Food Chemistry 43 (1992) 93–105

Comparative performance of chemically and enzymatically modified whey proteins

J. Lakkis & R. Villota

Department of Food Science, University of Illinois, 382 D, Agricultural Engineering Sciences Building, 1304 W. Pennsylvania Avenue, Urbana, Illinois 61801, USA

(Received 7 September 1990; accepted 7 January 1991)

Alternative approaches for enhancing the utilization of whey proteins were investigated. Chemical and enzymatic treatments were applied to induce some structural and electrostatic changes in the whey proteins. Chemical modification involved covalent attachment of succinyl and acetyl groups to the free amino sites of lysine residues. Enzymatic modification included deamidation of these active sites via trypsin and chymotrypsin under conditions unfavorable for potent proteolysis. Chemical treatments, in particular succinylation, resulted in better emulsifying capability and stability as evidenced by a well-balanced distribution of oil droplets within the emulsion ultrastructure. Foaming ability and stability were substantially enhanced by both treatments, although the effect was stronger in the chemically-treated proteins. Enzyme-treated whey proteins, however, showed superior solubility and in-vitro digestibility as compared to their chemically treated or native counterparts. In a simulated intermediate moisture food system, succinylation offered the best protective effect against browning development and loss of basic amino acids.

INTRODUCTION

Whey proteins have recently attracted considerable attention as potential food ingredients. First of all, their high nutritive value and well-balanced amino acid profile make them superior to all known food proteins except those of eggs. Secondly, the use of novel protein sources such as grain, leaf, single cell and others has often been found to be limited by the proteins' low biological value, undesirable organoleptic properties and, in many cases, poor solubility, which is reflected in low functionality.

Several attempts to incorporate whey proteins in food formulas have been made. However, most of these trials have not been very successful due to some physicochemical and compositional properties inherent to the protein components themselves. For example, the low content of proline and the uniform distribution of the acidic/basic and hydrophilic/hydrophobic residues along the polypeptide chains impose a certain globular conformation on the molecule and prevent it from flexible orientation at interfaces. In addition, the considerable number of the helical chains and the abundance of the -SH/-SS groups are thought to be directly related to the high susceptibility of whey proteins to severe denaturation upon heating (Morr, 1976). The high lysine content, although considered a great advantage of whey proteins from a nutritional point of view, is also a detrimental factor to their functionality. The ϵ-amino groups of lysine and their associated low pK result in high polarity and strong nucleophilic activity, which facilitate their interaction with the carboxyl groups in their vicinity. Upon processing and storage, this interaction has been found to result in some deteriorative effects such as nonenzymatic browning (NEB), transamidation, condensation reactions with dehydroalanine and cross-link formation. Therefore, additional methods for imparting functionality and enhancing stability of whey proteins upon processing and storage are needed.

Structural modifications have been considered for the alteration of the physicochemical properties of proteins and for improving their functionality (Kinsella, 1977). Protein modification can be used to block the reactivity of specific groups involved in some undesirable reactions, to enhance the digestibility and nutritional value and to improve the thermal stability of proteins. Covalent modification by acylation has been considered a very adequate treatment for food proteins due to many reasons. The reagents used for this type of modification, succinic and acetic anhydrides in particular, are easy to handle, are reasonably stable and their reactions take place under relatively mild conditions.

Food Chemistry 0308-8146/91/$03·50

This is an important prerequisite for their potential use in food systems. However, the most interesting attribute of protein acylation is its capability of preventing the formation of lysinoalanine (Freidman, 1984).

Deamidation is another novel area of protein modification for enhanced flexibility of globular proteins. Deamidation of proteins is usually achieved under mild acidic or alkaline conditions, although most effectively by means of enzyme action. This type of modification has been introduced by Kato *et al.* (1987) and was adopted by many other workers as a means of lowering inter- and intra-molecular interactions and increasing molecular flexibility.

Based on data available from the literature and from the technical problems reported by food industry experts, the lack of functionality of whey proteins has been mostly correlated with certain aspects of their chemistry. In this study, the role of lysine was investigated through two approaches: (a) protecting the reactive amino groups of lysine by means of covalent attachment of succinic or acetic groups to these sites (chemical modification), and (b) retarding the reactivity of these groups with their carboxyl counterparts, i.e. by deamidation (enzymatic modification).

MATERIALS AND METHODS

Materials

Whey protein isolates (WPI) were purchased from BioPro, Le Seuer Isolates (St. Paul, MN). All the chemicals used were reagent grade. Carbowax was obtained from Pharmacia (Piscataway, NJ) and Coomassie Brilliant Blue R-250 from BioRad (Richmond, CA). The rest of the chemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

Methods

Preparation of the modified whey proteins isolates

Succinylated whey protein isolates (SWPI) were prepared according to the method of Franzen & Kinsella (1976), while acetylation of WPI was done using acetic anhydride, according to the method of Hoagland (1966).

Deamidation was achieved using trypsin and chymotrypsin at pH 10·0, in order to avoid potent proteolysis of the protein, according to the method of Kato *et al.* (1987).

The modified proteins were recovered by dialysis against distilled water (24 h at 4°C), followed by freeze-drying.

The degree of proteolysis was determined as the ratio of absorbance (at 280 nm) of the treated samples (shaken with 10% trichloroacetic acid and filtered) to that of the native proteins. However, since this method is known to be suitable only for detecting small peptides and amino acids and not for large peptides, sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was also applied for this purpose.

The degree of modification was determined as the decrease in free amino groups of the lysine residues, using trinitrobenzenesulfonic acid according to the method of Fields (1972).

Conformational and structural changes

The effect of modification on induced structural conformations and flexibility of WPI was studied in terms of molecular weight changes (SDS–PAGE), fluorescence spectra, hydrophobicity (partition hydrophobicity), surface tension and disulfide bond cleavage.

SDS-PAGE was performed at pH 7·0 using 10% acrylamide gels, according to Lammeli (1970), using a slab gel unit (Hoefer Scientific Instruments, San Francisco, California). Electrophoresis was done at 25 mA for 4·5 h at 5°C. Staining was done with Coomassie Brilliant Blue R-250 in methanol, water and acetic acid. Destaining was followed in two steps, using acetic acid, methanol and water.

Fluorescence spectra of native and modified WPI (0·02 % w/v in pH 8·0 phosphate buffer) were obtained using an I.S.S. Greg PC spectrofluorimeter (I.S.S. Inc., Champaign, IL). Samples were excited at 295 nm and their corresponding emission between 300 and 400 nm was recorded. Slit widths were 8 nm for excitation and 16 nm for emission.

Hydrophobicity, expressed as partition hydrophobicity, was determined according to the method of Keshavarez & Nakai (1979). This method was used due to the good correlation it bears with functionality. Protein samples were partitioned between two phases: dextran and carbowax or its palmitate ester. Partition coefficients of the proteins were calculated as:

$$K = C_u/C_l$$

where C_u and C_l are the protein concentrations in the upper and lower phases, respectively. $\Delta\log K$ was calculated from

$$\Delta\log K = \log K_1 - \log K_2$$

and was used as an index of hydrophobicity of the protein. K_1 and K_2 are the partition coefficients in polyethylene glycol with and without the palmitate group attached, respectively.

Disulfide bond content was determined for native and modified whey proteins by using Ellman's reagent (5,5'-dithiobis-2-nitrobenzoic acid) according to Beveridge *et al.* (1974).

