

Figure 2. Amylograph curves of millet starches.

compared to all other starches. The final viscosity reading after 60 min at 35 °C was the lowest of all starches studied.

CONCLUSIONS

Proso and foxtail millet starches had higher water binding capacities and higher gelatinization temperatures than the wheat and rye starches. Swelling power values at 60 °C were higher for the wheat and rye starches than for the millet starches. With two exceptions, swelling power at 90 °C showed comparable values for wheat and millet starches, but lower values than observed for rye starch.

Millet starches were less soluble than wheat or rye starch at 60 °C. At 90 °C, solubilities of the millet starches were

lower than those of the wheat starch with one exception.

Amylograph viscosities of both proso and foxtail millet starches were higher than those of wheat starch at all reference points.

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Functional Properties of Succinylated and Acetylated Leaf Protein

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Succinylation of 84% of the ϵ -amino groups of lysine increased bulk density, improved flavor, and increased solubility of protein isolated from alfalfa leaves over tenfold. The succinylated leaf protein demonstrated a typical pH solubility curve except that it did not resolubilize below the isoelectric pH. The emulsifying activity was enhanced by 32% and the foaming capacity threefold. Succinylation had no effect on the viscosity of dilute solutions of leaf proteins nor on the amino acid composition. Acetylation of leaf protein improved solubility and foaming capacity to a much lesser extent.

The potential of leaves to provide significant amounts of protein for food use has been discussed by several authors (Pirie, 1970; Stahmann, 1968; Kinsella, 1970; Kohler et al., 1974). The amino acid compositions of several leaf protein preparations have been reviewed by Betschart and Kinsella (1974) and Wang and Kinsella (1976a,b). Furthermore, several researchers have demonstrated the effectiveness of properly prepared leaf protein as a protein supplement for protein deficient foods (Subba-Rau et al., 1972; Oke, 1971; Woodham, 1972; Kawatra et al., 1974). Unfortunately, varied and contradictory results concerning the composition and biological value of leaf proteins frequently emanate from the different methods used in their preparation, e.g., failure to remove saponins by washing the crude protein results in poor nutritional value.

Sufficient information has been accumulated so that it is now possible to prepare high quality protein concentrates from various leaves especially alfalfa (Pirie, 1971; Kohler et al., 1974; Betschart and Kinsella, 1974; Wang and Kinsella, 1976a,b).

However, for adoption by food manufacturers the isolated protein should display a range of critical functional properties, e.g., solubility, surface activity, gelation, etc. These will determine the successful application of novel proteins for the supplementation of foods and for the fabrication of new foods. Hence, research on novel food proteins should include evaluation of the functional properties of these proteins. The available information on functional properties of leaf proteins, e.g., solubility, gelation, foaming, and emulsifying properties, has been reported (Lu and Kinsella, 1972; Betschart and Kinsella, 1974; Wang and Kinsella, 1976a,b).

These studies indicated that methods used in preparation of leaf proteins cause denaturation which results in poor solubility, with subsequent deterioration in functionality. Hence, as with several other novel proteins, methods for improving functional properties are needed

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to successfully exploit this potentially copious source of food protein.

Several approaches have been used to improve the utility of denatured or nonfunctional proteins. These include hydrolysis by alkaline, acid, or enzymatic agents (Tannenbaum et al., 1970; Cheftel et al., 1971; Prendergast, 1974; Hermansson et al., 1974). Each of these methods is suitable for specific purposes but they possess inherent limitations. Thus, alkaline hydrolysis results in racemization of amino acids, the formation of potentially toxic compounds, lysinoalanine (DeGroot and Slump, 1969), and cross-linking of the proteins making them more resistant to digestion (Provencal et al., 1975). Acid hydrolysates are salty from the neutralization step and proteolysates are frequently bitter due to the formation of hydrophobic peptides (Arai et al., 1975). Furthermore, hydrolysates may lack certain functions, e.g., thickening power and gelling, foaming, or emulsifying abilities. Therefore, additional methods for imparting functionality to proteins are needed. A promising approach involves the acylation of specific functional groups into the protein and recently this has received some attention (Groninger and Miller, 1975; Chen et al., 1975; Melnychyn and Stapley, 1973).

In the present paper the effects of succinylation on some functional properties of protein preparations from alfalfa leaves were examined.

