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Controlled Hydrolysis of Cheese Whey Proteins Using Trypsin and α-Chymotrypsin

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Abstract

This study examined the production of protein hydrolysates with controlled composition from cheese whey proteins. Cheese whey was characterized and several hydrolysis experiments were made using whey proteins and purified β-lactoglobulin, as substrates, and trypsin and α-chymotrypsin, as catalysts, at two temperatures and several enzyme concentrations. Maximum degrees of hydrolysis obtained experimentally were compared to the theoretical values and peptide compositions were calculated. For trypsin, 100% of yield was achieved; for α-chymotrypsin, hydrolysis seemed to be dependent on the oligopeptide size. The results showed that the two proteases could hydrolyze β-lactoglobulin. Trypsin and α-chymotrypsin were stable at 40°C, but a sharp decrease in the protease activity was observed at 55°C.

Index Entries: Cheese whey; β-lactoglobulin; protein hydrolysates; trypsin; α-chymotrypsin.

Introduction

Cheese whey is the most abundant protein subproduct of the dairy industry, representing about 85–95% of the volume of the milk. It contains about 55 g/L of lactose and 7 g/L of proteins. The main proteins present in the whey are α-lactalbumin (16%, weight basis), β-lactoglobulin (49%), bovine serum albumin (BSA) (5%), and immunoglobulins (10%). Worldwide production of whey is about 145 million tons, with 60% recovered by

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several methods and 40% discarded directly into rivers without previous treatment. The development of products with high commercial value from this residue seems to be an attractive solution for the environmental problem caused by disposal of cheese whey *(1)*.

Controlled enzymatic hydrolysis of proteins (under mild conditions) leads to hydrolysates that possess improved functional properties or a pleasant taste, as well the elimination of allergenic characteristics. There are numerous published works in which these hydrolysates have been investigated *(2–5)*.

Cheese whey was initially characterized to determine appropriate pretreatment before reaction. Then, several hydrolysis experiments were made using whey proteins and purified β-lactoglobulin, as substrates, and trypsin and α-chymotrypsin, as catalysts. Temperatures were 40 and 55°C and pH was 8.0. The influence of enzyme concentration on the degree of hydrolysis, stability of the enzymes under operational conditions, and, finally, specificity of both proteases were studied. Maximum experimental hydrolysis degrees were compared to theoretical values and the resulting peptide compositions calculated.

Materials and Methods

Materials

Sweet cheese whey was donated by different cheese manufacturers of São Carlos, SP, Brazil. Trypsin (EC 3.4.21.4), from bovine pancreas, was donated by Novo Nordisk of Brazil or purchased from Sigma (treated to eliminate α-chymotrypsin activity). α-Chymotrypsin (EC 3.4.21.1), from bovine pancreas and treated to eliminate trypsin activity; purified β-lactoglobulin; and the synthetic substrates benzoyl-L-arginine ethyl ester (BAEE) and benzoyl-L-tyrosine ethyl ester (BTEE) were purchased from Sigma. All other reagents were of analytical grade from several commercial brands.

Methods

Lyophilization and Filtration of Whey

After cooling at –50°C in a Bio-Freezer (Forma Scientific), 200 mL of whey was placed in an Edwards lyophilizer (model L4RL) for 8 h, resulting in 0.03 g/mL of powder whey per volume of "*in natura*" whey. For small volumes, whey was filtered through filter paper and under vacuum in Millipore membranes with a diameter of 47 mm and several porosities (1.2, 0.45, and 0.22 μm). For volumes above 1 L, an ultrafiltration hollow-fiber unit was used 0.45 μ, CFP-4-E-6A; A/G Technology coupled to a microfiltration unit (model Pellicon; Millipore S/A).

Proteins

The protein contents in the liquid or lyophilized (after dissolution) cheese whey were analyzed by the Kjeldahl method using a Büchi unit (model 323).

Enzymatic Activity

First of all, two specifics solutions were made (for trypsin, 2 mL of 0.5 m*M* BAEE solution, dissolved in 50 m*M* phosphate buffer, pH 7.6; for α-chymotrypsin, 100 µL of 8 m*M* BTEE in ethanol and 2 mL of 100 m*M* phosphate buffer, pH 7.0). These solutions were transferred to two differents quartz cuvets (at 25°C, in a spectrophotometer, model Ultrospec 2000; Pharmacia Biotech). To these solutions, 100 and 200 µL of enzymatic solution (diluted, if necessary) of α-chymotrypsin and trypsin, respectively, were added. The increase in absorbance that occurs during the hydrolysis of the synthetic substrates BAEE at 253 nm, for trypsin, and BTEE at 254 nm, for α-chymotrypsin, was followed *(6,7)*. The activity was calculated after determining the initial reaction rate.

