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The effect of vegetable protein modifications on the microencapsulation process

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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

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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–