Effect of Fatty N-Acylamino Acids on Some Functional Properties of Two Food Proteins†

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The effect of fatty N-acylamino acids on the foaming, emulsifying, and gelling properties of egg white and whey protein isolate was investigated. The overrun, emulsifying activity, and gelling properties of these two protein products were generally enhanced by the addition of 0.1–0.5% of the fatty N-acylamino acids, while the foaming stability was lowered. Both lauroyl (C_{12}) and myristoyl (C_{14}) derivatives were highly effective, particularly the derivatives of phenylalanine and methionine. The palmitoyl (C_{16}) and stearoyl (C_{16}) derivatives were less effective, while the esters of active derivatives did not affect protein functionality. The thermal characteristics of the proteins were modified by fatty N-acylamino acids and were related to changes in functional properties. The results suggest the potential use of fatty N-acylamino acids as multifunctional food additives.

INTRODUCTION

Free fatty acids and their esters are known to have antibacterial activity (Kabara, 1981, 1982; Shibasaki, 1982), and lauric acid monoglycerol ester has been suggested for use as a food preservative (Shibasaki, 1982). Amides of fatty acids, particularly those of amino acids, also possess significant antibacterial properties (Fieser et al., 1956; Takehara et al., 1972; Paquet, 1977; Shibasaki, 1982; Paquet and Rayman, 1987). A series of fatty N-acylamino acids have been prepared (Paquet, 1974, 1979; Paquet and Sarwar, 1980) and investigated as potential food additives. The high nutritive value (Paquet and Sarwar, 1980), antimicrobial activity (Madhosingh et al., 1978; Paquet and Rayman, 1987), and good emulsifying properties (Fieser et al., 1956; Takehara et al., 1972) of these compounds have been recognized. In a previous study (McKellar et al., 1992), the antimicrobial activity of a series of fatty N-acylamino acids against Gram-positive food-borne pathogens was investigated. Myristoyl (C_{14}) derivatives gave the greatest activity, with derivatives of aromatic amino acids being more active, while the D-isomers and esters were inactive. In the present investigation, we study the effect of these fatty N-acylamino acids on the functional properties of two major food protein products: spray-dried egg white and whey protein isolate.

MATERIALS AND METHODS

Synthesis and Characterization of Long-Chain N-Acylamino Acids. Amino acids were purchased from Sigma Chemical Co. (St. Louis, MO) and fatty acid chlorides from Nu-Check Prep, Inc. (Elysian, MN). Melting points, optical rotation, and NMR spectral properties were measured according to methods described in a previous paper (McKellar et al., 1992).

Fatty N-acylamino acids were prepared from appropriate amino acids and succinimidyl esters of fatty acids (Paquet, 1974, 1980) or from fatty acids chlorides (Schroder and Lubke, 1965). The products were purified by repeated crystallization and characterized by physicochemical constants, elemental analysis, NMR spectra, and HPLC analysis as described previously (Paquet, 1980; McKellar et al., 1992) (Table I).

Functional Properties. Spray-dried egg white (Export Packers Co., Winnipeg, MB, Canada) and whey protein isolate (Le Suer Isolates, Le Suer, MN) were commercial products with over 90% protein. The whippability and foam stability of protein were determined according to the method of Phillips et al. (1987) using 5% (w/v) protein dispersions. The emulsification activity index (EAI) was determined according to a turbidimetric method (Pearce and Kinsella, 1978). The gelling properties were determined by methods described previously (Ma and Khanzada, 1987; Ma et al., 1990) using 10% (w/v) protein dispersions prepared in distilled water. Heat-induced gels were formed by heating the samples at 90 °C for 20 min. Gel hardness was determined according to the back extrusion method (Kramer and Hawbecker, 1966) using an Ottawa texture measuring system (OTMS, Canners Machinery Ltd., Simcoe, ON, Canada). The viscoelastic properties of the gels were assessed by dynamic oscillatory measurements using a Carri-Med controlled stress rheometer (Mitech Corp., Twinsburg, OH). Measurements were made over a frequency range of 0.02–10 Hz with a strain amplitude of 0.05. The storage modulus (G'), loss modulus (G''), and loss tangent ($\tan \delta = G''/G'$) were computed by software developed by Carri-Med Ltd. (Dorking, Surrey, U.K.).

