Spray-Dried Microcapsules of Cheese Whey and Whey Permeate as a Strategy to Protect Chia Oil from Oxidative Degradation

Running head: Dairy by-products as wall materials to prepare chia oil microcapsules

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SUMMARY

Research background. Cheese whey and whey permeate are dairy industry by-products usually sent to effluent treatment or incorrectly disposed in the environment, generating costs for the production of dairy products and environmental problems due to the high organic load. Cheese whey and whey permeate can be reused as wall materials to form chia oil microcapsules, which act as a barrier to pro-oxidants. This study aimed to develop encapsulation by spray-drying to protect chia oil using dairy by-products as wall materials.

Experimental approach. We evaluated cheese whey, whey permeate, and mixtures of cheese whey and whey permeate (5:5, 7:3, 8:2 m/m) as encapsulating agents with the spray dryer process. Initially, we characterized the chia oil and encapsulating materials. Chia oil emulsions were prepared using the encapsulating materials and an emulsifier. The stability of the emulsions was evaluated by creaming index, and they were characterized according to size distribution and polydispersity index.

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Emulsions were encapsulated in a spray dryer with inlet air temperature at 125 °C and outlet temperature at 105 °C. After encapsulation, we assessed chia oil for oxidative degradation over 30 days of storage by determining the peroxide index.

Results and conclusions. Emulsions presented creaming index between 51 and 83 % in all formulations, and the oxidative stability of microencapsulated chia oil was significantly higher than that of free chia oil after 30 days. Wall material combination affected both encapsulation efficiency and oxidation protection. The cheese whey and whey permeate (8:2) mixture exhibited the highest encapsulation efficiency (70.07 %) and ability to protect the chia seed oil. After 30 days, the peroxide value was below the maximum limit considered suitable for human consumption.

Novelty and scientific contribution. According to these results, dairy by-products can be used for encapsulation of oxidation–sensitive oils. This represents an alternative use for dairy by-products, which otherwise are discarded and can cause impact to the environment due to their high organic load levels. Our findings suggest dairy by–products can be effectively used as wall materials to generate value–added products.

Keywords: whey permeate; cheese whey; chia oil; microcapsules

INTRODUCTION

Chia (Salvia hispanica L.) is a plant species from the Lamiaceae family, native to Latin America. Approximately 28 % to 32 % of its oil is found in the seed. The oil has high levels of polyunsaturated fatty acid (PUFA), especially ω–3 and ω–6, with ω–3 representing about 61 to 70 % of total oil content. This plant is one of the richest plant sources of omega 3 and provides a highly-nutritional seed oil (1,2). Therefore, chia oil can be employed to develop functional foods for a diet rich in ω-3, being an alternative to fish for vegetarians (3). However, chia seed oil presents low stability and it is susceptible to oxidation, thus reducing its shelf life (1,2). The exposure of PUFA to factors such as oxygen, moisture, and temperature triggers reactions that cause oil to deteriorate, thus limiting its application in foods (4,5).

Encapsulation technologies can minimize chia oil degradation. Oil–in–water emulsions form the basis of numerous products. They enhance the physical properties of oils and facilitate encapsulation and preservation (6). Microencapsulation is the packaging of small particles (active substance) by wrapping it with a homogeneous matrix, forming small capsules (7). One of the most commonly applied encapsulation technologies is spray drying, which is fast, relatively inexpensive, and reproducible. Moreover, constant drying conditions stabilise powder specifications throughout the
dryer. As it is a continuous operation and adaptable to full automatic control, it can be used to
dehydrate heat–sensitive materials (8-11).

The choice of wall material is of utmost importance, as it determines microcapsule efficiency
and stability (12,13). Cheese whey (CW) and whey permeate (WP), natural and edible biopolymers,
may be alternatives for oil encapsulation. A large volume of CW is generated in the cheese making
process, ranging from 80 to 90 % of the milk volume used (13). Between 2017 and 2019, more than
230,000 kt of CW were produced. Global whey production is estimated to reach approximately
268,000 kt by 2029 (14). That means large amounts of CW are available from dairy industry processes
and can be reused instead of simply being discarded, as it has a high organic loading rate. CW is an
environmental pollutant that cannot be discarded without previous treatment (14,15). Alternatively,
CW can be ultrafiltrated to obtain whey retentate, a product rich in proteins with high added value.
However, this process generates another liquid waste, WP, comprised mostly of lactose, with high
organic loading (15-18).

