Functional Properties of Acylated Flax Protein Isolates

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Flaxseed protein isolates were prepared by sodium hexametaphosphate complexation and acylated with acetic or succinic anhydride to improve their functional properties. The degree of acylation of free amino groups was lower when succinic anhydride was used in place of acetic anhydride. The color of the acylated proteins became lighter as the degree of acylation was increased. Emulsification properties of protein preparations were improved due to acylation, particularly for succinylated products. While foaming properties of flax protein isolates were not improved by acylation, their solubility was markedly improved. Low degrees of acetylation improved fat binding capacity of flax protein isolates, but succinylation did not exhibit such an effect. Acylation also increased aromatic or surface hydrophobicity of the products, and the highest value was observed at the lowest degree of acetylation. The *in-vitro* enzymic hydrolysis of the isolated proteins was reduced due to the acylation process.

Keywords: *Acylation; acetylation; succinylation; flaxseed; functional properties; protein isolate*

INTRODUCTION

Flaxseed has been traditionally cultivated for its linen and oil. The meal, after oil extraction, has been used as an animal feed ingredient and sometimes as a fertilizer. Nonetheless, flaxseed meal may serve as a potential source of plant proteins for use in food products as it contains a high protein content and a reasonably well-balanced amino acid composition. Canada is the world's largest producer of flaxseed; however, flaxseed has not been exploited commercially as a plant protein source, perhaps due to lack of scientific and technical information. Previous papers from our laboratory have described a simple method to extract flaxseed proteins without contaminating polysaccharides and to prepare protein isolates with sodium hexametaphosphate (Wanasundara and Shahidi, 1996; 1997).

Flaxseed contains 34−37% protein on a defatted, dried weight basis (Oomah and Mazza, 1993). Vassel and Nesbitt (1945) reported the isolation of the major flax protein and named it linin. Madhusudhan and Singh (1983) reported that globulins constituted 70−85% of flaxseed proteins, two-thirds of which had a molecular mass of 250 kDa; the remainder was low molecular mass in nature. Amino acid composition of flaxseed proteins is comparable to those of soy and canola and contains high levels of arginine, aspartic acid, glutamic acid, and leucine, but its tryptophan content is marginal. Thus, flaxseed meal may be considered as a potential source of high-quality plant protein for incorporation into food products.

The food industry considers desired functional properties of new sources of proteins to include solubility characteristics, water and fat binding properties, and foaming, emulsifying, and viscoelastic properties. Treatments that modify one or more amino acids and involve pH modification or chemical changes may confer desired functional properties to protein products. Chemical changes of proteins may be carried out to change their functional properties, remove off-flavors or toxic or inhibitory substances, retard deteriorative reactions, and include other chemicals and nutrients (Feeney and Whitaker, 1985). Among these, acylation has been widely used to improve protein functionality of canola (Paulson and Tung, 1987), cottonseed (Child and Parks, 1976), oat (Ma and Wood, 1987), pea (Johnson and Brekke, 1983), and soybean (Franzen and Kinsella, 1976a). Acetylation and succinylation of plant proteins have been reported to increase protein solubility (Paulson and Tung, 1987, 1988a,b, 1989; Ma and Wood, 1987), improve emulsifying and foaming properties (Child and Parks, 1976), increase water holding and oil binding capacities (Ponnampalam *et al.*, 1990), and improve flavor quality (Franzen and Kinsella, 1976a,b) of plant protein products. According to Thompson and Cho (1984a) and Ponnampalam *et al.* (1990), succinylation enhances nitrogen extractability and emulsifying properties of canola flour. Protein isolates prepared from acylated canola flour contained low phytic acid, but the yield of protein recovery was low (Thompson and Cho, 1984b). Flaxseed meal exhibits a very good water absorptionn and emulsifying and foaming properties due to the chemical nature of its proteins and polysaccharides. Dev and Quensel (1986) have reported that functional properties of alkali-extracted flax proteins were comparable to those of soy protein isolates. Heat treatment improved water absorption but reduced fat absorption, nitrogen solubility, and foaming as well as emulsifying properties of the isolated flaxseed proteins (Madhusudhan and Singh, 1985). However, there are no reports of chemical modification of flaxseed proteins or of the functionality of such preparations. The objectives of the present study were to examine modification of sodium hexametaphosphate extracted flaxseed protein isolates using acetic and succinic anhydrides. The associated changes in functional properties of importance to the food industry were also examined.

MATERIALS AND METHODS

Low-mucilage flaxseed meal was prepared by soaking seeds in a 0.10 M sodium bicarbonate solution for 12 h (Wanasundara and Shahidi, 1997), followed by defatting with hexane. Sodium hexametaphosphate extraction was carried out under

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optimum conditions that were reported elsewhere (Wanasundara and Shahidi, 1996). A dry protein isolate was obtained after lyophilization of the neutralized protein extract. The flaxseed protein isolate so obtained contained 79% protein, 12% lipids, and 9% ash.

Preparation of Acylated Derivatives of Flax Protein Isolate. Flax protein isolate was acylated at room temperature with three levels of acetic or succinic anhydrides (0.05, 0.10, and 0.20 g/g of protein equivalents) at pH 8.5 ± 0.1 according to the method described by Thompson and Reyes (1980) for canola proteins. After the acylation reaction was terminated, proteins were precipitated, dispersed in distilled water, neutralized, and then dialyzed against distilled water over a 24-h period at 4 ± 1 °C. Dialyzed protein solutions were lyophilized and stored at 4 ± 1 °C.

Determination of the Degree of Acylation. The degree of acetylation or succinylation of free amino groups was determined according to the ninhydrin reaction (Moore and Stein, 1948). The free amino groups of unmodified and modified isolates were determined as lysine equivalents (micrograms per gram of protein). The ratio of the difference in the content of amino groups in the unmodified and modified isolates to that of the unmodified counterpart was calculated as percentage of amino groups of unmodified isolate and expressed as the degree of acylation as given below:

$$\text{degree of acylation, \%} = [(\text{no. of amino groups}_{\text{unmodified protein}} - \text{no. of amino groups}_{\text{modified protein}})/\text{no. of amino groups}_{\text{unmodified protein}}] \times 100 \quad (1)$$

Evaluation of Functional Properties of Modified and Unmodified Flax Protein Isolates. *Color.* Lyophilized protein preparations were evenly packed into glass Petri dishes (60-mm diameter, 15-mm height). The Hunter *L* (110, white; 0, black), *a* (+, red; −, green), and *b* (+, yellow; −, blue) values of samples were determined by surface reflectance measurement using a Colormet colorimeter (Instrumar Engineering Limited, St. John's, NF). The unit was standardized with a B-143 white calibration tile with Hunter values of *L*, 94.5 ± 0.2; *a*, −1.0 ± 0.1; and *b*, 0.0 ± 0.2.

Emulsifying Activity (EA). EA of protein isolates was determined in the pH range of 3.1−11.3 and NaCl concentrations of 0, 0.35, and 0.70 M according to the modified method of Pearce and Kinsella (1978) as described by Paulson and Tung (1988a) for succinylated canola protein isolates. EA of the modified and unmodified flax protein isolates was measured as absorbance of prepared emulsions at 500 nm.

