Functional and Electrophoretic Characteristics of Succinylated Peanut Flour Protein

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Defatted peanut flour protein was reacted with various amounts of succinic anhydride at pH 7.4–8.0. The functional and electrophoretic properties of treated flour were examined. Modifications as compared to untreated flour were as follows: electrophoretic mobility of major protein components in polyacrylamide gels was retarded and dissociation into subunits was observed; nitrogen solubility decreased at pH <4 and increased at pH 6–7; water-retaining capacity increased; water-adsorption capacity increased at equilibrium relative humidities of 68–97%, 21 °C; emulsion capacity generally increased; apparent viscosity increased; and slight darkening in color of wet flour was noted. These characteristics depended upon the succinic anhydride:protein ratio in the reaction mixture.

Succinic anhydride reacts principally with the ϵ-amino group of lysine in protein, converting it to a negatively charged residue by N-acylation. Other reaction products may also result from the treatment of protein with succinic anhydride. *O*-Succinyltyrosines and succinic derivatives of aliphatic hydroxy amino acids of pepsinogen have been reported (Gounaris and Perlmann, 1967). Succinic anhydride also reacts with the sulfhydryl groups of protein (Meighen and Schachman, 1970).

Succinylated proteins are known to have modified physical and functional properties. Unfolding and expansion result in larger molecules exhibiting higher intrinsic viscosity. Extensive succinylation, however, may cause proteins to dissociate into subunits (Klotz and Keresztes-Nagy, 1963). An increased negative charge on acylated proteins is thought to affect emulsion activity characteristics (Chen et al., 1975).

Modifications induced by acylation are apt to greatly affect the functionality of proteins when used in food systems. Gandhi et al. (1968) showed that acylated egg white had increased heat stability and Childs and Park (1976) reported that glandless cottonseed flour acylated with succinic and acetic anhydrides had increased functionality. Others (Groninger, 1973; Groninger and Miller, 1975) have investigated the functional properties of succinylated fish protein. This paper reports data on the functionality and electrophoretic characteristics of peanut protein as affected by treatment with various amounts of succinic anhydride.

MATERIALS AND METHODS

Flour. Peanut (*Arachis hypogaea* Linn.) flour was received from Dr. J. L. Ayres, Gold Kist Research Center, Lithonia, Ga. Preparation consisted first of blanching runner peanuts in a Bauer 341 split nut blancher followed by electronic sorting to remove darkened kernels. Peanuts were then conditioned with steam, flaked, dried, and extracted with hexane. The defatted peanuts were desolventized by passing them through a tube at 104 °C (residence time, 20 min). The meal was ground with a Kolloplex mill (Alpine American) at 11 200 rpm to yield on a percentage basis: protein (Kjeldahl N × 5.46), 53.7; fat (hexane extract), 1.8; ash, 4.8; fiber, 3.9; moisture, 1.2; and carbohydrate (by difference, excludes fiber), 34.6. Granulation (percentage to pass through U.S. mesh) was: 100, 100%; 200, 95.4%; 325, 91.2%; and 425, 65.4%.

Succinylation. A 15% (w/v) suspension of peanut flour was prepared in tap water and adjusted to pH 7.7. Succinic anhydride was added to the suspension over a 1–2-h period at levels 10, 40, 70, 100, and 130% of the weight of protein in the suspension. The pH was maintained at 7.7 ± 0.3 and the temperature ranged from 24 to 36 °C. Slurries were dialyzed against water at 2 °C for 24 h, freeze-dried, and passed through a Wiley mill twice.

Protein Solubilization. Flour was suspended in water (100 mg/ml) and centrifuged at 43 500*g* for 30 min. The protein in the supernatant fluid was quantitated by the method of Lowry et al. (1951), using bovine serum albumin as a standard. These preparations were used for electrophoretic analyses.

Polyacrylamide Gel Electrophoresis. Samples containing 200–500 μg of soluble protein (pH 7.7) were electrophoresed on low 10% polybisacrylamide disc gels according to the procedures outlined by Canalco (1973) and Cherry et al. (1970).