The literature shows studies on lipid oxidation of oils of different origins, such as anchovy oil
(19), pomegranate seed oil (20), carp oil (21), and fish oil (22,23). Studies on chia oil protection are
still recent, and there is no study on CW and WP efficiency in microencapsulation of chia seed oil.
Therefore, further studies are required in the production of stable and efficient chia oil microcapsules
using these dairy by-products. Using CW and WP as wall materials adds value to these by-products
and can reduce environmental effects of incorrect disposal. Thus, this study aimed to evaluate the
use of dairy by-products as wall materials for chia oil powders using spray drying. We evaluated
different CW and WP ratios in wall materials in chia seed oil for emulsion stability, encapsulation
efficiency, and the oxidative stability.

MATERIALS AND METHODS

Materials and characterisation

Cheese whey and whey permeate (powdered) were donated by regional dairy factories and
characterized following the methods of the Association of Official Agricultural Chemists (AOAC) for
lipid (2000.18), protein (991.20), moisture (990.20), carbohydrate (986.25), and ash contents (968.08)
(24).

The chia seed oil (Girioil Agroindústria, Entre-Ijuís, Brazil) was characterized according to the
methodology of the American Oil Chemists Society (AOCS) for iodine (Cd 1–25), acid (Cd 3d–63),
and saponification values (Cd 3b–76) (25). Peroxide value was determined according to a method
adapted from literature (26). Fatty acid composition was determined by gas chromatography
according to literature (27). We used Arabic gum (Labsynth, Diadema, Brazil) and soy lecithin (Bremil, Arroio do Meio, Brazil) as emulsification agents. All reagents use had analytical grade.

**Preparation and characterisation of chia oil–in–water emulsion**

Five emulsions were prepared using different wall materials in aqueous solutions. First, the Arabic gum was dissolved (3.0 % m/V) with distilled water at 60 °C. The wall material was added (23.0 % m/V) at 25 °C. CW, WP, and CW:WP mixtures (5:5, 7:3, and 8:2 m/m) were used as wall materials. The solution was constantly agitated at 300 rpm for 6 hours at 25 °C in an orbital shaker incubator (Marconi, Piracicaba, Brazil, MA 830), according to the methodology adapted from literature (28). The oil phase, chia oil (9.28 % m/V), and soy lecithin (0.5 % m/V) was added to the initial solution. Emulsions were obtained by phase homogenization using an Ultra–Turrax homogenizer (IKA, Campinas, Brazil, Ultra–Turrax S25N18GST) for 5 minutes at 8,000 rpm. The procedure and conditions were selected according to Lehn et al. (29).

Emulsion stability was determined using creaming index (CI), as proposed by literature (30). Emulsions were transferred to 10 mL test tubes and kept in a drying oven at 25 °C for 24 hours. CI was determined using the emulsion height, following Eq. 1:

\[
CI = \left(\frac{h_1}{h_0}\right) \times 100%
\]

where \(h_0\) is the initial height of the emulsion (cm), and \(h_1\) is the height of the whey–rich (upper) phase after 24 hours (cm).

Emulsions were characterized according to size distribution and polydispersity index (PDI) by dynamic light scattering (Anton Paar, São Paulo, Brazil, Litesizer 500). Morphology was observed using an optical microscope (Leica, Wetzlar, Germany, DM 500) with 40× magnification.

**Spray–drying process**

Emulsions were spray-dried immediately after preparation using a lab scale spray–dryer (LabMaq, Ribeirão Preto, Brazil, MD 0.5) with inlet and outlet drying temperature of 125 °C and 105 °C respectively, feed rate of 300 mL/h, two–fluid nozzle (0.7 mm diameter), and drying air and spray flow rates of 2.5 m³/min and 45 L/h, respectively (12).