Emulsion Stability (ES). For determination of ES, samples were prepared in a similar manner to those used for determination of EA. The absorbance at 500 nm was read as soon as the emulsion was formed, i.e. zero time. Subsequently, aliquots were removed at appropriate time intervals and absorbance values were read at 500 nm. ES was determined as the time, in minutes, required for the absorbance at 500 nm to reach half of that for the emulsion at zero time (i.e., half-life; Paulson and Tung, 1988a).

Fat Binding Capacity. Fat binding capacity of the unmodified and modified protein isolates was determined by employing the procedure described by Voutsinas and Nakai (1983).

Solubility. The effect of pH and salt concentration on solubility of modified and unmodified protein isolates was studied by preparing a 1% (w/v) protein dispersion. These were prepared by mixing 0.25 g of the isolate with 0, 2.0, or 4.0 mL of a 25.6% (w/v) NaCl solution to reach a final NaCl concentration of 0.0, 0.35, or 0.70 M. The pH (3.0−11.0) was then adjusted with 2 M NaOH or HCl followed by addition of distilled water to obtain a 25-mL volume solution. The protein dispersion was centrifuged at 20000*g* for 15 min. The protein content of the dispersion, before and after centrifugation (supernatant), was determined according to Lowry's method (Lowry *et al.,* 1951); bovine serum albumin was used as standard. The solubility index, expressed as percentage solubility, was determined as percent ratio of the protein content of the supernatant to that of the suspension.
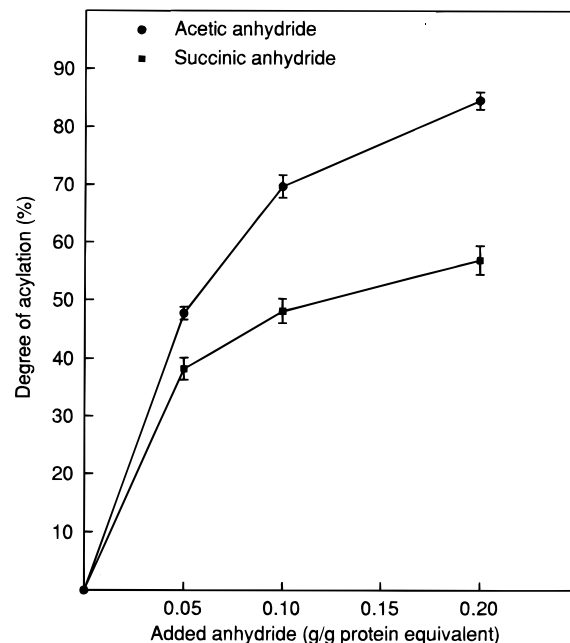

Figure 1. Degree of acylation of flaxseed protein isolates.

Table 1. Hunter *L*, *a*, *b* Color Values of Modified and Unmodified Flax Protein Isolates[a]

treatment	L	a	b
unmodified	68.8 ± 0.8[a]	1.0 ± 0.1	26.5 ± 0.9[c]
modified			
AA (g/g of			
protein equivalents)			
0.05	69.2 ± 1.0[a]	1.7 ± 0.1	23.6 ± 0.8[ab]
0.10	70.0 ± 2.2[ab]	1.2 ± 0.2	23.6 ± 0.9[ab]
0.20	71.8 ± 1.0[b]	0.9 ± 0.1	22.3 ± 0.9[ab]
SA (g/g of			
protein equivalents)			
0.05	72.0 ± 0.9[b]	0.5 ± 0.1	23.9 ± 0.8[ab]
0.10	72.9 ± 1.2[bc]	0.4 ± 0.1	22.6 ± 1.1[a]
0.20	74.2 ± 0.8[c]	1.0 ± 0.2	21.3 ± 0.8[a]

[a] The Colorimet unit was calibrated using a white tile with Hunter values of *L* = 94.5 ± 0.2, *a* = −1.0 ± 0.1, and *b* = 0.0 ± 0.2. Results are mean values of duplicate determinations of three samples ± standard deviation. Means followed by different superscripts within a column are significantly (*P* < 0.05) different from one another.

Whippability and Foam Stability. One hundred milliliters of a dispersion of protein isolates (1%, w/v) in distilled water was homogenized for 60 s using a Polytron homogenizer at 10 000 rpm and then transferred immediately into a 250-mL measuring cylinder, and the volume of the foam was recorded. The percentage ratio of the volume increase to that of the original volume of the protein solution was calculated and expressed as foam capacity or whippability (Naczk *et al.,* 1985). Foam stability was expressed (on the basis of 100 mL of a 3%, w/v, dispersion) as the volume of the foam remaining after 0.5, 5, 10, 15, and 20 min of quiescent period.

***In-Vitro* Digestibility.** *In-vitro* digestibility of protein isolates was determined using trypsin−pepsin or pepsin−pancreatin as described by Saunders *et al.* (1973) with the following modification. Nitrogen content released by enzyme was directly measured as the Kjeldhal nitrogen content of the supernatant. *In-vitro* digestibility was expressed as percentage enzymic digestion as given below:

$$\text{enzymic digestion, \%} = [\text{N (non-protein N) released by enzyme/total N content of undigested sample}] \times 100 \quad (2)$$

Surface (Aromatic) Hydrophobicity. Aromatic or surface hydrophobicity of proteins was determined fluorometrically (Perkin Elmer LS-5) as 1-anilino-8-naphthalenesulfonate

