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Some Functional Properties of Acylated Wheat Gluten

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Commercial wheat gluten was chemically modified by reaction with either succinic or citraconic anhydride. In the neutral pH range, the acylated glutens showed greater water solubility, emulsification capacity, and water holding capacity than an unmodified control sample. The modified glutens also showed greater water adsorption than the unmodified gluten. Gluten modified with succinic anhydride was similar in functionality to the gluten treated with citraconic anhydride except that after holding at pH 3, the latter showed evidence of substantial reversibility of the acylation.

A number of protein sources such as alfalfa leaf, fish, peanut flour, and soy flour have been investigated for possible incorporation into formulated foods. Chemical derivatization of groups such as the free ϵ-amino group of lysine in proteins with acid anhydrides has resulted in improved functional properties for various food proteins (McElwain et al., 1975; Childs and Park, 1976; Kinsella and Shetty, 1979). A reversible acylation could have advantages in that during digestion, amino groups such as those of lysine would be deacylated and the lysine would become nutritionally available. Brinegar and Kinsella (1979) have studied the deacylation of soy protein after modification with citraconic anhydride and found substantial reversibility under mild acidic conditions.

Wheat gluten is a readily available protein source that has been used extensively in baked products and pet foods (Sarkki, 1979). Gluten proteins are uniquely suited for dough-forming characteristics because of hydrophobicity, low water solubility, and the reactions of sulfhydryl groups. However, some of these same properties can limit other uses of gluten in foods. Gagen and Holmes (1972) and Grant (1973) have studied the acylation of wheat proteins, and certain characteristics were found to improve with the modification. Some functional properties of the acylated glutens have not been explored and the use of citraconic anhydride for forming reversible derivatives of gluten has not been previously investigated. It was the purpose of this research to evaluate the effect of acylation with succinic and citraconic anhydrides on some functional properties of wheat gluten and to determine the degree of reverisbility of the acylation of gluten.

MATERIALS AND METHODS

Acylation of the Gluten. Acylation with both succinic and citraconic anhydrides was performed in a procedure similar to the method of Friedman (1978). Commercially available vital gluten (Pro 80, Henkel Corp., Minneapolis, MN) was used throughout the study. Manufacturer's data indicated this material was 75% protein, which is typical for vital wheat gluten products (Kalin, 1979). The gluten was made into a slurry with a 1:4 gluten:water ratio, and the pH was adjusted to 7.5–8.5 with 1 N NaOH. To the slurry 0.5-g increments of either succinic anhydride or citraconic anhydride (Eastman Organic Chemicals) were added. The pH was readjusted to 7.5–8.5 with 2 N NaOH after each addition of the anhydride. The amount of anhydride added was varied in a preliminary experiment where the weight of anhydride was either half, the same as, or twice the weight of dry gluten. Because there was no significant change in nitrogen solubility of the modified protein samples with increasing levels of anhydride, a ratio of 1 part of anhydride to 2 parts of gluten was used throughout the remainder of the study. After the addition

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of the anhydride, the reaction mixture was dialyzed against running tap water at 10 °C water for 24 h to remove impurities and excess reagent. The chemically modified gluten product was recovered by lyophillization. The control gluten was obtained by using the above procedure except water rather than succinic or citraconic anhydride was added to the gluten slurry. The control gluten sample was adjusted to pH 7.5–8.5, dialyzed, and lyophilized. The nitrogen content was determined by Kjeldahl nitrogen analysis (Association of Official Analytical Chemists, 1975).

