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ure-Derivate. Faserforsch.

ler Eigenschaften von Pro-
2, 101-120.

ne. 3. Mitt. Isolierung und
und Rapssamen. Nahrung

M. 1975. Über Samenpro-
en und Albuminen aus den
und Raps (*Brassica napus*
n fernen UV. Studia Bio-

and LINOW, K.-J. 1985.
inflower seed (*Helianthus*
ein Res. 25, 345-354.

D. 1986. Modification of
flower (*Helianthus annuus*
lation. Nahrung 30, 263-

D. and RAAB, B. 1988.
modified low-molecular mass
sica napus L.). Biochem.

tionation of rapeseed meal
n. Soc. 58, 96-98.

1. 1967. Protein as random
efficients in concentrated
, 729-742.

composition and functional
i isolate. J. Food Sci. 49,

SOLUBILITY AND EMULSIFYING PROPERTIES OF BETA-CASEIN MODIFIED ENZYMATICALLY BY TRYPSIN

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ABSTRACT

Beta A1-casein was treated with TPCK-trypsin to give 3.2, 5.0, 5.8 and 7.4% hydrolysis of the peptide bonds. By sodium dodecyl sulfate-polyacrylamide gel electrophoresis the resulting peptides had apparent molecular weights in the range 8,000-4,000. Size-exclusion chromatography of hydrolyzed samples showed four major peaks near 15,000, 5,500, 3,500 and 2,500 molecular weights, representing 17, 15, 7 and 14% of the material, respectively, after 3.2% hydrolysis and 9, 6, 14 and 52% of the material, respectively, after 7.4% hydrolysis. Between the extremes 3.2% and 7.4% hydrolysis, a peak near 8,500 molecular weight was present until 5.8% hydrolysis then disappeared after 7.4% hydrolysis to be replaced by a peak near 12,000 molecular weight. Peptides recovered from reversed-phase high performance liquid chromatography were analyzed by determination of their amino acid composition and identified in the sequence of β -casein. After trypsin treatment, the solubility of β -casein hydrolysates was largely increased at pH 4.0-7.5. The emulsifying activity of the hydrolysates was higher than that of β -casein in the range of pH 1.5-3.5 and 6.5-10.0, but all the emulsions obtained with trypsin-treated β -casein were less stable than those obtained with original β -casein.

INTRODUCTION

Modification of a protein usually refers to physical, chemical, or enzymatic treatments changing its conformation and structure and consequently its physi-

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cochemical 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).

Of the many actual and potential uses of enzymes for modification of proteins and improvement of their functional and nutritional properties (Whitaker 1974, 1977), that of hydrolysis of proteins is the most widely used (Fox *et al.* 1982; Chobert *et al.* 1990). The peptides produced by proteolysis have smaller molecular sizes and less secondary structure than proteins and may be expected to have increased solubility near the isoelectric point, decreased viscosity, and perhaps significant changes in the foaming, gelling, and emulsifying properties from those of original proteins. The 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, b; Chobert *et al.* 1988a, b).

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 β -casein, whose primary structure has been well established (Ribadeau Dumas *et al.* 1972), has distinctive amphipathic structure in the molecule. Its N-terminal region is hydrophilic, while the C-terminal region is hydrophobic. β -Casein is, therefore, considered to be a good source to prepare peptides of different average hydrophobicity.

Enzymatically hydrolyzed caseins are used in candy manufacture (Fox 1970). Haggett (1974) reported that proteolysis improved solubility of lactic acid precipitated casein. Shimizu *et al.* (1983, 1986) have determined the emulsifying properties of bovine α_{s1} -casein and its peptides that were formed by limited proteolysis with pepsin or papain. Recently, Lee *et al.* (1987a) isolated two peptides, one hydrophilic and the other hydrophobic, from the hydrolysate (trypsin and chymosin) of β -casein. The emulsifying activity of both peptides depended on the pH range.

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 recently been described.

In this paper, we describe the changes in solubility and emulsifying properties (emulsifying activity and emulsion stability) of bovine β -casein digested with trypsin, in relation to the size distribution of the resulting peptides.

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Materials

Preparation of β -case
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Chemicals and Enzym
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Limited Proteolysis of β -

Beta-casein (2 g) was c
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A sample of β -casein (c
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In order to determine tha
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3b) or with trypsin (Chobert

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sulting peptides.

MATERIALS AND METHODS

Materials

Preparation of β -casein. Crude β -casein A1 was prepared as described in 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 $\times$ 50 cm; Pharmacia, Uppsala, Sweden). Elution of β -casein from the column was carried out with 0.02 M imidazole-HCl buffer (pH 7.0) containing 3.3 M urea under a linear gradient of NaCl (0.15 $\rightarrow$ 0.30 M). The β -casein fraction was dialyzed against distilled water, then freeze-dried. The homogeneity of the final product was checked by electrophoresis and RP-HPLC (as described below). The concentration of the β -casein solution was determined after filtration on 0.45 μ m filters, from the extinction coefficient $E_{280 \text{ nm}}/1 \text{ mg/mL} = 0.46$ 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. Trypsin treated with tosyl phenylalanine chloromethyl ketone (13,000–14,000 BAEE U/mg), sodium dodecyl sulfate (SDS), 2,4,6-trinitrobenzenesulfonic acid, L-leucine and electrophoresis calibration kit were obtained from Sigma Chemical Co. Rapeseed oil was from Carrefour, France.

Limited Proteolysis of β -Casein with TPCK-Trypsin

Beta-casein (2 g) was dissolved in distilled water (final concentration 4 or 2%, W/V) by adjusting to pH 8.0 with 1 N NaOH. TPCK-trypsin, previously solubilized in distilled water adjusted to pH 8.0, was added to the reaction mixture in order to give a final E/S ratio from 0.25 to 1%. The mixture was incubated at 37°C and gently stirred during the reaction. Addition of 0.1 N NaOH was monitored by using a pH-stat (Mettler DL 40 RC). At various times depending on the degree of hydrolysis to be reached, the enzymatic reaction was stopped by heating the casein hydrolysate for 15 min at 100°C. The solutions were then lyophilized and stored at -80°C .

