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Research note

Whey proteins solubility as function of temperature and pH

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Abstract

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 range of 40–60°C for temperature and 3.5–7.8 for pH. The results showed that, both temperature and pH influenced in the protein solubility, and these properties had great interaction. Besides, for whey proteins, the solubility values were minimum at the pH value of 4.5, which is the isoelectric point of whey proteins, for all temperature values. It was also observed that at pH 4.5, the solubility decreased as the temperature increased, which indicated that the protein denaturation occurred. This behavior was also noticed in the neutrality (pH = 6.8).

Keywords: Whey; Protein; Temperature; pH; Solubility

1. Introduction

Proteins, as macromolecules, perform important roles in functionality in foods and pharmaceuticals, as well in biological systems. Therefore, the growing demand for proteins as important ingredients in formulated food or in pharmaceutical and industrial mixtures has created a necessity for proteins with specific and consistent functional properties. Proteins exhibit many functional properties governed by their physicochemical activities in a bulk liquid phase. 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 have several definitions, since the proteins, in an aqueous spiritual medium, can form true or colloidal solution or insoluble particles suspension (Borderías & Monteiro, 1988). Thermodynamically, the protein solubility is the protein concentration in the solvent in a simple or two-phase system (protein solution in liquid–liquid or in liquid–solid phases) in balance state (Vojdani, 1996). Mathematically, the protein solubility degree of a protein is the amount of protein present in liquid phase in relation to the total amount of protein in liquid and solid phases in balance. The protein solubility also can be defined as a certain operational parameter for the protein retention in the supernatant after the solution centrifugation for certain time period and under certain force centrifuge (Morrisey, Mulvihill, & O'Neill, 1982).

The protein solubility is a function of many factors, such as the native or denatured state and environmental factors (i.e. pH, temperature). The pH of the solution affects the nature and the distribution of the protein's 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.

According with several authors (Kakalis & Regenstein, 1986; Wit, 1989; Mann & Malik, 1996;

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Vojdani, 1996; Wong, Camirond, & Pavlath, 1996), a protein usually has the least solubility at the isoelectric 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 values are in the range of 3.5 and 6.5. At extreme acidic or basic pH values, the protein may unfold, exposing more hydrophobic groups.

The temperature is also a factor that influences the protein solubility. In general, protein solubility is increased with temperature between 40°C and 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 noncovalent bonds involved is stabilization of secondary and tertiary structure; for e.g. hydrogen, hydrophobic and electrostatic bonds. When the secondary and tertiary structures of a protein are unfolded, the hydrophobic groups (i.e., the sulfhydril groups SH-, initially inside the protein molecules) interact and reduce water binding. 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). Caseins are not significantly affected by severe heating, while the whey proteins are completely denatured at high temperatures in few minutes. Various methods are used to determine when whey protein is denatured by heat, i.e. electrophoresis, gel filtration, centrifugation, immunodiffusion, optical rotatory dispersion and circular dichrome. The immunoglobulin fraction of the whey protein is denatured first followed by serum albumin, β-lactoglobulin is less affected under the same heating conditions, and α-lactalbumin is the most resistant of the whey protein fraction. The proteose peptone fraction is not sensitive to heat (Mutilang & Kilara, 1985).

2. Experimental procedure

2.1. Whey proteins

The product constituted of a whey protein isolate obtained from cow milk (ALACENTM 895), acquired by N.Z.M.P Brazil Ltd. It was acquired in enough quantity for the accomplishment of the determination of the centesimal composition and of the protein solubility analyses. 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 were 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 in the jacketed wall around them. These holding beakers were connected to a thermostatic bath (Nova Técinca), to maintain the set temperature. In this experiment, the temperatures varied from 40°C to 60°C, the maxim temperature allowed in the pH-meter (Mettler Toledo—model 320). The pH values varied from 3.5 to 7.8 as dictated by the experimental run, and maintained by adding NaOH 4.0 g/l or HCl 3.65 g/l solutions, when it was necessary. After 1 h agitation of the samples in a magnetic agitator (Fisatom—model 752A), the dispersion was transferred to a 0.05 l volumetric balloon, and the volume was completed with NaCl 5.85 g/l. Then the solution was centrifuged to 13 500 rpm for 30 min at 4°C, in a Sorvall Instruments (RC5C model) centrifuge with SS-34 rotor, and the supernatant was then filtered in Whatman paper no. 2. Aliquots of 0.002 l were taken and their soluble protein content 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 the average values were calculated.

The soluble protein percentage was calculated through the following equation:

$$\text{P.S.} = \left[\frac{A(\text{g/l})50}{W(\text{g})S/100}\right]100, \quad (1)$$

where P.S. is the soluble protein content in the sample (g/100 g), A the supernatant protein concentration (g/l), W the sample mass (g), S the sample protein concentration (g/100 g).

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

3. Results and discussion

3.1. Product characterization

The product lot used to calculate the protein solubility had the following whey centesimal composition characteristic: moisture = 3.70 g/100 g, total lipids = 0.30 g/100 g, ash = 1.50 g/100 g, and protein = 94.30 g/100 g.