Nitrogen Solubility. Nitrogen solubilities of control and succinylated flours were determined in water at various pH values. Each sample was combined with water at a 2% level, adjusted to pH 2.0–9.0 in 1-unit increments, and maintained at 24 °C for 45 min with occasional agitation. Dispersions were centrifuged at 9750*g* for 10 min and 25 ml of supernate was analyzed for nitrogen content by the Kjeldahl procedure (AOAC, 1975).

Water and Oil Retention. Four-gram samples of succinylated and control flours were combined with 20 ml of water or peanut oil (Gold Kist Ravo) in 30-ml centrifuge tubes. Slurries were stirred occasionally with a glass rod over a 30-min period at 24 °C and then centrifuged at 15 000*g* for 15 min. The volume of decanted supernatant fluid was measured, and milliliters of water or oil retained per gram of sample were calculated.

Water Adsorption. Equilibrium moisture contents (EMC) of succinylated and control flours at various equilibrium relative humidities (ERH) were determined at 21 °C using a method similar to that reported by Kilara et al. (1972). ERH of 68, 79, 85, 91, and 97% were maintained in closed chambers containing $CuCl_2$, $(NH_4)_2SO_4$, $LiSO_4$, $BaCl_2$, and K_2SO_4, respectively, as described by Rockland (1960). Duplicate 2-g samples were removed from the chambers after 10–12 days of equilibration, dried under vacuum at 65 °C for 24 h, and weighed. The EMC of samples prior to drying were then calculated.

Emulsion Capacity. Sixteen grams of flour was suspended in 100 ml of water in a jar and blended 30 s at low speed using an Osterizer blender. Peanut oil was added to the blending sample at a rate of 0.5 ml/s until the

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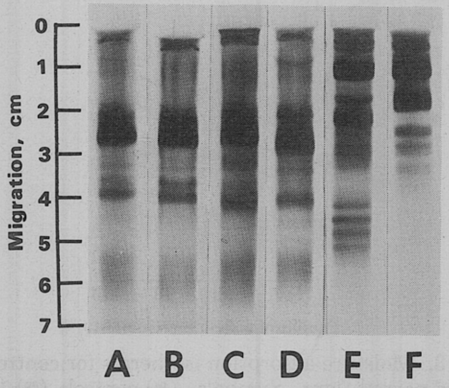

Figure 1. Polyacrylamide gel electrophoretic patterns of control and succinylated peanut flour. Letter designations: (A) control; (B) 10% (w/w, succinic anhydride/protein during succinylation procedure); (C) 40%; (D) 70%; (E) 100%; and (F) 130%.

emulsion breakpoint was reached. The breakpoint was defined subjectively as that point when emulsion coalescence broke to yield liquid separation and change in consistency.

Viscosity. Flours were dispersed in 400 ml of water at 1, 2, 5, and 10% (w/v) and allowed to remain at 24 °C with occasional agitation for 1 h. Viscosities were then measured using a Brookfield Synchro-lectric Viscometer (Model RVT). All measurements were made using a No. 1 spindle at a speed of 100 rpm. Since these dispersions have thixotropic properties, viscosity data should be considered as "apparent viscosity".

Color. Color and lightness of dried flours and pastes (flour:water, 1:2) were measured using a Gardner Color Difference Meter, Model C-4(L) set against a chromatic reflectance standard (No. SKC-SBC-31, Gardner Laboratory, Inc., Bethesda, Md.). Reference values for the standard were: $L = 93.6$ (lightness); $a = -1.3$ (green); and $b = +2.5$ (yellow).

RESULTS AND DISCUSSION

Gel Electrophoresis. Electrophoretic patterns of water-soluble (pH 7.7) proteins of control and succinylated flours are presented in Figure 1. The two main storage globulin proteins of peanuts, arachin and conarachin, are shown as major dark-staining components in the 2–3-cm region of the control (A) gel. These components did not change substantially upon treatment with up to 70% (D) succinic anhydride. Some minor distinct bands in the 3–4-cm region, however, became diffuse upon treatment with 40% (C) and 70% of the anhydride. Treatment of flour protein with an equal weight of succinic anhydride (100%, E) resulted in significant changes in the electrophoretic pattern. Major, more narrow, dark-staining bands are noted in 0.5–1.5- and 2–2.5-cm regions and several new bands are located in the 4–5.5-cm region. These bands appear to shift upward (less mobility) in flours treated with 130% succinic anhydride. The dissociation of peanut protein to yield subunits with greater mobility (E) confirms data reported by others. Succinylation has also been shown to cause dissociation of hemerythrin (Klotz and Keresztes-Nagy, 1963). The reduction in mobility of major peanut proteins treated with the highest levels of succinic anhydride (E, F) may be due to their unfolding and molecular expansion. Habeeb et al. (1958) observed that succinylated proteins exhibit decreases in sedimentation coefficients at neutral and alkaline pH. Extensive succinylation of bovine serum albumin results in increases in its Stokes radius (Habeeb, 1967).

