Food Hydrocolloids 41 (2014) 119–131

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Interaction of polyphenols and multilayered liposomal-encapsulated grape seed extract with native and heat-treated proteins

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ARTICLE INFO

Article history:
Received 2 November 2013
Accepted 19 March 2014
Available online 5 April 2014

Keywords:
Grape seed extract
Polyphenols
Precipitation
Multilayering
Liposomes
Proteins

ABSTRACT

Soy lecithin liposomes with incorporated grape seed extract (GSE) were prepared by high-pressure homogenization and coated with chitosan as a first layer and pectin as a second layer. The GSE and the uncoated and coated liposomes were mixed with native and heat-treated protein solutions of bovine serum albumin (BSA), whey protein isolate (WPI) and sodium caseinate (Na-caseinate) to investigate their interactions. Uncoated liposomes without GSE had the smallest particle size (34 nm); when GSE (0.1% w/w) was entrapped into liposomes; its size increased three-fold (102 nm). The particle sizes of chitosan-coated liposomes were 80 nm and 197 nm containing GSE as well as of pectin-chitosan-coated liposomes 227 nm and 157 nm, respectively. The ζ-potential changed from −23 over +49 to −31 mV (liposomes) and −26 over 46 to −29 mV after second layering (GSE-liposomes). Prior to centrifugation, the particle diameters of the chitosan-coated liposomes were smaller than in the other samples containing GSE and the ζ-potentials were the highest of all liposome−protein mixtures (~45 mV). After centrifugation, the polyphenols and proteins could be recovered in the supernatant, which proved that chitosan-coated liposomes have reduced precipitation properties. Additionally, only small protein concentrations too low for determination with SDS-PAGE electrophoresis were found in the residues. Na-caseinate, however, was precipitated by the positively charged chitosan-coated liposomes due to more hydrophobic forces. GSE and negatively charged liposomes (uncoated liposomes and chitosan-pectin-coated liposomes) precipitated and sedimented the proteins. This was demonstrated by increased particle sizes and reduced protein contents in the supernatants.

1. Introduction

Polyphenols from grape seed extract (GSE) have antioxidative capabilities and are able to protect cell components from oxidative stress (Scalbert, Manach, Morand, Remesy, & Jimenez, 2005). This stress is responsible for degenerative diseases, such as cancer, cardiovascular disease or osteoporosis (Scalbert et al., 2005). One disadvantage of these polyphenolic compounds is their capability to precipitate proteins and other food ingredients. Firstly, this effect can reduce the nutrient content of food products (Shi, Yu, Pohorly, & Kakuda, 2003). Secondly, due to interactions with proteins and mucopolysaccharides in the mouth, polyphenols cause an astringent sensation when they are consumed (Baxter, Lilley, Haslam, & Williamson, 1997). There is a great interest in the incorporation of plant polyphenols in food and beverages (Ferruzzi, Bordenave, & Hamaker, 2012). Due to the interactions of plant polyphenols with proteins, which can occur both in food products and in the oral cavity, their application forms need to be optimized in order to prevent undesired sedimentation and increase the bioavailability of the antioxidants (Ferruzzi et al., 2012).

Polyphenolic compounds bind non-covalently to proteins (Ferruzzi et al., 2012) which results in an irreversible precipitation of insoluble and soluble complexes (Hagerman & Butler, 1981). These interactions arise from hydrophobic interactions and hydrogen bonds at the phenolic hydroxyl groups (Baxter et al., 1997). The authors proved that the hydrophobic interactions were the main force which caused non-specific peptide/polyphenol bonds. The association between select polyphenols and salivary proline-rich proteins in the mouth results in conformational changes in protein structure, reduced solubility, and ultimately precipitation of salivary proteins in the oral cavity that creates a loss of oral lubrication as well as an astringent sensation occurs (Ferruzzi et al., 2012). An unpleasant astringent taste, as well as precipitates in products may be unappealing for consumers.

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http://dx.doi.org/10.1016/j.foodhyd.2014.03.024
0268-005X/

The type of polyphenols such as proanthocyanidin polymers, the size of polymer and the size, conformation and charge of a protein determine their affinity for one another (Hagerman & Butler, 1981). Small peptides with less than six residues and tightly coiled globularly arranged proteins have a low affinity for tannins, because multiple binding sites in protein molecules are necessary in order to have precipitation (Hagerman & Butler, 1981). A looser protein conformation enabled the tannins to bind at several sites (Hagerman & Butler, 1981). Proteins with a high content of proline and those with a random coil or helix structure had a high affinity for tannins (Hagerman & Butler, 1981; Jöbstl, O'Connell, Fairclough, & Williamson, 2004). Due to proline, the proteins are hydrogen bond acceptors as the nitrogen lies near the carbonyl group (Hagerman & Butler, 1981). This causes strong hydrogen bonds in tannin–protein complexes.