Enzymatic Hydrolysis

Hydrolysis experiments were developed in a Metrohn pHstat (model Titrino). The protein hydrolysis reaction was followed through base consumption (NaOH), which occurs to keep the reaction pH constant. When a peptide bond is broken, proton liberation occurs. Ionic equilibrium was taken into account in the calculations. The whey was transferred to a jacketed glass reactor at 40 or 55°C and the pH adjusted to 8.0. Then, the enzyme mass dissolved in 1 mL of 0.1 mol/L of $CaCl_2$ was added to the reactor, which contained a magnetic stirrer, and was coupled to a Neslab thermostatic bath, with recirculation (model RTE 111). In the sequential hydrolysis, trypsin was inactivated (80°C for 15 min). After cooling to 40°C, α-chymotrypsin was added.

Stability of Proteases

The stability of the enzymes was followed along the whole period of the reaction. At different times samples were withdrawn and assayed at 25°C, as described in the standard procedure.

Experimental Hydrolysis Degree

The experimental hydrolysis degree was calculated according to Adler-Nissen *(8)* using the following equation:

$$\%GH = C_b \times V_b \times \frac{1}{\alpha} \times \frac{1}{h_{\text{total}}} \times \frac{1}{M_p} \times 100\%$$

in which C_b = concentration of the base (mol/L); V_b = volume of the base (mL); $1/\alpha = 1.2$; $1/h_{\text{total}} = \sum$ mmol of individual amino acids by protein gram = 8.8; and M_p = protein mass.

Theoretical Hydrolysis Degree

The theoretical hydrolysis degree was calculated from knowledge of the primary sequence *(9,10)* and the specificity of the used proteases *(11)*. Trypsin is assumed to hydrolyze peptide bonds where lysine and arginine residues are in the carboxyl side of the bond; α-chymotrypsin, tryptophan,

phenylalanine, tyrosine, and leucine residues were taken into account. The theoretical hydrolysis degree for cheese whey was calculated as follows:

$$\%HD_{th} = \frac{n_{\alpha} \times C_{\alpha} + n_{\beta} \times C_{\beta} + n_{\mathrm{BSA}} \times C_{\mathrm{BSA}}}{n_{T\alpha} \times C_{\alpha} + n_{T\beta} \times C_{\beta} + n_{T\mathrm{BSA}} \times C_{\mathrm{BSA}}} \times 100$$

in which $n_{\alpha, \beta, \mathrm{BSA}}$ is the number of specific residues in the α-lactalbumin, β-lactoglobulin, and BSA proteins, respectively, for trypsin or α-chymotrypsin; $n_{T\alpha, T\beta, T\mathrm{BSA}}$ is the total number of residues in the α-lactalbumin, β-lactoglobulin, and BSA proteins, respectively; and $C_{\alpha, \beta, \mathrm{BSA}}$ is the concentration of α-lactalbumin, β-lactoglobulin, and BSA in the cheese whey, respectively.

The theoretical hydrolysis degree for cheese whey considered that the protein composition was 3.0 g/L of α-lactalbumin, 1.2 g/L of β-lactoglobulin, and 0.4 g/L of BSA. It is difficult to ascertain a more accurate value for this variable. The whey protein concentration depends on the source of the product; in addition, whey contains other proteins such as immunoglobulins, membrane proteins, and peptones in small concentrations. Table 1 shows the calculated values for whey proteins and purified β-lactoglobulin.

Theoretical Peptide Composition ($\%PC_{th}$)

The theoretical peptide distribution percentile was calculated for the maximum degree of hydrolysis as follows:

$$\%PC_{th} = \frac{p_{T\alpha} \times C_{\alpha} + p_{T\beta} \times C_{\beta} + p_{T\mathrm{BSA}} \times C_{\mathrm{BSA}}}{p_{\mathrm{th}\alpha} \times C_{\alpha} + p_{\mathrm{th}\beta} \times C_{\beta} + p_{\mathrm{th}\mathrm{BSA}} \times C_{\mathrm{BSA}}} \times 100$$