Extent of Modification. The trinitrobenzenesulfonic acid (TNBS) method of Habeeb (1966) was used to determine the extent of acylation of the gluten proteins. A borate buffer (pH 9) was used instead of sodium bicarbonate buffer and a water bath temperature of 60 °C instead of 40 °C was used to facilitate the TNBS reaction with the gluten proteins. The procedure involved the addition of 1 mL of a 0.1% TNBS solution to a protein suspension. The samples were heated in a 60 °C water bath for 2 h and then cooled to room temperature. One milliliter of 10% sodium dodecyl sulfate (Mallinckrodt, 95%) and 0.5 mL of 1 N HCl were added to the protein solutions. The absorbance of the solutions was read at 335 nm in a spectrophotometer against a reagent blank. The absorbance of the control gluten was set equal to 100% free amino groups, and the degree of acylation of the modified samples was calculated based on the decrease in absorbance because fewer amino groups were able to react with the TNBS reagent (Habeeb et al., 1958).

Functionality Tests. Based on previous work by Betschart (1974) and Balmaceda et al. (1976a,b), a procedure was developed to determine the solubility of acylated gluten derivatives at various pH levels. A 75-mg sample of modified or control gluten was placed in a screw-top polypropylene centrifuge tube, and water was added to make a 1% dispersion. The pH was adjusted to the desired level by using 1 N HCl or 1 N NaOH. The dispersions were shaken vigorously for 1 min and centrifuged at 12000*g* for 10 min at 5 °C. The supernatant was filtered through Whatman No. 1 filter paper, and the amount of nitrogen in the supernatant was determined by Kjeldahl nitrogen analysis. The nitrogen solubility was calculated as the amount of nitrogen in the supernatant divided by the amount of nitrogen in the original dispersion. The solubility profile was then obtained by plotting the means of the nitrogen solubility of duplicate samples vs pH.

While dough-forming properties were not the focus of this study on acylated glutens, doughing characteristics such as cohesiveness, adhesiveness, and extensibility were subjectively evaluated in a rehydrated sample of each acylated derivative and of the control gluten and native commercial gluten. The purpose of this evaluation was to detect any major changes in the dough-forming ability of the modified proteins. Bulk density was determined according to the method of Rasekh (1974).

The emulsification capacity of the acylated gluten was determined according to Webb et al. (1970) and Balmaceda et al. (1976a,b). A 0.2% protein dispersion was prepared, and 150 mL was put into a 1-pt mason jar fitted with electrodes to measure the resistance of an electrical current through the dispersion. The electrodes were placed opposite each other on the sides of the jar just above the blender blade. The jar and blender blade assembly were put onto a blender (Cycle-blend, Osterizer Co.), and the electrodes were connected to a volt–ohm meter (VOM) (Triplette Model 310, Type 2). The sample was blended for 30 s at high speed. At the end of this time, soybean oil was introduced into the rapidly blending protein dispersion through a hole in the jar via a buret at a rate of approximately 0.22 mL/s. The addition of oil was terminated when the resistance of the sample went from approximately 12.5 ohm/cm of distance between the electrodes to infinite resistance. This change in the VOM reading was considered the point of oil inversion in the emulsion since oil is unable to conduct an electrical current. Emulsification capacity was the amount of oil required by the protein dispersion to reach the phase inversion (or emulsion breakpoint). The emulsification capacity was calculated as milligrams of oil emulsified per 100 mg of protein. Four samples were run for each gluten sample at each pH level.

The method for determining water adsorption was adapted from the method of Beuchat (1977). Two grams of each sample was spread out evenly in a 5-cm diameter glass Petri dish. The samples were then brought to equilibrium in a water activity (a_w) chamber containing a saturated solution of $MgCl_2$ ($a_w = 0.34$) held at room temperature. After reaching equilibrium at this a_w, duplicate samples of each treatment were transferred to six chambers containing different saturated salt solutions to provide the following water activities: $a_w = 0.44$ (K_2CO_3), 0.65 ($NaNO_2$), 0.75 (NaCl), 0.80 (KCl), 0.85 (LiCl), and 0.88 (K_2CrO_4) (Labuza, 1975). After equilibrium was reached (1–2 weeks at room temperature), the percent moisture was determined gravimetrically after heating each sample in a vacuum oven (Association of Official Analytical Chemists, 1975).

