This is to certify that the

thesis entitled

FUNCTIONALITY OF LIQUID CYCLONE PROCESSED COTTONSEED FLOUR IN BREAD SYSTEMS

presented by

Mona A. El-Minyawi

has been accepted towards fulfillment of the requirements for

Ph.D. degree in Food Science & Human Nutrition

Major professor

Date 7/21/80

[illegible]	NOV 14 '90
[illegible]	Jan 4 [illegible]
[illegible]	Feb 4 '91
DEC 7 '89	[illegible]
Jan 8 '90	APR 7 '91
Feb 6 '90	[illegible]
[illegible]	Jul 10 '91
APR 4 '90	[illegible]
May 8 '90	[illegible]
Jun 20 '90	Oct 30 '91
Jul 12 '90	DEC 2 '91
	Jan 8 '92
D204	[illegible]
AUG 20 [illegible]	APR 1 '92
SEP 04 '90	[illegible]
OCT 19 '90	137
	JAN 13 '92

FUNCTIONALITY OF LIQUID CYCLONE PROCESSED COTTONSEED FLOUR IN BREAD SYSTEMS

By

Mona A. El-Minyawi

A DISSERTATION

Submitted to

Michigan State University

in partial fulfillment of the requirements

for the degree of

DOCTOR OF PHILOSOPHY

Department of Food Science and Human Nutrition

1980

ABSTRACT

FUNCTIONALITY OF LIQUID CYCLONE PROCESSED COTTONSEED FLOUR IN BREAD SYSTEMS

By

Mona A. El-Minyawi

The effects of Liquid Cyclone Processed cottonseed flour (LCP) substitution were studied in wheat dough systems. Farinograph studies showed increase in the water absorption and arrival time as well as decrease in stability as the level of cottonseed flour increased in dough systems. It was found that increasing the level of substitution with starch decreased farinograph water absorption, arrival time, and increased stability and resistance to mixing. LCP cottonseed flour breads were evaluated for acceptability; color was found to be the most objectionable character of the bread.

The effect of salt, conditioner and oxidant were studied in 0, 8 and 16% LCP cottonseed flour substituted dough systems. Mixograph studies showed that each additive added separately had strengthening effect on dough systems; this strength was much more noticeable in the substituted dough with 8 and 16% LCP cottonseed flour. Potassium bromate in combination with any other additive did not affect dough rheology; salt showed a strengthening effect in all the double and triple combinations of

additives and it increased with increasing the salt level.

Baking studies using 8% LCP cottonseed flour with single additives indicated that 1% salt produced high volume bread. The effect of double combinations of additives significantly increased crumb softness, and maximum softness was obtained with the combination of 1% salt, 30 ppm $KBro_3$. Triple combinations of additives had better effect on bread characteristics with combination of 1% salt, 1.5% dough conditioners (Tween 20), and 50 ppm $KBro_3$ producing the softest crumb.

The functionality of LCP cottonseed flour was studied in Egyptian "Baladi" bread system. Bread baked with 0, 4, 8, 12 and 16% cottonseed flour showed a decrease in bread volume as the cottonseed level increased. Sensory data indicated that bread was acceptable even at 12% level of substitution.

Mixograph studies showed the maximum effect of four types of dough conditioners on peak time, peak height and stability to be 0.5% conditioner. Egyptian bread baked with these conditioners, retained its softness after 3 days of storage when Tween 20 and Tandem 552 were added at 0.5% level. The softness was retained after 6 days when Tween 20 was added. The protein content of substituted bread increased significantly, as well as the lysine content of the substituted bread.

Mona A. El-Minyawi

Control and substituted doughs were modified using different chemical reagents. Low levels of urea, sodium-dodecyl-sulfate and succinic anhydride, caused slight strengthening to both dough systems. High concentrations of reagents caused weakening of dough. The ratio of SS/SH that are involved in dough mixing is higher in stronger doubh than weaker ones. This ratio also indicated that there was more disulfides involved in resistance to mixing than thiols in both control and LCP substituted systems.

Scanning electron microscopy of control and substituted dough systems shows that LCP cottonseed flour incorporated in dough gave thicker, less flexible gluten matrix than the control and some pockets were evident when dough conditioner (Tween 20) was added (0.5%), the protein matrix seemed more fluid, flexible and continuous. Fixation, dehydration and critical point drying as sample preparation procedures drastically altered the ultrastructure of both dough systems when compared to nitrogen freezing and freeze drying procedures.

To

My Mother, My Husband

and

My Children

ACKNOWLEDGMENTS

I wish to express my deepest appreciation to my major professor, Dr. Mary E. Zabik, for her patience, continuous support and encouragement during the course of this study, and for her assistance in the preparation of the thesis manuscript. Sincere appreciation is also extended to members of my committee, Dr. Wanda Chenoweth for her support and encouragement, Dr. J.R. Brunner and Dr. P. Markakis for their valuable advice and support, Dr. L. Minor of the Department of Hotel, Restaurant and Institutional Management for serving on the guidance committee and for his valuable advice. I also extend my deepest thanks to Dr. L.E. Dawson for attending my oral exam, and Dr. J. Gill of the Department of Dairy Science and Dr. C. Cress of the Department of Crop and Soil Science for their statistical advice and help during the preparation of this study. My deepest thanks go to Dr. W.T. Yamazaki of the Soft Wheat Lab, Wooster, Ohio for supplying the small farinograph bowl used in this study.

A special acknowledgment of appreciation goes to my mother, my husband and my children who provided encouragement, motivation and support to make this achievement possible.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The fortification of bread with oilseed proteins is expanding rapidly. At first the attention was focused on soy flour, and soy protein substitutes for bread and similar bakery foods. The initial purpose was to substitute other proteins for the nonfat dry milk in bread formulas. The rising price of nonfat dry milk made it economically feasible to search for alternative protein substitutes for white bread. Another major reason of the growing interest in the use of various nonwheat flour in bakery products is the desire to improve the nutritive value of wheat flour bakery foods by increasing their protein content, in particular their lysine level.

In many of the third world nations, not only is the level of food consumption inadequate by the American standards, but the optimum utilization of indigenous crops which are often of the oilseed variety, is hindered by a lack of proper technology. With cereal foods constituting the principal staple in the diet of many peoples of those nations, any nutritional improvement by means of protein substitution would represent a major step toward a dietary improvement. It should be remembered that any benefited results achieved

in the area of nutrition improvement, would also apply to America's aged and poor people whose quality of nutrition may not meet the U.S. recommended dietary standard (Pyler, 1979).

Cottonseed offers a potential solution to increase the human consumption of protein since its annual consumption was estimated as one hundred million tons of protein, and twice as much for feeding to livestock (Bressani, 1965; Lawhon et al., 1972). In 1970 approximately 24 million tons of cottonseed was readily available as a source of protein (Harden et al., 1975). The liquid cyclone processed (LCP) cottonseed, a relatively new processing procedure, yields a low gossypol cottonseed flour. A LCP plant has been installed in Texas, and the anticipated daily yield is 20-25 tons (Ziemba, 1972).

In this research, the rheological properties of wheat flour substituted with LCP cottonseed flour was studied. As a control the untreated wheat flour was substituted at 0, 4, 8, 12 and 16% levels of LCP cottonseed flour. Bread was baked to determine the effect of the different levels of LCP cottonseed flour on final product.

Salt (0, 1.0, 2.0%), potassium bromate (0, 30, 50 ppm) and dough conditioner polysorbate (20) (0, 0.5, 1.5%), were added to HRW wheat/LCP cottonseed flour dough and the effect on dough rheology was studied using the mixograph. The effect of these additives and the potential of their

interactions was studied in bread substituted with 8% cottonseed flour. The main objective was to determine the level of each additive that would produce optimum bread volume, grain texture, and tenderness.

Four dough conditions at three levels each were added to the HRW wheat/LCP cottonseed flour dough, and their rheological properties were studied using the mixograph. Egyptian bread was baked with 0, 4, 8, 12 and 16% LCP cottonseed flour, and the effect of dough conditions on the Egyptian bread were evaluated objectively, and for taste, appearance and tenderness after 3 different periods of storage. The objective was to test the functionality of LCP cottonseed protein in Egyptian bread, and the effect of different conditioners in retaining its freshness after 3 and 6 days of storage. The effect of LCP cottonseed substitution on the amino acid content of wheat flour was calculated.

Proteins in wheat/cottonseed flour systems as well as a control were modified using sodium dodecyl-sulfate, succinic anhydride, Urea, N-ethylmaleimide and dithiothreitol on flour bonding during mixing.

The effect of cottonseed flour on the ultrastructure of wheat dough was studied using a scanning electron microscope. Dough conditioner Tween 20 was added to the control and substituted dough and the electron micrographs were evaluated.