Differential Scanning Calorimetry (DSC). The thermal characteristics of egg white and whey proteins were studied by DSC according to the method described by Ma and Harwalkar (1988), using a Du Pont 1090 thermal analyzer equipped with a high-pressure DSC cell. Protein solutions (10% w/v) were prepared in distilled water, and 10-μL aliquots were pipetted into the pans followed by the addition of 0.05 mg of fatty N-acylamino acid as dry solid, giving a protein:additive ratio of 20:1. The pans were hermetically sealed and heated from 25 to 105 °C at 10 °C/min. The peak or denaturation temperature (T_d) and the heat of transition or enthalpy (ΔH) were computed from the thermograms by the 1090 analyzer.

Table I. Characteristics of Fatty N-Acylamino Acids

compound[a-c]	mp,[d] °C	$[\alpha]^{23}$,[e] deg	formula
lauroyl (C_{12})			
Phe	99–101	68.5	$C_{21}H_{33}NO_3$
Try	112	+25.5	$C_{23}H_{34}N_2O_3$
Met	69	+28.6	$C_{17}H_{33}NO_3S$
myristoyl (C_{14})			
Phe	90–91	65.5	$C_{23}H_{37}NO_3$
Try	106–107	+24	$C_{15}H_{38}N_2O_3$
Met	77–79	+27	$C_{19}H_{37}NO_3S$
palmitoyl (C_{16})			
Phe	91	+52	$C_{25}H_{41}NO_3$
Try	99–102	+18.7	$C_{27}H_{42}N_2O_3$
Met	84	+23.7	$C_{21}H_{41}NO_3S$
stearoyl (C_{18})			
Met	89–90	+22	$C_{23}H_{45}NO_3S$

[a] HPLC, elemental analysis, and NMR spectra were in accord with the structures. [b] In dimethylformamide. [c] Phe, phenylalanine; Try, tryptophan; Met, methionine. [d] mp, melting point. [e] $[\alpha]^{23}$, optical rotation.

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Table II. Effect of Fatty N-Acylamino Acids on the Whipping and Emulsifying Properties of Spray-Dried Egg White[a]

derivative	overrun, mL	foam stability, min	EAI,[b] m^2/g
control[c]	1120	48.4	4.53
Lau-Try	1520	30.9	14.9
Lau-Phe	1780	23.6	17.8
Lau-Met	1650	21.5	19.1
Lau-Met-OMe	1110	41.0	8.70
Myr-Try	1730	37.3	7.37
Myr-Phe	1540	22.5	11.3
Myr-Met	1680	21.8	14.3
Pal-Try	1490	57.5	5.51
Pal-Phe	1570	39.1	8.54
Pal-Met	1350	28.3	5.23
Ste-Met	1210	47.7	2.95

[a] Averages of two determinations. [b] EAI is the emulsification activity index. [c] No additive.

Table III. Effect of Fatty N-Acylamino Acids on the Whipping and Emulsifying Properties of Whey Protein Isolate[a]

derivative	overrun, mL	foam stability, min	EAI,[b] m^2/g
control[c]	750	23.0	59.5
Lau-Try	1155	9.80	79.5
Lau-Phe	1179	9.38	81.5
Lau-Met	1038	6.87	75.6
Lau-Met-OMe	750	21.5	55.4
Myr-Try	1008	13.6	73.4
Myr-Phe	615	4.20	109
Myr-Met	672	5.05	60.0
Pal-Try	835	23.8	92.3
Pal-Phe	853	7.40	61.4
Pal-Met	458	4.27	58.2
Ste-Met	593	4.00	71.4

[a] Averages of two determinations. [b] EAI is the emulsification activity index. [c] No additive.

