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Solubility of α_{S1} -, β - and κ -casein in water-ethanol solutions

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Abstract – The solubility and the selectivity of α_{S1} -, β - and κ -casein isolation from an industrially produced spray-dried sodium caseinate as a function of temperature (20–40 °C) and of ethanol volume fraction (0–0.6) at pH 5.0–7.5 were studied. Reverse-phase high-performance liquid chromatography and nitrogen balance were used as analytical methods. At 20 °C, casein solubility as a function of pH (5–7) and of the ethanol volume fraction (0–0.6) is quite well represented by an equation describing the ionisation process. α_{S1} -, β - and κ -casein solubility and β - and κ -casein extraction selectivity decrease both with a rise in the ethanol volume fraction to 0.45–0.50 and with temperature increase. For α_{S1} -casein, the selectivity increases to 67% for an ethanol volume fraction of 0.47 at 29 °C. This fraction could be used as an emulsifying agent or for cream liqueur production or further purification.

α_{S1} -casein / β -casein / κ -casein / solubility / purification / ethanol / temperature

摘要 – α_{S1} -, β - and κ -酪蛋白在乙醇-水溶液中的溶解性。以从工业上喷雾干燥法生产的酪蛋白酸钠中分离的 α_{S1} -, β -和 κ -酪蛋白为原料, 采用反相高效液相色谱法和氮平衡法研究了 α_{S1} -, β -和 κ -酪蛋白的溶解性和选择性 with pH (5.0 ~ 7.5)、温度 (20 °C ~ 40 °C) 和乙醇体积分数 (0 ~ 0.6) 之间的函数关系。在 20 °C 时, 酪蛋白的溶解性与 pH (5 ~ 7) 和乙醇体积分数 (0 ~ 0.6) 间的函数关系可以很好地用电离方程来表示。 α_{S1} -, β -和 κ -酪蛋白的溶解性及 β -和 κ -酪蛋白的提取选择性均随着乙醇体积分数 (0.45 ~ 0.50) 的提高和温度的升高而降低。在 29 °C 和乙醇的体积分数为 0.47 时, α_{S1} -酪蛋白的选择性提高到 67%。这一馏分的产品可以用作乳化剂或者用于奶酒产品中, 也可以用于进一步纯化。

α_{S1} -酪蛋白 / β -酪蛋白 / κ -酪蛋白 / 溶解性 / 纯化 / 乙醇 / 温度

Résumé – Solubilité des caséines α_{S1} -, β - et κ - dans des solutions d'éthanol-eau. La solubilité et la sélectivité de séparation des caséines α_{S1} , β et κ , à partir d'un caséinate de sodium industriel, en fonction de la température (20–40 °C) et de la fraction volumique d'éthanol (0 à 0,6) à pH 5,0–7,5, ont été étudiées. La chromatographie liquide de phase inverse haute performance et le bilan d'azote ont été utilisés comme méthodes analytiques. La solubilité de la caséine à 20 °C, en fonction du pH (5–7) et de la fraction volumique d'éthanol (0 à 0,6), est assez bien représentée par une équation qui décrit le processus d'ionisation. La solubilité des caséines α_{S1} , β et κ et la sélectivité d'extraction des caséines β et κ diminuent avec l'augmentation de la fraction volumique d'éthanol à 0,45–0,50

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et de la température. La sélectivité pour la caséine α_{S1} augmente jusqu'à 67 % à 29 °C pour la fraction volumique d'éthanol de 0,47. Cette fraction caséique pourrait être utilisée comme émulsifiant ou pour la fabrication de crèmes liqueurs, ou être purifiée davantage.

caséine α_{S1} / caséine β / caséine κ / solubilité / purification / éthanol / température

1. INTRODUCTION

A variety of methods have been used for the isolation of individual caseins. These are based on differences in the solubility of casein fractions in water, in concentrated urea solutions or in 50% ethanol.

Warner [54] was probably the first to propose a method of β -casein isolation, based on the differences in the solubility of casein fractions in cold water at 2 °C and at a pH value between 4.4 and 4.6. Later, the solubility of main casein fractions in water was studied as a function of temperature [8, 16], pH and temperature [6, 10, 11], micellar size and temperature [13], casein concentration, pH and ionic strength [17, 18], calcium and phosphate content and temperature [42], and calcium, phosphate, pH and temperature [43]. Based on these findings, industrially applicable methods have been proposed for β -casein enrichment and purification [27, 30, 38, 49].

Concentrated aqueous urea solutions were proposed by Hipp et al. [24] for the separation of α -, β - and γ -caseins by step-wise dilution of acid casein dissolved in 6.6 mol·L⁻¹ urea. β -Casein is soluble in 3.3 mol·L⁻¹ urea at pH 4.6 and precipitates in 1.7 mol·L⁻¹ urea at pH 4.9 and 30 °C [3]. The traces of α_{S1} - and α_{S2} -caseins present in β -casein can be removed by chromatography on DEAE cellulose [19]. κ -Casein is soluble in 6.6 mol·L⁻¹ urea at pH 1.3–1.5 but it precipitates when ammonium sulphate is added to its final concentration of 1 mol·L⁻¹ [56]. α_S -Casein is soluble at room temperature in 3 mol·L⁻¹ urea at pH 7, with 0.1% of tetraphosphate, but precipitates at pH 3.7 and 5 °C [33]. β -Casein and κ -casein obtained by the method of Aschaffenburg [3] and Zittle and

Custer [56] can be additionally purified with a calcium phosphate gel [20] or by chromatography [19, 50].