The degree of hydrolysis (DH) was determined spectrophotometrically by the trinitrobenzene sulfonic acid method according to Adler-Nissen (1979).

A sample of β -casein (control β -casein) was treated in the same manner but without addition of TPCK-trypsin, in order to determine the modifications only due to proteolysis.

In order to determine that the enzyme was actually killed by heating the casein hydrolysate for 15 min at 100°C, DH was measured again after the 30-min

equilibration period at room temperature, conditions used to measure solubility of the hydrolysates (see Solubility); no variation was detected.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE was carried out according to Swank and Munkres (1971), with slight modifications, on gel slabs (8 × 8 cm × 1 mm thick) with a running gel (12.5% acrylamide, 1.25% N,N'-methylenebis (acrylamide) containing 0.1% SDS with 8 M urea) and a stacking gel (6.2% acrylamide, 0.62% N,N'-methylenebis (acrylamide) containing 0.1% SDS with 4 M urea). The gel buffer was 1.65 M Tris, 0.925 M phosphoric acid and 1% SDS at pH 6.8. The electrode buffer was the gel buffer diluted ten-fold. The sample buffer was 9.25 mM phosphoric acid, adjusted to pH 6.8 with a 1 M Tris solution containing 8 M urea and 1% 2-mercaptoethanol. The samples were boiled in the buffer for 2 min and cooled prior to application to the gel slab. Electrophoresis was carried out at 15 mA for the stacking gel then 30 mA for the running gel, at room temperature, for 3 h. After fixation for at least 5 h in a 3% sulfosalicylic acid, 10% TCA solution, proteins were stained with 0.25% Coomassie Brilliant Blue R 250 in 7.5% acetic acid—5% methanol. The gels were destained with 7.5% acetic acid—5% methanol.

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 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 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 bovine β -casein tryptic digests was performed according to Carles and Ribadeau Dumas (1986), with some modifications (Ribadeau Dumas, personal communication). The μ Bondapak C18 column (2.0 mm i.d. × 30 cm; Waters), was equilibrated in solvent A (0.15% TFA in H₂O, 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 62.5 min.

Both the column and sol mL/min and the absorban

Following acid hydroly acid analyzer (Biotronik, × 3.2 mm i.d.).

Solubility

Control or enzyme-trea W/W) by mixing with a using HCl or NaOH of h bration period at room te determine emulsifying pr temperature (Lab Centrif the supernatant was dete bovine serum albumin or in percentage of total pro

Emulsifying Activity

To prepare the emulsion oil (ϕ (volume fraction of and homogenized at 20,00 Polytron equipped with a

With no protein present from experiment to expe formed. The presence of the stability of the emulsic are reported without corre was water, which had the 7.0.

Emulsifying activity of t to Pearce and Kinsella (1 diately pipetted from the in 0.1 M NaCl, pH 7.0. Tl mixtures, then absorbance glass cuvettes were used a between determinations. were measured and the in expressed as its emulsifyin

is used to measure solubility as detected.

Electrophoresis (SDS-PAGE)

and Munkres (1971), with (mm thick) with a running gel (acrylamide) containing 0.1% acrylamide, 0.62% N,N'-methylenebisacrylamide, 0.1% SDS at pH 6.8. The electrode buffer was 9.25 mM Tris solution containing 8 M urea. The gel was boiled in the buffer for 2 min. Electrophoresis was carried out in the running gel, at room temperature, in a 3% sulfosalicylic acid, 1% Coomassie Brilliant Blue G250. Gels were destained with 7.5%

model 680, two solvent delivery pumps, temperature control system, a fraction collector model 740 (Waters model 2150 and a fraction

on a Protein Pak 60 column (Waters) with 50 mM-K phosphate buffer, 0.5 mL/min. The absorbance was monitored with β -lactoglobulin (12,100), aprotinin (6,500), and polymyxin B sulfate

β -casein tryptic digests was used (1986), with some modification). The μ Bondapak C18 was calibrated in solvent A (115% solvent A) in 62.5 min.

Both the column and solvents were maintained at 40°C. The flow-rate was 1 mL/min and the absorbance was recorded at 220 nm.

Following acid hydrolysis, amino acids were quantified on an LC 5000 amino acid analyzer (Biotronik, Munich, FRG) equipped with a single column (20 cm $\times$ 3.2 mm i.d.).

Solubility

Control or enzyme-treated β -casein was dispersed in distilled water (0.1%, W/W) by mixing with a "Vortex". The pH was adjusted from 1.0 to 11.0 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. The protein content of the supernatant was determined by the method of Lowry *et al.* (1951) with bovine serum albumin or β -casein as a standard. The solubility was expressed in percentage of total protein concentration.

Emulsifying Activity

To prepare the emulsion, 21 mL of 0.1% protein solution and 7 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/l (A = absorbance at 500 nm and l = light path = 1 cm = 10^{-2} m), ϕ = oil phase volume = 0.25 and c is concentration of protein (0.1%) before the emulsion was 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:

$$\Delta E.A.I. \% = \frac{E.A.I. \text{ max} - E.A.I. 80^\circ\text{C}}{E.A.I. \text{ max}} \times 100$$

where $E.A.I. \text{ max}$ is the maximum value obtained either at $t = 0$ or $t = 24$ h. These values did not differ significantly. The smaller the value of $\Delta E.A.I. \%$, the better the stability.

RESULTS AND DISCUSSION

Limited Proteolysis of β -Casein With TPCK-Trypsin

Four hydrolysates were selected to determine solubility and emulsifying properties of the peptide mixtures: a 3.2% DH ($\beta 1$), a 5.0% DH ($\beta 2$), a 5.8% DH ($\beta 3$), and a 7.4% DH ($\beta 4$) obtained after 4-, 3-, 7-, and 180-min hydrolysis with E/S ratios of 0.25%, 0.50%, 1.00%, and 1.00%, respectively.

