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Colour stability of lutein esters in liquid and spray dried delivery systems based on Quillaja saponins

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ABSTRACT

Aim of the present study was to expand the existing knowledge on the functionality of Quillaja saponin extract by comparing micellar systems, nanoemulsions and the corresponding spray-dried formulations for the delivery of lutein esters as colouring agents to foods. A chemically well-defined saponin extract was used, and characterised by MALDI-TOF-MS and HPTLC-MALDI-TOF-MS. The composition of the extract comprised all major saponins described for Quillaja, but the relation between the individual constituents differed considerably from the literature. Colour intensity of lutein ester loaded systems was higher in nanoemulsions compared to micelles. Quillaja saponins provided good stability to lutein ester loaded systems during the process of microencapsulation by spray drying, as indicated by particle size analysis of the dispersed phases and colour determination. Colour stability upon storage was high in microencapsulated formulations. Results therefore prove the high functionality of Quillaja saponins for stabilisation of sensitive, natural food colourants.

1. Introduction

Colour is an important parameter to assess the quality of food. Considering the growing consumer demand for natural food ingredients, oil-soluble carotenoids receive increasing attention concerning their colouring and health-promoting properties. Natural food colourants and bioactive compounds are frequently isolated from plant materials. Consequently, when incorporating these compounds in foods, two important issues need to be considered: The isolated components may possess a poor solubility in the aqueous environment of the food matrix accompanied by their susceptibility to adverse environmental conditions, e.g. light and oxygen (Kuang et al., 2015). Lutein esters are easily degraded during processing and storage (Subagio, Shigemura, & Morita, 2001; Timberlake & Henry, 1986) and their degradation lowers the final product quality in terms of nutritional properties as well as their colour (Gharsallaoui, Roudaut, Chambin, Voilley, & Saurel, 2007). As a consequence colour is a well-suited parameter for monitoring oxidation processes (Antolovich, Prenzler, Patsalides, McDonald, & Robards, 2002).

To overcome solubility issues, micellar systems and nanoemulsions may serve as formulations to incorporate oil-soluble bioactive compounds in an aqueous environment. In a recent study, lutein esters were successfully solubilised via micelles based on saponins from the Chilean soapbark tree *Quillaja saponaria* Molina (Tippel, Lehmann, von Klitzing, & Drusch, 2016). Micellar systems are transparent and thus may be used for colouring clear non-alcoholic beverages, but are limited in their loading capacity. Nanoemulsions represent an alternative system allowing an increased carotenoid load. With respect to stabilisation against degradation, microencapsulation by spray drying is an important and common method, in which carotenoids are entrapped in a glassy carbohydrate-based matrix with very limited oxygen diffusion (Drusch et al., 2009; Subramaniam et al., 2013). The multitude of research articles concerning the microencapsulation of carotenoids shows the importance of this research (Kuang et al., 2015; Liang, Huang, Ma, Shoemaker, & Zhong, 2013; Wang et al., 2012). Many variables affect the functionality and stability of microcapsules prepared by spray drying. Apart from process parameters and the bulk carrier constituent also the type of emulsifier plays an important role to achieve high stability of the encapsulated material (Drusch, Regier, & Bruhn, 2012).

As the demand for natural surfactants increases this study focused on the use of Quillaja saponins (QS) as emulsifiers for carotenoid encapsulation. QS are extracted from the Chilean soapbark tree (*Quillaja saponaria* Molina). QS extracts comprise more than 100 triterpenoid saponins (Kite, Howes, & Simmonds, 2004). The surface-active properties derive from their molecular structure. Commonly saponins consist of a hydrophobic triterpenoid with hydrophilic sugar moieties attached at two positions. A characteristic of QS compared to saponins derived from other botanical origin is an acetyl group or fatty acyl chain acetylated to the fucose, a first sugar unit at C28 and an aldehyde group at

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position C4 (van Setten & van de Werken, 1996). However, the diversity of the chemical structures in a complex mixture such as a saponin-containing extract causes differences in functional, i.e. physicochemical properties. This becomes obvious, when comparing saponin extracts from different botanical origins as recently reported by Böttcher and Drusch (2015) with respect to the foaming properties of saponin extracts. Systematic data for various saponin extracts related to composition and functional properties is scarce and the majority of the available studies mentioned above does not report the composition of the saponin extract used. As a consequence, the correlation between saponin composition and solubilising properties is still poorly understood.