**Microcapsule characterisation**

The surface oil content, total oil content, and encapsulation efficiency of the microcapsules were characterized according to the methodology proposed by literature (12). The amount of unencapsulated oil was measured by adding hexane (15 mL) to 2 g of microcapsule powder and
shaking for 2 min. The suspension was filtered, and the residue was rinsed three times with 20 mL of hexane each time. The filtrate solution containing the extracted oil was then transferred to an oven at 70 °C where it remained for 6 h. Surface oil was calculated by the difference between initial and final slurry container mass, and encapsulation efficiency (% EE) was obtained using Equation 2:

\[
\% EE = \left(1 - \frac{\text{Surface oil}}{\text{Total oil}}\right) \times 100
\]

Water activity \((a_w)\) was measured using the AquaLab system (Meter Group Latam, São José dos Campos, Brazil, Lite) at 25 °C. Hygroscopicity values of the microcapsules were determined according method as described in the literature (31), and colour parameters were determined using a colorimeter (Minolta Corporation, Tokyo, Japan, CM–5) by measuring the three–dimensional Lab colour space (32).

The presence of functional groups of chia seed oil in the microcapsules, was determined using infrared spectroscopy (FTIR). Scanning electron microscopy (SEM) was used to determine morphological analysis and average size (Carl Zeiss, Oberkochen, Germany, EVO MA15). The samples were placed on stainless steel stubs and then sputter–coated with gold at 20 kV, with 300 to 5000× magnification was applied.

The stability of microencapsulated chia seed oil with CW, WP, and CW:WP mixtures (5:5, 7:3 and 8:2 m/m) was monitored at 25 °C, protected from light, for 30 days. Peroxide values of microcapsules and free chia oil were evaluated to analyse the protective effect of the encapsulating agents. A method was adapted to extract oil microcapsules (33). A microcapsule powder sample (1 g) was suspended in 1 mL of distilled water and stirred for 30 min in an incubator (Marconi, Piracicaba, Brazil, MA830) at 300 rpm and 20 °C. A 0.6 mL aliquot of this solution was homogenized with 1.5 mL of isooctane/isopropanol (2:1) solution. The supernatant was collected after centrifugation and rinsed with the solvent three times. An aliquot (0.5 mL) of the extracted product was used for peroxide index analysis. Samples were analysed after 0, 3, 7, 15, and 30 days for peroxide content in free and encapsulated chia seed oil (26).

Statistical analyses

Encapsulation experiments and analytical determinations were conducted in triplicate. Statistical data were analysed using the analysis of variance (ANOVA). Mean values were compared using the Tukey’s test at a significance level of 95 % \((p \leq 0.05)\) using Statistica® 7.1 software (34).

RESULTS AND DISCUSSION
Characterisation of dairy by-products

Based on CW and WP (powdered) chemical compositions, moisture on wall materials was approximately 3.0 %, ash content 5.0 %, and lipid content <0.5 %. However, both materials present different compositions depending on the production process. One particular difference between CW and WP is protein concentration. WP exhibited the lowest protein content (1.5 %) compared to CW (10.9 %) since proteins are retained in the membrane during CW ultrafiltration, which consequently increases carbohydrate content. Carbohydrate content—primarily consisting of lactose—was 90.1 % for WP and 78.5 % for CW (35,36). CW proteins provide emulsifying action, important in oil microencapsulation (37). This suggests that CW performs better as wall material.

Characterisation of chia seed oil

Table 1 shows the physicochemical characterisation of chia seed oil. The major fatty acids present in chia seed oil determined by gas chromatography (GC) analysis were 16:0 and 18:0 (10.9 %), 18:1 (6.7 %), 18:2 (17.1 %), and 18:3 (64.2 %). The high PUFA content (82.4 % total) indicates that chia seed oil is highly unsaturated, corroborated by the high iodine value (Table 1). This unsaturation degree makes chia seed susceptible to oxidation, particularly when exposed to adverse factors such as light, oxygen, moisture, and temperature (4,5).

Table 1

Chia seed oil presented a high saponification value (38) (Table 1), which is inversely proportional to fatty acid chain length, i.e. the index tends to be higher in oils with a higher level of short–chain triglycerides (39). Free fatty acids and peroxide values are parameters used to determine the quality of oils. There is specific legislation to determine the maximum limits of these indexes to ensure food quality. Free fatty acids and peroxides (Table 1) were within the limit established by CODEX Alimentarius (40), which should be lower than 0.6 mg oleic acid/100 g oil and 10 mEq O_2/kg oil, respectively.

