Food Hydrocolloids 41 (2014) 79–85

Contents lists available at ScienceDirect

Food Hydrocolloids

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

Evaluation of volatile characteristics in whey protein isolate–pectin mixed layer emulsions under different environmental conditions

Like Mao [a,b], Ludivine Boiteux [c], Yrjö H. Roos [b], Song Miao [a,*]

[a] Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland
[b] School of Food and Nutritional Sciences, University College Cork, Cork, Ireland
[c] Department of Bioengineering, Institute of Technology Engineering Industry, Auxerre, Bourgogne, France

ARTICLE INFO

Article history:
Received 28 November 2013
Accepted 19 March 2014
Available online 3 April 2014

Keywords:
Mixed layer emulsion
WPI
Pectin
Partition coefficient
Release rate
Artificial saliva

ABSTRACT

A mixture of whey protein isolate (WPI) and pectin was used to produce mixed layer emulsions, which were taken as delivery systems for volatile compounds. The interaction between WPI and pectin was confirmed by differential scanning calorimetry. At neutral pH (7 or 6) or NaCl $\geq$ 150 mM, severe droplet aggregation and creaming were observed in the emulsions. The emulsions were stable at pH $\leq$ 5 or NaCl $\leq$ 100 mM. When diluted with different artificial salivas containing salts, mucin, and/or α-amylase, the emulsions became phase separated. Volatiles had lower air-emulsion partition coefficients and release rates in mixed layer emulsions than in WPI stabilized emulsions. Change of pH from 5 to 7 did not significantly influence the partition coefficients of most volatiles ($p > 0.05$), but led to higher release rates of all the volatiles. Increase of NaCl concentration in the emulsions resulted in faster release and higher headspace concentrations of the volatiles. Volatiles had lower partition coefficients and release rates in emulsions treated with artificial salivas containing mucin and/or α-amylase than in emulsion treated with salts alone. These results indicated that WPI–pectin mixed layer emulsions can be potentially used to modulate volatile release by changing the environmental conditions.

1. Introduction

Aroma perception happens when receptor cells in the nasal cavity (olfactory epithelium) are in contact with volatile flavor compounds. The electrochemical signals produced based on the volatile-receptor interaction are then transmitted to the brain via the nervous system, giving "odor images" of different foods (Taylor, 2002). Prior to aroma perception, volatile compounds have to be emitted from the food matrix. Volatile release from food involves the partition of volatile molecules between phases and the transport of the molecules (such as diffusion) through food matrices. These processes are largely influenced by the interactions between volatile compounds and food ingredients and, by environmental conditions under which volatile release is triggered (Taylor, 1998).

Whey protein isolate (WPI) and pectin are common food ingredients, and they are widely used to stabilize emulsions (McClements, 2005). These two biopolymers have been individually reported to be able to interact with volatile compounds, and modify their release behaviors in food systems (Guichard, Issanchou, Descourvieres, & Etievant, 1991; Rogacheva, Espinosa-Diaz, & Voilley, 1999). The interactions could be due to adsorption (reversible or irreversible), complexation, and entrapment (Guichard, 2002). WPI (or β-lactoglobulin) can attach ketones and esters through hydrophobic interaction or covalent binding (Lubbers, Landy, & Voilley, 1998; Tavel, Andriot, Moreau, & Guichard, 2008; Wu, Pérez, Puyol, & Sawyer, 1999). The former attachment occurred in the hydrophobic pocket (the central calyx) of β-lactoglobulin, as well as the crevice near the alpha helix, and the dimer interface (Kontopidis, Holt, & Sawyer, 2004; Wu et al., 1999), while the latter could happen at a protein surface (Lübke, Guichard, & Le Quéré, 2000). According to Harrison and Hills (1997), binding reduced the concentration of free volatiles in the aqueous phase and consequently, the volatiles released into headspace. Protein adsorbed at the interface can act as a barrier to slow mass transfer of volatile compounds, leading to reduced release rate (Harvey, Druaux, & Voilley, 1995). For example, the presence of β-lactoglobulin at the miglyol–water interface increased resistance to the transfer of benzaldehyde across the interface (Rogacheva et al., 1999). Pectin was also able to slow the release of many volatiles by pectin–volatile interactions. The adsorption of volatiles to pectin could proceed through van der Waals interactions between the alkyl patch of a volatile molecule and the hydrophobic region of pectin (Maier, 1970). The interactions may also occur through hydrogen

