Food Chemistry 199 (2016) 667–674

Contents lists available at ScienceDirect

Food Chemistry

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

β-Lactoglobulin as nanotransporter for allicin: Sensory properties and applicability in food

Sandra Catharina Wilde *, Julia Katharina Keppler, Kalpana Palani, Karin Schwarz

Institute of Human Nutrition and Food Science, Food Technology, Christian-Albrechts-Universität zu Kiel, Heinrich-Hecht-Platz 10, 24118 Kiel, Germany

ARTICLE INFO

Article history:
Received 31 August 2015
Received in revised form 26 November 2015
Accepted 10 December 2015
Available online 11 December 2015

Keywords:
β-Lactoglobulin
Allicin
Whey protein isolate
Garlic powder
Functional food
Covalent binding
Sensory properties
Spray drying

ABSTRACT

The thiosulfinate allicin is a labile, bioactive compound of garlic. In order to enrich allicin in a functional food, a delivery system which stabilises the compound and masks its intense flavour is necessary. In the present study allicin was covalently bound to the whey protein β-lactoglobulin and the incorporation of this transporter in a food matrix was tested. The sensory properties of the pure functional ingredient as well as of an enriched beverage were characterised by quantitative descriptive analysis. The concentration of volatile compounds was analysed by headspace gas chromatography–mass spectrometry. The garlic-related organoleptic properties of garlic powder were significantly improved by the binding of allicin in combination with spray drying. After purification of the modified β-lactoglobulin the garlic taste and smell were barely perceptible. β-Lactoglobulin modified with allicin provided a stable functional ingredient that can be used to enrich a broad range of food products.

1. Introduction

The continuing demand of consumers for health and well-being promoting products has led to an enormous increase in the number of functional foods that contain specific bioactive compounds (Benshitrit, Levi, Tal, Shimoni, & Lesmes, 2012). The enrichment and fortification of these compounds in food is a major scientific and technological challenge because many bioactive ingredients are relatively labile, resulting in a fast inactivation or degradation during food processing, storage and digestion. To ensure their high bioaccessibility and bioavailability, delivery systems have been developed to protect the compounds from degradation and enable their release at the desired absorption site (de Vos, Faas, Spasojevic, & Sikkema, 2010). In addition, bioactive compounds, such as phytochemicals, can cause bitter or astringent tastes or unpleasant off-flavours. Since consumers are not willing to compromise on taste for health benefits, potential adverse effects on sensory properties need to be overcome (Verbeke, 2006).

The whey protein β-lactoglobulin (β-LG) provides structural and physicochemical properties that facilitate the transport of small, hydrophobic ingredients. The globular protein is folded into a hydrophobic calyx which functions as the major non-covalent binding site beside hydrophobic pockets on the surface of the protein (Qin et al., 1998). Furthermore, β-LG has diverse technofunctional properties, GRAS (generally recognised as safe) status, a high nutritional value, and is soluble over a wide pH range (de Wit, 1998). Additionally, the protein can reduce the sensory perception of hydrophobic or volatile compounds due to its binding properties (Seuvre, Diaz, & Voilley, 2002; Shpigelman, Cohen, & Livney, 2012). However, due to its compact globular structure and its resistance to gastric conditions, it is the main allergen in bovine milk (del Val et al., 1999). The use of β-LG as a transporter for non-covalently bound ligands has been frequently reported, but the targeted covalent binding of bioactive compounds is a more recent approach (Keppler et al., 2014; Rade-Kukic, Schmitt, & Rawel, 2011; Shpigelman et al., 2012; Teng, Li, Luo, Zhang, & Wang, 2013).