Surface tension was determined by using a Wilhelmy plate balance consisting of a thin platinum plate attached to an ordinary analytical balance. Protein dispersions (0·5%) in phosphate buffer (pH 8·0) were used.

Measurements were recorded over a 16 h period, by monitoring the change in weight of the platinum plate due to the amount of adhering solution.

Functional properties

The effect of modification on the functional properties of WPI was evaluated in terms of solubility, emulsifying and foaming characteristics and their performance in an intermediate moisture food (IMF) system.

Solubility was determined according to the turbidimetric method of Damodaran & Kinsella (1982). Emulsifying properties were determined according to the method of Yamauchi *et al.* (1982). The surface structure of native and modified WPI stabilized emulsions was examined by means of cryoscanning electron micrography (AmRay 1000A). Droplets of freshly prepared emulsions of 20:80 (w/w) protein (1%) to corn oil (Puritan, Procter and Gamble, Cincinnati, OH) were prepared. Samples (10 μl) were placed in wells of copper specimen stubs and quench-frozen by plunging them into liquid nitrogen-chilled propane (*c.* −183°C). The surface of the samples was coated with *c* 10 nm of gold/palladium (40/60) after surface fracturing. Foaming ability was determined according to Damodaran & Kinsella (1982).

Performance in an IMF system

The effect of protein modification on the shelf-life stability of an IMF system was measured in terms of browning development as due to Maillard-type reactions. Protein powders and their corresponding sugar mixtures (2:1 protein to D-glucose) were dispersed in distilled water (2%) to enhance the interactions between the free amino groups of the protein and the free aldehyde groups in the sugar molecules, and then freeze-dried. Native WPI was used as a reference. Samples with/without sugar were stored in a desiccator at 75% RH provided by a supersaturated solution of NaCl. The test samples were stored at room temperature or at 50°C (to accelerate the rate of NEB) for different periods of time and were examined periodically. Two attributes were monitored: (a) color development expressed as Browning Index, rate of development and induction time, and (b) changes in the amino acid profile. Color development was determined according to the method of Clark & Tannenbaum (1970). The rate of browning was calculated according to Schnickels *et al.* (1976) as the slope of the line after measurable browning started to appear. The induction time was determined as the period up to which the amount of browning started to increase by a zero order reaction. Changes in lysine and other basic amino acid residues were determined by means of amino acid analysis and were expressed as a percentage of that of the native, unmodified and unstored WPI.

In-vitro *digestibility*

In-vitro digestibility of the modified proteins was measured and compared to that of the native WPI. A mixture of trypsin and chymotrypsin was chosen for this test due to the high sensitivity of both enzymes to changes in the protein (substrate) conformation. Aliquots of protein dispersion (3 mg/ml) were flushed with nitrogen and their pH was adjusted to 8·0 prior to addition of the enzyme mixture (100 μl of enzyme of 1·2 mg/ml). Samples were incubated at 37°C for a period of 1 h. Aliquots of the protein preparation were added to an equivalent volume of 10% TCA, shaken for 30 min, centrifuged and filtered. Digestibility was expressed as the ratio of absorbance (280 nm) of the modified proteins to that of the native, multiplied by 100.

RESULTS AND DISCUSSION

Physicochemical properties of modified WPI

The degree of proteolysis assessed by the TCA test showed that, compared to the NaOH-treated samples (control), trypsin and chymotrypsin were not very effective in hydrolyzing WPI under the prevailing conditions (Table 1).

The degree of modification, expressed as the change (reduction) in ϵ-amino groups of lysine, showed that the succinylated whey protein (SWPI) and acetylated whey protein (AWPI) resulted in 77 and 56.3% blockage of the ϵ-amino groups of lysine, respectively (Table 2). Deamidated whey proteins, on the other hand, showed a low degree of modifi-cation (12·5% for CWPI and

Table 1. Degree of proteolysis of native whey proteins upon deamidation by trypsin (TWPI) and chymotrypsin (CWPI) compared to NaOH-treated whey proteins (pH 8·0)

Treatment	Proteolysis (%)
NaOH-Treated WPI	1·3
TWPI	7·8
CWPI	6·2

Table 2. Reduction in the free amino groups of native (WPI), acetylated (AWPI), trypsin-treated (TWPI) and chymotrypsin-treated (CWPI) whey protein isolates (expressed as % of the concentration in the native protein.)

Protein	NH_2 groups (%)
Native (WPI)	100·0
AWPI	43·7
SWPI	23·0
CWPI	87·5
TWPI	84·6

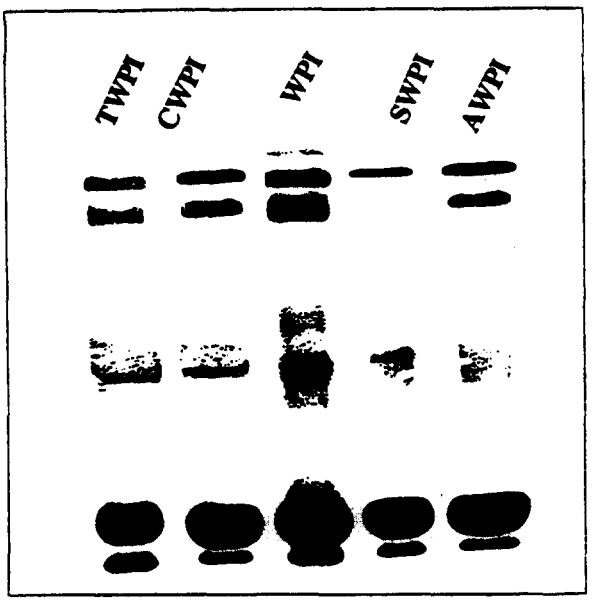

Fig 1. Vertical SDS–PAGE of native (WPI), acetylated (AWPI), succinylated (SWPI), trypsin-treated (TWPI) and chymotrypsin-treated (CWPI) whey protein isolates.

15·4% for TWPI). These results show that succinylation was the most effective modifying treatment for whey protein isolates under the conditions of this study. The introduction of the bulky groups of succinates to the WPI molecule, as well as the considerable denaturation and the consequent polypeptide chain repulsion, may have enhanced the placement of additional groups on sites which were inaccessible in the native protein, and so resulted in the highest degree of modification.

Table 3. Reduction in the disulfide bond content of native (WPI), acetylated (AWPI), succinylated (SWPI), trypsin-treated (TWPI) and chymotrypsin-treated (CWPI) whey protein isolates (expressed as % of the concentration in the native protein.)

