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J.M. Chobert, A. Touati, C. Bertrand-Harb, Michèle Dalgalarrrondo, M.G. Nicolas. Solubility and emulsifying properties of kappa casein and its caseinomacropeptide. *Journal of Food Biochemistry*, 1989, 13 (6), pp.457-473. hal-02722039

HAL Id: hal-02722039

<https://hal.inrae.fr/hal-02722039>

Submitted on 1 Jun 2020

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SOLUBILITY AND EMULSIFYING PROPERTIES OF KAPPA CASEIN AND ITS CASEINOMACROPEPTIDE

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Received for Publication April 25, 1989
Accepted for Publication July 25, 1989

ABSTRACT

Kappa-casein A was treated with chymosin in order to isolate the caseinomacropепptide corresponding to the C-terminal 106-169 residues of κ -casein. Whole casein, κ -casein and the caseinomacropепptide (CMP) were studied for their water solubility and emulsifying activity. The CMP was soluble over the range of pH from 1 to 10, with a "minimum" solubility (88%) in the range of pH 1-5 and a "maximum" solubility (98%) in the range of pH 5-10. For whole casein and κ -casein, at pH values above 5.5, the emulsifying activity increased when pH increased and the maximum value was obtained for very alkaline solutions; for pH values below 4.5, the increase in emulsifying activity was much more pronounced at pH 2.5; below pH 2.5, emulsifying activity decreased. For CMP, the increase in emulsifying activity was much more pronounced in the acidic range than in the alkaline range. After 24 h storage and heating of the emulsion, a large pH-dependant decrease of emulsifying activity (22-60%) was observed for CMP for pH values below 4.0; under the same conditions, the emulsifying activity of whole casein and κ -casein showed a 5-19% and a 1-21% decrease, respectively. For pH values above 6.0, a 22-59% decrease was observed for CMP as compared to a 1-12% and a 4-17% decrease with whole casein and κ -casein, respectively.

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INTRODUCTION

Modification of a protein usually refers to physical, chemical, or enzymatic treatments changing its conformation and structure and consequently its physicochemical and functional properties. This subject has been widely reviewed (Kinsella 1976; Cheftel 1977; Friedman 1977, 1979; Feeney and Whitaker 1977; Kinsella and Shetty 1979; Phillips and Beuchat 1981; Morr 1982; Whitaker and Puigserver 1982; Cheftel *et al.* 1985; Kilara and Sharkasi 1986; Feeney 1987; Chobert and Mesnier 1988; lung and Linden 1988).

Enzymatic hydrolysis of food proteins frequently destroys their functionality. However, in some cases, the hydrolysis provides a useful means for improving the functional properties of the proteins. For example, increases in the emulsifying capacity, emulsion stability and foaming capacity of various proteins by enzymatic hydrolysis have been reported (Kuehler and Stine 1974; Horiuchi *et al.* 1978; Adler-Nissen and Olsen 1979; Jost *et al.* 1982; Rahma and Rao 1983).

The peptides, which have smaller molecular sizes and simpler structures than proteins, can be expected to have different functional properties from those of proteins. The functional peptides may be useful in various food processing operations, but very little information has so far been available on the functional properties of peptides produced by proteolysis (Adler-Nissen 1976, 1984; Adler-Nissen and Olsen 1979; Olsen and Adler-Nissen 1979; Gunther 1979; Adler-Nissen *et al.* 1983; Shimizu *et al.* 1986; Lee *et al.* 1987a and b; Chobert *et al.* 1988a and b; Chobert *et al.* 1989).

Milk proteins, the molecular structure of which has been extensively studied, can be used as models for determining some relationships between structure and functionality. Bovine κ -casein, whose primary structure has been well established (Mercier *et al.* 1973), has a distinctive amphipathic structure. Its N-terminal region is hydrophobic, while the C-terminal region is hydrophilic. This distinct separation of the hydrophobic and charged domains has been appreciated for some time due to the specific chymosin-catalyzed hydrolysis which releases the caseinomacropptide resulting in the clotting of para- κ -casein; the only bond hydrolyzed during the primary action of chymosin is Phe 105—Met 106. Shimizu *et al.* (1983, 1986) have determined the emulsifying properties of bovine α_1 -casein and its peptides that were formed by limited proteolysis with pepsin or papain. They demonstrated that the α_1 -CN (f 1–23) peptide fraction showed an emulsifying activity index (E.A.I.) value similar to that of α_1 -casein at neutral pH levels. However, such a high E.A.I. value was due to the coexistence of some peptides in the fraction. By mixing α_1 -CN (f1–23) with α_1 -CN (f154–199) or the caseinomacropptide a synergistic effect on the emulsifying properties of α_1 -CN (f1–23) was observed. Lee *et al.* (1987a) isolated two peptides, one hydrophilic, β -CN (f1–25) by action of trypsin on β -casein and the other hy-