in which $p_{\mathrm{th}\alpha, \mathrm{th}\beta, \mathrm{th}\mathrm{BSA}}$ is the theoretical number of peptides obtained from α-lactalbumin, β-lactoglobulin, and BSA hydrolysis, respectively, using trypsin and α-chymotrypsin alone or for sequential action (α-chymotrypsin after trypsin action), for several molecular mass ranges; $p_{T\alpha, T\beta, T\mathrm{BSA}}$ is the total number of peptides obtained from the maximum hydrolysis degree of each situation; and $C_{\alpha, \beta, \mathrm{BSA}}$ is the concentration of α-lactalbumin, β-lactoglobulin, and BSA in cheese whey, respectively.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of β-lactoglobulin after hydrolysis with α-chymotrypsin at different reaction times was performed on a Hoefer SE 200 vertical slab gel according to the manufacturer's instructions. The gel was stained with Coomassie blue and fixed with methanol. Molecular weights were estimated using a Pharmacia PMW calibration kit (14,000–94,000 Daltons).

Table 1
Theoretical Hydrolysis Degree (*HDth*) Calculated for Main Proteins in Cheese Whey[a]

	Trypsin			α-Chymotrypsin							
				woLeu[b]			wLeu[c]		woLeu[d]		wLeu[d]	
Protein	*Nt*	*Ns*	*HDth* (%)	*Nt*	*Ns*	*HDth* (%)	*Ns*	*HDth* (%)	*Ns*	*HDth* (%)	*Ns*	*HDth* (%)
α-Lactalbumin	123	14	11.38	123	12	9.76	24	19.51	11	8.94	21	17.07
β-Lactoglobulin	162	18	11.11	162	10	6.17	32	19.75	7	4.32	27	16.67
BSA	582	82	14.09	582	49	8.42	110	18.30	41	7.04	90	15.46
Whey[e]	—	—	11.96	—	—	7.39	—	19.48	—	5.84	—	16.41

[a]*Nt* = total number of residues; *Ns* = number of specific residues for each protease; *HDth* = (*Ns*/*Nt*) × 100; wLeu and woLeu = *Ns* and *HDth* calculated with and without considering peptide bonds where leucine is present at the bond C-side.
[b]Not considering leucine residues.
[c]Considering leucine residues.
[d]After total hydrolysis with trypsin.
[e]α-Lactalbumin (1.2 g/L), β-lactoglobulin (3.0 g/L), BSA (0.4 g/L).

Table 2
Proteic Content in *In Natura*
and Filtrate Whey and in Retentate

Material analyzed	Concentration of protein (g/L)
In natura whey	8.6 ± 0.12
Filter paper	0.63
Membrane (1.2 mm)	0
Membrane (0.45 μm)	0
Membrane (0.22 μm)	0.24
Filtrate whey	8.1 ± 0.10

Results and Discussion

Characterization of Cheese Whey

Cheese whey contains many insoluble particles represented mainly by insoluble globules of fat and casein. To verify whether the particulate protein could be hydrolyzed, *in natura* whey was submitted to successive microfiltration steps using membranes of different porosities. Observation under microscope of all the obtained permeates (×400 magnification) showed that most of the particulate material, as well visible fat, was retained on filter paper. Table 2 presents the results of protein analysis of the different whey fractions obtained during the filtration steps. It can be observed that only the fractions retained in membranes of 1.2 (insoluble globules of casein) and 0.22 μm (bacteria, probably) had protein content. Two kinds of experiments were then made. In the first, the particulate material was submitted to hydrolysis with trypsin and no base consumption was observed. In the second, *ïn natura* and filtrated whey were hydrolyzed using trypsin. The hydrolysis degree obtained at the same operational conditions for the two substrates and the protein content of the retained fractions on the filter paper, after hydrolysis, were compared. The results (not shown here) indicated that the particulate material (probably casein precipitated that is kept in the whey after the separation of the solid fraction in the process of cheese production) is inaccessible to the catalytic attack of trypsin. Therefore, there is no advantage to keeping those particles in the whey and two steps of microfiltration are recommended: the first one using a membrane with high porosity to remove casein and fat, and the second one, to remove bacteria. Although the low-porosity membrane could retain both solids, that would imply a great increase in the filtration time, with consequent risk of whey contamination.