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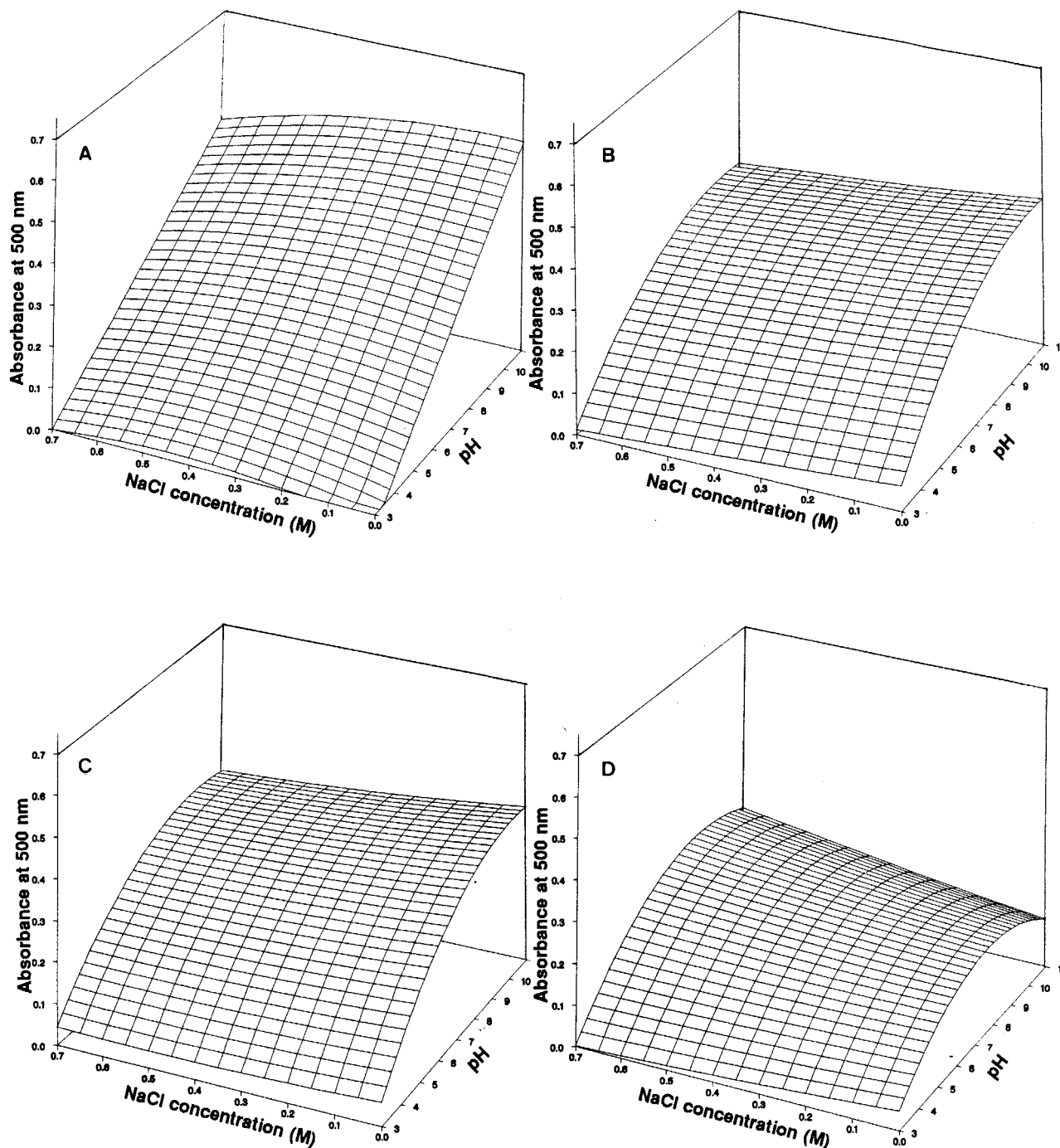
Figure 2. EA (measured as absorbance at 500 nm) of acetylated flax protein isolates as a function of pH and NaCl concentration: (A) unmodified; (B) 5% AA; (C) 10% AA; (D) 20% AA.

(ANS)−protein conjugate as described by Paulson and Tung (1987) for canola protein isolates.

Statistical Analyses. All experiments in this study were replicated at least three times. Mean values with standard deviations (SD) were reported when and where necessary. Analysis of variance (ANOVA) was performed, and differences in mean values were determined using Tukey's Studentized test at $P < 0.05$ and employing ANOVA and TUKEY procedures of statistical analysis system (SAS, 1990), respectively. Simple linear and multiple regression analyses were also performed using the same software in the general linear model (GLM) and response surface regression (RSREG) procedures, respectively.

RESULTS AND DISCUSSION

Figure 1 exhibits the degree of acylation of flaxseed protein isolate with increasing quantities of acetic or

succinic anhydride. The degree of acylation of flax protein isolate was determined as percentage loss of ϵ-amino groups of lysine residues. The extent of acetylation (47.4, 70.0, and 84.5%) was higher than that of succinylation (38.2, 45.5, and 56.9%) at the three concentrations of anhydride reacted, that is, 0.05, 0.10, and 0.20 g of anhydride/g of protein equivalents, respectively. Results of this study indicated that all free amino groups were not acylated. Incomplete acylation of reactive amino acid residues by an acetyl, succinyl, or any other acyl group is common for storage proteins of oilseeds (Child and Parks, 1976; Franzen and Kinsella, 1976a; Schwenke *et al.*, 1993; Ponnampalam *et al.*, 1990). Nitecka *et al.* (1986) have also reported that acetic anhydride (AA) is more effective than succinic anhydride (SA) in blocking ϵ-amino groups of rapeseed
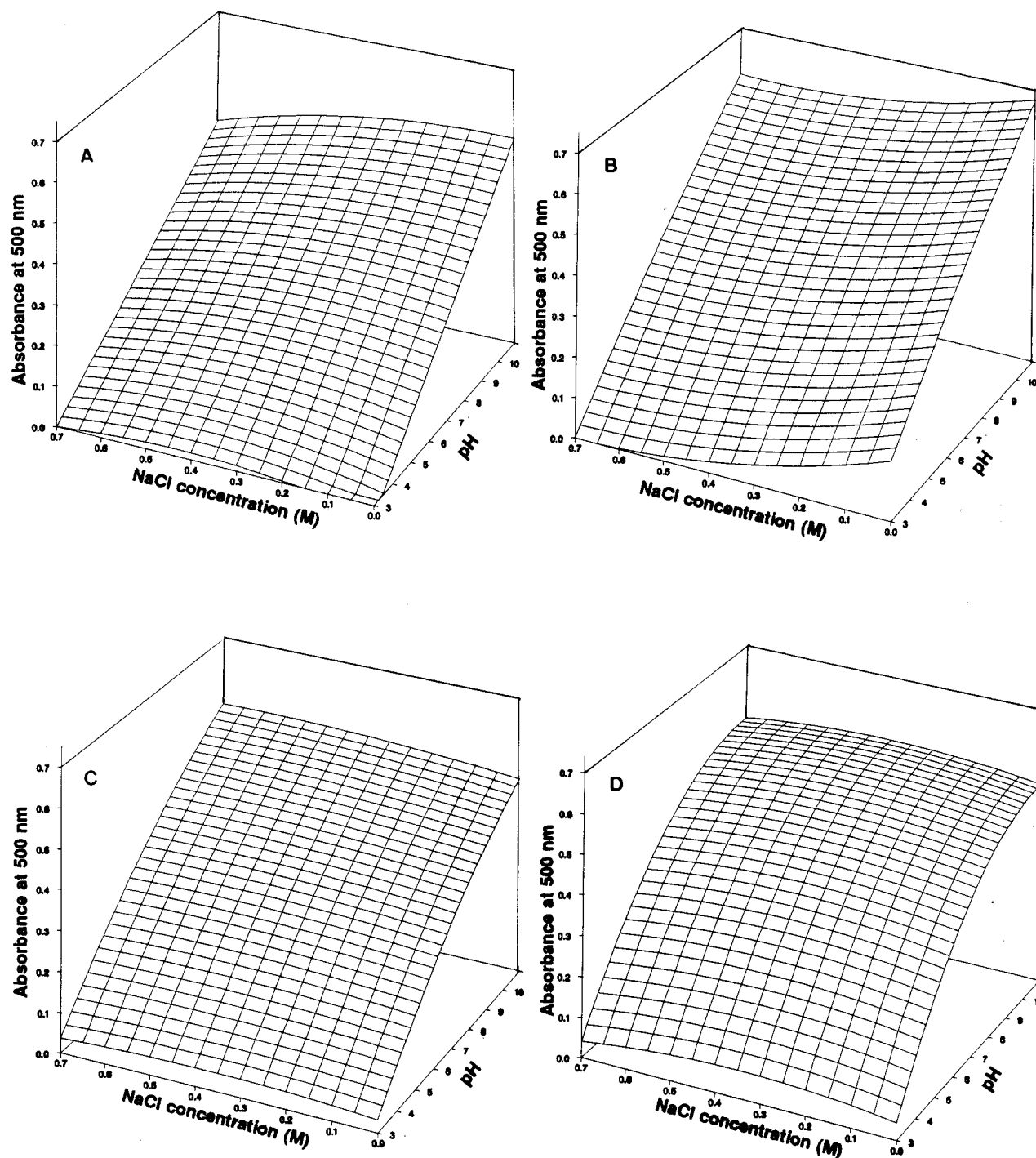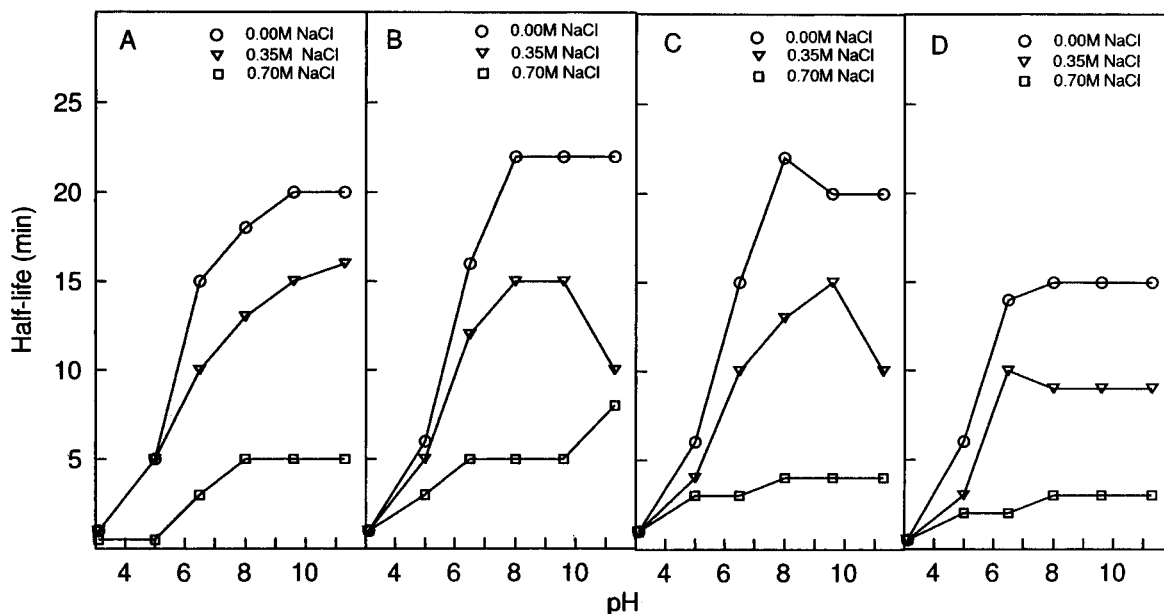
Figure 3. EA (measured as absorbance at 500 nm) of succinylated flax protein isolates as a function of pH and NaCl concentration: (A) unmodified; (B) 5% SA; (C) 10% SA; (D) 20% SA.