drophobic, β -CN (f193–209) by action of chymosin on β -casein. The emulsifying activity of both peptides was low at a neutral pH. In acidic or alkaline conditions, however, β -CN (f193–209) showed high emulsifying activity values; β -CN (f1–25) also showed high emulsifying activity values at acidic pH. These peptides were shown to be more surface active at pH 3 than at pH 7.

Peptide size control is essential if optimum and reproducible changes in functional properties are to be achieved. This control might be accomplished by use of highly specific proteases (Whitaker and Puigserver 1982). Changes in solubility and emulsifying activity of whole bovine casein digested either with *Staphylococcus aureus* V8 protease (Chobert *et al.* 1988b) or with trypsin (Chobert *et al.* 1988a) have been described. Recently, we have studied the changes in solubility and emulsifying activity of β -casein modified enzymatically by trypsin (Chobert *et al.* 1989).

In this paper, we describe the changes in solubility and emulsifying properties (emulsifying activity and emulsion stability) of bovine κ -casein and its caseinomacropptide.

MATERIALS AND METHODS

Preparation of κ -casein

Crude κ -casein A was prepared as described by Zittle and Custer (1963) from the milk of a cow homozygous at the four casein loci. This fraction was then chromatographed, according to Mercier *et al.* (1968), on a Q-Sepharose Fast-Flow column (5 × 50 cm; Pharmacia, Uppsala, Sweden). The elution of κ -casein from the column was carried out with 0.02 M imidazole-HCl buffer (pH 7.0) containing 3.3 M urea, 0.16 M NaCl and 1% 2-mercaptoethanol. The κ -casein fraction was dialysed against distilled water, then freeze-dried and stored at –80°C. The homogeneity of the final product was checked by electrophoresis and size-exclusion chromatography (as described below). The concentration of the κ -casein solution was determined after filtration on 0.45 μ m filters, from the extinction coefficient $E_{280\text{nm}}^{\text{1mg/mL}} = 0.95$ given by Swaisgood (1982).

Chemicals and Enzyme

Organic solvents from Carlo Erba (Italy) were used for HPLC. All other reagents were of analytical grade. Buffers and solvents for HPLC were filtered through Millipore 0.45 μ m filters (Millipore Corp., Bedford, MA) and degassed under vacuum before use. Chymosin (E.C. 3.4.23.4) and sodium dodecyl sulfate (SDS) were obtained from Sigma Chemical Co. Bicinchoninic acid (BCA) Protein Assay Reagent, 6 N hydrochloric acid, phenyl isothiocyanate (PITC) and

amino acid standards were from Pierce Chemical Co. Rapeseed oil was from Carrefour, France.

Isolation of the Caseinomacropeptide (CMP) from κ -casein

Limited cleavage of the κ -casein was carried out by using chymosin, according to Lee *et al.* (1987b), with slight modifications. To a 1.5% κ -casein solution (w/v) in 50 mM imidazole-HCl buffer (pH 6.5) was added chymosin to give an enzyme/substrate ratio of 0.5/1000 (w/w) and the mixture was incubated at 35°C, for 10 min. After incubation, the solution was immediately centrifuged at 4,000 g for 10 min at 15°C. The supernatant (CMP fraction) was acidified to 4.0 and without delay chromatographed on a Trisacryl SP (IBF, France) column (33 × 5.5 cm) in 50 mM sodium acetate-acetic acid buffer, pH 4.0; the flow-rate was 3.2 mL/min. The first fraction passed through the column was collected and desalted on a Sephadex G-15 (Pharmacia, Uppsala, Sweden) column (95 × 3.2 cm) in distilled water with a flow rate of 3.2 mL/min. The CMP, detected at 226 nm, was collected, freeze-dried and stored at -80°C.

