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Beta-lactoglobulin removal from whey protein concentrates Production of milk derivatives as a base for infant formulas

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Abstract

A commercial whey protein concentrate (WPC65, supplied by ILAS S.A., Spain) was used as raw material to obtain an alpha-lactalbumin (α-La) enriched solution. Firstly, α-La was simultaneously precipitated with bovine serum albumin (BSA) and immunoglobulins (Igs) by adding lactic acid as Ca^{2+} ion sequestrant at a pH value around 4.0. The α-La precipitation process was observed to be reversible. Therefore, when the original pH was recovered the protein became soluble again. The proposed process consists of the following steps: (1) precipitation, (2) centrifugation (I), (3) precipitate washing (twice), (4) centrifugation (II) and finally (5) solubilization of the precipitate. Steps 1 and 2 were studied in a previous work by the same authors. Steps 3–5 were investigated in this work. These steps were performed and optimized at laboratory scale. The final α-La enriched solution obtained had a purity of about 74%, while the recovery of this protein was about 86%. More than 99% of the β-lactoglobulin (β-Lg) present in the WPC was removed in the process. This α-La enriched solution can be considered as a good base to prepare infant formulas. This product is very interesting because of its high water solubility and the very low β-Lg concentration. The concentration of β-Lg in the supernatant fractions was higher than 85% (on a dry basis); therefore these fractions could be further concentrated by membrane technology (ultrafiltration) to obtain a β-Lg enriched concentrated product.

Keywords: Alpha-lactalbumin; Beta-lactoglobulin; Whey protein concentrate; Infant formulas

1. Introduction

Most of the infant adapted milks are produced using cow's milk or its derivatives as a standard base. There are similarities between human milk and cow's milk, but also clear differences. With respect to the protein profile, alpha-lactalbumin (α-La) is a common protein present in both milks. This protein has a high demonstrated nutritional quality (protein efficiency ratio of 4.0, compared to 3.6 for whey and 2.9 for casein) and an excellent biological value (higher than whey protein concentrates (WPCs) and caseins) [1,2].

On the other hand, beta-lactoglobulin (β-Lg) is the main protein in whey (around 50% of total whey proteins), but it is not present in human milk. This protein has been demonstrated to be one of the main sources of infant allergy that limits the use of cow's milk for the preparation of infant formula [3]. In the case of highly sensitive infants this protein can induce allergic reactions even at very low concentrations (generally lower than μM) [4]. Hydrolysed milk formulas are an attempt to avoid exposure to cow's milk proteins early in life. Hydrolysed casein and whey formulas are suggested as a replacement for or supplement to breast-feeding [5]. Proteins can be extensively or partially hydrolysed in infant formula and some authors agree that only extensive hydrolysate should be used to avoid any reaction in highly sensitive infants [6,7]. Hydrolysis of whey proteins (especially β-Lg) is under study in order to cause an extensive protein hydrolysis avoiding even β-Lg traces.

Another approach to produce infant formulas similar to human milk is to try to remove β-Lg from cow's milk or its derivates. However, many of the commercial products to feed infants contain great amounts of β-Lg [8,9]. This is due to the difficulty to design an economic process to remove this pro-

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tein from milk or whey, while maintaining the properties of the rest of proteins. Apart from the nutritional properties of these proteins, it is convenient to maintain their functional properties (especially the solubility if they are going to be incorporated into liquid products).

Whey protein concentrates are standard products obtained in whey processing plants. Their protein concentration (on a dry basis) ranges between 35% and 80% and they are widespread used as food ingredients. Whey protein isolates (WPIs) are protein concentrates with higher protein purity (higher than 85% on a dry basis) and they are usually used as ingredients to prepare high added value products (pure protein as source of peptides or specific food products—sports formulas, medical diets, etc.). Many products are obtained from whey (mainly sweet whey) nowadays, but whey companies try to find new markets for whey and WPCs. Infant formulas represent an interesting market that absorbs great amounts of WPCs. Many companies intend to produce adapted milks with characteristics and composition similar to those of human milk.

Different methods have been used to fractionate proteins or to remove β-Lg from fresh whey (mainly sweet whey) [10–12]. Important efforts have also been made to produce protein streams enriched in α-La and other proteins (bovine serum albumin (BSA), immunoglobulins (Igs), etc.). The most advanced works can be found in the field of ion exchange and ion exchange chromatography [13]. By means of these techniques it is possible to obtain quite high purity proteins at pilot plant and laboratory scales. Ion exchange shows the classic problems associated to resin regeneration and water and chemicals consumption, but it can be considered as a good technique if there is not a competitive alternative.

Membrane processes are considered as clean techniques very useful to treat biological feeds. Due to recent developments in membrane science and technology, new membrane materials and designs as well as membranes with narrower pore size distribution can be found. These developments can have a significant impact on the utilisation of membranes to fractionate proteins if obtaining native state proteins is considered as a key objective [14,15].