Sample preparation for electron microscopy involves fixation, dehydration and drying. Chobat (1979) reported the effect of some sample preparation methods on the image formed. The objective of this section was to compare two different sample preparation methods on the control and LCP cottonseed flour substituted doughs.

REVIEW OF LITERATURE

Wheat Proteins

The protein content of wheat which is an important index of its quality for the manufacture of different food products is influenced by climate, weather, soil and the variety of grain. The quantity of protein is influenced to a large extent by environmental factors; the quality of protein is heritable. For many years it has been believed that differences in protein content among varieties of wheat grown under the same conditions were small as compared to the environmental differences. In recent years, it has been shown that the protein content of wheat can be increased greatly by selective breeding (Pomeranz, 1980).

The wheat proteins include water-soluble proteins (albumins), salt-soluble proteins (globulins), alcohol-soluble proteins (prolamins or gliadins), and acid- and alkali-soluble proteins (glutelins). The wheat endosperm proteins (gliadin and glutenin) form a colloidal complex known as gluten, when water is added to them. The gluten complex is responsible for the superior performance of wheat over other cereals for the manufacture of leavened products, because of its ability to retain carbon dioxide produced by

yeast or chemical leaving agents (Pomeranz, 1980).

In 1745 Becarri reported the first separation of gluten from the starch of flour by adding 60 to 65% water to a hard wheat flour, mixing and allowing the dough to rest for 30 minutes, then washing out the bulk of starch under steady stream of water. An elastic, rubberlike material holding, roughly, two thirds of its weight of water was obtained (Sullivan, 1954). The gluten protein can be about equally divided into two classes - gliadin which is soluble in 70% ethanol and glutenin which is insoluble in ethanol. The cohesive and elastic properties of gluten are a composite of these two fractions. The gliadin when hydrated is fluid, whereas glutenin is cohesive and elastic but much tougher than the gluten formed.

Gluten proteins compose about 80-85 percent of the wheat flour proteins, while the water soluble albumins and salt-soluble globulins constitute the remaining 15-20 percent (Krull and Wall, 1969). For a single wheat variety, it is well known that loaf volume is directly proportional to the protein content, however, there is disagreement as to which wheat protein fraction is responsible for the variation in loaf volume among wheat varieties (Khan, 1978). Pomeranz (1965) published one of the first reports on the functionality of glutenin in bread making. He reported that there was an inverse relationship between the wheat proteins solubilized by 3M urea and baking quality, while loaf

volume was directly related to the proteins insoluble in 3M urea. The baking studies on reconstituted flour (Shorgan et al., 1969) confirmed these findings. Conversely, Orth and Bushuk (1972) concluded that glutenin is responsible for the variations in loaf volume (at constant protein content), while Hoseney et al. (1969) had concluded that gliadin proteins control the loaf-volume potential of a wheat flour. Thus, this apparent controversy remains unsolved.

The key to the functional behavior of glutenin lies in its physiochemical properties, molecular size, shape, tendency to aggregate and its amino acid composition and sequence (Khan and Bushuk, 1978). The molecular weights range from 150,000 to 3 million (Jones et al., 1961; Nielsen et al., 1962; Taylor et al., 1973). The gliadin proteins have a relatively narrow molecular weight distribution from 20,000 to 50,000 (Jone et al., 1961). The work of Pence and Olcott (1952) on the effect of disulfide reducing agents on the viscosity of gluten led to the idea that glutenin molecules consists of polypeptide subunits. Later, Woychik et al. (1964) showed by starch gel electrophoresis that some of the subunits obtained by reducing glutenin resembled gliadin but glutenin also contained subunits that were different which indicate that glutenin is not a polymer of gliadin as was believed at that time. Bietz and Wall (1973) and Orth and Bushuk (1973) both

demonstrated the uniqueness of glutenin proteins by applying sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The technique separated a highly complex mixture of subunits ranging in molecular weight from 12,000 to 134,000. Gel filtration studies by Wall (1974) and Khan (1977) showed that glutenin is composed of three groups of subunits. Group I contained subunits of MW of 68,000 to 12,000 and therefore exhibited a strong tendency to aggregate, group II contained the largest glutenin subunits, while group III contained the two major gliadin proteins (MW 35,000 and 45,000). Each group may contribute to the functional properties of glutenin.

The amino acid composition of gliadin and glutenin are very similar. Wheat proteins are composed of 21 different amino acids, and a single protein chain may contain more than 150 amino acids. Different proteins contain different proportions and sequences of the 21 amino acids, which result in variation in protein structure (Krull et al., 1969). Glutamine constitutes about 37 percent of all amino acids in single protein (Kasarda et al., 1976), glycine, alanine, valine, isoleucine, phenylalanine and proline compose 40 to 50 percent of the residues of wheat proteins. Proline, an amino acid which occurs in high levels in gluten, is of interest because of its cyclic side chain which interferes with helix formation by causing the polypeptide chain to bend (Wall and Beckwith, 1969). It is

well known that wheat proteins have an abundance of hydrophobic (e.g. valine, leucine) and hydrophilic (e.g. glutamine) residues, and a scarce amounts of charged residues (aspartic acid and arginine). As a result of this amino acid composition, the wheat proteins are compactly folded to minimize hydrophobic residue exposure to aqueous solutions thus a very small number of charged amino acid occur in the surface of the protein. In fact there are sufficient hydrophobic amino acids that all cannot be buried in the interior of the molecule and some of them must be exposed to the solvent at the surface of the protein.

The low charge density on the surface of the proteins combined with hydrophobic areas and an abundance of residues capable of forming hydrogen bonds, result in a high sensitivity to salt concentration, even at low concentration (0.005 M NaCl) (Berardin, 1978).

As a class, cereal proteins are not as high in biological value as are certain legumes, nuts, or animal proteins. The limiting amino acid in wheat endosperm is lysine (Pomeranz, 1980).

Dough Formation

The amino acid studies of different wheat varieties failed to explain the difference in their breadmaking characteristics (Pomeranz et al., 1970). The viscous and elastic properties of dough are primarily due to the

properties of its continuous phase or gluten phase. It has been reported that the viscoelasticity of gluten and dough is considered to be due to a network of protein molecules, while the rheological properties of such a network greatly depend on the number and strength of the cross-links between the protein molecule (Pomeranz, 1978). Kneading of wheat proteins with water causes hydration of the polypeptides folded in the aleurone granules, permits their partial uncoiling (particularly, the glutenins), and facilitates extensive intra- and intermolecular association of these polypeptides via several types of chemical and physical forces which together influence size, shape and subunits of wheat proteins hence their functionality (Krull and Wall, 1969; Kinsella, 1976).

Chemical bonds

The chemical bonds are divided into two major types: covalent and noncovalent forces. The only covalent bonds that are known to be significant in dough structures are the disulfide linkage between proteins, which have an energy of 49 Kcal per mole and are not broken at room temperature except by chemical reaction (Wehrli and Pomeranz, 1969). The amino acids cystine or cysteine form about 1.4% of the amino acids of gluten protein and supply the disulfides or sulfhydryls, respectively. In glutenins the disulfide bonds bind protein subunits of MW 20,000 together to form a giant structure with molecular weight of some millions,

while the disulfides in gliadin are intramolecular bonds. As early as 1936, Balls and Hale suggested that the stiffening of a dough made by oxidative flour improve is due to the formation of cross-linked (SS) from thiol groups (SH), corresponding the conversion of the amino acid cysteine into cystine.

$$2\ Pr\text{-}CH_2\text{-}SH \rightleftharpoons Pr\text{-}CH_2\text{-}SS\text{-}CH_2\text{-}Pr + 2\ H$$

Cysteine Cystine

Total disulfide and thiol contents of wheat flour are of the order of 10 and 1 μmole per g of flour respectively (Mecham, 1968), but only 1-2% of the disulfides of gluten protein are crucial to the rheological properties (Mauretzen, 1967). Goldstein (1957) was the first to suggest that the thiol-disulfide interchange reactions can explain the opening of the rigid cross-linked structure to give the viscous flow which characterizes the bread dough. The SS-SH interchange reaction can also explain the effect of oxidizing agents on the rheological properties of dough. Bloksma (1968) explained that effect as first the breaking disulfide crosslinks, and secondly their concurrent reformation by an exchange reaction with a free sulfhydryl during which the velocity of the reaction would be expected to be proportional to the combined number of sulfhydryl and

disulfide groups. The baking quality of wheat is governed by protein content and SS:SH ratio; optimum breadmaking results were obtained if the ratio SS:SH is around 15 (Belderok, 1967). This SS:SH ratio increases during flour storage. The ratio of reactive to total SS groups increase with decreasing mixing strength, and the ratio of reactive to total SH groups also increases with decreasing flour strength. Thus mixing strength appears inversely related to reactive SH and SS contents (Tsen and Bushuk, 1968).

