Contents lists available at ScienceDirect

Food and Bioproducts Processing

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

Partitioning of bovine lactoferrin in aqueous two-phase system containing poly(ethylene glycol) and sodium citrate

Angélica Ribeiro da Costa[a,b], *Jane Sélia dos Reis Coimbra*[b,e,*], *Luísa Alexandre Ferreira*[c], *João Carlos Marcos*[d], *Igor José Boggione Santos*[b], *Marleny D.A. Saldaña*[e], *José António Couto Teixeira*[c]

[a] *Departamento de Tecnologia Rural e Animal, Universidade Estadual do Sudoeste da Bahia, Praça Primavera, 40, Bairro Primavera, 45700-000, Itapetinga, BA, Brasil*

[b] *Departamento de Tecnologia de Alimentos, Universidade Federal de Viçosa, Campus Universitário, s/n, Centro, 36570-000, Viçosa, MG, Brasil*

[c] *IBB–Institute for Biotechnology and Bioengineering, Centre for Biological Engineering, Universidade do Minho, Campus de Gualtar, 4710-057, Braga, Portugal*

[d] *Centro de Química, Universidade do Minho, Campus de Gualtar, 4710-057, Braga, Portugal*

[e] *Department of Agricultural, Food and Nutritional Science (AFNS), University of Alberta, T6G 2P5, Edmonton, AB, Canada*

ARTICLE INFO

Article history:
Received 23 March 2014
Received in revised form 21 March 2015
Accepted 8 April 2015
Available online 25 April 2015

Keywords:
ATPS
Molar mass
PEG
pH
Phase diagram
Whey protein

ABSTRACT

The partitioning of the whey protein lactoferrin, which is an iron transporter glycoprotein, in an aqueous two-phase system composed of poly(ethylene glycol) (PEG) and sodium citrate was evaluated. Equilibrium data at 25 °C were determined for each system studied using PEG with a molar mass of 1000 and 4000 g mol^{-1} at pH values of 5.5, 6.5, and 7.5. An increase in the molar mass of the polymer promoted the expansion of the two-phase region and caused the migration of the lactoferrin to the salt-rich bottom phase. An increase in pH also led to the expansion of the biphasic region. However, changing the pH over the tested range slightly affected protein partitioning. Lactoferrin recovery percentages greater than 94% were observed for all of the systems evaluated. The results indicated that lactoferrin can be successfully partitioned in an aqueous two-phase system formed of 14% (w/w) PEG and 10% (w/w) sodium citrate at pH 5.5 and 25 °C. The protein was concentrated 1000-fold in the salt-rich bottom phase in this system.

1. Introduction

New insights into purification strategies for biotechnological products are in demand to reduce production costs. Alternative bioseparation techniques are needed to (i) maintain biologically active molecules; (ii) achieve a high degree of purity and recovery; and (iii) be efficient, effective, and economical for large-scale processes. Aqueous two-phase systems (ATPS) are an alternative for separating, concentrating, and purifying biologically active molecules such as proteins in complex mixtures. ATPS are formed by mixing either two polymers (such as poly(ethylene glycol)/dextran) or one polymer

* Corresponding author at: Departamento de Tecnologia de Alimentos, Universidade Federal de Viçosa, Campus Universitário, s/n, Centro, 36570-000, Viçosa, MG, Brasil. Tel.: +55 31 38991618; fax: +55 31 38992208.
E-mail addresses: jcoimbra@ufv.br, janeseliacoimbra@gmail.com (J.S.d.R. Coimbra).

http://dx.doi.org/10.1016/j.fbp.2015.04.002

and one salt (such as poly(ethylene glycol)/sodium phosphate) in water at a certain concentration and temperature. The two phases consist primarily of water (between 70% and 90%) and provide an adequate environment with favourable conditions for biomolecule distribution, in addition to a low cost of implementation and ease of scale-up (Albertsson, 1986; Coimbra and Teixeira, 2010; Zaslavsky, 1995).

ATPS are used to concentrate and purify whey proteins (Alves et al., 2000; Anandharamakrishnan et al., 2005; Capezio et al., 2005; Silva et al., 2009). These proteins have high biological and functional value and confer technological and nutritional properties to food products. Lactoferrin (Lf) is an important whey protein because of its (i) anti-inflammatory, antimicrobial, antioxidant, and immunomodulatory properties; (ii) association with other biological functions, such as anticancer, proteolytic, and enzymatic activities; and (iii) use as a food supplement, such as in infant formula, yoghurt, skim milk, drinks, and pet foods, in skin care and personal hygiene products, such as lotions, creams, and face wash, moisturisers, and antioxidants; and in oral care products, such as mouthwash, mouth gels, toothpaste, and chewing gum (Baker and Baker, 2005; Coimbra and Teixeira, 2010; Moore et al., 1997; Steijns and van Hooijdonk, 2000; Ward et al., 2005).