Protein	Intermolecular -SS (%)	Intramolecular -SS (%)
WPI	100·0	100·0
AWPI	38·2	46·5
SWPI	36·1	48·0
CWPI	49·8	59·0
TWPI	44·6	65·3

Conformational and structural changes

Molecular weight

The electrophoretic pattern of whey proteins was not found to be drastically affected upon acylation (Fig. 1). For the case of succinylation, there was a slight tendency towards fragmentation, which was reflected in a faster movement of some low molecular weight bands towards the buffer line. This behavior was expected due not only to the electrostatic effects of the covalent attachment of the succinyl group, but also to a possible reaction between the anhydrides and the ruptured disulfide bonds, which are usually involved in the maintenance of the macromolecule in the undissociated state (Grants, 1973; Klotz, 1976). Table 3 shows the effect of both chemical and enzymatic treatment on the retention of disulfide bonds. The decrease in intermolecular disulfide bond content upon acylation was more drastic than in the enzyme deamidation. This could be due to

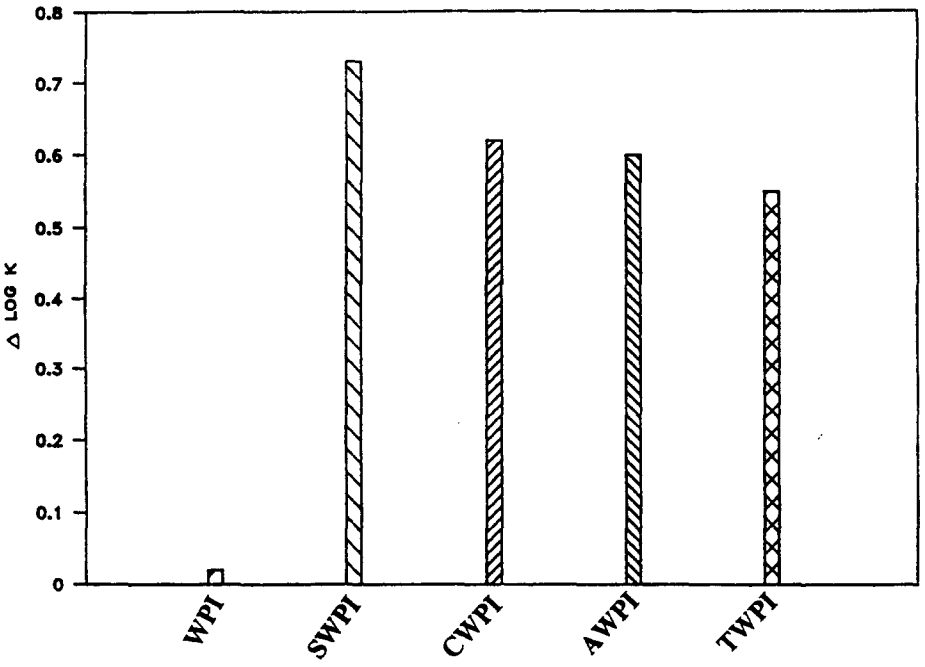

Fig 2. Partition hydrophobicity coefficient of native (WPI), acetylated (AWPI), succinylated (SWPI), trypsin-treated (TWPI) and chymotrypsin-treated (CWPI) whey protein isolates.

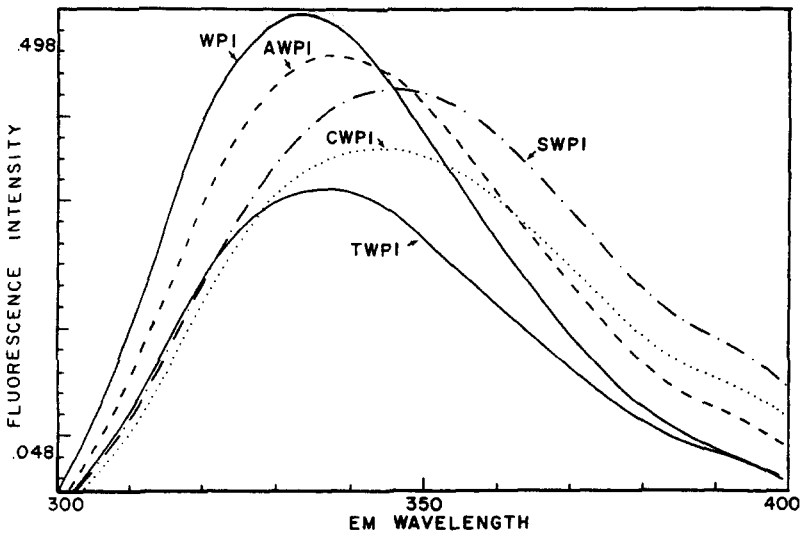

Fig 3. Fluorescence spectra of native (WPI), acetylated (AWPI), succinylated (SWPI), trypsin-treated (TWPI) and chymotrypsin-treated (CWPI) whey protein isolates (0·02% in pH 8·0 phosphate buffer).

the well-known high affinity of anhydrides for the free sulphur moieties on the polypeptide chains.

Hydrophobicity

Hydrophobicity was enhanced drastically upon modification of WPI. Figure 2 shows that, compared to the native proteins, SWPI resulted in a 25-fold increase in the hydrophobicity coefficient, followed by CWPI, AWPI and TWPI. Hydrophobic side chains of globular proteins are usually located in the interior of the molecule. Any effect which forces to open/unfold this structure should result in a more flexible conformation, thus causing the hydrophobic groups buried inside the compact structure to appear on the 'surface' (Graham & Phillips, 1976). It seems that the conformational changes which occurred upon modifying WPI were due to a depression in some inter- and intra-molecular protein–protein interactions. In the case of deamidation, removal of the hydrophilic polar ϵ-amino groups from the lysine residues, and probably from some arginine and glutamine sites, would lower the changes of interaction between these groups and the neighbouring carboxyl groups. Upon acylation, the increase in hydrophobicity could also be associated with other conformational effects. The presence of the bulky acyl group might have forced the globular structure of the protein molecules towards a more disordered conformation and thus, to greater flexibility (Habeeb *et al.*, 1958; Riordan & Vallee, 1964). For SWPI, the introduction of the excess negative charges is expected to affect the conformational changes in the protein by means of lowering the degree of interaction between the free amino and adjacent carboxyl groups, and also by increased repulsion between active groups (Habeeb *et al.*, 1958; Grants, 1973). In the case of acetylation, the charge effect was not of considerable importance due to the neutrality of the attached group.

Fluorescence spectra

Steady-state fluorescence spectra of proteins usually reflect their degree of unfolding, i.e. the extent of exposure of the 'buried' tryptophan residues. As shown in Fig. 3, SWPI resulted in the greatest red shift followed by CWPI, AWPI, TWPI and native WPI. This may indicate that the SWPI molecule was the most denatured and had the highest degree of destabilization of hydrophobic interaction. For the particular case of SWPI, it seems that, as discussed earlier, the attachment of the bulky succinate group, with all the associated negative charge, had imposed a very flexible structure on the molecule. Earlier studies on succinylation of soy proteins have shown similar results (Kim & Kinsella, 1986).

Surface tension

Figure 4 shows the effect of modification on the change in surface tension of WPI solutions. Native and modified WPI followed the same trend, i.e. a sharp drop at the beginning due to possible packing and conformational changes in the protein adsorbed at the interface. This was followed by a steady-state behavior with time. In all cases, modification was found to lower surface tension significantly. SWPI showed the greatest depression in surface tension, which was in good correlation with hydrophobicity and reduction of the disulfide bond content. Molecules with large hydrophobic groups on the surface are more adsorbable on a surface than those having small ones. The reason may lie in the strong tendency of these hydrophobic groups to avoid the surrounding water. This correlation between the amount of disulfide bonds and increased molecular flexibility, expressed in terms of the decrease in surface tension and enhanced red shift, indicates that these bonds serve as an energy barrier for the rearrangement of the peptide chains.