3.2. Solubility values

Table 1 shows the protein solubility average values of four replicates, calculated from Eq. (1). for the ALA-CENTM 895. The values of the whey proteins solubility are illustrated in Fig. 1, which indicates that the solubility values for any temperature were minimum at the pH of 4.5; in those conditions the protein–protein interactions increase because the electrostatic forces are lowest and less water interact with the protein molecules. It was also noticed that the minimum solubility did not happen at the isoelectric point of β-lactoglobulina (5.2) and such a deviation was because the product is not a pure protein, but a mixture of the whey proteins present in the milk, where the precipitation happens at the isoelectric point of its proteins, and not at the β-lactoglobulin pI. At 40°C, where the protein structure was less affected due to heat action, it was observed that for pH values below and above the isoelectric point (4.5) 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 was observed that at the isoelectric point of whey proteins (pH = 4.5) 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. In the neutrality (pH = 6.8), it was observed that the solubility decreased with the temperature, indicating that the thermal protein denaturation occurred. At the pH of 5.65 protein solubility increased with the temperature

Table 1
Protein solubility (P.S) values of whey proteins

Temperature (°C)	pH	P.S. (g/100 g)
	3.50	87.13 ± 0.03
	4.50	81.76 ± 0.12
40	5.65	86.29 ± 0.02
	6.80	87.67 ± 0.02
	7.80	92.76 ± 0.49
	3.50	87.71 ± 0.28
	4.50	78.78 ± 0.41
43	5.65	88.96 ± 0.88
	6.80	83.85 ± 0.26
	7.80	88.94 ± 0.35
	3.50	87.13 ± 0.06
	4.50	72.17 ± 0.26
50	5.65	89.60 ± 0.05
	6.80	74.56 ± 0.01
	7.80	88.56 ± 0.02
	3.50	81.95 ± 0.20
	4.50	64.93 ± 0.16
57	5.65	87.58 ± 0.50
	6.80	72.62 ± 0.08
	7.80	85.20 ± 0.32
	3.50	80.74 ± 0.18
	4.50	62.35 ± 0.06
60	5.65	92.38 ± 0.10
	6.80	68.16 ± 0.15
	7.80	87.75 ± 0.05

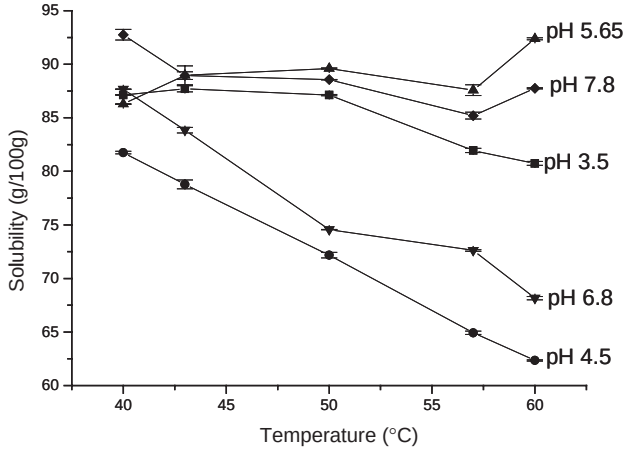

Fig. 1. Effect of pH and temperature on the whey protein solubility.

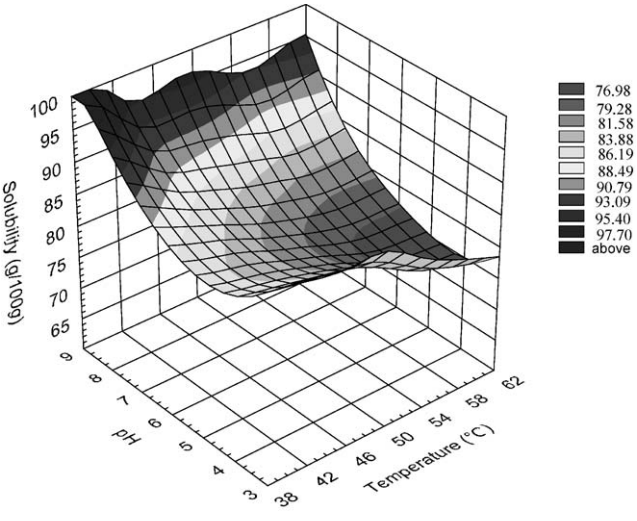

Fig. 2. Three-dimensional plot generated using correlation for whey protein solubility as function of temperature and pH.

Table 2
Confidence intervals on parameters in correlation

Variable	Squares sum	Degrees of freedom	Medium Square	F	F_{tab} ($\alpha = 1\%$)
Temperature	1309.88	4	327.4700	439.258	3.6
pH	4640.00	4	1160.2960	1556.382	3.6
Temperature–pH	1315.78	16	82.2360	110.308	2.3
Error	55.913	75	0.7455	—	
Total	7321.57	99	—	—	

indicating that there was neither coagulation nor aggregation between the protein molecules, possibly because in this pH value the β-lactoglobulin is a dimer that is dissociated in monomers at 50°C and only above 60°C the proteins unfold and the hydrophobic groups react. The three-dimensional graphs of protein solubility as temperature and pH function and the correlation parameters also will be presented (Fig. 2 and Table 2).

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 functional property. Besides, it was also observed that an interaction between the temperature and pH on whey proteins solubility, being minimum at their isoelectric point.

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