MATERIALS AND METHODS

Acylation of Protein. Succinylation was done by the procedure of Hoagland (1966). Alfalfa leaf protein concentrate (2 g), prepared by the method of Betschart and Kinsella (1973), was added to 0.075 M phosphate buffer (250 ml), and 0.5-g increments of succinic anhydride (Eastman Organic Chemicals, Rochester, N.Y.) were added to a total of 2 g with stirring. During the succinylation procedure the pH was maintained between 7 and 8 with NaOH (3.5 M). When acylation was complete the solution was dialyzed, using seamless cellulose dialyzer tubing with a pore diameter of 4.8 nm (Fisher Scientific Co., Pittsburgh, Pa.), against distilled water (4 °C for 24 h) to remove impurities and excess reagents. The chemically modified protein was recovered by lyophilization (Virtis Gardiner, New York, N.Y., freeze-drier). Soy isolate was treated similarly (Franzen and Kinsella, 1976) to use as a standard for comparison.

The method of Hoagland (1966) which was adopted from the method of Fraenkel-Conrat et al. (1949) was employed in the acetylation procedure. Saturated aqueous sodium acetate solution (120 ml) was prepared at 25 °C by dissolving sodium acetate (55 g) in distilled water (120 ml). Leaf protein concentrate (LPC) (2 g) was added to this solution and acetic anhydride was added in 0.2-ml increments to a total of 2.4 ml. After 1 h the solution was dialyzed against distilled water at 4 °C for 24 h, and the acetylated protein was recovered by lyophilization.

Determination of Extent of Chemical Modification. Succinylation occurs mostly on the ϵ -amino group of lysine residues. Dinitrophenyl (Dnp) derivatives of native and succinylated LPC were prepared according to the method of Wofsky and Singer (1963) to quantify the extent of chemical modification. When native LPC is treated with fluorodinitrobenzene, the ϵ -amino group of the lysine becomes acylated with the Dnp group, and this linkage is resistant to the acid hydrolysis procedure used in the analysis of amino acids. With succinylated LPC, however, the Dnp group attaches to the succinate moiety. Upon acid hydrolysis the succinate moiety is cleaved from the lysine ϵ -amino group, and this amino acid is then determined as lysine. Hence, the difference in peak areas of lysine be-

tween the Dnp derivatives of native and succinylated LPC was used as an index of the degree of acylation of the lysine residues.

Thus, protein (50 mg) in 2 ml of aqueous solution was denatured by addition of ethanol (4 ml). After 1 h NaHCO₃ (0.4 g) and fluoro-2,4-dinitrobenzene (0.2 ml, Eastman) were added. The mixture was shaken in a water bath (Evapo-mix, Buchler Instruments, Fort Lee, N.J.) at 25 °C for 2 h. The insoluble protein was recovered by centrifugation, and two washes were performed successively using alcohol and diethyl ether. The Dnp derivatives of native and succinylated LPC (approximately 25 mg) were then subjected to routine amino acid analysis for quantification of lysine.

Amino Acid Analysis. Samples of native and succinylated LPC were each placed in a 10-ml glass ampule to which was added concentrated HCl (5 ml), distilled water (4 ml), and norleucine solution (1 ml). Each sample was then frozen in an acetone/dry ice mixture followed by evacuation of oxygen from its headspace. The samples were sealed with an acetylene torch and incubated at 110 °C for 22 h. After acid hydrolysis each sample was quantitatively filtered through a Millipore filter, and 2-ml portions were evaporated to dryness under NaOH in a vacuum desiccator. The residue was dissolved in 4 ml of sodium citrate buffer (pH 2.2). An aliquot (1 ml) of the solution was analyzed on a Beckman Model 120C amino acid analyzer.

pH-Solubility Profile. An aqueous solution (0.3 g in 30 ml of water) of LPC was prepared, adjusted to approximately pH 12 with 3.5 M NaOH, and centrifuged for 15 min at 27300g (Sorvall Superspeed RC2-B Centrifuge, Norwalk, Conn.). The supernatant was considered to have 100% nitrogen solubility. Aliquots (3 ml) were placed in centrifuge tubes (10 ml size) and the pH values were adjusted to those covering the range 1.5–12.0 using 1 M HCl, 1 M NaOH, and 0.1 M NaOH. The samples were shaken for 30 min at 25 °C in a water bath and then centrifuged for 15 min at 20200g. Aliquots (2 ml) of the supernatant from each pH sample were withdrawn and analyzed for protein by the micro-Kjeldahl method.