Lactoferrin and bovine lactotransferrin belong to the group of glycoproteins that are known as transferrins, which have a molar mass of approximately 80,000 Da with 689 amino acid residues, including all of the essential amino acids. Because of its capacity to reversibly bind to iron ions, lactoferrin occurs in two forms: hololactoferrin, which is saturated with iron, and apolactoferrin, which does not contain metal and is predominantly found in milk (Baker and Baker, 2005; Moore et al., 1997; Steijns and van Hooijdonk, 2000; Ward et al., 2005). Lactoferrin has a highly alkaline character with a pH between 8 and 9, probably due to three important concentrations of positive charges in its basic residues: (1) at the N-terminal region (residues 1–7); (2) along the outside of the first helix (residues 13–30); and (3) in the inter-lobe region, close to the connecting helix (Baker and Baker, 2005).

Knowledge of the equilibrium data for a system is required to successfully use ATPS to partition lactoferrin. The literature contains equilibrium data for various polymer-polymer ATPS, such as poly(ethylene glycol) (PEG) + dextran (Albertsson, 1986), PEG + maltodextrin (Machado et al., 2012), PEG + (ficoll, ucon, or hydroxypropyl starch) (Madeira et al., 2008); and polymer-salt, such as PEG + potassium phosphate (Silva et al., 1997), PEG + citrate salts (Marcos et al., 1999; Oliveira et al., 2008b; Tubío et al., 2006), PEG + sulphate salts (Oliveira et al., 2008a). Polymer-salt ATPS are the most commonly used systems for biomolecule partitioning because of the greater differences in density and viscosity among the phases, which reduces the phase splitting time and allows for the easier handling of the phases. ATPS equilibrium parameters are affected by the nature of the added salt and polymer, by the pH and temperature of the system, and by the presence of other compounds (Albertsson, 1986; Coimbra and Teixeira, 2010; Zaslavsky, 1995). An aqueous two-phase system that has potential for lactoferrin partitioning is a system composed of PEG and sodium citrate. It should be emphasised that sodium citrate is biodegradable and non-toxic and can be discharged from plants for biological wastewater treatment (Silva et al., 2009). The transfer of lactoferrin between the phases can be evaluated by using the protein partition coefficient, which is defined as the ratio of the protein concentration in the top phase to that in the bottom phase. Several factors, such as temperature, pH, system composition, the molar mass of the polymer, and the ionic species, as well as the specific characteristics of the lactoferrin (size, hydrophobicity, and surface charge) influence the protein transfer between the phases (Ferreira et al., 2011; Nascimento et al., 2010; Oliveira et al., 2009; Porto et al., 2011).

The objectives of this study were to evaluate the partitioning of lactoferrin in aqueous two phase systems composed of poly(ethylene glycol) and sodium citrate and to characterise the system according to the polymers with average molar masses of 1000 and 4000 g mol^{-1}, a polymer concentration between 14% and 21% (w/w), and pH values of 5.5, 6.5, and 7.5 at 25 °C. Binodal curves and tie-lines for the studied systems were also obtained.

2. Materials and methods

2.1. Chemicals

Lactoferrin was donated by FrieslandCampina DMV (Veghel, Nederland). Poly(ethylene glycol) (PEG) with average molar masses of 1000 g mol^{-1} (PEG1000) and 4000 g mol^{-1} (PEG4000) were purchased from Sigma-Aldrich (St. Louis, MA, USA) and Merck (Darmstadt, Germany). Sodium citrate was supplied by Riedel-de Haën (Seelze, Germany), and citric acid monohydrate was obtained from Pronalab (Lisbon, Portugal). All chemicals were of analytical grade and used without further purification. Double distilled and deionised water was used in all experiments (Milli-Q, Millipore Inc., Bedford, MA, USA; 18.2 MΩ cm).

2.2. Equilibrium data

Phase diagrams were characterised by binodal curves and tie-lines. Binodal curves were determined using the cloud point method (Ferreira and Teixeira, 2011). Stock solutions of PEG (1000 and 4000; 50% w/w) were prepared and then stored at 4 °C. Concentrated solutions (35.3%, w/w) of sodium citrate at pH values of 5.5, 6.5, and 7.5 were prepared by adding a citric acid monohydrate solution (1.2 mol kg^{-1}) to tri-sodium citrate dehydrate solution (1.2 mol kg^{-1}) until the desired pH was reached (CRISON, MicroTT 2050, Spain). The system pH was measured and was close to the pH of the stock citrate solution.

Appropriate amounts of PEG solution, citrate solution, and water were mixed in glass tubes to obtain a heterogeneous system (2 g total mass). The tubes were vigorously shaken and kept in a thermostatic water bath (Grant Instruments, England) at (25 ± 0.1) °C for 20 min. Subsequently, a known amount of water was added to the tube until a homogeneous system was obtained. Additional binodal points were obtained by adding sufficient PEG stock solution dropwise to produce turbidity, followed by the addition of a small amount of water to clarify the system. Weighing was done using an analytical balance (Ohaus, Explorer Pro, Model EP214DC, Switzerland) that was accurate to ±0.2 mg.

The initial composition of the mixture used to construct the binodal curve (PEG, citrate, and water) and the amount of PEG or water added (to produce turbidity or to clarify the system) to the mixture were used to calculate the total system composition, which provided the points in the binodal curve. Each observed binodal curve was adjusted to the empirical Eq. (1) as follows (Merchuk et al., 1998):

$$y = aexp\left(bx^{0.5} - cx^{3}\right) \quad (1)$$