oils meaning that these oils are prone to react with oxygen and undergo degradation. While green coffee showed the presence of α- and β-tocopherols (no γ- and δ-tocopherols were detected) and a higher concentration of total tocopherols, walnut oil showed γ- and δ-tocopherol (no α- and β-tocopherols were detected) and a lower amount of total tocopherols. The prevalence of these two isoforms in green coffee oil (González et al., 2001) and walnut oil (Uzunova et al., 2015) have been previously reported.

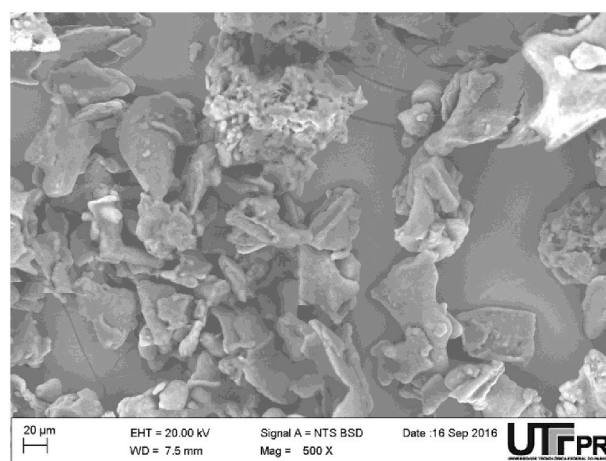
3.2. Microparticles characterization

Encapsulation efficiency (%) for linoleic acid and α-linolenic acid, melting enthalpy (ΔH) and transition temperatures (DSC curves are included in Supplementary Material, Fig. S2) and particle sizes (D_{10} , D_{50} and D_{90}) are shown in Table 2. Fig. 1 shows SEM images of the oil-loaded

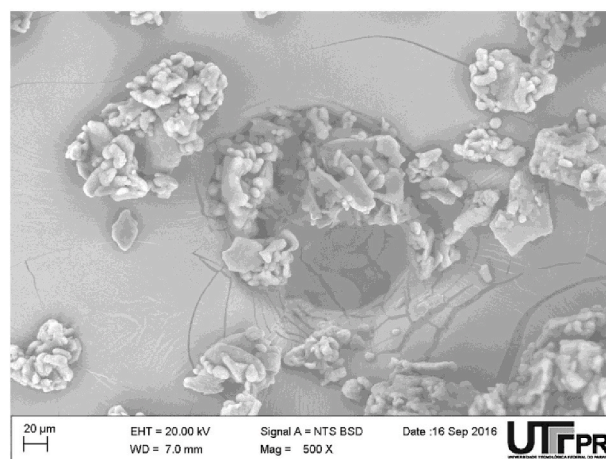
Table 2

Encapsulation efficiency of linoleic acid and α-linolenic acid, microparticles particle size, melting enthalpy and melting temperature for the produced microparticles.

	Oil-loaded microparticles	Green coffee	Walnut
		Content in %w/w	
Encapsulation efficiency (%)	18:2n6 (linoleic acid) 18:3n3 (α-linolenic acid)	94 ± 1 103 ± 1	92 ± 3 92 ± 3
D_{10} (μm)		8.3 ± 0.9	5.8 ± 0.1
D_{50} (μm)		39 ± 3	32 ± 1
D_{90} (μm)		86 ± 5	82 ± 2
Span		2.0	2.4
ΔH (J.g ⁻¹)		190 ± 10	170 ± 20
T (°C)		73 ± 2	74 ± 6



(a)



(b)

Fig. 1. Scanning electron microscopy of the oil-loaded microparticles (500×). (a) Green coffee oil-loaded microparticles. (b) Walnut oil-loaded microparticles.

microparticles. Infrared spectra for oils and oil-loaded microparticles are shown in Fig. S3 (Supplementary Material).