RESULTS

Preliminary experiments showed that a minimum concentration of the fatty N-acylamino acids was required to alter the functional properties of the two proteins. The concentrations selected were 0.1% (w/v) for EAI measurement, 0.2% for whipping tests, and 0.5% for gelling and DSC experiments. Most of the fatty N-acylamino acids have limited solubility in aqueous media used for functionality measurements. However, with careful mixing, a stable dispersion (mixture of protein and fatty N-acylamino acid) can be prepared. Further mixing (whipping and emulsifying experiments) or heating (gelling experiments) during the functionality tests ensured the formation of homogeneous mixtures.

Whipping and Emulsifying Properties. The effects of fatty N-acylamino acids on the whipping and emulsifying properties of spray-dried egg white and whey protein isolate are shown in Tables II and III, respectively. For egg white, there was a general increase in overrun but the foam stability was lowered. Lau-Phe and Myr-Try provided the highest overrun, followed closely by Lau-Met and Myr-Met. Palmitoyl and stearoyl derivatives were less effective. Similarly, the lauroyl and myristoyl derivatives, particularly methionine and phenylalanine, gave the greatest increases in emulsification activity for egg white (Table II). For whey protein, only the lauroyl derivatives and myristoyl tryptophan provided an increase in overrun, and the foam stability was markedly reduced by all derivatives (except Pal-Try). The emulsifying activity was generally enhanced by the fatty N-acylamino acids, with Myr-Phe and Pal-Try being the most effective (Table III). For both egg white and whey protein, Lau-Met-OMe, the methyl ester of the highly active compound, did not affect the whipping and emulsifying properties of the two protein products.

Table IV. Effect of Fatty N-Acylamino Acids on the Gelling Properties of Spray-Dried Egg White[a]

derivative	gel hardness, N	G',[b] Pa	G'',[c] Pa	tan δ
control[e]	4.57	6 470	884	0.137
Lau-Try	6.58	11 400	2200	0.148
Lau-Phe	6.60	14 100	2740	0.194
Lau-Met	6.92	13 000	2320	0.178
Lau-Met-OMe	4.92	6 550	887	0.135
Myr-Try	6.71	12 700	2340	0.184
Myr-Phe	6.33	10 900	1980	0.182
Myr-Met	6.40	14 700	2470	0.168
Pal-Try	6.40	10 200	1750	0.172
Pal-Phe	6.50	12 900	2290	0.178
Pal-Met	6.60	11 800	2100	0.178
Ste-Met	5.56	9 580	1680	0.176

[a] Averages of two determinations. [b] G' is the storage modulus. [c] G'' is the loss modulus. [d] tan $\delta = G''/G'$. [e] No additive.

Table V. Effect of Fatty N-Acylamino Acids on the Gelling Properties of Whey Protein Isolate[a]

derivative	gel hardness, N	G',[b] Pa	G'',[c] Pa	tan δ[d]
control[e]	11.5	11 000	1610	0.146
Lau-Try	16.1	13 200	1860	0.141
Lau-Phe	17.2	14 300	2200	0.154
Lau-Met	19.0	14 500	2240	0.155
Lau-Met-OMe	11.3	10 500	1470	0.140
Myr-Try	11.5	10 600	1150	0.109
Myr-Phe	13.4	11 800	1310	0.111
Myr-Met	14.4	11 400	1360	0.119
Pal-Try	11./2	10 600	1150	0.109
Pal-Phe	18.0	14 700	1750	0.119
Pal-Met	12.1	10 600	1220	0.115
Ste-Met	7.03	8 210	972	0.118

[a] Averages of two determinations. [b] G' is the storage modulus. [c] G'' is the loss modulus. [d] tan $\delta = G''/G'$. [e] No additive.

