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The effects of acylation on the functionality and performance of cottonseed flour

Firyal B. Al-Dabbagh

To the Graduate Council:

I am submitting herewith a thesis written by Firyal B. Al-Dabbagh entitled "The effects of acylation on the functionality and performance of cottonseed flour." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

H. O. Jaynes, Major Professor

We have read this thesis and recommend its acceptance:

S. L. Melton, J. L. Collins

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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Hugh O. Jaynes

H. O. Jaynes, Major Professor

We have read this thesis
and recommend its acceptance:

Sharon L. Melton

J. L. Collins

Accepted for the Council:

Hilton A. Smith

Vice Chancellor
Graduate Studies and Research

THE EFFECTS OF ACYLATION ON THE FUNCTIONALITY AND PERFORMANCE OF COTTONSEED FLOUR

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Firyal B. Al-Dabbagh

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ABSTRACT

The purposes of this study were to acylate glandless cottonseed flour by using succinic anhydride and then to study the functionality and performance of acylated flour.

Glandless cottonseed flour was acylated by adding one-tenth gram of succinic anhydride per gram flour. The anhydride was added to the flour suspension over a period of 60 minutes with constant stirring at room temperature (23-24°C.). The pH of the reaction was maintained at pH 8 (±0.5) by addition of 50 percent NaOH. After the reaction was finished, the pH of the flour suspension was lowered to pH 4 (±0.5). The acylated flour was recovered by centrifugation, washed with water to remove residual succinate, and freeze-dried.

Physical functionality of acylated cottonseed flour was measured. Water and oil holding capacities were increased while emulsifying capacity was reduced.

Acylated cottonseed flour and untreated cottonseed flour were used as a replacement for 6 or 12 percent of the wheat flour in white layer cakes. The quality of these cakes was inferior to controls made with all wheat flour.

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER I

INTRODUCTION

The world requirement for protein for human consumption is 100 million tons and twice that amount for feeding livestock. Oilseed proteins such as cottonseed offer a partial solution to this need. In 1970, about 24 million tons of cottonseed were produced in the world.

The presence of the chemically reactive antinutritional factor, gossypol, or pigment glands, which is toxic for nonruminant animals in regular glanded cottonseed has been a handicap to its usage. This problem has been overcome with production of higher quality, edible protein concentrate by solvent extraction of glandless cottonseed and with a new method of producing deglanded cottonseed flour from glanded cottonseed. This method is called the Liquid Cyclone Process (LCP) and was developed at USDA's Southern Regional Research Laboratory in New Orleans. Basically, LCP removes gossypol and lipids from cottonseed material to produce a flour. Flours, protein concentrates and isolates are prepared from cottonseed. The flour contains about 60 percent protein, less than 1 percent fat and 3 percent fiber.

Cottonseed flour has been used in yeast breads by substituting it for wheat flour at various levels. Cottonseed bread was found to be heavier, more compact, coarse textured and had a color comparable to that of a whole wheat bread.

Extension of cottonseed flour utilization to other types of food products might be feasible if the flour itself or a chemically modified flour were found to have high functionality. A technique which has been successfully utilized with fish protein concentrates, eggs and cottonseed flour to improve functionality is acylation of the protein with acidic anhydrides. Acylation of proteins has been found to cause an increase in the electrostatic repulsion forces in the protein resulting in an expansion of the molecule.

The purposes of this study were to investigate the functionality of acylated glandless cottonseed flour; (a) water holding capacity, (b) oil holding capacity, (c) emulsifying capacity. Then the modified flour was used in a food product--white layer cake.

CHAPTER II

REVIEW OF LITERATURE

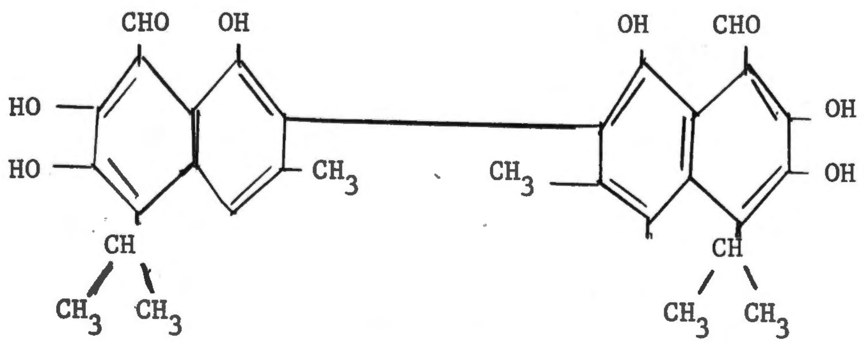
Cottonseed represents an outstanding example of conversion of an agricultural product into a source of valuable oil, protein, and other by-products (3).

The two principal elements of the seed are the spermaderm and the embryo; the third part is a membrane, which completely envelops the embryo. Cottonseed is dicotyledonous; it contains two large flat cotyledons and axial organs, the radical, the hypocotyl and the epicotyl. The cotyledons are folded around the radical and over the top of the hypocotyl in the embryo (3).

Oil is found in all the tissue and organs of the embryo but is most abundant in the mesophyll of the cotyledons and the parenchymatous cells of the hypocotyl. It is embedded in the tissue in minute droplets. Cottonseed oil is of major economic importance and occurs in the seed in quantities approximately equal to the amount of protein (3). Cottonseed oil consists primarily of triglycerides of fatty acids such as linoleic, 50 percent; oleic, 23 percent; palmitic, 23 percent; and small quantities of myristic, stearic and palmitoleic (6). It is liquid at ordinary temperatures, has good flavor and stability to oxidative rancidity, and it is an edibel vegetable oil (7, 8).

