The effect of vegetable protein modifications on the microencapsulation process

A. Nesterenko [a,b], I. Alric [a,b], F. Violleau [c], F. Silvestre [a,b], V. Durrieu [a,b,*]

[a] Université de Toulouse, Institut National Polytechnique de Toulouse — Ecole Nationale Supérieure des Ingénieurs en Arts Chimiques et Technologiques, Laboratoire de Chimie Agro-Industrielle, F-31030 Toulouse, France
[b] INRA, UMR 1010 CAI, F-31030 Toulouse, France
[c] Université de Toulouse, Institut National Polytechnique de Toulouse — Ecole d'Ingénieurs de Purpan, Département Sciences Agronomiques et Agroalimentaires, UPSP/DGER 115, 75, voie du TOEC, BP 57611, F-31076 Toulouse Cedex 03, France

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ABSTRACT

The use of soy proteins (SoyP) and sunflower proteins (SunP) in the microencapsulation by spray-drying technique of α-tocopherol (T) with a core/wall ratio of 2/1 was studied. SoyP and SunP were used as wall material in an unmodified and modified state. The enzymatic (hydrolysis and cross-linking) and chemical (acylation and cationization) modifications were carried out on vegetable proteins in order to improve their encapsulating properties. The results obtained demonstrated that in the native state, SunP showed higher retention efficiency for T microencapsulation (92.6%) compared to SoyP (79.7%), which could be connected to the different composition of protein extracts. Hydrolysis, acylation and cationization of protein resulted in reduced emulsion viscosity. The retention efficiency of T was improved up to 94.8—99.5% after protein acylation, which was attributed to improved affinity between core and wall material.

1. Introduction

Vegetable proteins have been extensively studied in recent years, because of their renewable and biodegradable character, and good functional properties, such as emulsifying capacity, filmogenic properties and water solubility (Nunes, Batista, Raymundo, Alves, & Sousa, 2003). Our recent review suggests that vegetable proteins represent a highly suitable microencapsulation wall material (Nesterenko, Alric, Silvestre, & Durrieu, 2013) with potential applications in foods, medicines and cosmetics. Proteins extracted from soybean, pea, wheat, corn and barley have already proved their ability to efficiently protect various sensitive ingredients by microencapsulation, mainly using a spray-drying technique. As stated in the literature, microencapsulation efficiency, microparticle size and morphology are strongly affected by active core and wall material concentrations, drying temperature and use of additives (Gharsallaoui, Roudaut, Chambin, Voilley, & Saurel, 2007). For example, the incorporation of polysaccharides in the protein matrix, involves emulsion stability and protection of active core against oxidation (Young, Sadra, & Rosenberg, 1993).

Soybeans are recognized as an excellent source of low-cost proteins with good functionality that can be used in the food and packaging industry. Soy proteins (SoyP) can act as barriers to the transfer of oxygen, oil, and carbon dioxide, increasing the interest of using them as microencapsulation wall material. The effectiveness of SoyP to protect different active substances by spray-drying microencapsulation has been reported in several studies (Augustin, Sanguansri, & Bode, 2006; Charve & Reineccius, 2009; Favaro-Trindade, Santana, Monterrey-Quintero, Trindade, & Netto, 2010; Rascon, Beristain, Garcie, & Salgado, 2011; Rusli, Sanguansri, & Augustin, 2006; Tang & Li, 2013; Yu, Wang, Yao, & Liu, 2007).

Proteins extracted from sunflower seeds show interesting physico-chemical properties, in particular water solubility, gelling, emulsifying and foaming capacities (Gonzalez-Perez & Vereijken, 2007; Gonzalez-Perez, Vereijken, Koningsveld, Gruppen, & Voragen, 2005; Molina, Petruccelli, & Anon, 2004). However, compared to SoyP, which is widely used in food and non-food applications, sunflower proteins (SunP) are mainly used for animal foods. The quality of SunP is affected by the presence of phenolic compounds, especially chlorogenic acid and caffeic acid, because they impact protein digestibility and organoleptic properties (Gonzalez-Perez et al., 2007). Nonetheless, there is increasing worldwide demand for proteins of plant origin, and sunflower seeds are particularly interesting in view of their availability in places where soy is not

* Corresponding author. INRA, UMR 1010 CAI, F-31030 Toulouse, France.
E-mail address: vanessa.durrieu@ensiacet.fr (V. Durrieu).

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produced. Thus some processes of phenolic free SunP extraction have recently been reported (Pickardt et al., 2009; Salgado et al., 2012).

The demand for multi-functional products has increased the need for industry and researchers to develop new and original modification techniques to enhance and diversify protein functionalities. And modification of proteins offers the possibility of altering their physico-chemical properties, such as solubility, amphiphilic properties, oil and water binding. Concerning microencapsulation, modification of protein chains allows microparticles with new properties to be obtained, different from those produced with other wall materials.

One of these modifications which is gaining acceptance as a valuable way to improve the functional properties of vegetable proteins, is enzymatic hydrolysis. The latter improves protein solubility and their emulsifying and foaming abilities (Chabanon, Chevalot, Framboisier, Chenu, & Marc, 2007; Lamsal, Jung, & Johnson, 2007; Ortiz & Wagner, 2002). In addition, hydrolysis can increase the protein surface hydrophobicity, because of the exposure of hydrophobic groups buried in the core of native proteins (Yust, Pedroche, Millan-Linares, Alcaide-Hidalgo, & Millan, 2010).

