Whey proteins solubility curves at several temperatures values

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Summary

This work showed the whey proteins solubility curves, according with temperature and pH conditions. The product constituted of a whey protein isolate obtained from cow milk (ALACENTM 895), acquired by New Zeland Milk Products Ltd. There is a straight analogy between fouling and protein unfolding when milk derived fluids are processed in equipments of heat exchangers, where whey proteins are unfolded in an irreversible way, exposing hidrophobic groups, and they become insoluble and form aggregates. An integrated study was conducted on the effects of temperature and pH on the solubility of whey proteins. The solubility was determined experimentally in the temperature range of 40-90 °C, and pH range of 5.0 - 6.8. The results showed that, both the temperature and pH influenced in the protein solubility; besides, the solubility values were minimum at the pH 5.0 for all temperature values. It was also observed that solubility decreased with temperature increased.
Keywords: whey, protein, temperature, pH, solubility.

Resumo

No presente trabalho foram esboçadas as curvas de solubilidade das proteínas presentes no soro do leite, em função da temperatura e do pH. O produto utilizado para tal análise consistiu de um isolado protéico obtido a partir do soro do leite de vaca (ALACENTM 895), adquirido junto a New Zeland Milk Products Ltd. Existe uma ligação direta entre incrustação e desnaturação das proteínas quando os fluidos derivados do leite são processados em equipamentos de troca térmica, onde as proteínas do soro se desnaturam de uma maneira irreversível, expondo os grupos hidrofóbicos livres até que se tornem insolúveis e formem agregados. No presente trabalho foi conduzida uma análise completa dos efeitos da tem-

peratura e pH na solubilidade das proteínas presentes no soro do leite. Tal solubilidade foi determinada experimentalmente para temperaturas na faixa de 40-90 °C, e pHs entre 5,0 e 6,8. Os resultados mostraram que tanto a temperatura quanto o pH influenciaram na solubilidade protéica; além disto, os valores da solubilidade protéica foram mínimos no pH de 5,0, em todas as temperaturas estudadas. Também observou-se que a solubilidade protéica decresceu com o aumento da temperatura.
Palavras-chave: soro do leite, proteína, temperatura, pH, solubilidade.

1. Introduction

There are about 4000 species of mammal, the milk from each of which is species-specific. Although milk of 5 or 6 species are used by man, in addition to human milk, only bovine milk is used as a source of functional properties of bovine milk proteins (Fox, 1993).

Whey proteins represent 20% of the nitrogen in bovine milk. These proteins can be fractionated into two principal groups by making acid whey to 50% saturation with $(NH_4)_2SO_4$, i.e. an insoluble b-lactoglogulin fraction (~10% of whey protein N) and a soluble a-lactalbumin fraction (most components of which are not true albumins). Both whey protein fractions are heterogeneous. The lactalbumin fraction contains b-lactoglobulin, a-lactalbumin, blood serum albumin and several minor proteins. The principal globulins in bovine milk belong to the immunoglobulin G class with smaller amounts of IgA, IgM and IgE. The concentrations (g/L) of b-lactoglobulin, a-lactalbumin, blood serum albumin and immunoglobulins, in normal bovine milk are 2-4, 1-1.5, 0.1-0.4 and 0.6-1.0, respectively (Wong et al, 1996).

Among the functional properties of proteins, solubility is of primary importance due to its significant influence on the other functional properties of proteins. In general, proteins used for functionality are required to have high solubility, in order to provide good emulsion, foam, gelation and whipping properties (Nakai & Chan, 1985, Wit, 1989). In other words, a decrease in protein solubility affects in unfavorable manner its functionality (Vojdani, 1996). Solubility of proteins relates to surface hydrophobic (protein-protein) and hydrophilic (protein-solvent) interaction; in food case, such solvent is the water, and therefore the protein solubility is classified as a hydrophilic property.

The protein solubility is a function of many factors, such as environmental factors, mainly pH and temperature. The pH of the solution affects the nature and the distribution of the protein net charge. Generally, the proteins are more soluble in low (acids) or high (alkaline) pH values because of the excess of charges of the same sign, producing repulse among the molecules and, consequently, contributing to its largest solubility (Fox, 1989).

According with several authors, a protein usually has the least solubility at the isoeletric point (pI), i.e., protein-protein interaction increases because the electrostatic forces of the molecules are at a minimum and less water interacts with the protein molecules. This is a favorable condition for protein molecules to approach each other and aggregate, and possibly precipitate. At pH values above and below the pI, where a protein has a net negative or positive charge, more water interacts with the protein charges. Net charges and charge repulsion contribute to greater protein solubility and the protein may stay in the solution. For a great number of proteins, their pI are in the range of 3.5 and 6.5 (Vojdani, 1996). At extreme acidic or basic pH values, the protein may unfold, exposing more hydrophobic groups (Daniele et al., 1994, Vojdani, 1996, Wong et al, 1996, Mann & Malik, 1996, Helm and Alicia, 2004, Pelegrine and Gasparetto, 2004).