The water holding capacity (WHC) procedure was based on methods described by Childs and Park (1976) and Balmaceda et al. (1976a,b). A 5% aqueous dispersion of each sample was adjusted to pH 3, 5, 7, 9, or 11 with either 1 N HCl or 1 N NaOH. After centrifugation at 500*g* for 25 min the supernatant was removed, and the water remaining with the gluten in the centrifuge tube was determined by difference. The WHC was calculated as grams of H_2O per gram of gluten sample. Duplicate samples were run at each pH for each gluten product. Data for WHC, solubility, and emulsification capacity were subjected to an analysis of variance to determine significant differences between treatments.

For study of the reversibility of the acylation reaction with gluten, a 0.5 mg/mL protein dispersion was made in 0.5 mL of 0.2 M citrate buffer (pH 3.0). Samples were placed in a shaker at room temperature for either 0, 6, 12, or 24 h. After the designated time period the samples were immediately adjusted to pH 8.8 with 0.1 N NaOH and 0.1 M borate buffer. The TNBS colorimetric test was used to determine the amount of liberated amino groups, as described earlier. For each of the four reaction times, gluten samples were prepared and analyzed in triplicate.

RESULTS AND DISCUSSION

Treatment of wheat gluten with succinic anhydride at a 1:2 anhydride:gluten ratio resulted in acylation of approximately 60% of the free amino groups as measured by the TNBS reaction. Reaction of gluten with citraconic anhydride at the same 1:2 ratio led to a 40% modified derivative. The extent of modification of the wheat gluten proteins was somewhat lower than the level of acylation found with other food proteins reacted under similar conditions. Amino group modification of 80% or more has been reported with a variety of proteins (Hoagland, 1966; Franzen and Kinsella, 1976a,b; Eisele and Brekke, 1981). The difference in extent of acylation may be due to the hydrophobic character and poor solubility of the gluten, which could possibly hinder the reaction of the acylating

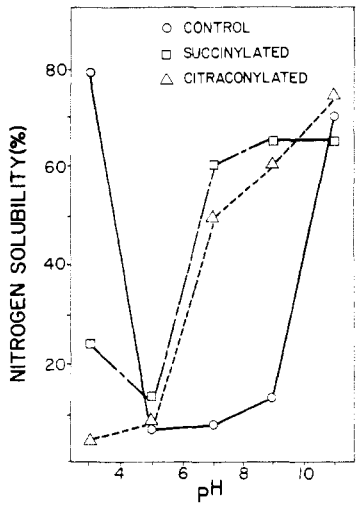

Figure 1. Influence of pH on the solubility of control and acylated gluten.

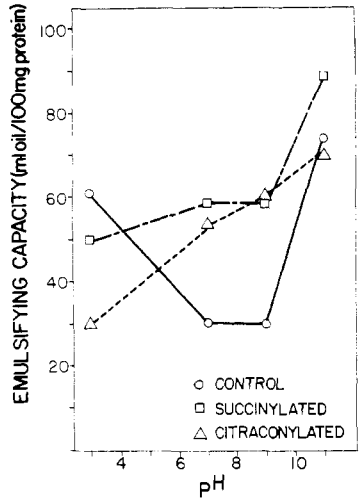

Figure 2. Emulsifying capacity of control and acylated gluten at four pH levels.

reagents with the amino groups. To obtain higher modification levels of gluten, it may be necessary to alter the procedure by using a reaction medium other than water. Grant (1973) found that dioxane could increase the extent of acylation of wheat proteins apparently by increasing the solubility of the proteins during the modification reaction.

The preliminary studies of anhydride levels showed there was no significant change in the nitrogen solubility between the gluten samples acylated with 1:2, 1:1, 1:0.5 anhydride:gluten ratios (w/w). Even though the gluten proteins were not fully acylated by the succinic or citraconic anhydrides, as determined by the TNBS reaction, it appears that higher levels of the anhydride do not produce a more soluble protein. The results suggest that the point where no further increase in solubility is observed for wheat gluten occurs at or below the 1:2 anhydride:gluten ratio.