Allicin, the major thiosulfinate in fresh crushed garlic, is mainly responsible for the characteristic taste and smell of garlic (Bautista et al., 2005; Salazar et al., 2008). The organosulfur compound exerts various health-promoting effects, such as the reduction of the risk of certain cancers and cardiovascular diseases (Borlinghaus, Albrecht, Gruhlke, Nwachukwu, & Slusarenko, 2014; Fleischauer, Poole, & Arab, 2000). With respect to functional foods, these effects are two of the most important health-related properties for consumers; therefore allicin is an interesting functional ingredient (Kraus, 2015). However, allicin is fairly unstable (e.g. at pH values above 6, at higher temperatures, in the presence of oil) and rapidly degrades

* Corresponding author.
E-mail address: info@foodtech.uni-kiel.de (S.C. Wilde).

http://dx.doi.org/10.1016/j.foodchem.2015.12.055
0308-8146/

during food processing and storage, which limits its bioaccessibility (Lee, Kim, & Kyung, 2014). For example, spray drying has been reported to cause 25–70% degradation of allicin (Rodriguez-Jimenes et al., 2014). Beside the instability of allicin, a decisive shortcoming is its smell. Frequent garlic consumers associate garlic with its health-promoting effects, whereas the aversion of seldom and non-users is mainly caused by the malodorous odour, especially in breath (Rosin, Tuorila, & Uutela, 1992).

Through the covalent binding of allicin to β-LG a stable, non-volatile S-allylmercapto- derivative of the free cysteinyl residue is formed. The digestibility of β-LG modified by allicin is not affected and *S*-allylmercaptocysteine could be released and absorbed like other amino acids (Wilde et al., 2016a). *S*-Allylmercaptocysteine is a metabolite of allicin and acts as a stable reservoir of the *S*-allyl moiety to mediate and prolong its activity. Therefore the health-related effects of *S*-allylmercaptocysteine were suggested to be similar to those of allicin (Miron, Listowsky, & Wilchek, 2010; Rabinkov et al., 1998). So far, the transfer to a food-grade level and the sensory properties of the bound allicin have not been tested. Therefore, the objective of the present study was to produce β-LG modified with allicin at a food-grade level by taking the influence of various process parameters into account. Further, a suitable food matrix for the enrichment of the functional ingredient was developed. Finally, the sensory properties of the modified protein and the functional food were assessed.

2. Materials and methods

2.1. Materials

For the production of the modified β-LG and the study drink (beverage model for experiments) only food and pharmaceutical grade ingredients were used. Whey protein isolate (WPI) was from BiPRO, Davisco Foods International, Inc., Eden Prairie, MN, with 97.7% protein and 75% β-LG in dry matter. Fresh garlic bulbs, instant coffee, sugar, lactose, cocoa powder, coffee whitener (main ingredients: glucose syrup, vegetable fat) and cream were purchased from a local grocery store. Carrageen Satiagum ADC 25 (Cargill Deutschland GmbH, Krefeld, Germany) and vanilla flavour (Symrise AG, Holzminden, Germany) were generous gifts. Sodium hydroxide (Panreac Applichem, Darmstadt, Germany) and hydrochloric acid (Merck, Darmstadt, Germany) were food and pharmaceutical grade, respectively.

All chemicals used for chemical analysis were analytical grade. Allicin was synthesised according to a modified procedure from Small, Bailey, and Cavallito (1947) as described by Wilde, Keppler, Palani, and Schwarz (2016b).

2.2. Preparation of garlic powder

Garlic cloves of five different garlic cultivars (white and purple-type, from China, Spain and France) were separately processed to garlic powder. At first garlic cloves were manually peeled and cut into 3–4 mm thick slices. In a perforated plastic bag the slices were frozen in liquid nitrogen and freeze dried afterwards (laboratory freeze dryer, Gamma 1–16 LSCplus; Martin Christ Gefriertrocknungsanlagen GmbH, Osterode, Germany). The dried slices were ground by an analysis mill. The allicin content of the garlic powder was measured by RP-HPLC with an Agilent 1200 Series system (Agilent Technologies, Santa Clara, CA). The powder was dissolved in water (5 mg/mL) and filtered by a syringe filter (0.2 μm pore size). RP-HPLC analysis was conducted with a C-18 column Nucleodur Gravity (100 mm × 2 mm i.d., 1.8 μm particle size, Macherey–Nagel GmbH & Co. KG, Düren, Germany). The mobile phase consisted of 5 mM ammonium acetate dissolved in water, pH 6.6 (eluent **A**) and acetonitrile with 0.1% formic acid (eluent **B**) at a flow rate of 0.2 mL/min with a gradient program as follows: 40% **B** (0–10 min), 100% **B** (15–19 min), 5% **B** (20–22 min), 40% **B** (25–35 min). The injection volume was 5 μL, the UV detector operated at 205 nm and the column temperature was 25 °C. Quantification of allicin was done by calibration with allicin standard.