The temperature is also the factor that has influence in the protein solubility. In general, protein solubility is increased with temperature between 40-50°C. When the temperature of the solution is raised high enough for a given time, the protein is denatured. Proteins are denatured by the effect of temperature on the non-covalent bonds involved is stabilization of secondary and tertiary structure; for example, hydrogen, hydrophobic and electrostatic bonds (Wong et al, 1996). When the secondary and tertiary structures of a protein are unfolded, the hydrophobic groups (i.e, the sulfidril groups SH -, initially inside the protein molecules) interact and reduce water binding. Any hydrophobic interactions lead to aggregation, followed by coagulation and precipitation. Denaturation decreases protein solubility compared to native protein, and leads to aggregation and difficulty of reversal upon cooling (Mine, 1995, Kim, 1998, Langendorff et al., 1999, Pelegrine and Gasperetto, 2005). Whey proteins are completely denatured at high temperatures in about ten minutes. The immunoglobulin fraction of the whey protein is denatured first followed by serum albumin, b-lactoglobulin is less affected under the same heating conditions, and a-lactalbumin is the most resistant of the whey protein fraction (Mutilang & Kilara, 1985). The proteose peptone protein fraction is not sensitive to heat (Mutilang & Kilara, 1985).

2. Material and methods

2.1. Whey proteins

The product constituted of a whey protein isolate obtained from bovine milk (ALACENTM 895), acquired by New Zeland Milk Products Brazil Ltd. Before solubility analysis, some physical-chemistry analyses were

accomplished for the characterization of the product, such as the moisture (A.O.A.C, 1980, method 16192), total lipids (Bligh & Dyer, 1959), ashes (A.O.A.C, 1980, method 16196) and protein contents (A.O.A.C, 1980, method 38012).

2.2. Protein Solubility

Morr et al. (1985) developed a collaborative study and reliable procedure for determining the solubility of food protein products. Whey proteins solubility determination followed this methodology where, about 0.5 g of dry protein product was accurately weighed in Bosch-SEA200 semi-analytic scale, into separate 0.1 L standard beakers and several aliquots of 5.85 g/L NaCl solution were added with stirring to form a smooth paste. Additional 5.85 g/L NaCl solution was then added to bring the total volume of the dispersion to about 0.04 L. Soon after, the mixture was transferred to holding beakers, which circulated hot water inside of them. These holding beakers were coupled to a thermostatic bath (Nova Técnica), and the temperature was maintained in agreement with the interest of each experiment. In this experiment, the referring temperatures varied from 40 to 90°C, the maximum temperature allowed in the pHmether. The pH values varied from 5.0 to 6.8, and it was maintained in agreement with the interest of each experiment by adding NaOH 4.0 g/L or HCl 3.65 g/L solutions, when it was necessary, after the reading pHmether (Marconi – model PA200). The photo of that procedure is illustrated in fig. 1. After agitation of the samples, during 1 hour, in a thermostatic agitator (Tecnal - model Dubnoff), the dispersion was transferred to a 0.10 L volumetric balloon, and the volume was completed with NaCl 5.85 g/L. Then the solution was centrifuged to 13500 rpm during 30 minutes at 4?C, in a Lodan (17R model) centrifuge with SS-9 rotor, and the supernatant was then filtered in Whatman paper nº2. Aliquots of 0.002 L were taken and their soluble protein contents was determined using the micro-Kjeldahl method (A.O.A.C., 1980–Method 38012). For each temperature and pH case, the experiments were carried out four times and it was calculated the average values of them.

The soluble protein percentage was calculated through the following equation:

$$P.S. = \frac{\left[A(g/L)\cdot 50\right]}{\left[W(g)\cdot S/_{100}\right]}\cdot 100 \qquad (1)$$

where:

$P.S.$ = soluble protein content in the sample [g/100g];

A = supernatant protein concentration [g/L];

W = sample weigh [g];

S = sample protein concentration [g/100g].

Each experiment was accomplished in duplicated, being the soluble protein content the resulting average of the two values.

Figure 1. Schematic diagram to adapt the temperature and pH conditions.

3. Results and discussion

3.1. Product characterization

The product lot (6949) used to calculate the protein solubility presented the whey centesimal composition characteristic, and the results are summarized in table 1.

Table 1. Centesimal composition of ALACENTM 895.