So far QS are described as powerful emulsifiers that can be used at very low concentrations to prepare emulsions containing orange oil for spray drying (Subramaniam et al., 2013). However, Subramaniam et al. (2013) focused on the effect of the dextrose equivalent of maltodextrin on the stability of the encapsulated oil, but did not investigate the process of spray drying. Until now there has been no publication concerning the physical characterisation of saponin-stabilised dispersions during the multiple steps during the spray drying process. Atomisation goes along with mechanical stress in the system and the increase in surface area due to droplet formation may lead to relocation of surfactant molecules affecting the interfacial characteristics. Particularly, for micellar solutions it might be expected that a change in surfactant concentration facilitates a disaggregation of the micelles (Dhara & Shah, 2001) or a structural change (Daful, Avalos, & Mackie, 2012) resulting in loss of solubilised material. Also compositional changes in the drying droplet due to water evaporation may affect the physical integrity of the dispersed system.

Thus, aim of the present study is to provide an integrated approach, in which the composition of the saponin extract from the Chilean soap bark tree (*Q. saponaria* Molina) is linked to data on the functionality concerning the solubilisation of lutein ester extract into micelles and nanoemulsions. Expecting future studies on saponins from different botanical origins or manufacturers, consolidated findings may allow an in-depth understanding of the functionality of various saponins depending on their chemical structure. In order to identify saponins in the Quillaja extract, matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF-MS) as well as the coupling with high-performance thin-layer chromatography (HPTLC) are applied. Investigations concerning the functionality include monitoring of the physical and chemical stability of lutein ester-loaded saponin micelles and nanoemulsions during atomisation, spray drying and storage.

2. Material and methods

2.1. Material

Quillaja saponin (QS) extract was obtained from Ingredion Germany GmbH (Hamburg, Germany). The QS extract contained about 65% saponin on dry basis. Lutein OSC 350 (3.25 wt.% lutein ester) was a kind gift from Christian Hansen A/S (Hørsholm, Denmark). Spray dried glucose syrup with a dextrose equivalent of 38 (C*Dry GL 01934) was obtained from Cargill (Krefeld, Germany). Ammonium sulphate was purchased from AppliChem Chemie GmbH (Steinheim, Germany), ammonia (≥25% p.a.) from Carl Roth GmbH & Co. KG (Karlsruhe, Germany), acetic acid (p.a.) and sulphuric acid (≥95%) from Th. Geyer GmbH & Co. KG (Renningen, Germany). Hydrochloric acid (37%) was purchased from Grüssing GmbH (Filsum, Germany) and *p*-anisaldehyde (4-methoxybeanzaldehyde) from Merck KGaA (Hohenbrunn, Germany) respectively. 2,5-Dihydroxybenzoic acid (DHB) was used as matrix for (HPTLC-)MALDI-TOF-MS analysis and was provided by Bruker Daltonik GmbH (Bremen, Germany). The standard substance soyasaponin I (Glycine max, ≥95%) was acquired by Sigma-Aldrich Chemie GmbH (Steinheim, Germany). The polypropylene columns (Chromabond C18, 500 mg/6 mL) were obtained from Macherey-Nagel GmbH & Co. KG (Düren, Germany). Chromatographic analysis of saponin-rich fractions was performed on 10 × 10 cm silica gel 60 aluminium and/or glass plates (Merck KGaA, Darmstadt, Germany). All solvents were of analytical grade and water was double distilled (double-distilled H_2O).