proteins. The acylated flax protein products are identified as the amount of acyl anhydride reacted, 5, 10, and 20% AA and 5, 10, and 20% SA for acetylated and succinylated products, respectively.

The chemical modification by acylation consists of blocking of the reactive amino groups of proteins by an acyl residue. Due to high nucleophilic character, low pK, and steric hindrance, the ϵ-amino group of lysine is most reactive. Therefore, the degree of protein modification is expressed as the percentage of blocked amino groups of lysine (Schwenke, 1990). Acetylation of cationic amino groups renders them electrically neutral, while succinylation introduces a negative charge in each residue.

Effect of Acylation on Color of Protein Isolates. The flax protein isolate prior to acylation had a light yellow color and a fluffy texture. Table 1 shows that Hunter L and b values changed with increased degree of acylation of the isolates. The increase in Hunter L value, up to 6 units (from 68.8 to 74.2), indicated a lighter color for products as compared to the unmodified isolate. The increase in Hunter L value at the highest degree of succinylation was larger than that of the highest degree of acetylation. The decrease in Hunter b values of acylated products indicates a less yellowish color product, which was also noticeable by the naked eye. Flax protein isolates with the highest degree of succinylation (20% SA) had the highest L and the lowest

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Figure 4. ES (time required to reach 50% absorbance at 500 nm) of acetylated flax protein isolates as a function of pH and NaCl concentration: (A) unmodified; (B) 5% AA; (C) 10% AA; (D) 20% AA.

b values and were almost white in color. However, *a* values of the protein products did not exhibit any specific pattern of change. No off-odors were detected in any of the products so obtained.

Effect of Acylation on Emulsifying Properties of Protein Isolates. Emulsifying activities (EA) of acetylated and succinylated flax protein isolates, measured as a function of pH and NaCl concentration, are presented in the form of response surfaces in Figures 2 and 3, respectively. The EA of unmodified flax protein isolate was increased as the pH and concentration of NaCl in the medium increased. The resultant response surfaces for acetylated proteins were different from response surfaces for their succinylated counterparts, thus indicating that the effect of pH and NaCl concentration on EA was different for the acyl groups involved in modification of proteins. As a general trend, EA showed a decrease when degree of acetylation increased from 0.0 to 84.5% (Figure 2B–D). For a given degree of acetylation, EA decreased as NaCl concentration in the medium increased from 0.0 to 0.70 M (Figure 2B,D). As the pH of the medium changed to acidic or basic, EA of the acetylated proteins was lowered when compared to EA values at the neutral pH.

Emulsifying activities of acetylated protein isolates were different from their succinylated counterparts; succinylation showed an increasing influence on the EA of protein isolates (Figure 3). As the degree of succinylation increased, a higher EA was observed, in contrast to the acetylated flax protein isolates. An increase in the pH of the medium resulted in an increase in the EA values, but an increase in the NaCl concentration gave lower EA values for succinylated flax protein products (Figure 3B). Response surfaces obtained for succinylated products showed that as the degree of succinylation increased, the EA improved at low pH values (Figure 3B–D).

ES of the prepared products is given in Figures 4 and 5 as half-life of the emulsion (time required to obtain a 50% absorbance at 500 nm compared to time zero) for acetylated and succinylated proteins, respectively. ES increased with the degree of acetylation (Figure 4B,C) and succinylation (Figure 5B–D), except at the highest degree of acetylation (Figure 4D). As the pH of the

medium increased, ES was improved; however, increased salt concentration in the medium exerted an opposite effect on the acylated products.

Effect of Acylation on Fat Binding Capacity (FBC) of Protein Isolates. The FBC of flax protein products is presented in Table 2. The unmodified flax protein isolate was able to bind 93.0 ± 1.7 mL of corn oil/100 g of material. Acylation changed the FBC of the protein isolate. The highest FBC was observed at the lowest degree of acetylation (5% AA), and the lowest value was obtained at the highest degree of succinylation (20% SA). Succinylated protein products (at all degrees of succinylation) had lower FBCs than their acetylated counterparts.