The thiol and disulfide groups in dough can be affected by some compounds other than oxidants. The effects of cysteine, glutathione, mercaptoacetic acid, gammaglutamyl cysteine, thioctic acid and dithiothreitol, as thiol compounds on dough consistency were reported by Pomeranz (1978). The decrease in dough consistency, an increase in extensibility and acceleration of stress and structural relaxations were explained by the increase in the rate of SS-SH interchange reactions brought about by these additions. Thiol blocking compounds such as N-Ethylmalermide were reported to hinder the interchange reaction, which results in a higher resistance to extension.

Three types of non-covalent chemical bonds (such as ionic, hydrogen and VanderWaals) also occur in the dough system. The importance of ionic bonds is demonstrated by several effects of adding salt to the dough. Ions in dough may complex with some groups of lipids (Fullington, 1967),

proteins (Bennet, 1965), and pentosans (Neukom et al., 1967). Low salt concentrations (e.g. 0.005M NaCl) can effectively mask the repulsion of one charged protein molecule for another of like charge which allows a sufficiently close approach of one molecule to another to allow hydrophobic and hydrophilic interactions to form. Therefore by altering the salt concentration, the protein-protein interactions may be increased or decreased. The effect of salt concentration on protein aggregation is straight forward and it follows the ionic strength. Trivalent ions would be expected to be more effective than divalent ions which are more effective than monovalent ions (Bernardin, 1978).

Hydrogen bonds result from the affinity of hydrogens of hydroxyl, amide, or carboxyl groups for the oxygen of carbonyl or carboxyl groups. The energy of this bond, however, is low (about 8 Kcal per mole) but it is compensated for by their abundance, which is due to the high amide content of the gluten proteins (Beckwith and Wall, 1963). Hydrogen bonds cause the insolubility of gluten proteins due to the intermolecular bonds (Wehrli & Pomeranz, 1969). Reagents like urea, acetamide and sodium salicylate can salinate the hydrogen bonding capacity of a single chain and by competition with other peptide chains, prevent the formation of intermolecular hydrogen bonds. As a consequence, these reagents readily dissolve gluten (Bloksma, 1978). Hydrogen bonds are among the factors that determine

the rheological properties of dough in spite of their low energy. This importance was clearly demonstrated by replacing the water (hydrogen oxide) by deuterium oxide (higher bond energy than hydrogen bonds), resulting in a strengthened gluten as was shown by farinogram and extensigram studies (Tkachuk, 1968).

Van der Waals bonds and dipole-induced-dipole interactions provide very weak bonds (up to 0.5 Kcal per mole). They are not significant in the presence of stronger bonds, and at distances longer than 5 Angstroms. They may play a role in attraction between nonpolar amino acid residues or fatty acid side chain in systems with limited water where hydrophobic bonds are impossible. They play a role in stabilizing starch-glyceride complex which has been postulated to affect baking and bread characters.

Hydrophobic bonds may contribute to both plasticity and elasticity. The bond energies are low enough to allow rapid interchange at room temperature and to contribute to dough plasticity. On the other hand they might stabilize conformations with small surfaces. Hydrophobic bonds could also be important in early stages of baking. Hydrophobic bond formation, in contrast to all the other chemical bond formations, is an endothermic process favored by increasing temperature up to 60^{o}C (Wehrli and Pomeranz, 1969).

Cottonseed Flour

Cottonseeds, in contrast to cereal seeds, are composed primarily of embryo tissue with a very thin layer of residual endosperm. These seeds store oil as the major energy source. The cotyledons of the cottonseed embryo typically contain three major classes of cells: epidermal, palisade and spongy mesophyll (Wall, 1965). In addition to these cell types, glanded cottonseed varieties contain intercellular structures called pigment glands which are the deposition sites of gossypol pigments. These glands are distributed throughout the colyledons and the periphery of the axial tissue. They vary in diameter from 50-400 μ, with the majority being 80-120 μ (Boatner, 1948). Moore and Rollins (1961) showed that the center of the pigment glands consists of a complex net-like structure holding the globules of gossypol which were found to be less than 1 μ in diameter. The intracellular structure of the cottonseed is highly organized, where lipid, protein and phosphorus are neatly packed and embedded in the cytoplasm, in addition to the other basic cell structures (Engleman, 1966; Yatsu, 1965). This natural organization of the cell is extremely important to the processing of edible cottonseed products.

Cottonseed flour processing

Very early in the processing history of cottonseed it was recognized that cottonseed could be a multiple source of edible products - both protein and oil (Richardson,

1917). However the presence of the chemically reactive, antinutritional factor - gossypol (Berardi and Goldblatt, 1969) affected any rapid development of edible protein products, therefore cottonseed processing became a compromise between oil quality, gossypol inhibition, and protein quality, where the economically important oil and potentially toxic gossypol were given major emphasis. As a result, the full potential of the quality and functionality of the protein of cottonseed was seldom evident in commercially produced meals (Altschul et al., 1958; Bailey, 1948). The two major developments that renewed the interest in the potential of edible cottonseed protein products were: the elimination of gossypol through breeding (McMichael, 1959; Miravall, 1959) and the development of the liquid Cyclone method (Gastrock et al., 1969) for processing glanded cottonseed.

Cottonseed flour as defined by Martinez et al. (1970) is the finely ground material produced from dehulled and defatted seed. The heat, moisture, and pressure used in the process of oil removal also affect the protein constituents of the seed, as well as the product's color (Martinez, 1969; Vix, 1968). Further concentration of the proteins of the cottonseed and the removal of gossypol can be accomplished by either wet or dry processing of the defatted flour. Ninety percent ethanol was used for the optimum extraction of lipid and sugar with a minimum loss of

nitrogen (Berardi et al., 1968). Aqueous extraction at neutral pH (6.3-6.8) is another wet procedure for preparation of cottonseed protein concentrate (Martinez, 1969), where water or a very dilute divalent cationic salt solution (0.008M $CaCl_2$) can be used to extract the defatted flour. The outer structure of the pigment glands is fairly rigid, thus allowing properly conditioned kernels to be processed without appreciable damage and subsequent ejection of gossypol. The differences in density, size, and shape of pigment glands and of other extraglandular seed material, particularly in the solvent media, have been employed in a number of procedures to obtain a gland free, low gossypol protein product. However there are two major difficulties associated with wet operations: the processing of the extract, the byproduct in an economically feasible manner, and the drying of the major product. The first difficulty involves low-yield product recovery, solvent recovery, and pollution problems. The second difficulty involves denaturation of the major product during drying, which is more severe when alcohol is part of the extracting solvents. These two problems hinder the applicability of wet operations commercially (Kadan et al., 1979).

The dry procedure of air classification has neither of these difficulties (Martinez, 1969; Martinez et al., 1967). It is based on the mechanical separation of particles, clusters of unruptured cells, cell wall fragments with

adhering residual cytoplasm, and residual sphersome membranes are separated from free, intact protein bodies. It is also used for the removal of pigment gland (Meinke & Reiser, 1962, 1964; Vix et al., 1972). The free gossypol of the air-classified product was not low enough to meet the standards of Food & Drug Administration (1974). Graza (1959) and Schaller and Lapple (1971) showed that air classification of solids results in a fine fraction and a coarse fraction that have overlapping particle sizes. Successive air classifications for the removal of intact and partially broken pigment were suggested by Kadan (1979) to produce an edible cottonseed protein product, without affecting its nutritional value.

The non-aqueous, liquid classification procedure of Liquid Cyclone Procedure (LCP) was developed primarily for the removal of the pigment glands (Gastrock et al., 1969). Appropriately defatted flakes are ground in hexane to remove the cellular tissue from the pigment gland, which was then separated from the flour particles in a liquid centrifuge called a Liquid Cyclone. Cottonseed flour contains about 0.8% free-gossypol which is responsible for the toxicity associated with the protein as a food or feed for non-ruminants. According to the Protein Advisory Group of the United Nations Systems (FAO-PAG, 1975), free gossypol content of edible-grade cottonseed flour should not exceed 0.06%.

El-Tinay et al. (1980) studied the effect of pH, salts and the combined effect of pH and salts, on the extractability of protein and free-gossypol from fully gossypolised cottonseed flour. These researchers reported that a high salt concentration (0.25 M) was needed to bring about reasonably high protein extractability, and alkaline extraction at pH 10 is the best method to obtain a protein isolate totally free from free-gossypol with a high protein content. Several workers (Jonassen, 1955; Smith, 1970; Smith and Clawson, 1970) have shown that the addition of ferrous salts to nonruminant animal rations containing cottonseed meal reduces the toxicity of gossypol to varying degrees. Bressani et al. (1964), Jarquin et al. (1966), Braham et al. (1967) have reported the addition of iron in the presence of calcium hydroxide reduces the free gossypol in meals containing cottonseed flour, to low levels. Mayorga et al. (1975) found that the addition of 1% $Ca(OH)_2$ or 0.1% $FeSO_4$ reduced free gossypol in prepress solvent cottonseed meal slightly. When both were added together, the free gossypol was reduced to a greater extent. The combined treatment of calcium hydroxide and ferrous salts plus heating the meal up to 130^{o}C for 90 min was the most effective in reducing 60% of the initial free gossypol, with no reduction in the available lysine.