Acylated proteins have been shown to possess improved functional properties, including increased hydrophobicity and enhanced surface functionality (Matemu, Kayahara, Murasawa, Katayama, & Nakamura, 2011). The acylation affects protein conformation by promoting unfolding of the quaternary structure, facilitating its arrangement at the oil–water interface and thus improving emulsion stability.

Enzymatic cross-linking of vegetable proteins by transglutaminase has been extensively studied to improve the texture, rheological and gelling properties of food preparations (Gan, Latiff, Cheng, & Easa, 2009; Gujral & Rosell, 2004; Sun & Arntfield, 2012; Wang, Zhao, Yang, Jiang, & Chun, 2007). This enzymatic treatment makes it possible to enhance thermal stability of proteins and increase the denaturation temperature (Shand, Ya, Pietrasik, & Wanasundara, 2008; Sun & Arntfield, 2011; Tang, Chen, Li, & Yang, 2006).

Cationization is another technique used to improve functional properties of biopolymers. The resultant cationic derivatives from different polysaccharides (Channasanon, Graisuwan, Kiatkamjornwong, & Hoven, 2007; Wang et al., 2012) and animal proteins (Kiick-Fischer & Tirrell, 1998; Zohuriaan-Mehr, Pourjavadi, Salimi, & Kurdtabar, 2009) show enhanced solubility, swelling power and water absorption.

As no single wall material possesses all the properties required of an ideal encapsulating material, the focus of the current work was to compare the encapsulating properties of SoyP and SunP in unmodified and modified states. The effects of hydrolysis, acylation, cross-linking and cationization of proteins as well as their composition, on the microencapsulation of T by spray-drying technique, were investigated. The properties of the oil-in-water emulsions and spray-dried microparticles obtained were compared before and after modification of proteins.

2. Materials and methods

2.1. Materials

Soy protein isolate was purchased from Lustrel Laboratoires SAS (Saint Jean de Vedas, France) and sunflower protein concentrate was provided by CVG (Dury, France). All other chemicals were of analytical grade. α-Tocopherol, alcalase (2.4 U/g activity), sodium hydroxide, hydrochloric acid (37%), dodecanoyl chloride, glycidyltrimethylammonium chloride, sodium chloride and cyclohexane (HPLC grade) were purchased from Sigma (Saint-Quentin Fallavier, France). Microbial transglutaminase (MTG) used for protein cross-linking was an Activa enzyme preparation (99% maltodextrine and 1% MTG) with an activity of approximately 100 U/g donated by Ajinomoto Co., Inc. (Tokyo, Japan).

2.2. Protein characterizations

2.2.1. Composition

SoyP and SunP vegetable proteins were analyzed for proximate composition using the following procedures. The protein content was found using the Kjeldahl method ($N \times 6.25$). The ash content and moisture content were determined by heating a sample in an oven to constant weight at 550–600 °C for organic matter degradation and at 105 °C for water evaporation, respectively (AOAC., 1995). The lipid percentage was found using conventional Soxhlet extraction in cyclohexane for 7 h. Polysaccharide content was calculated as 100% less the combined percentages of crude protein, ash, moisture and lipid.

SoyP and SunP amino acid composition was determined after total acid hydrolysis of protein under a nitrogen atmosphere in a sealed tube (5.37 M HCl, 105 °C, 24 h). The sample obtained was concentrated by evaporation and dissolved in a trisodium citrate buffer (pH 2.2). After filtration (0.45 μm PTFE membrane), protein amino acids were analyzed using a Biochrom 30 amino acid analyzer (Serlabo Technologies, Entraigues sur la Sorgue, France). All analyses were performed in triplicate.

2.2.2. Solubility

The pH-dependent solubility profile of proteins was obtained using the method described previously (Nesterenko, Alric, Silvestre, & Durrieu, 2012; Nesterenko, Alric, Violleau, Silvestre, & Durrieu, 2013b). Briefly, an aqueous solution of SoyP and SunP was prepared, and the necessary quantity of 4 M NaOH or 4 M HCl added to obtain a given pH (from 1 to 13). After stirring at 70 °C for 1 h, the protein suspensions were centrifuged at 10,000 × *g* for 15 min (Sigma Laborzentrifugen, Osterode, Germany). The soluble protein content in the supernatant was analyzed in triplicate using the Kjeldahl method and solubility (S%, w/w) was defined as follows:

$$S(\%) = \frac{\text{protein content in the supernatant}}{\text{total protein content in solution}} \times 100 \quad (1)$$

2.2.3. Size distribution

Size distributions of two protein extracts were examined by Asymmetrical Flow Field-Flow Fractionation (AsFlFFF) as detailed in a previous study (Nesterenko et al., 2013b). To summarize, the protein-based solutions (0.5% w/w, pH 9.0) were analyzed using an Eclipse 2 System AsFlFFF apparatus (Wyatt Technology Europe, Dernbach, Germany) connected to an Agilent 1100 UV HPLC system (Agilent Technologies, Waldbronn, Germany) with UV detection (at 280 nm) used for quantitative detection. The eluent used for analysis was deionized water at pH 9.0 filtered at 0.1 μm before use.