Protein occurs in aleurone grains and as a component of the cytoplasm. It is found in all the organs of the embryo. About 80 percent of the nitrogen in carefully defatted cottonseed is soluble in dilute sodium chloride solution; 90 percent is soluble in dilute alkali; about 10 to 12 percent is soluble in water; and approximately 4 to 10 percent is non-protein nitrogen (3, 16).

Gossypol is the major pigment of cottonseed. It is a yellow pigment and was once considered for use as a textile dye (3). It reacts as an aldehyde and as a polyphenol and has fairly strong acidic characteristics. Adams and co-workers (2) showed the following structure for one of the tautomeric forms of gossypol:



Gossypol

Gossypol has physiological properties which influence the utilization of cottonseed meal for monogastric animals. For many years cottonseed meal was not used to any large extent for such animals because of the fear of toxic effects from gossypol (7, 8). The development of glandless cotton varieties and Liquid Cyclone Processing (LCP) techniques may allow production of higher quality, edible meals that have very low or zero concentrations of gossypol (7, 30, 46).

The LCP method was developed at USDA's Southern Regional Research Laboratory in New Orleans. Basically, LCP removes gossypol and lipids from finely ground cottonseed to produce a flour (17, 55).

Cottonseed flour was offered for sale in the United States as early as 1876. It contains about 58 to 60 percent protein, 3 percent fiber, and less than 1 percent fat (3, 29, 30).

The amino acid composition indicates that the lysine and threonine values of cottonseed flour are lower than those of soybean meal or casein. The lysine content is slightly decreased after the removal of gossypol (14) but not below the value of Food and Agricultural Organization (FAO) amino acid pattern (1965) or the value of the glandless cottonseed flour (21, 29). All cottonseed flours contain adequate quantities of valine, leucine, tyrosine and phenylalanine as compared with FAO values, although some cottonseed flours are slightly low in methionine and isoleucine (3, 29)(see Table I). The use of cottonseed protein in the diet of humans, especially young children, has been frequently proposed.

Altschul (3) interpreted data presented at a meeting of United Nations Children's Fund and showed that at low levels of protein in the diet, cottonseed protein concentrate is better nutritionally than soybean meal or fish concentrate. No ill effects were noted when cottonseed protein concentrate of acceptable quality compared to soybean protein was fed.

Cottonseed, fish, and soy protein have been characterized both functionally and nutritionally as a partial replacement for wheat flour with non-wheat flour in yeast breads (38, 39, 41, 42, 49).

TABLE I

COMPARISON OF COTTONSEED AND SOY PROTEIN TO THE FAO ESSENTIAL AMINO ACID PATTERN[1]

	FAO Pattern	Cottonseed Flours			Soybean Meal	Casein
		LCP	Glanded	Glandless		
	g./100 g.					
Lysine	4.2	5.4	5.6	4.4	8.6	7.7
Threonine	2.8	4.1	4.4	4.7	4.9	4.9
Valine	4.2	4.8	5.1	5.1	4.6	5.0
Methionine	2.2	1.8	2.2	2.0	1.8	3.5
Isoleucine	4.2	3.6	5.0	3.6	4.5	4.1
Leucine	4.8	7.3	8.8	7.6	9.2	10.2
Tryosine	2.8	3.9	3.6	3.9	4.3	6.3
Phenylalanine	2.8	6.8	6.8	6.6	6.1	5.7

[1]M. L. Harden and S. P. Yang, 1975 (29).

Bacigalupo et al. (5) found that up to 5 to 10 percent replacement of wheat flour by non-wheat flour was successful. However, at higher replacement levels, loaf volume was severely decreased along with serious deterioration of crumb color, grain, and texture (42, 43, 49).

Dendy et al. (18) and Felming et al. (20) pointed out that the maximum level of replacement depends on the type of non-wheat flour, the strength of the wheat flour gluten, the baking procedure and the dough stabilizing compounds used. Tsen and co-workers (51) used sodium stearoyl-2-lactylate and calcium stearoyl-2-lactylate to produce sponge dough bread with 12 percent soy flour. The dough conditioners increased loaf volume and the organoleptic properties of the soy bread were comparable to bread with 100 percent wheat flour. The dough conditioners permit other non-wheat flours to replace wheat flour without loss of bread quality (20, 30, 51).

Defatted oilseed flour and non-wheat cereal flours were used by Kim and DeRuiter (35) in 1968 to produce enriched breads by using yeast-leavened bread without wheat. Bread from non-wheat flour mixtures could be made with appropriate procedures and emulsifying agents, but the organoleptic properties were unacceptable.

Rooney et al. (48) studies the functional properties of heat-treated and non heat-treated flour from cottonseed, peanut, sesame, and sunflower for bread production. They used the straight dough method. The loaf volume and crumb color of the bread baked from the oilseed-wheat flour blend were compared to a recipe using all wheat flour. The baking properties of the non heat-treated oilseed flours varied in compatibility with the wheat flour. Sunflower and cottonseed flours were the least compatible because of the reduction in loaf volume. Peanut and sesame flours were more compatible with wheat flour for baking and produced acceptable volume and interior properties in the bread (42). Using peanut and sesame flours, the best bread was produced with non-heated material, while the best bread with cottonseed and sunflower was produced with heat-treated flours.

The question arises now as to whether the use of these products with wheat flour would increase the protein quality of the resulting baked products.

