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Effect of hydrolysed sunflower lecithin on the heat-induced coagulation of recombined concentrated milk emulsions

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ABSTRACT

In an attempt to decrease the degree of heat-induced intermolecular protein interactions in recombined concentrated milk, the effect of sunflower lecithin, a natural source of phospholipids, was examined. The obtained results proved that lecithin supplemented milk emulsions demonstrated a less pronounced increase in viscosity as well as particle size, upon severe heating. Additionally, enzymatic hydrolysis was performed using a phospholipase A1. Phosphorus nuclear magnetic resonance and high performance liquid chromatography revealed that the lysophospholipid content reached a maximum after 20 min of hydrolysis and gradually declined as the hydrolysis reaction duration was extended. This work showed that sunflower lecithin largely improves the heat stability of whey protein containing solutions and emulsions due to its high phospholipid content. As the phospholipid molecular structure is important, it follows that the heat stability of whey protein containing products may be optimised by appropriate processing, such as enzymatic hydrolysis of intact lecithins.

1. Introduction

From a physico-chemical point of view, milk is considered as a natural oil-in-water (O/W) emulsion, where fat droplets are dispersed in an aqueous continuous phase. Whereas the interface of the fat globules in raw milk is covered by the so-called milk fat globule membrane (MFGM), the interface in homogenised and recombined milk is mainly covered by the main milk proteins, i.e., casein micelle particles and whey proteins.

To obtain a microbiologically stable product, intense heating is mostly applied. A number of studies have shown that upon heating β-lactoglobulin, the major globular protein of the whey fraction, its dimeric configuration dissociates into monomeric. In addition, structural rearrangements occur making accessible the hydrophobic residues and the thiol group, which were previously enclosed in the globular structure; they can therefore be involved into aggregates formation via thiol-disulphide exchange reactions with κ-casein, as well as with other denatured whey protein molecules (Anema & Li, 2003; Pelegrine & Gasparetto, 2005; Verheul, Pedersen, Roefss, & de Kruif, 1998).

During heating of milk, several reactions take place, including denaturation of whey proteins and complex formation between denatured whey proteins, casein micelles and fat droplets, giving rise to a phenomenon known as heat coagulation (Corredig & Dalgleish, 1996; Dissanayake & Vasiljevic, 2009; Jeurnink & Dekruif, 1993; Kasinos, Tran Le, & Van der Meeren, 2014; Oldfield, Singh, Taylor, & Pearce, 2000; Singh, 2004; Verheul et al., 1998).

Previous research demonstrated that lecithin, a natural low molecular weight surfactant composed of a mixture of various phospholipids, can influence the heat stability of whey protein containing emulsions (Cruijsen, 1996; Hardy, Sweetsur, West, & Muir, 1985; McSweeney, Healy, & Mulvihill, 2008; Tran Le et al., 2007). However, the effects have been described either as positive or negative, depending on the type and amount of added lecithin (Yamamoto & Araki, 1997) and consequently, an in-depth analysis is required.

Cabezas, Madoery, Diehl, and Tomás (2011), McCrae (1999), Tran Le et al. (2011), and Van der Meeren, El-Bakry, Neirynck,

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and Noppe (2005) described the improved heat-stabilising effects of hydrolysed over native lecithin, after its incorporation in O/W emulsions. In addition, Tran Le et al. (2007) demonstrated that the effect of hydrolysed soybean lecithin in decreasing heat-induced whey protein–casein interactions was significant, whereas the effect of non-hydrolysed lecithin was almost negligible. Similarly, particle size analysis performed by Cabezas et al. (2011) and Sünder, Scherze, and Muschiolik (2001), as well as heat stability evaluation by McCrae (1999), found hydrolysed lecithins to be most effective, whereas Jost, Dannenberg, and Rosset (1989) mentioned that hydrolysed lecithin incorporation, before or during emulsification, reduced the strength of a heat-set whey protein emulsion gel.

The aim of this contribution was to study the effect of sunflower lecithin, a by-product from the degumming process of crude sunflower seed oils (Pan, Tomás, & Añón, 2002), after its incorporation prior to emulsification, on the heat induced coagulation of recombined concentrated milk emulsions, subjected to intense heating. The main justification for sunflower lecithin utilisation emerges from its classification as a non-GMO product and it can therefore be considered as an attractive replacement for soybean lecithin.

In addition, intact sunflower lecithin was enzymatically treated by Lecitase® Ultra, a protein-engineered phospholipase A1 (Mishra, Kumaraguru, Sheelu, & Fadnavis, 2009). The enzymatic treatment was carried out to establish the potential of hydrolysed lecithin to further reduce the coagulation tendency of concentrated milk. Finally, hydrolysis of lecithin was performed for varying time intervals, to determine the significance of hydrolysis on heat stability.

2. Materials and methods

2.1. Materials

High-heat skimmed milk powder (SMP) was obtained from FrieslandCampina (Deventer, The Netherlands). According to the manufacturer, the SMP contained 37.3% (w/w) protein, 0.5% (w/w) fat and 0.15% (w/w) phospholipids. Whey protein isolate (WPI) was obtained from Davisco Foods International, Inc. (BiPro®, Le Sueur, MN, USA). Kjeldahl analysis revealed that this WPI contained 92.6% of protein, whereas polyacrylamide gel electrophoresis (PAGE) indicated that approximately 85% of the total protein consisted of β-lactoglobulin. Besides, the WPI contained 1.6% ash (by incineration at 525 °C), 5.0% moisture (from weight loss at 102 °C) and 0.8% fat.