Characterisation of emulsions

The CI (after 24 h storage), size distribution, and PDI of the emulsions are shown in Table 2. None of the emulsions was particularly resistant to cream formation, and after storage time, the emulsions were divided into an opaque white layer at the top and a cloudy layer at the bottom.
According to Owens et al. (41), a low repulsive electrostatic force between droplets leads to rapid creaming. It is common for polysaccharides and proteins to form complexes between the PI of the protein and the pKa of the polysaccharide.

Table 2

In addition, Table 2 shows that the highest CI values occurred in the capsules that used pure WP or the highest proportions of WP (5:5 and 7:3). These differences in CI may be correlated with protein content in the emulsion. Proteins facilitate the formation of a stable interface between the oil and the aqueous phase. Milk proteins adsorb the oil–water interface, forming micelles that coat the oil phase (41, 42). Noello et al. (43) studied the emulsion of chia seed oil using whey protein concentrate and pectin. They observed that the emulsion containing only whey protein concentrate remained stable for a period of 24 hours, indicating the ability of whey proteins to confer stability to the emulsion.

CI can provide indirect information on the extent of droplet aggregation in an emulsion: the higher the CI, the larger the droplets and/or the higher the aggregation (44), confirmed by size distribution and high PDI values (above 0.2), which indicate a tendency towards agglomeration (Table 2). These values are confirmed in Fig. 1, which shows the morphology of the emulsions. Fig. 1 correlates diameter, uniformity, and distribution of emulsion droplets to emulsion stability, and consequently, to the quality of the microcapsules (45). Emulsions were spray-dried to check the viability of dairy wall materials to prepare microcapsules and protect chia oil, considering that wall materials can create a physical barrier to the air, without chemical interactions with the core oil.

Figure 1

Characterisation of microcapsules

Table 3 shows encapsulation efficiency (% EE), water activity ($a_w$), and hygroscopicity (%) of samples. Based on the results in Table 3, it is evident that encapsulation efficiency is directly related to the type of wall material used. The highest value (~70 %) was obtained using the CW:WP mixture (8:2), indicating good encapsulation. Adding fractions of permeate to the wall material decreases the hydrophobicity of whey proteins, preventing the migration of hydrophobic compounds from the core material to the microcapsule surface (46). Rodea–González et al. (47) encapsulated chia seed oil using spray-drying with CW concentrate and Arabic gum, and obtained an encapsulation efficiency of 70 %. Gallardo et al. (5) microencapsulated flaxseed oil using CW isolate, maltodextrin, and Arabic gum as wall materials and obtained ~87 % of encapsulation efficiency. The encapsulation efficiency
results shown in Table 3 indicate that CW and WP perform as well as the whey concentrates and isolates used in the studies mentioned.

Table 3

Water activity ($a_w$) indicates the amount of water available for microbiological development and degradation reactions. Table 3 shows the water activity of microcapsule powders was lower than 0.3, within the range for atomized products (48). The ability to absorb water from the environment indicates hygroscopicity, which is directly associated with the preservation of the material. Products with low hygroscopicity may exhibit longer preservation periods (37). Hygroscopicity values were higher in the experiments with higher whey contents (CW and CW:WP 8:2) (Table 3). This difference is associated with wall material composition. During emulsification and drying, lactose derived from both CW and WP can assume different forms since there is no lactose crystallization before drying. According to Hargrove et al. (49), lactose crystallization, which may occur during the drying process, reduces the hygroscopicity in whey powder, thus explaining the reduced hygroscopicity in wall materials that have higher lactose content. The lactose content of CW was approximately 50 %, and that of WP 76 %. The stability of these powdered depends on hygroscopicity, which is influenced by the lactose form present, which may be in the alpha form (less hygroscopic) or the beta form (more hygroscopic).