A 50 μL of sample solution was injected, and elution (separation) started at the 12th min of analysis. During separation, channel flow rate was fixed at 1 mL/min and cross-flow rate was variable. Elution mode was started at a cross-flow rate of 2.5 mL/min for 4 min, then reduced linearly for 10 min to a rate of 0.2 mL/min. Elution was stopped at the 26th min of analysis. Using the recovery % (the difference between injected and detected mass) for each sample, the percentage of non-fractionated particles/molecules with a size equal or less than 10 kDa (i.e. which passed through the membrane during analysis) was calculated. AsFlFFF separated particles/molecules according to differences in diffusion coefficient *D*, which can be converted to the hydrodynamic radius R_h using the Stokes–

Einstein relationship (Yohannes, Wiedmer, Tuominen, Kinnunen, & Riekkola, 2004).

2.3. Protein modifications

2.3.1. Enzymatic hydrolysis, acylation and cationization

SoyP and SunP modifications were carried using methodologies described in previous studies (Nesterenko et al., 2012; Nesterenko et al., 2013b). The enzymatic hydrolysis of vegetable proteins was carried out using Alcalase at pH 7.0, 50 °C for 15 min with an enzyme/protein ratio of 0.002 U/G. The acylation reaction with dodecanoyl chloride (DDC) was performed at pH 10.0 and 50 °C for 180 min with a DDC/NH_2 molar ratio of 0.5/1. Protein cationization using glycidyltrimethylammonium chloride (GTMAC) was made at pH 10.0 and 70 °C for 60 min with a GTMAC/NH_2 molar ratio of 4/1.

2.3.2. Enzymatic cross-linking

The enzymatic cross-linking reaction was carried out according to the method proposed by Shand et al. (2008) with some modifications. An aqueous solution of protein (8% w/w, pH 7.5) was prepared using 1% NaCl and incubated in a water bath at 50 °C. Cross-linking of protein was induced by the addition of MTG (1 U/g enzyme/protein ratio), and after 15 min reaction time, this was inactivated by heating at 90 °C for 5 min. For microencapsulation, a cross-linking reaction was carried out after the emulsion homogenization step.

2.3.3. Modification efficiency

All samples with modified proteins were freeze-dried using Cryo-Rivoire equipment at 20 Pa (Cryonext, Saint Gely du Fesc, France) and stored at 4 °C. Samples obtained from unmodified soy and sunflower proteins were named respectively as follows: SoyP-H and SunP-H for hydrolyzed proteins, SoyP-A and SunP-A for acylated proteins, SoyP-C and SunP-C for cationized proteins, and SoyP-CL and SunP-CL for cross-linked proteins. The degree of hydrolysis (DH), acylation (DA), cationization (DC) and cross-linking (DCL) were evaluated using the o-phtaldialdehyde method (OPA) (Church, Swaisgood, Porter, & Catignani, 1983; Goodno, Swaisgood, & Catignani, 1981) and calculated using the following equations:

$$\mathrm{DH}(\%) = \frac{(N_h - N_0)}{(N_t - N_0)} \times 100 \quad (2)$$

$$\mathrm{DA, DC, DCL}(\%) = \frac{(N_0 - N_m)}{N_0} \times 100 \quad (3)$$

where N_h is the molar quantity of amino groups per gram of partially hydrolyzed protein, N_0 the molar quantity of amino groups per gram of non-modified protein, N_t the molar quantity of amino groups per gram of totally hydrolyzed protein and N_m the molar quantity of amino groups per gram of modified (acylated, cationized or cross-linked) protein. Analyses were performed in triplicate.

2.4. Preparation and characterization of microparticles

A two-step procedure was adopted to prepare protein-based microparticles. Firstly, an aqueous solution of protein (unmodified, hydrolyzed, acylated or cationized) was mixed with active core material (α-tocopherol (T)). Then, the oil-in-water emulsion obtained was spray-dried to give dry microparticles.

2.4.1. Emulsion preparation

The wall material was dissolved in deonized water (8% w/w) at 70 °C for 1 h under constant mechanical stirring (1000 rpm). In order to allow maximum protein solubilization, solution pH was fixed at 10.5 for SoyP and at 8.5 for SunP (except for the pH of the solution used for the cross-linking reaction which was fixed at 7.5). Active material (T) was then mixed with protein solution at the protein/active core ratio of 2/1 or 11.5% of total solids (the ratio of solid mass, i.e. protein and T, to total emulsion mass). The pre-emulsion obtained was stabilized using a high-pressure homogenization device (APV Systems, Albertslund, Denmark) at 50 MPa with double circulation through the homogenizer. Freshly homogenized emulsion was spray-dried or kept for the cross-linking reaction. Samples obtained with T were named SoyP/T (SoyP-H/T, SoyP-A/T, SoyP-C/T and SoyP-CL/T) and SunP/T (SunP-H/T, SunP-A/T, SunP-C/T and SunP-CL/T) for unmodified (hydrolyzed, acylated, cationized and cross-linked) soy and sunflower proteins, respectively.