Gelling Properties. Tables IV and V show the effect of fatty N-acylamino acids on the gelling properties of spray-dried egg white and whey protein isolate, respectively. Similar to whipping and emulsifying properties, lauroyl and myristoyl derivatives, particularly phenylalanine and methionine, provided the highest increases in gel hardness and storage modulus, a measure of gel rigidity. Again, palmitoyl and stearoyl derivatives were less effective agents for enhancing the gelling properties of the two proteins, and the ester of Lau-Met was ineffective. All of the gels showed a relatively low tan δ value, indicating the formation of a viscoelastic gel structure. The loss tangent value (tan δ, G'',G'), a measure of the relative importance of viscous and elastic processes in the materials (Ferry, 1980), of egg white gels was increased by most fatty N-acylamino acids tested, indicating a lowering in viscoelasticity, For whey protein gels, however, the tan δ value was lowered by myristoyl and palmitoyl derivatives, suggesting an increase in viscoelasticity.

Figures 1 and 2 show the frequency dependence of storage and loss moduli of egg white and whey protein

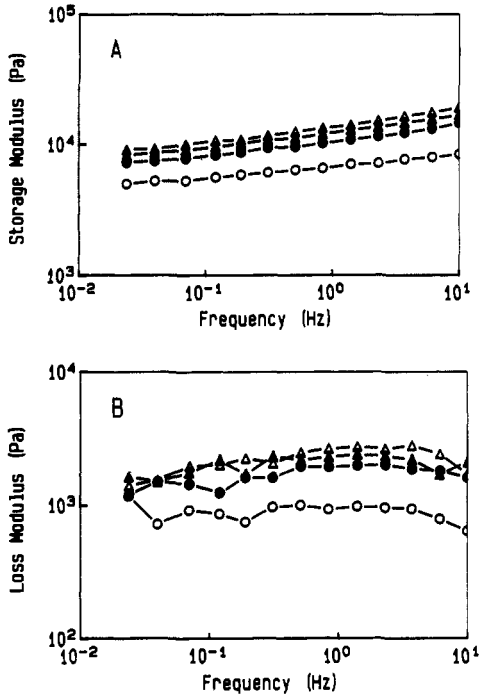

Figure 1. Frequency dependency of storage (A) and loss (B) moduli of egg white gels. Measurements were made at 25 °C with a strain amplitude of 0.05. ○, Control (no additive); △, Lau-Phe; ●, Myr-Phe; ▲, Pal-Phe.

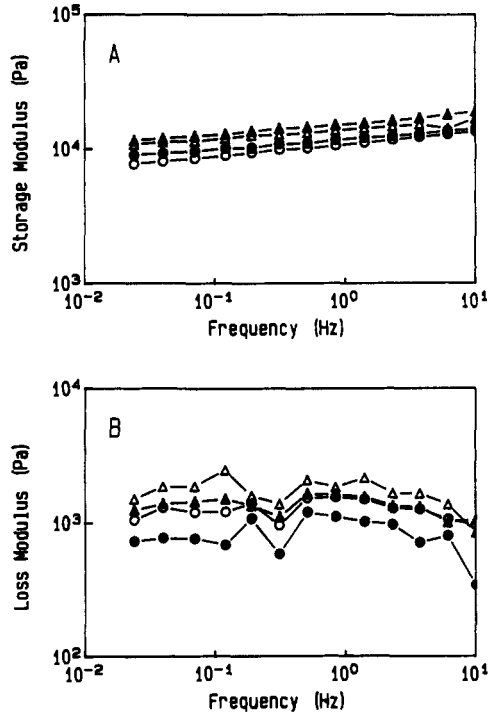

Figure 2. Frequency dependency of storage (A) and loss (B) moduli of whey protein gels. Measurements were made at 25 °C with a strain amplitude of 0.05. ○, Control (no additive); △, Lau-Met; ●, Myr-Phe; ▲, Pal-Phe.