50% ethanol was used as a solvent for casein fractionation [24]. The whole casein is completely soluble at pH 7 in 50% ethanol. At pH 6.5, a fraction containing 92% of α -casein precipitates was determined by free-flow electrophoresis. Pierre [41] examined sodium caseinate solubility in 0 to 66% ethanol at pH 3 to 8. In our previous article [35], we proposed a physicochemical analysis of the whole casein solubility as a function of the ethanol concentration (0–75%) and pH values between 3 and 9. This analysis indicated the possibility of partial separation of casein fractions in ethanol solutions.

The aim of this work is to analyse the solubility of α_{S1} -, β - and κ -caseins as a function of ethanol concentration, stirring time, pH, calcium content and temperature.

2. MATERIALS AND METHODS

Industrially produced, spray-dried sodium caseinate (Armor Protéines, Saint Brice-en-Cogles, France) was reconstituted as described previously [35]. The pH of sodium caseinate solutions in water was adjusted to values in the 5.2–6.6 range with 0.5 mol·L⁻¹ HCl and left for 1 h at room temperature. Then ethanol was slowly added and continuously stirred, to reach the final ethanol volume fraction of up to 0.6. The solutions were then kept in tightly closed bottles for 1 h in a water bath at a specified temperature. The casein concentration in final solutions was 10 g·L⁻¹. The pH of water and ethanol solutions was measured with a pH meter equipped with a combination electrode.

Calibration was performed with buffers in aqueous solution.

The relative dielectric constants of the aqueous ethanol solutions were calculated according to Åkerlöf [1], Harvey and Prausnitz [21] and Smith et al. [44].

Soluble caseins were separated from precipitate by centrifugation at $2000\times g$ for 30 min. Casein content in the initial sodium caseinate samples, solutions and supernatants was estimated from nitrogen determinations using Kjeldahl techniques with 6.38 as a nitrogen to protein conversion coefficient [28, 29]. Supernatants were evaporated under vacuum to eliminate alcohol and the concentrates were freeze-dried and kept at $-20\text{ }^{\circ}\text{C}$ until analysed. Casein fraction contents in supernatants were determined by reverse-phase high-performance liquid chromatography (RP-HPLC).

Sodium caseinate or freeze-dried supernatants were dissolved at $1\text{ mg}\cdot\text{mL}^{-1}$ in $8\text{ mol}\cdot\text{L}^{-1}$ urea with $2\text{ mg}\cdot\text{mL}^{-1}$ of β -mercapto-ethanol and incubated for 1 h at $25\text{ }^{\circ}\text{C}$. For dilution of protein samples, a buffer A composed of $1\text{ g}\cdot\text{L}^{-1}$ of trifluoroacetic acid (TFA) in MilliQ water was used. The samples were injected into a RP-HPLC system equipped with a Vydac C_4 $5\text{ }\mu\text{m}$ column i.d. 4.6 mm, 250 mm length (Touzart et Matignon, Vitry-sur-Seine, France). Eluate was monitored at 214 nm using a M490 spectrophotometer detector (Waters, Saint Quentin-Yvelines, France). The flow rate was maintained at $1\text{ mL}\cdot\text{min}^{-1}$ with the gradient presented in Table I.

Solubility and selectivity of a given casein fraction (x) were calculated as follows:

Solubility of x -casein = x -casein concentration in the supernatant / x -casein concentration in the initial solution.

Selectivity or purity = x -casein in the supernatant / (α_{S1} -casein + α_{S2} -casein + β -casein + κ -casein) in the supernatant.

Table I. Gradient of RP-HPLC. Buffer A = TFA 0.1% in water, buffer B = TFA 0.1% in acetonitrile/water 80/20 (v/v).

Time (min)	Buffer A	Buffer B
0	63	37
5	55	45
15	45	55
20	20	80
27	63	37

A central composite design was adopted to assess the combined effect of temperature ($20\text{--}40\text{ }^{\circ}\text{C}$) and ethanol volume fraction ($0.3\text{--}0.6$) on the solubility and the selectivity of α_{S1} -, β - and κ -caseins. The levels of variables tested were chosen on the basis of preliminary experiments. A polynomial model was used to quantify the relationship between the solubility or the selectivity and the temperature (T) in $^{\circ}\text{C}$ and the ethanol volume fraction (E):

$$Y = b_0 + b_1 \cdot T + b_2 \cdot E + b_3 \cdot T^2 + b_4 \cdot E^2 + b_5 \cdot T \cdot E + \text{Err} \quad (1)$$

where: b_0 is the intercept; b_1 to b_5 are the model coefficients reflecting the simple and interactive effects, and Err is the error term following a normal distribution. The accuracy of the model was evaluated using the correlation coefficient (R^2) and the statistical significance using the F-test. The effect of each term in the model was tested using a t-test procedure. Only the variables with significant t-test values (probability lower than the chosen P -value, 0.05 in most cases) were taken into account in the final model.