Emulsifying Activity and Emulsifying Stability. The method of Yasumatsu et al. (1972) was used for determining emulsifying activity and the heat stability of emulsions. Protein (0.7 g) was suspended in water (10 ml), and corn oil (10 ml) (Mazola, CPC International, Englewood Cliffs, N.J.) was added. This mixture was homogenized in an Eberbach semi-micro blender container at high speed (20000 rpm) for 1 min. The emulsion was divided evenly into two 10-ml centrifuge tubes and centrifuged at 1300g for 5 min. The emulsifying activity was calculated as (height of emulsified layer (mm)/height of whole layer in centrifuge tube) \times 100. The heat stability of these emulsions was measured by recentrifugation following heating at 80 °C for 30 min.

Foam Expansion and Foam Stability. Protein solutions (0.3 g in 30 ml of distilled water) were prepared in 100-ml graduated cylinders. Stoppers were inserted, and they were placed horizontally in a mechanical shaking device described by Wang and Kinsella (1976b). Following agitation for 1 min at 25 °C the cylinders were removed from the shaker, and a filter paper disk, having a diameter equal to that of the internal diameter of the cylinder, was gently pushed onto the top of the foam layer to catch any residual foam remaining on the sides and to eliminate large air pockets. The foams resisted gentle compression by this disk at a point which was consistent among replicate samples of the same protein foam. The height of the foam

was recorded, labeled as foam expansion, and taken as an index of the foaming power of the protein. The height of the foam was again recorded after 30 min of undisturbed standing at room temperature, and the value was labeled as foam stability.

Egg albumin (Nutritional Biochemicals Corp., Cleveland, Ohio) was used as a standard for comparison.

Viscosity and Heat Stability. The effects of several parameters influencing the viscosity of native and succinylated LPC, i.e., protein concentration, pH, temperature, and salts, were determined. All viscosity measurements were made using a Brookfield Viscometer Model LVT at 60 rpm fitted with a no. 4 spindle.

The viscosities of 1 and 2% aqueous protein solutions adjusted to pH 7 were measured to investigate the effects of protein concentration on viscosity. First, the no. 4 spindle was properly immersed in the protein solution and allowed to rotate for 30 s with the viscometer brake on. Viscosity readings were then made after rotation of the spindle for 5 s. After eight readings were taken in this manner for each protein sample, the spindle was removed from the solution and rinsed with distilled water. This procedure was repeated, and the average value of the 16 readings was calculated for each sample.

To obtain an estimation of the effects of heat on the viscosity of native and succinylated LPC, the samples were placed in an agitating water bath at 80 °C for 30 min. The tubes were cooled to 4 °C with ice and then allowed to warm up to 25 °C. The viscosity of each sample was then determined by the procedure described above.

Because of the replacement of positive charges by negative charges upon acylation it was surmised that succinylated LPC might form electrostatic bonds with calcium or other cations and possibly form gels. Therefore, the effects of calcium on the viscosity of dispersions of native and succinylated LPC were noted by adding calcium chloride dihydrate ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) at 0.5% levels to 1% protein solutions at pH 7 and 10. The agitated mixture of protein and water was adjusted to the proper pH. The $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ was then added followed by adjustment of the pH since it was slightly depressed by this salt. This order was used since earlier experiments with 10% levels of protein and 2% levels of CaCl_2 at pH 12 indicated that gelation only occurred if the salt was added after adjustment to the higher pH. After the viscosity measurements were recorded, the heat stability of each sample was determined.

The effects of cation size were compared by adding 0.2% $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ or KCl to 1% aqueous solutions of succinylated LPC at pH 10. As in earlier experiments, the pH was adjusted to 10 before addition of the salt. Heat stability was also observed.

RESULTS

Treatment of leaf protein concentrate with succinic anhydride acylated 84% of the lysine ϵ -amino groups. Succinylation greatly increased the bulk density (i.e., volume/unit weight) of leaf protein concentrate and lightened its color. LPC has a green color and gritty texture; however, succinylated LPC had a lighter green color and exceedingly fluffy texture. No odors nor flavors were imparted by the succinylation process. Acetylation lightened the color and increased the bulk density of LPC but to a much lesser extent than that produced by succinylation.