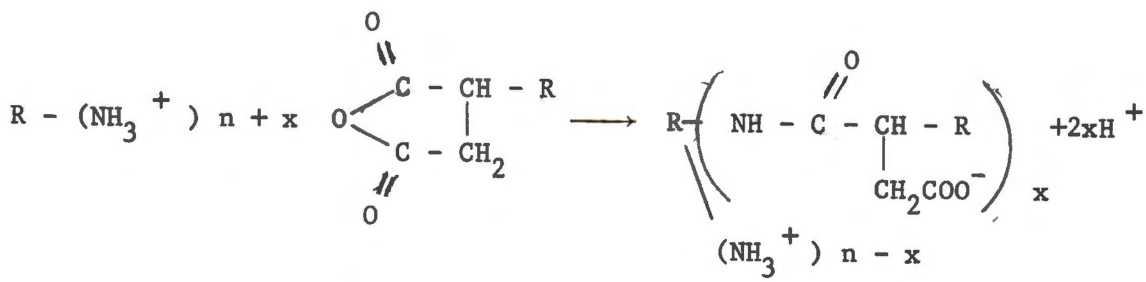
Jones and Divine (33) demonstrated that the growth-promoting value of a mixture of five parts of cottonseed flour to ninety-five parts of wheat flour was higher than that of wheat flour alone when fed to rats at a level of 9.1 percent protein in the ration.

In 1954, Womack, Marshall, and Summers (53) studied the nutritive value of bread and cookies containing cottonseed flour. They used weanling male rats in their study. Bread containing 10 parts of cottonseed flour per 100 parts of wheat flour (white) gave significantly higher rates of gain per gram of nitrogen consumed than bread without the cottonseed flour when fed to young rats at levels furnishing 10 percent protein. They also tested the effect of the addition of cottonseed flour to bread containing non-fat milk solids. They found that no significant increase in the nitrogen efficiencies was brought about by the cottonseed flour when the breads were both fed at the same protein level. However, when the breads were fed at the same percentage by weight, the higher protein content of bread containing cottonseed flour brought about significantly higher rates of gain than the same amount of bread without cottonseed flour. As a conclusion from this study they showed that the incorporation into white bread of either 10 parts of cottonseed flour or 4 parts of milk solids will improve nutritive value to about the same extent. The protein quality was measured by rat growth. Where protein supplies are limited and where milk solids are not available, cottonseed flour of the quality used in these experiments might prove useful to enhance the protein quality of bread.

Extension of cottonseed flour utilization to other types of food products might be feasible if the flour itself or a chemically modified flour were found to have high functionality. A technique which has been successfully utilized with fish protein concentrates, egg white, and wheat to alter functionality is acylation of protein with acidic anhydrides (23, 25, 26).

Succinylation is one specific type of acylation reaction that may provide special advantages in some circumstances. The reagent required, succinic anhydride, being a nonvolatile, reasonably stable solid, is easy to handle experimentally. Succinic anhydride provides a nucleus for the attachment of various functional groups and thus makes it possible to introduce such groups into proteins and other macromolecules under mild, simple circumstances (36).

The reaction in general may be represented as follows:



Depending on the ratio of moles of reagent x to protein, R - $(NH_3^+)n$, the number of groups introduced may vary from a small number all the way up to n, i.e., up to complete coverage of amino groups of the protein (28). The use of succinic anhydride as a modifying agent in the study of proteins was first described by Habeeb et al. (28). Since then the technique has been investigated and discussed by several others (24, 31, 37, 47). Succinic anhydride reacts with free amino groups, tyrosyl hyroxyl groups, and sulfhydryl groups in proteins, forming amide ester and thioester linkages, respectively. The tyrosyl ester and thioester linkages are hydrolyzed spontaneously in aqueous media within a few hours (24, 47). Little reaction occurs with alcoholic hydroxyl groups of serine and threonine (24, 32).

As a result of the reaction, each amino group with its potential positive charge is blocked, while at the same time an additional carboxyl group is introduced. Thus the pattern of inter- and intramolecular electrostatic forces is markedly altered.

McElwain et al. (44) studied the functional properties of succinylated single cell protein concentrate. The purpose of modification of single cell protein (NSI) by succinylation was to alter the protein in such a way as to improve its functional properties which include solubility, emulsification stability and apparent viscosity. Single cell protein concentrate was prepared by extracting dried Condida utilis with 0.2 N NaOH at 95°C. for 10 minutes. The soluble protein was precipitated at pH 3.5, washed and lyophilized. Portions of the protein isolates were succinylated so that 84 percent of the free amino groups were blocked. As a conclusion from this study, they found that the functional properties of succinylated (SI) and non-succinylated (NSI) single cell protein concentrate exhibited similar solubilities above pH 4, the apparent isoelectric point. At pH values below 4, SI was quite insoluble whereas NSI was very soluble at pH 2. Stabilities of emulsions prepared from NSI and SI tended to be comparable with NSI being slightly better. Apparent viscosities of emulsions increased with increasing oil content and increasing emulsifier concentration.

Gandhi et al. (23) used 3, 3-dimethylglutaric anhydride to modify egg white protein. They studied the heat stability of the glutarated egg white by using it in angel food cake. The heat stability and emulsifying ability increased significantly at certain pH and temperatures. These results may offer the egg products technologist a way to control the effect of heat on liquid egg white and to permit pasteurization at temperatures more useful for eliminating pathogens from the products.