An encapsulation efficiency >100% was found for α-linolenic acid possibly due to the low amount of this FA in green coffee oil. Overall, encapsulation efficiency showed high values due to the affinity between

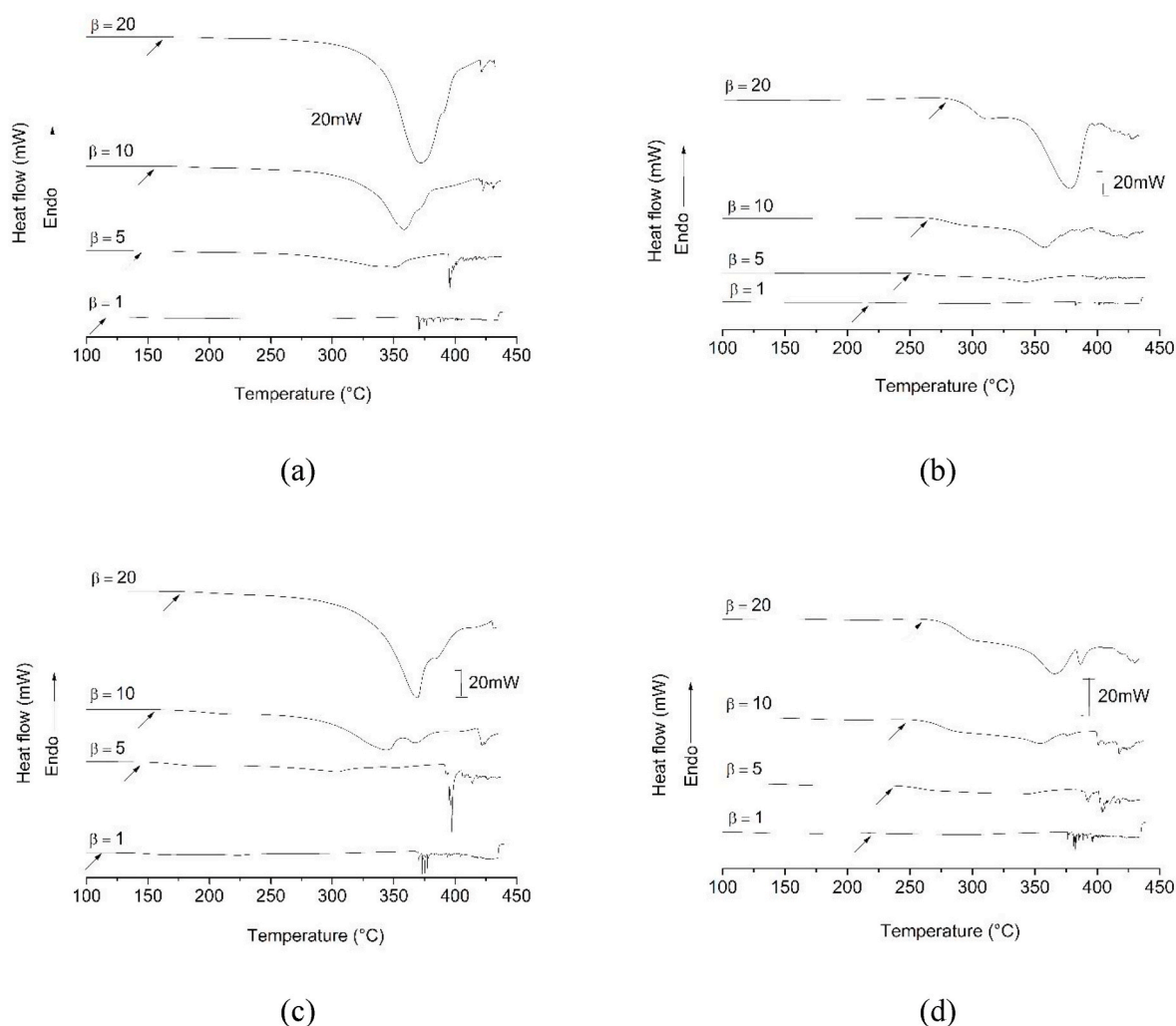


Fig. 2. Non-isothermal DSC curves for free and encapsulated oils: (a, b) Green coffee and (c, d) Walnut (onset oxidation temperature is indicated for each curve; β = heating rate in $^{\circ}\text{C min}^{-1}$). (a) Green coffee oil. (b) Green coffee-oil loaded microparticles. (c) Walnut oil. (d) Walnut oil-loaded microparticles.

the oils and the encapsulant matrix (stearic acid). This was also observed for spearmint essential oil (Ciftci & Temelli, 2016) and vitamin E (Albertini et al., 2008). High encapsulation efficiencies were reported for chia seeds oil, pumpkin seeds oil and baru oil using stearic acid as the encapsulant (Rojas et al., 2019). Particle shapes (Fig. 1) were irregular and most of the particles showed sizes $< 86 \mu\text{m}$ which may be satisfactory in the case of encapsulated food additives because larger particles could impact acceptance.