Hydrolysis of Cheese Whey Proteins with Trypsin and α-Chymotrypsin

The hydrolysis of whey proteins using increasing enzyme:substrate ratios (E:Ss) was an important point to determine which E:S ratio would

Table 3
Experimental Hydrolysis Degree (*HDexp*) for Filtrate Whey Using Trypsin[a]

Time (s)	(E:S)	Cenz (U/mL)[b]	%*HDexp*	*HDth* (%)	η[b] (%)[c]
0	1:100	12.96	—	—	—
1800	—	11.55	5.57 ± 0.61	11.96	46.57
1860	1:50	—	—	—	—
3600	—	20.98	8.8 ± 0.95	11.96	73.58
0	1:20	64.78	—	—	—
1800	—	54.98	8.13 ± 0.48	11.96	67.98
1860	1:16.7	—	—	—	—
3600	—	60.38	11.28 ± 0.6	11.96	94.31
0	1:4	323.89	—	—	—
1800	—	256.41	11.93 ± 1.19	11.96	99.75

[a]E:S = enzyme:substrate ratio; η = (*HDexp*/*HDth*) × 100; NaOH = 0.027 *M*.
[b]The yield was not calculated at the time when the enzyme was added.
[c]Calculated yield.

Table 4
Experimental Hydrolysis Degree (*HDexp*) for Filtrate Whey Using α-Chymotrypsin

Time (s)	(E:S)	Cenz (U/mL)[b]	%*HDexp*	*HDth* woLeu (%)[c]	*HDth* WLeu (%)[d]	η (%)[e]
0	1:100	11.98	—	—	—	—
1800	—	10.28	7.53 ± 0.56	7.39	19.48	38.66
1860	1:50	—	—	7.39	—	—
3600	—	18.62	11.20 ± 0.79	7.39	19.48	57.34
0	1:20	56.92	—	7.39	—	—
1800	—	49.17	7.60	7.39	19.48	39.01
1860	1:16.7	—	—	7.39	—	—
3600	—	54.15	10.71	7.39	19.48	54.97
0	1:4	284.62	—	7.39	—	—
1800	—	241.83	8.56	7.39	19.48	43.89
1860	1:3.8	—	—	7.39	—	—
3600	—	234.63	10.62	7.39	19.48	54.52

[a]E:S = enzyme:substrate ratio; η = (*HDexp*/*HDth*) × 100; NaOH = 0.027 *M*.
[b](BTEE-U)/mL of solution.
[c]Not considering leucine residues.
[d]Considering leucine residues.
[e]Calculated yield.

lead to the maximum experimental hydrolysis degree, for a predetermined time, i.e., one that would allow us to control the reaction degree. Experiments with three E:S ratios were accomplished, using trypsin and α-chymotrypsin, separately; the results are given in Tables 3 and 4.

For trypsin, the maximum experimental hydrolysis degree was reached within 0.5 h of reaction with an initial E:S of 0.25 (initial concentration of enzyme about 324 BAEE-U/mL of solution). The agreement between experimental and theoretical values of the maximum hydrolysis degree indicates a high specificity of trypsin for lysine and argentine residues, as reported in the literature *(11)*. However, because the theoretical value calculated for whey is not exact, this conclusion has to be confirmed using purified β-lactoglobulin as the substrate. In the case of α-chymotrypsin, the maximum experimental hydrolysis degree seems to be, in fact, about 11%, reached after 60 min of reaction. This value did not change significantly, within the experimental error, when enzyme concentrations were four times higher.

Concerning α-chymotrypsin specificity, Abeles et al. *(11)* stated that this enzyme attacks preferentially peptide bonds with bulky nonpolar aromatic groups, but it also attacks, although more slowly, nonpolar groups such as leucine. The experimental value obtained for the maximum hydrolysis degree is smaller than the theoretical calculated value, when the possibility of hydrolysis of all peptide bonds with leucine residues on the carboxyl side of the bonds is considered. A possible explanation would be that a fraction of the enzyme molecules binds to the proteins at the points where phenylalanine, tyrosine, and tryptophan residues are in the required positions and breaks the peptide bonds quickly. Meanwhile, other enzyme molecules bind to protein molecules at leucine residues, but the reaction rate is small in this case. That behavior could explain the observed experimental hydrolysis degree. However, if the reaction rate were the only restriction, when the enzyme concentration is increased, the experimental degree should also be higher, which was not observed. For all the investigated conditions, the hydrolysis degree reached approx 56% of the maximum theoretical degree. Therefore, this "kinetic" explanation for the uncompleted hydrolysis is not reasonable. In addition, thermal inactivation of the enzyme cannot be responsible for this behavior. The activities of the proteases were followed during the reaction and were equal to 100% of the initial activity for the whole reaction time, at 40°C.

Another possible explanation for this phenomenon could be the incomplete hydrolysis of β-lactoglobulin (about 50% of the protein content of cheese whey). Reddy et al. *(12)* affirm that α-chymotrypsin does not attack this protein, owing to its high conformational stability. To verify this hypothesis, α-chymotrypsin was used to hydrolyze purified β-lactoglobulin.