The nitrogen content of the modified glutens on a weight basis was less than that of the control (11.7 vs. 12.4%). This was likely due to the addition of non-nitrogen-containing succinyl and citraconyl groups to the protein or the presence of these compounds due to noncovalent binding or incomplete removal by dialysis.

The pH–solubility profile of the control gluten showed maximum solubility at pH 3 and 11 and a much lower solubility at pH 5, 7, and 9 (Figure 1). This minimum solubility range corresponds with other reported values that indicate the isoelectric point and lowest water solubility of gluten is in the range of pH 5–9 (Kasarda et al., 1976; Sarkki, 1980).

Upon acylation with succinyl and citraconyl groups, there was a significant increase in solubility at pH 7 and 9 over that of the control gluten ($P = 0.05$) (Figure 1). The change in solubility at these pH levels is likely due to the electrostatic repulsions of the acyl anions among themselves and with other carboxyl groups on the protein. It has been reported by several workers that by breaking the strong hydrogen bonds of the gliadin and glutenin, which are the major protein fractions in gluten, the number of protein–protein interactions is reduced and the ability for water molecules to interact with the protein increases (Gagen and Holmes, 1972; Kasarda et al., 1976).

Both the succinylated and citraconylated derivatives were less soluble than the control gluten at pH 3 ($P = 0.05$). The lack of solubility of the acylated gluten at acid pH is likely due to the alteration of the cationic amino groups and a lowering of the isoelectric point. By the elimiantion of this positive charge, there was probably an insufficient number of hydrophilic cationic groups at pH 3 to overcome the aggregating forces resulting from the hydrophobic bonds of the alkyl and aromatic groups of the amino acid residues, as suggested by Franzen and Kinsella (1976a). While the acylation with citraconic anhydride may be somewhat reversible at pH 3, the citraconylated sample showed low solubility at pH 3, indicating nonreversibility at least under the conditions of the solubility testing.

At pH 11, there was no significant difference in solubility between any of the gluten samples ($P = 0.05$). One explanation of this phenomenon is that disaggregation and denaturation of both the acylated and control samples at high pH result in the exposure of hydrophilic groups from the protein interior. In addition to the protein being well above the isoelectric point, interaction of the water molecules with the exposed hydrophilic groups may be to such an extent that equal solubility of both acylated and gluten and unacylated gluten results.

Compared to that of the control wheat gluten, the bulk density of the modified gluten was lower. Acylation with succinic anhydride resulted in a dried product with a bulk density of 0.27 g/cm^3 compared to 0.30 g/cm^3 for the citraconic treatment and 0.41 g/cm^3 of the control. While a number of factors such as drying conditions can affect bulk density, the relative differences due to modification were noticeable when the control and modified samples were handled in the same manner. The change could be due to altered conformation of the proteins caused by electrostatic repulsions of the negatively charged acyl groups. Similar changes in bulk density have been noted for modified soy and leaf protein (Franzen and Kinsella, 1976a,b).

When rehydrated, none of the gluten products including the control showed the doughing properties that were observed in the native gluten. The observed loss of characteristic gluten doughing properties in the control sample indicates that the sample handling procedures (i.e., extensive mixing, contact with alkali, and/or dialysis) contributed to the decrease in doughing properties in addition to any effects the acylation reaction may have had. The treatments may have disrupted hydrogen and disulfide bonds in the gluten resulting in the loss of dough extensibility (Kasarda et al., 1976; Huebner et al., 1977).

Emulsification capacity of the acylated gluten was about double that of the control sample at pH 7 and 9 (significant at $P = 0.05$) (Figure 2). At pH 3, however, both succinylated and citraconylated derivates displayed decreases in emulsification capacity compared to that of the control

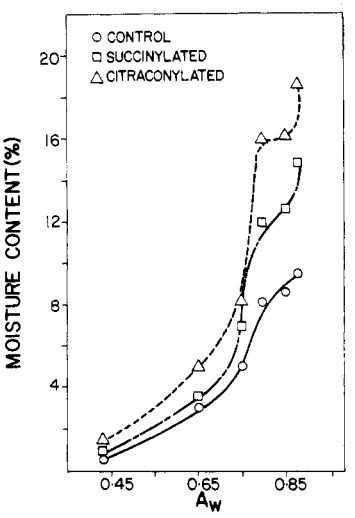

Figure 3. Water adsorption of control and acylated gluten.