Ultrafiltration is the most appropriate membrane technique to fractionate the proteins present in whey. Nevertheless, in spite of the last advances, it is quite difficult to reach a good fractionation of the main whey proteins only by means of ultrafiltration. The molecular weights of the main proteins in whey (α-La = 14,000 Da and β-Lg = 18,000 Da (monomer)) are too close to be efficiently separated. Their isoelectric points (IP) are also very similar (α-La = 4.5–4.8 and β-Lg = 5.2). Therefore it is quite difficult to take advantage of the electrostatic interactions between the membrane and the proteins to improve the separation, at least at industrial scale [12,16–18].

Selective precipitation of one or some of these proteins has been investigated by several authors. Some of these processes involve irreversible protein denaturation (due to extreme temperatures, strongly acidic media or combinations of both) or the addition of different chemicals that must be subsequently removed [19–21]. In those cases the proteins are not obtained in their native state and, although they maintain most of their nutritional interest, they show low solubility what limits their uses as food ingredients.

WPCs obtained by ultrafiltration (alone or combined with diafiltration) seem to be a good starting material to perform whey protein fractionation (or β-Lg removal). They can be partially desalted or treated to reduce the amount of lactose. Products with low phospholipids content can also be obtained by means of thermocalcic treatments.

In a previous work by the authors it was demonstrated that, by means of the combination of a moderately acidic medium (pH 4.0) and low Ca^{2+} concentration (by adding a Ca^{2+} sequestrant (lactic acid), or by removing part of the Ca^{2+} ion with different techniques), the reversible precipitation of α-La was achieved [22]. The proposed process was based in previous research works where the reversible precipitation of α-La takes place by the presence of an intermediate compound (*apo*-α-La) [23–25]. Lactic acid, a common product from whey industries, was able to precipitate α-La, BSA and Igs simultaneously, maintaining most of β-Lg in solution. The best starting material was WPC65, which was a WPC with 65% protein content on a dry basis. Temperature, pH, α-La initial concentration and Ca^{2+}/lactic acid concentration ratio were optimized in that work. However, in spite of the fact that most of the β-Lg remains in solution (the supernatant obtained after centrifugation contains 85% β-Lg on a dry basis), part of this protein was detected in the precipitate, reducing the purity of the α-La fraction.

In this work, the steps that follow the precipitation of α-La were investigated. First, the precipitate was rinsed to remove β-Lg and afterwards the solubilization of α-La was studied. The aim of this work was to obtain a liquid stream enriched in native α-La with low β-Lg concentration.

2. Materials and methods

Commercial WPC65 supplied by ILAS Reny Picot (Asturias, Spain) was used as raw material to perform the quantitative precipitation of α-La. Its composition is shown in Table 1. The WPC was centrifuged and maintained at 4 °C prior to be used. Jacketed glass vessels of 2 L capacity and equipped with temperature control devices were used to carry out the precipitation steps. The operating conditions considered to perform the precipitation experiments were the following: a pH value of 4.0, temperature of 55 °C, continuous stirring for 90 min, and lactic acid concentration of about 0.75 M. These operating conditions were selected from the results obtained in a previous work by the authors [22]. Afterwards the glass vessels content was centrifuged and then the precipitate was washed to remove as much as the adsorbed β-Lg as possible. The precipitate was first washed with NaCl solutions of different concentration and afterwards fresh water acidulated to pH 4.0 with HCl was used. Up to three successive washing steps were performed with each washing solution. Temperature was varied between 15 and 60 °C to study the effect of this variable on protein solubilization degree. After each washing step the product was centrifuged. Finally, the optimum conditions to solubilize the precipitate were investigated. As α-La precipitation was demonstrated to be reversible,

Table 1
Composition of the raw materials and the different fractions obtained

	Sweet whey[a]		WPC65		Precipitate		Washed precipitate		α-La enriched fraction	
	(g/L)	(%)	(g/L)	(%)	(g/L)	(%)	(g/L)	(%)	(g/L)	(%)
Volume (L)	–	–	2	–	0.8	–	0.8	–	1.6	–
α-La (g/L, %[b])	0.7	14.6	12.0	13.6	29.1	27.0	28.6	55.4	12.9	74.2
β-Lg (g/L, %[b])	3.0	62.5	57.0	64.5	58.0	53.8	3.8	7.4	0.95	5.4
BSA (g/L, %[b])	0.5	10.4	9.0	10.2	5.06	4.7	4.7	9.1	0.74	4.3
Igs (g/L, %[b])	0.6	12.5	10.3	11.7	15.6	14.5	14.6	28.3	2.76	15.9
Total protein (g)	4.8	–	176.6	–	86.2		41.3	–	27.8	–
(α-La/β-Lg) ratio	0.23	–	0.21	–	0.5		7.5	–	13.7	–
α-La recovery (g, %)	–	–	–	–	23.3	97.0	22.9	95.3	20.6	86.0
BSA recovery (g, %)	–	–	–	–	4.04	22.0	3.76	20.9	1.18	6.6
Igs recovery (g, %)	–	–	–	–	12.48	60.6	11.7	56.7	4.42	21.4

[a] Data included as reference.
[b] Percent on a dry basis.

an increase in the pH value and in Ca^{2+} ion concentration was expected to cause the solubilization of the precipitated α-La [22]. To perform this step, $CaCl_2$ solutions of different concentration were used, while temperature was varied between 25 and 55 °C. The pH of the solutions was maintained at 7.5 by means of the addition of NaOH.

