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Formation, characterization, and stability of encapsulated hibiscus extract in multilayered liposomes

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ABSTRACT

Transparent liposomes (1, 2 and 5% w/w soy lecithin) containing anthocyanin-rich hibiscus extract (*Hibiscus sabdariffa*) (0.2, 0.4 and 0.8% w/v) were prepared by high pressure homogenization (microfluidization) at 22,500 psi in acetate buffer (0.25 mol/L, pH 3.5). Liposomes containing extract had mean particle diameters of less than 46 nm, similar to control liposomes (32–46 nm). The encapsulated amount of hibiscus extract in liposomes was analyzed between 63 and 72% w/v. Transparent liposomes containing hibiscus extract were oxidatively stable over a period of 145 days, i.e. significantly less hexanal (<90 μmol/L) was generated in comparison to liposomes containing no extract (>1100 μmol/L). A layer-by-layer electrostatic deposition method was used to prepare multilayered liposomes with interfacial membranes consisting of chitosan and pectin. *z*-Average particle diameter of control liposomes increased to 65 nm after chitosan coating. After deposition of the second layer pectin, the *z*-average particle diameter increased to approximately 200 nm. The ζ-potentials of the different liposomes changed from −26 mV to 70 mV for the chitosan coating and to approximately −20 mV for the second pectin coating. The highest lecithin concentration of liposomes containing hibiscus extract coated with pectin formed aggregation and was physically unstable. All coated liposomes containing hibiscus extract had higher particle diameters compared to control liposomes. These multilayered liposomes were physically stable for about 30 days. Our results, thus, demonstrate that multilayered polymer coatings of liposomes are highly competent carrier systems for anthocyanins. Moreover, the incorporation of hibiscus extract reduced the fat oxidation in liposomal systems.

1. Introduction

Commercially available lecithin from natural sources, such as chicken egg yolk or soybeans, is classified as a food ingredient which is generally recognized as safe (GRAS), because it is biodegradable, biocompatible and nontoxic. Pure lecithin as an emulsifier is lipid soluble and can form either water-in-oil or oil-in-water emulsion (Nieuwenhuyzen van & Tomas, 2008; Ogawa, Decker, & McClements, 2003). Lecithin is mainly used in the food industry due to its emulsification properties (Nieuwenhuyzen van & Tomas, 2008). Soy lecithin is a mixture of phosphatidylcholine, -ethanolamine and -inositol as the main components. Liposomes are formed when phospholipids are dispersed in water (Mozafari, Johnson, Hatziantoniou, & Demetzos, 2008). Liposomes are spherical, single or multiple layer lipid vesicles. They can be classified according to their size or structure. These spherical vesicles that contain only a single bilayer membrane are known as small (<30 nm; Takahashi et al., 2006) or large (30–100 nm) unilamellar vesicles (Mozafari et al., 2008; Taylor, Davidson, Bruce, & Weiss, 2005), respectively. Liposomes that contain more than a single bilayer membrane are called multilamellar vesicles (Albasarah, Somavarapu, Stapleton, & Taylor, 2010; Taylor et al., 2005). In general, liposomes are useful microscopic carriers for nutrients and have a great potential for applications in both food and pharmaceutical industries (McClements & Li, 2010; Taylor et al., 2005). Due to their ability to simulate the behavior of natural cell membranes, liposomes have been recognized as a powerful tool in the treatment of diseases by the pharmaceutical industry (Nieuwenhuyzen van & Tomas, 2008; Takahashi et al., 2006; Taylor et al., 2005). They are used as drug delivery vesicles and for medical applications, such as in anticancer therapy (Kijima, Phung, Hur, Kwok, & Chen, 2006), vaccination, gene therapy (Mohammadabadi, El-Tamimy, Gianello, & Mozafari, 2009; Taylor et al., 2005), and diagnostics (Lasic, 1998; Lu, Nielsen, Timm-Heinrich, & Jacobsen, 2011). A liposomal dispersion is extremely biocompatible, which results in a fast ingestion of drugs (McClements, 2010). They can entrap