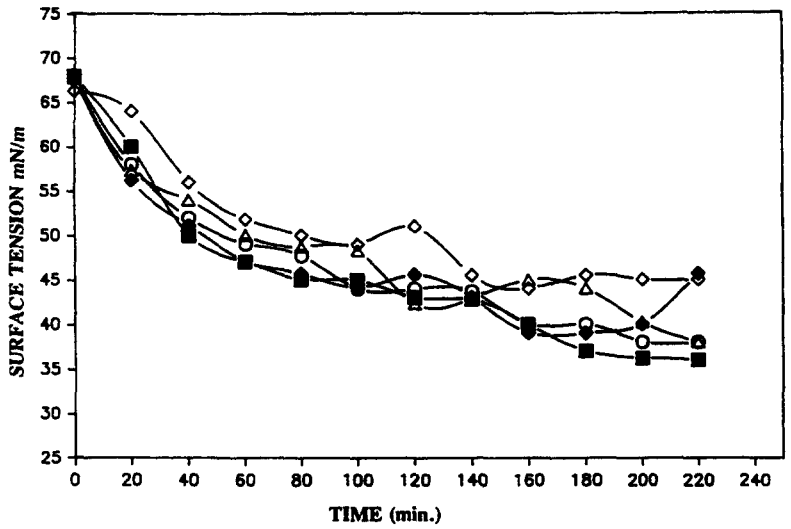

Fig. 4. Time dependence of interfacial tension of the air-water interface of native (◇), acetylated (◆), succinylated(■), trypsin-treated (○) and chymotrypsin-treated (Δ) whey protein isolates (0·05% in pH 7·0 phosphate buffer).

Functional properties

Solubility

The effect of modification on solubility of WPI is shown in Fig. 5. The solubility profile (turbidity) was found to follow the typical protein solubility behavior, i.e. minimum solubility at the isoelectric point (IEP) and resolubility in both regions away from the IEP. Enzymatic deamidation was found to enhance solubility significantly over a wide pH range. For acylated samples, the change in solubility was not very dramatic as compared to that of the native proteins. SWPI and AWPI solubility was intermediate in magnitude between the enzyme-treated and the native WPI. For SWPI, the high negative charge on the succinylated sites and the consequent conformational effects may have made it easier for the water molecules to penetrate in between the polypeptide chains and thus encouraged the stabilization of more soluble protein structures over a wide pH range. Earlier studies on modifications of some food proteins have shown conflicting results. Kim & Kinsella (1986) reported that the covalent attachment of succinyl groups to the glycinin protein was very beneficial to its solubility. The product was found to be completely soluble in the pH range 4·0 – 8·0, while the unmodified protein was insoluble in that region. Chobert *et al.* (1978) showed that the covalent attachment of various hydrophobic amino acid residues to casein resulted in decreased water solubility, although it improved other functional properties.

Emulsifying ability

The emulsifying ability of WPI was found to improve significantly in both the chemically and the enzymatically modified WPI (Fig. 6). All protein samples followed the same trend, i.e. reduced stability with time.

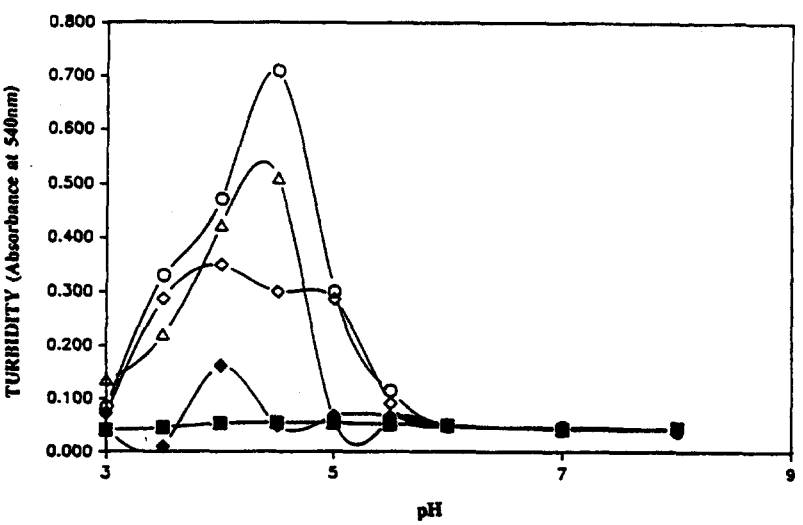

Fig. 5. Solubility profile of native (○), acetylated (Δ), succinylates (◇), trypsin-treated (■) and chymotrypsin-treated (◆) whey protein isolates (turbidity at 540 nm as a function of pH).

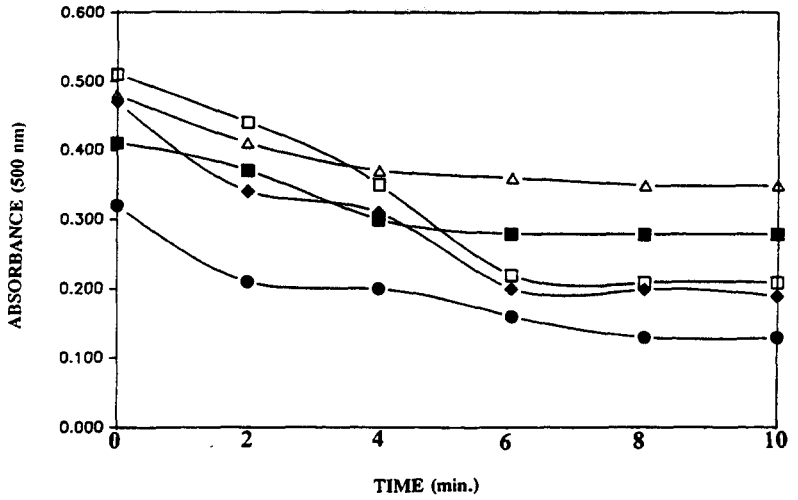

Fig. 6. Changes in the turbidity of emulsions produced from native (●), acetylated (■), succinylated (Δ), trypsin-treated (□) and chymotrypsin-treated (♦) whey protein isolates (pH 9·0).

This behavior could be explained on the basis of peptide rearrangement, and inter and intra-molecular interactions, pushing some of the covalently attached groups into the interior of the molecule with subsequent protein folding and masking of the few hydrophobic peptides essential for emulsion stability.

Succinylation was found to result in improved emulsifying ability and longer time stability, followed by acetylation. Trypsin-treated samples showed a better emulsifying ability and stability than the chymotrypsin-treated and native proteins, although both had lower emulsifying stability than the acylated types. Chen *et al.* (1975) had earlier reported the positive effect of succinylation on the emulsifying ability of succinylated fish protein concentrates. Similarly, the emulsifying activity of soy proteins was found to increase substantially upon acetylation (Groninger & Miller, 1975). Two approaches may be considered for explaining the emulsifying capacity of different proteins, namely, solubility and hydrophobicity. As the protein becomes more soluble, it forms layers around the fat droplet and so facilitates the association with the aqueous phase by means of decreased surface tension (Pearson *et al.*, 1965). However, in other cases, a poor correlation was found between solubility and emulsifying ability of proteins (Aoki *et al.*, 1980). Hence, hydrophobicity has been claimed to be the most important contributor to emulsifying ability, as a result of hydrophobic interactions between polypeptide chains and lipids in the vicinity of the proteins. In the case of succinylation, for example, the molecule is made more flexible, more disordered and more negatively charged. In fact, these aspects have made caseins the preferred emulsifying food proteins.