3. RESULTS AND DISCUSSION

3.1. Composition

Sodium caseinate contains 10%, 8%, 28% and 35% of κ -, α_{S2} -, α_{S1} - and

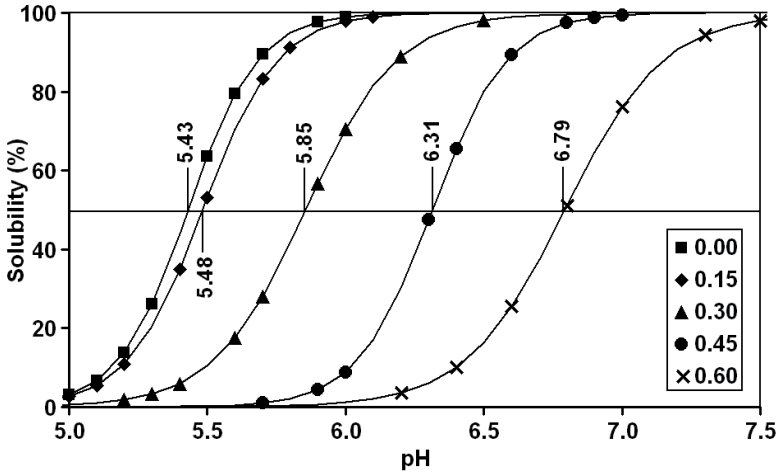


Figure 1. Casein solubility at 20 °C, as a function of pH (5.0–7.5) and the ethanol volume fraction (0–0.6). Solid lines represent Equation (2) with coefficients pH_i and D presented in Figure 2.

β -caseins, respectively, as determined by RP-HPLC. Around 19% are represented by other non-identified fractions. Similar levels of the main casein fractions in milk and caseinate were observed by other authors [9, 10, 12, 14, 31, 46–48, 53].

The α_{S1} - to β -casein ratio is, however, relatively low (0.8), being on a lower level (0.82 to 1.25) than that observed by Dalgleish and Law [9] in different sodium caseinate samples.

3.2. Solubility in water-ethanol mixtures

For pH values between 5.0 and 7.5, casein solubility (Fig. 1) can be represented by the equation describing the ionisation process [25]. This equation can be presented in the form:

$$S = \frac{S_{\min} + S_{\max} \cdot 10^{[(pH-pH_i)/D]}}{1 + 10^{[(pH-pH_i)/D]}} \quad (2)$$

where: pH_i is the pH of the inflexion point or the pH corresponding to 50% casein solubility; D is the pH increase causing

the $\text{Log}_{10}[(S - S_{\min})/(S_{\max} - S)]$ to rise by 1 unit, $S_{\min} = 0\%$ and $S_{\max} = 100\%$ are, respectively, the minimal and maximal casein solubility, and S is the solubility at a given pH and ethanol concentration.

The inflection point pH increases from 5.43 for water solutions to 6.79 for 0.6 ethanol volume fraction (Fig. 1). As we previously observed [35], the pH_i and D are inversely proportional to the relative dielectric constant (ϵ) of the solvent (Fig. 2):

$$pH_i \text{ or } D = A + B/\epsilon + \text{Err} \quad (3)$$

where: A is the intercept or the pH level for the hypothetical $\epsilon = \infty$, the slope coefficient B indicates the hypothetical pH increase for unitary increase in $1/\epsilon$, and Err is the standard error term following a normal distribution. More evocative is the reciprocal value of the coefficient B , which indicates the change in $1/\epsilon$ causing the pH or the apparent pK to change by 1 unit. For the solubility profile presented in Figure 1, the numerical values of the coefficients A , B and Err are given in Figure 2. An identical type of relation can be deduced for the pH increase when ethanol is added to

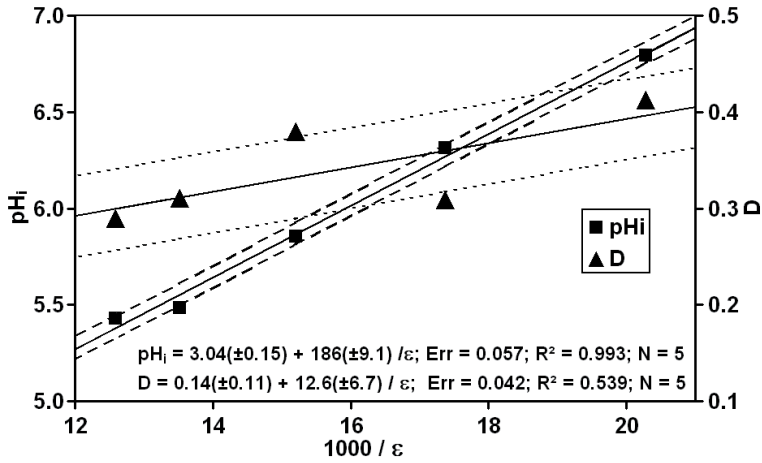


Figure 2. Evolution of pH_i and D coefficients from Equation (2), as a function of the reciprocal relative dielectric constant (ϵ). Solid lines represent Equation (3) with coefficients A and B given in the figure. Broken and dotted lines show standard deviation limits for pH_i and D , respectively.

milk or to sodium caseinate dissolved in water [26, 34, 35, 40, 41].