Cottonseed flour use in foods

The edible use of cottonseed proteins is influenced by its functionality, color, flavor and nutritive value. The full range of cottonseed products from flour to isolates have been tested by the American Institute of Baking in both sponge, dough and continuous bread formulations. With slightly reduced mixing times and the addition of 30 ppm bromate, the flour and air-classified concentrate at 3% and 2.6% levels substitution respectively, produced bread generally comparable in all characteristics to the control containing 3% nonfat dry milk. The bread substituted with the air-classified concentrate had reduced break and shred, and firmer texture. Neither the flour nor the concentrate performed satisfactorily in continuous bread formulation. Volume, bread characteristics, and shock resistance were all lacking (Martinez et al., 1970).

There are two cottonseed protein isolates obtained by the two-step procedure of isolation. The major isolate of glandless cottonseed proteins has a low moderate flavor profile and its acid solubility suggests its use in citric acid based beverages. The minor isolate has an interesting whippability at acid pH and produces foam volumes as much as nine times that produced by sodium caseinate at pH 4, and 75% of that produced by sodium caseinate at pH 7 (Berardi et al., 1969).

Matthews et al. (1970) studied the effect of both glandless and glanded cottonseed flour, peanut, safflower and full fat soy at 25% level of replacement of wheat flour on bread characteristics. They reported the need for more research to determine the maximum amount of the oilseed flour that can be used to meet consumer acceptance standards. When liquid Cyclone Processed cottonseed flour was used substituting 13% of cake flour, it gave cakes and doughnuts with a very desirable yellow color comparable to that of an egg rich product. In devils food cake, a substitution of 10 percent LCP flour for cake flour produced an excellent product with good color, flavor and texture. The LCP imparted an undesirable greenish color to waffles and pancakes and a grayish cast to white cake.

Cottonseed flour was also used at levels up to 8% in beef patties and up to 6% in sausage. Research on extruded products containing LCP flour at Grain Processing Corporation and Texas A&M University showed that cottonseed flour materials have promise for use in meat products and cereals (Olsen, 1973; Gardner et al., 1973). Cottonseed proteins were used for replacing 10% and 30% of the meat in frankfurters (Terrell et al., 1979). Texturized cottonseed products with good nutritional and functional properties were obtained by an extrusion cooking process using cottonseed flour (Cabrera et al., 1979).

Cottonseed products are influenced by pigments present in seeds, or the glands contained in the seed (Hedin et al., 1976). The basic pigment groups that affect the quality of cottonseed flour or proteins are:

Terpenoids: of which gossypol has been the compound of the greatest interest, and is concentrated in discrete glands.

Flavonoids: The need for a color-free cottonseed protein led to the study of pigments other than gossypol in cottonseeds. Blouin et al. (1978) reported that there are six major flavonoids in both glanded and glandless seeds which were present in the surrounding embryo meats in both varieties. The flavonoids added to biscuit gave yellow-brown color (Blouin and Cherry, 1978).

Flavor is another important factor in the acceptance of a vegetable protein for human food applications. Cottonseed flour is notably bland compared to other vegetable sources, but there are several free phenolic acid fractions which have been identified and might contribute to taste in cottonseed flour (Jones, 1979).

Nutritional Value

The nutritional value of cottonseed proteins is influenced by the reaction of free gossypol with available epsilon amino group of lysine, and in this state is much less physiologically active in the animal gut (Conkerton, 1959). The gossypol that is thus reacted is called "bound

gossypol" and that gossypol that is still not bound to protein is designated as "free gossypol". The main concern of nutritionists is the amount of free gossypol consumed by animal, which has some toxic effects on different animal species (van Sumere et al., 1975; Sabir et al., 1974; Sosulski et al., 1969). Bressani et al. (1969) conducted long term feeding studies with rats to determine the effect of constant intake of gossypol in cottonseed flour. He found that rats were able to detoxify the ingested gossypol, since the amounts consumed daily were relatively small (0.011-0.028%), however they do not resist the toxic effects of higher levels of pigment as reported by Bressani and Elias (1968). Hale and Lyman (1957) and Sharma et al. (1966) have reported the effect of high protein levels of diet on counteracting gossypol toxicity. The phenol compounds in seeds when oxidized by atmospheric or enzyme-catalyzed oxidation, results in quinoidal production and the formation of hydrogen peroxides, which are both destructive to labile amino acids, denature proteins, and inhibit enzymes such as indole acetic acid oxidase (Rabin and Kein, 1957), trypsin and lipase (Milic et al., 1968) and arginase (Muszynska and Reifer, 1970). Cinnamic acids and their esters are of particular significance in oilseeds because they are a preferred substrate for phenol oxidase. Caffeic and chlorogenic acids are oxidized to o-quinones by a copper containing enzyme which occur in plants. Once

o-quinones are formed they react nonenzymatically to polymerize, and are reduced or bound covalently to amino thiol and methylene groups. The epsilon-amino group of lysine and the thioether group of methionine are commonly attacked to render them nutritionally unavailable to monogastric digestive system (Sosulski, 1979). Lysine is considered to be the first limiting amino acid in cottonseed, therefore, the air-classified concentrates and the liquid cyclone processed flour were lower in lysine than the parent flours and their PERs were 2.3 and 2.6, respectively, as compared to 2.5 for the standard sodium caseinate (Martinez et al., 1970; Ridlehuber and Gardner, 1974).

Supplemental Value of Plant Proteins on on Wheat Flour

Because of the universal acceptance of bread and the potential of its fortification with proteins, amino acids, vitamins, and minerals, the measurement of the effect of added proteins on dough formation and bread's physical and nutritional quality have been extensively studied.

Effect of plant proteins on the functionality of wheat proteins in dough and bread

The dough forming capacity of protein mixtures and the properties of doughs are routinely determined by the farinograph and mixograph which measure development time, dough strength, consistency and stability. Soy flour has

traditionally been added to wheat flour as a source of lipoxygenase, which aids maturation and bleaching. In addition small amounts of soy proteins are being increasingly added to bakery products for several claimed functions (Wolf, 1970). Replacement of wheat flour by oilseed flour up to 5-10% has been successful (Bacigalupo et al., 1967). However at higher levels of replacement, loaf volume is severaly decreased along with serious deterioration of crumb color, and grain texture (Matthews et al., 1970; Sidwell and Hammerle, 1970). The maximum level of replacement depends on the type of nonwheat flour, the strength of wheat flour, the baking procedure, and the dough stabilizing compounds used (Dendy et al., 1970; Pringle et al., 1969). Tsen and co-workers (1971) used sodium and calcium stearyl-2-lactylate to produce sponge dough breads with 12% soy flour. The dough conditioners increased loaf volume, and the organoleptic properties of soy bread were comparable to bread with 100% wheat flour. Rooney et al. (1972) compared the functional bread-making properties of heat-treated and non-heat-treated flour from cottonseed, peanut, sesame and sunflower flours. The oilseed flour replaced wheat flour to produce blends at two protein levels, 17.5 and 20.5 percent. He reported that the farinograph water absorption increase with the increased levels of replacement of wheat flour with oilseed flour at both protein levels. Moreover, the heat treated flours had

higher water absorption than the non-heat-treated ones. The type of oil seed did not significantly influence the water absorption of the various flour/oilseed blends. The sunflower substitution drastically weakened the dough structure while cottonseed destroyed the dough stability. Peanut flour incorporation at the high protein blend (20.5%) caused noticeable weakening of dough structure, whereas sesame showed only a slight decrease in mixing strength. The heat treatment of oilseed flours improved mixing strength and stability. The heat-treated cottonseed flour was more compatible with the proteins of wheat which accounts for the dramatic increase in loaf volume. The functional properties of sunflower were improved by heating while sesame and peanut flours responded negatively to the heat-treatment and the bread baked had poor internal properties.

Khan et al. (1976) studied the baking properties of cottonseed protein concentrate (CSPC) spray dried at different pH levels, and compared the influence of Ca^{++} and Na^{+} on its baking properties, in an attempt to optimize the CSPC processing method. They reported that acidic pH (4.5) adversily affected the baking properties of spray-dried concentrate. pH adjustment with $Ca(OH_2)$ significantly reduced the loaf volume and bread crumb grain score. Acceptable loaves were obtained with spray-dried concentrates at near neutrol pH adjusted with NaOH, and they

were similar in quality to breads baked with parent glandless cottonseed flour or commercial soy flour.