Sunflower lecithin was obtained from Oleaginosa Moreno (Bahia Blanca, Argentina). According to the manufacturer, this lecithin contains 66.9% acetone insoluble matter, 0.5% moisture and 32% oil.

Lecitase® Ultra was purchased from Novozymes (Bagsværd, Denmark). This is a protein-engineered phospholipase A1 containing 6.5% protein and is a carboxylic ester hydrolase from *Thermomyces lanuginosus*/*Fusarium oxysporum* produced by the submerged fermentation of a genetically modified *Aspergillus oryzae* (Mishra et al., 2009).

The high oleic sunflower oil (Hozol, Contined, Bennekom, The Netherlands) contained maximum 0.05% free fatty acid as oleic. Its melting point is at 0 °C and hence the oil remains clear even after 10 h storage at 4 °C.

Ca-imidazole buffer containing 20 mM imidazole, 5 mM $CaCl_2.H_2O$, 30 mM NaCl and 1.5 mM NaN_3 was prepared according to Anema (1997). Its pH was adjusted to 6.55 with 1 M HCl. This buffer was selected to have a pH and calcium activity that resembled those of cows' milk.

2.2. Enzymatic hydrolysis

Non-hydrolysed sunflower lecithin dispersion was prepared by adding 4% (w/w) lecithin in distilled water and was stirred with a magnetic stirrer at 50 °C, until homogeneous. HCl (0.1 M) was used to adjust the pH of the reaction medium to 5.0. The mixture was transferred into a water bath to control and maintain the temperature at 50 °C and subsequently, 0.05% (v/v) Lecitase® Ultra was added to the dispersion. As free fatty acids are released upon enzymatic hydrolysis, NaOH addition is required to keep pH constant. For that purpose, an automatic titrator (Dosimat 765, Metrohm, Herisau, Switzerland) was used to measure pH and add the required volume of NaOH. This method involves the continuous titration of the fatty acids released during enzymatic hydrolysis of the phospholipids.

The samples remained in the water bath for 10, 20, 30, 40 and 60 min. To stop the reaction, the mixtures were subsequently incubated at 100 °C for 5 min to inactivate the enzyme.

2.3. Nuclear magnetic resonance measurement

Quantitative phosphorus nuclear magnetic resonance (^{31}P NMR) was used to analyse non-hydrolysed and hydrolysed sunflower lecithin. Samples were prepared with distilled water and D_2O (99.8% AtomD, Armar Chemicals, Gottingen, Switzerland) at a 1:1 ratio, containing 20 mg mL^{-1} of either non-hydrolysed or hydrolysed sunflower lecithin, 10% (w/w) sodium deoxycholate (Sigma–Aldrich, Steinheim, Germany), 2 mM Na-EDTA (Acros Organics, Geel, Belgium) and 1 mM glyphosate (Sigma–Aldrich) as internal standard for quantification. All samples were mixed using a Sonifier 250/450 (Branson Ultrasonics, Danbury, CT, USA), as well as Shaker SM-30 (Edmund Bühler, Hechingen, Germany) at 40 °C alternately, whereas the pH was adjusted to 8.0 with NaOH before measurements. ^{31}P NMR spectra were performed on a Bruker Avance 500 (Rheinstetten, Germany) operating at a ^{31}P frequency of 202.46 MHz, equipped with a BBI 5 mm probe. Inverse gate proton decoupling was used for suppression of Nuclear Overhauser Effect (NOE). Measurements were performed at 40 °C and the temperature was controlled to within ±0.01 °C with a Eurotherm 3000 VT (Ashburn, VA, USA) digital controller. The following instrument settings were used: 65180 data points, 90° excitation pulse, number of scans of 256 with a 12.25 s relaxation delay time and a decay acquisition time of 3.22 s. Phospholipon 90G, a highly purified soybean lecithin containing at least 90% PC (Rhône-Poulenc, Köln, Germany) and pure lysophospholipids standards (NOF Corporation, Tokyo, Japan) were used for peak identification.

2.4. High performance liquid chromatography analysis

Phospholipids analysis was carried out with a Shimadzu high performance liquid chromatography (HPLC) System (Tokyo, Japan) with an evaporative light scattering detector (ELSD, Alltech-3300, Alltech Associates Inc., Lokeren, Belgium). The analysis procedure was performed according to the method described by Nguyen et al. (in press). All analyses were executed in duplicate.

2.5. Emulsion preparation

Recombined concentrated milk emulsions were prepared using 16.5% (w/w) SMP, 6.5% (w/w) oil, as well as 0.0–0.3% of (hydrolysed) sunflower lecithin. The lecithins were first added into 0.02% NaN_3 (Acros Organics, Geel, Belgium) aqueous solution, to prevent microbial contamination, and the solutions were subsequently stirred over night until a homogeneous dispersion was obtained. Finally, SMP powder and high oleic sunflower oil were added and