The melting enthalpy and the melting temperature for particles obtained using the same experimental conditions, but without oil (blank microparticles), were reported with values of 249 J g^{-1} and $76.4 \text{ }^{\circ}\text{C}$, respectively (Rojas et al., 2019). Although the melting temperature did not change due to the presence of the oils, the melting enthalpy showed a sharp decrease of $\sim 70\%$, which is in accordance with the amount of oil used and the encapsulation efficiency. This behavior was also found for encapsulated oils like andiroba oil (Senhorini et al., 2012), chia seeds oil (Inácio et al., 2018) as well as other hydrophobic substances (Özdemir & Gökmen, 2015).

Characteristic absorption bands of $\text{C}=\text{C}-\text{H}$ and $\text{C}=\text{O}$ of the oils were found at 3010 and 1740 cm^{-1} , respectively. These bands may be attributed to chain unsaturation in the FA and to carbonyl groups of the ester groups of triacylglycerol structures (Freiberger et al., 2015; Vidal et al., 2014). The band at 3010 cm^{-1} was intense in the pure oils but it was attenuated in the oil-loaded microparticles. The decrease in intensity was greater than expected if only caused by the low

concentration of oils in the microparticles, being also related with the saturated nature of the shell material used (stearic acid), consistent with the effective entrapment of the oils inside the microparticles. The higher attenuation observed with green coffee oil, compared to walnut oil, is consistent with the encapsulation efficiency (Table 2).

3.3. Thermal stability

Fig. 2 shows the non-isothermal DSC curves for the oils (green coffee and walnut) and for the oil-loaded microparticles (regression curves are shown in Fig. S4, Supplementary Material).

Activation energy values found for green coffee oil and the corresponding oil-loaded microparticles were 82 ± 4 and $1.05 \pm 0.09 \text{ } 10 \text{ kJ mol}^{-1}$, respectively, and for walnut oil and the corresponding oil-loaded microparticles were 90 ± 5 and $149 \pm 1 \text{ kJ mol}^{-1}$, respectively. Lipid oxidative degradation is initiated by a free radical with the formation of radicals occurring throughout the reaction. Oil oxidation is usually evaluated using the kinetic parameters at the beginning of the reaction (Micić et al., 2015) where higher activation energy denotes an increased stability. Lipid degradation is complex due to the presence of primary reaction products such as hydroperoxides, which increases degradation rates at low temperatures, but also because intermediary products such as aldehydes and acids may evaporate when temperature increases (Micić et al., 2015). Oxidation onset temperatures increased as expected for all samples whilst it was higher for the oil-loaded microparticles