gels, respectively, under the influence of some fatty *N*-acylamino acids. For all samples, G' increased linearly with log frequency, and the increases were relatively small (Figures 1A, 2A). The data agreed with many studies demonstrating a flat frequency dependence over wide frequency ranges for hydrogels (Clark and Lee-Tuffnell, 1986). Both egg white and whey protein gels exhibited some frequency dependence for loss modulus, with changes in G'' (increases and/or decreases) between the frequency

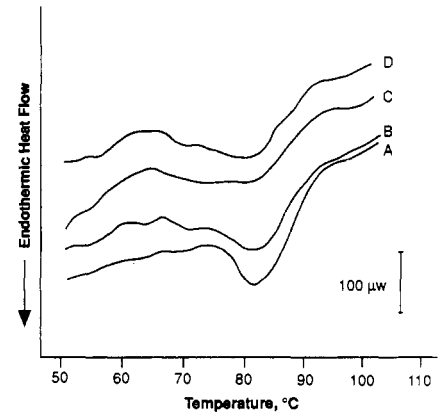

Figure 3. Effect of myristoyl derivatives on differential scanning calorimetric thermograms of spray-dried egg white. Protein samples (10%) were prepared in distilled water, and 0.05 mg of fatty *N*-acylamino acid was added. A, Control (no additive); B, Myr-Try; C, Myr-Met; D, Myr-Phe.

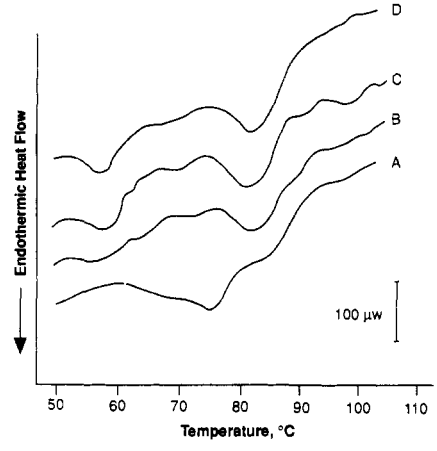

Figure 4. Effect of lauroyl derivatives on differential scanning calorimetric thermograms of whey protein isolate. Protein samples (10%) were prepared in distilled water, and 0.05 mg of fatty *N*-acylamino acid was added. A, Control (no additive); B, Lau-Try; C, Lau-Met; D, Lau-Phe.

range 0.1–0.3 Hz (Figures 1B, 2B). There seems to be a shift in the G'' minimum to either lower or higher frequency with the addition of some fatty *N*-acylamino acids. The frequency dependence of loss tangent (data not shown) was similar to those of G'' due to relatively flat G' curves. Such changes in G'' or tan δ values at low frequency were attributed to entanglement coupling of gel network in which extended linear fragments interact in a specific frequency range (Ferry, 1980; Gill and Tung, 1978).

Thermal Characteristics. The effects of fatty *N*-acylamino acids on the thermal characteristics of egg white and whey proteins are illustrated in Figures 3–5 and summarized in Table VI. The DSC thermograms of spray-dried egg white (Figure 3A) and whey protein isolate (Figures 4A, 5A) showed the major protein components, ovalbumin and β-lactoglobulin, respectively, similar to those reported by other workers (Donovan et al., 1975; De Wit and Swinkels, 1980; Paulsson et al., 1985). The minor proteins, conalbumin and lysozyme in egg white and α-lactalbumin and bovine serum albumin in whey isolate, were not clearly distinguished. Hence, only the changes in the T_d of the major peaks and the total enthalpy of denaturation were monitored.