Lawhon et al. (1972) demonstrated that solvent-extracted cottonseed proteins prepared from glandless cottonseed flour and spray-dried at pH 4.5 had significantly poorer baking properties than a comparable concentrate spray-dried near neutral pH (6.8).

Recent studies on flours from faba beans (McConnell et al., 1974) showed that the addition of faba bean flour to hard red spring (HRS) wheat flour at the rate of 10, 20, 30, and 40% resulted in a progressive decrease in loaf volume and a deterioration in crumb grain, even in the presence of the conditioner SSL. D'Appolonia (1977) studied the physical dough properties and baking potential of five legumes including: mung bean, faba bean, navy bean, pinto bean and lentil, substituting HRS wheat at 5, 10, and 20% levels. Results indicated that mung bean, and lentil had the least water holding capacity while pinto and navy bean flours retained the water better than the other legume flours. Farinograph water absorption also increased with increasing the level of pinto and navy beans in flour blends, while the dough development times for all bean flour combinations were less than the all wheat flour and stability decreased.

Fleming and Sosulski (1977), using 15 percent faba bean and field pea, found it necessary to add 2 percent vital

gluten, and about 1 g of dough conditioner per 100 g flour to produce acceptable bread quality. Yellow peas (raw and cooked) have been studied as a bread fortifier by Jeffers et al. (1978). D'Appolonia (1977) reported that as the level of legume flour in the blend was increased, the crust color of the bread became increasingly darker; the 20% blend level of the mung bean bread produced the darkest crust color. The whitest crumb color was noted with the 5 and 10% legume flour breads and these were whiter than the control bread. The 5 and 10% navy bean flour blends produced the best grain and internal appearance of the various legume flour-containing breads.

They substituted raw pea, cooked pea and soy flour for 5, 10, 15 and 20% of wheat flour and they reported the significant difference between the cooked and raw yellow pea on physical dough and bread making where raw pea flour was better. At 5% level of substitution the functional baking performances of pea-substituted flours were superior to soy-substituted flours. At the 15 percent level, yellow pea flours produced acceptable breads, and no dough conditioners were needed to produce acceptable bread with up to 15 percent yellow pea flour.

The supplementary value and protein quality of oilseed flours.

Great emphasis has been placed on the use of composite flour in bread-baking in the last 10 to 15 years. Composite flour refers to flour containing blends of wheat flour with

nonwheat flours (D'Appolonia, 1977). The nutritional value or protein quality of a food protein depends not only on its content of amino acids, but on their physiological availability. This availability varies with the protein source, processing method, and interaction with other diet components. The availability also depends on the condition of the consuming animal (Boloorforooshan, 1977). The biological value of a protein is one way of expressing its nutritional value. The biological value is based on the amino acid content of a protein food as well as the essential amino acid balance, that is the relative proportions of the amino acids present in a protein. Thus, any factor that affects the protein food and causes the amino acid balance to change, would also alter the biological value of the protein (Boloorforooshan, 1977).

The storage proteins of cereals, oilseeds and legumes are variable in their amino acid composition in contrast to the similar amino acid distribution of proteins in all the metabolitically active tissues whether they are animal or plant or microorganism (Bressani, 1968). Plant storage proteins are deficient in one or more essential amino acids. Furthermore, the ratio of essential to total amino acids is smaller in plant storage protein in comparison to that of animal protein, which causes lower utilization of some plant proteins, even after the essential amino acid pattern is corrected by substitution (Harper and DeMuelenaere,

1963).

Evans and Bandemer (1967) studied the nutritive values of peanuts, safflower, sesame, soybean and sunflower seeds and found that methionine and lysine are the most limiting amino acids in the seeds studied. Only soybean proteins had high nutritive value, but these were deficient in the sulfur amino acids, while sesame seed proteins have an abundance of the sulfur amino acids (Evans et al., 1967) but are deficient in lysine.

Effect of Processing on the Protein Quality

The loss in nutritional value of a protein due to thermal processing is measured by determining the available lysine loss (Lea and Hannan, 1950; Carpenter, 1973). The available lysine destruction rates for pure proteins stored at a_w = 0.68 and temperature = 35°C, and included in model food systems containing 10% glucose varied with the type of protein. Albumin was the most stable protein while soy protein was the least (Schnickels et al., 1976). The effect of heat on the nutritive value of proteins was observed as early as 1917 by Osborne and Mendel in their attempts to destroy the toxic pigment gossypol in cottonseed by steaming the seeds for a long period of time. These researchers reported heats effect on reducing the nutritive value of seeds. Hopkins (1967) determined the PER of cottonseed flour and found it equal in nutritive value to casein control. Lysine is considered to be the

first limiting amino acid in cottonseed flour. Therefore, the air-classified concentrates were slightly lower in lysine than the parent flour. However, the residues from the air classification process were equal in PER to the parent flours (Martinez et al., 1970). The available lysine in the wet-processed cottonseed concentrate was lower than in its parent flour (Lawhon et al., 1972). Solvent extraction of glandless cottonseed was recommended by Vix et al. (1968) as the defatting method because of the mineral effects on protein quality and versatility of use with different oilseeds. Desolventization is the most important step for the protein, because high residual solvent will cause off-flavors. However the use of heat and added moisture in the absence of fat resulted in disruption of cellular integrity and produced rapid browning, coagulation of water-soluble proteins, and reduction in lysine availability (Martinez et al., 1970).

The potential use of edible cottonseed protein products could be extensive. The inherent differences between cottonseed and soy in protein products should complement rather than compete to fulfill the spectrum of needs in the food industry (Martinez, 1970). Bressani et al. (1966), and Squibb et al. (1959) have supplemented corn, sesame flour and sorghum with cottonseed flour in formulating a vegetable mixture to upgrade their protein content. Jones and Divine (1944) substituted white wheat flour with cotton-

seed flour, and found that the addition of 5 parts of cottonseed flour to 95 parts of wheat flour produced mixtures containing 16 to 19% more protein than the wheat flour alone, and a protein combination that was definitely superior in its growth promoting value in rats when compared to feeding the same amount of wheat flour.

The more recent liquid cyclone process technique (Gastrock et al., 1969) yields a flour superior in quality and higher in protein. The liquid cyclone processed cottonseed flour had PER value (2.3) very close to casein (PER = 2.5). Castro et al. (1976) investigated the substitution effect of LCP cottonseed flour on the protein quality of soybean concentrates and isolates, triticale, wheat, and rye. They found a significant improvement in the protein quality of all the grains investigated which suggests that LCP cottonseed flour is a valuable supplement to these proteins and possibly to other grain products as well. Breads made with 18.8% LCP cottonseed flours substituted for wheat flour were tested for their nutritional value by amino acid analyses, and biological tests (Harden and Yang, 1975). Amino acid analyses of breads indicated that lysine content was higher than the comparable wheat breads.

Protein Modification and Functional Properties

Protein modification usually refers to the intentional alteration of the protein structure by physical, enzymatic, or

chemical agents, to improve functional properties. Thus, modification of food protein may involve alterations in structure or conformation at all levels of organization (primary, secondary, and tertiary structures). It may include disruption and reformation of covalent bonds and secondary forces using physical (thermal and pressure), chemical, or enzymatic treatments. Dough formation is one example of protein modification (Kinsella, 1976). Modification procedure may also result in improvements of flavor, color, elimination of off-flavors, and destruction of undesirable enzymes, antinutritive factors, hemagglutinins, and allergens.

Salts.

In breadmaking, salt (sodium chloride) forms a part of the dough ingredients (Bloksma, 1978). Altering the salt content in a protein solution causes the breadkown of one of the physical bonds (electrostatic bond) between protein molecules in a food system. The role of salt can be explained by the action of salt ions on reducing the repulsion of the alike charges on protein molecules, which causes protein to aggregate and then to precipitate (Krull and Wall, 1969). The effect of salt ions on the protein aggregation have also been described by Bernardin, 1978. He reported that the close approach of one protein molecule to another resulting from the masking effect of salt ions on the repulsion of the similarly charged protein molecule

allows hydrophobic and hydrophilic interactions to form, causing protein to aggregate. This effect of salt ions was also found at very low concentrations (0.005 M NaCl). The effect of salt concentration on protein aggregation is straightforward, and follows directly the ionic strength. Trivalent will be more effective than di and mono valent ion in reducing the charge-charge repulsion of similarly charged protein molecules.