Results of Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE results of control and enzyme-treated β -casein in the presence of SDS, urea and 2-mercaptoethanol are shown in Fig. 1. Separation is based primarily on size. A plot of log (molecular weight) versus mobility by using the reference proteins (Fig. 1a,g) provided some information on the apparent size of peptides or peptides mixtures formed after hydrolysis.

In the absence of enzyme treatment (Fig. 1b), β -casein exhibits a major band (MW ~ 23,000) and some minor bands (from 21,000 to 17,000). After 4 min enzyme treatment with an E/S ratio of 0.25% (Fig. 1 c, sample $\beta 1$) the major band of MW ~ 23,000 completely disappeared and 4 main bands were visible

with apparent molecular increased degree of hydr ~7,000 had decreased in two remaining bands at dye staining.

In summary, after tryp mobility and correspond 8,000.

Size-Exclusion Chromat

Size-exclusion chromat to obtain more quantitative of β -casein with trypsin, a from 15,000 to 2,500.

A first group of peptide had a decreased relative c (17, 14, 14 and 9% for β peptides having an elution samples $\beta 1$, $\beta 2$ and $\beta 3$ (44

ENZ

a

FIG. 1. SDS-PAGE OF PROT derived from the cleavage of t top to bottom, myoglobin (po myoglobin (Fragment 1, 8.16 2.510). b, control β -casein; c (5

3 A/I (A = absorbance at 500
oil phase volume = 0.25 and
emulsion was formed.

ld at room temperature (20°C)
turbidity measured as described
were then heated at 80°C for 30
g, turbidity was again measured
was calculated by the formula:

$$\frac{I_{80^\circ\text{C}}}{I_{20^\circ\text{C}}} \times 100$$

ed either at $t = 0$ or $t = 24$ h.
smaller the value of $\Delta E.A.I.$ %,

DISCUSSION

Trypsin

olubility and emulsifying prop-
a 5.0% DH (β_2), a 5.8% DH
-, 7-, and 180-min hydrolysis
00%, respectively.

Immune Gel Electrophoresis

ted β -casein in the presence of
Fig. 1. Separation is based
t) versus mobility by using the
formation on the apparent size
hydrolysis.

β -casein exhibits a major band
1,000 to 17,000). After 4 min
Fig. 1 c, sample β_1) the major
and 4 main bands were visible

FIG. 1. SDS-PAGE OF PROTEINS AND PEPTIDES a and g, reference proteins are five polypeptides derived from the cleavage of the polypeptide chain of horse heart myoglobin (apomyoglobin). From top to bottom, myoglobin (polypeptide backbone, 16,950); myoglobin (Fragment I + II, 14,400); myoglobin (Fragment I, 8,160); myoglobin (Fragment II, 6,210) and myoglobin (Fragment III, 2,510). b, control β -casein; c, sample β_1 (3.2% DH); d, sample β_2 (5.0% DH); e, sample β_3 (5.8% DH) and f, sample β_4 (7.4% DH).

with apparent molecular weights ranging from $\sim 8,500$ to $\sim 4,200$. With an increased degree of hydrolysis, the band with an apparent molecular weight of $\sim 7,000$ had decreased intensity and disappeared with sample β_4 (Fig. 1f); the two remaining bands at MW $\sim 4,500$ and $\sim 3,700$ had the major intensity of dye staining.

In summary, after trypsin treatment of β -casein the main bands have a high mobility and correspond to material with apparent molecular weights below 8,000.

Size-Exclusion Chromatography

Size-exclusion chromatography was used to verify the SDS-PAGE results and to obtain more quantitative data on the size distribution (Fig. 2). After hydrolysis of β -casein with trypsin, all the resulting peptides had molecular weights ranging from 15,000 to 2,500.

A first group of peptides having an elution time of 14.0 min (MW $\sim 15,000$) had a decreased relative concentration as the degree of hydrolysis was increased (17, 14, 14 and 9% for β_1 , β_2 , β_3 and β_4 , respectively). A second group of peptides having an elution time of 16.0 min (MW $\sim 8,500$) was observed with samples β_1 , β_2 and β_3 (44, 38 and 28%, respectively); this peak was not obtained

FIG. 2. SIZE-EXCLUSION CHROMATOGRAPHY 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 0.20 M NaCl. The arrows indicate elution time of standard proteins: 1, β -lactoglobulin (18,000 MW); 2, α -lactalbumin (14,400 MW); 3, cytochrome c (12,100 MW); 4, aprotinin (6,500 MW); 5, insulin chain B (3,500 MW); 6, insulin chain A (2,500 MW); 7, polymixin B sulfate (1,447 MW).

FIG. 3. SEPARATION OF THE BOVINE β -CASEIN ON A PROTEIN PAK COLUMN (2.0 mm $\times$ 30 cm). Solvent: 40% solvent A. Flow rate: 1 ml/min. The arrows indicate elution time of standard proteins: A (1-25); B (26-28); C (29-32); D (33-169); J (170-176); K (1-169).

FIG. 2. SIZE-EXCLUSION CHROMATOGRAPHY 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 0.20 M NaCl. The arrows indicate elution time of standard proteins: 1, β -lactoglobulin (18,000 MW); 2, α -lactalbumin (14,400 MW); 3, cytochrome c (12,100 MW); 4, aprotinin (6,500 MW); 5, insulin chain B (3,500 MW); 6, insulin chain A (2,500 MW); 7, polymyxin B sulfate (1,447 MW).

FIG. 3. SEPARATION OF THE BOVINE β -CASEIN TRYPTIC HYDROLYSATES ON A μ BOND-PAK COLUMN (2.0 mm $\times$ 30 cm). Solvent A: 15% TFA in H_2O , pH 2.50; solvent B: 60% acetonitrile and 40% solvent A. Flow rate: 1 ml/min. Temperature 40°C. Identification of the peaks in the sequence of bovine β -casein according to Ribadeau-Dumas *et al.* (1972).

A(1-25); B(26-28); C(29-32); D(33-48); E(49-97); F(100-105); G(106-107); H(108-113); I(114-169); J(170-176); K(177-183); L(184-202); M(192-202); N(203-209)

with sample $\beta 4$ but another one with an apparent molecular weight $\sim 12,000$ appeared, with a relative concentration of 8%.