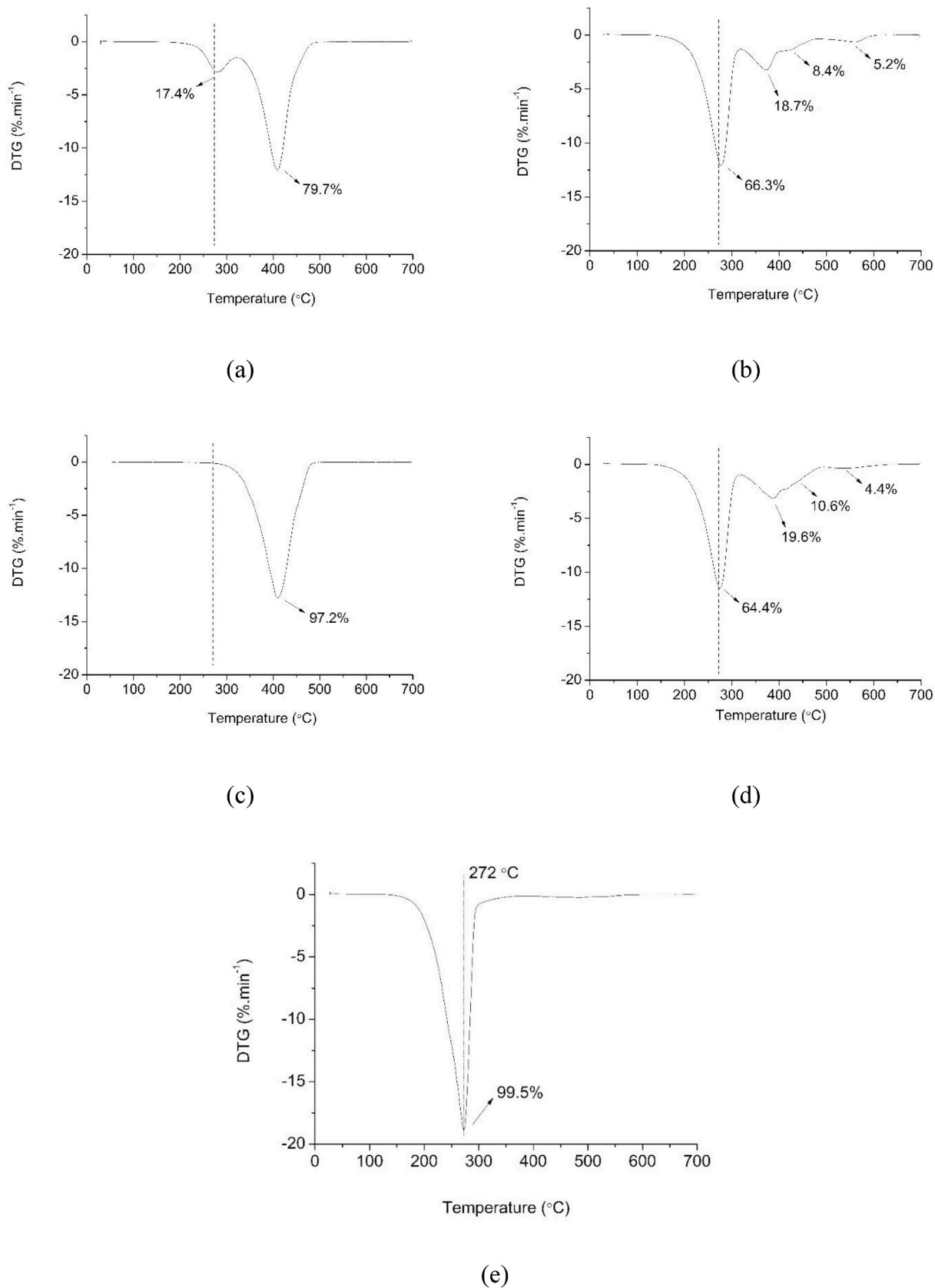


Fig. 3. Differential thermogravimetric analyses curves for free and encapsulated oils: (a, b) Green coffee; (c, d) Walnut; and (e) Blank microparticles (no oil added). Dotted lines indicate the temperature of maximum weight loss of the encapsulant. (e) Blank microparticles (no oil added). (a) Green coffee oil. (b) Green coffee-oil loaded microparticles (c) Walnut oil. (d) Walnut oil-loaded microparticles. (e) Blank microparticles (no oil added).