Hydrolysis of β-Lactoglobulin: Specificity of Proteases Trypsin and α-Chymotrypsin

Table 5 presents the results obtained in independent experiments using trypsin and α-chymotrypsin. It is observed that, for trypsin, the maximum experimental hydrolysis degree is approached. An experiment with trypsin, treated to eliminate α-chymotrypsin activity, was also

Table 5
Experimental Hydrolysis Degree (*HDexp*)
of β-Lactoglobulin Using Trypsin and α-Chymotrypsin[a]

Trypsin[b]				
Time (s)	%*HDexp*	*HDth* (%)	η (%)[c]	
0	—	11.11	—	
3600	7.29 ± 0.06	11.11	65.62	
7200	8.77 ± 0.06	11.11	78.94	
10,800	9.51 ± 0.44	11.11	85.60	
14,400	10.05 ± 0.33	11.11	90.46	
α-Chymotrypsin				
Time (s)	%*HDexp*	*HDth* woLeu (%)[d]	*HDth* wLeu (%)[e]	η (%)[c]
0	—	—	—	—
3600	12.62	6.17	19.75	63.90
7200	14.96	6.17	19.75	75.75
10,800	14.96	6.17	19.75	75.75
14,400	14.96	6.17	19.75	75.75

[a]Mass of protein = 0.175 g; mass of enzyme = 0.002 g; C_{enz} = 12.5 (BAEE- or BTEE-U/mL of solution in the reactor); NaOH = 1 *M*.
[b]Trypsin from Novo Nordisk of Brazil.
[c]Calculated yield.
[d]Not considering leucine residues.
[e]Considering leucine residues.

performed to verify the influence of protease purity on hydrolysis degree. The results showed that the experimental hydrolysis degree is similar to the calculated, within the experimental error, confirming the previous results and validating, therefore, the methodology used in this work to study protease specificity.

The results using α-chymotrypsin allowed us to conclude that this enzyme is able to attack peptide bonds of β-lactoglobulin protein, within its specificity. A yield of 75.75% was observed, and this value is higher than that obtained for cheese whey, in which other proteins are also present. Figure 1 shows the disappearance of the β-lactoglobulin band in SDS-PAGE after 4 h of reaction, confirming that this protein is hydrolyzed by α-chymotrypsin. Thus, the inaccessibility of the protein to this protease is not the reason for the low yields that were observed. Proceeding with the experimental investigation, sequential hydrolysis using the two proteases was performed. First of all, cheese whey and purified β-lactoglobulin were submitted to trypsin action. After inactivation of this protease, the substrate was submitted to α-chymotrypsin. The experimental hydrolysis degrees obtained were compared with the theoretical values calculated for

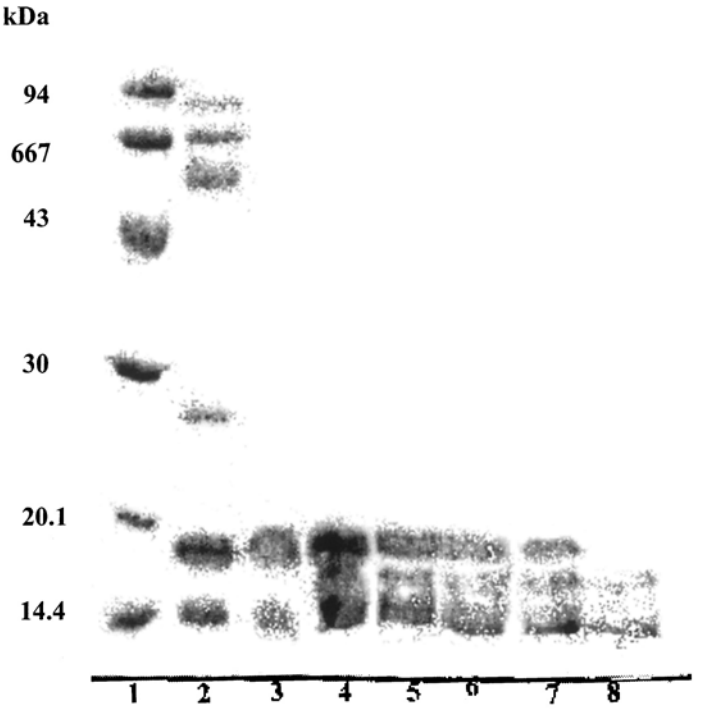

Fig. 1. Incubation of β-lactoglobulin with α-chymotrypsin. Lane 1: Molecular weight marker (14,000–94,000 Daltons); lane 2: unhydrolyzed cheese whey; lane 3: unhydrolyzed β-lactoglobulin (4 g/L); lanes 4, 5, 6, 7, and 8: hydrolysates after 30 min, 2 h, 4 h, 6 h, and 24 h, respectively.

the sequential action. Table 6 presents the obtained results, for filtrated whey and β-lactoglobulin.