2.4.2. Emulsion characterization

The analysis of oil droplet size distribution in homogenized oil-in-water emulsions was performed using dynamic light scattering (Zetasizer Nano-ZS, Malvern Instruments, Worcestershire, UK). Analyzed emulsions were diluted 100 times with deionized water before measurements in order to avoid multiple scattering effects. The relative refractive index used to analyze the data was $\eta_{oil}/\eta_{water} = 1.12$ ($\eta_{oil} = 1.49$, $\eta_{water} = 1.33$). The volume particle diameter (D_{43} or D_v), assuming that all droplets had spherical shape, was used as an indicator of the mean emulsion droplet size. Emulsions were also visualized using an Eclipse E600 optical microscope (Nikon, Sendai, Japan), linked to a digital video camera (DXM1200, Nikon, Sendai, Japan) at a magnification of 1000×, to check good dispersion of T droplets and their uniformity.

The analysis of apparent viscosity of obtained emulsions with Newtonian behavior, was performed at 20 °C with shear stress of 0–1 N/m^2 for 3 min using a CSL100 Rheometer (Carri-Med LTD, Dorking, UK) equipped with a plate-cone geometry (6 cm diameter, 0.035 rad cone angle).

2.4.3. Microparticle preparation

Emulsions containing proteins and T were spray-dried using a Mini Spray Dryer B-290 (Büchi, Flawil, Switzerland) under stable process conditions as follows: inlet air temperature at 124 ± 4 °C and outlet at 74 ± 4 °C, drying air flow rate of 470 L/h, liquid feed flow rate of 0.33 L/h and aspiration of 100%. Dry microparticles were taken from the container and stored in opaque and hermetic packaging at 4 °C. The spray-drying yield was calculated from the following equation:

$$\text{Spray-drying yield}(\%) = \frac{m_{Pow}}{m_{Prot+T}} \times 100 \quad (4)$$

where m_{Pow} is the mass of powder collected, and m_{Prot+T} the initial mass of solid content in emulsion including protein and T.

2.4.4. Microparticle characterization

The amount of active core (T) retained during spray-drying was evaluated using UV/VIS spectroscopy (Faria, Mignone, Montenegro, Mercadante, & Borsarelli, 2010). Briefly, about 5 mg of microspheres containing the T to be determined were dissolved in 10 mL of cyclohexane. The solution was stirred for 10 min and filtered through a 0.2 μm PTFE membrane filter. The absorbance of the solution was measured using a UV Spectrometer (UV-1800, Shimadzu, Kyoto, Japan) at 298 nm. The retention efficiency (RE) was defined as the percentage of estimated active core content in particles obtained (T_{exp}) over theoretical core content (T_{theo}).

$$RE(\%) = \frac{T_{exp}}{T_{theo}} \times 100 \quad (5)$$

The difference between experimental and theoretical values was caused by T loss during spray-drying. The loading efficiency (LE), corresponding to T content per 100 g of powder, was calculated as follows:

$$LE(\%) = \frac{m_{T_{exp}}}{m_m} \times 100 \quad (6)$$

where $m_{T_{exp}}$ is the estimated mass of core in microparticles, and m_m the mass of the analyzed sample of microparticles.

Microparticles obtained were characterized in the dry state for size distribution by laser diffractometry using the Scirocco 2000 equipment (Malvern Instruments, Worcestershire, UK). The volume particle diameter (D_{43} or D_v) was calculated as the mean of three measurements per sample. The morphology of the microparticles was examined by scanning electron microscopy (LEO435VP, Electron microscopy Ltd., Cambridge, UK). To see the internal structure, the microparticles were first broken up in liquid nitrogen using a mortar and the samples deposited on conductive double-sided adhesive tape and sputter-coated with silver. Both broken and intact microparticles were observed.

2.5. Statistical analysis

All statistical analyses were conducted using Minitab 16 software (State College, USA). Experimental data was studied using one-way analysis of variance (ANOVA). Statistical significance was accepted at the $P < 0.05$ level. The multiple comparison procedure used Tukey's test.

3. Results and discussions

3.1. Vegetable protein characterization

3.1.1. Composition

The analysis of the contents of protein extracts, was made in order to evaluate the effect of wall material composition on the microencapsulation process. From the results shown in Table 1, the content of crude protein in SoyP sample was higher than those in SunP sample (82.3% and 73.5%, respectively). This variation could be due to the isolation method, but also to the initial composition of soybean and sunflower seeds, although SoyP and SunP had similar contents of moisture, lipid and ash. However, a difference in polysaccharide fraction in SunP (15.9%) compared to SoyP (3.5%) was observed, and this higher SunP polysaccharide content resulted in the lower protein content found.

From the results obtained (Fig. 1), SoyP and SunP had comparable amino acid composition. Both proteins studied were composed mainly of 16 amino acids with four major ones, i.e. glutamic acid, aspartic acid, arginine and leucine which accounted for about 51–58%, and this composition is characteristic of vegetable proteins (Conde, Escobar, Pedroche, Rodriguez, & Rodriguez, 2005; Zamora, 2005).

Table 1
Composition of SoyP and SunP constituents.