2.2. Methods

2.2.1. Extraction and purification of saponins from the QS extract for chemical characterisation of saponins

The commercial extract (3 mL) was mixed with 0.4 M ammonium sulphate (1/2, v/v) and left on an orbital shaker overnight to precipitate non-saponin constituents. After centrifugation (3225 ×*g*, 30 min) the supernatant was evaporated to dryness under nitrogen. For further purification and concentration of saponins a solid phase extraction (SPE) was conducted. For this purpose Chromabond C18 SPE columns were preconditioned with methanol (6 mL) and equilibrated with double-distilled H_2O (6 mL). The dried samples were redissolved in 6 mL double-distilled H_2O and loaded on the adsorbent material. Polar compounds, i.e. carbohydrates were washed off by 3 mL of MeOH/double-distilled H_2O (5/95, v/v). Subsequently, saponins were eluted with an ascending methanol–water gradient at alkaline conditions, prepared using a 17% ammonia solution as described elsewhere with slight modifications (Guo & Kenne, 2000a; Reim & Rohn, 2014). In sum, five fractions (I–V) were collected and dried under a stream of nitrogen and dissolved in 1 mL of the same eluent used for SPE before.

2.2.2. Chemical characterisation of saponins by mass spectrometry

2.2.2.1. Coupling of high-performance thin-layer chromatography with matrix-assisted laser desorption ionisation time-of-flight mass spectrometry. The saponin fractions were analysed using a coupling of HPTLC with MALDI-TOF-MS. The combination of HPTLC with MALDI-TOF-MS provides a first assessment of the composition of saponins in the extract. Briefly, 10–25 μL of sample were applied as 6 mm bands under a flow of nitrogen in an autosampler (ATS4, CAMAG AG, Muttenz, Switzerland) on HPTLC Silica gel 60 F254 MS-grade aluminium sheets (7.5 × 5.0 cm, Merck KGaA, Darmstadt, Germany). The mobile phase used for chromatographic separation was chloroform/acetic acid/methanol/double-distilled H_2O (6.3/3.2/1.2/0.8; v/v/v/v) (Wagner & Bladt, 1996). The development of the plates was carried out in twin-trough chambers until a solvent front of 70 mm was reached. Excess solvent was evaporated overnight (12 h). Development and derivatisation were done in duplicate. One plate served as template, which was immersed in a *p*-anisaldehyde sulphuric acid staining solution (2 s). Subsequently, the plate was dried for 10 min at room temperature and heated at 70 °C for 5 min. Detection was carried out using a photodocumentation system (TLC visualizer, CAMAG AG, Muttenz, Switzerland) at ultraviolet light (366 nm) and white light. The second non-derivatised plate was covered with a matrix-solution consisting of 2,5-dihydrobenzoic acid (DHB) (c = 200 mg/mL in acetonitrile/dH_2O (90/10, v/v) with 0.1% trifluoroacetic acid (30/70, v/v; TA30) and 10 mM ammonium dihydrogen phosphate). The plate was immersed in the matrix solution (DHB solution) for 1 s and dried for 90 s under the airstream of a blow-dryer. Dipping was repeated and the plate was dried for another 4 min. The plate was stored in a vacuum oven at 60 °C ensuring complete desiccation of the matrix coating. The measurement was performed with a MALDI-TOF/TOF mass spectrometer (ultrafleXtreme, Bruker Daltonik GmbH, Bremen, Germany) in positive reflector ion mode within a mass range of 700-3200 Da. External calibration was done with digested β-lactoglobulin (Sigma-Aldrich Chemie GmbH, Steinheim, Germany). Spectra were acquired by taking shots of 0.7 mm per step for all sample lanes with a laser intensity of 37–40%. Seven individual shots were summed up and evaluation of the data achieved was performed with the specified software flexAnalysis v. 3.3 (Bruker Daltonik GmbH, Bremen, Germany).