For the pH range between 5 and 7.5, the pH_i increase with the rise in the ethanol volume fraction is mainly due to the increase in the apparent pK of γ - and δ -carboxyl groups of aspartic and glutamic acids [35, 53]. The average B coefficient from Equation (3) is 133 for the spontaneous pH shift when ethanol is added to the water solution of the sodium caseinate [35, 39, 41]. The same coefficient is 186 for the pH_i (Fig. 2), while for 10% and 90% of protein solubility it is 174 and 198, respectively. To achieve half solubility in 45% ethanol solution, the pH has to be increased by 0.27 over the spontaneous pH shift. For 90% solubility, the pH increase has to be 0.61. The addition of ethanol increases pH, i.e. decreases the ionisation of the carboxyl groups. As protein solubility is governed by the ionisation process, additional energy has to be introduced to increase the ionisation.

The regression line between the experimental (S_{EXP}) and model (S_{MOD}) values of casein solubility for $N = 42$ experimen-

tal points gives a standard error (Err) of around 5%, which is quite satisfactory:

$$S_{\text{MOD}} = 0.48 (\pm 1.32) + 0.995 (\pm 0.021) \cdot S_{\text{EXP}}; \\ \text{Err} = 5.2; R^2 = 0.983; N = 42.$$

3.3. Casein fractions

3.3.1. α_{S1} -Casein

For the analysed ranges of ethanol volume fraction (0.3–0.6) and temperature (20–40 °C) the solubility of α_{S1} -casein varies between 48 and 87%, with its lowest point being 0.47 of ethanol volume fraction at 20 °C (Fig. 3). Only coefficients b_0 , b_2 and b_4 from Equation (1) are statistically significant. The effect of ethanol is parabolic, while that of temperature is statistically insignificant. The standard error for the set of data analysed is 4.5%.

The maximal selectivity of soluble α_{S1} -casein is about 67%, which is obtained for an ethanol volume fraction of 0.47 at 29 °C (Fig. 4). Only the interactive effect of alcohol and temperature (b_5) is statistically insignificant. The maximal level

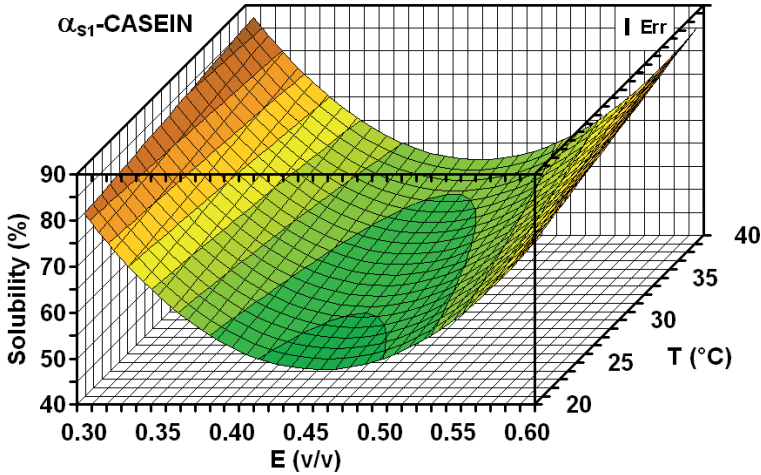


Figure 3. The solubility profile of α_{S1} -casein as a function of ethanol concentration and temperature, calculated by Equation (1) with the coefficients (b_x) given in Table II. Err = standard error bar.

of α_{S1} -casein purity is within the minimal solubility region. The α_{S1} -casein content in the sodium caseinate was $28 (\pm 1)\%$.

The highest selectivity of soluble α_{S1} -casein ($\sim 75\%$) was observed for the pH range between 6.3 and 6.5 (Fig. 5) and for the lowest calcium level. The increased precipitation of milk proteins by calcium from 35% ethanol solutions was observed by Sommer and Binney [45] and the calcium sensitivity of α_S -casein was studied by Zittle [55].

The solubility of α_{S1} -casein in 0.45 ethanol volume fraction at 30 °C and at pH 6.4 does not change during prolonged stirring of up to 24 h of the sodium caseinate suspension (Fig. 6). The selectivity of α_{S1} -casein seems to follow a power-law type relation (Fig. 6) and increases from 66% to 76% during 24 h of stirring. To reach 70, 80 or 90% selectivity, the stirring time would be 6.6, 59 or 407 h, respectively. This means that the diffusion of casein fractions between small and bigger aggregates is relatively slow and several weeks would be needed to reach the equilibrium state.