The native leaf protein concentrate was insoluble; however, upon succinylation LPC hydrated instantaneously and remained dispersed in solution. Acetylated LPC was more water soluble than LPC and thus remained more

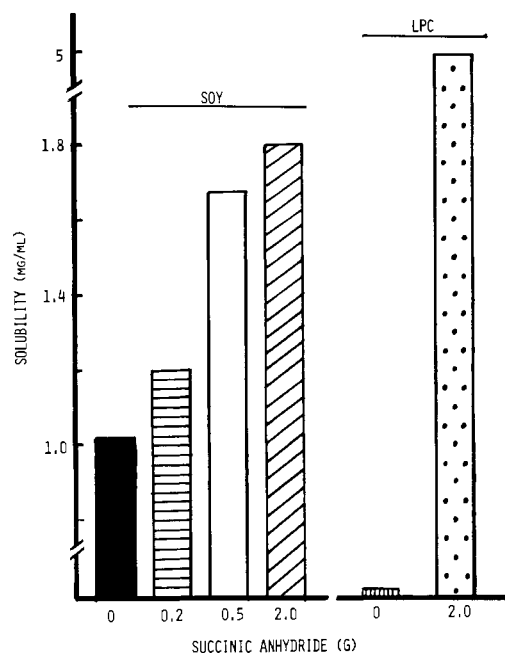


Figure 1. The effects of succinylation on the solubility of soy isolate protein and leaf protein.

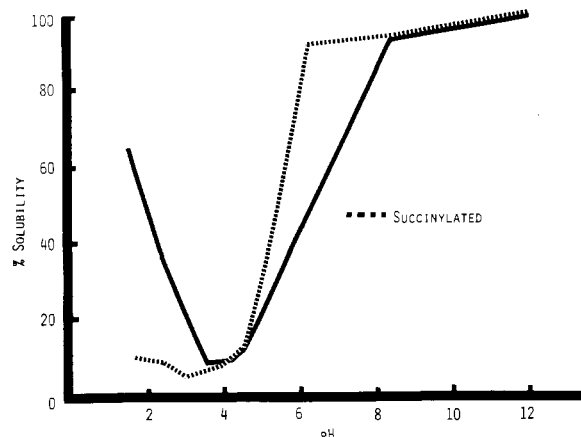


Figure 2. The solubility of unmodified and succinylated leaf protein concentrate at various pH values.

evenly suspended in solution.

Succinylation enhanced the solubility of LPC over tenfold (Figure 1). The improvement in solubility of the leaf protein which was insoluble was much more dramatic than that shown by soy protein which was reasonably soluble initially. The solubility of acetylated LPC was intermediate in magnitude between that of LPC and succinylated LPC.

The native form of LPC exhibited the typical pH-solubility profile, i.e., decreasing solubility with decreasing pH, minimum solubility at the isoelectric point (IEP), and resolubilization at pH values acidic to the IEP (Figure 2). Upon succinylation (i.e., 2 g of succinic anhydride/2 g of protein) the solubility at alkaline pH values was high and decreased to a minimum at the IEP. However, succinylated LPC did not resolubilize at pH values acidic to the IEP. Acetylated LPC exhibited a pH-solubility behavior similar to that of succinylated LPC.

The emulsifying activity and emulsifying stability of succinylated LPC were 79.5 and 72.4% compared with respective values of 60.3 and 53.1% for the unmodified LPC (Figure 3). Hence, the increases in the emulsifying activity and emulsifying stability were 31.8 and 36.3%.

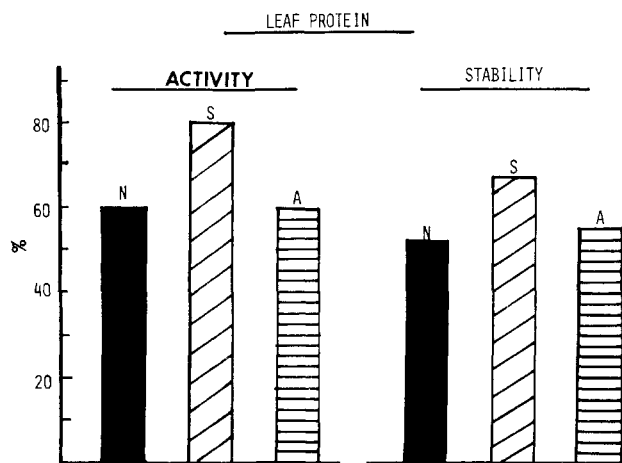


Figure 3. Emulsifying activity and emulsifying stability of unmodified (N), succinylated (S), and acetylated (A) leaf protein concentrate.