Effect of Acylation on Foaming Properties of Protein Isolates. Foaming capacity and foam stability of flax protein isolates are presented in Table 3. The highest foaming capacity was observed for the unmodified protein isolate and the isolates with the lowest degree of modification. As the degree of acetylation or succinylation increased, the foaming capacity decreased and isolates with the highest degree of acylation had the lowest foaming capacity. The foams of acylated protein isolates were less stable than those of the unmodified isolate (Table 3). The foam stabilities of the modified protein isolates were related to the pattern of foaming capacity.

Effect of Acylation on the Solubility of Flax Protein Isolates. Figures 6 and 7 represent response surfaces of unmodified, acetylated, and succinylated protein isolates due to solubility changes with pH and NaCl concentration, respectively. Both acetylation (Figure 6B–D) and succinylation (Figure 7B–D) increased the solubility of isolated proteins as compared to their unmodified counterpart. A decreased solubility was observed at acidic (low) pH values for all protein isolates and their modified products. Increased concentration of NaCl had a negative effect on the solubility of modified protein products, which was completely clear at alkaline (high) pH values. Compared to acetylated or unmodified proteins, succinylated products exhibited marked solubility increases at pH values between the isoelectric pH of flax protein (3.5 ± 0.1) and pH 7.0, similar to that observed for other plant proteins such

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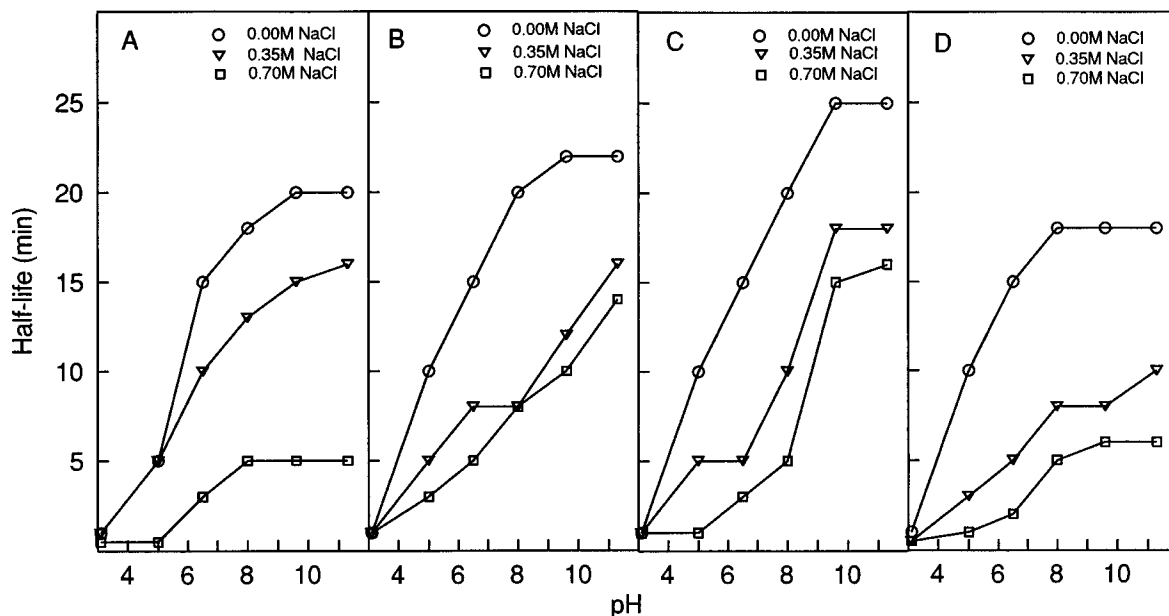
Figure 5. ES (time required to reach 50% absorbance at 500 nm) of succinylated flax protein isolates as a function of pH and NaCl concentration: (A) unmodified; (B) 5% SA; (C) 10% SA; (D) 20% SA.

Table 2. Fat Binding Capacity of Acylated Flax Protein Isolates[a]

treatment	FBC (mL/100 g)
unmodified	93.0 ± 1.7^d
modified	
AA (g/g of protein equivalents)	
0.05	105.0 ± 2.1^f
0.10	98.7 ± 1.6^e
0.20	94.7 ± 1.3^d
SA (g/g of protein equivalents)	
0.05	82.5 ± 1.9^c
0.10	72.6 ± 1.4^b
0.20	63.9 ± 1.5^a

[a] Results are mean values of duplicate determinations of three samples $\pm$ standard deviation. Means followed by different superscripts within a column are significantly ($P < 0.05$) different from one another.

Table 3. Foaming Properties of Modified and Unmodified Flax Protein Isolates[a]

treatment	foam expansion[b] (%)	foam stability[c] (%)
unmodified	112.0 ± 5.0^d	51.0 ± 3.0^e
modified		
AA (g/g of protein equivalents)		
0.05	97.0 ± 2.5^d	36.1 ± 2.0^c
0.10	92.5 ± 1.0^c	28.5 ± 1.0^b
0.20	74.5 ± 1.0^b	14.6 ± 1.0^a
SA (g/g of protein equivalents)		
0.05	98.2 ± 2.0^d	44.3 ± 1.0^d
0.10	88.1 ± 3.4^c	40.3 ± 1.4^c
0.20	66.9 ± 2.0^a	16.0 ± 2.0^a

[a] Results are mean values of duplicate determinations of three samples $\pm$ standard deviation. Means followed by different superscripts within a column are significantly ($P < 0.05$) different from one another. [b] At pH 7.0, percentage volume increase after whipping 50 mL of 1% (w/v) protein solution. [c] Percentage foam remaining after 15 min as a percentage of original foam volume.

as pea (Johnson and Brekke, 1983), canola (Paulson and Tung, 1987), and soybean (Franzen and Kinsella, 1976a). The general trend of protein solubility of modified flax protein isolates was similar to those of other acylated plant protein isolates (Shukla, 1982).

Surface (Aromatic) Hydrophobicity. Surface hydrophobicity of flax protein isolates, measured as fluo-

rescence intensity per milligram of protein, is presented in Figure 8. Acetylation brought about an increase in surface hydrophobicity of flax protein isolates. Thus, as the degree of acetylation increased, hydrophobicity values also increased. Exposure of the hydrophobic interior and modification of positively charged lysine residues with uncharged acetyl groups resulted in a decrease in net charge of proteins and hence increased surface hydrophobicity (Kim and Rhee, 1989). Unfolding of protein molecules may also make hydrophobic sites accessible for binding the ANS fluorescence probe. Schwenke *et al.* (1993) have reported increased surface hydrophobicity of pea proteins at low and moderate levels of succinylation. According to these authors a decrease in surface hydrophobicity was observed after the critical level (70%) of N-succinylation was passed, perhaps due to the effect of high negative charge density that inhibits the ANS molecule from approaching and binding to the protein surface.