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hydrophobic as well as hydrophilic compounds within their structure. Furthermore, liposomes can protect the incorporated compounds in their aqueous interior core or within their bilayer membrane from external destructive conditions such as light, pH or enzymes, allowing them to be released at designated targets (Shehata, Ogawara, Higaki, & Kimura, 2008). However, liposomes often rapidly destabilize due to highly flexible and fragile bilayer membranes. In addition, unsaturated fatty acids, as parts of membrane structure, may undergo oxidation processes leading to the formation of hydroperoxides which further break down in typical fat degradation by-products, such as malondialdehyde, hexanal and other very rancid olfactory substances (Panya et al., 2010).

Extracts of hibiscus (*Hibiscus sabdariffa*) contain a high amount of polyphenolic compounds, such as anthocyanins, protocatechuic acid and other polyphenols exhibiting significantly reducing potential (Sayago-Ayerdi, Arranz, Serrano, & Gontili, 2007). These polyphenolic compounds can trap reactive oxygen species in the interstitial fluid of the arterial wall or plasma and they probably inhibit low density lipoprotein (LDL) oxidation, therefore, suppressing an atherosclerosis onset (Hou, Zhou, Yang, & Liu, 2004). Hibiscus extract is supposed to have a number of positively relevant effects on lipid metabolism, antihypertensive activity and apoptosis (Kumar, Kumar, & Prakash, 2008; Sayago-Ayerdi et al., 2007) leading to its use in pharmacological mixtures and teas. Moreover, a free radical scavenging activity against hydroxyl, superoxide and hydrogen peroxide radicals, and reduced nitric oxide formation was observed in foods (Kumar et al., 2008). Flowers of *H. sabdariffa* are widely used in Latin America as beverages and 66% of the total extractable polyphenolic compounds present in the hibiscus flower are known to have a high antioxidant capacity (Sayago-Ayerdi et al., 2007). Both the inhibition of the formation of heterocyclic amines as an additive in marinades (Gibis & Weiss, 2010) and an antimutagenic effect was described for colon carcinogens such as heterocyclic amines and azoxymethane in the *Salmonella* mutation assay (Chewonarin et al., 1999).

A major challenge for scientists is the design of encapsulated and delivery systems that can resist the harsh conditions existing in the stomach and upper gastrointestinal tract to provide an effective target in the colon (McClements & Decker, 2005; McClements & Li, 2010). Therefore, a variety of polysaccharides can be used as protective coatings in the acidic milieu of the stomach as well as triggered release systems in the colon, such as pectin (Sriamornsak et al., 2008), chitosan (Henriksen, Smistad, & Karlsen, 1994; Laye, McClements, & Weiss, 2008; Weiss, McClements, & Decker, 2009) or combined pectin/chitosan coatings (Ghaffari et al., 2008; Ghaffari, Oskoui, Helali, Bayati, & Rafiee-Tehrani, 2006; Gibis, Vogt, & Weiss, 2012; Ogawa, Decker, & McClements, 2004). The human colonic bacteria produce enzymes which can break down such undigested polysaccharides (Aoki, Decker, & McClements, 2004; McClements & Decker, 2005; McClements & Li, 2010).

Liposomes have been investigated for their ability to protect and deliver water-soluble functional compounds in foods. The objective of the present study was to encapsulate hibiscus extract into uncoated and coated liposomes. For this purpose, we studied the formation of encapsulated anthocyanin-rich hibiscus extract in primary (lecithin), secondary (chitosan-coated) and tertiary (chitosan–pectin-coated) liposomes. We hypothesized that the encapsulation of hibiscus extract may prevent the fat oxidations of primary liposomes. Furthermore, the formation of resistant multilamellar coatings may stabilize the liposomes against time-induced aggregation. For this reason, the possibility of using chitosan and pectin as feasible layer materials was investigated.