3.3.2. β -Casein

The solubility of β -casein varies between 13 and 76%, with the minimum level occurring at 0.45 of ethanol volume fraction (Fig. 7). The minimal solubility of β -casein coincides with the maximal purity of α_{S1} -casein (Fig. 4). Only coefficients b_0 , b_2 and b_4 from Equation (1) are statistically significant. This means that within the range of variables analysed, β -casein yield depends only on ethanol concentration. The standard error for the set of data analysed is 6.2%.

Only at low temperatures (0 to 5 °C) is β -casein much more soluble than other casein fractions [2, 6, 8, 10, 13, 16, 24, 42, 43, 54]. Beyond 20 °C, temperature is not a screening factor in β -casein preparation.

β -Casein selectivity varies between 18 and 52% and its minimum level is for 0.45 ethanol volume fraction at 30 °C (Fig. 8). Contrary to α_{S1} -casein, the lowest purity level of β -casein coincides with its minimal solubility. The precipitation of β - and κ -caseins in 50% ethanol was used to purify α_S -casein obtained by an urea-sodium chloride method [56].

Table II. Coefficients b_0 to b_5 from Equation (1) expressing the influence of ethanol volume fraction and temperature (in °C) on the solubility and the selectivity of α_{S1} -, β - and κ -casein extraction. Number of experimental points $N = 13$, P = probability value, Err = standard error, R^2 = correlation coefficient.

Coefficient	Solubility (%)		Selectivity (%)	
	Value	P	Value	P
α_{S1}-casein				
b_0	352 ± 62	$7.28 \text{ E-}04$	-293 ± 51	$6.85 \text{ E-}04$
b_1	-1.1 ± 2.5	$6.64 \text{ E-}01$	5.7 ± 2.1	$2.68 \text{ E-}02$
b_2	-1300 ± 165	$1.02 \text{ E-}04$	1192 ± 136	$5.11 \text{ E-}05$
b_3	0.01 ± 0.03	$7.62 \text{ E-}01$	-0.073 ± 0.028	$3.64 \text{ E-}02$
b_4	1326 ± 152	$5.27 \text{ E-}05$	-1176 ± 125	$3.27 \text{ E-}05$
b_5	2.0 ± 3.0	$6.56 \text{ E-}01$	-3.2 ± 2.5	$2.37 \text{ E-}01$
Err	4.51		3.72	
R^2	0.928		0.934	
β-casein				
b_0	645 ± 84	$1.23 \text{ E-}04$	346 ± 44	$9.98 \text{ E-}05$
b_1	-6.2 ± 3.4	$1.13 \text{ E-}01$	-5.4 ± 1.8	$1.79 \text{ E-}02$
b_2	2362 ± 226	$1.62 \text{ E-}05$	-1089 ± 118	$3.53 \text{ E-}05$
b_3	0.07 ± 0.05	$2.05 \text{ E-}01$	0.069 ± 0.024	$2.52 \text{ E-}02$
b_4	2343 ± 209	$9.89 \text{ E-}06$	1103 ± 108	$1.89 \text{ E-}05$
b_5	6.0 ± 4.2	$1.95 \text{ E-}01$	3.0 ± 2.2	$2.12 \text{ E-}01$
Err	6.18		3.21	
R^2	0.954		0.939	
κ-casein				
b_0	505 ± 57	$4.49 \text{ E-}05$	47.3 ± 12.7	$7.50 \text{ E-}03$
b_1	-5.43 ± 2.8	$4.87 \text{ E-}02$	-0.3 ± 0.5	$6.18 \text{ E-}01$
b_2	-1682 ± 152	$1.07 \text{ E-}05$	-104 ± 34	$1.89 \text{ E-}02$
b_3	0.08 ± 0.03	$4.47 \text{ E-}02$	0.004 ± 0.007	$6.26 \text{ E-}01$
b_4	1603 ± 140	$8.53 \text{ E-}06$	74 ± 31	$5.15 \text{ E-}02$
b_5	3.1 ± 2.8	$3.07 \text{ E-}01$	0.3 ± 0.6	$6.77 \text{ E-}01$
Err	4.14		0.93	
R^2	0.973		0.932	

3.3.3. κ -Casein

The κ -Casein solubility varies between 14 and 87% and its minimum level corresponds to 0.50 ethanol volume fraction at 20 °C (Fig. 9). The solubility profile of κ -casein is similar to that of α_{S1} - and β -caseins. This is mainly a parabolic function of the ethanol concentration with only a minor, yet statistically significant temperature effect.

The selectivity of κ -casein decreases from 21% to 12% for the increase in the ethanol volume fraction from 0.3 to 0.6 (Fig. 10). In the initial sodium ca-

seinate, κ -casein represents about 12% of all caseins. This level is attained in 60% ethanol solutions, but in 30% ethanol, the purity of κ -casein is almost twice as high.