Each of the fractions obtained in the different steps of this work were analysed by means of high performance liquid chromatography (HPLC) and gel electrophoresis. These analyses were performed on the liquid fractions. The concentration and the amount of proteins in the precipitate fractions were calculated by mass balance, taking into account the amount of proteins in the feed and in the supernatant fraction. The results were expressed in terms of precipitation yield, which is the percentage of precipitated protein with respect to the initial amount of protein in the feed. By means of HPLC it was possible to measure the concentration of proteins in the liquid streams. Gel electrophoresis was used in this work as a qualitative technique to identify the proteins that are present in the liquid samples.

The concentration of whey proteins was determined according to the Resmini's method [26] using a HP 1050 series HPLC chromatograph (Hewlett Packard, Agilent Technologies, Spain). It was equipped with a Zorbax reverse phase column (HPLC300 Stable bond C-18, Agilent Technologies, Spain) of 4.6 ID × 150 mm and 5 μm particle size. The chromatograph was equipped with a UV detector, which was set to 210 nm. Peak area values were calculated using a highly sensitive ChemStation system (Agilent Technology, USA). It was even able to integrate very small peaks that were not visible.

Sodium dodecyl (lauryl) sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was also used to confirm the presence of proteins in the different samples. The analyses were performed according to the method proposed by Laemmli [27]. A Bio-Rad Mini Protean III Electrophoresis Cell (Bio-Rad Laboratories, USA) was used. It was connected to a Power Pac 300 electric supply. A constant voltage (200 V) was fixed during the whole analysis process (50 min).

3. Results and discussion

The complete process followed for the production of an α-La enriched fraction is shown in Fig. 1. In this figure, the experimental range studied for each variable is included. The optimum value selected is indicated between brackets. The first steps (centrifugation–precipitation–centrifugation) were performed and optimized in a previous work by the authors [22]. Washing, centrifugation and solubilization steps were investigated in this work. In the figure, the α-La purity and recovery as well as the α-La/β-Lg concentration ratio obtained in each step are indicated. The main objective of this work is the production of an α-La enriched fraction with the highest amount of α-La and the lowest amount of β-Lg. A high recovery of α-La is desired as well. This figure will be commented throughout the text.

The precipitation of α-La at a pH value of about 4.0 and in the presence of a Ca^{2+} sequestrant as lactic acid (0.75 M) is a temperature dependent process. As can be seen in Fig. 2, the two main proteins present in WPC (β-Lg and α-La) can be separated depending on the temperature value. At room temperature the separation is not possible, but when temperature increases α-La precipitation yield increases much faster than β-Lg precipitation yield. At temperatures higher than 50 °C, the precipitation yield of α-La (together with BSA and Igs) is much higher than β-Lg precipitation yield, what makes possible their efficient separation. The precipitation of α-La is almost quantitative at a temperature of 55 °C, meanwhile β-Lg co-precipitates to a much lower extent. Theoretically, β-Lg stability increases at low Ca^{2+} concentration [25], however, in Fig. 2 a slight increase in the precipitation yield with temperature is observed. In a previous work [22] it was suggested that β-Lg can be adsorbed on the precipitate.

The precipitate obtained was separated from the supernatant by centrifugation. The next step was to try to remove the adsorbed β-Lg from the precipitate. According to several authors [28,29], β-Lg does not become unstable by the lack of Ca^{2+} ion, however it is present in the precipitate obtained. The washing of this precipitate demonstrated that this protein was in fact adsorbed on the precipitate. The presence of β-Lg in the pre-