The concentration of salt in most dough systems is 2% (Miller et al., 1947). The addition of sodium-chloride to dough makes it stiff and less sticky, as confirmed by the measurements with the extensigraph (Fisher et al., 1949; Grogg et al., 1967; Calvel, 1969; Margulis and Campagne, 1955). They reported that the curves with added salt show a higher resistance and increase extensibility. Farinograph studies usually fail to show this trend, showing only decrease in consistency (viscosity) (Bennett et al., 1953; Hlynka, 1962; Tanaka et al., 1967). This observed decrease in consistency has been explained as being due to the decrease in stickiness rather than in stiffness (Bennett et al., 1953). Mixographs were also used to show the increase of stiffiness with salt addition (Bennett et al., 1953).

The specific effects brought about by ions, particularly organic acids and lipid molecules, which bind to the protein surface and bear a charge and which can reduce or increase the net charge on the proteins have been studied by

Bernardin (1978). Bernardin reported their effect on the aggregation of A-gliadin. The use of salts of L-ascorbyl G-palmitate (AP) and D-isoascorbyl 6-palmitate (IAP) in breadmaking has been reported by Ofelt (1958) to be as effective as monoglyerides on bread-crumb softening. Nevertheless it was found that at levels of 0.4% based on flour, the compounds darkened bread crumb. Hoseney et al. (1977) used the salts of 6-acyl esters of L-ascorbic acid and D-isoascorbic acids in breadmaking. These researchers found that both salts behaved similarly in breadmaking because of their closely related structure. They reported that 2-acyl esters of L-ascorbic acid did not give a desirable dough-conditioning effect because of their effect on tightening the dough structure.

Oxidizing Agents.

Oxidants are used to modify and control dough consistency and strength (Kinsella, 1976). The effect of oxidizing agents is twofold: flour improvement which refers to changes in rheological properties of the dough and flour bleaching, which is due to destruction of its yellow pigments; and results in whiter flour and bread (Bloskma, 1978). It is generally believed that the agents function by controlling disulfide bond rupture and the extent of disulfide interchange reactions (Ewart, 1972; Wall, 1964, 1971). Bromate and iodate improve dough properties without bleaching, while nitrogen dioxide and benzoyl peroxide bleach only effects

are exerted. Chlorine dioxide and acetone peroxides both improve flour and bleach. The reaction of bromate has been explained by Bloksma (1978) as the formation of reactive groups on the protein chains that form cross-links only during structural activation. He said that it is possible that the reactive groups are too far removed from one another during dough resting and they only come together by the work of rounding and shaping. The exact nature of the assumed reactive groups is still unknown; there is little doubt, however, that bromate and iodate act via the oxidation of thiol groups. The reaction of bromate is slow except at elevated temperatures (Dempster et al., 1956).

Surfactants.

Surfactants have been used in commercial bakeries for many years. The first ones to be used were monoglycerides, introduced in the 1930's, and lecithin. Research during the 1950's and 1960's resulted in more sophisticated and effective products. The need for these sophisticated surfactants was caused by the use of high speed mixing equipments and large scale production in the baking industry (Tenney, 1978). Surfactants are sometimes referred to as dough conditioners which have a number of distinct actions on dough and bread.

Dough conditioners have been reported to improve the handling of properties of dough, to increase the loaf volume, to increase the water absorption of the dough, to

replace shortening in the formula, to counteract the deleterious effect of foreign proteins, and to retard the rate of firming of bread. Basically, surfactants are esters composed of polyhydric alcohols and long-chain fatty acids. Thus they are characterized by both hydrophilic and lipophilic groups. Their performance in breadmaking requires a proper balance between both groups, which is affected by the presence of ionic charges on the hydrophilic groups and even by the counter-ion associated with that charge (Hoseney et al., 1976).

The use of softeners and conditioners in breadmaking is controlled by government regulation. The FDA approved bread softeners included: mono- and diglycerides, diacyl tartaric acid esters of mono- and diglycerided and propylene glycol mono- and diesters of fat forming fatty acids. The regulations state that the total weight of the individual softeners may not exceed 20 percent of the combined weight of the softeners and the shortening in the bread. The dough conditioners that were approved by the FDA regulations included: polysorbate 60, calcium and sodium stearoyl-2-lactylate, lactylic stearate, sodium stearyl fumerate and succinylated mono- and diglycerides. The regulation further stated that conditioners may be used alone or in combination of both a conditioner and a softener at the level not to exceed 0.5 percent based on flour weight for each additive separately (Newbold, 1976).

Scanning Electron Microscope

The direct examination of a tissue structure was limited to the images obtained in the light microscope, and at the ultrastructural level in the transmission electron microscope which are both two-dimentional images. Only the low-power stereascopic light microscope reproduced the object in a manner corresponding to man's eye. The standard light microscope is limited by its low resolving power (2000 A) because of the wavelength of the visible light. The transmission election microscope (TEM) also is limited by the power of the electron beam which requires the use of complex and time consuming specimen preparation and sectioning techniques, at the same time only extremely small areas of the specimen can be viewed.

The scanning electron microscope (SEM) was commercially produced in the 1960's. The instrument combines the advantages of the stereoscopic light microscope for producing a three-dimensional image with a better resolution (200 A), and the transmission electron microscope of producing images with very high magnification. The SEM has an additional advantage over both types of microscopes, that is the possibility of viewing a larger specimen area (1 cm^2) than the TEM. Since the SEM is a surface examining method, the observed image is limited by the details preserved in the viewed structure (Aranyi and Hawrylewicz, 1969).

Principle of Operation.

In the scanning electron microscope (SEM) the sample surface is scanned by an electron beam, and the emitted secondary electrons create an image of the structure viewed. As the scanning beam of electrons moves across the specimen, another electron beam of a standard cathode ray tube is driven in synchrony with it. The brightness of the cathode ray tube is modulated by a signal produced from the secondary electrons emitted from the specimen surface and amplified by a scintillator-photomultiplier system. The magnification is determined by the ratio of the two synchronized electron scans. The electron micrographs of the viewed images can be taken instantly with a Polaroid camera (Hayes et al., 1966; McDonald et al., 1967; Pease, 1968; Pease et al., 1968).

Application.

The goal of the food scientist is to understand structural features of a material that are important in its functional role in a food system. Despite the rapid advances in instrumental design of scanning electron microscopes, there are still limits to the image resolution set by the biological specimen preparation problems. Food science samples are biological materials that differ in nature, hardness, form, moistness, and size, so the viewing of their structure is limited by the two major problems that limit the biological sample resolution in SEM. These

are: the removing of water from the specimen without destroying the structural relationships, and making the specimen conductive; by coating with metal layer deposited by high-vacuum-evaporation. The applications of SEM in the food science area has proved to be useful in some areas more than others (Pomeranz, 1976). Aranyi et al. (1968) found scanning electron microscopes were well suited for the study of wheat flours and doughs, because of the absence of fixation, embedding, and thin-sectioning techniques, which permit the sample to be viewed in their natural state. The fine structure of developing and mature wheat endosperm has been investigated by Buttrose (1963), Seckinger et al. (1967), and Simmonds (1972). The structural relationships of protein and starch in a good quality bread flour at various dough stages and in bread crumb have been studied by Khoo et al. (1975) using scanning and transmission microscopy. All microscopic studies on wheat kernel structure, flour components, and dough formation showed that only wheat flour produces the type of loaf that is now expected for bread. Both gluten forming proteins and starch granule size and morphology are important, since other starch or proteins drastically alters crumb structure. An understanding of the precise functional relationships between all the components in a loaf of bread is important not only from a need for basic knowledge of foods, but also aiding attempts at adding other nutrients such as single

cell proteins (Evans et al., 1977) or fiber (Pomeranz et al., 1977).

Sample preparation for SEM:

The routine techniques of fixation, dehydration and drying have been critically discussed by Chabot, 1979. He concluded from a study of fixation conditions on bread morphology that it produced profound changes in structure. These occurred precisely in the area of the interaction between protein and starch which have attracted the most attention. Dehydration in alcohol resulted in non-uniform shrinkage of starch protein connections. Varriano-Marston (1977) compared different dough preparation procedures for scanning electron microscopy and reported a definite effect of fixatives on the dough structure. He reported that the method of drying had affected the structure where air-drying caused more distortions to the dough structure than freeze drying.

EXPERIMENTAL DESIGN

The purpose of this research was to study the influence of liquid cyclone processed cottonseed flour on dough rheology and bread baking performance in American and Egyptian breads. Bread formulas were optimized with dough conditioners. Specific chemical and physical effects were followed through measurement of chemical bonds and scanning electron microscopy. Thus the study was divided into five sections.

The first section included an overall physical and rheological characterization of hard red winter (HRW) wheat flour substituted with liquid cyclone processed (LCP) cottonseed flour and with unmodified wheat starch at 0, 4, 8, 12 and 16% of flour weight. Testing in this section included an evaluation of dough rheology using farinograph and viscoamylograph. Baking studies including sensory evaluations were also conducted. Proximate analyses on flour and bread were performed.