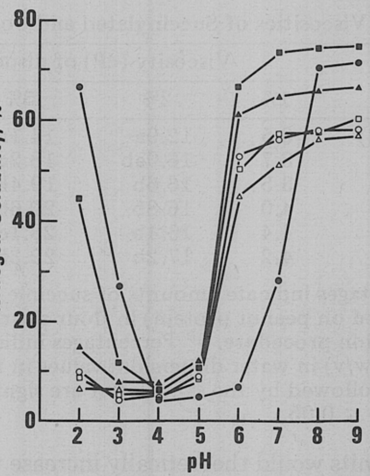

Figure 2. Nitrogen solubility profiles for control and succinylated peanut flour. Symbols: (●) control; (■) 10% (w/w, succinic anhydride/protein during succinylation procedure); (▲) 40%; (○) 70%; (□) 100%; and (△) 130%.

Table I. Liquid Retention and Emulsion Capacity Properties of Succinylated and Control Flours

Sample[a]	Liquid retention, ml/g of sample[b] H_2O	Liquid retention, ml/g of sample[b] Oil	Emulsion capacity, ml of oil/g of sample[b]
A (control)	0.94a	2.07a	23.1bc
B (10%)	1.12b	2.62c	23.9c
C (40%)	1.38c	2.52c	26.7d
D (70%)	1.50d	2.35b	23.7c
E (100%)	1.56d	2.41b	22.6ab
F (130%)	1.75e	2.42b	21.5a

[a] Percentages indicate amounts of succinic anhydride (w/w, based on peanut protein) in flour slurries during succinylation procedure. [b] Values in the same column not followed by the same letter are significantly different at $p \leq 0.05$.

Nitrogen Solubility. Solubility profiles for nitrogen in control and test flours are plotted in Figure 2. Succinylation caused a slight increase in the nitrogen (proteins, peptides, amino acids, nonprotein nitrogen) solubility of flours in the isoelectric pH range (4–5) of most peanut proteins. On the acid side of the isoelectric point, nitrogen solubility decreased progressively in flours treated with increasing levels of succinic anhydride. At 70% and above, solubilities at pH 2 were essentially the same as those at pH 4. Solubility of nitrogen at pH 6–7 was increased dramatically by succinylation. Positively charged amino groups of protein are converted to negatively charged residues by N-acylation with succinic anhydride (Means and Feeney, 1971). Thus, extensive succinylation would in effect shift the apparent isoelectric point to a more acidic pH. Data presented here on the solubility of succinylated peanut protein at various pH values tend to confirm this shift. At pH 6–7, nitrogen solubilities of flours treated with succinic anhydride are, although increased over the control flour, not directly proportional to the level of treatment. This indicates that protein solubility is also affected by other characteristics such as molecular size and configuration in addition to charge. The reduction in nitrogen solubility of fish (Chen et al., 1975) and single cell (McElwain et al., 1975) protein concentrates at pH values below their isoelectric points also has been reported.

Water and Oil Retention. The capacity of peanut flour to retain water increased with increased levels of treatment with succinic anhydride (Table I). This is probably due to both physical and chemical modification. The unfolding of protein molecules would presumably enhance entrapment of water while dissociation of protein

Table II. Viscosities of Succinylated and Control Flours

Sample[a]	Viscosity (cP) of dispersions[b] 1%	2%	5%	10%
A (control)	3.5	12.9a	14.1a	22.4a
B (10%)	3.7	16.0ab	18.2ab	35.7b
C (40%)	3.5	16.6b	19.4b	46.1c
D (70%)	4.0	16.8b	20.6bc	45.8c
E (100%)	4.4	16.4b	23.1c	47.0c
F (130%)	4.2	17.2b	22.3bc	47.2c

[a] Percentages indicate amounts of succinic anhydride (w/w, based on peanut protein) in flour slurries during succinylation procedure. [b] Percentages indicate amounts of flours (w/v) in water dispersion; values in the same column not followed by the same letter are significantly different at $p \leq 0.05$.

into subunits would theoretically increase the number of potential water-binding sites.