Grant (25) modified wheat flour proteins by the succinylation process. He treated wheat flour with succinic anhydride according to Habeeb's method (28) with some modification. Solid succinic anhydride was added in small portions to an aqueous protein solution. The pH was maintained near 8.5 by the addition of 1.0 or 2.0 M NaOH solution from a burett over a period of 30 minutes. The reaction mixture was stirred continuously at ice bath temperatures. A 5 percent solution of succinic anhydride in 1, 4-dioxane was added gradually from a burett. The insoluble residue was removed by centrifugation. Dioxane and other low molecular weight components were removed by dialysis of the supernatant against water. Solutions were concentrated by pervaporation. As a conclusion of this study, Grant showed that the removal of dioxane and excess reagents by dialysis against water did not cause any precipitation of the succinylated flour protein. Also, he observed that 5 minutes of heating in a boiling water bath caused no coagulation of the succinylated protein in aqueous solution. The apparent viscosity of succinylated protein was markedly higher than nonacylated material.

Groninger (26) studied the properties of succinylated fish-myofibrillar protein. The purpose of his study was to prepare a stable dry product that possessed desirable functional properties by succinylation of myofibrillar protein from fish muscle. Rockfish (Sebastes sp.) fillets of high quality were used in this experiment. Fish myofibrillar protein was reacted with succinic anhydride at 0°C. and a pH of 7.5-8.5 to form succinylated myofibrillar protein. The modified protein showed no coagulation or precipitation occurred during heating at 100°C. due to heat stability, rapid rehydration, relatively good dispersion in the pH range of 6.0-8.5, relatively high emulsification capacity and had a relatively bland odor and flavor. Groninger also showed that the dried protein was organoleptically stable when stored at ambient temperatures with no special precautions to protect it from atmospheric oxygen and light.

Myofibrillar protein could be succinylated at various levels, and the degree of succinylation was related to functional properties such as emulsification capacity. The protein efficiency ratio for succinylated protein was somewhat lower than that of unsuccinylated fish protein.

Groninger and Miller (27) studied the properties and uses of an enzyme-modified succinylated fish protein. They used high quality Rockfish (Sebastes sp.) and succinylated the myofibrillar protein according to the method of Groninger (26). Fish protein was hydrolized and the degree of hydrolysis was measured by the Lowery method (40). The hydrolyzed, succinylated protein was substituted for egg white in various foods on the basis that egg white contained 88 percent moisture. The protein was incorporated into a dessert topping, a soufflé', and both a chilled and a frozen dessert in order to test the performance of this protein under a variety of conditions. These foods were subjected to taste panel evaluation by a small expert panel. No detectable fishlike odors or flavors were found in the four foods tested. These four foods were as acceptable to the panel as similar foods that contained no fish protein.

Groninger and Miller (27) also showed that samples of the hydrolyzed, succinylated protein that were stored at ambient temperature in air and unprotected from light for a period of 4 months did not show any evidence of deterioration in aeration or organoleptic properties.

Recently Chen et al. (11) studied some functional properties of succinylated proteins from fish protein concentrate (FPC). FPC was prepared from hake by extraction with hot isopropanol, partially solubilized by succinylation with succinic anhydride followed by heating at pH 9 for 30 minutes. The yield was 73 percent of the FPC, and the degree of succinylation of this protein isolate was 90 percent. FPC was also extracted at pH 11.5. The yield was approximately 50 percent. The isoelectric point of succinylated fish protein was about pH 4, whereas that of fish protein extracted at high pH was about pH 5. The functional properties of FPC were altered by succinylation. Chen et al. (11) showed that succinylated fish protein generally has a greater emulsion capacity and stability than did fish protein extracted at high pH which in turn had a greater emulsion capacity and stability than did gelatin. They also showed that succinylated fish protein formed a strong cheese-like curd upon quiescent acidification, whereas fish protein extracted at a high pH formed a very fragile curd.

Childs and Park (13) studied the functional properties of acylated glandless cottonseed flour. Glandless cottonseed flour was acylated according to the method of Habeeb et al. (28). Succinic or acetic anhydride was added to the flour suspension over a period of 60 minutes with constant stirring in an ice bath at 0°C. The pH of the reaction was maintained at pH 7.5-8.5 by addition of 4 N NaOH. The acylated material was dialyzed overnight at 4°C.

against distilled water and freeze dried. Acylation of cottonseed flour caused an increase in the specific viscosity of the flour and an increase in its functionality. Water-holding, oil-holding, emulsifying and foam capacities were increased from 1.2 to tenfold over nonacylated flour (12, 13). These workers adapted a method for emulsifying capacity developed for meat protein (10).

CHAPTER III

MATERIALS AND METHODS

I. ACYLATION OF COTTONSEED FLOUR

Source of Cottonseed Flour

One lot of glandless cottonseed flour was provided by National Cottonseed Products Association, Memphis, Tennessee. This was stored at 4°C. in a sealed plastic bag until used.

Acylation of Cottonseed Flour

Acylated cottonseed flour was prepared by a modification of the method described by Habeeb et al. (28). Two kg. of flour were suspended in 40 l. of water. Two hundred g. of succinic anhydride were added to the flour suspension over a 60 minute period with constant stirring at room temperature. The succinic anhydride was added in small portions, e.g. 20 g. every 6 minutes. The pH of the reaction was adjusted to 8.0 (± 0.5) with 50 percent NaOH after each addition.

When all the anhydride had been added the pH was lowered to 4.0 (± 0.5) by the addition of 6 N HCl. Then the suspension was centrifuged at 1000 x g. for 10 minutes to sediment the acylated flour.

The acylated flour was resuspended in 5 l. of deionized water to remove residual succinic anhydride. The flour suspension was then centrifuged at 1000 x g. for 10 minutes. The washing technique was repeated twice.

The acylated flour was lyophilized and held at room temperature in sealed brown glass jars until used.