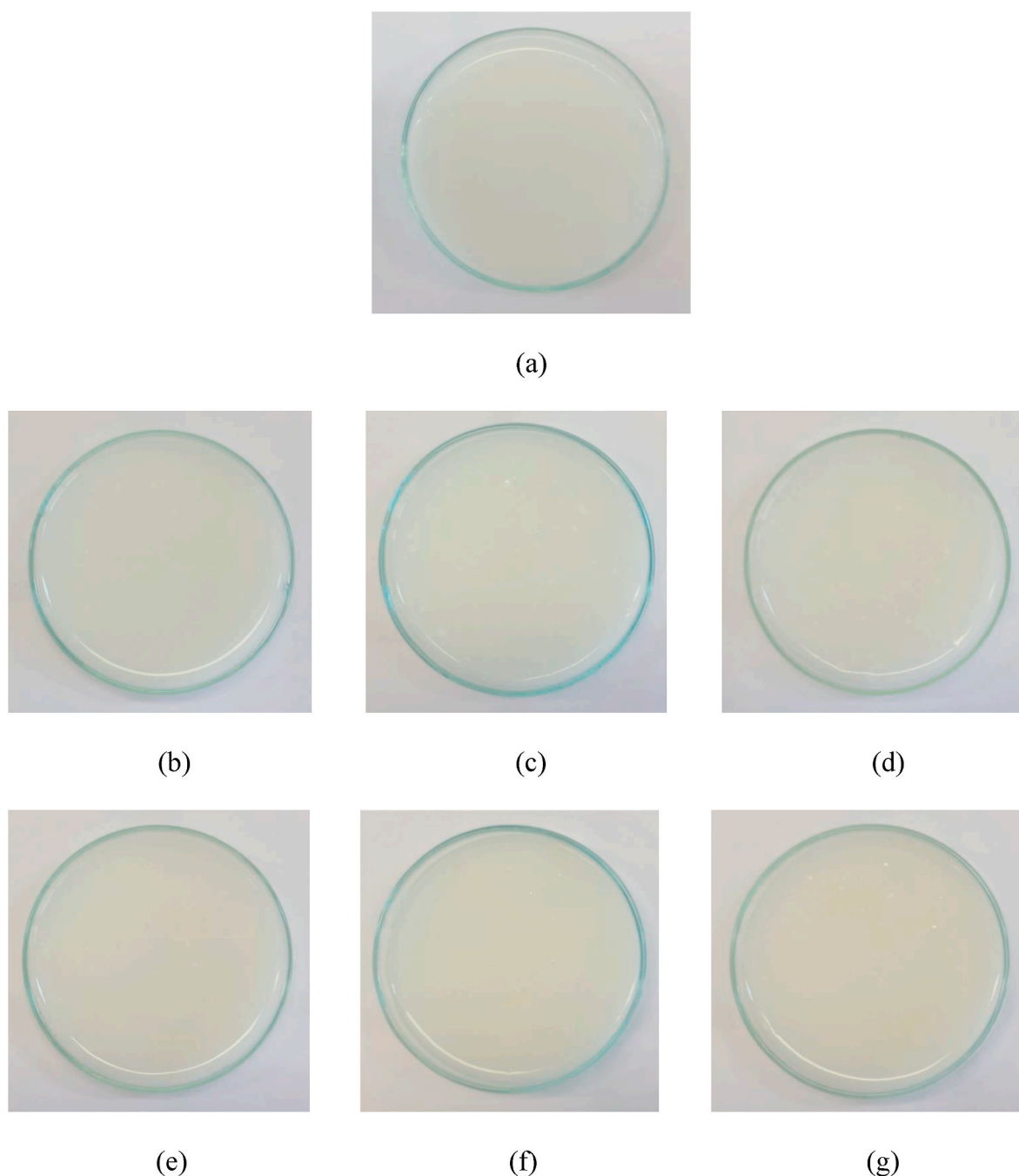


Fig. 4. Images of the whey protein: (a) Control sample (no microparticles added); (b), (c) and (d) Walnut oil-loaded microparticles; and (e), (f) and (g) Green coffee oil-loaded microparticles. (a) Control sample (whey protein without microparticles). (b) 2.5% w/w walnut oil-loaded microparticles. (c) 5.0% w/w walnut oil-loaded microparticles. (d) 7.5% w/w walnut oil-loaded microparticles. (e) 2.5% w/w green coffee oil-loaded microparticles. (f) 5.0% w/w green coffee oil-loaded microparticles. (g) 7.5% w/w green coffee oil-loaded microparticles. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

when compared with the corresponding free oils at the same heating rate. Moreover, activation energy was higher for the microencapsulated oil with a significant improvement for walnut oil. This is an improvement of the oil stability which was previously reported for other oils and encapsulants (Grampone et al., 2013; Inácio et al., 2018).

Differential TGA (DTGA) curves of the oils, oil-loaded microparticles and blank microparticles (no oil added) are shown in Fig. 3. DTGA curves of the walnut oil showed that weight loss occurred at ~ 410 °C (97.2% weight loss) while for coffee oil weight loss was found at 278 °C (17.4%) and thereafter at 410 °C (79.7%). This difference may be attributed to the higher concentration of low molar mass FA (mainly

palmitic acid) in green coffee oil, when compared to walnut oil. The encapsulant (stearic acid) showed the maximum weight loss at 272 °C (17.4%). This peak was also observed in the microparticles as expected.

Results showed that the oils were successfully encapsulated and their oxidative stability was improved due to encapsulation. This means that the microparticles may successfully protect the oils in the preparation of enriched foods.