A third group of peptides was obtained at 17.3 min (MW $\sim 5,500$) representing nearly 15% for samples $\beta 1$, $\beta 2$ and $\beta 3$, and only 6% for sample $\beta 4$. A fourth group of peptides, eluted after 18.5 min (MW $\sim 3,500$) was obtained with a relative concentration of 7% for samples $\beta 1$ to $\beta 3$, and of 14% for sample $\beta 4$. A last group of peptides with an apparent molecular weight of $\sim 2,500$ had an increasing relative concentration as the degree of hydrolysis increased (14, 26, 30 and 52% for $\beta 1$, $\beta 2$, $\beta 3$ and $\beta 4$, respectively).

Reversed-Phase High Performance Liquid Chromatography (RP-HPLC)

Peptide mixtures obtained after trypsin hydrolysis of β -casein were submitted to RP-HPLC (Fig. 3). The peaks eluted were identified in the sequence of bovine $\beta A1$ -casein according to Ribadeau Dumas *et al.* (1972), on the basis of retention times and amino acid composition (Carles and Ribadeau Dumas 1986; Ribadeau Dumas, personal communication).

After a 3.2% DH ($\beta 1$), nine of the fifteen possible peptides were recovered: peptides A (1–25), B (26–28), C (29–32), D (33–48), E (49–97), F (100–105), I (114–169), J (170–176) and K (177–183). After a 5.0% DH ($\beta 2$), one more peptide was obtained H (108–113). After a 5.8% DH ($\beta 3$), the C-terminal peptide N (203–209) was eluted. After a 7.4% DH ($\beta 4$), the last two peptides L (184–202) and M (191–202) were obtained. The dipeptide 106–107, eluting early in the chromatography with the unretained compounds, was not identified. The time course of tryptic digestion of β -casein was in agreement with the results of Leonil *et al.* (1988).

The different fractions obtained by gel filtration were submitted to RP-HPLC. This chromatography on a hydrophobic matrix showed us that during the gel filtration reassociation of peptides occurred since several peptides of different molecular weights eluted as a single peak by gel filtration, which did not enable us to accurately determine the molecular weight of the different peptides (data not shown).

Solubility

After partial hydrolysis (3.2%, 5.0%, 5.8% and 7.4%) of β -casein with trypsin, none of the peptide solutions had a minimum solubility in contrast to the minimum solubility observed at pH 5.20 for the control β -casein (Fig. 4). Solubility in the pI (isoelectric point) range increased from 10% to 82%, 90–95% for control β -casein, $\beta 1$ and $\beta 2$, $\beta 3$, $\beta 4$, respectively.

FIG. 4. SOLUBILITY OF CONTROL β -CASEIN. pH Solubility was measured after a 30 min incubation at 37°C, expressed as percent total protein (0.1%) for control β -casein (open triangles); filled symbols: $\beta 1$ (filled circles), $\beta 2$ (filled squares), $\beta 3$ (filled stars), $\beta 4$ (filled diamonds).

Emulsifying Activity

Emulsions were prepared from the peptide solutions. The emulsifying activity was measured at pH 4.5–5.5 (Fig. 5). For pH values below 4.5, the emulsifying activity increased and the maximum value was observed at pH 3.0; below pH 3.0, E.A.I. decreased.

After trypsin treatment, no emulsion was formed at pH 4.0–6.5, 4.0–6.0, 4.0–6.0. The baseline absorbance is not significantly different from the control.

arent molecular weight $\sim 12,000$

3 min (MW $\sim 5,500$) representing only 6% for sample $\beta 4$. A fourth $\sim 3,500$ was obtained with a $\beta 3$, and of 14% for sample $\beta 4$. Molecular weight of $\sim 2,500$ had an of hydrolysis increased (14, 26, ely).

Chromatography (RP-HPLC)

olysis of β -casein were submitted identified in the sequence of bovine (1972), on the basis of retention Ribadeau Dumas 1986; Ribadeau

ossible peptides were recovered: 33–48), E (49–97), F (100–105), after a 5.0% DH ($\beta 2$), one more DH ($\beta 3$), the C-terminal peptide b), the last two peptides L (184–peptide 106–107, eluting early in ounds, was not identified. The as in agreement with the results

on were submitted to RP-HPLC. t showed us that during the gel ice several peptides of different d filtration, which did not enable t of the different peptides (data

und 7.4%) of β -casein with tryptum solubility in contrast to the e control β -casein (Fig. 4). Sol- sed from 10% to 82%, 90–95% ctively.

FIG. 4. SOLUBILITY OF CONTROL AND PROTEASE MODIFIED β -CASEIN 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: — + —, control β -casein; O—O, sample $\beta 1$; ☆—☆, sample $\beta 2$; Δ — Δ , sample $\beta 3$; □—□, sample $\beta 4$.

Emulsifying Activity

Emulsions were prepared from control β -casein 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. 5). 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 4.5, the increase in E.A.I. was much more pronounced at pH 3.0; below pH 3.0, E.A.I. decreased.

After trypsin treatment, no reproducible emulsions were obtained in the ranges pH 4.0–6.5, 4.0–6.0, 4.0–6.0 and 4.0–5.5 for $\beta 1$, $\beta 2$, $\beta 3$ and $\beta 4$, respectively. The baseline absorbance is not 0, since the oil/protein/water system had a small absorbance in relation to the blank. Most of the absorbance values giving an

FIG. 5. EMULSIFYING ACTIVITY INDEX (E.A.I.) OF CONTROL AND PROTEASE MODIFIED β -CASEIN AS A FUNCTION OF pH. See METHODS for conditions. Symbols as in Fig. 4.

E.A.I. below $75 \text{ m}^2 \cdot \text{g}^{-1}$ were not significant. At pH 2.0–3.0, an increase of E.A.I. was observed as compared to the control β -casein; the maximum value was obtained with $\beta 1$ and the minimum value with $\beta 3$. For pH values above 7.0, the E.A.I. of the hydrolysates was larger than the control β -casein; the minimum improvement was observed with $\beta 1$ in the range pH 7.0–9.0.