Additionally, the interactions occur between liposomes and proteins which are influenced by the surface charge, the hydrophobicity and the rheological characteristics of liposomes and proteins (Taylor, Davidson, Bruce, & Weiss, 2005). Proteins are able to adsorb liposomes and destabilize the membrane which results in leakage of encapsulated material (Dimitrova & Matsumura, 1997; Tantipolphan, Rades, McQuillan, & Medlicott, 2007). Bridges between proteins and liposomes formed as opposite charges are attracted to each other (Dimitrova, Tsekov, Matsumura, & Furusawa, 2000). In addition to electrostatic attraction, hydrophobic and van der Waals' attractions were also involved in this process, and finally, flocculation may occur due to an increase of the particle sizes of the aggregates of liposomes and proteins (Dimitrova et al., 2000). The interactions between proteins and lecithin in a dispersed system may lead to a decrease in surface activity, conformation changes, such as the unfolding of the tertiary structure of proteins, enhanced molecular flexibility, alterations in viscoelasticity, and the net charge and incorporation of protein into surfactant micelles and vesicles (van Nieuwenhuyzen & Tomás, 2008). Recent studies have shown that liposomes are particularly well suited to incorporate polyphenols of GSE with high mean encapsulation efficiency up to 83.5% and 87%, respectively (Gibis, Rahn, & Weiss, 2013; Gibis, Vogt, & Weiss, 2012). In this connection, the most part of polyphenols was entrapped in bilayer membrane and after the multilayering with chitosan and pectin, a loading capacity of GSE polyphenols was found of 91% for the chitosan-coated and 61% for the pectin chitosan coated liposomes, respectively (Gibis et al., 2012, 2013).

The objective of this study was to investigate the interactions of a polyphenolic plant extract, uncoated and coated soy lecithin liposomes containing GSE, with several food proteins. Native and heat-treated (denatured) states of bovine serum albumin, whey protein isolate and sodium caseinate (Na-caseinate) which have different sizes, molecular weights, structures, and characteristics were used in the experiments. For practical applications in industry, there is a huge interest for developing dairy beverages in particular whey beverages, yoghurt drinks or milk shakes containing fruit juice from concentrate up to 30% with very low pH values (3.5–4.5). One problem is the high aggregation affinity in connection with whey protein or caseinate in preparation of these products at low pHs and high content of polyphenols. Among others, Na-caseinate possesses strong hydrophobic properties due to its non-polar residues (Dickinson, 1999). As a working hypothesis, we postulate that coated liposomes with the same surface charge show reduced interaction or no interaction at all and inhibit the extensive precipitations of the proteins. The principles of the potential interactions depending on the electrostatic surface charges are schematically presented in Fig. 1. At a pH value of 3.60, the negatively charged extract, lecithin liposomes and chitosan-pectin coated liposomes should precipitate with all proteins due to opposite charges. A positively charged chitosan coating should prevent interactions with the similarly charged proteins.

2. Materials and methods

2.1. Materials

Acetic acid glacial (100%, p.a.), bovine serum albumin (BSA, fraction V, ≥98%), Coomassie Brilliant Blue G-250, hydrochloric acid (32%, p.a. ISO), methanol (≥99.9%, HPLC Grade), pre-stained protein marker and sodium lauryl sulfate (SDS, ≥99%) were obtained from Carl Roth GmbH & Co. KG (Karlsruhe, Germany). 2-Mercaptoethanol (for electrophoresis, ≥98%), ferric chloride

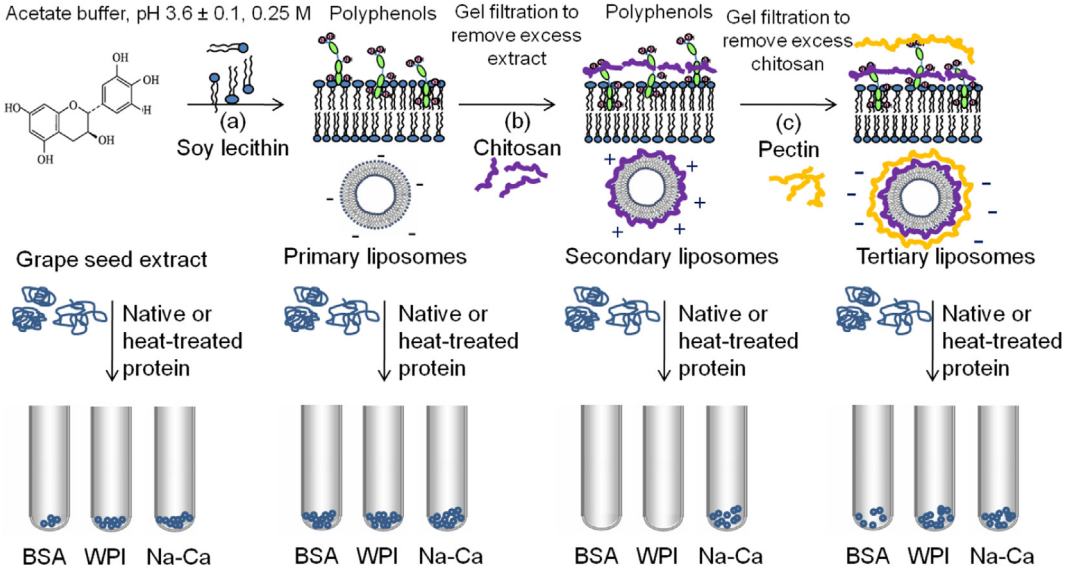

Fig. 1. Schematic overview of possible interactions between GSE polyphenols and liposomes containing GSE (0.1% w/w) and various proteins due to their electrostatic surface charges, (a): 1% w/w lecithin, high-pressure homogenization, 22,500 psi, five passes, primary liposomes (b): Secondary liposomes coated with chitosan, (c): Tertiary liposomes coated with chitosan-pectin, BSA: Bovine serum albumin, WPI: Whey protein isolate, Na–Ca: Na-caseinate, all proteins in native and heat-treated (denatured) states (80 °C for 6000 s, BSA: molten globule state 80 °C for 600 s), modified (Gibis et al., 2012).