The ultrastructures of emulsions stabilized by native and modified WPI are shown in Fig. 7. The micrograph of the native WPI emulsion shows a distinguished uneven distribution of oil droplets, accompanied by the formation of plaque-like bodies of proteins (flocculation). Of all the emulsions studied, acylated WPI showed the best oil droplet distribution, followed by the enzyme-treated proteins. SWPI resulted in a uniform distribution of fine oil droplets. Similar results were obtained for AWPI, although the distribution was less even. Due to the assumed flexibility and strong repulsion between the polypeptide chains, the protein films formed around the oil droplets may have formed a barrier to their close approach and thus prevented them from collapsing. For SWPI, its high hydrophobicity might have resulted in immobilizing the liquid in the continuous phase, thus stabilizing its drainage. Enzyme-treated samples showed an improved emulsion ultrastructure compared to that of the native WPI emulsions, though the oil droplets occupied large pores. The high solubility of CWPI and TWPI might be responsible for the generation of large void spaces in the structure of the emulsion.

Foaming ability and stability

Data on the foaming properties of native and modified WPI are presented in Fig. 8. Foaming ability and stability were found to increase drastically upon modifying WPI for all the treatments studied. SWPI experienced the highest foaming activity (5-fold increase in foaming ability and 12-fold in stability over the native WPI). CWPI resulted in an intermediate degree of foaming, following by AWPI and TWPI. Introduction or uncovering of the hydrophobic groups of proteins is usually a good criterion for their enhanced foaming activity (Kinsella, 1976; Halling, 1981; Kato *et al.*, 1987). Correlating foamability data to surface tension (Fig. 4) shows evidently that the more denatured the protein molecule, and consequently the more flexible, the easier it spreads on interfaces. The

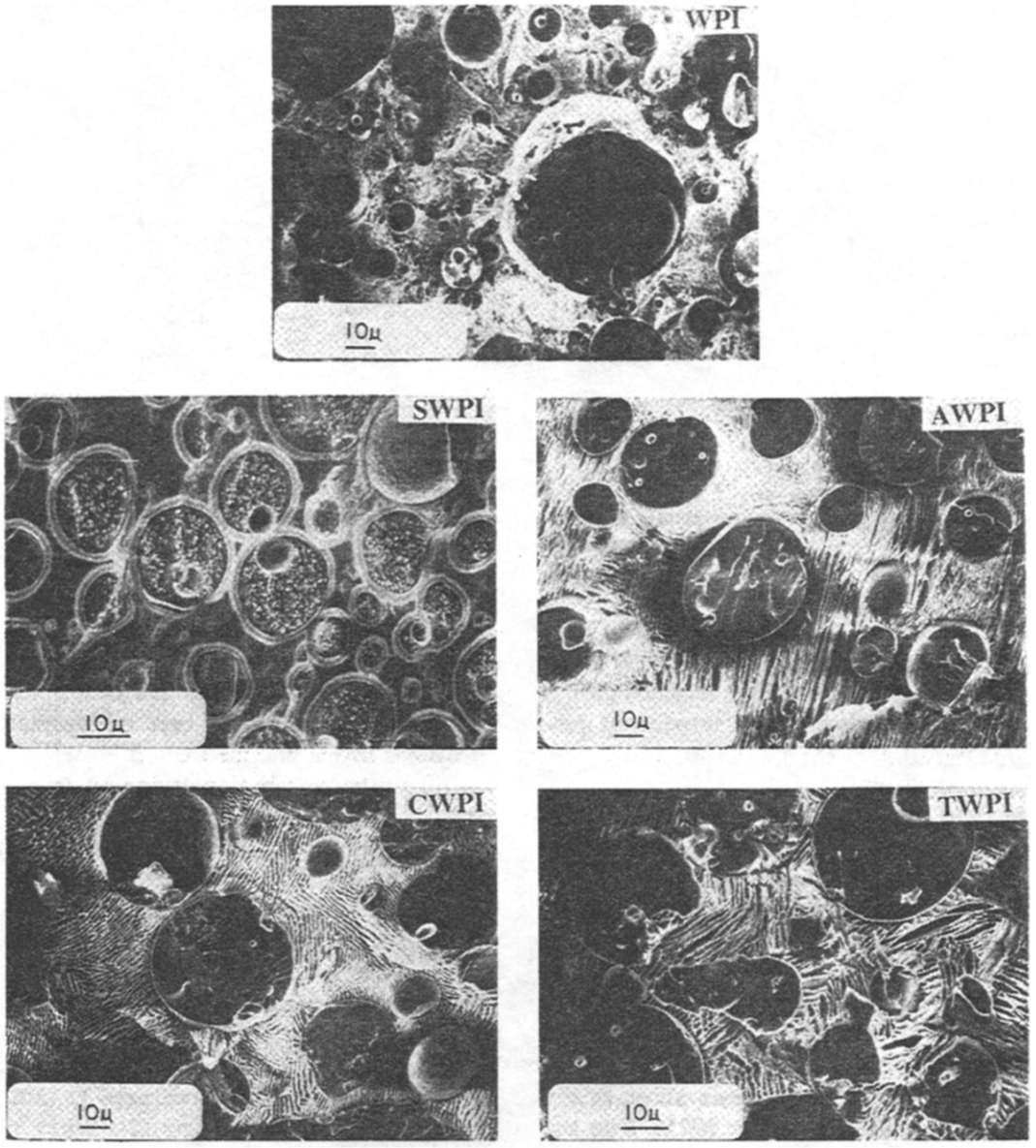

Fig. 7. Scanning electron micrographs of native (WPI), acetylated (AWPI), succinylated (SWPI), trypsin-treated (TWPI) and chymotrypsin-treated (CWPI) whey protein isolates/corn oil emulsions (12:8, w/w) using the cryo-method.

same reasoning mentioned earlier on the combined effect of solubility and hydrophobicity applies here.

Performance of modified WPI in an IMF system

Color Development

Results of color development patterns in terms of browning rate are presented in Figs 9 and 10. The most interesting feature of these results is the delayed browning of acetylated WPI under all conditions of storage. This observation may involve one or all the following possibilities. First, the SWPI color was considerably lighter than the enzyme-treated and the native proteins, and so was expected to darken less. Secondly, the successful acylation, as reflected in less available free amino groups at the lysine residues, might have been a good protective factor to the availability of the nucleophilic amino sites, and hence prevented, or at least delayed, their interaction with the electrophilic carbonyl sites of glucose (Table 1). Enzyme-treated WPI did not show a definite pattern, though they delayed the onset of browning as compared to the native proteins. This may be due to the high retention of the lysine residues upon deamidation which is reflected in the persistence of the interaction between the amino and carbonyl groups involved in Maillard reactions. Schnickels *et al.* (1976) showed that a casein–glucose model system at 35°C and a water activity of 0·82 reached maximum browning. Normally, the ideal water activity required for maximum browning development is between 0·3 and 0·7. However, it seems that this phenomenon still depends on the type of sugar and the specific food system (Eichner, 1975).