Water-Holding Capacity

For the determination of water-holding capacity (WHC), the method of Childs and Park (13) was used. One-tenth g. of flour was weighed into a tared 15 ml. glass centrifuge tube and 5.0 ml. of 0.02 M citrate buffer (pH 3.5) was added. The contents of the centrifuge tube were mixed 2 minutes on a vortex mixer. The centrifuge tube and its contents were centrifuged 15 minutes at 500 x g. The supernatant was decanted and the tube was allowed to drain for 5 minutes and the inside of tube above the pellet was wiped with absorbent paper. The weight of the pellet was determined and bound water was calculated as WHC (g. HOH/g. flour).

Oil-Holding Capacity

Oil-holding capacity (OHC) of the acylated flour was determined the same way as water-holding capacity, except cottonseed oil was used instead of citrate buffer. The bound oil was calculated as oil-holding capacity of flour (g. oil/g. flour).

Emulsifying Capacity

The method of Webb et al. (52) was followed to determine emulsifying capacity. One-tenth g. of flour was blended into 30 ml. of 5 percent NaCl solution for 30 seconds at 12,000 rpm. Then 20 ml. of cottonseed oil was added from a burette and blended 30 seconds at 12,000 rpm. With continuous blending, oil was added slowly until an ohmeter attached to the cell indicated a sharp break in resistance. The volume of oil was recorded and calculated as emulsifying capacity (ml. oil/g. flour).

Three replicates were used for WHC, OHC, and emulsifying capacity.

II. USE OF ACYLATED COTTONSEED FLOUR IN WHITE LAYER CAKE

Preparation of White Layer Cake

The basic cake recipe was that of Brockmole and Zabik (9):

Ingredient	wt. (g.)
Flour	84.34
Sugar	118.08
Shortening	42.17
Nonfat Dry Milk	10.12
Dried Egg Whites	7.59
Salt	2.53
Water	130.73
Baking Powder	4.42

Cake Preparation

The cakes were prepared according to AACC specifications method 10-90(1). The preweighed dry ingredients, shortening, 60 percent of the distilled water and baking powder were mixed in a Kitchen-Aid mixer, model K-5, at low speed (145 rpm) for 1/2 minute. The bowl was scraped and mixing was continued for an additional 4 minutes at medium speed (249 rpm). Half of the remaining water was added and the batter was mixed for 1/2 minute at low speed, the bowl scraped, and the batter mixed 2 minutes at medium speed. The remaining water was added and the batter was again mixed for 1/2 minute at low speed, the bowl scraped, and the batter mixed for 2 minutes at medium speed.

Weighed to the nearest gram, 400 g. of batter was scaled into an 8 inch round cake pan. The cakes were placed on the middle shelf of a Dispatch rotary oven and baked for 25 minutes. The oven temperature was maintained at 178 ±3°C. After removal from the oven the cakes were allowed to cool in the pan for 30 minutes. They were then removed from the pan onto racks and allowed to cool for an additional 30 minutes before evaluation.

Cakes were prepared according to the following schedule, using cottonseed flour to replace wheat flour:

1. 100 percent Wheat Flour (WF).
2. 88 percent WF, 12 percent Cottonseed Flour (CF).
3. 88 percent WF, 12 percent Acylated Cottonseed Flour (ACF).
4. 94 percent WF, 6 percent CF.
5. 94 percent WF, 6 percent ACF.
6. As #1 except plus Na-stearoyl-2-lactylate (0.5 percent of the weight of shortening).
7. As #2 except plus Na-stearoyl-2-lactylate (0.5 percent of the weight of shortening).
8. As #3 except plus Na-stearoyl-2-lactylate (0.5 percent of the weight of shortening).
9. As #4 except plus Na-stearoyl-2-lactylate (0.5 percent of the weight of shortening).

10. As #5 except plus Na-stearoyl-2-lactylate (0.5 percent of the weight of shortening).

Two replicate cakes were prepared with each flour or flour combination.

III. MEASUREMENTS RELATED TO CAKES OR BATTERS

Consistency of Cake Batter

Consistency was determined by the use of a horizontal flow consistometer, the Bostwick Consistometer. The consistometer was filled, leveled, then the batter was allowed to flow for 5 minutes at 23-24°C. Flow distance (cm.) was recorded.

Density of Cake Batter

Density was determined according to the Platt and Kratz method (45). Water was pipetted into a weighing pan to determine its volume. The weight of the empty pan was determined, and then it was filled with batter, leveled and weighed with batter. The density of the batter was calculated as weight per unit volume (g./cc.).

pH of Cake Batter

The method of Ash and Colmey (4) was used to determine pH of cake batter. Fifteen g. of batter was weighed in a beaker, 100 ml. of deionized water was added and allowed to stand for 30 minutes at room temperature with occasional agitation. The solids were allowed to settle and the liquid was poured off. The pH was then read on a pH meter and recorded.

Direction for tests applicable to cake batters was gained from the work of Donelson and Wilson (19).

Determination of Alkaline Water Retention Capacity (AWRC) of the Flour in the Cake

The method by Yamazaki et al. (54) was used to determine AWRC. A 1.00 g. flour sample was weighed into 100 x 15 mm. culture tube with a screw cap and 5.0 ml. of 0.1 N sodium bicarbonate solution was added to the flour sample. The tube was closed and shaken vigorously by hand to suspend the flour. The culture tube with its contents was allowed to stand for 20 minutes to hydrate the flour. The suspension was shaken every 5 minutes, then centrifuged for 15 minutes at 1000 x g. The centrifuge was allowed to stop without braking. The supernatant liquid was decanted, the tube was drained, and the gel pellet was weighed.