Emulsifying Stability

After 24 h storage and heating of the emulsion, a large pH-dependent decrease of emulsifying activity index (3–50%) was observed for pH values below 4.0,

TABLE I.
EMULSION STABILITY OF β -CASEIN AND TRYPSIN-TREATED β -CASEIN AFTER 24 H AT 20°C AND 30 MIN AT 80°C

pH	control	$\beta 1$	$\beta 2$	$\beta 3$	$\beta 4$
	β -casein				
1.0	6 ± 0.93	6 ± 0.83	7 ± 0.31	3 ± 1.44	n.d.
2.0	9 ± 1.67	18 ± 1.51	12 ± 2.04	9 ± 1.13	11 ± 0.44

for enzyme-treated β -casein; ur β -casein showed a 6–16% decrease. 60% decrease was observed for decrease with control β -casein. stability but was less stable than

OF CONTROL AND PROTEASE
H See METHODS for conditions.
4.

At pH 2.0–3.0, an increase of
rol β -casein; the maximum value
ie with β 3. For pH values above
er than the control β -casein; the
in the range pH 7.0–9.0.

on, a large pH-dependent decrease
erved for pH values below 4.0,

TABLE 1
EMULSION STABILITY OF β -CASEIN AND TRYPSIN-TREATED β -CASEIN AFTER
24 H AT 20°C AND 30 MIN AT 80°C

pH	control	β 1	β 2	β 3	β 4
	β -casein				
1.0	6 $\pm$ 0.93	6 $\pm$ 0.83	7 $\pm$ 0.31	3 $\pm$ 1.44	n.d.
2.0	9 $\pm$ 1.67	18 $\pm$ 1.51	12 $\pm$ 2.04	9 $\pm$ 1.13	11 $\pm$ 0.44
3.0	16 $\pm$ 2.87	47 $\pm$ 0.20	44 $\pm$ 2.73	35 $\pm$ 4.66	14 $\pm$ 0.10
4.0	—	—	—	—	—
5.0	—	—	—	—	—
6.0	25 $\pm$ 0.98	—	—	52 $\pm$ 4.83	34 $\pm$ 1.02
7.0	23 $\pm$ 18.80	56 $\pm$ 3.20	61 $\pm$ 0.86	39 $\pm$ 1.50	30 $\pm$ 0.19
8.0	17 $\pm$ 0.92	57 $\pm$ 5.18	57 $\pm$ 4.38	33 $\pm$ 2.55	31 $\pm$ 0.93
9.0	18 $\pm$ 1.59	50 $\pm$ 2.90	57 $\pm$ 2.36	36 $\pm$ 2.99	24 $\pm$ 4.79
10.0	19 $\pm$ 4.29	62 $\pm$ 0.44	59 $\pm$ 0.11	55 $\pm$ 2.50	38 $\pm$ 2.18
11.0	28 $\pm$ 1.55	48 $\pm$ 2.34	51 $\pm$ 1.62	50 $\pm$ 0.68	41 $\pm$ 1.09

Results are expressed as % difference between E.A.I. maximum value and that after thermal processing (see Methods).
—: not determined because of emulsion collapse.
n.d.: not determined.

for enzyme-treated β -casein; under the same conditions, E.A.I. of control β -casein showed a 6–16% decrease (Table 1). For pH values above 7.0 a 25–60% decrease was observed for modified β -casein as compared to a 15–30% decrease with control β -casein. The most hydrolyzed sample showed the best stability but was less stable than control β -casein.

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. After partial proteolysis of β -casein with trypsin, solubility was increased particularly in the pI range, with 10%, 82%, 90–95% solubility for control β -casein, β 1 and β 2, β 3, β 4, respectively. In the pH range around the pI, it was not possible to obtain a reproducible emulsion. These results near the pI show that increasing the solubility did not increase the emulsifying activity. Similar results were obtained with whole casein treated with either *Staphylococcus aureus* V8 protease (Chobert *et al.* 1988b) or trypsin (Chobert *et al.* 1988a).

Solubility of β -casein was improved in the pI range after partial hydrolysis. However, no emulsion activity was obtained in this range of pH. For pH values below and above pI, when solubility was increased for β -casein derivatives, an improvement of emulsifying activity was observed. However, the emulsifying stability of the β -casein derivatives was lower than that of control β -casein. There is the possibility that the majority of the peptides are not amphiphilic enough as is needed for good emulsifying stability. In a preceding paper (Chobert *et al.* 1988b) we showed that after partial hydrolysis (2.0 and 6.7% DH) of whole casein with *Staphylococcus aureus* V8 protease, emulsifying activity and stability of casein derivatives decreased as compared to control casein. After partial hydrolysis (4.3%, 8.0% and 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 whole casein. In both cases (enzymic modification with *S. aureus* V8 protease or trypsin), the size of the resulting peptides seems to be in the same range, according to the results obtained by SDS-PAGE and by size-exclusion chromatography. Therefore, the specificity of the protease is quite important. Such differences were also observed by Adler-Nissen and Olsen (1979) with soy proteins. They reported that 3 to 8% hydrolysis of the peptide bonds of soy proteins with Alcalase at pH 8.0 increased the solubility of the product at pH 5.0 by 5 to 20 times over that of the original soy protein. The limited proteolysis increased the whipping expansion (12-fold at 3% hydrolysis) of the product. After 3% hydrolysis of the same soy protein with Neutrase, the whipping expansion was only 4-fold larger than the untreated protein.

Although functional properties could be improved by controlled limited proteolysis of proteins, further work is needed in order to predict the relationships between the structure of a protein and its functional properties. Separation and characterization of the peptides will be required. Shimizu *et al.* (1986) demonstrated that a peptide of 23 residues, purified from the peptic hydrolysate of α _{s1}-casein, also had very complex emulsifying properties, being affected by

coexistent peptides, emulsification *et al.* (1987) isolated two peptide from the hydrolysate (trypsin and activity of both peptides depended on of peptide functionality is essential).