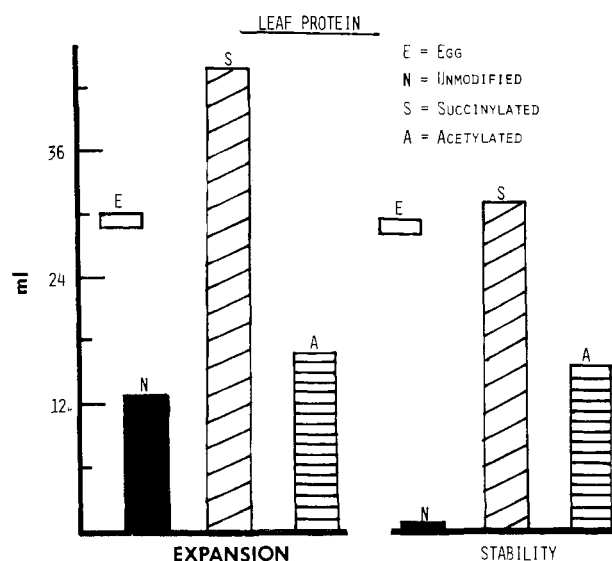


Figure 4. The foam expansion and foam stability of unmodified (N), succinylated (S), and acetylated (A) leaf protein concentrate compared to egg albumin (E) showing a foam expansion and foam stability of 30 ml.

The emulsifying activity and emulsifying stability of acetylated LPC were 58.0 and 56.3%, respectively.

Succinylation increased the foam expansion and the foam stability of LPC (Figure 4). The foam expansion and foam stability values of acetylated LPC were intermediate in magnitude between those of the native and succinylated forms, i.e., 15.8 and 12.9 ml, respectively.

The effects of pH, protein concentration, temperature, and the addition of salts, i.e., CaCl_2 and KCl , on the viscosities of LPC and succinylated leaf protein concentrate were investigated (Tables I and II). Succinylation, protein concentration, and heating did not significantly alter the viscosity of LPC at pH 7. No significant changes in viscosity were observed upon the addition of calcium to solutions of native and negatively charged succinylated LPC at both pH 7 and 10. Calcium cations have larger ionic radii than potassium cations, and it was initially thought that steric effects perhaps prevented viscosity increases by the calcium cations; however, no significant changes occurred in the viscosity of succinylated LPC upon addition of KCl at pH 10.

The amino acid compositions of native and succinylated LPC were similar. Hence, as expected, there was no

Table I. Effects of Protein Concentration and Temperature on the Viscosities of Native and Succinylated Leaf Protein at pH 7

| Protein | Concn, % | Viscosity ^a | |
|---------------------------------------|----------|------------------------|------------------------------|
| | | 25 °C | After heating, 80 °C, 30 min |
| Leaf protein concentrate | 1 | 0.719 | 0.700 |
| | 2 | 0.707 | 0.654 |
| Succinylated leaf protein concentrate | 1 | 0.700 | 0.628 |
| | 2 | 0.647 | 0.691 |

^a Brookfield viscosity, Model LVT no. 4 spindle.

Table II. Effects of Calcium Chloride and Potassium Chloride on the Viscosities of Aqueous Solutions of Native and Succinylated Leaf Protein Concentrate

| Protein | Viscosity ^a | | | |
|---------------------------------------|------------------------|------------------------------|-------|------------------------------|
| | pH 7 | | pH 10 | |
| | 25 °C | After heating, 80 °C, 30 min | 25 °C | After heating, 80 °C, 30 min |
| Leaf protein concentrate | 0.619 | 0.669 | 0.669 | 0.732 |
| + CaCl_2 (0.5%) | 0.629 | 0.675 | 0.722 | 0.669 |
| Succinylated leaf protein concentrate | 0.710 | 0.669 | 0.657 | 0.679 |
| + CaCl_2 (0.2%) | | | 0.703 | 0.663 |
| + CaCl_2 (0.5%) | 0.775 | 0.729 | 0.710 | 0.728 |
| + KCl (0.2%) | | | 0.594 | 0.635 |

^a Brookfield viscosity, Model LVT no. 4 spindle.

apparent destruction of amino acids during the succinylation process.