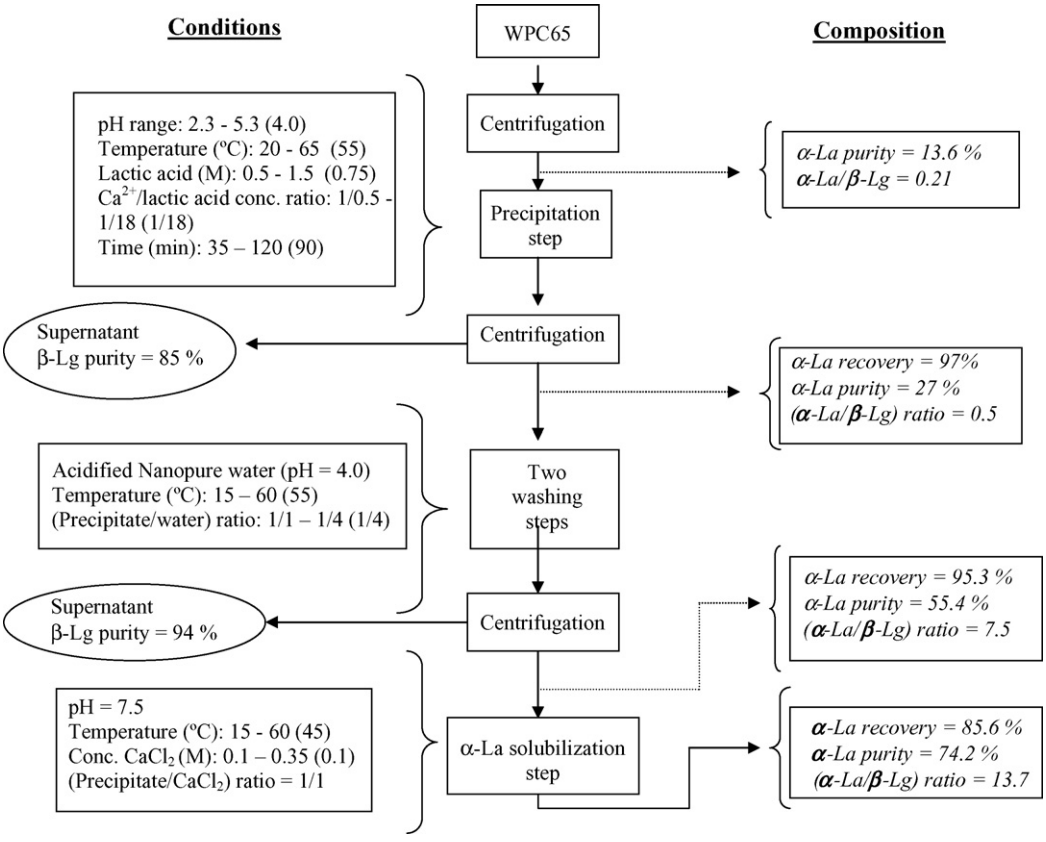

Fig. 1. Flowsheet of the proposed process to obtain a liquid stream enriched in α-La. The values between brackets represent the optimum value of the corresponding variable.

cipitate fraction was probably enhanced by the high protein concentration used in this work (WPC65 as starting feed), while it could be less significant if the initial protein concentration was lower. In order to remove as much of the β-Lg adsorbed on the precipitate as possible, some of the washing steps were carried out with NaCl solutions of different concentration, as they have been recommended in previous works [17]. Apparently, an increase in the ionic strength improved the recovery of β-Lg from the precipitate, but great amounts of other proteins (Igs and α-La) were also dissolved. However BSA remained in the precipitate almost completely.

According to those experiments, washing with NaCl solutions is not recommended as α-La is partially solubilized together with β-Lg, thus reducing the recovery of α-La with respect to the initial amount of this protein. The results obtained are similar to those obtained by other authors [24,25], however some discrepancies in the amount of α-La and Igs lost in the supernatant phase can be found. Losses of α-La and Igs are slightly higher in this work. The discrepancies can be explained as a result of the different starting material used to perform the experiments. In this work WPC65, with 12 g/L of α-La, was used, while less concentrated WPCs or slightly concentrated sweet whey were used in the cited literature.

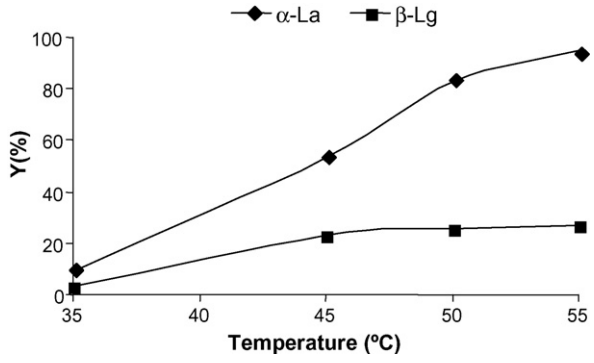

Fig. 2. Precipitation yield (Y %) of α-La and β-Lg *vs*. temperature. Operating conditions: pH 4.0; lactic acid concentration = 0.75 M.

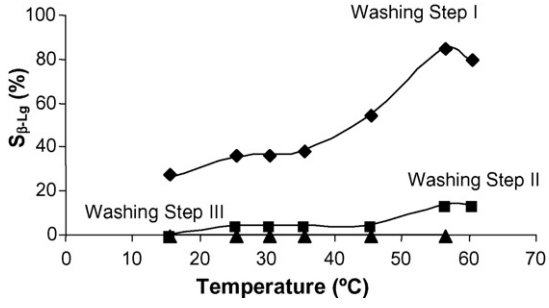

Fig. 3. Influence of temperature on the solubilization degree of β-Lg (S) after three successive washing steps performed with acidulated fresh water at a pH value of 4.0.