Polyacrylamide Gel Electrophoresis (PAGE)

PAGE was carried out on gel slabs (8 cm × 8 cm × 1 mm; Biometra, Minigel, Gottingen, RFA) with a running gel (10% acrylamide, 0.26% N,N'-methylenebis (acrylamide) containing 7 M urea) and a stacking gel (3.5% acrylamide, 0.10% N,N'-methylenebis (acrylamide) containing 7 M urea). The gel buffer was 2 M Tris, pH 8.8, for the running gel and 0.5 M Tris, pH 6.8, for the stacking gel. The electrode buffer was 0.04 M Tris, 0.4 M glycine, pH 8.3. The sample buffer was 0.5 M Tris, 7 M urea, 0.5% 2-mercaptoethanol, pH 6.8. The samples were boiled in the buffer for 2 min and cooled prior to application to the gel slab. Electrophoresis was carried out at 10 mA for the stacking gel then 20 mA for the running gel, at room temperature, for 90 min. Proteins were stained with 0.2% Coomassie Brilliant Blue R 250 in 5% acetic acid—30% ethanol. The gels were destained with 5% acetic acid—30% ethanol.

High Performance Liquid Chromatography

The equipment consisted of a system controller model 680, two solvent delivery systems model 510, a U6K injector, a temperature control system, a variable wavelength U.V. monitor model 455, an integrator model 740 (Waters Associates, Milford, MA, USA); a peak separator model 2150 and a fraction collector (Isco, Lincoln, Nebraska).

Size-exclusion chromatography of κ -casein and its CMP was performed on a Protein Pak 60 column (7.8 mm i.d. × 30 cm; Waters), equilibrated in a 50 mM K phosphate buffer, pH 7.0, containing 3.3 M urea and 0.20 M NaCl.

The flow rate was 0.5 mL/min. The absorbance was recorded at 214 nm. The column was calibrated with β -lactoglobulin (18,000), α -lactalbumin (14,400), cytochrome c (12,100), aprotinin (6,500), insulin chain B (3,500), insulin chain A (2,500), and polymyxin B sulfate (1,447).

Reversed-phase HPLC (RP-HPLC) of CMP was performed on a μ Bondapak C18 column (2.0 mm i.d. × 30 cm; Waters), equilibrated in solvent A (0.15 M NaCl/HCl, pH 2.50) and the elution was obtained by using a linear gradient from solvent A to solvent B (60% acetonitrile and 40% solvent A) in 30 min. Both the column and solvents were maintained at 40°C. The flow rate was 0.5 mL/min and the absorbance was recorded at 214 nm.

Following acid hydrolysis in a Pico-Tag Station (Waters), amino acids were quantified by RP-HPLC on a Pico-Tag column (Waters) after derivatization with PITC according to Bidingmeyer *et al.* (1984).

Solubility

Whole casein, κ -casein and CMP were dispersed in distilled water (0.1%, w/w) by mixing with a "Vortex". The pH was adjusted from 1.0 to 10 by using HCl or NaOH of high normality to limit dilution. After a 30-min equilibration period at room temperature (20°C), a part of each solution was used to determine emulsifying properties; the rest was centrifuged for 15 min at room temperature (Lab Centrifuge MLW T5) at 5,500 rpm (3,000 g). The protein content of the supernatant was determined by using the Bicinchoninic Acid Protein Assay (Pearce Chemical 1986) with whole casein, κ -casein or CMP as a standard. The solubility was expressed in percentage of total protein concentration.

Isotonic Point

The mixed-bed deionization procedure developed by Janus *et al.* (1951) and used by Ho and Waugh (1965) was employed. Ten milligrams of κ -casein or CMP were dissolved in 10 ml of deionized water with stirring. One gram of mixed-bed ion-exchange resin (AG 501-X8; Bio-Rad, Richmond, CA) was added to the protein or peptide solution and the pH monitored until it became constant. The isotonic point of κ -casein or CMP was considered to be this pH, and proteins of known isotonic points were used as standards.

Emulsifying Activity

To prepare the emulsion, 3 mL of 0.1% protein solution and 1 mL of rapeseed oil (ϕ , volume fraction of the dispersed phase = 0.25) were shaken together and homogenized at 20,000 rpm for 30 s at room temperature (Kinematica GmbH Polytron equipped with a Reco 20 T speed and time control system).

With no protein present, the emulsions were unstable and the turbidity varied from experiment to experiment. In a few cases water-in-oil emulsions were formed. The presence of as little as 0.1% protein caused a marked increase in the stability of the emulsion and improved reproducibility. Therefore, all results are reported without correction for turbidity in the absence of protein. The blank was water, which had the same absorbance as 0.1% SDS in 0.1 M NaCl, pH 7.0.