Fig. 2 shows the FTIR spectrum of chia seed oil and dairy by-products. Some bands at 3430, 1383, 1296, 1260, 1142, 1117, 1035, 915, 898, and 632 cm$^{-1}$ are typical of lactose, with the highest content in CW and WP (50). There was a peak at 3100 cm$^{-1}$ attributed to a deformation associated with a hydrogen bonded to an unsaturated fatty acid carbon. The bands at 2920 and 2850 cm$^{-1}$ represented the –CH$_2$– symmetric and asymmetric stretching vibrations, respectively. The double bond between carbon and oxygen (C=O) was represented at 1740 cm$^{-1}$. There was a tendency towards forming a band near 1250 cm$^{-1}$ associated with the C–O bond in the esters of fatty acids. The band near 950 cm$^{-1}$ was associated with the overlapping of out-of-plane angular vibrations of the –HC=CH– (cis and trans) groups (51). According to the spectrum of microcapsules (not shown), the nature of the peaks did not vary in oil combined with wall material, which indicates lack of any significant chemical interactions between them. All the microcapsule spectra were similar, as the main bonds of the materials used were similar, and no new bonds were formed via chemical reactions. This indicates the absence of chemical interactions between the components of the microcapsules.

Figure 2
Table 4 shows data on the colour of microcapsules with different wall materials. CW in its original form had a hue angle of 85.3° and chromaticity of 13.9, indicating a tendency towards light yellow coloration. WP had a hue angle of 72.3° and a chroma of 26.1, and therefore a darker brown coloration, justified by the higher chroma value. Chroma values indicate colour intensity, which increased according to wall material, probably due to reactions such as caramelization and Maillard related to emulsion drying. Even so, the capsules did not show intense coloration. Furthermore, hue values between 70 and 90° refer to yellow coloration, which was expected considering the wall material applied. The experiment conducted with WP and CW:WP 5:5 showed a significant change in hue angle, associated with the wall material. Therefore, the relationships between $H^\circ$ and $C^*$ lead to the conclusion that the capsules produced are yellow–whitish, although darker hues may be obtained by darkening reactions due to heating during the drying process.

Table 4

Fig. 3 shows the SEM images of the microcapsules obtained with dairy by-products and their mixtures (5:5, 7:3, and 8:2), respectively. Fig. 3a indicates that microencapsulated chia oil with CW has a higher tendency to have a spherical shape compared with WP and the CW:WP mixtures. Fig. 3 does not indicate fissures and broken microcapsules. The difference between concentrations of microcapsule components, such as lactose and protein, may be associated with the behaviour shown in the formation and distribution of these microcapsules. The aggregation observed in Fig. 3b could be explained because WP has a higher lactose content compared to CW, contributing to adhesiveness effects due to exposure to the environment. Lactose, depending on its content and form in the product, may provide greater hygroscopicity to the powder. Humidity and low molecular weight sugars, such as lactose, are the main sources of instability of spray-dried milk powders.

Figure 3

The oxidative stability of microencapsulated chia oil can be observed in Fig. 4 (storage at 25 °C). Fig. 4 shows positive effects of chia oil microcapsule protection during storage. Free chia oil showed increased peroxide value. These results indicate a relationship between encapsulation efficiency and the maintenance of peroxide values in the microcapsules. The CW:WP (8:2) mixture exhibited higher encapsulation efficiency, providing higher oxidation resistance and stability. Furthermore, the wall material provided better protection for the encapsulated oil after the drying process, the primary reason for changes in peroxide value compared to free oil on day 0. Due to their flexibility and amphiphilic nature, milk proteins rapidly adsorb the emulsion interface, where they self-aggregate and form continuous and homogeneous membranes around oil droplets through
intermolecular betasheet interactions. By coating oil droplets with charged layers, protein films provide an electrostatic barrier against flocculation and coalescence, allowing the formation of efficient microcapsules after spray drying (52). Possibly the surface oil is responsible for the first result (in the case of microcapsules with CW:WP 8:2 mixture) of the peroxide value, as an effect of the exposure to the drying temperature. The other particles collected from day 3 onwards had less exposure to air.

Figure 4

The use of CW and WP in emerging technologies such as microencapsulation represents an alternative and beneficial use of these low-cost waste products with high organic load. Moreover, microcapsules formed with these dairy by-products as wall materials can be used to develop high-added value food.

