Food Hydrocolloids 63 (2017) 716–726

Contents lists available at ScienceDirect

Food Hydrocolloids

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

Improved heat stability of whey protein isolate stabilized emulsions via dry heat treatment of WPI and low methoxyl pectin: Effect of pectin concentration, pH, and ionic strength

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ARTICLE INFO

Article history:
Received 24 August 2016
Received in revised form
8 October 2016
Accepted 14 October 2016
Available online 25 October 2016

Keywords:
Whey protein isolate
Low methoxyl pectin
Dry heat treatment
Heat stability
Emulsion
Conjugate

ABSTRACT

In this study, Whey Protein Isolate (WPI) was conjugated with Low Methoxyl Pectin (LMP) as a means to improve the heat stability of whey protein stabilized oil in water (o/w) emulsions. Hereby, the emulsifying activity and heat stability of the emulsions (10% w/w of oil) stabilized by 0.5% WPI-LMP conjugates were compared to the stability of emulsions stabilized by either 0.5% WPI, 0.5% of a mixture of WPI-LMP, or 0.5% dry heated WPI. The influence of LMP concentration, pH (i.e. 6.5 versus 5), and ionic strength (i.e. 0 and 30 mM of NaCl) on the stability of the emulsions was studied. It was found that unheated emulsions stabilized by WPI-LMP conjugates had a relatively small droplet size and were stable against creaming, irrespective of the pH and ionic strength of the emulsions. Results also showed that not only the pH sensitivity, but also the heat-sensitivity of WPI-stabilized emulsions could be significantly improved by application of pectin. Upon heating at 80 °C and 120 °C up to 20 min, there was almost no change in the particle size distribution of the conjugate-stabilized emulsions after heating. Viscosity measurements showed a similar trend; heat treated emulsions stabilized by conjugates did not show any change in consistency. This pronounced heat stabilizing effect of LMP, however, was not observed in emulsions stabilized by a mixture of WPI-LMP. With regard to the dry heat incubation time, a 2 days incubation period was sufficient to produce a WPI-derived emulsifier with superior stability towards both pH and heat treatment.

1. Introduction

Oil in water (o/w) emulsions exist in many food products such as milk, mayonnaise, or vinaigrette. In addition, this type of emulsion is also often prepared to encapsulate some functional compounds such as fat soluble vitamins and β-carotene for health purposes and for drug delivery (Almeida & Souto, 2007; Mao, Dubot, Xiao, & McClements, 2013; Muhoza, Karangwa, Zhang, & Xia, 2016). An emulsifier is required in this system in order to stabilize the emulsion. Nowadays, natural emulsifiers (such as egg yolk, lecithin, and proteins) are mostly preferred (Dickinson, 1993). According to Garti (1999) and Dickinson (2011), food grade ingredients such as proteins and polysaccharides are promising natural compounds that can be used to replace synthetic emulsifiers (Dickinson, 2011; Garti, 1999).

Whey Protein Isolate (WPI) is a byproduct of the milk industry, particularly cheese processing. Based on its high protein content, WPI has been widely used as food ingredient due to its various functionalities (Bryant & McClements, 2000). Thus, it has been known that WPI is a good emulsifier (Damodaran & Paraf, 1997; Gunasekaran, Ko, & Xiao, 2007; Schmitt, Sanchez, Desobry-Banon, & Hardy, 1998), foaming agent (van der Ven, Gruppen, de Bont, & Voragen, 2002), and gelling agent (Gunasekaran et al., 2007). Nevertheless, the application of WPI as an emulsifier should not only consider the ability of WPI to stabilize the emulsions against destabilization e.g. by flocculation and coalescence during storage (Damodaran, 2005), but also during processing. Many food product are heat treated to prolong the shelf life of these products (Wijayanti, Bansal, Sharma, & Deeth, 2014), which eventually also affects the structure of the emulsions during storage and transportation (Leal-Calderon, Thivilliers, & Schmitt, 2007). Thus, in the application of WPI, it is important that WPI can withstand a heat treatment while at the same time stabilizing the emulsions.

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http://dx.doi.org/10.1016/j.foodhyd.2016.10.025
0268-005X/

However, having its denaturation temperature at around 75–80 °C (Bernal & Jelen, 1985), WPI is very heat labile and can easily denature. This well-known heat induced denaturation of whey proteins in its turn leads to the alteration of their functional properties (Kessler & Beyer, 1991), whereby heat treatment of WPI stabilized emulsions may cause protein aggregation and droplet flocculation (Leal-Calderon et al., 2007), thus altering the characteristics of the emulsions, such as their consistency which may be transformed from liquid to highly viscous fluid and, in the worst case, to a gel, which is undesirable. This limited heat stability is still a major problem for the application of WPI in food industry (Dissanayake, Ramchandran, Donkor, & Vasiljevic, 2013).

Previous research has shown that combining the amphiphilic properties of proteins and the hydrophilic properties of polysaccharides can help improving the functional properties, including the heat stability of whey proteins (Jiménez-Castaño, López-Fandiño, Olano, & Villamiel, 2005; Zhu, Damodaran, & Lucey, 2008). This can be achieved through conjugation of proteins and polysaccharides using the dry heat treatment method (Aoki et al., 1999; Bu et al., 2015; Einhorn-Stoll, Ulbrich, Sever, & Kunzek, 2005; Kato, 2002). In this method, a dry mixture of proteins and polysaccharides is exposed to heat at a controlled relative humidity (Kato, 2002). This method is considered to be safe as no chemical additives are needed (Damodaran, 2005; Kato, 2002). Upon incubation, the proteins and polysaccharides will be covalently linked to each other via a Maillard type reaction (Dickinson, 2008).