3.4. Whey protein supplement containing the microparticles

Nutritional composition of the whey protein before the addition of

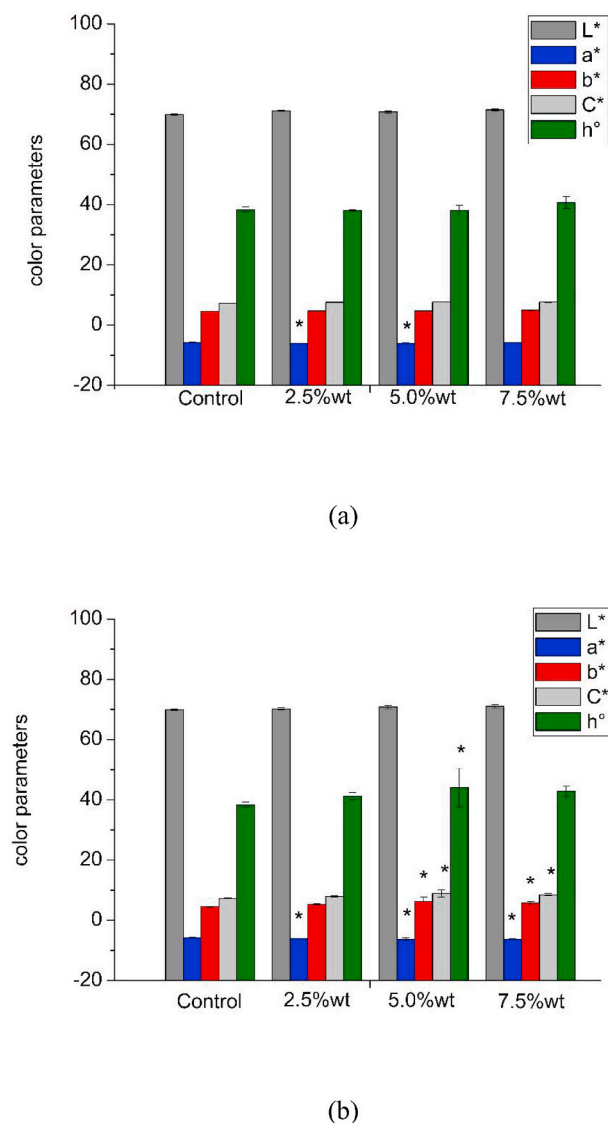


Fig. 5. Color parameters of the whey protein containing 0% w/w (control sample) 2.5% w/w, 5.0% w/w and 7.5% w/w of oil-loaded microparticles (units for L*, a*, b* C* are arbitrary and unit for h° is degree). (a) Whey protein containing walnut oil-loaded microparticles. (b) Whey protein containing green coffee oil-loaded microparticles. Results were compared using Tukey test with 5% significance. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

the microparticles was determined as follows: $4.58 \pm 0.03\%$ humidity, $1.09 \pm 0.02\%$ ash, $77.5 \pm 0.1\%$ crude proteins, $3.0 \pm 0.1\%$ total lipids, $13.9 \pm 0.1\%$ carbohydrates (by difference). These values are consistent with the nutritional data provided on the product label.

Microparticles were added to the whey protein and then the final product dispersed in water as recommended by the manufacturer for the whey supplement. Images of the whey protein containing the green coffee and walnut oil-loaded microparticles are shown in Fig. 4 showing the general aspect of the supplements. Fig. 5 shows the color parameters determined for each formulation.

High luminosity, low yellow (+b*) and green (- a), and low color intensity (C*) were found for all samples. WPS with 2.5 and 5.0% w/w of walnut oil-loaded microparticles showed the a parameter statistically different from control samples ($p \geq 0.05$) while no difference was found for the 7.5% w/w. This difference was not observed visually by the authors (Fig. 4). For the green coffee oil, statistically significant differences were found for particles concentrations of 5.0 and 7.5% w/w

meaning that these concentrations influenced the color of the whey protein supplement. Although images in Fig. 4 do not provide enough visual contrast, it was possible to observe this difference visually by the authors in the original samples.

Rheology curves were obtained at 25 and 37 °C for the whey protein containing the oil-loaded microparticles and then fit to the mathematical models of Casson, Bingham and Herschel-Bulkley. These results are shown in Table S1 and Figs. S5 and S6 (Supplementary Material).