CONCLUSIONS

Combinations of dairy by-products (CW and WP) were used to microencapsulate chia oil using spray-drying. CW with low proportions of WP were good alternatives as additional wall materials to form emulsions. Higher values of CI were found in capsules with the highest amounts of WP related to larger particle diameter. The powder was spherical, with low water activity and hygroscopicity, which is typical of microcapsules formed using spray-drying.

The oxidative stability of microencapsulated chia oil was significantly higher than that of free chia oil after 30 days in all formulations. Wall material combination affected both encapsulation efficiency and oxidation protection. The CW:WP mixture (8:2) had the highest encapsulation efficiency and ability to protect the chia seed oil. The process generated microcapsules with positive effects in protection against lipid oxidation. These results indicate the viability of using dairy by-products as wall materials to generate value-added products.

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CONFLICT OF INTEREST
The authors declare that there is no conflict of interest in carrying out this work.

AUTHORS’ CONTRIBUTION

Daniel Neutzling Lehn: conception of the work, drying, peroxide determination, MEV image analysis, critical revision, paper drafting, final approval of the version to be published

Clauzia Fernanda Volken de Souza: conception of the work, performing the statistical analysis, paper drafting, critical revision, final approval of the version to be published.

Luiz Antonio de Almeida Pinto: paper drafting, critical revision.

Marcos Aurélio Dahlen Júnior: creaming index determination and powder characterisation, data analysis and interpretation.

Wendell Dal’Agnol: performing the analysis of peroxide, iodine, and saponification, data analysis and interpretation.

Natália Neitzke: performing the analysis of peroxide, and water activity.

Adriani Cristina Felipe dos Santos: performing the analysis of peroxide values, preparing of emulsions, data analysis and interpretation.

Vanessa Mendonça Esquerdo: drafting the article, performing the polydispersity index analysis, fatty acid composition, and FTIR analysis, critical revision.

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### Table 1. Physicochemical characterisation of commercial chia seed oil

<table>
<thead>
<tr>
<th>Characterisation index</th>
<th>Chia seed oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iodine value</td>
<td>173.41±5.28 (gI₂/100 g oil)</td>
</tr>
<tr>
<td>Saponification value</td>
<td>196.53±2.84 (mgKOH/g oil)</td>
</tr>
<tr>
<td>Peroxide value</td>
<td>4.00±0.17 (mmol O₂/kg oil)</td>
</tr>
<tr>
<td>Acid value</td>
<td>0.34±0.04 (mg oleic acid/100 g oil)</td>
</tr>
</tbody>
</table>

Mean ± standard deviation (n = 3)

### Table 2. Effect of wall materials on the characteristics of chia oil-in-water emulsions

<table>
<thead>
<tr>
<th>Sample</th>
<th>Creaming index/%</th>
<th>d/μm</th>
<th>Polydispersity index</th>
</tr>
</thead>
<tbody>
<tr>
<td>cheese whey (CW)</td>
<td>(51.7±1.2)c</td>
<td>(3.21±0.25)b</td>
<td>(0.27±0.04)a,b</td>
</tr>
<tr>
<td>whey permeate (WP)</td>
<td>(83.5±0.4)a</td>
<td>(3.96±0.17)a</td>
<td>(0.25±0.01)b</td>
</tr>
<tr>
<td>CW:WP (5:5)</td>
<td>(82.1±0.3)b</td>
<td>(3.74±0.20)a</td>
<td>(0.31±0.03)a</td>
</tr>
<tr>
<td>CW:WP (7:3)</td>
<td>(80.3±1.1)c</td>
<td>(4.02±0.16)a</td>
<td>(0.29±0.03)a</td>
</tr>
<tr>
<td>CW:WP (8:2)</td>
<td>(78.1±1.2)d</td>
<td>(3.93±0.32)a</td>
<td>(0.29±0.02)a</td>
</tr>
</tbody>
</table>

Mean ± standard deviation (n = 3). Different letters in the same column indicate a significant difference (p < 0.05)
Table 3. Characterisation parameters of chia seed oil microcapsules