Emulsifying activity of the caseins was evaluated by spectroturbidity according to Pearce and Kinsella (1978), with slight modification. Aliquots were immediately pipetted from the emulsion and diluted 500-fold into 0.1% (w/v) SDS in 0.1 M NaCl, pH 7.0. The tubes were inverted 3 times to obtain homogeneous mixtures, then absorbance at 500 nm was recorded. Identical 1-cm pathlength glass cuvettes were used and were rinsed with a jet of distilled water and dried between determinations. Absorbancies of duplicate aliquots of each emulsion were measured and the individual values plotted. The emulsifying activity was expressed as its emulsifying activity index (E.A.I.).

E.A.I. = $2T/\phi c$, where T = turbidity = $2.3 A/1$ (A = absorbance at 500 nm and 1 = light path = $1 \text{ cm} = 10^{-2} \text{ m}$), ϕ = oil phase volume = 0.25 and c is concentration of protein (0.1%) before the emulsion is formed.

Emulsion Stability

The stock emulsions prepared above were held at room temperature (20°C) for 24 h. After stirring, aliquots were diluted and turbidity measured as described above (E.A.I., 20°C). The 24 h-old emulsions were then heated at 80°C for 30 min. After cooling to room temperature and stirring, turbidity was again measured as above (E.A.I., 80°C). The emulsion stability was calculated by the formula:

$$\Delta \text{EAI} \% = \frac{\text{EAI}_{\text{max}} - \text{EAI}_{80^\circ\text{C}}}{\text{EAI}_{\text{max}}} \times 100$$

where E.A.I. max is the maximum value obtained either at t_0 or $t_{24\text{h}}$. These values did not differ significantly, except for CMP. The smaller the value of Δ E.A.I. %, the better the stability.

RESULTS AND DISCUSSION

Purification of κ -casein

The homogeneity of the κ -casein fraction obtained after purification on a Q-Sepharose Fast-Flow column was checked by PAGE (Fig. 1), size-exclusion chromatography (data not shown) and amino acid composition (Table 1).

FIG. 1. PAGE OF CASEINS

See Methods for complete details. 1, whole bovine casein; 2, κ -casein; 3, κ -casein after chymosin action.

Isolation of the Caseinomacropeptide

The CMP was obtained from the chymosin hydrolysate of κ -casein. The peptide fraction showed no band on PAGE (Fig. 1) but produced one peak by size-exclusion chromatography (Fig. 2A) and by RP-HPLC analysis (Fig. 2B). The amino acid composition of this fraction (Table 2) indicates that this peptide corresponded to the C-terminal 106–169 residues of κ -casein.

Solubility

The typical water solubility curve of whole casein as a function of pH was also obtained for κ -casein; however, the minimum solubility was observed at pH 5.30 for κ -casein, instead of 4.60 for whole casein (Fig. 3). By contrast, the CMP was soluble over the range of pH from 1 to 10, with a "minimum" solubility (88%) in the range of pH 1–5 and a "maximum" solubility (98%) in the range of pH 5–10.

Emulsifying Activity

Emulsions were prepared from whole casein, κ -casein and CMP solutions over a range of pH values. The emulsifying activity index (E.A.I.) increased at pHs above and below pH 4.5–5.5 (Fig. 4 and Table 3). For whole casein and κ -casein, for pH values above 5.5, E.A.I. increased when pH increased and the maximum value was obtained for very alkaline solutions; for pH values below

TABLE I.
AMINO ACID COMPOSITION OF κ -CASEIN FRACTION

Amino acid	Molar ^a ratio	Nearest Integer	κ -Cr ^b
Asx	10.97	11	12
Glx	28.07	28	27
Ser	12.55	13	13
Gly	2.48	2	2
His	3.11	3	3
Arg	5.30	5	5
Thr	14.10	14	15
Ala	14.30	14	14
Pro	20.16	20	20
Tyr	9.27	9	9
Val	11.00	11	11
Met	2.25	2	2
Ile	12.05	12	12
Leu	8.97	9	8
Phe	4.36	4	4
Lys	9.46	9	9
Cys	nd		2
Trp	nd		1

nd = not determined

^a = values obtained after 60 min hydrolysis at 150°C with 6 N HCl

^b = according to Mercier *et al.* 1973.