3.3.4. Low temperature

For 24 h of stirring of the sodium caseinate suspension in 0.45 ethanol volume fraction at -24 to $+21$ °C and at pH 6.4, the selectivity of casein fractions seems to follow (Fig. 11) the Arrhenius type relation:

$$\text{Log}_{10}(\text{Selectivity}) = A + E/(R \cdot T) \quad (4)$$

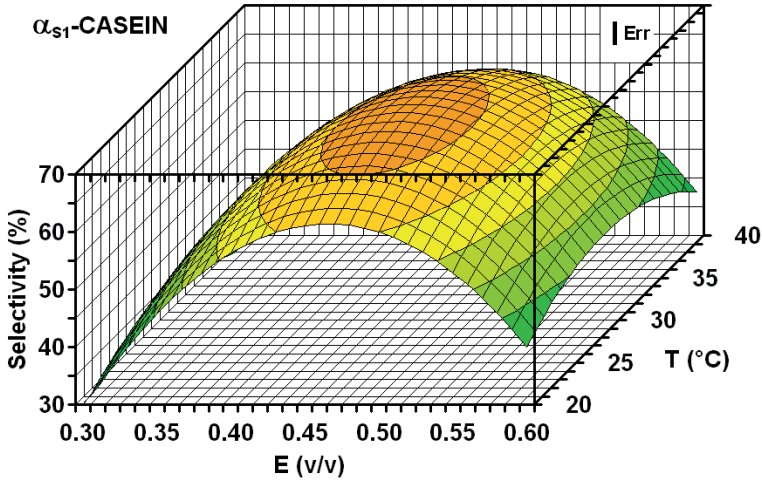


Figure 4. The selectivity profile of α_{S1} -casein extraction as a function of ethanol concentration and temperature, calculated by Equation (1) with the coefficients (b_x) given in Table II. Err = standard error bar.

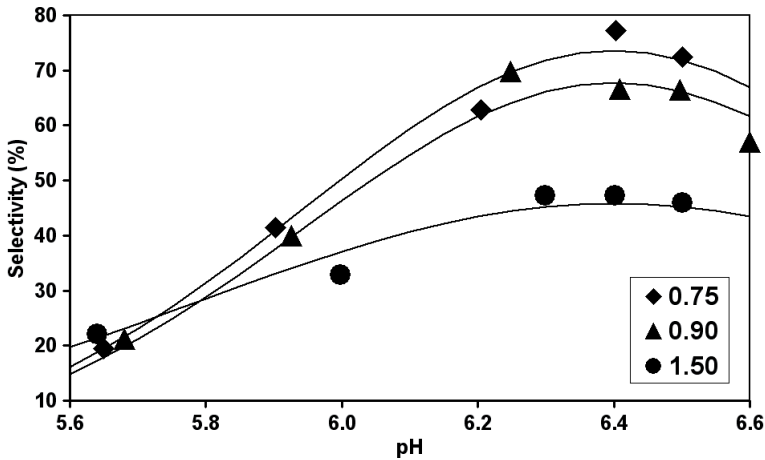


Figure 5. Effect of pH (5.6–6.6) and of calcium concentration (0.75–1.50 $\text{mmol}\cdot\text{L}^{-1}$) on the selectivity of α_{S1} -caseins isolated from the suspension of 10 $\text{g}\cdot\text{L}^{-1}$ sodium caseinate in 0.45 ethanol volume fraction at 30 °C.

where: A is the hypothetical level of $\text{Log}_{10}(\text{Selectivity})$ for $T = \text{infinity}$, E is the activation energy in $\text{J}\cdot\text{mol}^{-1}$, R is the gas constant = $8.314 \text{ J}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$ and T is absolute temperature (K).

For α_{S1} -casein, the selectivity increases from 60% at $-24 \text{ }^\circ\text{C}$ to 74% at $+21 \text{ }^\circ\text{C}$. For β - and κ -caseins, the selectivity rises

by 10% when the temperature is reduced from $+21 \text{ }^\circ\text{C}$ to $-24 \text{ }^\circ\text{C}$.

Among the three casein fractions analysed, α_{S1} -casein is the most electrically charged and the least hydrophobic, while β -casein is the most hydrophobic casein and has a relatively low electrical charge [46, 53]. The presence of