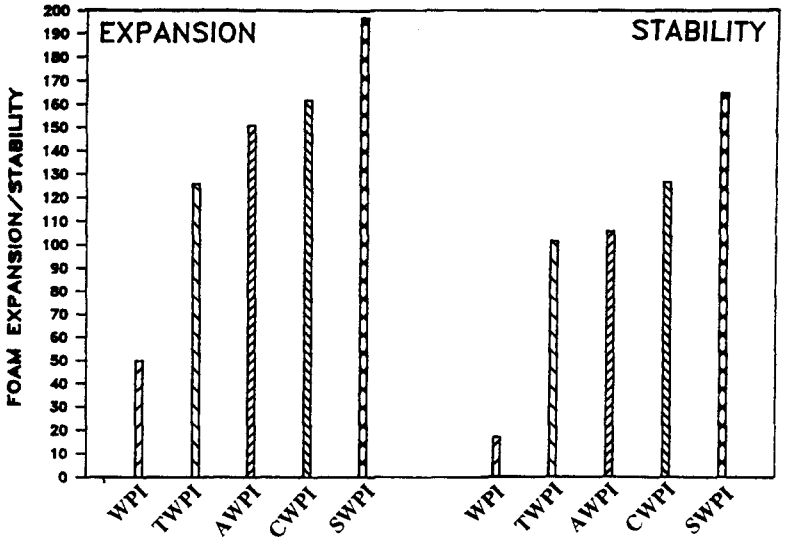

Fig. 8. Foaming ability and stability of native (WPI), acetylated (AWPI), succinylated (SWPI), trypsin-treated (TWPI) and chymotrypsin-treated (CWPI) whey protein isolates (0·5% in distilled water, blended at *c.* 20 000 rpm).

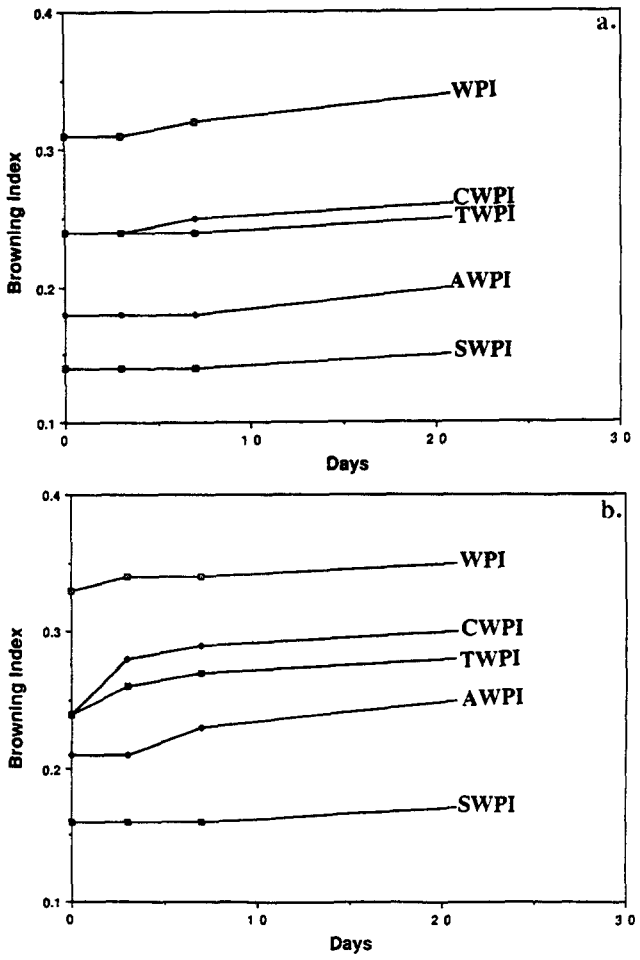

Fig 9. Browning Index of native (WPI), acetylated (AWPI), succinylated (SWPI), trypsin-treated (TWPI) and chymotrypsin-treated (CWPI) whey protein isolates without (a) or with (b) glucose (samples were stored at room temperature and 75% RH for periods of 0, 3, 7 and 21 days).

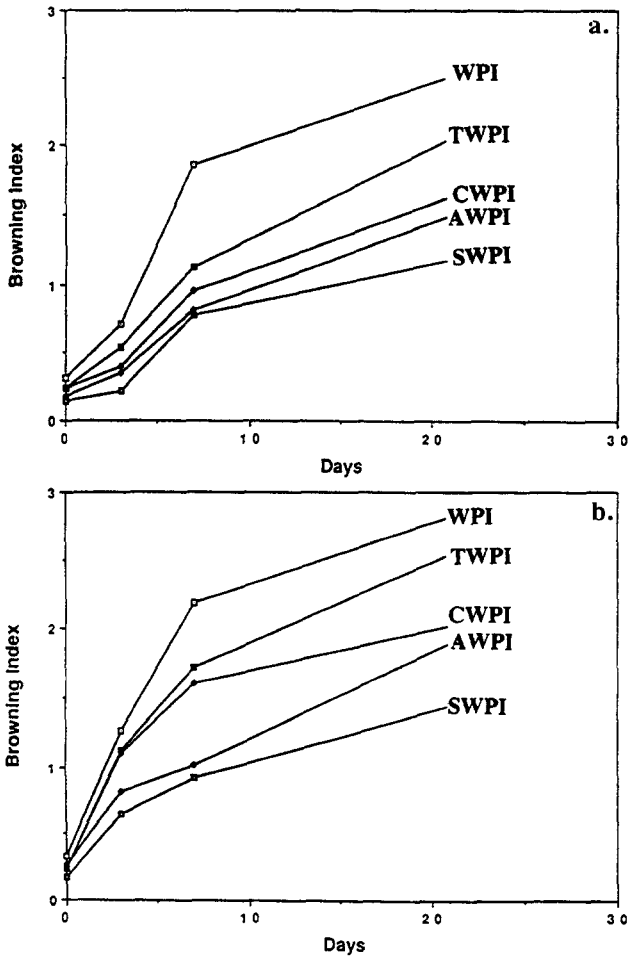

Fig. 10. Browning Index of native (WPI), acetylated (AWPI), succinylated (SWPI), trypsin-treated (TWPI) and chymotrypsin-treated (CWPI) whey protein isolates without (a) or with (b) glucose (Samples were stored at 50°C and 75% RH for periods of 0, 3, 7 and 21 days).

Table 4. Induction time and browning rate for samples of native (WPI), acetylated (AWPI), succinylated (SWPI), trypsin-treated (TWPI) and chymotrypsin-treated (CWPI) whey protein. Samples were stored at room temperature and 50°C (75% RH)

Protein	Induction time (days)	Browning rate (OD/Day) × 10^3
Room Temperature		
WPI	3	2·50
SWPI	7	0·71
AWPI	7	1·40
CWPI	3	2·50
TWPI	7	0·91
50°C		
WPI	<3	100·00
SWPI	<3	26·70
AWPI	<3	53·30
CWPI	<3	56·70
TWPI	<3	133·00

Table 5. Induction time and browning rate for samples of native (WPI), acetylated (AWPI), succinylated (SWPI), trypsin-treated (TWPI) and chymotrypsin-treated (CWPI) whey protein in a sugar mixture. Samples were stored at room temperature and 50°C (75% RH)

Protein	Induction time (days)	Browning rate (OD/Day) × 10^3
Room Temperature		
WPI	<3	3·30
SWPI	7	0·71
AWPI	3	5·00
CWPI	3	1·20
TWPI	3	6·70
50°C		
WPI	<3	293·00
SWPI	<3	160·00
AWPI	<3	183·00
CWPI	<3	283·00
TWPI	<3	310·00

In another study on browning applied to a casein-glucose system, Lea & Hannan (1950) noted the effects of pH of the medium on browning, besides those of temperature and relative humidity. Clark & Tannenbaum (1970) suggested that browning development involves the formation of some low molecular weight polypeptides which are responsible for the pyrazine pigment formation.