The yields obtained with the sequential action of α-chymotrypsin are even smaller than those obtained using this protease alone. For whey, HDexp of 11 and 6.4% (yields of about 56 and 39%) were obtained for individual and sequential action of α-chymotrypsin, respectively. For β-lactoglobulin, HDexp of 15 and 5.14% (yields of 75.75 and 30.83%) were obtained, respectively. Since treated proteases were used and trypsin has a high specificity, an increase in the yield was expected after the sequential action experiments. However, a reduction was observed for whey and β-lactoglobulin (note that for the purified protein the theoretical degree can be calculated with accuracy).

We finally conclude that the decrease in length of the polypeptide chain prevents the hydrolysis of some bonds, probably the ones that have leucine residues, since the affinity of the enzyme for this residue may be smaller than for the aromatic ones. Positive confirmation of this conclusion will require size identification of the liberated oligopeptides after the hydrolysis reaction. This analysis should also include the identification of peptides' terminal residues.

Table 6
Sequential Hydrolysis of Cheese Whey Proteins
and Purified β-Lactoglobulin Using Trypsin and α-Chymotrypsin[a]

Filtrated whey						
Enzyme	Time (h)	Cenz	%*HDexp*	*HDth* (%)		η (%)[b]
Trypsin	4	23.57	11.09	11.96		92.73
				HDth woLeu (%)[c]	*HDth* wLeu (%)[d]	
Chymotrypsin (after action of the trypsin)	4	10.92	6.43	5.84	16.41	39.18
β-Lactoglobulin						
Enzyme	Time (h)	Cenz	%*HDexp*	*HDth* (%)		η (%)[b]
Trypsin	4	12.25	9.82	11.11		88.39
				HDth woLeu (%)[c]	*Hdth* wLeu (%)[d]	
Chymotrypsin (after action of the trypsin)	4	10.98	5.14	4.32	16.67	30.83

[a]NaOH = 1 *M*.
[b]Calculated yield.
[c]Not considering leucine residues.
[d]Considering leucine residues.

Theoretical Peptide Compositions (%PC_{th}*)*

Probable distributions of the molecular mass of the peptides for maximum degree of hydrolysis with trypsin, chymotrypsin, and chymotrypsin after previous action of trypsin (sequential hydrolysis) were calculated. This assessment was based on the primary sequence of proteins, on the specificity of the proteases, and on the assumed protein composition for cheese whey. Figure 2 shows the action of chymotrypsin after total hydrolysis of purified β-lactoglobulin. Table 7 presents the simulated percentile compositions calculated for different cases. These simulated results were compared with those reported in the literature *(13–15)*, in which the authors analyzed, by high-performance liquid chromatography and mass spectrometry, the molecular masses of the peptides obtained under similar operational conditions.

Chobert et al. *(16)* studied the hydrolysis of β-lactoglobulin with trypsin. They found that 20% of the resulting peptides were about 2700 Daltons.

