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Partitioning of α-lactalbumin and β-lactoglobulin from cheese whey in aqueous two-phase systems containing poly (ethylene glycol) and sodium polyacrylate

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ABSTRACT

Partitioning behavior of the whey proteins α-lactalbumin (α-la) and β-lactoglobulin (β-lg) in aqueous two-phase systems prepared with poly ethylene glycol (PEG) and sodium polyacrylate (NaPA) was investigated as a function of pH and polymer concentrations. It was observed that α-la concentrated in the PEG phase while β-lg concentrated in the NaPA phase. Response surface methodology was applied to optimize protein partitioning and to achieve the best conditions for their fractionation. Thermodynamic analysis based on isothermal titration microcalorimetry indicated that the partitioning of α-la was accompanied by endothermic heat and was entropically driven, while β-lg partitioning was accompanied by exothermic heat and was enthalpically driven at low polymer concentrations and entropically driven at high polymer concentrations. Purification and yield parameters were determined using fresh whey and the results allowed for conclusion of the great applicability of this new system for α-la and β-lg fractionation.

Keywords: Aqueous two-phase system; Partitioning; Whey protein; Optimization; Microcalorimetry

1. Introduction

Whey is a co-product of the dairy industry and continues to be discarded as an effluent despite the presence of important biomolecules. Among whey components, β-lactoglobulin (β-lg) and α-lactalbumin (α-la) account for about 80% of the total protein content (Boutin et al., 2007). Due to their great functional and nutritional properties, these biomolecules are largely applied in the food industry and as a consequence, possess high commercial values (Siso, 1996; Sliwinski et al., 2003; Chatterton et al., 2006; Boutin et al., 2007). However, it has been claimed that β-lg can induce allergenic reactions, especially in children (De Luis et al., 2009). Therefore, the α-la fraction, free of β-lg, can be widely applied as an ingredient for infant food formulations. The β-lg rich fraction can be used in food products where its functional and technological properties (gelling and foaming) are desired.

Different techniques have been applied for β-lg fractionation, including ionic exchange, ultrafiltration, and selective precipitation (Roger et al., 1984; Bottomley, 1991; Kristiansen et al., 1998; Kiesner et al., 2000; Wu, 2001; Muller et al., 2003). However, some problems are associated with such

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operations, such as high costs and water consumption of chromatographic processes, difficulties in obtaining high purity fractions related to membrane separation and precipitation, and residue generation related to precipitation. Liquid–liquid extraction using aqueous two-phase systems (ATPS) is an interesting alternative in this case, since this method presents low costs when compared to the above-mentioned techniques, as well as the potential for easily scale up and possibility to recycle the phase constituents (Albertsson, 1971; Picó et al., 2006; Dembczynski et al., 2013). Partitioning of biological molecules occurs due to Van der Waals force, ionic and hydrophobic interactions and hydrogen bonding (Picó et al., 2006; Saravanan et al., 2008). Therefore, the partition coefficient depends on different variables such as pH, temperature, concentration of the system components, and molecular weight of the polymers (Johansson et al., 2008a; Faria et al., 2009; Alcântara et al., 2011; Mokhtarani et al., 2011; Dembczynski et al., 2013; Priyanka et al., 2012).

PEG–salt and PEG–dextran are the most common ATPS used for biomolecule partitioning. The negative aspects of PEG–salt systems are economic and environmental associated with large consumption of phase-forming materials which are not easily regenerated. On the other hand, the major drawback of PEG–dextran systems is economic due to the high cost of dextran (Saravanan et al., 2006). Alternative economical polymers have been studied, including polyethylenimine – PEG (Gupta et al., 2002), the PEG–maleic acid copolymer (Kajiuchi et al., 2002) and poly (acrylic acid) (Saravanan et al., 2006, 2008). The poly (acrylic acid)-PEG aqueous system is interesting in that it can form a two-phase polymer–polymer system at pH levels above 5.0 when a sufficient quantity of salt is added to the system, in order to facilitate the compartmentalization of highly charged polyelectrolytes in the phases. A polymer–water system is formed at pH levels below 5.0 (Johansson et al., 2008b). This ATPS separates relatively fast and has relatively low viscosity in contrast to other traditional two-phase systems (Sampaio et al., 2012; Minim et al., 2010). The two polymers PEG and poly (acrylic acid) are environmentally harmless, relatively inexpensive and easily handled (Johansson et al., 2008a).