Constituent	Content (w/w %)	
	SoyP	SunP
Crude protein	82.3 ± 1.6	73.5 ± 1.3
Moisture	8.0 ± 0.3	6.0 ± 0.2
Lipid	1.3 ± 0.1	1.6 ± 0.1
Ash	5.0 ± 0.3	3.0 ± 0.2
Polysaccharide	3.5	15.9

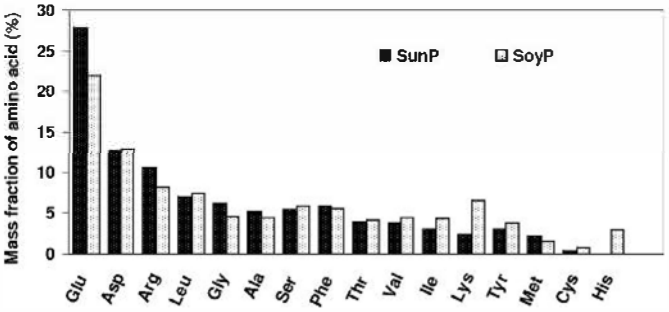

Fig. 1. Amino acid composition of SoyP and SunP obtained after total acid hydrolysis.

3.1.2. Solubility profiles and size distributions

Knowing the solubility profiles of protein-based products is important to evaluate their functional properties, in order to screen them for potential applications. Solubility of vegetable proteins is highly dependent upon the physico-chemical states of protein molecules, and in this study the effect of solution pH on protein solubility was studied (Fig. 2). The vegetable proteins had a U-shaped protein solubility pH profile, with the lowest solubility observed at their isoelectric point (pH 4–5) which agreed with literature data (Kabirullah & Wills, 1983; Lee, Ryu, & Rhee, 2003). The presence of glutamic and aspartic amino acids in large quantities could explain this position of the isoelectric point. Solubility reached a maximum at pH 9–10 for SunP and pH 11–12 for SoyP, corresponding to conditions where solvent/biopolymer interactions were optimal.

SoyP solubility was lower than that of SunP especially under acidic (pH 2) and neutral (pH 7–8) conditions, and this result could be related to the difference in composition of protein extracts (Table 1). The polysaccharide fraction was significantly higher in SunP compared to SoyP (15.9% and 3.5%, respectively), and it has been reported that in the presence of polysaccharides, the solubility of globular proteins is enhanced due to favorable protein/polysaccharide interactions (Guo & Narsimhan, 1991).

The size distributions of SoyP and SunP were studied using the AsFlFFF technique (Fig. 3). Fractograms obtained, showed that a major population of particles/molecules of Sun Poccurred at a retention time of 13–15 min. The same population can be observed

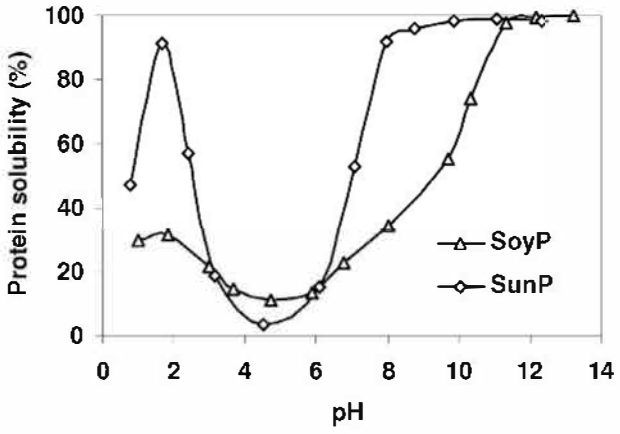

Fig. 2. Solubility profiles of SoyP and SunP as function of pH determined by the Kjeldahl method. The error bars are not visible because they are smaller than the points plotted.

for SoyP, but the major population for this protein extract was located at a retention time of 17–19 min. The R_h of SunP particles/molecules varied from 20 to 100 nm with the major fraction at 40–50 nm. In contrast, SoyP had higher R_h values ranging from 20 to 200 with the majority of particles/molecules at 130–180 nm. Using the recovery mass of samples, the fraction of small particles/molecules with a molecular weight equal to or less than 10 kDa, corresponding to non-fractionated particles/molecules passed through the membrane during analysis, was determined. This population varied from 8.3% to 20.5% for SoyP and SunP, respectively, confirming that SunP had lower particle/molecule sizes compared to SoyP, which might partially explain the higher solubility of SunP.

3.2. Vegetable protein modifications

The degrees of vegetable protein modifications are summarized in Table 2. The results obtained after SoyP and SunP modification by enzymes or chemicals, showed that two proteins had relatively comparable values concerning degree of modification after hydrolysis, acylation and cross-linking. However, the degree of cationization was significantly different for SoyP and SunP (92% and 67%, respectively), which could be related to the different protein conformation and NH_2 group accessibility (Matemu et al., 2011) under these experimental conditions.

Table 2
Modification degree of SoyP and SunP.

Sample name	Nature of protein modification	Degree of modification (%)
SoyP-H	Enzymatic hydrolysis with Alcalase	4 ± 1.0
SunP-H		5 ± 1.1
SoyP-A	Acylation with DDC	32 ± 0.8
SunP-A		40 ± 0.6
SoyP-CL	Enzymatic cross-linking with MTG	23 ± 0.7
SunP-CL		18 ± 1.9
SoyP-C	Cationization with GTMAC	92 ± 1.2
SoyP-C		67 ± 0.6

3.3. Microencapsulation with unmodified and modified vegetable proteins

3.3.1. Effect of protein modification on emulsion properties

All emulsions obtained with T from unmodified and modified protein solutions were characterized in terms of morphology and viscosity (Table 3 and Fig. 4).