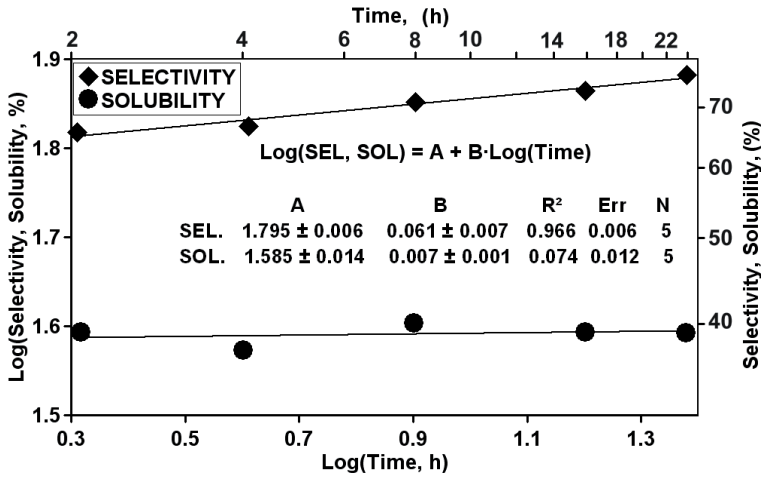


Figure 6. Effect of the stirring time of 10 g·L⁻¹ sodium caseinate suspension in 0.45 ethanol volume fraction at 30 °C and pH 6.4 on the selectivity and the solubility of α_{S1} -casein.

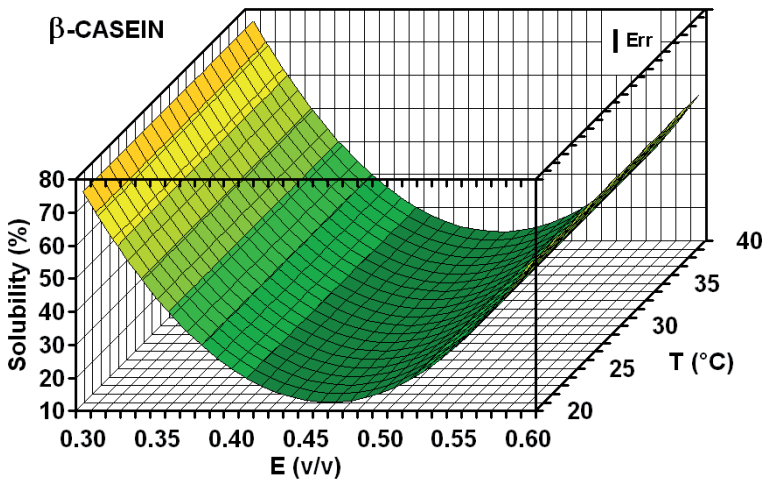


Figure 7. The solubility profile of β -casein as a function of ethanol concentration and temperature, calculated by Equation (1) with the coefficients (b_x) given in Table II. Err = standard error bar.

1 to 3 N-acetylneuraminic acid molecules attached to the galactosylated threonyl residues slightly reduces the overall hydrophobic character of κ -casein [46, 53]. Having a hydrophobic N-terminal tail and a hydrophilic, glycosylated “head” close to the C-terminal polar domain, κ -casein behaves as a surfactant and is mainly situated outside the casein mi-

celles. In the experimental conditions of this work, κ -casein is almost electrically uncharged [35]. For these reasons, κ -casein behaves similarly to β -casein (Fig. 11), i.e. as a typical hydrophobic protein. The hydrophobic bonding strongly increases with temperature [52]. On the other hand, the associations of α_{S1} -casein are mostly governed by the ionic interactions which

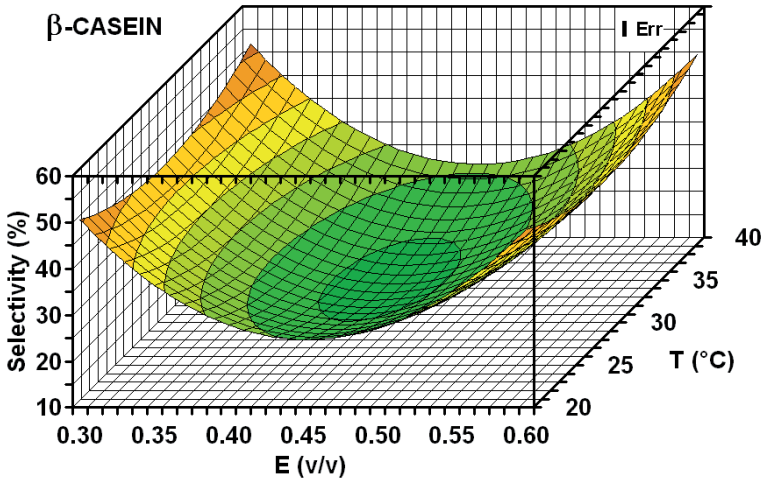


Figure 8. The selectivity profile of β -casein extraction as a function of ethanol concentration and temperature, calculated by Equation (1) with the coefficients (b_x) given in Table II. Err = standard error bar.