The second section was a study of the effects of additives on the rheology of dough substitued with 0, 8, and 16% cottonseed flour. Sodium chloride 0, 1 and 2%, potassium bromate 0, 30 and 50 ppm and conditioner

polysorbate (20) (Tween 20) 0, 0.5, 1.5% of flour weight were added and the effect of each additive at their three levels were investigated.

The potential for all the possible interactions among the three additives used and their three levels were conducted on doughs prepared with 0, 8, 16% cottonseed substitutions and breads prepared with the 8% level of LCP cottonseed substitution. The mixograph was used to measure dough rheology for these additive studied. Bread was baked with single, double and triple combinations of additives according to a 3^3 factorial design.

Baking studies were repeated twice, and breads were evaluated by 32 panelists for the sensory characters of interest. These data were subjected to Multiple Regression analyses, and the optimum level of each additive to produce an 8% cottonseed flour-substituted bread with an optimum volume and texture was calculated. Optimum bread was baked and evaluated for sensory characteristics and objectively for texture.

The third section of testing was a study of the functionality of 0, 4, 8, 12 and 16% LCP cottonseed flour substitution for wheat flour in Egyptian bread. Bread was evaluated for the characteristics of interest by 6 middle-eastern panelists, and the data were subjected to analysis of variance. Proximate analyses were performed on these breads.

A 4^3 factorial experiment was designed to test the effects of four dough conditioners at three levels each on the Egyptian bread character. The effect of conditioners on bread softness was tested after 0, 3 and 6 days of storage.

The fourth section was a mixograph and farinograph investigation of the effects of sodium dodecyl-sulfate, succinic anhydride, Urea,N-ethylmaleimide and dithiothreitol on the flour bonding during dough mixing.

The final section was a scanning electron microscope study of the untreated and 8% substituted doughs. For the first part of this microscopic study, the effects of 0.5% conditioner on both dough systems were investigated. For the second part, samples were prepared by two different methods to evaluate the effect of preparation procedures on SEM electron micrographs.

Materials

Straight grade, and 85% extraction hard red winter (HRW) untreated wheat flour was purchased from Department of Grain Science, Manhattan, Kansas. Liquid cyclone processed (LCP) cottonseed flour was supplied by the Southern Regional Research Center, New Orleans, Louisiana, sample no. 85400-A. Red Star Active Dry Yeast was purchased from Universal Foods, Milwaukee, WI. Sugar was supplied by Michigan Food Company, Saginaw, MI. Analytical reagents potassium bromate

and sodium chloride were purchased from Mallinckrodt Chemical Works, St. Louis, MO. Dough conditioners Polysorbate 20 (Tween 20), Polyoxyethylene Sorbitan Mono-Stearate (Tandem 552) were supplied by ICI United States Inc. Specialty Chemical Division, Wilmington, DE. Polysorbitan, Mono and Diglycerides Polysorbate 60 (36%) and Polyoxyethylene 10 stearyl ether were purchased from Sigma Chemical Company, St. Louis, MO.

Dough Rheology Studies

Farinograph Testing:

A C.W. Brabender Instruments, Inc. Farinograph was used, and it was equippped with a Type Pl-2H Dynamometer and a Type 3-S-300 Measuring Head. Temperature of this instrument was kept constant at 30 ± 0.1°C by a Heat-Transfer Circulator Type T-60-B.

The AACC Constant Dough Weight Procedure 54-21 B (1962) was followed using a 300 g bowl maintained at 30 ± 0.1°C. On "as-is" moisture basis, 300 g flour as flour/cottonseed mixtures were weighed to ± 0.5 g, then mixed in the Farinograph bowl for 1 min. starting at the 9.0 minute mark on the chart paper. At zero minute, the water was added from a fast delivery burette (within 25 seconds), the bowl sides were scraped with plastic spatula and then covered with plastic plate to prevent evaporation. When the peak was centered on the 500 BU-line the Farinogram was run for 20

minutes. If the curve was not centered at the peak on the 500 BU-line, re-estimation of absorption was done according to the approximate relationship: 20 BU = 0.6 ml water.

From Table 54-28 of the AACC handbook, the weight of flour and water which correspond to the estimated "as-is" absorption were used to run triplicate Farinograms for each level of supplementation with cottonseed flour. Farinograms produced were evaluated for the following parameters:

Water Absorption: The amount of water necessary to center the peak on the 500 BU-line. It was corrected to 14% moisture basis as illustrated in Note No. 2 of Table 54-29 of the AACC Test procedure.

Arrival Time: The difference between zero minute and the point where the top of the curve first intersects the 500 BU-line.

Peak Time: The interval from the first addition of water (zero time) to the top of the curve (peak).

Stability: The difference between the departure time and the arrival time.

Departure: The time from zero to the point where the top of the curve leaves the 500 BU-line.

Mixing Tolerance Index (M.T.I.): The difference in BU from the top of the curve at the peak to the top of the curve measured at 5.0 minutes after the peak.

Twenty Minutes Drop (T.M.D.): The difference in BU from the 500 BU-line to the center of the curve measured at

twenty minutes from the addition of the water.

Mixograph Testing.

Mixographic studies were performed on a National Mfg. Co. Mixograph equipped with 35 g bowl, and Gra-Lab timer for automatically stopping mixograms. The spring was set on position number A on the damping arm.

The procedure outlined in AACC 54-40 (1955) was followed where 30 ± 0.1 g flour on 14% moisture basis were placed in the mixograph bowl, mixer head was lowered to operation position, the pen was lowered to the chart about 0.25 to 0.5 minutes before it reached a vertical line. The mixer was turned on and the amount of water needed to give the required absorption was added when the pen reached a vertical line. The curve was run for 9 minutes, then was evaluated for the following characters:

Peak Time: The time from the first addition of water to the highest point of the curve, in minutes.

Peak Height: The distance in cm between the center of the peak to the base line.

Curve Height After 9 Minutes: The distance between the center of the curve at the 9-minute point and the base line.

Area Under Curve: The area under the curve measured by a Compensating Polar Planimeter expressed in cm^2.

Viscoamylograph Testing.

Tests were performed on a Brabender Viscoamylograph type VA-VL equipped with a sensitivity cartridge 700 cm-g, 7 pin style stirrer, 8 pin style stainless steel bowl of 500 ml capacity, and a thermoregulator 20-97 °C and a programmed heating rate 1.5°C/min.

The diastic activity of flour was determined according to AACC method 22-10 (1960), where 100 g flour was weighed on 14% moisture basis, and was put in 1000 ml flask to which 360 ml diluted anhydrous sodium phosphate buffer (pH 5.3-5.35) were added and mixed well with flour by shaking. The flour slurry was put into the viscoamylograph bowl and the flask was washed with 100 ml diluted phosphate buffer, which was added to the slurry. The starting temperature was adjusted by hand, then the heating stage started with the bowl in motion. The viscosity was recorded as temperature increased from 30°C to 95°C at 1.5°C/min. to reach the maximum viscosity, after which a holding at ambient temperature for 15 minutes followed. A rapid cooling at 1.5°C/min followed the holding period and was run for 30 minutes. The viscoamylograms were evaluated for peak viscosity in BU and the viscosity after 60 minutes in BU.

Baking Studies

A Hobart Kitchenaid K5-A mixer which was equipped with a dough hook was used for mixing dough; fermentation was performed in Cres-Cor Model 210-1828 fermentation cabinet. A National Manufacturing Company Sheeter and Holder was used for white bread shaping, after which the breads were baked in 9x5.3x5.8 cm pans. All breads were baked in Reel Type Test Baking Oven, National MFG Company, Lincoln, Nebraska. Bread volume was measured by a National Mfg. Company loaf volumeter; texture was evaluated by Food Technology Corporation Texture Test System, Rockville, MD., which was equipped with TP-2 Texturpress and FT-100 force transducer, TC-1 compression test cell and TR-3 Texturecorder with thermal writing chart paper. Bread tenderness was measured on an Instron Universal Testing Instrument Standard-low speed-Metric Floor Model TT-B, Instron Corporation, Canton, Massachusetts. Cross head speed was 5 units. The single blade test cell of Food Technology Corporation Texture Test System CA-1 was used for tenderness. The color of baked loaves was evaluated on a D25-2L Hunter Lab equipped with automatic reading for the color difference and a Tri scale simultaneous display. It was standardized with a yellow tile.

Baking Procedure for American Bread.

The formula for baking bread was 100 g flour, 2 g dry yeast, 2 g sugar and 2 g salt. The amount of water added was

the Farinograph water absorption for each mix.