Analyses	Content (g/100g)
Moisture	4.940
Total Lipids	0.279
Ashes	3.540
Protein	80.33

3.2. *Solubility values*

The table (2) shows the protein solubility average values of two replicates, for the ALACENTM 895. The values present in that tables were calculated from equation (1). The values of the whey proteins solubility are illustrated in fig. 2.

Table 2. Protein solubility values of whey proteins.

$T_o{}^oC$	pH	*P(g)*	$\psi(g/ml)$	*P.S. (%)*
40	5.00	1.015	0.00690.0067	68.2866.27
	6.00	1.023	0.00790.0081	77.5678.76
	6.80	1.015	0.01100.0108	100.00100.00
50	5.00	1.001	0.00310.0030	31.3030.50
	6.00	1.002	0.00520.0048	51.9648.04
	6.80	1.026	0.00880.0088	85.4287.36
60	5.00	1.004	0.00610.0050	55.1854.22
	6.00	1.006	0.00790.0073	78.1571.96
	6.80	1.002	0.00680.0068	67.8867.94
70	5.00	1.009	0.00560.0055	61.1059.42
	6.00	1.002	0.00870.0089	86.5289.67
	6.8	1.020	0.00800.0078	78.4976.20
80	5.00	1.003	0.00220.0024	22.3124.04
	6.00	1.003	0.00310.0029	45.1951.85
	6.80	1.026	0.00740.0076	73.2975.62
90	5.00	1.007	0.00150.0013	14.5912.54
	6.00	1.002	0.00450.0051	30.7429.84
	6.8	1.004	0.00330.0034	32.8134.33

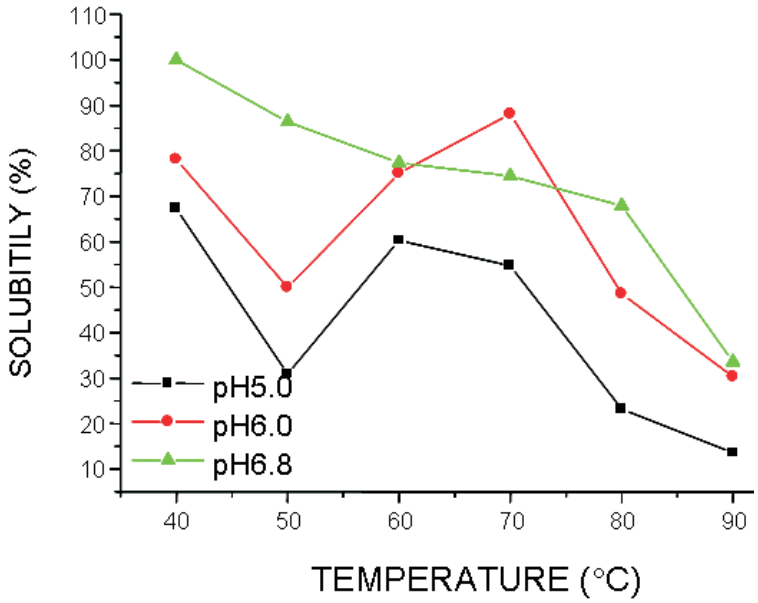

Figure 2. Effect of pH and temperature on the whey protein solubility.

From table 2 and fig.2 it could observe that, for any temperature, the solubility values were minimum at pH of 5.0 (near isoelectric point of whey proteins); in those conditions the protein-protein interactions increase because the electrostatics forces are minimum and less water interact with the protein molecules. At 40°C, where the protein structure was less affected due to heat action, it was observed that for pHs above 5.0 (isoelectric point of b-lactoglobulin) the solubility increased, because in those conditions the proteins had positive or negative net charges, and more water interacted with the protein molecules. About the previous illustration it could be observed that near to neutral pH (pH=6.8) the solubility decreased with the temperature due to the effect of the temperature in the bonds involved in the secondary and tertiary structures stabilization, where its unfolding favors the interaction among the hydrophobic groups, reducing the protein-water interactions, indicating that the thermal protein denaturation occurred. At the pH of 5.00 and 6.00 protein solubility increased with the temperature where temperature increased from 50°C to 60°C (pH 5.00) and from 50°C to 70°C (pH 6.00), indicating that there was not coagulation nor aggregation between the protein molecules, possibly because in those pH value the b-lactoglobulin is a dimmer that is dissociated in monomers at 50°C and only above 60°C (at pH 5.0) or 70°C (at pH 6.0) the proteins unfold and the hydrophobic groups react.

4. Conclusion

The integrated study provided valuable information for whey protein solubility analysis. Solubility of whey proteins could be altered by temperature and pH changes, concluding that both the temperature and the pH influenced in this property. Besides, it was also observed an interaction among the temperature and pH on whey proteins solubility, being minimum at their isoelectric point.

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