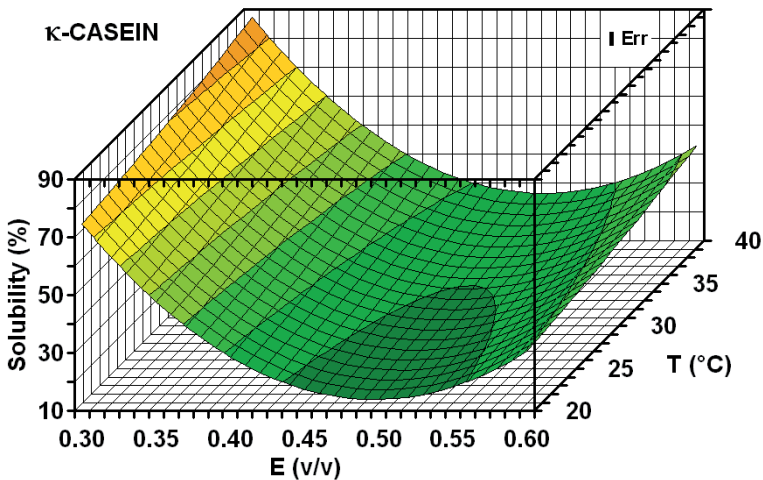


Figure 9. The solubility profile of κ -casein as a function of ethanol concentration and temperature, calculated by Equation (1) with the coefficients (b_x) given in Table II. Err = standard error bar.

depend on the electrostatic charge, the ionic strength, the activity coefficient and the dielectric constant, themselves being functions of temperature [23, 46, 51–53]. This important difference in basic casein fraction behaviour in water solutions, first discovered by Warner [54], is also observed and even enhanced in water-ethanol

mixtures for quite a wide temperature range.

In this work, we presented the solubility of α_{S1} -, β - and κ -caseins as a function of the ethanol volume fraction, the temperature, the calcium concentration and the pH. Analysis of the results confirms that within the 6 to 7 pH range at 20 to 40 °C and for an

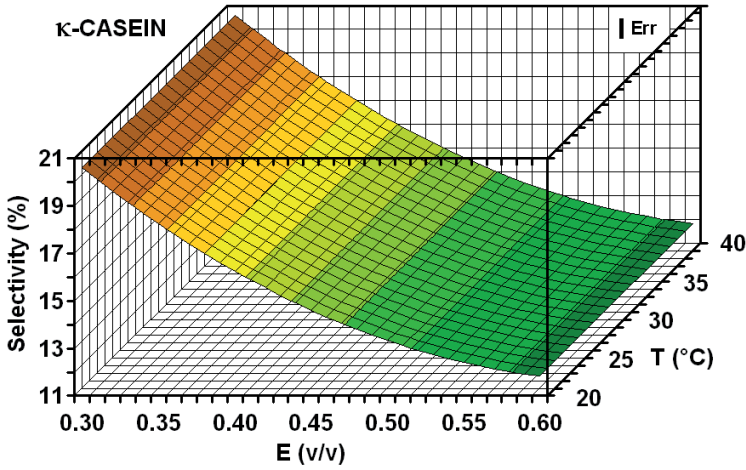


Figure 10. The selectivity profile of κ -casein extraction as a function of ethanol concentration and temperature, calculated by Equation (1) with the coefficients (b_x) given in Table II. Err = standard error bar.

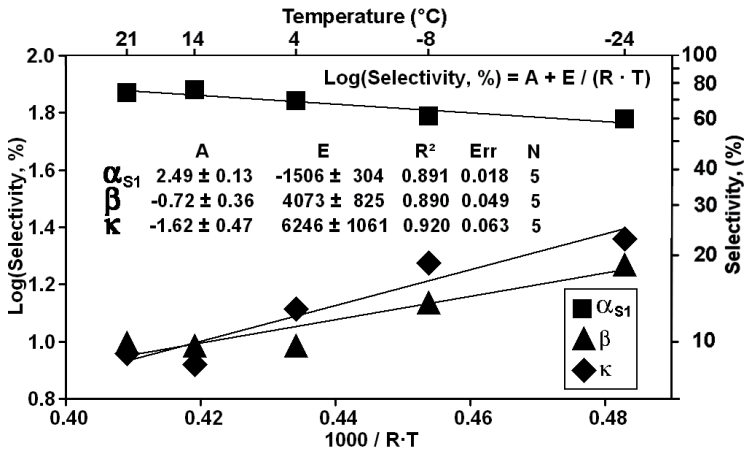


Figure 11. Effect of temperature on the selectivity of α_{S1} -, β - and κ -caseins isolated from the suspension of $10 \text{ g}\cdot\text{L}^{-1}$ sodium caseinate in 0.45 ethanol volume fraction at pH 6.4.

ethanol volume fraction between 0 and 0.6, the caseins form aggregates whose composition depends on the electrical properties of the molecules [6, 9, 22, 25, 26, 38–41, 46, 53, 57].

Taking into account the good solubility in ethanol, the α_{S1} -casein enriched fraction could be used for cream liqueur production [4, 5, 15, 32, 36, 37, 39] or could be further purified either by the ethanol-pH

method [24, 56] or by chromatographic methods [7, 50].

4. CONCLUSIONS

The solubility of the main casein fractions is governed by the ionisation process of acidic and amino groups, being a function of pH, the solvent's dielectric constant, ionic strength, calcium content and temperature.

The enhancement of the differences in the solubility between the casein fractions by ethanol could be employed for the preparation of enriched or purified α_{S1} -casein.

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