* Corresponding author. Tel.: +353 25 42468; fax: +353 25 42340.
E-mail address: song.miao@teagasc.ie (S. Miao).

http://dx.doi.org/10.1016/j.foodhyd.2014.03.025
0268-005X/

bonding involving the hydrogen atoms in the undissociated carboxyl group of pectin and the unshared electron pairs of heteroatoms, oxygen atoms of volatile molecules (Braudo et al., 2000). In pectin-water systems, volatiles could be trapped in pectin gel network, leading to slowed diffusion of the volatiles (Guichard et al., 1991).

A protein and a polysaccharide can interact with each other either chemically (covalent bonding) or physically (electrostatic bonding, etc.) (Dickinson, 2011). Protein–polysaccharide mixtures have been used industrially to stabilize food systems (e.g., beverage, yogurt). In comparison to emulsions stabilized by a protein alone, emulsions stabilized by a mixture of a protein and a polysaccharide (termed "mixed layer emulsions" as opposed to "multilayer emulsions", where the protein and polysaccharide are added sequentially) could have better stability against higher salt concentrations, wider pH range, thermal treatment, freeze-thawing, etc. (Benichou, Aserin, & Garti, 2002; Dickinson, 2011). Therefore, a protein–polysaccharide mixed layer emulsion can be used as a delivery system for volatile compounds under adverse conditions.

The main purpose of the present study was to investigate the release behaviors of volatile compounds in WPI–pectin mixed layer emulsions, when triggered by pH, salt and artificial salivas. WPI and pectin solutions were mixed at pH 5, and the mixture was used to stabilize emulsions. As emulsion properties (e.g., droplet size, emulsion stability) were greatly dependent on environmental conditions, effects of emulsion properties on volatile release were also considered. Five volatile compounds with different physicochemical properties were added into emulsions, and their release behaviors were investigated through GC headspace analysis.

2. Materials and methods

2.1. Materials

WPI (BiPro) was kindly donated by Davisco Food International (Le Sueur, MN, USA), and it contained 71 wt% β-lactoglobulin and 12 wt% α-lactalbumin. Apple pectin (degree of esterification 70–75%) was purchased from Sigma–Aldrich (St. Louis, MO, USA). Sunflower oil was purchased from a local supermarket and used without further purification. The following chemicals were also used in this study: 1-propanol (>99.5% purity), diacetyl (>99.5% purity), 2-pentanone (>99% purity), ethyl butyrate (>99% purity), 2-heptanone (>99% purity), mucin (from porcine stomach, Type II), α-amylase (from porcine pancreas, Type VI-B, 22 units/mg solid), sodium azide, sodium chloride, sodium hydroxide, sodium phosphate dibasic, citric acid, potassium phosphate monobasic, sodium thiocyanate, urea (Sigma–Aldrich); potassium chloride, sodium sulfate, hydrogen chloride, sodium hydrogen carbonate, calcium chloride (BDH Laboratory Supplier, Poole, UK).

2.2. Solution preparation

WPI solutions and pectin solutions were prepared separately by adding WPI and pectin powder into phosphate buffer (pH 5). Sodium azide (0.01 wt%) was added to prevent microorganism growth. The solutions were kept overnight to ensure complete hydration. WPI and pectin solutions were then mixed (1:1) to form a water phase, and the pH was readjusted to 5. Stock phosphate buffer solutions (pH 3–7), NaCl solutions (0–400 mM, pH 3–7), 0.1 M HCl and 0.1 M NaOH were also prepared. Deionized water was used to prepare all these solutions.