The AWRC was expressed as percent weight gained over 1.00 g. and the result was adjusted to the flour moisture basis.

$$\text{AWRC} = \text{percent } \frac{\text{wt. of flour}}{\text{wt. of gel}} - \text{percent moisture of flour}$$

Moisture Content of the Flour

A 2.0 g. sample of each flour used in the cake batter preparation was weighed into a tared pan. The flour sample was dried to a constant weight in vacuum oven at 65°C. and 380 torr. The weight of the dried sample was recorded. The weight of dried flour was subtracted from the initial weight of flour (or before drying) to get the weight of moisture in the flour. The percent moisture of flour was calculated.

Measuring Cake Volume

Volume of each cake was measured with a cake volume meter using rapeseed displacement. Specific cake volume was determined as:

$$SCV = \frac{\text{vol. of cake (cc.)}}{\text{wt. of cake (g.)}}$$

Protein Analysis

Protein was analyzed by Kjeldahl method using a sample of 2 g. and a conversion factor of 5.7 x percent N.

Sensory Evaluation

Overall acceptability of the cakes was assessed by an expert panel of 6 people (22). The criteria for judgments were listed on a score card, Figure 1, adapted from a form developed at Cornell University (15). Judges examined portions of each cake in individual carrels under cool-white fluorescent lighting and scored the cakes using score cards (Figure 1) which did not have the number-value designations (see footnote, Figure 1). Application of the number-values after the fact would have given data which could have been subjected to statistical analysis. Then the judges, as a group, examined the half of each cake remaining from the individual assessments and came to a consensus judgment of each cake, again using the criteria listed on the score card.

Data Analysis

All objective data were analyzed statistically by means of analysis of variance. Where appropriate, means separation was measured by Duncan's Multiple Range Test (50). Consistency,

	Score[1]
Size of cells	
large	3
medium	10
small	10
very fine	3
compact	1
very compact	1
Distribution of cells	
uniform	6
irregular	4
tunnelled	2
Crumb characteristics	
velvety	10
slightly harsh	8
very harsh	2
Tenderness	
crumbly	3
very tender	6
tender	10
slightly tender	8
tough	3
Moisture	
moist	10
dry	5
wet	2
Flavor	
well-balanced	10
sweet	5
salt	2
bitter	2
Off-flavor	
slight	2
definite	1
Color	

Figure 1

Score Card for Assessing Appearance and Palatability Qualities of Cakes[1]

[1]The number values did not appear on the judges' score cards (15).

density, pH, volume, and protein measurements were determined on 2 replicates. Three replicates were used for AWRC and moisture on the flour mixtures.

CHAPTER IV

RESULTS AND DISCUSSION

The functionality of acylated cottonseed flour at different levels of acylation was studied. The results are shown in Table II.

The degree of acylation by succinic anhydride was essentially complete when 0.1 g. anhydride per gram of flour was added. Attempts were made to measure the degree of acylation by measuring residual epsilon-amino groups of lysine (34). Wide experimental variations were encountered which could not be eliminated; therefore, that method was abandoned. No significant changes in functionality were noted when levels of succinic anhydride in excess of 0.1 g. per gram of flour were added. Hence acylation was assumed to be complete with that treatment and it was used to acylate flour for product application.

As shown in Table II acylation of glandless cottonseed flour increased its water holding capacity greatly. Succinylated cottonseed flour (0.1 g./g. acylation level) bound 1.6 times more water than unmodified glandless flour. Oil holding capacity was markedly increased by acylation. Succinylated flour bound 1.75 times more oil than unmodified flour. The reaction of succinic anhydride with free amino groups, tyrosyl hydroxyl groups and sulfhydryl groups in proteins, forming amide ester and thioester linkages, respectively, will block the potential positive charge, while at the same time an additional carboxyl group is introduced. This evidently did affect the water holding capacity of the protein.

TABLE II

FUNCTIONALITY OF ACYLATED COTTONSEED FLOUR AT DIFFERENT LEVELS OF ACYLATION[1,2]

Succinic Anhydride (g./g. flour)	Water-holding Capacity (g./g.)	Oil-holding Capacity (g./g.)	Emulsifying Capacity (ml./g.)
0.0 (control)	2.921c	3.280b	990a
0.02	4.312b	6.187a	500c
0.1	4.768ab	5.718a	680b
0.2	4.487ab	5.351a	700b
0.4	4.911a	7.113a	570c
1.0	3.097c	6.087a	560c

[1]All data are the mean of triplicate samples.

[2]Means in columns followed by the same letter are not significantly different at 5 percent level.

The increase in oil holding capacity could be due to the unfolding of the polypeptide chain of the protein.

Emulsifying capacity of the succinylated flour was lowered as shown in Table II. The chemical and physical changes in the protein worked to reduce the capacity of the flour to hold droplets of oil in suspension. This was unexpected, since molecular unfolding would be expected to increase emulsifying capacity of a protein. Perhaps the reversal was due to influences from changes in surface charges or to some undetermined effect on flour components other than protein. Water and oil holding capacity results agreed with (13), emulsifying capacity did not.

Defatted oilseed flour and non-wheat cereal flours have been used as partial replacements for wheat flour in yeast bread to increase the nutritional value (39, 41, 42, 49). Bacigalupo et al. (5) found that up to 5 to 10 percent replacement of wheat flour by non-wheat replacement levels severely decreased loaf volume. Others have found serious deterioration of crumb color, grain and texture (42, 49).

Since some of the physical functionalities of cottonseed flour were improved by acylation as shown in Table II, further research was designed to determine the differential effects of the substitution of varying levels of cottonseed and acylated cottonseed flour for wheat flour on the physical and sensory quality characteristic of white layer cakes.