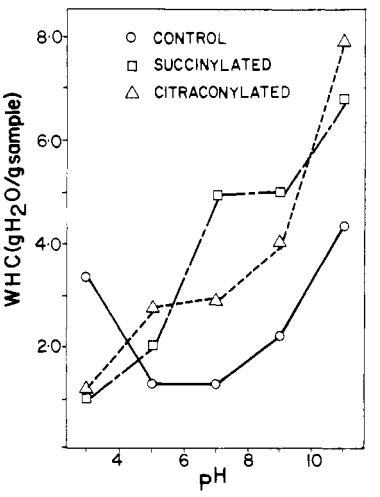

Figure 4. Influence of pH on the water holding capacity of control and acylated gluten.

gluten. This trend is similar to that observed in the solubility of the gluten samples, and the relationship of solubility to emulsification capacity has been reported in other protein systems (Franzen and Kinsella, 1976a,b; Crenwelge et al., 1974). An increase in protein solubility would encourage a rapid migration to and adsorption at the water–oil interface. This adsorption of the protein would, in turn, lower the interfacial tension between the water and oil and stabilize the emulsion (Becher, 1965). At pH 11 succinylated gluten appears to have a higher emulsion capacity than the other two samples, but this cannot be explained on the basis of solubility.

Figure 3 shows the water adsorption curves for the control and modified glutens. The shape of the curves is similar up through $a_w = 0.75$ with the modified samples containing slightly higher levels of water. From $a_w = 0.75$ to $a_w = 0.80$ the water uptake is more dramatic followed by a leveling off from $a_w = 0.80$ to 0.85. Above $a_w = 0.85$ the modified proteins show a greater increase than the unmodified control. The relationship of moisture uptake with increasing a_w for gluten is similar to that for soy protein isolate and succinylated peanut flour protein (Kinsella, 1979; Beuchat, 1977). The increased water adsorption by the modified gluten proteins over that of the control gluten may be due to the charge effects of the added acyl groups. The results show that the acylation treatment of gluten can enhance water adsorption, and this may mean better water binding properties in food systems.

The WHC (ability to hold water against gravity) was increased with the modification treatment above pH 5 as shown in Figure 4. At pH 5 there was no significant difference in the samples, and at pH 3 the control sample had a significantly higher water holding capacity ($P = 0.05$). The modification apparently alters protein structure to allow better interaction and binding with water at neutral and alkaline pHs. The decrease in isoelectric point caused by the modification may play a role in the higher WHC of the acylated glutens.

WHC and solubility properties appear to correlate at pH 9 and lower. However, at pH 11, the control has less WHC even though the water solubility (Figure 1) is similar for all three gluten samples. This effect at pH 11 may be due to altered structure caused by alkaline hydrolysis. While treatment with succinic anhydride appears to be more effective for increasing WHC than treatment with citraconic anhydride, the trend of increased WHC over the unmodified gluten in the pH range above 5 suggests that the acylation would make the modified gluten better able to hold water in most food systems.

Another aspect of using acylated proteins in food systems involves the potential reversibility of the modification, especially at the pH found in acid foods or during human digestion. When the acylated gluten samples were held in dispersion at pH 3.0, tests for free amino groups with TNBS showed that after 6 h 70% of the citraconyl groups had hydrolyzed from the modified protein, after 12 h 80% had been removed, and after 24 h the gluten was fully deacylated. A similar rate of deacylation of the citraconylated soy proteins was reported by Brinegar and Kinsella (1979). The succinylated wheat gluten did not deacylate at pH 3.0 when held for up to 24 h. Previous studies with other proteins have indicated the nonreversibility of the succinic reaction (Bjarnason and Carpenter, 1969; Gounaris and Perlmann, 1967). The results of this study show that wheat gluten treated with citraconic anhydride can deacylate at low pH, providing an opportunity for recovery of free amino groups during digestion. The possibility of eventually obtaining free amino groups on gluten may make citraconic anhydride more nutritionally acceptable as a modifying agent.