Fig. 3 shows the recovery rate of β-Lg at different temperatures after three successive washing steps performed with acidulated fresh water (no NaCl added). The successive washing steps were carried out at temperatures between 15 and 60 °C. The pH value was maintained at 4.0 in order to minimise the solubilization of α-La. The recovery of β-Lg was almost 100% after two washing steps at temperatures around 55 °C. The third washing step was not necessary as no β-Lg recovery was observed. At temperatures higher than 55 °C the recovery of β-Lg in the supernatant fraction decreased.

In Table 1 the protein content of the different fractions obtained in each step is shown. Sweet whey has been included in the table only as a reference. It can be observed that α-La/β-Lg concentration ratio is around 0.21–0.23 both in sweet whey and in WPC65. After the precipitation step this ratio increases up to 0.5 and to 7.5 after the washing step. The washing step is compulsory when high α-La purity is the main objective. The retention of β-Lg in the precipitate is higher if it is obtained from a highly concentrated starting raw material, as in the case of WPC65.

The composition of the washed precipitated is shown in Table 1 as well. It can be observed that 55% of the proteins (on a dry basis) correspond to α-La. This protein is now the dominant protein and together with the Igs fraction, both account for 80% of the total proteins. Additionally, more than 95% of the α-La and about 90% of the Igs present in WPC65 were recovered in the precipitated fraction. This product can be very interesting by itself as a base to prepare infant formulas or to perform a subsequent protein hydrolysis to obtain valuable peptides.

Native proteins are characterised by their solubility in a wide pH range. Thus, they can be added to a number of different foods (acidic and alkaline drinks, baby foods, special sports formulas, etc.) in which the proteins must be soluble. On the other hand, many of the processes reported to obtain pure proteins from whey have the drawback of causing protein denaturation due to different reasons, such as thermal degradation, addition of non-food-compatible chemicals, etc. [3]. The next set of experiments had the objective of investigating the conditions to solubilize the previously obtained precipitate.

The precipitate was submitted to a change in pH and in Ca^{2+} ion concentration in order to confirm the reversibility of the conversion of α-La into *apo*-α-La, which was responsible for the precipitation of this protein in the first step of this process. Theoretically, if the original conditions are recovered, α-La will become soluble again. In Fig. 4 the solubilization degree (S) of each protein in different conditions is shown. All the experiments were performed at a pH value of 7.5 and at a temperature of 55 °C. As can be seen, α-La solubilization is very high in all cases. Therefore, it can be suggested that the protein recovers its native state in those conditions. However, when a mineral acid such as HCl was used, the precipitation of the protein was possible [22], but the solubilization of α-La afterwards was very poor. Increasing Ca^{2+} concentration (in the form of $CaCl_2$) had no significant effect in the solubilization of the proteins. BSA and Igs solubilization was only partial (around 40%) and β-Lg did not show a clear trend, probably due to the analytical

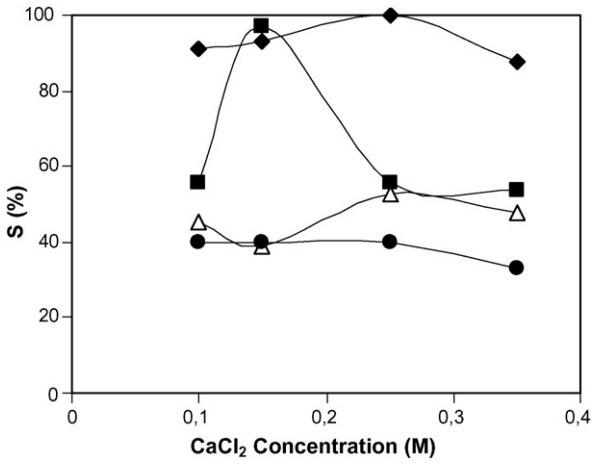

Fig. 4. Influence of $CaCl_2$ concentration on protein solubilization degree (S). Operating conditions: pH 7.5; temperature = 45 °C ((♦) α-La; (■) β-Lg; (△) BSA; (●) Igs).

difficulties when a very low amount of protein is present in the sample (less than 0.8 g/L in the precipitate).

However the influence of temperature on the solubilization degree of α-La was significant as can be seen in Fig. 5. The optimum temperature to perform the solubilization was observed to be around 45 °C. Higher temperatures reduced the recovery of α-La. As can be seen, a great amount of α-La was recovered in the solution (higher than 90%).

After the solubilization step, α-La purity increased, as can be observed in Table 1 and in Fig. 1. The final recovery of soluble α-La was around 85% and a purity of 75% was achieved. The presence of β-Lg in the final product was very low (around 5%). The final (α-La/β-Lg) concentration ratio was observed to be around 14, what means that the initial ratio was multiplied by a factor of 60.