2.3. Preparation of β-LG modified with allicin

For the binding reaction of allicin from garlic powder to β-LG from WPI, the WPI was dissolved and stirred for one hour. The powder of the garlic cultivar with the highest allicin yield, rose garlic (Ail rose de Lautrec), was used for the binding experiments with β-LG. The garlic powder was dissolved separately and filtered before it was added to the protein solution. The pH of the mixture was adjusted to 8.5 by 0.1 M NaOH and the final concentration of WPI was 26 g/L (corresponds to 1000 μM β-LG). To test different ligand–protein ratios the final concentration of garlic powder was varied between 2.9 and 5.8 g/L (corresponding to 250–500 μM allicin). For the control sample no garlic powder was added. The solution was stirred for one hour and incubated at 4 °C for 24 h. Two different drying techniques were tested. For the freeze drying the solution was filled in dishes after incubation, frozen at −25 °C and freeze dried using the same freeze dryer (see Section 2.2). For spray drying two different pH values of the solution during the drying process were tested. A part of the samples was left unchanged after incubation and had a pH value of about 8.0. The other part was adjusted to pH 6.0 by 0.1 M HCl. Finally, the solutions were spray dried on a pilot plant spray dryer (Mobile Minor 2000; Niro A/S, Copenhagen, Denmark) using a rotating atomiser disc at a flow rate of 47 mL/min, at 180 °C/70 °C inlet/outlet temperature and an outlet pressure of 4 bar.

For the analysis of the thermal stability of unmodified and modified β-LG, pure β-LG and allicin were used. The samples were prepared as described by Wilde et al. (2016b) by using different molar ratios (β-LG/allicin: 1:0 mol/mol; 2:1 mol/mol; 1:1 mol/mol).

2.4. Characterisation of modified β-lactoglobulin

Degree of denaturation

The influence of the covalent modification by allicin on the thermal stability of β-LG was analysed. Therefore, pure β-LG modified by pure allicin was diluted to 100 μM β-LG and heated in a water bath for 30 min at different temperatures (70, 75, 80, 85, 90 °C). Afterwards the samples were cooled down in an ice bath and the degree of denaturation was determined. In addition, the degree of denaturation was measured in the samples prepared with β-LG from WPI and with allicin from garlic powder, before and after drying processes.

The determination was done by the content of acid-soluble β-LG in the samples according to methodical provision of the German Industrial Standard (DIN 10473) (German Industrial Standard, 1997). The method is based on the isoelectric precipitation of denatured β-LG at pH 4.6. The β-LG concentration was analysed by RP-HPLC using an Agilent 1100 Series HPLC with a diode-array detector and PLRP-S column (300 Å, 8 μm, 150 × 4.6 mm, Agilent Technologies, Santa Clara, CA). The injection volume was 20 μL at a flow rate of 1.0 mL/min and a column temperature of 40 °C using eluents **A** (0.1% (v/v) TFA in water) and **B** (0.1% TFA (v/v) in ACN). The elution used gradient steps of 35–38% **B** (1–8 min), 38–42% **B** (8–16), 42–46% **B** (16–22 min), 46–100% **B** (22–22.5 min) and 100–35% **B** (23–23.5 min). The detection wavelength was 205 nm. The relative difference of the β-LG concentration in the sample before and after precipitation corresponded to the degree of denaturation.

Degree of modification

The area of the unmodified and modified β-LG A and B (genetic variants) in the RP-HPLC chromatogram was used to determine