***In-Vitro* Digestibility.** *In-vitro* digestibility values of flax protein products with pepsin–trypsin and pepsin–pancreatin are summarized in Figure 9. Both acetylation and succinylation reduced the digestibility of proteins. At the highest degree of acetylation or succinylation, a significant ($P < 0.05$) decrease in pepsin–pancreatin and pepsin–trypsin digestibility was observed from 90 to 78 and 80%, respectively. Pepsin–pancreatin and pepsin–trypsin digestion simulates gastrointestinal digestion of food proteins (Saunders *et al.,* 1973).

Ponnampalam *et al.* (1987) have reported that acetylation decreased tryptic hydrolysis but pepsin hydrolyzed acylated canola proteins more effectively. *In-vitro* enzymic digestion of pea protein isolates was not impaired even when up to 95% of ϵ-amino groups were acylated (Johnson and Brekke, 1983). However, reduced availability of lysine is expected to be due to the susceptibility of ϵ-amino groups of proteins to chemical modification. This may be regarded as nutritionally unfavorable but may be advantageous to certain processing conditions by preventing the formation of Maillard reaction products.

Effect of Acylation on Overall Functional Properties of Flax Protein Isolates. Structural changes

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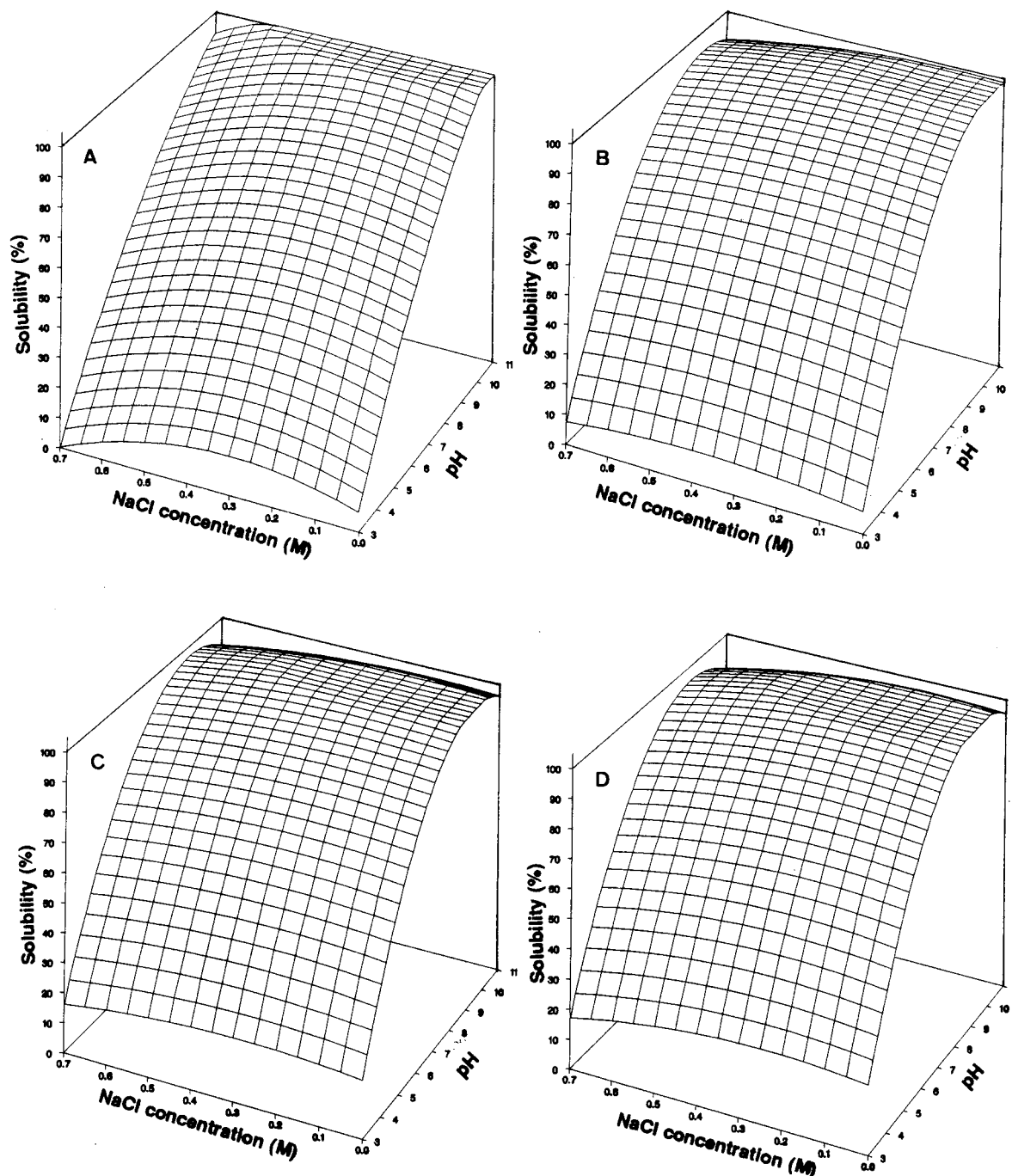*J. Agric. Food Chem.,* Vol. 45, No. 7, 1997 **2437**
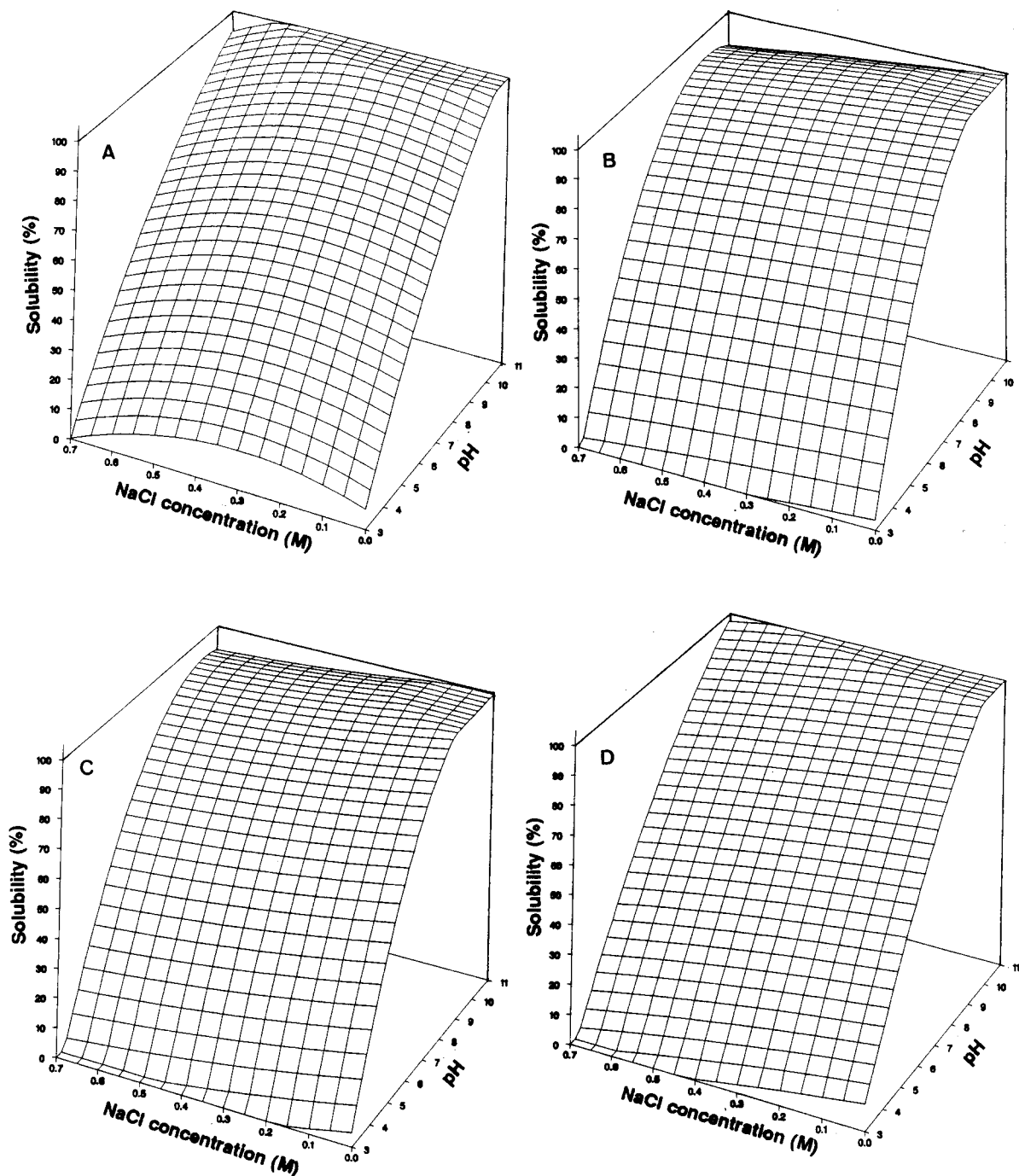
Figure 6. Solubility of acetylated flaxseed protein isolate as a function of pH and NaCl concentration: (A) unmodified; (B) 5% AA; (C) 10% AA; (D) 20% AA.