The oil-retaining properties of peanut flour were not influenced in the same manner as were those of water retention. Greatest increases in retention were noted for proteins treated with 10 and 40% succinic anhydride. Succinylation has also been reported to increase the water- and oil-holding capacities of glandless cottonseed flour (Childs and Park, 1976).

Water Adsorption. The capacity of control and succinylated flours to adsorb water when equilibrated with the atmosphere at various relative humidities is shown in Figure 3. Isotherms show that succinic anhydride treatment substantially increased the water-adsorbing properties of peanut flour. As with the case of water retention, the ability to adsorb water is undoubtedly due in part to the general unfolding and expansion of protein molecules. In previous studies (Beuchat et al., 1975; Quinn and Beuchat, 1975; Beuchat, 1977a, b) we demonstrated a marked increase in the capacity of fermented and enzymatically hydrolyzed peanut flour protein to adsorb and absorb water. These properties were attributed mainly to an increase in the number of potential water-binding sites. The increase in water-adsorption capacity of succinylated peanut protein observed in the present study is also influenced by the extent of protein dissociation resulting from succinylation.

Emulsion Capacity. A significant increase in emulsion capacity of peanut flour was noted to occur at the 40% (C) succinic anhydride level (Table I). The highest treatment (130%, F) resulted in a significant reduction in emulsion capacity. It is reported that the type of vegetable oil does not significantly affect the emulsion characteristics of succinylated single cell protein concentrate (McElwain et al., 1975). However, the presence of ionic charge and nonprotein constituents may influence emulsion properties adversely. Since extensively succinylated protein would be more negatively charged than the control flour protein at pH 7.7 due to the increased number of free carboxyl groups and since fat globules are also negatively charged, the reduced emulsion capacity noted in sample F (130%) may be due to repulsion of the protein and oil phases. Improved emulsion capacities in succinylated soy isolate and alfalfa leaf protein (Franzen and Kinsella, 1976a,b) and in fish protein concentrate (Chen et al., 1975) have been noted. Apparently the type of dispersion medium, the concentration of sample present in the test mixture, the quality and quantity of nonprotein constituents in the sample, and the extent of succinylation greatly influence the emulsion characteristics of succinylated proteins.

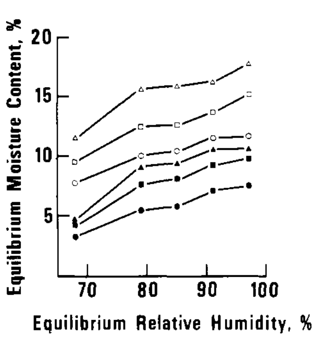

Figure 3. Moisture adsorption isotherms for control and succinylated peanut flour. Symbols: (●) control; (■) 10% (w/w, succinic anhydride/protein during succinylation procedure); (▲) 40%; (○) 70%; (□) 100%; and (△) 130%.

Viscosity. The apparent viscosities of water dispersions of control and succinylated peanut flour proteins are given in Table II. Significant increases in viscosities were observed in 2 and 5% dispersions (40% treatment, C) and in 10% dispersions (10% treatment, B) as compared to the control flour at respective levels of dispersion. Oppenheimer et al. (1966, 1967) reported that succinylation of chicken myosin resulted in increased viscosity. Later, succinylated myofibrillar fish protein dispersions (Groninger, 1973), single cell protein concentrate emulsions (McElwain et al., 1975), and glandless cottonseed flour (Childs and Park, 1976) were reported to have increased viscosities over respective control materials. Again, the extent of unfolding of protein molecules as a result of succinylation undoubtedly affects changes in viscosity just as it also affects other functional and electrophoretic characteristics.