Induction time

The induction time of browning, determined as the period where no brown color could be detected, was found to be longer for all the modified samples than for the native proteins (Tables 4 and 5). SWPI resulted in the longest induction time accompanied by the lowest rate of brown color development. At room temperature, the induction time was found to be 7 days for all the modified samples as compared to 3 days for the native WPI systems. In the presence of glucose and at room temperature, only the SWPI system showed a signifi-cant resistance to browning. Upon heating, however, the induction time was less than 3 days for all the systems. Although the induction stage showed no apparent brown color development, it might bear some effect on the initiation rate of browning in terms of pigment intermediates formation as discussed in earlier studies (Mohammed *et al.*, 1949; Schnickels *et al.*, 1976).

Changes in basic amino acid content

The free amino acid profiles of native and modified whey protein isolates upon incorporation into an IMF system are presented in Tables 6 and 7. Although succinylation and acetylation had some drastic effect of the amino acid composition of whey proteins upon preparation, this was not the case upon storage both at room temperature and at 50°C. WPI samples stored for 3 days at 50°C resulted in almost 50% loss in the original lysine content. However, SWPI and AWPI lost only 21

Table 6. Basic amino acid profile of native (WPI), acetylated (AWPI), succinylated (SWPI), trypsin-treated (TWPI) and chymotrypsin-treated (CWPI) whey protein samples stored for 0, 3 and 7 days at (i) room temperature and (ii) 50°C (75% RH)

	0 days					3 days					7 days				
	WPI	AWPI	SWPI	CWPI	TWPI	WPI	AWPI	SWPI	CWPI	TWPI	WPI	AWPI	SWPI	CWPI	TWPI
Room temperature															
Lysine	100·0	84·0	76·3	91·3	86·6	72·1	70·4	65·2	67·3	65·0	63·0	61·5	60·0	53·4	58·0
Arginine	100·0	86·3	81·7	90·3	87·7	80·0	73·1	72·0	69·0	68·3	67·3	68·1	65·2	60·0	62·1
Histidine	100·0	88·1	82·0	80·3	81·0	82·3	74·1	72·7	67·0	69·5	71·0	63·9	68·0	61·0	56·8
Phenylalanine	100·0	89·9	82·5	88·0	87·0	79·9	76·0	73·4	69·3	74·0	65·3	72·4	70·4	57·3	57·9
50°C															
Lysine	100·0	84·0	76·3	91·3	86·6	50·4	58·3	55·0	52·3	52·0	26·7	43·9	48·7	39·3	44·2
Arginine	100·0	86·3	81·7	90·3	87·7	62·1	54·0	57·1	54·9	56·1	47·4	46·1	51·0	51·0	47·1
Histidine	100·0	88·1	82·0	80·3	81·0	62·4	66·0	64·3	64·0	59·8	28·7	57·0	57·2	53·0	52·1
Phenylalanine	100·0	89·9	82·5	88·0	87·0	55·7	60·9	65·6	59·0	64·9	24·3	47·8	56·3	39·0	45·1

Table 7. Basic amino acid profile of native (WPI), acetylated (AWPI), succinylated (SWPI), trypsin-treated (TWPI) and chymotrypsin-treated (CWPI) whey protein samples in a sugar mixture stored for 0, 3 and 7 days at (i) room temperature and (ii) 50°C (75% RH)

	0 days					3 days					7 days				
	WPI	AWPI	SWPI	CWPI	TWPI	WPI	AWPI	SWPI	CWPI	TWPI	WPI	AWPI	SWPI	CWPI	TWPI
Room temperature															
Lysine	100·0	84·0	76·3	91·3	86·6	94·5	80·0	71·4	86·4	80·2	73·8	68·1	67·9	74·3	69·3
Arginine	100·0	86·3	81·7	90·3	87·7	96·6	84·1	77·0	85·1	81·0	90·1	74·3	73·0	74·0	74·4
Histidine	100·0	88·1	82·0	80·3	81·0	96·0	83·2	78·2	75·1	74·4	79·6	71·0	75·0	73·1	68·1
Phenylalanine	100·0	89·9	82·5	88·0	87·0	97·1	85·4	77·4	84·5	84·3	73·5	75·1	75·9	77·0	76·3
50°C															
Lysine	100·0	84·0	76·3	91·3	86·6	62·1	63·0	67·4	59·7	60·0	35·2	55·1	62·1	44·2	49·4
Arginine	100·0	86·3	81·7	90·3	87·7	64·0	59·8	62·1	61·0	59·0	55·0	54·4	58·7	56·7	52·9
Histidine	100·0	88·1	82·0	80·3	81·0	75·3	71·0	72·1	70·0	55·1	36·3	62·0	66·6	62·0	51·1
Phenylalanine	100·0	89·9	82·5	88·0	87·0	63·8	62·3	69·4	65·3	60·0	39·0	56·6	64·0	58·0	53·0

and 36%, respectively. Freshly prepared enzyme-treated whey proteins, on the other hand, although they had a better retention of the essential amino acids upon preparation, were found to undergo greater loss in the IMF system upon storage. In all cases, heating was found to be the most detrimental factor for the rate of decomposition of the amino acids upon storage, although the effect of glucose enhanced this trend. It seems that, under the prevailing conditions, even in the absence of glucose, the protein has been hydrolyzed and this has resulted in a faster rate of amino acid loss. Tanaka *et al.* (1975) reported that, upon storage of glucose–egg albumin mixtures at 35°C and 68% RH, a fast rate of decomposition was perceived. Similar results were obtained by Schnickels *et al.* (1976).

In-vitro digestibility

The in-vitro digestibility profile of native and modified WPI is shown in Fig. 11. The digestibility of the enzyme-treated WPI was superior to chemically treated and to native proteins. As expected, SWPI and AWPI showed the lowest digestibility. Introduction of the bulky succinyl and acetyl groups to the whey protein molecule, and the corresponding change in its conformation, may have reduced the availability of the protein substrate for enzymatic attack. On the other hand, enzyme-treated whey proteins were found to be more soluble, which may have accounted, to a considerable extent, for the accessibility of the enzymes to their target sites along the polypeptide chains.

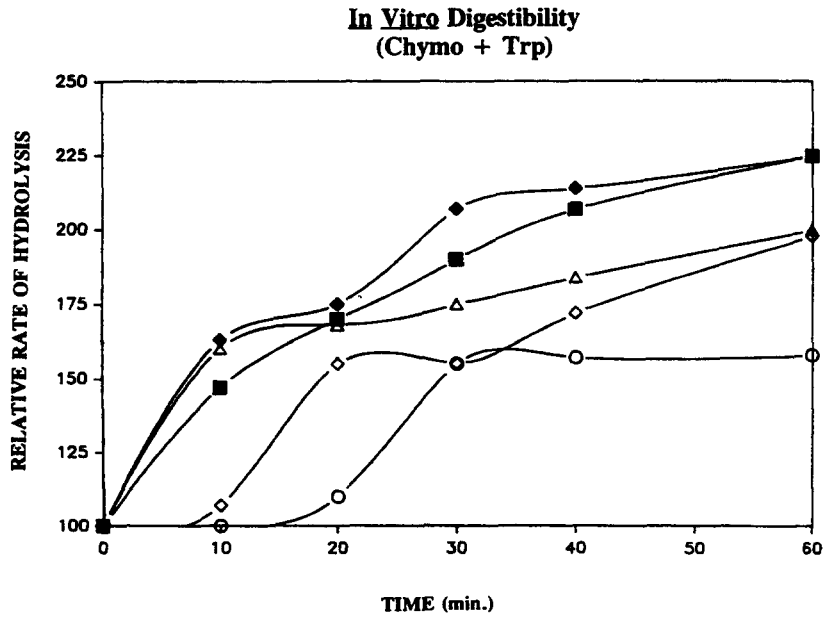

Fig. 11. In-vitro digestibility of native (Δ), acetylated (◇), succinylated (○), trypsin-treated (◇) and chymotrypsin-treated (■) whey protein isolates (enzyme–substrate, 1:128).