of proteins such as those in molecular mass (dissociation), shape (unfolding), and charge due to acetylation or succinylation have been used to explain variations in functional properties of chemically modified storage proteins of seeds. These alterations change hydration properties and surface activities of proteins and affect their solubility and surface activity-related properties (emulsifying and foaming) when compared to the native proteins.

According to Habeeb *et al.* (1958) replacement of short-range attractive forces (ammonium−carboxyl) with short-range repulsive forces (succinate carboxyl−carboxyl) due to succinylation may alter the molecular conformation. The resultant negative charge, in combination with the electrostatic repulsion due to the introduction of succinate anion, causes looser texture, higher bulk density, and lighter color of the succinylated

proteins as compared with their unmodified counterparts. Light colored products have been obtained from succinylation of fish muscle (Groninger, 1973), alfa-alfa leaf (Franzen and Kinsella, 1976b), and soybean (Melnychym and Stapley, 1973) proteins. The exact mechanism by which this change is brought about remains unknown. According to Franzen and Kinsella (1976a,b) inclusion of water molecules may be facilitated due to the repulsion of adjacent polypeptide molecules, thus increasing the solubility of succinylated proteins.

Studies of Gueguen *et al.* (1990) on rapeseed 12S globulin suggest that succinylation induces a stepwise dissociation and leads to unfolding of its subunits. The combination of intra- and intermolecular charge repulsion also promotes protein unfolding and induces fewer protein−protein and more protein−water interactions. The unfolding of protein molecules, and their dissocia-

Figure 7. Solubility of succinylated flaxseed protein isolate as a function of pH and NaCl concentration: (A) unmodified; (B) 5% SA; (C) 10% SA; (D) 20% SA.

tion to subunits, shifts the isoelectric point of proteins to lower values; thus acylated proteins were more soluble in the acidic pH range as compared to their unmodified counterparts. As the net negative charge is proportional to the extent of succinylation, solubility of flax protein isolates would increase as the number of succinylated groups increases (Figure 7). Solubility is a very important property of functional proteins as it is a critical prerequisite for using proteins in beverages and fluid foods and for emulsion and foam formation. In fact, solubility reflects the balance of charge and hydrophobicity of protein molecules (Hayakawa and Nakai, 1985), which affects their interaction with the solvent and other protein molecules (Nakai *et al.,* 1991). The presence of NaCl in the medium results in a change of the protein solubility in both the acidic and alkaline pH regions, due to the salting-in and salting-out effects,

which also indirectly affects other functional properties such as emulsifying ability of the protein.

Acetylation reduces the extent of electrostatic attraction between neighboring cationic amino and anionic carboxyl groups due to substitution of amino groups with neutral acetyl groups. As a result, acetylated flax proteins behave differently from succinylated products and exhibit lower solubility when compared to their succinylated counterparts.

Succinylation has been reported to improve the emulsifying properties of oilseed proteins (Child and Parks, 1976; Franzen and Kinsella, 1976a,b; Thompson and Cho, 1984a; Paulson and Tung, 1988a). As a reflection of increased solubility and looser structure of succinylated proteins, diffusion/migration of protein molecules to the oil/water interface and rearrangement within the interfacial film is facilitated (Waniska and