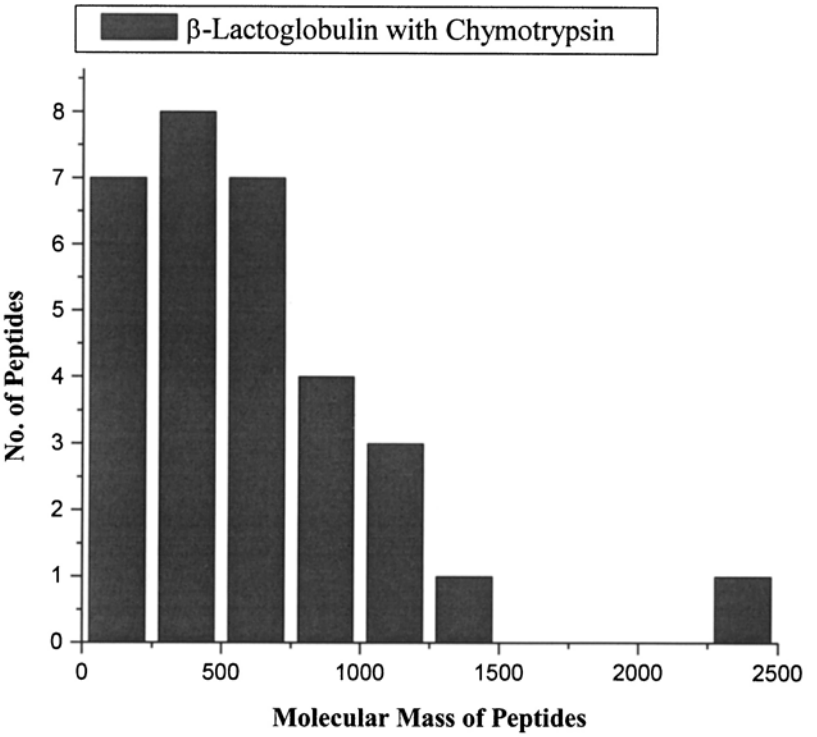

Fig. 2. Simulated distribution of peptide size after the hydrolysis of β-lactoglobulin with chymotrypsin.

Our theoretical distribution shows 10.5% of peptides in the range 2500–2750 Daltons. However, probably the value of 20% obtained by Chobert et al. *(16)* also took into account the peptides obtained in the 1250- to 2750-Daltons range, because the molecular weight standards usually have a larger range of values. It is also possible that they did not achieve the maximum degree of hydrolysis in their experiments.

After 4 h of hydrolyzing β-lactoglobulin with trypsin, a peptide with molecular mass of 1658 Daltons was identified by Otte et al. *(13)* using mass spectrometry. This fragment was also theoretically identified when simulating the peptide composition in this work.

Iung et al. *(17)*, after hydrolysis of β-lactoglobulin with trypsin, obtained two fragments with molecular mass between 4000 to 5000 Daltons and 14,000 Daltons (10% yield); molecular mass was estimated by SDS-PAGE. These components were not identified in our maximum hydrolysis profile. Iung et al.'s *(17)* experiments probably did not achieve maximum reaction conversion, either.

The analytical characterization of the peptide size distribution obtained after controlled proteolytic hydrolysis will be made in continuing studies of this work.

Table 7
Probable Distributions of Molecular Mass of Peptides for Maximum Degree of Hydrolysis[a]

Molecular mass range (Daltons)	Theoretical peptide composition (%)											
	α-Lactalbumin			β-Lactoglobulin			BSA			Cheese whey		
	Tryp	Chymo	Chymo after trypsin action	Tryp	Chymo	Chymo after trypsin action	Tryp	Chymo	Chymo after trypsin action	Tryp	Chymo	Chymo after trypsin
0–250	13.3	24.0	36.1	15.8	22.6	41.3	8.4	23.4	30.6	13.1	23.1	37.5
250–500	20.0	36.0	41.7	10.5	25.8	19.6	26.5	31.5	43.4	17.0	29.1	30
500–750	20.0	20.0	11.1	15.8	22.6	26.1	20.5	15.3	17.9	17.9	20.2	21.2
750–1000	20.0	8.0	5.6	21.1	12.9	8.7	12.1	13.5	5.7	18.1	12.2	7.3
1000–1250	13.3	4.0	2.8	15.8	9.7	2.2	7.2	2.7	1.2	12.8	6.8	2.0
1250–1500	—		—	5.3	3.2	2.2	13.3	8.1	1.2	6.8	3.9	1.5
1500–1750	6.7	4.0	2.8	5.3	—		7.2	1.8	—	6.1	1.2	0.5
1750–2000	—		—	—	—		3.6	—	—	1.1	—	—
2000–2250	—		—	—	—		—	3.6	—	0	1	—
2250–2500	—	4.0	—	—	3.2		1.2	—	—	0.4	2.5	—
2500–2750	—		—	10.5	—		—	—	—	5.5	—	—
4750–5000	6.7		—	—	—		—	—	—	1.1	—	—

[a]Tryp, trypsin; Chymo, chymotrypsin.

Table 8
Experimental Hydrolysis Degrees
of Filtered Whey at 40 and 55°C Using Trypsin and α-Chymotrypsin

Trypsin					
Time (s)	%*HDexp*		*HDth* (%)	η (%)[a]	
	T = 55°C	*T* = 40°C		*T* = 55°C	*T* = 40°C
0	—	—	—	—	—
3600	7.51	4.23	11.96	62.79	35.37
7200	7.91	5.51	11.96	66.14	46.07
10,800	8.18	6.71	11.96	68.40	56.10
14,400	8.43	8.05	11.96	70.48	67.31

α-Chymotrypsin						
Time (s)	%*GHexp*		*HDth* (%)		η (%)[a]	
			woLeu (%)[b]	wLeu (%)[c]		
	T = 55°C	*T* = 40°C			*T* = 55°C	*T* = 40°C
0	—	—	—	—	—	—
3600	8.70	7.07	7.39	19.48	44.66	36.29
7200	9.29	8.76	7.39	19.48	47.69	44.97
10,800	9.58	9.82	7.39	19.48	49.18	50.41
14,400	9.71	10.60	7.39	19.48	49.85	54.41

[a]Calculated yield.
[b]Not considering leucine residues.
[c]Considering leucine residues.