Tsen and co-workers (51) used sodium stearoyl-2-lactylate (SSL) and Ca-stearoyl-2-lactylate to produce sponge dough bread with 12 percent soy flour. The dough conditioners increased loaf volume and improved the organoleptic properties of the soy bread compared to bread made with 100 percent wheat flour. Their results induced as to use SSL in the cakes, hopefully to overcome anticipated detrimental effects from the CF and ACF.

Two series of batters and cakes were prepared with two levels (6 and 12 percent) of cottonseed flour (CF) and acylated cottonseed flour (ACF). One cake series was prepared without SSL added and the other series was prepared with 0.5 percent SSL added to the shortening.

Physical measurements (consistency, pH, density, loaf volume, specific cake volume, and protein contents) of cakes and cake batters are shown in Table III.

Table IV shows which measurements were significant by analysis of variance. From the analysis of variance, it was found that consistency and density of the cake batter prepared with 100 percent wheat flour was not significantly changed by the substitution of either cottonseed flour or acylated cottonseed flour. However, according to Table III, the consistency of the cake batters prepared with either 6 or 12 percent acylated cottonseed flour with SSL added tended to be more viscous.

The pH of the cake batters decreased when either 6 or 12 percent ACF was added (Table III). Loaf volume and specific volume of cakes prepared with 6 or 12 percent of CF or ACF, with or without emulsifier SSL are also shown. By increasing the level of ACF to 12 percent the volume was decreased even with SSL added. But at

TABLE III

MEANS[1] FOR CONSISTENCY, pH, DENSITY, VOLUME, SPECIFIC VOLUME, AND PROTEIN CONTENT OF WHITE CAKES OR BATTERS PREPARED WITH DIFFERENT FLOUR COMBINATIONS

	Batter			Cakes		
Flour Combination (%)	Consistency (cm.)	pH	Density (g./cc.)	Loaf Volume (cc.)	Specific Cake Volume (cc./g.)	Protein (%)
100% WF[2]	1.58	7.1a[3]	0.776	955ab	2.38	4.18g
88% WF, 12% CF[4]	1.73	7.0abc	0.772	838bc	2.10	5.55abc
88% WF, 12% ACF[5]	1.05	6.75cdef	0.787	763cd	1.89	5.86a
94% WF, 6% CF	1.95	7.0abc	0.768	840bc	1.59	4.53def
94% WF, 6% ACF	1.10	6.75cdef	0.759	775cd	2.01	4.99cde
100% WF, SSL[6]	1.83	6.95abcd	0.777	965a	2.36	4.22defg
88% WF, 12% CF, SSL	1.95	7.0abc	0.747	835bcd	2.06	4.77de
88% WF, 12% ACF, SSL	0.88	6.55f	0.731	713d	1.72	5.63ab
94% WF, 6% CF, SSL	1.70	7.05ab	0.758	863abc	2.16	5.02cde
94% WF, 6% ACF, SSL	0.78	6.8bcde	0.716	832bcd	2.05	5.14bcd
Standard Deviation	0.52	0.20	0.03	85.03	0.325	0.643

[1]All data are the mean of two replicates.

[2]WF = wheat flour.

[3]Means in columns followed by the same letter are not significantly different at 5 percent level.

[4]CF = cottonseed flour.

[5]ACF = acylated cottonseed flour.

[6]SSL = Na-stearoyl-2-lactylate (0.5 percent of the weight of shortening).

TABLE IV

ANALYSIS OF VARIANCE FOR OBJECTIVE MEASUREMENTS ON BATTERS AND CAKES

Source	d.f.	Mean Square[1]
Consistency	9	0.413
Error	10	0.151
pH	9	0.069*
Error	10	0.0112
Density	9	0.00099
Error	10	0.00078
Loaf Volume	9	12417.92*
Error	10	2561.25
Specific Cake Volume	9	0.1254
Error	10	0.0874
Protein	9	1.338*
Error	30	0.136

[1]An asterisk indicates significance at the 5 percent level.

the lower substitution level, 6 percent, and SSL added, the volume was improved. It was found that substitution of wheat flour by even the low level of either CF or ACF significantly decreased cake volume. Although specific volume varied rather widely, the variation was not significant.

The performance of wheat for baking purposes is due largely to the unique property of the proteins of the wheat endosperm in forming gluten when wetted with water. When wheat flour is mixed with water the proteins hydrate, forming gluten, a typically elastic, coherent mass which makes possible the formation of a dough. Such a dough will retain the carbon dioxide produced by yeast or chemical leavening agents, e.g. baking powder (3). The addition of cottonseed flour in either form, ACF or CF, interfered with formation of gluten and then a gluey type batter was formed. This affected volume and texture of the final product.

Alkaline water retention capacity (AWRC) of a flour gives some indication of how a flour will perform in baked products (54). This property of the cake flour mixtures was determined. The results are shown in Table V.

It was found that AWRC and moisture content of WF, CF, and ACF were significantly different from each other (see Table VI). The AWRC of WF mixtures was decreased by the substitution of ACF but not by CF. The AWRC values were lower for both CF and ACF than for wheat flour. It is interesting to note that, in combination with wheat flour, untreated cottonseed flour had no effect on AWRC. However, both levels of succinylated cottonseed flour lowered AWRC

TABLE V

MOISTURE AND ALKALINE WATER RETENTION CAPACITY OF FLOUR IN DIFFERENT COMBINATIONS[1]

Flour Combination	Moisture (%)	AWRC[2,3]
Wheat Flour (WF)	10.70a	42.54a
Cottonseed Flour (CF)	7.20d	22.71d
Acylated CF (ACF)	1.11e	26.89cd
88% WF, 12% CF	9.96bc	43.04a
88% WF, 12% ACF	9.47c	32.68bc
94% WF, 6% CF	10.39ab	42.46a
94% WF, 6% ACF	9.68c	38.39ab

[1]All data are means of three replicates.