In Figs. 6 and 7 HPLC and electrophoresis profiles of different samples obtained from each step are shown, respectively. In Fig. 6 (line I), the four proteins studied can be clearly observed in the protein profile of the original WPC65. Line II shows that β-Lg is the dominant protein in the supernatant (additionally the shape of the peak seems to suggest that this protein is in

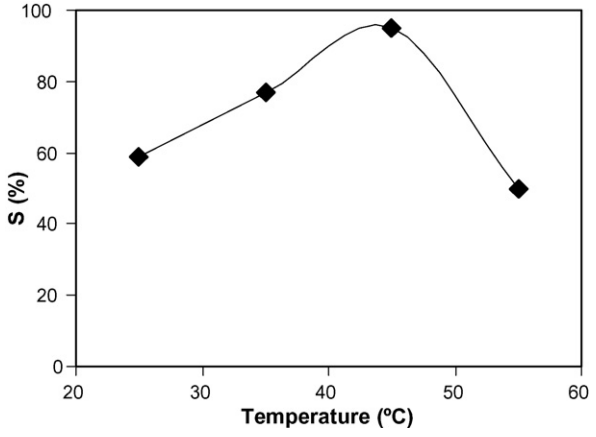

Fig. 5. Influence of temperature on the solubilization degree (S) of α-La when the precipitate was washed with a solution of 0.1 M $CaCl_2$ at a pH value of 7.5.

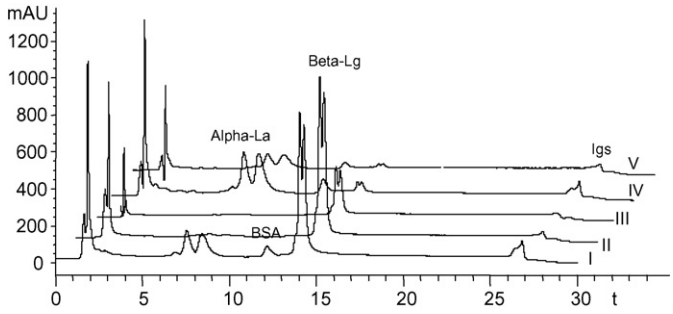

Fig. 6. HPLC profile obtained after each process step. Line I: WPC65; line II: supernatant after precipitation + centrifugation; line III: supernatant after precipitate washing; line IV: precipitate solubilized in 0.1 M $CaCl_2$ (first washing) (chromatograms IV and V are five times less attenuated than the rest of the chromatograms in order to be able to see all the protein peaks); line V: precipitate solubilized in 0.1 M $CaCl_2$ (second washing) (chromatograms IV and V are five times less attenuated than the rest of the chromatograms in order to be able to see all the protein peaks).

its native state). When this line was analysed, a small amount of BSA was detected (in spite of the fact that the peak was not observed on the chromatogram). This point was confirmed from the electrophoresis pattern shown in Fig. 7 (line 3). It can be observed a band that corresponds to β-Lg and a very thin BSA band.

The HPLC and the electrophoretic analyses performed on the supernatant obtained after washing the precipitate are shown in Figs. 6 (line III) and 7 (line 4). Both techniques revealed that there was a great amount of β-Lg in this fraction. In the case of HPLC, a small peak indicated partial losses of Igs, which accounted for about 40% of losses with respect to the initial amount of Igs in WPC65. Lines IV and V in Fig. 6, and 5 and 6 in Fig. 7 show the protein profile of the final solution obtained,

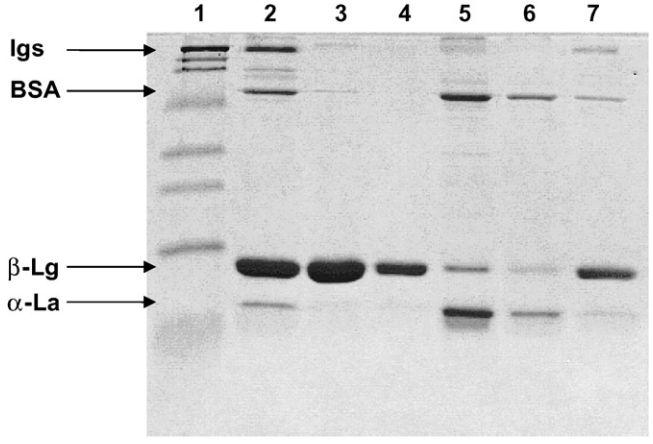

Fig. 7. Gel electrophoresis of proteins from the samples obtained after each process step. Line 1: protein reference (molecular weight (Da) of the proteins used as reference: α-La: 14,000; β-lg: 18,000; BSA: 69,000; Igs: 150,000); line 2: WPC65 (1/100 dilution); line 3: supernatant after precipitation + centrifugation; line 4: supernatant after precipitate washing; line 5: precipitate solubilized in 0.1 M $CaCl_2$ (first washing) (lines 5 and 6 were obtained from samples five times less diluted in order to be able to see the bands); line 6: precipitate solubilized in 0.1 M $CaCl_2$ (second washing) (lines 5 and 6 were obtained from samples five times less diluted in order to be able to see the bands); line 7: WPC65 (1/200 dilution).