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REFERENCES

- ADLER-NISSEN, J. 1976. Enzymic modification of food proteins. *Food Technology*, 30, 10–15.
- ADLER-NISSEN, J. 1979. Determination of protein hydrolysates by trinitrobenzoyl chloride. *Food Chemistry*, 27, 1256–1262.
- ADLER-NISSEN, J., ERIKSEN, S. 1978. The functionality of vegetable protein concentrates. In *Plant Proteins for Human Food*, (Eds. Nijhoff, M.; Junk, W.), pp. 219–230. Nijhoff, M.; Junk, W., Publishers.
- ADLER-NISSEN, J. 1984. Control of bitterness in protein hydrolysis products. *Food Chemistry*, 215–222.
- ADLER-NISSEN, J. and OLSEN, H. 1979. Length on taste and functional properties of soy protein hydrolysates. In *Functionality and Protein Structure*, (Eds. Ser. 92; American Chemical Society, Washington, DC), pp. 219–230.
- CARLES, C. and RIBADEAU DUMAS, P. 1984. High-performance liquid chromatography for the separation of peptides. *Dairy Research*, 53, 595–600.
- CHEFTEL, J. C. 1977. Chemical and physical properties of proteins due to processing and storage. In *Food Chemistry*, (Eds. Tannenbaum, eds.) pp. 401–405, Van Nostrand Reinhold, New York.
- CHEFTEL, J. C., CUQ, J. L. and LAFITE, J. 1984. *Caractérisation des propriétés fonctionnelles des protéines alimentaires*. Lavoisier, Paris.

Under ideal conditions, soluble protein at the interface once the interfacial electrical potential is an important prerequisite for adsorption at the interface is with trypsin, solubility was increased to 90–95% solubility for control β -casein in the pH range around the pI, it was observed. These results near the pI show the emulsifying activity. Similar results were obtained with either *Staphylococcus aureus* or *Alcalase* (Chobert *et al.* 1988a).

In the pH range after partial hydrolysis, the emulsifying activity was observed. However, the emulsifying activity was lower than that of control β -casein. The peptides are not amphiphilic in nature. In a preceding paper (Chobert *et al.* 1988a), the emulsifying activity and compared to control casein. After 2.0 and 6.7% DH of whole casein with trypsin was improved but emulsifying stability was not improved. In both cases, the size of the particles was in the same range, according to the results of electron microscopy. Therefore, the differences were also observed with proteins. They reported that 3 to 5.0 by 5 to 20 times over that of control casein. The whipping expansion after 3% hydrolysis of the same soy protein was only 4-fold larger than the

improved by controlled limited proteolysis in order to predict the relationships between chemical and functional properties. Separation and identification. Shimizu *et al.* (1986) demonstrated from the peptic hydrolysate of casein, being affected by

coexistent peptides, emulsification conditions and other factors. Recently, Lee *et al.* (1987) isolated two peptides, one hydrophilic and the other hydrophobic, from the hydrolysate (trypsin and chymosin) of β -casein. The emulsifying activity of both peptides depended on the pH range. In summary, more knowledge of peptide functionality is essential.

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REFERENCES

- ADLER-NISSEN, J. 1976. Enzymatic hydrolysis of proteins for increased solubility. *J. Agric. Food Chem.* 24, 1090–1093.
- ADLER-NISSEN, J. 1979. Determination of the degree of hydrolysis of food protein hydrolysates by trinitrobenzenesulfonic acid. *J. Agric. Food Chem.* 27, 1256–1262.
- ADLER-NISSEN, J., ERIKSEN, S. and OLSEN, H. S. 1983. Improvement of the functionality of vegetable proteins by controlled enzymatic hydrolysis. In *Plant Proteins for Human Food*, (C. E. Bodwell and L. Petit, eds.) pp. 207–219, Nijhoff, M.; Junk, W., Publishers, The Hague.
- ADLER-NISSEN, J. 1984. Control of the proteolytic reaction and the level of bitterness in protein hydrolysis processes. *J. Chem. Technol. Biotechnol.* 34B, 215–222.
- ADLER-NISSEN, J. and OLSEN, H. S. 1979. The influence of peptide chain length on taste and functional properties of enzymatically modified soy protein. In *Functionality and Protein Structure*, (A. Pour-El, ed.) pp. 125–146, Adv. Chem. Ser. 92; American Chemical Society, Washington, DC.
- CARLES, C. and RIBADEAU DUMAS, B. 1986. Determination of gradient elution conditions for the separation of peptide mixtures by reversed-phase high-performance liquid chromatography: bovine β -casein tryptic digest. *J. Dairy Res.* 53, 595–600.
- CHEFTEL, J. C. 1977. Chemical and nutritional modifications of food proteins due to processing and storage. In *Food Proteins*, (J. R. Whitaker and S. R. Tannenbaum, eds.) pp. 401–405, Van Nostrand Reinhold/AVI, New York.
- CHEFTEL, J. C., CUQU, J. L. and LORIENT, D. 1985. Altération ou amélioration des propriétés fonctionnelles. In *Protéines Alimentaires*, (Technique et Documentation—Lavoisier, ed.) pp. 278–297, Paris.