Color. The effects of succinic anhydride on color of dry and wet peanut flour are shown in Table III. Succinylated dry flours were generally lighter (*L*) at the highest treatment levels. Significant yellowing (*b*) was detected in pastes from which succinylated (10 through 100%, B through F, respectively) flours were prepared. Succinylation also resulted in darkening (*L*) of wet flours. These color and lightness changes are not dramatic, however, and would probably not be detectable in food systems in which succinylated peanut flour might be incorporated.

Table III. Objective Color and Lightness Values for Succinylated and Control Flours and Pastes

	Dry flour[b]				Paste[b]			
Sample[a]	−*a*	*b*	*L*	*E*[c]	−*a*	*b*	*L*	*E*
A (control)	2.95	13.3ab	75.4b	21.5	1.65a	13.1b	65.3d	30.5
B (10%)	1.50	13.9ab	73.9b	22.9	2.25a	13.9c	58.8c	36.9
C (40%)	5.40	14.6b	71.8a	25.5	4.50b	14.5c	56.3b	39.6
D (70%)	2.75	12.4ab	79.3c	17.6	1.45a	14.6c	59.0c	37.0
E (100%)	6.80	11.3ab	80.9d	16.6	1.45a	14.3c	57.0b	38.7
F (130%)	4.40	10.5a	78.2c	17.8	1.00a	10.3a	53.4a	41.3

[a] Percentages indicate amounts of succinic anhydride (w/w, based on peanut protein) in flour slurries during succinylation procedures. [b] Values in the same column not followed by the same letter are significantly different at $p \leq 0.05$. Data for *E* were not subjected to statistical analyses. [c] $E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2}$, where ΔL, Δa, and Δb are respective differences between standard and sample.

In summary, data are presented which demonstrate that changes in functional properties of peanut protein are inducible by treatment with succinic anhydride. The uniqueness and value of these modified properties can be determined only through incorporating succinylated flours into food or beverage systems.

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Effects of Amylose Content on Some Characteristics of Parboiled Rice

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Forty-nine rice varieties have been analyzed to examine the influence of amylose content on parboiled and canned rice qualities. Statistical analysis shows a high positive correlation between amylose content and firmness of cooked or canned parboiled rice and appearance of canned rice. A parabolic-type relation was found between amylose content and milling yield. Rice with high amylose content is recommended for use by the canning industry. Formation of a chalky texture due to starch retrogradation may be avoided in cold meal by increasing the water content of rice.

The amylose content of starch is usually considered to be the main parameter of the eating characteristics of rice (Juliano, 1972). The texture of cooked rice is improved by increasing the amylose–amylopectin ratio (Juliano et al., 1965). According to Zakiuddin and Bhattacharya (1972), the difference in behavior between differently processed parboiled rices is due to the gelatinization and the retrogradation of the starch granules. The characteristics of the parboiled rice are dependent on the processing conditions: steeping time, steaming pressure, steaming time (Roberts et al., 1954; Bhattacharya and Subba Rao, 1966; Subrahmanyan, 1971; Dimopoulos and Muller, 1972; Feillet and Alary, 1975a,b).

According to Webb et al. (1968), amylose content is an indicator of dry matter (solids) loss during canning of parboiled rice. Varietal differences in the textural quality of canned rice have been attributed, at least partially, to variation in amylose content (Burns, 1972). This study was conducted to determine the effect of amylose content on other parboiled and canned rice characteristics.

MATERIALS AND METHODS

Rice. Forty-nine rice varieties, listed in Table I, with amylose contents ranging from 21 to 29% (moisture free basis), were grown under controlled conditions at the experimental rice field of Mas d'Adrien (Station d'Amélioration des Plantes de Montpellier, Institut National de la Recherche Agronomique). After harvesting, paddy rices were stored in a room at 18 °C and 72–75% relative humidity; the final moisture content after 2 weeks was around 14.5% (wet basis).

Parboiling. Samples were parboiled in a fully automatic laboratory-scale apparatus in which time, pressure, and temperature of vacuum, steeping, and steaming can be programmed (Feillet and Alary, 1975a). In regard to our previous results, the following parboiling conditions were used: vacuum for 10 min under 1 bar; steeping for 30 min at 65 °C under 3.1 bars; steaming for 30 min at 120 °C. Parboiled rice was dried like paddy rice under very

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