For egg white, the presence of myristoyl derivatives (Figure 3) and lauroyl derivatives (not shown) led to a broadening of the ovalbumin peak and a decrease in the ΔH value (Table VI), suggesting partial denaturation of

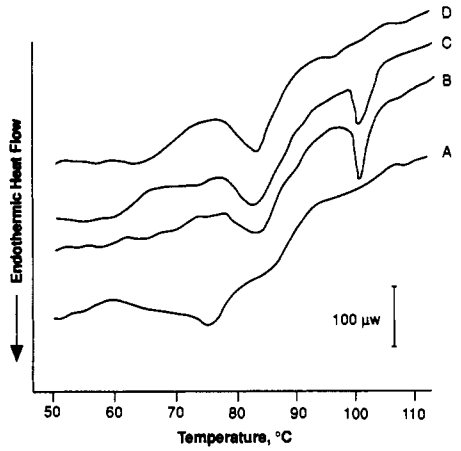

Figure 5. Effect of palmitoyl derivatives on differential scanning calorimetric thermograms of whey protein isolate. Protein samples (10%) were prepared in distilled water, and 0.05 mg of fatty N-acylamino acid was added. A, Control (no additive); B, Pal-Try; C, Pal-Met; D, Pal-Phe.

Table VI. Effect of Fatty N-Acylamino Acids on the Thermal Characteristics of Spray-Dried Egg White and Whey Protein Isolate[a]

	spray-dried egg white		whey protein isolate	
derivative	T_d,[b] °C	ΔH, J/g	T_d, °C	ΔH, J/g
control[d]	83.3	12.2	76.3	11.0
Lau-Try	82.7	6.42	84.3	6.82
Lau-Phe	84.5	10.1	83.5	7.63
Lau-Met	81.9	8.76	83.5	5.09
Lau-Met-OMe	82.9	8.35	76.6	5.64
Myr-Try	83.7	8.43	85.1	7.74
Myr-Phe	83.2	9.40	84.8	6.16
Myr-Met	82.8	8.80	82.2	6.84
Pal-Try	83.7	12.1	84.5	9.58
Pal-Phe	83.3	11.7	84.4	8.90
Pal-Met	84.3	9.00	82.9	8.18
Ste-Met	83.0	9.83	81.5	8.85

[a] Averages of two determinations. [b] T_d is the denaturation temperature of major protein (ovalbumin in egg white and β-lactoglobulin in whey isolate). [c] ΔH is the total enthalpy of denaturation. [d] No additive.

the proteins. The denaturation temperature of ovalbumin, however, was not shifted markedly. With the exception of Myr-Met, myristoyl derivatives did not cause significant lowering in total enthalpy of egg white and the T_d of ovalbumin was also not shifted. Both Ste-Met and Lau-Met-OMe caused a decrease in total enthalpy of egg white.

For whey protein isolate, the T_d of β-lactoglobulin was markedly increased by the addition of fatty N-acylamino acids, while the total enthalpy was lowered to various extents (Figures 4, 5; Table VI). The methyl ester of lauroyl methionine also caused a decrease in ΔH, but T_d was not changed.

An additional peak near 60 °C was observed when lauroyl derivatives were added to whey protein isolate (Figure 4) or egg white (not shown). This could be attributed to the transition of these derivatives which showed a sharp endothermic peak between 55 and 60 °C when heated alone (5% w/v in distilled water) (data not shown). The addition of palmitoyl tryptophan (Figure 5B) and methionine (Figure 5C) to whey protein isolate and spray-dried egg white (not shown) led to the appearance of an additional sharp endotherm near 100 °C. These derivatives showed a sharp endothermic peak at around 80 °C when heated alone (in distilled water) (data now shown), and the extra endotherm could be an interaction product between the palmitoyl derivatives and proteins. The origin and identity of these additional endothermic peaks were not known, and further investigation would require the use of purified proteins (e.g., ovalbumin and β-lactoglobulin) under a wider range of conditions (pH, derivative concentrations, etc.).