As can be seen from Fig. 4, the morphology of emulsions varied for the different types of proteins used. The agglomeration of matrix chains around droplets was higher for SunP based emulsions and for SoyP-CL samples. Nevertheless, good, uniform dispersion of active core droplets was observed for all the emulsions. The presence of large size, cross-linked protein aggregates did not allow accurate measurement of emulsion oil droplet size and viscosity. No significant tendency, with droplet diameter changing after protein modifications (hydrolysis, acylation and cationization), was observed. The volume average diameter of emulsion droplets was close to 1–2 μm (Table 3). However, these modifications resulted in a fall in emulsion viscosity compared to emulsions with unmodified proteins (SoyP/T and SunP/T).

For protein enzymatic hydrolysis, the lower emulsion viscosity is simply due to the reduction of protein molecular chain length. Concerning acylation, the attachment of fatty acid chains to the protein enhanced its amphiphilic character and its surface-active properties, resulting in a decreased emulsion viscosity (Derkatch et al., 2007). Finally, cationization of proteins also resulted in increased amphiphilic properties due to grafting of polar groups. Moreover, the enhanced hydrophilic character favored the mobility of protein chains in water and, apparently, could contribute to the emulsion viscosity reduction.

3.3.2. Effect of protein modification on microparticle properties

The different microparticle characterizations are summarized in Table 3. The results obtained showed that unmodified protein-based wall materials were effective encapsulating agents with high RE (79.7–92.6%). This indicated that SoyP and SunP had the capacity to bind T droplets and keep them efficiently inside the microparticle matrix. Indeed, SunP was significantly more efficient for T encapsulation than SoyP. Microencapsulation of T with two protein extracts was carried out under the same experimental conditions, apart from the protein solution pH value. This difference in pH value might affect the protein/polysaccharide interactions and the microencapsulation efficiency. Nevertheless, a number of other factors, such as protein size distribution, amount of different protein fractions and presence of additives, could cause the difference between the two samples. For example, the significantly higher amount of polysaccharides in SunP compared to SoyP (15.9% and 3.5%, respectively). As the major polysaccharide fraction in sunflower meal is pectin (Marechal & Rigal, 1999), interactions

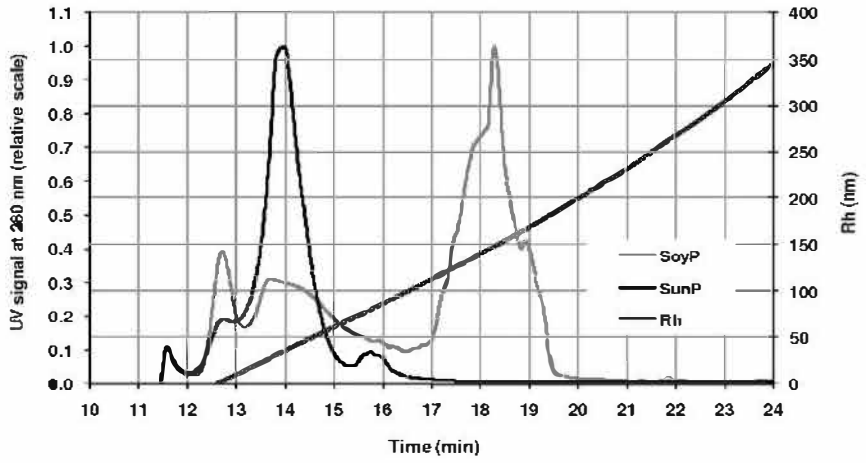

Fig. 3. Hydrodynamic radius and UV signal (280 nm) measured by AsFlFFF technique as a function of analysis time for SoyP and SunP samples (0.5% w/w solution with pH of 9.0).

Table 3
Properties of SoyP/T and SunP/T based emulsions and spray-dried microparticles.

Sample name[a]	Emulsion droplet size (μm)	Emulsion viscosity (mPa·s)	Spray-drying yield (%)	RE[b] (%)	LE[c] (%)	Particle size (μm)
SoyP/T	1.1 ± 0.02^{c}	15.0 ± 0.2^{b}	65	79.7 ± 1.0^{d}	26.3 ± 0.3^{c}	9.3 ± 0.1^{b}
SunP/T	1.3 ± 0.05^{b}	36.1 ± 0.4^{a}	70	92.6 ± 1.8^{bc}	30.5 ± 0.6^{b}	6.9 ± 0.03^{e}
SoyP-H/T	0.5 ± 0.03^{f}	3.6 ± 0.1^{h}	57	38.9 ± 2.4^{f}	12.8 ± 0.8^{f}	6.3 ± 0.1^{f}
SunP-H/T	1.7 ± 0.07^{a}	5.2 ± 0.3^{f}	67	80.2 ± 1.1^{d}	26.5 ± 0.4^{c}	6.9 ± 0.08^{e}
SoyP-A/T	0.7 ± 0.04^{e}	8.0 ± 0.2^{d}	62	94.8 ± 2.2^{ab}	31.3 ± 0.7^{ab}	7.7 ± 0.2^{cd}
SunP-A/T	0.8 ± 0.03^{de}	9.5 ± 0.2^{c}	70	99.5 ± 2.4^{a}	32.9 ± 0.8^{a}	7.7 ± 0.2^{cd}
SoyP-CL/T	n/d	n/d	69	78.3 ± 2.3^{d}	24.0 ± 0.7^{d}	10.6 ± 0.2^{a}
SunP-CL/T	n/d	n/d	56	87.6 ± 3.3^{c}	27.6 ± 1.1^{c}	8.1 ± 0.1^{c}
SoyP-C/T	0.9 ± 0.02^{d}	4.1 ± 0.3^{g}	61	38.3 ± 1.0^{f}	12.6 ± 0.3^{f}	7.6 ± 0.3^{d}
SoyP-C/T	1.1 ± 0.03^{c}	5.8 ± 0.2^{e}	63	60.1 ± 2.4^{e}	19.8 ± 0.7^{e}	7.3 ± 0.2de