Considering the limited number of studies using PEG and polyacrilate systems and their attractive characteristics for protein partitioning, this work sought to study the partitioning of β-lg and α-la in ATPS composed of polyethylene glycol (PEG) and sodium polyacrilate (NaPA). In order to investigate the forces governing the partitioning of both proteins, the enthalpy of transfer was determined by isothermal titration microcalorimetry. Optimization of the partition coefficient was carried out using a central composite rotatable design (CCRD) with response surface methodology (RSM). The optimized system was applied for extraction experiments using fresh whey.

2. Materials and methods

2.1. Chemicals

PEG (average molar mass of 6000 g/mol) and NaPA (average molar mass of 8000 g/mol) were purchased from Sigma (St. Louis, USA). α-La and β-lg (purity greater than 90%) were obtained from Davisco (USA) and NaCl (analytical grade) was purchased from Sigma (St. Louis, USA). Acetonitrile (HPLC grade) and coomassie brilliant blue were also purchased from Sigma (St. Louis, USA).

2.2. Partitioning studies

Initially, stock solutions of PEG 40% (w/w) and NaPA 25% (w/w) were prepared and pH levels were adjusted to the desired condition using NaOH (1 mol/L) or HCl (1 mol/L). The aqueous two-phase systems weighing 10 g (AUX220, Shimadzu, USA) were prepared in 15 mL centrifuge tubes by mixing the proper amounts of deionized water (Milli-Q, Millipore, France) with the polymers in order to achieve the concentrations defined in the experimental design. The tubes were agitated in a vortex shaker, centrifuged at 2000 × *g* for 20 min (Eppendorf 5804, Germany) to accelerate the phase separation, and then left in an incubator at constant temperature (Tecnal TE-184, Brazil) for 16 h to reach equilibrium. Subsequently, a volume of 2.5 mL was taken from the top and bottom phases and added to a 10 mL centrifuge tube for the partitioning experiments. Volumes of 50 μL of the protein solution (50 mg/mL) were added to the systems which were mixed in a vortex shaker, and then centrifuged at 2000 × *g* for 20 min for phase separation. The tubes were incubated for 16 h to reach equilibrium. Samples from the bottom and top phases were collected with syringes and proteins were quantified by spectrophotometry in order to determine the partition coefficient (Eq. (1)). In all experiments the temperature was maintained at 25 °C and the NaCl concentration was maintained at 1% (w/w)

$$K_P = \frac{C_T}{C_B} \quad (1)$$

where K_p is the partition coefficient, and C_T and C_B are the protein concentrations in the top and bottom phases, respectively.

2.3. Enthalpy of transfer determination

In this study, the enthalpy of transfer (ΔH_t) was measured by isothermal titration microcalorimetry (ITC) using a CRC titration microcalorimeter (model ITC 4200, USA). For ITC measurements, the microreaction system consisted of a 1.8 mL stainless steel ampoule which was filled with the bottom and top pre-equilibrated phases (0.9 mL each). When thermal equilibrium between the ampoule and heat sink was reached, the system was titrated (10 μL) using the PEG phase containing β-lg or the salt phase containing α-la with a Hamilton syringe, fitted with a stainless steel needle and driven by a computer-controlled pump. The output signal was collected as energy, *P* (μJ), versus time, *t* (s), and was integrated to obtain the total heat Q_T for each injection. To obtain the net heat of transfer (Q_{Net}), i.e., the energy associated only with protein transfer, energy associated with the formation of micro-phases from the upper phase dispersed in the lower phase (Q_{Ph}) was discounted from the total heat of transfer (Q_T), according to Eq. (2)

$$Q_{Net} = Q_T - Q_{Ph} \quad (2)$$