Hydrolysis of Cheese Whey Proteins with Trypsin and α-Chymotrypsin at 55°C

The use of temperatures higher than 40°C is important to avoid contamination as well as to increase reaction rates. Studies comparing evaporation and ultrafiltration to concentrate raw whey concluded that the latter displayed higher economic viability *(11)*. The permeation rate, however, is a critical variable. Viotto *(18)* studied several whey pretreatments in order to sustain high permeation flows during the concentration process. Keeping whey at 55°C, for 44 min, was found to be the most effective treatment. For this reason, hydrolysis experiments using the two proteases were conducted at 55°C; the results are given in Table 8. Values obtained at 40°C are also presented to facilitate discussion.

We can note that the values of hydrolysis degree obtained in the first hour of reaction are higher at 55°C, as expected, for the two proteases. Since the same trypsin concentration was used, it was expected to reach a higher hydrolysis degree for the highest temperature, provided that enzymatic deactivation was negligible. Nevertheless, the conversion achieved for both temperatures, 40 and 55°C, after 4 h of reaction, is very close—and still far from the theoretical maximum degree. The explanation for these results is

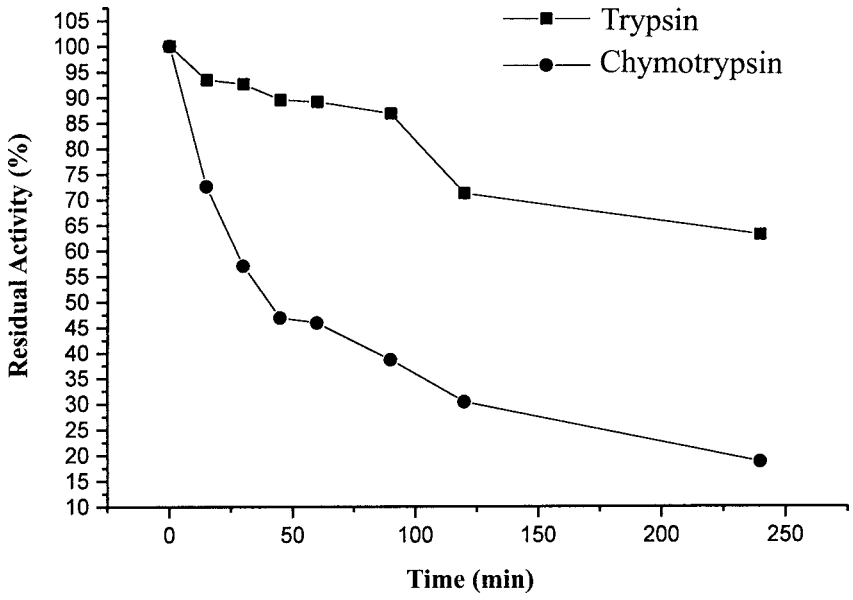

Fig. 3. Residual activity of proteases during hydrolysis of cheese whey proteins at 55°C.

the sharp decrease in the protease activity at 55°C, as can be seen in Fig. 3. The use of high temperatures for this kind of process is strongly recommended to avoid contamination, but the low operational stability of the enzymes in such conditions has to be overcome. Multipoint immobilization of the proteases on insoluble supports as agarose might significantly improve the thermal stability of these enzymes, and this will be investigated in the continuation of this work.

Conclusion

The maximum hydrolysis degree for sweet cheese whey (11.9%) using trypsin was reached after 1800 s of reaction, with an initial concentration of enzyme equal to 324 (BAEE-U)/mL of solution; one hundred percent yield with respect to the theoretical value was obtained, confirming the high specificity of this protease. Using α-chymotrypsin the obtained yield was 56% for cheese whey and 75.75% for β-lactoglobulin, showing that the enzyme does hydrolyze this protein. SDS-PAGE analysis confirms this result. The sequential use of α-chymotrypsin after trypsin indicated that the action of α-chymotrypsin seems to depend on the size of the oligopeptide: the proteases were stable when operating at 40°C but had severe thermal inactivation at 55°C.

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