[a–g] Different letters in the same column indicate a statistically difference between the mean values ($P < 0.05$).
[a] SoyP and SunP: non-modified soy and sunflower proteins; SoyP-H and SunP-H: hydrolyzed proteins; SoyP-A and SunP-A: acylated proteins; SoyP-CL and SunP-CL: cross-linked proteins; SoyP-C and SunP-C: cationized proteins; T: α-tocopherol.
[b] RE: retention efficiency determined by UV spectroscopy.
[c] LE: loading efficiency or α-tocopherol content per 100 g of powder.

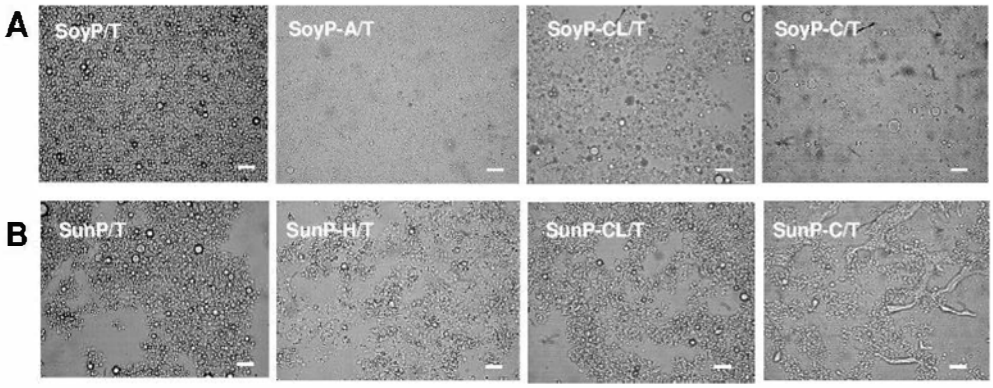

Fig. 4. Optical micrographs of oil-in-water emulsions obtained using high-pressure homogenization at 50 MPa with protein/T ratio of 2/1 (SoyP and SunP for unmodified proteins, SunP-H for hydrolyzed proteins, SoyP-A for acylated proteins, SoyP-CL and SunP-CL for cross-linked proteins, and SoyP-C and SunP-C cationized proteins). Scale bar — 10 μm.

occurring between this negatively charged molecule and protein chains, could affect the degree of active core protection and the RE value.

The combination of proteins with polysaccharides, depending on the nature of biopolymers and the degree of complexing, can result in enhanced oil-in-water emulsion stabilization (Liu, Elmer, Low, & Nickerson, 2010). Emulsion stability is favored by steric repulsive forces between droplets for a protein/polysaccharide stabilized interface. At relatively high polysaccharide contents (about 30%), this 'steric stabilization' can be further improved by the formation of a 'network-like' structure within a continuous phase.

It has been reported that in the case of a pea globulin/gum arabic stabilized emulsion, the increased hydrophobic nature of the protein/polysaccharide complex formed, involved the enhancement of the polymers' adsorption at the oil/water interface, compared to pea globulin alone (Ducel, Richard, Popineau, & Boury, 2005). Electrostatic attractive interactions between protein and polysaccharide alter the conformation of protein chains at the oil-water interface, and help strengthen the viscoelastic film protecting oil droplets (Martinez, Carrera Sanchez, Pizones Ruiz-Henestrosa, Rodriguez Patino, & Pilosof, 2007).

Thus, the combination of polysaccharides with proteins enhanced the protection of the hydrophobic active core in emulsion, but also improved the drying properties of the wall material. This resulted in faster formation of a dry crust around the drying droplets and reduced T losses during this drying (Gharsallaoui et al., 2007). The RE values indicated the effectiveness of a protein/polysaccharide-based wall system in the case of the SunP/T sample. Additionally, the difference in the protein chain size may also influence the retention efficiency of active core during spray-drying. The higher mobility of small protein chains in SunP explain the fast adsorption of proteins at the air-liquid interface of the drying droplets, and enhanced protection of the active core.

Microparticles prepared with hydrolyzed protein had reduced RE of active core compared to unmodified protein-based microparticles (from 79.7% to 38.9% for SoyP and from 92.6% to 80.2% for SunP). This can be explained by the fact that short protein chains of hydrolyzed proteins cannot efficiently enwrap the T droplets in emulsion, and thus their protection during drying was significantly lower.