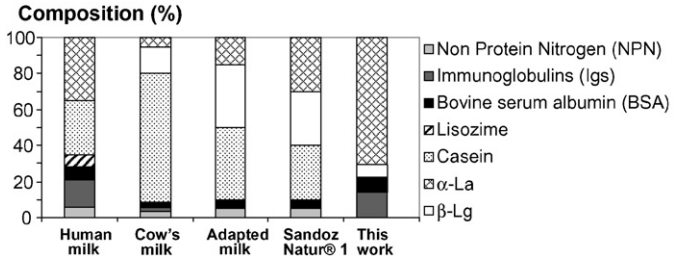

Fig. 8. Comparison among protein content in human milk, cow's milk, commercial adapted milks and in the α-La enriched fraction obtained in this work.

which was enriched in α-La. The major bands correspond to α-La. A BSA band was also observed in line 5 (Fig. 7) and it was also detected in HPLC chromatograms. Lines 5 and 6 in Fig. 7 were obtained from samples that were less diluted in order to be able to see the bands. For the same reason, chromatograms IV and V in Fig. 6 were less attenuated thus being able to see the peaks. Therefore the size of the peaks and the intensity of the bands obtained from the corresponding samples are not comparable with those obtained from the rest of samples. The values of protein concentration shown in Fig. 1 and Table 1 were calculated taking into account the peak areas obtained from the chromatograms using samples with the same concentration and in the same conditions.

Finally, the α-La enriched fraction had a purity of about 74% (α-La recovery of about 86%). Other proteins present were Igs (16% purity and 21% recovery rate) and BSA (4.3% purity and 6.6% recovery rate). The protein that showed the lowest concentration was β-Lg (around 5%) and more than 99% of this protein was removed from the WPC65.

Fig. 8 compares protein concentration in human milk, cow's milk and commercial infant adapted milks with protein concentration in the α-La enriched fraction obtained in this work. As can be seen in Fig. 8, human milk has great amounts of α-La and caseins, but it does not have β-Lg. The ratio of casein to whey proteins is completely different in human and cow's milk. The amount of β-Lg in whey and WPC (compared to the rest of the proteins) is even higher than in cow's milk. When infant formulas are produced using cow's milk or whey as a base, most of the commercial products obtained contain β-Lg (see the protein composition of standard infant adapted milks and that of the commercial adapted milk Sandoz Natur® 1 in Fig. 8). The product obtained in this work at laboratory scale contains α-La as the dominant protein. This protein has the same properties in cow's and human milk. The product contains also significant amounts of Igs, while β-Lg is present in a much lower extent.

4. Conclusions

An α-La enriched soluble fraction (74% purity) was obtained at laboratory scale using a commercial WPC as raw material (WPC65). The recovery of α-La was around 86%. An interesting characteristic of this product is the very low amount of β-Lg (about 5% of total protein content). More than 99% of this protein was removed from the initial product (WPC65). In the precipitation step studied in a previous work by the authors, it

was observed that part of the β-Lg was adsorbed on the precipitate. In this work, this protein was removed from the precipitate by means of an adequate washing process. Two washing steps performed with acidified water at 55 °C were enough to remove almost 100% of the adsorbed β-Lg. The best conditions to perform the solubilization of α-La were a pH value of about 7.5 and a temperature of 45 °C. Part of the BSA and Igs that co-precipitated together with α-La were also solubilized in this step.

However, there are some points that must be further investigated, such as the process scale up and the reasons why only part of the precipitated BSA and Igs were solubilized in the last step of the proposed process. The final precipitate, which contained about 50% of the initial BSA and Igs, could be also considered as an interesting product. Once the process is studied at pilot plant scale, the last step that must be investigated is the protein concentration in the α-La enriched solution, as well as the protein concentration in the different supernatant phases obtained, which are rich in β-Lg.