In this research WPI was covalently linked to Low Methoxyl Pectin (LMP) through dry heat treatment. Pectin was chosen due to its abundant availability which makes it a cheap source of polysaccharides. The performance of WPI-LMP conjugates as emulsifier in o/w liquid emulsions as well as their heat stability were evaluated. The focus of the study was set on the capability of the WPI-LMP conjugates to create stable emulsions against creaming and to protect the emulsions against heat induced denaturation and aggregation. Since the heat stability of WPI stabilized emulsions is influenced by external factors, such as pH and ionic strength, these factors were also taken into consideration in this study. Moreover, different concentrations of LMP in the conjugates, as well as different durations of the incubation time were applied to evaluate their influence on the emulsifying activity of the conjugates and the heat stability of the emulsions stabilized by the conjugates.

2. Materials and methods

2.1. Materials

WPI was purchased from Davisco Foods International Inc. (Le Sueur, MN, USA). Protein analysis revealed that the WPI contains approximately 92.6% protein, whereby 85% of the protein is β-Lactoglobulin (Van der Meeren, El-Bakry, Neirynck, & Noppe, 2005). Low Methoxyl Pectin (LMP) (Unipectin OB700) was obtained from Cargill (Ghent, Belgium) and contained 89.6% of dry matter. The LMP was used without further purification. Oil in water emulsions were prepared using sunflower oil purchased from a local supermarket.

2.2. Conjugate preparation

Conjugates were prepared through dry heat treatment at controlled relative humidity. The conjugates were prepared from a 5% (w/v) protein solution and 1% (w/v) LM Pectin solution. Correction on the protein content and dry matter content of the WPI and LMP, respectively, was performed when preparing the solutions. The solutions were prepared in distilled water and the pH of the solutions was adjusted to 7 with 1 N HCl to avoid formation of ionic complexes that might form at lower pH during mixing (Mishra, Mann, & Joshi, 2001). Subsequently, the solutions were kept at refrigerator temperature overnight before mixing. Solutions were mixed at a WPI to LMP ratio of 1:0, 4:1, 2:1 and 1:1 (on weight basis) and were frozen afterwards. The concentration of WPI was kept constant at all mixing ratios, while the concentration of pectin was varied.

The frozen samples were lyophilized (Alpha 1-2 LD plus, Christ) to remove all the water and obtain a dry product. The freeze dried products were then dry heat treated by incubation at a temperature of 60 °C for up to 16 days in a desiccator containing a saturated NaCl solution to keep the relative humidity at ±74% (Greenspan, 1977).

2.3. Emulsion preparation

To investigate the influence of dry heat incubation time on the emulsifying activity of the conjugates and the heat stability of the emulsions, emulsions stabilized by a WPI-LMP mixture (ratio 2:1, day 0) and WPI-LMP conjugates incubated at different incubation times (ratio 2:1, day 1–16) were prepared. These emulsions were compared to emulsions stabilized by native WPI (WPI), freeze dried WPI (ratio 1:0, day 0) and dry heated WPI (ratio 1:0, day 4–16) as controls. The emulsions were prepared at both pH 6.5 and pH 5.0 to investigate the influence of pH. Furthermore, at pH 5.0, the emulsions were prepared in the absence and presence of 30 mM NaCl to study the effect of ionic strength on the heat stability of protein-stabilized emulsions around the protein's isoelectric point. Emulsions stabilized by WPI-LMP mixtures and conjugates at different LMP concentration (WPI:LMP ratio 4:1, 2:1, and 1:1) were prepared to study the influence of LMP concentration on the emulsifying activity of the WPI-LMP mixtures and conjugates as well as on the heat stability of the emulsions.

Initially, the aqueous phase containing 0.5% of WPI or 0.5% WPI-LMP mixture or conjugates was prepared. The aqueous phase was then kept overnight in the fridge prior to emulsification to fully hydrate the hydrocolloids. An appropriate amount of oil was added to the aqueous phase to have 10% (w/w) of oil in the final emulsion. The mixture was premixed using an IKA Ultra-turrax TV45 (Janke Kunkel, Staufen, Germany) at the highest speed (24,000 rpm) for 1 min. Subsequently, the premix was homogenized using a Microfluidizer 110S for 2 min at 4 bar of compressed air pressure corresponding to 560 bar of liquid pressure. The heat exchanger coil of the Microfluidizer was immersed in a water bath set at 55 °C during emulsification.

2.4. Heat coagulation test

The heat coagulation test was conducted based on the method developed by Kasinos, Karbakhsh, and Van der Meeren (2015). The test was performed at both 80 °C and 120 °C in an oil bath. To ensure a homogenous temperature distribution in the oil bath, an IKA RW20 stirrer with a 3 bladed metallic propeller set at 250 rpm was put in the corner of the oil bath. The temperature of the oil bath was monitored using a thermometer and a thermocouple connected to an electronic digital thermometer (Agilent 34970A, Diegem, Belgium).

Approximately 9 ml of emulsion was brought into 20 ml headspace vials (75.5 × 22.5 mm, 1 st hydrolytic class, Grace, Deerfields, IL, USA) and sealed tightly with a metallic cap. The emulsions were then placed in a metallic rack and immersed in the oil bath. Heating of the emulsions was carried out for 20 min. Afterwards, the emulsions were cooled and kept at room temperature before further measurement. All the measurements were performed within one day after the emulsion preparation.