The protein cationization also had a significant effect on the microencapsulation process. After grafting of cationic functions onto protein chains, the retention level of encapsulated T was reduced from 79.7% to 38.3% for SoyP and from 92.6% to 60.1% for SunP. These results could be related to the enhanced hydrophilic character of cationized proteins (Kiick-Fischer et al., 1998; Zohuriaan-Mehr et al., 2009), and thus the reduced affinity with hydrophobic T, with the repulsion forces between core and wall materials preventing efficient microencapsulation.

Conversely, the RE values of T were positively affected by protein acylation and reached maximum levels of 94.8—99.5%. The grafting of hydrophobic moieties to protein chains enhanced their hydrophobicity and affinity for the hydrophobic active core. During spray-drying, acylated proteins were efficiently adsorbed at the droplet surface, suggesting enhanced protection of active core inside the wall matrix (Nesterenko, Alric, Silvestre, & Durrieu, 2014).

The cross-linking of protein chains did not positively or negatively affect microencapsulation efficiency. The increase in

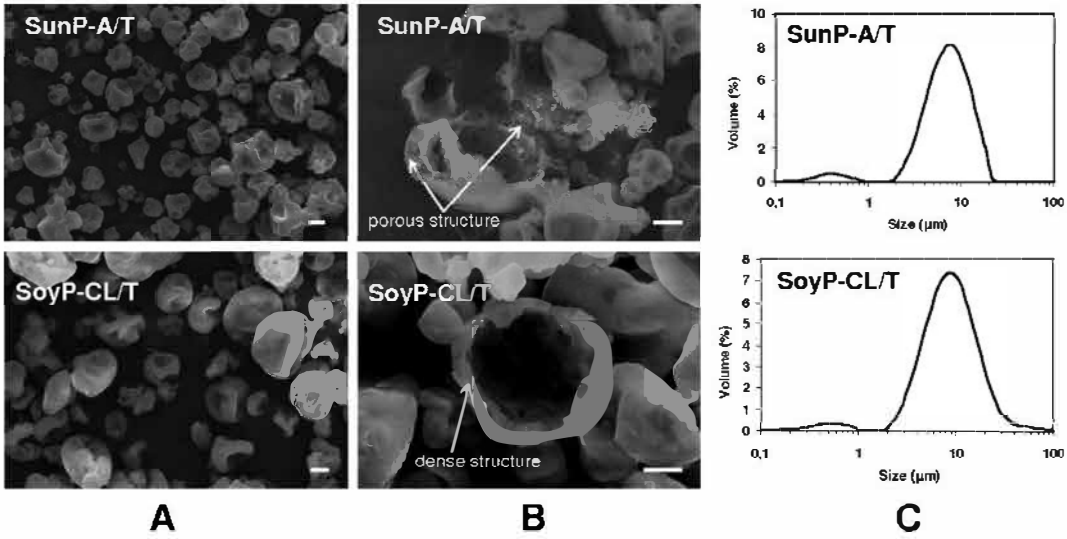

Fig. 5. Scanning electron micrographs ((A) external and (B) internal structures, scale bar – 2 μm), and (C) particle size distributions of SunP-A/T and SoyP-CL/T microparticles obtained by spray-drying technique.

microparticle size from 6.9 to 9.3 μm to 8.1–10.6 μm was observed after protein cross-linking, which confirmed the formation of protein agglomerates. From the results shown in Table 3, there was no significant effect of protein modification on spray-drying yield values.

The SEM micrographs of microparticles obtained (Fig. 5A and B) revealed the absence of fissures or disruptions, which is fundamental to guaranteeing higher protection of active core. Microparticles were of assorted sizes, between 1 and 12 μm, and those of Microparticles SunP-A/T exhibited inner morphology with a porous wall structure. Small pores were well distributed inside the protein matrix, indicating that proteins formed a protective thin layer membrane around encapsulated active core. Nevertheless, cross-linked SoyP-CL/T microparticles had a dense non-porous wall, which could be due to formation of a compact protein network after cross-linking.

The mean volume size distribution curves for the microparticles are shown in Fig. 5C. Particles had a size distribution with the mean diameter between 6.9 and 10.6 μm, and such values are to be expected for vegetable protein-based microparticles produced by spray-drying (Favaro-Trindade et al., 2010; Pierucci, Andrade, Farina, Pedrosa, & Rocha-Leao, 2007).

4. Conclusions

The present study describes the use of two types of proteins extracted from plant seeds, for the microencapsulation of a hydrophobic active core using the spray-drying technique. In the unmodified state, sunflower proteins (SunP) showed a significantly higher efficiency level for α-tocopherol (T) encapsulation (92.6%) compared to 79.7% obtained with soy proteins (SoyP). This difference can be due to various factors including protein size distribution, amount of different protein fractions and presence of additives. After protein modifications, the interactions occurring between wall material and active core also altered the retention efficiency values. Enzymatic hydrolysis and cationization of SoyP and SunP resulted in reduced microencapsulation efficiency of T. Conversely, protein acylation led to enhanced affinity between core and wall and thus increased retention efficiency. Findings from this paper may lead to efficient microencapsulation of hydrophobic cores using vegetable proteins. Further research is in progress to study the effect of protein modifications on release properties of microparticles.

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