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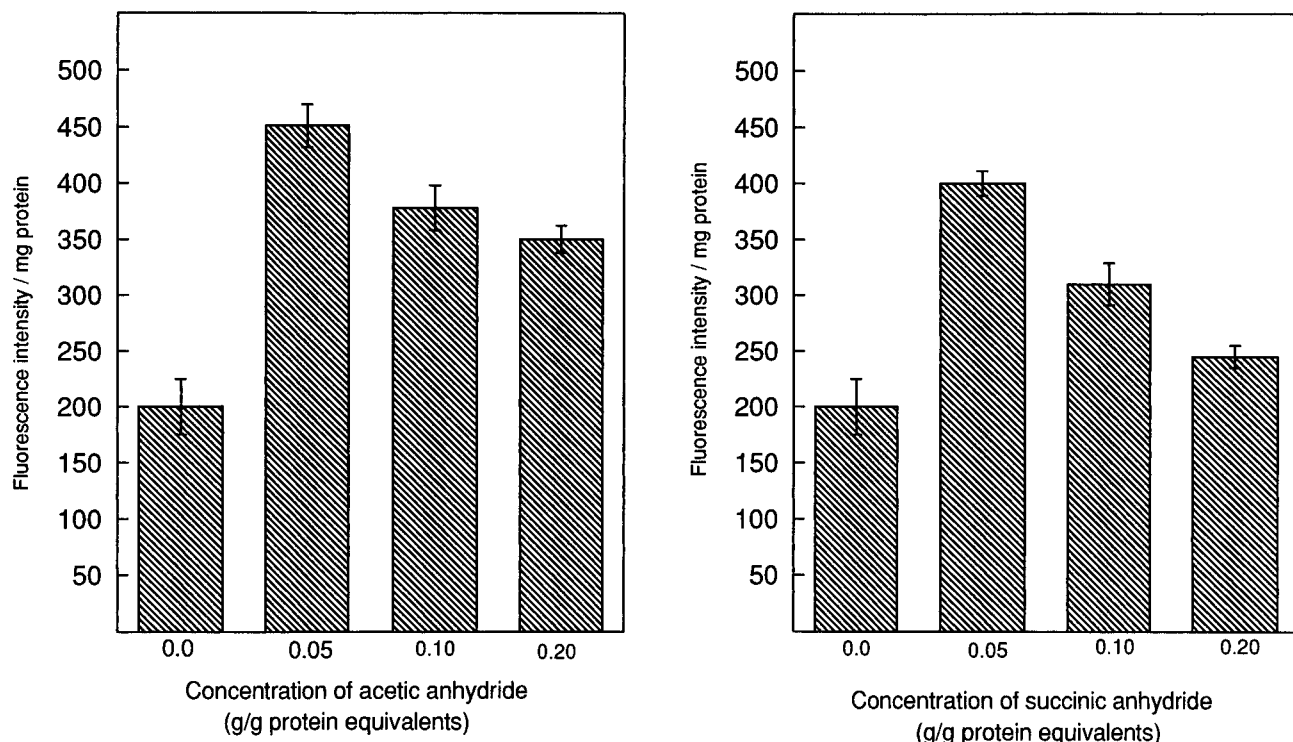
Figure 8. Surface (aromatic) hydrophobicity of modified and unmodified protein isolates.
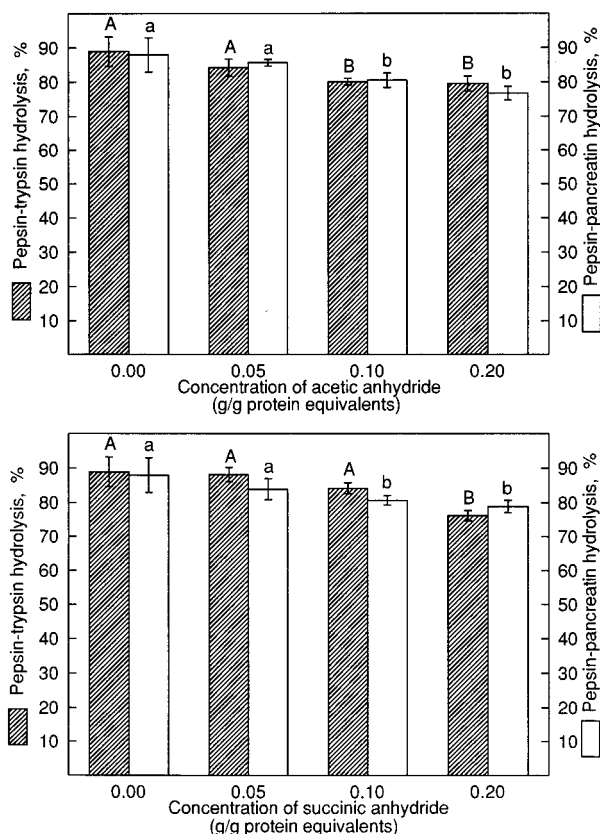
Figure 9. *In-vitro* digestibility of modified and unmodified protein isolates.

Kinsella, 1979). Therefore, good solubility of a protein is essential for the formation of emulsions (Nakai and Li-Chan, 1988). Pearce and Kinsella (1978) reported that succinylation of yeast proteins affords smaller droplets in oil-in-water emulsions when compared to unmodified proteins; the decrease in droplet size increases interfacial area and hence increases the emulsifying ability of proteins. Watanabe and Arai (1982)

have also reported that increased surface area increases surfactant properties of proteins. As proteins become more soluble, they form layers around the fat globule and facilitate association with the aqueous phase which encloses the fat globule and renders the emulsion more stable and resistant to coalescence (Halling, 1981). A positive relationship existed between solubility and ability of succinylated plant proteins to emulsify oil (Franzen and Kinsella, 1976a,b; Thompson and Cho, 1984a; Ponnampalam *et al.,* 1990). However, emulsifying properties of acylated proteins do not depend solely on solubility. The hydrophilic–lipophilic balance (HLB) of a particular protein is also important but does not necessarily increase linearly with the increase in protein hydrophobicity (Nakai, 1983). The unfolding of the protein structure due to succinylation may expose more hydrophobic groups buried in the molecule and could change hydrophobicity and hence the HLB value. Halling (1981) has suggested that increased rheological strength of protein films could reduce mechanical deformation and desorption of the interfacial proteins to give more emulsified droplets. Therefore, emulsion formation may be favored by protein–protein (hydrophobic) interaction as well as rheological properties of the interfacial protein film that encapsulates the oil droplets.

The effect of NaCl on EA may be related to its effect on protein adsorption at the oil/water (o/w) interface. The presence of electrolyte may have a favorable or an unfavorable effect on ES depending on whether it diminishes charge repulsion or increases the electrical potential of the ionized layer of the interfacial film. At low ionic strength, high charge repulsion at the o/w interface decreases the amount of protein adsorbed at the interface and at intermediate ionic strength, neutralization of surface charges reduces electrostatic repulsion and facilitates and increases the rate of protein adsorption and protein–protein interaction. However, at high ionic strength, EA is expected to

decrease due to reduced rate of protein transfer to the o/w interface (Paulson and Tung, 1988a).

FBC of proteins is important as it enhances flavor retention and improves mouthfeel. The mechanism of fat absorption of a protein was suggested as physical entrapment of oil by protein particles (Wang and Kinsella, 1976). However, the method used in this study was proposed by Voutsinas and Nakai (1983) to minimize any physical entrapment effect and to estimate only the absorbed fat by the protein. The protein–lipid interactions are affected by protein conformation, protein–protein interaction, and the spatial arrangement of the lipid phase resulting from lipid–lipid interaction. Noncovalent interactions such as hydrophobic, electrostatic, and hydrogen bonding forces are also involved in protein–lipid interactions. The aromatic hydrophobicity indicates the exposed aromatic (hydrophobic) residues of the protein.

A good foam-forming protein should reduce the surface and interfacial tension of the liquid by forming a structural, continuous, and cohesive film around air bubbles (Kinsella, 1976). Increased negative charge of the protein could hinder protein–protein interactions due to changes in structure and hydrophobicity and may lead to low foaming ability of the modified protein products; however, solubility is directly related to foaming ability. Gueguen *et al.* (1990) have suggested that unfolding of the protein molecule due to succinylation may increase both the viscosity and interactions between polypeptide chains to overcome the repulsive effects of the negative charges. Therefore, better foam forming and stabilizing effects of highly succinylated proteins may be related to electrostatic repulsive forces between the air bubbles, as they have increased charge density due to adsorbed proteins. However, such a behavior was not observed for succinylated protein isolates.

Conclusions. Acetylation and succinylation improved color and solubility of flax protein isolates in the low pH range, indicating potential use of such products in acid foods. Improved emulsifying properties and FBC of protein isolates due to acetylation and succinylation might avail them for use in food products where such functions are required. Acylation had a negative effect on foaming properties and *in-vitro* digestibility of flax protein isolates.

ABBREVIATIONS USED

AA, acetic anhydride; SA, succinic anhydride; FBC, fat binding capacity; ANS, 1-anilino-8-naphthalene-sulfone; HLB, hydrophobic–lipophilic balance; EA, emulsifying activity; ES, emulsion stability.

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