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α-Lactalbumin precipitation from commercial whey protein concentrates

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Abstract

The precipitation of α-lactalbumin (α-La) by acidification of whey and whey protein concentrates (WPCs) was studied in this work. Three different acids (HCl, citric acid and lactic acid) were considered to perform the precipitation step. Sweet whey, WPCs with different protein concentration and whey protein isolates (WPIs) were used as feed solutions. The two organic acids were able to complexate the Ca^{2+} ions. However, when hydrochloric acid was used, protein precipitation was due to the irreversible denaturation of the proteins. The precipitation process was performed at different operating conditions (protein concentration, pH, temperature and acid/Ca^{2+} molar ratio) at laboratory scale in order to be optimised. The optimum initial protein concentration was observed to be around 12 g/L. A temperature of 50 °C, a pH value of 4.0, and an organic acid/Ca^{2+} molar ratio higher than 9 were the optimum values to perform the precipitation at laboratory scale. When the precipitation process was carried out at a pH value close to the isoelectric point (IP) of α-La and it was combined with calcium ion complexation, α-La was observed to precipitate together with BSA and immunoglobulins. However, β-lactoglobulin (β-Lg) remained in solution due to the stabilisation of this protein at low Ca^{2+} concentrations.

Keywords: Whey protein concentrates (WPCs); α-Lactalbumin; Protein precipitation

1. Introduction

Whey protein concentrates (WPCs) are the most important products obtained in whey processing industries. Commercial WPCs may have different protein concentration (between 35 and 80% on a dry basis). The products with higher protein concentration are called whey protein isolates (WPI, protein content higher than 85% on a dry basis). The first group of products (WPCs) are obtained by ultrafiltration with spiral wound or flat organic membranes and their uses are widespread, being added to a great number of foods due to their functional properties. On the other hand, WPI are obtained by ultrafiltration (UF) + diafiltration (DF) and they are included in sports formulas, infant formulas or medical formulas, mainly due to their high protein concentration. Moreover, WPI can be used as a source of pure proteins or they can be hydrolysed to obtain valuable peptides [1,2].

However, whey industries must open the markets and find new applications for WPCs. Their utilization as ingredients in many foods is not enough to absorb total production. Moreover, WPC production is expected to grow in the future due to the increasing amount of whey that is being processed, especially in medium-developed countries.

Infant formula production is other important market that has been explored for the last two decades. Most of the commercial infant formulae contain bovine whey platform mixed with other components (vitamins, minerals, taurine, nucleotides and others) to obtain a product resembling human milk. The main problem with these whey derived products to be used as main ingredients in infant formulae is the presence of β-Lactoglobulin (β-Lg). This protein, absent in human milk, has been demonstrated to be an important source of infant allergy that limits the use of cow's milk as a raw material for the production of milk devised for

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infants. Nevertheless, some commercial products to feed infants are based on whey proteins and most of them have important amounts of β-Lg [3,4].

The separation of the main whey proteins has been an important research topic for many years. Extensive literature (research papers and patents) on this topic has been published in recent years. Different approaches were reported to perform whey protein fractionation by means of different technologies (ion exchange, ion exchange chromatography, selective heat aggregation, chemical additives and membrane technology), but many of them have low efficiency (low protein purity and/or low protein recovery) and others present difficulties at industrial or even pilot plant scale [5–17].

The interest of many researchers in obtaining native proteins from whey limits the technologies that can be used to fractionate or to recover whey proteins. Some of the methods proposed for the fractionation include heat irreversible denaturation that can be an important drawback to use the products in soluble form [7]. Additionally, the fractionation by means of the addition of different chemicals can contaminate the final products.

Membrane technology is considered as a "clean technology" that can overcome part of these problems. However, UF is not selective enough to efficiently separate α-lactalbumin (α-La) and β-Lg, the two main proteins in whey, due to their similar molecular weight (α-La = 14,000 Da and β-Lg (monomer) = 18,000 Da). In order to improve the fractionation process, the pH or the ionic strength must be changed to take advantage of the electrostatic interaction between the proteins and the membrane surface [18]. However, these electrostatic effects usually have much less influence as feed concentration increases (due to the dominant effect of polarization concentration).

Basic studies on the physico-chemical behaviour of whey proteins can give the keys to combine different techniques to improve the fractionation of proteins from whey or WPC. In this sense, Ca^{2+} ion has been demonstrated to play an important role in the stabilisation of α-La and β-Lg [19–21].

The present research work is focused on the investigation of different ways to perform the precipitation of α-La. Different chemicals (inorganic acids, citric acid and lactic acid) were added to acidify the WPC and information on the behaviour of the main whey proteins (α-La, β-Lg, bovine serum albumin (BSA) and immunoglobulins (Igs)) was obtained. The main objective of this work is to optimise the precipitation yield. It is also desirable that the resolubilization of the precipitated protein is as high as possible, what could indicate that the protein is in its native state. The experiments were performed at laboratory scale as an initial step to further research.

2. Experimental

2.1. Feed

Different commercial liquids: sweet whey, WPC and WPI (supplied by Reny Picot, Asturias, Spain) were used as raw material. Their composition is shown in Table 1. WPC and WPI were obtained by UF with spiral wound organic membranes at standard industrial conditions. The feed suspensions were centrifuged and microfiltered with a 20 μm pore size filter and afterwards sodium azide (0.1 wt.%) was added to prevent microorganisms growth. Finally, they were maintained at 4 °C until they were processed.

Fresh sweet whey was demineralised by ion exchange and nanofiltration in order to reduce the amount of soluble calcium. Thus, it was possible to investigate the effect of calcium concentration on the precipitation of whey proteins. The demineralisation was carried out by means of a cationic resin (Lewatit S2568, Bayer Chemicals, Germany) and an anionic resin (Lewatit S3428, Bayer Chemicals, Germany). The nanofiltration process was carried out with a 200 Da molecular weight cut-off spiral wound membrane (DK2540 model, Filtration Engineering, EEUU). The nanofiltered whey was then diluted with water to obtain the same protein concentration as in the demineralised whey produced by ion exchange.

2.2. Analytical methods

The amount of whey proteins (α-La, β-Lg, BSA and Igs) was measured by HPLC (Hewlett-Packard 1050 series, EEUU) according to the method proposed by Resmini et al. [19,22]. A Zorbax HPLC 300 Stable bond C18 column (4.6 mm i.d. × 150 mm and 5 μm particle size, Agilent Technologies,

Table 1
Composition of the commercial liquids used as feed

	Sweet whey	WPC (35%)	WPC (65%)	WPI (80%)
pH	6.2	5.9	5.6	5.6
Dry matter (%)	6.2	13.4	18.0	19.6
Proteins (% dry basis)	10.0	30.1	58.0	78.1
Density (g/L)	1027.3	1062.9	1097.0	1098.0
Lactose (g/L)	49	52	48	23
Ca^{2+} (g/L)	0.61	0.73	0.87	0.85
Na^{+} (g/L)	0.91	0.80	0.79	0.75
K^{+} (g/L)	2.06	2.04	2.00	1.98
α-La (g/L)	0.7	5.6	12.0	15.1
β-Lg (g/L)	3.0	23.0	57.0	71.0
BSA (g/L)	0.5	3.8	9.0	11.5
Igs (g/L)	0.6	3.8	10.3	11.0
α-La/β-Lg	0.23	0.24	0.21	0.21