DISCUSSION

Acylation by succinate is possible on all nucleophilic groups of amino acid residues, e.g., ϵ -amino groups of lysine, hydroxyl groups of serine and threonine, the sulfhydryl group of cysteine, the phenol group of tyrosine, and the imidazole group of histidine (Gounaris and Perlmann, 1967). The lysine ϵ -amino group reacts more readily, however, because of its relatively low pK and steric availability for contact with the applied reagent. The ϵ -amino group of lysine is hydrophilic in nature and projects from the molecular surface into the aqueous media (Tanford, 1973).

Succinylation of amino acid residues, e.g., α - and ϵ -amino groups, has three major effects on the physical character of proteins: it increases net negative charge (Grant, 1973; Hoagland, 1966; Habeeb et al., 1958); changes conformation (Riordan and Vallee, 1964; Gounaris and Perlmann, 1967; Oppenheimer et al., 1967; Habeeb, 1967; Hoagland, 1966; Hass, 1964; Oppenheimer et al., 1966; Habeeb et al., 1958); and increases the propensity of proteins to dissociate into subunits (Grant, 1973; Frist et al., 1965; Polyanovski, 1965; Hass, 1964; Klotz and Keresztes-Nagy, 1963). The change in the physicochemical properties of leaf protein concentrate upon chemical modification resulted in an alteration of its appearance, solubility, and emulsifying and foaming abilities.

The effects of succinylation on the color and texture of proteins have been cited by other investigators. According to Melnychyn and Stapley (1973), succinylated soy protein had improved whiteness and dispersibility characteristics making it suitable for incorporation into coffee whiteners. Groninger (1973) described succinylated fish myofibrillar protein as being light and fluffy in appearance with a high

bulk density and characterized it as possessing moderately rapid rehydration and relatively good dispersion in the pH range of 6.0–8.5.

Electrostatic repulsion(s) resulting from the introduction of succinate anions alter the conformation of the protein and penetration by water molecules is physically easier because of the expanded loosened state of the polypeptides. The consequences of a high distribution of negative charges are important. According to Habeeb et al. (1958) the structural instability of succinylated proteins is the result of their high net negative charge and the replacement of short range attractive forces in the native molecule with short range repulsive ones with subsequent unfolding of polypeptide chains. This occurs if two negatively charged carboxyl groups are juxtaposed in the succinylated molecule where an ammonium and carboxyl group had been formerly apposed in the unmodified form. This probably accounts for the looser texture, higher bulk density, lighter color, and enhanced solubility of succinylated LPC.

Upon acetylation cationic ϵ -amino groups of lysine were substituted with neutral acetyl groups $-C(=O)CH_3$. There were fewer electrostatic attractions between neighboring cationic amino and anionic carboxyl groups than in native LPC; however, there were no electrostatic repulsions between juxtaposed carboxyl groups as in the succinylated form. Hence, acetylated LPC was not as soluble as succinylated LPC.

The limited solubility of LPC at acidic pH values probably reflected the removal of cationic ammonium groups from lysine by succinylation and acetylation. Conceivably, there were an insufficient number of hydrophilic cationic groups to exceed the aggregate forces resulting from hydrophobic bonds between the alkyl and aromatic groups of constituent amino acid residues. This effect was also demonstrated in the succinylated forms of β -casein (Hoagland, 1966), myosin (Oppenheimer et al., 1967), bread flour proteins (Grant, 1973), and fish protein concentrate (Chen et al., 1975).

Succinylation increased the emulsifying activity and emulsifying stability of leaf protein concentrate most likely because of the enhanced solubility of succinylated LPC. Acetylated LPC, although slightly more soluble than native LPC, was not as soluble as the succinylated form. The emulsifying activity of acetylated LPC was similar to that of the native form while its emulsifying stability was slightly greater than that of native LPC.

The positive correlation between solubility and the ability of a protein to emulsify is well documented (Crenwelge et al., 1974; Yasumatsu et al., 1972; Pearson et al., 1965; Swift and Sulzbacher, 1963). As the protein becomes more soluble, it forms layers around the fat droplet to facilitate association with the aqueous phase. Granular, insoluble proteins, however, separate from the oil phase or just float on the oil surface where they remain inert and contribute little toward emulsification. Similarly, soluble proteins enclose the fat globule and render the emulsion more stable to heat treatment.