The whey protein supplement (base product and product with oil-loaded microparticles) showed non-Newtonian, pseudoplastic behavior, and thixotropic flow with small hysteresis for all samples. The Herschel-Bulkley model showed the best correlation coefficient and the smaller error thus being the most suitable model to represent the data for both temperatures. The supplement was highly fluid in all cases with low shear stress (all samples were below 4.0 Pa). This is expected because whey proteins were not gelatinized in this type of supplement (Kilara and Vanghela, 2004). The samples analyzed at 25 °C showed a shear stress higher than 37 °C samples, when compared at the same strain rate (Fig. S5) which was expected (Tabilo-Munizaga & Barbosa-Cánovas, 2005). As observed in Figs. S5 and S6, the curves coincide with each other, indicating a similar rheological behavior between the samples, which was also confirmed by the results shown in Table S1. Parameters K_c , η_a and K (Table S1) were also representative of low viscosity systems. Low viscosity and non-Newtonian flow were also observed for partially denaturated whey protein (Zhang et al., 2016). The samples parameters did not show a definite tendency related to the increase of the concentration of microparticles. To understand this behavior, a comparative analysis was done between the averages using the Tukey test and no significant difference ($p < 0.05$) of the microparticles' addition to the whey supplement was found for both encapsulated oils. Due to the similarity of the results, the rheological analysis indicated that the oil-loaded microparticles probably did not alter the three-dimensional structure of the protein and did not promote thermodynamic incompatibility.

There was no significant difference ($p < 0.05$) between the preference for the whey protein samples enriched or not with walnut oil microparticles. Thus, it is possible to add up to 5% w/w of walnut oil microparticles in whey protein based beverages, without affecting the preference of the samples and obtaining the nutritional gain resulting from this addition. It was determined that the addition of green coffee microparticles had a negative impact on the preference of the whey protein beverages. The most preferred was the beverage without enrichment, while the least preferred sample was the beverage with 5% w/w of green coffee oil microparticles.

Several factors can cause the reduction of food preference such as changes in appearance, taste or other sensory characteristics. As the preference ranking test is a non-specific test, it is not possible to state which factor promoted the preference reduction. It is suggested that the color change caused by the addition of the microparticles of green coffee oil, as shown by the colorimetric analysis, may be responsible for this preference decrease. Research evaluating additions of green coffee extract in soymilk (Sęczyk et al., 2017) and ground green coffee beans in breads (Zain et al., 2018) found that color is the sensorial attribute whose acceptance is negatively influenced by the addition.

4. Conclusion

Whey protein supplements containing oil-loaded microparticles were produced using walnut oil or green coffee oil. Encapsulation resulted in an efficient protection of the oils against oxidative degradation as determined using DSC and TGA. High encapsulation efficiencies were found using GC and further confirmed using spectroscopic and thermic characterization. The main FA present in walnut oil were linoleic acid and α -linolenic acids, while linoleic and palmitic acids were predominant in green coffee oil. This last oil also showed more tocopherols with the prevalence of α - and β -tocopherols.

Concentrations of 2.5, 5.0 and 7.5% of oil-loaded microparticles were added to the whey protein supplement. Rheology analysis showed that the microparticles did not influence flow behavior or viscosity, which is important from a commercial point of view. For the walnut oil microparticles, no appreciable change in color was found, even when 7.5% of microparticles were added. However, for the green coffee oil microparticles at this concentration changed the color of the supplement. Sensory analysis was carried out to determine if consumer's preference would be influenced by the presence of the microparticles at 2.5 and 5.0%, it being concluded that it was not affected when walnut oil microparticles were added, for all the tested concentrations, but decreased when green coffee oil-loaded microparticles were used, particularly at 7.5%.

Results showed that it is feasible to produce a whey protein supplement enriched with microencapsulated polyunsaturated FA, which might be an interesting ingredient.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Valquíria Maeda Rojas: Methodology, Investigation, Writing - original draft. **Alexandre Guimarães Inácio:** Methodology, Investigation, Writing - original draft. **Isabel Patricia Martins Fernandes:** Methodology, Writing - original draft. **Fernanda Vitória Leimann:** Conceptualization, Methodology, Writing - original draft, Writing - review & editing. **Angela Maria Gozzo:** Conceptualization, Writing - original draft. **Renata Hernandez Barros Fuchs:** Conceptualization, Methodology, Writing - original draft. **Maria Filomena Filipe Barreiro:** Methodology, Writing - original draft. **Lillian Barros:** Methodology, Writing - original draft. **Isabel C.F.R. Ferreira:** Methodology, Writing - original draft, Writing - review & editing. **Ailey Aparecida Coelho Tanamati:** Conceptualization, Methodology, Writing - original draft. **Odinei Hess Gonçalves:** Conceptualization, Methodology, Writing - original draft, Writing - review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fbio.2020.100690>.

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