In summary, the results of this study indicate that acylation using succinic and citraconic anhydride can modify and generally improve the functional properties of wheat gluten over those of the native form. The increase in solubility, emulsification capacity, water adsorption, and water holding capacity at neutral pH makes the modified gluten protein a potentially functional ingredient in formulated foods where these properties may be required. Citraconylated wheat gluten provides the added advantage of reversibility under acid conditions, and this may improve the nutritional utilization of the protein.

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Stabilization of Carotene and Xanthophyll in Alfalfa Leaf Protein Concentrates

Cameron K. Lyon* and George O. Kohler

Carotene and xanthophyll are valuable constituents of alfalfa leaf protein concentrate (Pro-Xan), which is now prepared commercially. The effects of moisture content, pH, pelleting, inert atmosphere storage, and the addition of an antioxidant, fat, or alfalfa-soluble solids on the storage stability of these carotenoids were investigated. Earlier work on storage at low temperatures and in the dark is discussed. Greatest stability is obtained by addition of the antioxidant ethoxyquin (0.05%), storage in an inert atmosphere, and, if economically feasible, cold storage. Increases in stability, particularly of carotene, are also obtained by drying to a lower moisture content, addition of fat, or addition of the soluble solids remaining after separation of the alfalfa protein. It is essential that any added fat be stabilized with antioxidants and free of peroxides that promote the oxidation and destruction of carotenoids.

Alfalfa leaf protein concentrate (Pro-Xan), a feed product containing 50–60% protein and high levels of xanthophyll and β-carotene, is prepared by wet fractionation of fresh alfalfa. Early work on leaf protein concentrates was reviewed by Pirie (1971) and Kohler et al. (1978). Superior large-scale processes for its preparation have now been developed, and the yields and quality of Pro-Xan have been significantly improved (Edwards et al., 1978). Pro-Xan is now being produced commerically in this country by the Valley Dehydrating Co., Sterling, CO (Kohler and Edwards, 1980; Edwards et al., 1979).

Pro-Xan is a good source of protein and energy for poultry (Kuzmicky and Kohler, 1977) and because of its high xanthophyll content is particularly valuable as a pigment source for boilers and laying hens (Kuzmicky et al., 1977). To maintain this value, it is important that the carotenoid pigments be protected from oxidative degradation during storage, and means of accomplishing this have been investigated (Witt et al., 1971, 1972; Livingston et al., 1980). However, the earlier investigations were carried out before the current, preferred Pro-Xan process was developed so that the products had different exposures to heat and air during drying, different contents of water-soluble compounds, etc. Moreover, the effects of variables such as moisture content, added oil, or water-soluble compounds from alfalfa were not always consistent, so it seemed desirable to investigate the factors that could improve the stability of the carotenoids in Pro-Xan as currently prepared.

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EXPERIMENTAL SECTION

Leaf Protein Concentrates. In general, the concentrates were prepared in the pilot plant as described by Edwards et al. (1978) by pressing juice from ground alfalfa, adjusting its pH to 8.5 with ammonia, coagulating protein in the juice at 80 °C or higher by direct injection of steam, separating the protein curd in a continuous centrifuge, and drying the protein. Drying required air temperatures of about 220 °C for a few minutes in a fluidized bed dryer (No. 2052-1, Witte Co., Washington, NJ) or 70 °C for 2–3 h in 1.0–1.5-cm layers in a tunnel dryer. In one experiment, the concentrates were prepared on a small scale as described by Lyon et al. (1976) and dried in a force draft