2.3. Differential scanning calorimetry

Thermal behaviors of WPI (in solutions with and without pectin) were analyzed using a DSC Q2000 differential scanning calorimeter (TA Instruments, Crawley, UK). 15–20 mg of each sample was prepared in a Tzero pan, which was sealed with a Tzero hermetic lid. An empty pan was used as a reference. The DSC sample pans were heated from 25 to 90 °C at 2.5 °C/min to track the heat induced denaturation of protein. The DSC was calibrated with indium at a heating rate of 2.5 °C/min.

2.4. Artificial salivas preparation

Five different artificial salivas were used (W, S, SM, SA, SMA). The compositions of the salivas followed the recipe described by Hur, Decker, and McClements (2009) with some modifications. Saliva W consisted of distilled water only. Saliva S contained a mixture of salts (10 mL KCl 89.6 g/L, 10 mL NaSCN 17 g/L, 10 mL KH_2PO_4 100.6 g/L, 10 mL Na_2SO_4 129.33 g/L, 20 mL $NaHCO_3$ 84.7 g/L, 1.7 mL NaCl 175.3 g/L, 5 mL $CaCl_2$ 22.2 g/L, 8 mL urea 25 g/L). Saliva SM contained salts (same composition as saliva S) and 25 mg mucin. Saliva SA contained salts (same composition as saliva S) and 20 mg α-amylase. Saliva SMA contained salts (same composition as saliva S), 25 mg mucin and 20 mg α-amylase.

2.5. Emulsion preparation

Emulsion was prepared by mixing the water phase (mixture of WPI solution and pectin solution, 90 wt% of final emulsion) and sunflower oil (10 wt% of final emulsion) at 10,000 rpm for 2 min using a ULTRA-TURRAX (IKA, Staufen, Germany) to form a coarse emulsion, which was further homogenized using an M110-EH Microfluidizer with a 75 μm Y-type ceramic interaction chamber (Microfluidics International Corp., Newton, MA, USA) at 50 MPa for three passes. The emulsions were immediately cooled to room temperature in a water bath (25 °C) and then stored in an incubator at 25 °C for future use. The mixed layer emulsions contained 0.5 wt % WPI, 10 wt% oil, and 0.4 or 0.8 wt% pectin. For comparison purpose, an emulsion with WPI only (0.5 wt% WPI, 10 wt% oil) was also prepared.

To study the effects of environmental stresses, pH of the emulsions were adjusted using 0.1 M HCl or 0.1 M NaOH, and either salt solutions (0–400 mM NaCl, 1:1 dilution), or different artificial salivas (1:1 dilution) were added. The subsequent characterization of emulsion properties was performed within 1 h. All of the work was carried out at 25 °C unless otherwise stated.

For the flavoring of emulsions, the procedure was described in a previous study (Mao, Roos, & Miao, 2013). Briefly, volatile solution in ethanol (5% v/v for each volatile) was added into emulsions in gastight glass vials (20 mL, silicone/PTFE seals) (La-pha-pack GmbH, Langerwehe, Germany) to reach a concentration of 500 mg/L for each volatile, and equilibrated for >1 h. Emulsions were stored at 25 °C before headspace analysis.

2.6. Emulsion characterization

Oil droplet size and ζ potential of the emulsions were determined by dynamic light scattering using a Zetasizer Nano-ZS90 (Malvern Instruments, Worcestershire, UK) at a fixed detector angle of 90°. To minimize multiple scattering effect, emulsions were diluted with buffer solutions (same pH and salt concentration as the measured sample) prior to each measurement.

Emulsion stability was evaluated using a multisample analytical centrifuge (Lumifuge, LUM GmbH, Berlin, Germany). The principle of the method was detailed in a previous study (Mao, O'Kennedy, Roos, Hannon, & Miao, 2012). In the present study, samples were centrifuged at 1500 rpm at a scanning rate of once every 10 s for 1 h (25 °C), and scanning light transmitted through the sample was detected. The result was determined by graphing the integral