4.5, the increase in E.A.I. was much more pronounced at pH 2.5; below pH 2.5, E.A.I. decreased. For CMP, the increase in E.A.I. was much more pronounced in the acidic range than in the alkaline range; the dip in the E.A.I. occurred at its pI (pH 4.10–4.20), regardless of its solubility.

Emulsifying Stability

After 24 h storage and heating of the emulsion, a large pH-dependent decrease of emulsifying activity index (22–60%) was observed for CMP for pH values below 4.0; under the same conditions, E.A.I. of whole casein and κ -casein showed a 5–19% and a 1–21% decrease, respectively (Table 4). For pH values above 6.0 a 22–59% decrease was observed for CMP as compared to a 1–12% and a 4–17% decrease with whole casein and κ -casein, respectively. These data

FIG. 2A. SIZE-EXCLUSION CHROMATOGRAPHY OF CMP ON A PROTEIN PAK 60 COLUMN (7.8 mm i.d. $\times$ 30 cm) Chromatography was performed in 50 mM phosphate buffer, pH 7.0, containing 3.3 M urea and 0.20 M NaCl.

FIG. 2B. REVERSED-PHASE HPLC OF CMP ON A μ -BONDAPAK C18 COLUMN (2.0 mm i.d. $\times$ 30 cm) Solvent A: 0.15 M NaCl, pH 2.50; solvent B: 60% acetonitrile and 40% solvent A. Flow rate: 0.5 mL/min; temperature 40°C.

suggest that there is no relationship between E.A.I. and emulsion stability. A protein (or a peptide) can have a good emulsifying activity and a good emulsion stability (whole casein or κ -casein) or a good emulsifying activity and a poor emulsion stability (CMP).

During the formation of an emulsion under ideal conditions, soluble protein diffuses to and concentrates at the oil-water interface once the interfacial electrostatic barrier is overcome. Solubility of protein is an important prerequisite for film formation because rapid migration to and adsorption at the interface is critical. Peptides have simpler structures compared with proteins. Peptides of low molecular weight are considered to have fewer tertiary structures and a less rigid conformation. The contribution of the primary structure to the emulsifying

TABLE 2.
AMINO ACID COMPOSITION OF THE PEPTIDE OBTAINED FROM THE CHYMOSIN HYDROLYSATE OF κ -CASEIN

Amino acid	Molar ratio ^a	Nearest integer	CMP ^b κ (106-169)
Asx	5.30	5	5
Glx	10.87	11	10
Ser	5.57	6	6
Gly	0.74	1	1
His	0	0	0
Arg	0	0	0
Thr	11.17	11	12
Ala	4.59	5	5
Pro	8.44	8	8
Tyr	0	0	0
Val	5.58	6	6
Met	0.92	1	1
Ile	5.81	6	6
Leu	1.09	1	1
Phe	0.28	0	0
Lys	2.82	3	3
Cys	nd		0
Trp	nd		0

nd = not determined

^a = values obtained after 60 min hydrolysis at 150°C with 6 N HCl

^b = according to Mercier *et al.* 1973.

property could be more pronounced in peptides. In order to find a peptide having emulsifying activity, hydrolysis of κ -casein by chymosin was surveyed in this study. Removal of the hydrophobic para- κ -casein after chymosin hydrolysis of κ -casein gave a peptide (CMP) with a solubility largely improved in the pH range as compared to the native κ -casein. However, no improvement of emulsion activity was obtained for CMP in this range of pH. For pH values below and above its pI, CMP showed a similar E.A.I. to that of intact κ -casein, indicating that a peptide whose molecular weight is approximately 7,000 can emulsify oil to a certain extent. It is well known that pH often influences the solubility of proteins, thereby affecting the emulsifying properties. However, the solubility of CMP did not change within the range of pH 1-10, suggesting that the emul-

FIG. 3. SOLUBILITY OF WHOLE-, κ -CASEIN AND CMP AS A FUNCTION OF pH
Solubility was measured after a 30 min equilibration period at room temperature (20°C).
The solubility is expressed as percent total protein (0.1%) in solution. Symbols used: $\square$ — $\square$, whole casein; $\circ$ — $\circ$, κ -casein; Δ — Δ , CMP.