<table>
<thead>
<tr>
<th>Wall material</th>
<th>Encapsulation efficiency/%</th>
<th>Water activity ($a_w$)</th>
<th>Hygroscopicity/%</th>
</tr>
</thead>
<tbody>
<tr>
<td>cheese whey (CW)</td>
<td>(56.71±2.61)$^b$</td>
<td>(0.11±0.04)$^b$</td>
<td>(9.25±0.15)$^a$</td>
</tr>
<tr>
<td>whey permeate (WP)</td>
<td>(29.46±0.64)$^d$</td>
<td>(0.24±0.06)$^a$</td>
<td>(3.70±0.42)$^d$</td>
</tr>
<tr>
<td>CW:WP (5:5)</td>
<td>(44.39±2.94)$^c$</td>
<td>(0.22±0.03)$^{ab}$</td>
<td>(5.52±0.81)$^c$</td>
</tr>
<tr>
<td>CW:WP (7:3)</td>
<td>(43.25±4.33)$^c$</td>
<td>(0.26±0.06)$^a$</td>
<td>(5.86±0.61)$^c$</td>
</tr>
<tr>
<td>CW:WP (8:2)</td>
<td>(70.07±1.07)$^a$</td>
<td>(0.20±0.03)$^{ab}$</td>
<td>(7.16±0.13)$^b$</td>
</tr>
</tbody>
</table>

Different letters in the same column indicate significant difference ($p \leq 0.05$).

Table 4. Colour parameters of microcapsules

<table>
<thead>
<tr>
<th>Wall material</th>
<th>$L^*$ ± SD</th>
<th>$a^*$ ± SD</th>
<th>$b^*$ ± SD</th>
<th>$C^*$ ± SD</th>
<th>$H/\circ$ ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>cheese whey (CW)</td>
<td>(85.23±0.01)$^a$</td>
<td>(3.84±0.02)$^c$</td>
<td>(23.35±0.02)$^c$</td>
<td>(23.33±0.03)$^a$</td>
<td>(80.72±0.01)$^c$</td>
</tr>
<tr>
<td>whey protein (WP)</td>
<td>(68.27±0.02)$^c$</td>
<td>(7.73±0.01)$^a$</td>
<td>(29.12±0.01)$^a$</td>
<td>(30.28±0.02)$^c$</td>
<td>(75.13±0.03)$^a$</td>
</tr>
<tr>
<td>CW:WP (5:5)</td>
<td>(74.22±0.02)$^b$</td>
<td>(5.87±0.02)$^b$</td>
<td>(26.96±0.01)$^{ab}$</td>
<td>(26.80±0.01)$^b$</td>
<td>(75.33±0.02)$^a$</td>
</tr>
<tr>
<td>CW:WP (7:3)</td>
<td>(73.24±0.01)$^b$</td>
<td>(4.92±0.00)$^{bc}$</td>
<td>(25.31±0.01)$^{bc}$</td>
<td>(26.00±0.00)$^{ab}$</td>
<td>(79.27±0.00)$^{bc}$</td>
</tr>
<tr>
<td>CW:WP (8:2)</td>
<td>(76.68±0.02)$^b$</td>
<td>(4.68±0.02)$^{c}$</td>
<td>(25.16±0.03)$^{bc}$</td>
<td>(25.44±0.04)$^{a}$</td>
<td>(79.63±0.03)$^{bc}$</td>
</tr>
</tbody>
</table>

Mean of three independent experiments ± standard deviation. Different letters in the same column indicate significant difference ($p \leq 0.05$).
Fig. 1. Images of chia oil emulsions with 40× magnification. Wall materials: (a) cheese whey, (b) whey permeate, (c) CW:WP (5:5), (d) CW:WP (7:3), (e) CW:WP (8:2)
Fig. 2. Infrared spectroscopy spectrum of chia seed oil, whey permeate, and cheese whey
Fig. 3. SEM images of microcapsules with (a) cheese whey, (b) whey permeate, (c) CW:WP 5:5, (d) CW:WP 7:3, and (e) CW:WP 8:2
Fig. 4. Stability of free commercial and microencapsulated chia seed oil for 30 days at 25 ºC: (■) chia seed oil, (●) cheese whey (CW), (▲) whey permeate (WP), (▼) CW:WP (5:5), (◄) CW:WP (7:3), (►) CW:WP (8:2)