- CHOBERT, J. M., BERTRAND-HARB, C. and NICOLAS, M. G. 1988a. Solubility and emulsifying properties of caseins and whey proteins modified enzymatically by trypsin. *J. Agric. Food Chem.* **38**, 883-892.
- CHOBERT, J. M. and MESNIER, D. 1988. Procédés d'amélioration des propriétés fonctionnelles des protéines alimentaires. In *Propriétés Fonctionnelles des Macromolécules Alimentaires*, Les Cahiers de l'ENS.BANA; (Technique et Documentation—Lavoisier, ed.) pp. 175-198, Paris.
- CHOBERT, J. M., SITOBY, M. Z. and WHITAKER, J. R. 1988b. Solubility and emulsifying properties of caseins modified enzymatically by *Staphylococcus aureus* V8 protease. *J. Agric. Food Chem.* **36**, 220-224.
- CHOBERT, J. M., SITOBY, M. Z. and WHITAKER, J. R. 1990. Proteolytic degradation of proteins. In *Marine Product, Composition and Properties*, ACS Symposium Series; American Chemical Society, Washington, DC. In Press.
- FEENEY, R. E. 1987. Chemical modification of proteins: comments and perspectives. *Int. J. Peptide Protein Res.* **29**, 145-161.
- FEENEY, R. E. and WHITAKER, J. R., Eds. 1977. *Food Proteins: Improvement Through Chemical and Enzymatic Modification*. Adv. in Chem. Ser. **160**, 312 pp., American Chemical Society, Washington, DC.
- FOX, K. K. 1970. Casein and whey products. In *Byproducts from Milk*, 2nd ed., (B. H. Webb, and E. O. Whittier, eds.) p. 331, Van Nostrand Reinhold/AVI, New York.
- FOX, P. F., MORRISSEY, P. A. and MULVIHILL, D. M. 1982. Chemical and enzymatic modification of food proteins. In *Developments in Food Proteins-1*, (B. J. F. Hudson, ed.) pp. 1-60, Applied Science Publ., London.
- FRIEDMAN, M. 1977. Effects of lysine modification on chemical, physical, nutritive, and functional properties of proteins. In *Food Proteins*, (J. R. Whitaker and S. R. Tannenbaum, eds.) pp. 446-483, Van Nostrand Reinhold/AVI, New York.
- FRIEDMAN, M. 1979. Alkali-induced lysinoalanine formation in structurally different proteins. In *Functionality and Protein Structure*, (A. Pour-Ei, ed.) pp. 225-235, ACS Symposium Series 92; American Chemical Society, Washington, DC.
- GUNTHER, R. C. 1979. Chemistry and characteristics of enzyme-modified whipping proteins. *J. Amer. Oil Chem. Soc.* **56**, 345-349.
- HAGGETT, T. O. 1974. Hydrolysis of lactic casein by proteolytic enzyme. In *Proceedings, 19th International Dairy Congress, Part 1E, International Dairy Congress*, p. 339, Melbourne, Australia.
- KILARA, A. and SHARKASI, T. Y. 1986. Effects of temperature on food proteins and its implications on functional properties. *CRC Crit. Rev. Food Sci. Nutr.* **23**, 323-395.
- KINSELLA, J. E. 1976. Functional properties of proteins in foods: a survey. *CRC Crit. Rev. Food Sci. Nutr.* **7**, 219-280.
- KINSELLA, J. E. and SHETT 1976. Improving functional properties of α_1 -casein. *Protein Structure*, (A. Pour-Ei, ed.) American Chemical Society, Washington, DC.
- LEE, S. W., SHIMIZU, M., KAMIYAMA, Y. and KINSELLA, J. E. 1983. Emulsifying properties of peptidic hydrolyzates of bovine caseins. *Agric. Biol. Chem.* **51**, 161-164.
- LEE, S. W., SHIMIZU, M., KAMIYAMA, Y. and KINSELLA, J. E. 1983. Emulsifying properties of a peptidic hydrolyzate of bovine caseins. *J. Food Sci.* **48**, 100-104.
- LEONIL, J., MOLLE, D. and M. 1983. Hydrolysis of β -casein by trypsin. *J. Food Sci.* **48**, 100-104.
- LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. and RANDALL, R. D. 1951. Protein measurement with Folin phenol reagent. *J. Biol. Chem.* **193**, 265-275.
- MERCIER, J. C., MAUBOIS, J., MAS, B. 1968. Fractionnement par chromatographie sur DEAE. *Bull. Soc. Chim. Biol.* **50**, 521-524.
- MORRISSEY, P. A. 1982. Functional properties of food ingredients. In *Developments in Food Proteins-1*, (B. J. F. Hudson, ed.) pp. 375-399, Applied Science Publ., London.
- OLSEN, H. S. and ADLER-NISSEN, R. 1972. Purification and properties of a soluble enzyme. *Biochem. J.* **14**, 6-11.
- PEARCE, K. N. and KINSELLA, J. E. 1983. Evaluation of a turbidimetric technique for the determination of α_1 -casein. *J. Food Sci.* **48**, 100-104.
- PHILLIPS, R. D. and BEUCHAT, J. 1977. Functional properties of food proteins. In *Protein Functionality in Food*, (J. E. Kinsella, ed.) pp. 1-10, American Chemical Society, Washington, DC.
- RIBADEAUDUMAS, B., BRIGNON, J. C. 1972. Structure primaire de α_1 -casein. *Bull. Soc. Chim. Biol.* **54**, 505-514.
- SHIMIZU, M., TAKAHASHI, T., KAMIYAMA, Y. and KINSELLA, J. E. 1983. Adsorption onto an oil surface of α_1 -casein in relation to its molecular weight. *J. Food Sci.* **48**, 100-104.
- SHIMIZU, M., LEE, S. W., KAMIYAMA, Y. and KINSELLA, J. E. 1983. Functional properties of a peptidic hydrolyzate of α_1 -casein: change in the peptide. *J. Food Sci.* **48**, 100-104.
- SWAISGOOD, H. E. 1982. Chemical and functional properties of food proteins. In *Food Proteins: Improvement Through Chemical and Enzymatic Modification*, (R. E. Feeney and J. R. Whitaker, eds.) pp. 1-10, American Chemical Society, Washington, DC.