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References

[1] E. Fosum, Nutritional evaluation of whey protein concentrates and their fractions, J. Dairy Sci. 57 (1973) 665–670.
[2] E. Renner, Milk Dairy Products in Human Nutrition, Volkswirtsch Verlag, München, 1983.
[3] Y. Uchida, M.M. Shimatani, T. Mitsuhashi, M. Koutake, Process for preparing a fraction having a high content of α-lactalbumin from whey and nutritional compositions containing such fractions, US Patent 5,503,864, 1996.
[4] M.T. Veledo, M. Fuentes, J.C. Diez-Masa, Analysis of trace amounts of bovine β-lactoglobulin in infant formulas by capillary electrophoresis with on-capillary derivatization and laser-induced fluorescence detection, J. Sep. Sci. 28 (2005) 941–947.
[5] S.H. Arshad, Primary prevention of asthma and allergy. Current reviews of allergy and clinical immunology, J. Allergy Clin. Immunol. 115 (2005) 1–14.
[6] Y.H. Chan, L.P. Shek, A.M. Qwak, B.W. Lee, Use of hypoallergenic formula in the prevention of atopic disease among Asian Children, J. Pediatr. Child Health 38 (2002) 84–88.
[7] Y. Pouliot, M.C. Wijers, S.F. Gauthier, L. Nadeau, Fractionation of whey protein hydrolysates using charged UG/NF membranes, J. Membr. Sci. 158 (1999) 105–114.
[8] B. Lönnerdal, S. Atkinson, Human milk proteins, in: R.G. Jensen (Ed.), Handbook of Milk Composition, Academic Press, San Diego, 1995.
[9] S. Rudloff, C. Kunz, Protein and nonprotein nitrogen components in human milk, bovine milk and infant formula: quantitative and qualitative aspects in infant nutrition, J. Pediatr. Gastroenterol. Nutr. 24 (1997) 328–344.
[10] L. Roger, J.L. Maubois, G. Brulé, M. Piot, Process for obtaining an α-lactalbumin enriched product from whey, and uses thereof, US Patent 4,485,040, 1984.
[11] R.C. Bottomley, Process for obtaining concentrates having a high α-lactalbumin content from whey, US Patent 5,008,376, 1991.
[12] A. Muller, B. Chaufer, U. Merin, G. Daufin, Purification of alpha-lactalbumin from a prepurified acid whey: ultrafiltration or precipitation, Lait 83 (2003) 439–451.
[13] K.R. Kristiansen, J. Otte, R. Ipsen, K.B. Qvist, Large-scale preparation of β-lactoglobulin A and B by ultrafiltration and ion-exchange chromatography, Int. Dairy J. 8 (1998) 113–118.
[14] A. A. Zydney, Protein separations using membrane filtration: new opportunities for whey fractionation, Int. Dairy J. 8 (1998) 243–250.
[15] P. Punidadas, S.S.H. Rizvi, Separation of milk proteins into fractions rich in casein or whey proteins by cross flow filtration, Food Res. Int. 31 (1998) 265–272.
[16] D. Lucas, M. Rabiller, L. Millesime, B. Chaufer, G. Daufin, Extraction of α-lactalbumin from whey protein concentrate with modified inorganic membranes, J. Membr. Sci. 148 (1998) 1–12.
[17] B. Gésan-Guiziou, G. Daufin, M. Timmer, D. Allersma, C. van der Horst, Process steps for the preparation of purified fractions of α-lactalbumin and β-lactoglobulin from whey protein concentrates, J. Dairy Res. 66 (1999) 225–236.
[18] B. Cheang, A. Zydney, Separation of α-lactalbumin and β-lactoglobulin using membrane ultrafiltration, Biotech. Bioeng. 83 (2003) 2001–2009.
[19] C. Kiesner, I. Clawin-Rädecker, H. Meisel, W. Buchheim, Manufacturing of α-lactalbumin-enriched whey systems by selective thermal treatment in combination with membrane processes, Lait 80 (2000) 99–111.
[20] C. Wu, Whey treatment process for achieving high concentration of α-lactalbumin, US Patent 6,312,755 B1, 2001.
[21] L. Da Fonseca, L.R. Bradley, Fractionation of whey proteins by complex formation, US Patent 10,095,856, 2002.
[22] M.E. Lucena, S. Alvarez, C. Menéndez, F.A. Riera, R. Alvarez, Alpha-lactalbumin precipitation from commercial whey protein concentrates, Sep. Purif. Technol., in press.
[23] R.J. Pearce, Whey protein recovery and whey protein fractionation, in: J.G. Zadow (Ed.), Whey and Lactose Processing, Elsevier Applied Science, New York, 1992.
[24] C. Bramaud, P. Aimar, G. Daufin, Thermal isoelectric precipitation of α-lactalbumin from whey protein concentrate: influence of protein calcium complexation, Biotech. Bioeng. 47 (1995) 121–130.
[25] C. Bramaud, P. Aimar, G. Daufin, Optimisation of a whey protein fractionation process based on the selective precipitation of α-lactalbumin, Lait 77 (1997) 411.
[26] P. Resmini, L. Pellegrino, R. Andreini, F. Prati, Determinacione delle sieroproteine solubili del latte per HPLC in fase inversa, Sci. E. Technica Latterio-Casearia 50 (1989) 7–23.
[27] U.K. Laemmli, Cleavage of structural proteins during the assembly of the head of bacteriophage T4, Nature 227 (1970) 680–685.
[28] V.R. Harwolker, Measurement of thermal denaturation of β-lactoglobulin at pH 2.5, J. Dairy Sci. 63 (1980) 1043–1051.
[29] L.A. Tedford, C.J. Schaschke, Induced structural change to β-lactoglobulin by combined pressure and temperature, Biochem. Eng. J. 5 (2000) 73–76.