DISCUSSION

The present data showed that some fatty N-acylamino acids can be used to enhance the whipping power, emulsifying activity, and gelling properties of egg white and whey protein. Previous study (McKellar et al., 1992) has demonstrated that fatty acid derivatives of amino acids such as aspartate, threonine, and alanine were much less effective antimicrobial agents than the derivatives of aromatic amino acids and methionine. Capric (C_{10}) and stearic (C_{18}) acid derivatives were less effective than lauric (C_{12}), myristic (C_{14}), and palmitic (C_{16}) acid derivatives. The influence of these compounds on the functional properties of egg white and whey proteins seems to follow a similar trend. Overall, the lauroyl derivatives, especially phenylalanine and methionine, were the most effective compounds in enhancing the whipping, emulsifying, and gelling properties of the two proteins. These were followed by myristoyl and palmitoyl derivatives.

Surface and interfacial properties of proteins play a vital role in the formation and stabilization of foams and emulsions (Kinsella, 1976). N-Acylamino acids are composed of hydrophobic fatty acids and polar amino acids and have been known as surface active agents. Kester (1949) reported that long-chain N-acylglutamic acids could be used as wetting agents, foaming agents, or detergents. The emulsifying property of N-acylamino acids has been examined in a series of synthetic emulsifying agents (Fieser et al., 1956). Aqueous solutions of long-chain N-acylglutamic acids were shown to have low surface tensions and good foaming properties (Takehara et al., 1972). Fatty N-acylaspartic acids were also shown to lower the surface tension of water (A. Paquet and J. Holme, unpublished data). The observed enhancement in overrun and emulsifying activity of the two proteins can therefore be attributed to the presence of these surface active agents. The varying effectiveness of different derivatives suggests that a proper lipophilic–hydrophilic balance in the fatty N-acylamino acids is required for optimal foaming and emulsifying characteristics. The ineffectiveness of the esters of active derivatives was probably due to the blocking of the hydrophilic carboxyl group of amino acids, upsetting the lipophilic–hydrophilic balance.

The formation of protein-based foams and emulsions also depends on partial denaturation of protein at air–liquid and oil–water interfaces, respectively, and foam stability depends on the ability of the unfolded protein to form an elastic film at the interface. The DSC data showed that the total enthalpy of the two protein products was generally lowered by the fatty N-acylamino acids, suggesting partial denaturation. This would enhance the foaming and emulsifying ability of the proteins. It is not clearly understood why the foam stability was decreased by the derivatives. It is possible that with expansion in foam volume the air cell membrane was weakened and collapsed at a faster rate.

The hardness and rigidity of the heat-induced protein gels were increased by some N-acyl derivatives. Previous studies showed that some fatty acid salts also improve the gel-forming ability of myosin (Egelandsdal et al., 1985) and oat protein (Ma et al., 1988). It was suggested that the increase in gel strength was due to binding of these

amphiphiles to protein, causing repulsion between protein chains and a more ordered aggregation (gelation) upon heat treatment (Egelandsdal et al., 1985). It is interesting to note that medium-chain-length fatty acid salts (C_{11} and C_{12}) were also found to be much more effective than longer-chain-length compounds (C_{17} and C_{18}) (Ma et al., 1988). However, the effect of these fatty acid salts on the thermal characteristics of proteins was different from that of fatty *N*-acylamino acids. Fatty acid salts lowered thermal stability of proteins without significant changes in denaturation enthalpy (Egelandsdal et al., 1985; Ma et al., 1988).

CONCLUSION

The present data demonstrate that fatty *N*-acylamino acids can enhance the emulsification activity, whipping power, and gel formability of egg white and whey protein, two major food protein products for the food industry. The high nutritive value and antimicrobial activity of these compounds have been demonstrated previously. These suggest that the fatty *N*-acylamino acids may serve as multifunctional food additives, particularly in systems (e.g., comminuted meat products) in which both antimicrobials and functional agents (e.g., emulsifiers) are needed. It should be noted that these compounds have varying effects on different functional properties and on different proteins, and the selection should be based on specific functionality and protein source of the products.

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