- ARB, C. and NICOLAS, M. G. 1988a. Properties of caseins and whey proteins modified by laccase. *J. Food Chem.* **15**, 881-892.
- ARB, C. 1988. *Procedures d'amélioration des propriétés fonctionnelles*. In *Propriétés Fonctionnelles Les Cahiers de l'INRA*: (Technique pp. 175-195, Paris).
- ARB, C. and WHITAKER, J. R. 1988b. Solubility of caseins modified enzymatically by *Staphylococcus aureus*. *J. Food Chem.* **15**, 221-224.
- ARB, C. and WHITAKER, J. R. 1990. Proteolytic modification of proteins: comments and perspectives. *J. Food Sci.* **55**, 101-104.
- ARB, C., Eds. 1987. *Food Proteins: Improvements in Food Processing*. Adv. in Chem. Ser. 250, American Chemical Society, Washington, DC.
- ARB, C. and WHITAKER, J. R. 1987. *Food Proteins: Improvements in Food Processing*, 2nd ed., eds. J. R. Whitaker and J. R. Whitaker, Reinhold Publishing Co., New York.
- ARB, C. and MULVILL, D. M. 1982. Chemical modification of proteins. In *Developments in Food Processing*, (P. F. Fox, ed.) pp. 1-60, Applied Science Publ., London.
- ARB, C. and WHITAKER, J. R. 1987. Lysine modification in chemical, physical, and enzymatic processes. (J. R. Whitaker, ed.) pp. 241-255, Van Nostrand Reinhold, New York.
- ARB, C. and WHITAKER, J. R. 1987. Lysine modification in structurally modified proteins. (A. Pour-El, ed.) pp. 1-10, American Chemical Society, Washington, DC.
- ARB, C. and WHITAKER, J. R. 1987. Lysine modification of enzyme-modified proteins. *J. Food Sci.* **52**, 101-104.
- ARB, C. and WHITAKER, J. R. 1987. Lysine modification of lactic acid bacteria. In *International Dairy Congress*, (A. Pour-El, ed.) pp. 1-10, American Chemical Society, Washington, DC.
- ARB, C. and WHITAKER, J. R. 1987. Lysine modification of proteins: a survey. *J. Food Sci.* **52**, 219-220.
- KINSELLA, J. E. and SHETTY, K. J. 1979. Chemical modification for improving functional properties of plant and yeast proteins. In *Functionality and Protein Structure*, (A. Pour-El, ed.) pp. 37-63, ACS Symposium Series 92; American Chemical Society, Washington, DC.
- LEE, S. W., SHIMIZU, M., KAMINOGAWA, S. and YAMAUCHI, K. 1987a. Emulsifying properties of peptides obtained from the hydrolyzates of β -casein. *Agric. Biol. Chem.* **51**, 161-166.
- LEE, S. W., SHIMIZU, M., KAMINOGAWA, S. and YAMAUCHI, K. 1987b. Emulsifying properties of a mixture of peptides derived from the enzymatic hydrolyzates of bovine caseins. *Agric. Biol. Chem.* **51**, 1535-1540.
- LEONIL, J., MOLLE, D. and MAUBOIS, J. L. 1988. Study of the early stages of tryptic hydrolysis of β -casein. *Lait* **68**, 281-294.
- LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. and RANDALL, R. S. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**, 265-275.
- MERCIER, J. C., MAUBOIS, J. L., POZNANSKI, S. and RIBADEAU DUMAS, B. 1968. Fractionnement préparatif des caséines de vache et de brebis par chromatographie sur DEAE-cellulose en milieu urée et 2-mercaptoéthanol. *Bull. Soc. Chim. Biol.* **50**, 521-530.
- MORR, C. V. 1982. Functional properties of milk proteins and their use as food ingredients. In *Developments in Dairy Chemistry—1—Proteins*, (P. F. Fox, ed.) pp. 375-399, Applied Science Publ., London.
- OLSEN, H. S. and ADLER-NISSEN, J. 1979. Industrial production and applications of a soluble enzymatic hydrolyzate of soya protein. *Process Biochem.* **14**, 6-11.
- PEARCE, K. N. and KINSELLA, J. E. 1978. Emulsifying properties of proteins: evaluation of a turbidimetric technique. *J. Agric. Food Chem.* **26**, 716-723.
- PHILLIPS, R. D. and BEUCHAT, L. R. 1981. Enzyme modification of proteins. In *Protein Functionality in Foods*, (J. P. Cherry, ed.) pp. 275-298, ACS Symposium Series 147; American Chemical Society, Washington, DC.
- RIBADEAU DUMAS, B., BRIGNON, G., GROSCLAUDE, F. and MERCIER, J. C. 1972. Structure primaire de la caséine β bovine. *Eur. J. Biochem.* **25**, 505-514.
- SHIMIZU, M., TAKAHASHI, T., KAMINOGAWA, S. and YAMAUCHI, K. 1983. Adsorption onto an oil surface and emulsifying properties of bovine α_{s1} -casein in relation to its molecular structure. *J. Agric. Food Chem.* **31**, 1214-1218.
- SHIMIZU, M., LEE, S. W., KAMINOGAWA, S. and YAMAUCHI, K. 1986. Functional properties of a peptide of 23 residues purified from the peptic hydrolysate of α_{s1} -casein: changes in the emulsifying activity during purification of the peptide. *J. Food Sci.* **51**, 1248-1252.
- SWAISGOOD, H. E. 1982. Chemistry of milk protein. In *Developments in*

- Dairy Chemistry—1—Proteins*. (P. F. Fox, ed.) pp. 1–59, Applied Science Publishers, London.
- SWANK, R. T. and MUNKRES, K. D. 1971. Molecular weight analysis of oligopeptides by electrophoresis in polyacrylamide gel with sodium dodecyl sulfate. *Anal. Biochem.* 39, 462–477.
- WHITAKER, J. R., ed. 1974. *Food Related Enzymes*. Adv. Chem. Ser. 136; 365 pp., American Chemical Society, Washington, DC.
- WHITAKER, J. R. 1977. Enzymatic modification of proteins applicable to foods. In *Food proteins: Improvement through Chemical and Enzymatic Modification* (R. E. Feeney, and J. R. Whitaker, eds.) pp. 95–155, Adv. Chem. Ser. 160; American Chemical Society, Washington, DC.
- WHITAKER, J. R. and PUIGSERVER, A. J. 1982. Fundamentals and applications of enzymatic modifications of proteins—an overview. In *Modifications of Proteins: Food, Nutritional and Pharmacological Aspects*, (R. E. Feeney and J. R. Whitaker, eds.) pp. 57–87, Adv. Chem. Ser. 198; American Chemical Society, Washington, DC.
- ZITTLE, C. A. and CUSTER, J. H. 1963. Purification and some of the properties of α_s -casein and κ -casein. *J. Dairy Sci.* 46, 1183–1188.

THE 7S PR

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¹Research supported by the Vermont State Project VT 970.

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