2. Materials and methods

2.1. Materials

The plant extract (*H. sabdariffa*) was provided from Nature Network, Martin Bauer Group, Plantextrakt GmbH & Co. KG (Vestenbergsgreuth, Germany). This extract was produced by spray-drying a water extract of hibiscus flowers. Acetic acid (purity $\geq$ 99.7%), anhydrous sodium acetate, triton X 100 (laboratory grade), sephadex G-50, and hexanal (purity $\geq$ 97%) were obtained from Sigma–Aldrich (St. Louis, MO, USA). The dialysis membrane Spectra/Por® 7 (MWCO 50,000) was applied from Carl Roth AG (Arlesheim, Germany). The soy lecithin used was Lipoid S75 from Lipoid (Ludwigshafen, Germany). The contents of phosphatidylcholine, phosphatidylethanolamine and lysophosphatidylcholine were 69.3, 9.8 and 2.1%, respectively, as well as a fatty acid composition containing palmitic (17–20%), stearic (2–5%) oleic (8–12%) linoleic (58–65%), and linolenic (4–6%) acid, according to the company's specifications. The concentration of antioxidant DL-α-tocopherol was 0.18% (company's specification). Chitosan with a degree of esterification of 79% and medium molecular weight (Sigma–Aldrich, St. Louis, MO, USA) was applied. Pectin was provided from Herbstreith & Fox KG (Neuenbürg, Germany) with a degree of esterification of 55% (Betapec RU 301). The capillary gas column HP-FFAP (30 m, ID 0.32 mm, 0.25 μm) was used from Agilent Technologies (Waldbronn, Germany) and a Varian gas chromatograph GC CP 3800 (Varian Instrument Group, Walnut Creek, CA, USA) connected with autoinjector QHSS 40 (QUMA, Wuppertal, Germany). The following equipment was also used: a light microscopy Axio Scope (Axio Lab.A1, Carl Zeiss Jena GmbH, Jena, Germany) with Axio Vision Software (Carl Zeiss MicroImaging GmbH, Jena, Germany), Zetasizer Nano ZS (Malvern Instruments, Worcestershire, UK), a UV–Vis spectral-photometer HP 8453 (Agilent Technologies, Waldbronn, Germany), a Vortex mixer (Scientific Industries Inc., New York, USA), a high-shear disperser Ultra-Turrax (Janke & Kunkel, Staufen, Germany), and a microfluidizer M110 EH-30 as homogenizer (microfluidics, Newton, USA).

2.2. Preparation of hibiscus extract solutions

Spray-dried hibiscus extract was used at concentrations of 0.2, 0.4 and 0.8% w/v. For this purpose, the water-soluble extract was dissolved in acetate buffer. The acetate buffer (pH 3.5 ± 0.1; 0.25 mol/L) was prepared by using 14.21 g/L acetic acid and 1.81 g/L anhydrous sodium acetate. The antioxidative capacity of the extract concentrations used was analyzed in a study published earlier (Gibis & Weiss, 2010). Each concentration of dissolved extract was adjusted to pH 3.5 with sodium hydroxide (0.1 mol/L).

2.3. Preparation of primary liposomes

Soy lecithin at concentrations of 1, 2 and 5% w/w dissolved in acetate buffer (0.25 mol/L, pH 3.5) or in hibiscus extract solutions with a concentration of 0.2, 0.4 and 0.8% w/v, respectively, was pre-emulsified by using a high-shear disperser with the rotational speed of 24,000 rpm. The liposomes were then passed five times through a microfluidizer at a pressure of 22,500 psi. The diamond interaction chamber was cooled during homogenization using an ice water bath to prevent overheating of the samples.

2.4. Preparation of multilayered liposomes

2.4.1. Preparation of polymer solutions

For the coating, 1% w/w each of chitosan and pectin solutions was prepared in acetate buffer (0.25 mol/L, pH 3.5 ± 0.1). The chitosan and pectin solutions were placed in an ultrasound bath and