sifying properties of the peptides did not relate to the solubility. The structure of CMP is considered to be affected by changing pH, since it contains 2 Asp, 7 Glu, 1 phospho-Ser and 3 Lys residues. At pH 3, dissociation of the Glu residues must be suppressed, which makes the C-terminal portion of κ -casein (CMP) less hydrophilic. The amphipathic property of the CMP induced by such a change in the ionizable groups might have caused the similar emulsifying activity as compared to κ -casein. CMP has a carbohydrate moiety, which might play some role in stabilization of the emulsified oil droplets. Lee *et al.* (1987b) have observed, by using lectins, that the carbohydrate moiety of CMP is on the outer surface of the oil droplets. CMP showed a great decrease in E.A.I. at alkaline pH after 24 h storage; in this range of pH this peptide might be too hydrophilic to stabilize the emulsion. Shimizu *et al.* (1986) demonstrated that a peptide of 23 residues, purified from the peptic hydrolysate of α - κ -casein, also had very complex emulsifying properties, being affected by coexistent peptides, emulsification conditions and other factors. Lee *et al.* (1987a) isolated two

FIG. 4. EMULSIFYING ACTIVITY INDEX (EAI) OF WHOLE- κ -CASEIN AND CMP AS A FUNCTION OF pH. See Methods for conditions. Symbols as in Fig. 3.

peptides, one hydrophilic and the other hydrophobic, from the hydrolysate (tryptsin and chymosin) of β -casein; the emulsifying activity of both peptides depended on the pH range.

In preceding papers we had shown that after partial hydrolysis (2.0 and 6.7% DH) of whole casein with *Staphylococcus aureus* V8 protease (Chobert *et al.* 1988b) emulsifying activity and stability of casein derivatives decreased as compared to control casein. After partial hydrolysis (4.3% to 9.9% DH) of whole casein with trypsin (Chobert *et al.* 1988a), emulsifying activity was improved but emulsifying stability of casein derivatives was lower than that of control

TABLE 3.
EMULSIFYING ACTIVITY INDEX (MEAN AND STANDARD DEVIATION OF FOUR MEASURES IN DUPLICATE) OF CASEINS AND PEPTIDE AT t_0 AND AFTER 24 H AT 20°C AND 30 MIN AT 80°C (°C)

pH	whole casein	κ -Cn	CMP
	t_0	t_0	t_0
2	197 $\pm$ 5.89	184 $\pm$ 3.26	190 $\pm$ 10.32
3	193 $\pm$ 6.00	171 $\pm$ 19.00	202 $\pm$ 11.50
7	232 $\pm$ 12.50	217 $\pm$ 21.00	204 $\pm$ 12.85
8	275 $\pm$ 5.00	257 $\pm$ 19.00	210 $\pm$ 7.92
9	281 $\pm$ 6.00	256 $\pm$ 7.79	236 $\pm$ 11.03
	t_0	t_0	t_0
2	197 $\pm$ 2.03	157 $\pm$ 9.28	210 $\pm$ 2.03
3	193 $\pm$ 6.40	106 $\pm$ 18.71	219 $\pm$ 6.40
7	232 $\pm$ 20.31	126 $\pm$ 15.68	194 $\pm$ 20.31
8	275 $\pm$ 14.95	135 $\pm$ 13.20	200 $\pm$ 14.95
9	281 $\pm$ 27.78	113 $\pm$ 17.00	224 $\pm$ 27.78

TABLE 4.
EMULSION STABILITY OF CASEINS AND PEPTIDE*

pH	whole casein	κ - Cn	C M P
2	5.23<6.60<7.88	1.00<3.16<5.55	21.70<25.24<28.85
3	4.52<11.39<18.71	2.82<11.39<20.94	44.44<51.60<59.15
7	2.45<6.46<10.90	5.53<6.37<7.33	33.64<35.05<36.78
8	1.42<6.54<11.85	3.67<6.19<8.91	31.16<32.50<34.05
9	8.01<8.89<9.82	7.29<11.86<16.89	48.41<49.55<51.02

*Results are expressed as percent difference between EAI max and that after thermal processing (see Methods).

whole casein. After partial hydrolysis (3.2% to 7.4% DH) of β -casein with trypsin (Chobert *et al.* 1989), when solubility was increased for β -casein derivatives, an improvement of emulsifying activity was observed while their emulsifying stability was lower than that of control β -casein. The present study demonstrates that a peptide of 64 residues also had very complex emulsifying properties. The hydrophilic C-terminal region of κ -casein showed similar emulsifying activity as compared to the amphiphilic κ -casein but a large decrease of emulsion stability particularly in the neutral and alkaline range of pH. In order to utilize peptides as functional ingredients for various food products, more knowledge of the peptide functionality is essential.

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