Groninger (1973) reported a direct relationship between the emulsifying capacity of succinylated fish myofibrillar protein and the extent of amino acylation. Chen et al. (1975) observed that succinylated fish protein concentrate generally had a greater emulsifying capacity, emulsifying activity, and emulsifying stability than alkaline-extracted FPC.

As with emulsifying properties the ability of a protein to foam is also positively related to its solubility (Eldridge et al., 1963). Hence, succinylation of LPC substantially

enhanced its foam expansion and foam stability and produced foam volumes greater than those of egg albumin. The foam expansion and foam stability values of acetylated LPC were intermediate in magnitude between those of native and succinylated LPC.

Groninger and Miller (1975) observed that succinylated fish myofibrillar protein, partially hydrolyzed by bromelain treatment, had improved dispersibility, aeration capacity, and foam stability. Succinylated fish protein was successfully incorporated into products requiring the formation of stable foams, e.g., dessert toppings, soufflés, and chilled and frozen desserts.

The acylation of protein may reduce the availability of lysine and conceivably some serine and threonine. Some conflicting data have been reported in the literature though there is definitely loss of some nutritive value (Creamer et al., 1971; Groninger and Miller, 1975; Franzen and Kinsella, 1976).

Thus, succinylation may afford a practical approach for expanding the usefulness of novel proteins by improving their functional properties.

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Physical-Chemical Methods for the Recovery of Protein from Waste Effluent of Potato Chip Processing

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Simple physical-chemical methods for the recovery of protein from potato chip processing were evaluated. It was estimated that an average potato chip plant, processing 31 metric tons of potatoes per day, could daily recover approximately 170 kg of dried potato protein (550 kg of food containing 30% of protein). Sedimentation or centrifugation after heating, adjusting pH, or both yielded similar amounts of protein. Heat (80-90 °C) at pH 4-4.5 was most effective for protein recovery but total dry matter reduction was highest if no heat was applied. Protein yields were improved if the waste was kept in motion during floc formation. Recovery was similar when pH was raised to 11.5 and then lowered with either H₃PO₄ or FeCl₃ to pH 9. Approximately 30-40% of the crude protein or 80-90% of the coagulable protein, presently wasted, could easily be recovered.

The potato processing industry is one sector of the food industry where serious waste problems are caused by potentially valuable food materials. Chip manufacturers need simple economical methods to minimize losses and to meet local and federal standards for the effluent discharged (Federal Register, 1973).

Traditionally potato chip plants are located in urban areas where space for conventional treatment or agricultural use of the effluent is not available. Peel, potato fragments, and other particulate solids can be readily removed by screening or settling (Ballance, 1964). Recovery of dissolved and suspended solids in the waste effluent, which results from peeling and slicing, is still inadequate.

Most research on potato waste has been done on effluent from starch manufacturing where only starch is retained. However, Vlasblom and Peters (1958) have patented a process to recover protein. Xander and Hoover (1959) precipitated proteins from potato juice by heat coagulation and recovered amino acids and amides with a strongly basic anion exchanger. Using ion exchange, Heisler et al. (1972) investigated the recovery of amino acids, proteins, and potassium and Schwartz et al. (1972) studied the

recovery of organic acids and phosphate. However, Stabile et al. (1971) found that protein recovery followed by removal of other constituents using ion exchange is not economically feasible. Reverse osmosis treatment of waste was investigated by Porter et al. (1970), but great difficulties were encountered in a pilot plant study with potato chip effluents (Seifert, 1974). In the small potato chip plants simple methods for by-product recovery are required. Hydrocyclones for the recovery of starch are available (Pettay, 1975). The purpose of this study is to examine optimal conditions for recovery of proteins by simple means. Special emphasis is given to heat treatments, since waste heat generated by the cooker could be recovered in a heat exchanger.

MATERIALS AND METHODS

(I) Preparation of Protein Water. Preliminary work was initiated with processing water from a potato chip factory. Because of the inconvenience of transporting a dilute solution and possible compositional changes, it was decided to simulate processing water in the laboratory. Russet Burbank potatoes were washed thoroughly, peeled, and ground in a Waring Blendor. The slurry was diluted with tap water (composition, Table I) to 10 times its volume. This, after filtering the juice through several layers of cheesecloth followed by settling for 30 min, produced protein water of a composition similar to the

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