CONCLUSIONS

Results from this study showed, in general, that both the enzymatic and the chemical modifications employed were beneficial to the functional properties of whey proteins. The most important features could be summarized as follows:

1. Enzymatic deamidation resulted in better retention of the basic amino acid profile of whey proteins than did chemical acylation.
2. Chemical and enzymatic treatments of whey proteins have resulted in enhanced molecular flexibility as reflected in improved hydrophobicity, enhanced denaturation and depressed surface tension.
3. Functional properties of the modified proteins were found to be highly dependent on conformational changes in the molecule while their resistance to browning was directly related to the degree of blocking of the ϵ-amino groups of lysine (this was more obvious in the chemically treated proteins).
4. Blocking the ϵ-amino groups of lysine was found to preserve these residues from depletion upon storage in an IMF system.
5. In-vitro digestibility of enzyme-treated whey proteins was greater than those of the native and the chemically modified counterparts.

Clearly, chemical modification appears to be a very promising approach for improving the functional properties of food proteins. Additional information is needed on the nutritional value and the possible toxicological implications of protein modification in order to facilitate the potential use of modified proteins.

REFERENCES

Aoki, H., Taneyma, O. & Inami, M. (1980). Emulsifying properties of soy proteins: Characteristics of 7S and 11S proteins. *J. Food Sci.*, **45**, 534–8.

Beveridge, T., Toma, S. J. & Nakai, S. (1974). Determination of -SH and -SS groups in some food proteins using Ellman's reagent. *J. Food Sci.*, **39**, 49–51.

Chen, J. Y., Pira, M. & Labuza, T. P. (1975). Evaluation of water binding capacity (WBC) of food fiber sources. *J. Food Sci.*, **49**, 59–63.

Chobert, J. M., Bertrand-Harb, C., Nicoles, M. G., Gaertner, H. H. & Puigserver, A. J. (1978). Solubility and emulsifying properties of casein chemically modified by covalent attachment of L-methionine and L-valine. *J. Agric. Food Chem.*, **35**, 638–44.

Clark, J. H. & Tannenbaum, S. R. (1970). Isolation and characterization of pigments from protein-carbonyl browning systems. Isolation, purification and properties. *Food Chem.*, **18(5)**, 891–4.

Damodaran, S. & Kinsella, J. E. (1982). Effect of conglycinin on the thermal aggregation of glycinin. *J. Agric. Food Chem.*, **30**, 812–17.

Eichner, M. (1975). The influence of water content on nonenzymic browning reactions in dehydrated foods and model systems and the inhibition of fat oxidation by browning intermediates. In *Water Relations in Food*, ed. R. B. Duckworth. Academic Press, London, pp. 417–34.

Fields, R. (1972). The rapid determination of amino groups with TNBS. *Methods in Enzymology*, **25**, 464–8.

Franzen, K. L. & Kinsella, J. E. (1976). Functional properties of succinylated and acetylated leaf protein. *J. Agric. Food Chem.*, **24(5)**, 914–19.

Freidman, M. (ed.) (1984). *Proteins: Nutritional Quality of Foods and Feeds. Nutrition and Clinical Nutrition* Vols I and II, Marcel Dekker, New York.

Graham, D. E. & Phillips, M. C. (1976). In *Foams*. ed. R. J. Akers. Academic Press, New York. pp. 237–55.

Grants, D. (1973). A modification of wheat flour proteins with succinic anhydride. *Cereal Chem.*, **50**, 417–28.

Groninger, H. & Miller, R. (1975). The modification of wheat flour protein with succinic anhydride. *J. Food Sci.*, **40**, 327–30.

Habeeb, A. F., Kassidy, H. & Singer, S. (1958). Molecular structural effect produced in proteins by reaction with succinic anhydride. *Biochim. Biophys. Acta.*, **29**, 587–93.

Halling, P. G. (1981). Protein-stabilized foams and emulsions. *CRC Crit. Rev. Food Sci. Nutr.*, **15**, 155–203.

Hoagland, P. (1966). Acetylated-caseins: Electrostatic interactions and aggregation. *J. Dairy Sci.*, 49, 783–7.

Kato, A., Tanaka, A., Matsudoni, N. & Kobayashi, K. (1987). Deamidation of food proteins by proteases in alkaline pH. *J. Agric. Food Chem.*, **35**, 285–8.

Keshavarez, E. & Nakai, S. (1979). The relationship between hydrophobicity and interfacial tension of proteins. *Biochim. Biophys. Acta*, **576**, 269–79.

Kim, S. & Kinsella, J. E. (1986). Effect of progressive succinylation on some molecular properties of soy glycinin. *Cereal Chem.*, **63(4)**, 342–5.

Kinsella, J. E. (1976). Functional properties of proteins in foods: A survey. *CRC Crit. Rev. Food Sci. Nutr.*, 7, 219–80.

Kinsella, J. E. (1977). Functional properties of novel proteins. *Chem. Ind.*, 5, 177–80.

Klotz, I. M. (1976). Succinylation. *Methods in Enzymology*, **11**, 576–80.

Lammeli, V. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. *Nature (London)*, **227**, 680–5.

Lea, C. H. & Hannan, R. S. (1950). Studies on the reactions between proteins and reducing sugars in the dry state. *Biochim. Biophys. Acta*, **5**, 433–53.

Mohammed, A., Frankel-Conrat, H. & Olcoh, H. S. (1949). Browning reactions of proteins with glucose. *Arch. Biochem. Biophys.*, **24**, 270–80.

Morr, C. V. (1976). Whey proteins—An update. *Food Technol.*, **30(3)**, 18–22.

Pearson, A. M., Spooner, M. E., Hegarthy, G. R. & Bratzel, L. L. (1965). Stability of soy, Na-protein caseinate, K-caseinate and NFDM. *Food Technol.*, **19**, 103–7.

Riordan, J. F. & Vallee, B. L. (1964). Succinylcarboxypeptidase. *Biochem.*, **3**, 1768–74.

Schnickels, R. A., Warmbeir, H. C. & Labuza, T. P. (1976).

Effect of protein substitution on non-enzymatic browning in an Intermediate Food System. *J. Agric. Food Chem.*, **24**, 901–3.

Tanaka, M., Lee, T. & Chichester, C. O. (1975). Effect of browning on chemical properties of egg albumin. *Agric. Biol. Chem.*, **39**, 863–66.

Yamauchi, F., Ogawa, Y., Kamata, Y. & Shibasaki, K. (1982). Emulsifying properties of soybean conglycinin and glycinin: Evaluation by turbidimetry. *Agric. Biol. Chem.*, **46**(3), 615–21.