[2]Means in columns followed by the same letter are not significantly different at 5 percent level.

[3]AWRC--Alkaline Water Retention Capacity.

TABLE VI

ANALYSIS OF VARIANCE FOR ALKALINE WATER RETENTION CAPACITY (AWRC) AND MOISTURE OF FLOURS AND FLOUR MIXTURES

Source	d.f.	Mean Square[1]
Moisture	6	34.58*
Error	14	0.09
AWRC	6	199.90*
Error	14	13.64

[1]An asterick indicates significance at the 5 percent level.

when mixed with wheat flour. An interaction is apparent which differs depending on the type of cottonseed flour. These data also imply that the water holding capacity of acylated cottonseed flour is pH dependent. At pH 3.5 (see Table II, page 26) acylation increased water holding capacity about 1.6 fold.

The protein content of cakes are shown in Table III, page 29. The cakes prepared with 12 percent CF or ACF had significantly higher protein content than the other cakes with one exception. All wheat flour cakes had lower protein content as shown in Table III, but the cakes prepared with 6 percent CF or ACF added had protein levels which were intermediate between all WF and WF + 12 percent CF or ACF. Protein content was determined for the cakes, not on a dry weight basis. It seemed more appropriate to measure protein in the cakes as they would be eaten. Thus, the moisture content of the cakes would affect the protein content due to the difference in moisture content of the cakes. By adding 12 percent CF, total protein content of the cakes was increased about 30 percent. However, the increase was from about 4.2 percent to 5.6 percent.

Sensory evaluation of the two replicate sets of cakes was carried out by using an expert panel and generating a "flavor profile" of each treatment. Six people were chosen who had had considerable experience with bakery products. Two training sessions were conducted. For each session cakes were baked which spanned the sensory range expected in the experimental cakes. The panel judged the cakes using a score card (15) which described cake quality. The score card is shown in Figure 1, page 23. Some modifications were added to fit the present situation, particularly with regard to off-flavor and color. After assigning the scores that the panelists judged were right for each cake, the group discussed each cake and came to a common conclusion about each one of the quality items.

When each of the two sets of 10 experimental cakes were baked, samples of each cake were presented to the panelists in individual booths along with score cards. The samples were examined and scored. Cool-white flourescent lights were used in all cases. One-half of each cake was used for this purpose.

After examining the samples in private, the group examined the other half of all the cakes together and came to an agreed description of each of the 10 samples. The individual scoring resulted in data which could have been analyzed. However, differences in the treatments were rather obvious, and it was felt that in this case the group's opinion, or the "flavor profile," of each treatment would have more meaning. In the opinion of the group, cakes from the two replicate sets were similar.

The cakes prepared with wheat flour (with or without SSL) were judged to have good volume, to be white in color, and had very fine but irregular air cells. They were also tender and moist cakes. The crumb characteristic of the cake was velvety and the flavor was well-balanced.

The cakes prepared with either CF and ACF substitution were judged inferior to the cakes prepared with wheat flour. Cake volume was reduced, the size of cells were between compact and very compact, cells were irregularly distributed, and the moisture characteristic of the cakes was high and the cakes were wet. There was an off flavor in the cakes prepared with CF or ACF but the cake with ACF added had less off-flavor compared to those prepared with CF. Also the color of the cakes was changed. The color of the cakes with CF had a grayish color but cakes prepared with ACF had an off-white to yellowish creamy color.

There was no significant changes in the cakes' characteristics by adding SSL. It had been hoped that the addition of SSL, an emulsifier, to the shortening would help to overcome the effect of the CF and ACF on the physical character of the cakes. Such was found to be true with the addition of soy flour to bread (51). In this instance, however, SSL did not make up for changes brought about by the substituted flours.

CHAPTER V

SUMMARY AND CONCLUSIONS

In this study, glandless cottonseed flour was acylated by using succinic anhydride. Based on the results the following conclusions were made:

1. One-tenth gram succinic anhydride per gram of glandless cottonseed flour completed acylation of the protein in the flour. The functionality of acylated cottonseed flour was significantly affected.

2. Water holding capacity of acylated cottonseed flour was significantly increased by acylation, approximately 1.6 fold.

3. Oil holding capacity of acylated flour was significantly increased, 1.75 fold, by acylation.

4. Emulsifying capacity of the acylated flour was decreased by acylation, regardless of the level of succinic anhydride utilized.

Acylated and untreated cottonseed flours were used in the preparation of white layer cakes as a partial replacement of wheat flour. Physical characteristics of cakes and cake batters were studied.

1. Consistency of cake batters prepared with wheat flour did not significantly change when either ACF or CF was added at 6 or 12 percent of the wheat flour.

2. pH of cake batters was significantly decreased by adding ACF but not CF.

3. ACF and CF did not influence the density of cake batter prepared when substituted for 6 or 12 percent of the wheat flour.

4. Loaf volume of cake prepared with WF was significantly decreased by adding either CF or ACF even when SSL was added to the shortening. There was no influence on the specific volume of the cake.

5. Alkaline water retention capacity of wheat flour was decreased by the addition of ACF. The addition of CF did not significantly change AWRC of flour mixtures.

6. Protein content of cakes prepared with all wheat flour was significantly increased by the addition of CF or ACF.

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