The baking procedure was optimized for the 0% formula according to the AACC method 10-10 (1961). The yeast was hydrated in water for 5 minutes in the mixing bowl, then the dry ingredients were added and mixed at speed 2 for 2 minutes, after which the bowl sides were scraped with a rubber spatula. Mixing was completed at speed 6 (standardized to 144 rpm) for 5 minutes; after which the dough was transferred to a floured board where it was shaped into a ball by folding in half for six times. The dough balls were transferred to slightly greased stainless steel bowls and fermented for 90 minutes at 88^{o}F ± 2 and 90% relative humidity. The fermented dough was scaled to 150 g, then degassed with a dough sheeter set at 7/32 in, molded into loaves and panned. After it was given a 10 minute bench rest at room temperature, loaves were proofed for 60-70 minutes at 88 ± 2^{o}F and 90% relative humidity. The bread was then baked at 425^{o}F reel oven for 20 minutes. Bread was cooled to room temperature before being weighed and wrapped in plastic wrap. Volume was measured by rapeseed displacement. Bread was then stored in a freezer until objective and sensory evaluations were done.

Testing Procedure for American Bread.

pH of Dough. pH was measured at 0, 45, 90 minutes during fermentation by weighing 3 g of dough and dispensing it into 10 ml distilled water.

Compressability. To measure the bread texture, a 3 cm high slice of each loaf was cut with a round cutter of 5.0 cm diameter, and was compressed to 30% of its original height, using a Food Technology Corporation Texture Test System. The compressability was expressed as lbs force.

Color. Hunter Lab color meter was standardized with a yellow tile for L=lightness (Black & white), a=redness and greenness, b=yellowness and blueness. A round slice of 5 cm diameter bread was read for the 3 scales.

Sensory Evaluation. Bread substituted with cottonseed flour was judged for color, crust and crumb characters, grain texture and for overall acceptability by six judges. A linear scale was applied, where the score card was designed so that a line 10 cm long represents each character to be tested. Minimum and maximum descriptions were printed on the 0 and 10 cm mark on the line. Judges were asked to put a (X) mark along the horizontal line indicating their evaluation for each character. The sensory evaluation tests were repeated five times before the data were subjected to statistical analyses.

The same bread score card was used for evaluation of bread baked with different levels of additives and for the interaction between the additives. The data were used for applying Multiple-Regression analyses to optimize the levels of additives in bread substituted with 8% cottonseed flour.

A slice of this optimized bread was presented to each of 16 panelists, and scored for color, grain texture, tenderness, flavor and overall acceptability. These tests were duplicated. Samples of score cards appear in the Appendix.

Baking Procedure for Egyptian Bread.

Bread was substituted with cottonseed flour at 0, 4, 8, 12 and 16% of the wheat flour weight. The effect of four conditioners; Polysorbate 20 (Tween 20), Polyoxyethylene Sorbitan Mono Stearate (Tween 60), Mono and Diglyceride Polysorbate 60 (Tandem 552) and Polyoxyethylene 10 Stearyl Ether, each at 0, 0.5 and 1.5% of flour weight, on bread quality was tested. The effect of these conditioners on tenderness was tested after different storage periods.

The bread formula was 200 g of 85% HRW wheat flour, 3 g salt, 9 g dry yeast, and distilled water, 20% more than the Farinograph absorption. Dry yeast was hydrated for 5 minutes in the mixer bowl, then the dry ingredients were added and mixed at speed 2 for 1 min. The bowl sides were scraped down with a rubber spatula, after which the mixing was continued on speed 6 for 7 minutes. The dough was then transferred to a slightly greased stainless steel bowl and fermented at 88 $\pm$ 2^{o}F for 4 hrs. The fermented dough was scaled into two 150 g loaves, which were put on a cookie sheet previously dusted with shorts. The loaves were shaped by padding by hand to a round loaf of 18 cm in

diameter and 1 cm high. Loaves were proofed for 30 minutes at 90°F and 88% relative humidity. Bread was baked at 550°F in a reel-oven for 5 minutes, cooled to room temperature, then weighed, wrapped in plastic wrap and volume was measured with rapeseed displacement.

Testing Procedure for the Egyptian Bread:

Sensory Evaluation. A score card was developed for judging bread substituted with cottonseed flour where crust color, crumb color, flavor, texture, aroma, and general acceptability were evaluated on a scale of 1-7 where 1 is very poor to 7 excellent for flavor, texture, aroma and general acceptability. Crust and crumb color, descriptions were given for each of the seven numbers. A sample score card appears in the Appendix.

The bread was presented to six middle eastern panelists for evaluation 1 hour after baking. The test was repeated five times. The data were subjected to statistical analyses.

Tenderness. Bread tenderness was tested with an Instrom equipped with single blade tenderness test cell after 0, 3 and 6 days of storing bread at 10°C. Bread was left at room temperature for 1 hour before tenderness was measured each day. A round slice of bread 5 cm diameter was sheared with the single blade. Tenderness was expressed as g force/ 5 cm of sample diameter.

Chemical Analysis

All chemicals used were reagent grade, and deionized water was used for all the chemical analyses. Mercuric sulfate, boric acid, potassium sulfate, sodium borohydride and sodium lauryl sulfate were all supplied by Fisher Chemical Company, New Jersey, Di Na-EDTA, dithiothreitol, 5,5'dithiobis-(2-nitrobenzoic acid) and succinic anhydride were purchased from Sigma Chemical Company, St. Louis, MO. N'ethylmaleimide was supplied by Aldrich Chemical Company, Milwaukee, Wisconsin.

Moisture

The AACC method 44-40 (1961) was followed for moisture determination in flour. A well mixed sample of 2 g weighed to the nearest 0.001 g was weighed into a predried and weighed aluminum dish. Samples were dried at 90^{o}C under vacuum equivalent to 25-30 mm Hg in a Hotpack #633 vacuum drying oven to a constant weight. Samples were cooled in a desiccator to room temperature then were reweighed. The percentage loss in weight was reported as percent moisture.

Bread moisture was determined according to the AACC method 62-05 (1961) where a representative loaf of bread was weighed to ±0.2 g, placed on a waxed paper and sliced to 2-3 mm thick slices. Cut slices were left at room temperature for 18 hours, then were weighed and the percent loss of weight was reported as moisture at air drying.

Dried bread was ground to pass 20-mesh sieve and moisture was determined according to the AACC method 44-40. Percentage weight loss in the two drying procedures was expressed as percent moisture.

Kjeldahl Total Nitrogen (Micro-Method).

The method of McKenzie (1970) was followed. Reagents prepared for this micro Kjeldahl method included:

Mercuric Sulfate Solution: In a total volume of 100 ml of 2 M sulfuric acid, 13.7 g mercuric sulfate were dissolved.

Sodium Hydroxide-Sodium Thiosulfate Solution: In 400 ml deionized water, 200 g sodium hydroxide and 12.5 g sodium thiosulfate were dissolved.

Boric Acid Indicator Solution: Twenty grams boric acid were dissolved in 800 ml deionized water, 6.67 mg methylen blue in 50 ml deionized water and 13.3 gm methyl red in 10 ml ethyl alcohol, were all combined and brought up to 1 liter with deionized water.

Hydrochloric Acid Standard Solution 0.02 N: This solution was prepared diluting 1.65 ml of 37% hydrochloric acid to 1000 ml.

A dry sample of approximately 30 mg, 1.5 g powdered potassium sulfate, 2 ml sulfuric acid and 0.5 ml mercuric sulfate, were added to a narrow mouthed, 100 ml non-transfer

micro Kjeldahl flask, and digested on a Lab con-co digestion rack #21621 for micro Kjeldahl digestion. Flasks were cooled after digestion and the digest was diluted with about 15 ml deionized water before distillation.

A scientific Glass Associates micro-Kjeldahl distillation apparatus was used, where flask mouth was greased and transferred to the apparatus. When the boiling water started to distill over, 10 ml of sodium hydroxide-sodium thiosulfate solution was added. The steam distilled mixture was collected in 50 ml beaker containing 5 ml of the boric acid indicator solution. Distillation was continued until 45 ml of sample were collected, then the beaker was lowered and the tip of condenser was rinsed with deionized water and 5 more ml of distillable were collected, and titrated with 0.02 N hydrochloric acid solution to a grey-lilac end point.

Nitrogen recoveries were determined with dl-tryptophan that had been dried in a dessicator. A blank was run to correct for nitrogen contamination. The percentage protein was calculated by multiplying the percentage nitrogen by 5.70 for wheat flour and 6.25 for cottonseed flour.

Lipid.

Lipid was determined in flour and bread as crude fat according to the AACC method 30-10. The following reagents were used for crude fat analyses:

- Ethyl alcohol 95%.
- Ethyl ether, free from residue on evaporation.
- Hydrochloric acid solution 25 + 11 (v/v)
- Petroleum ether b.p. below 60°C.

A 2 g flour sample was weighed in a 50 ml beaker and moistened with 2 ml alcohol and 6 ml hydrochloric acid solution were added and mixed well. The beakers were held in a water bath at 70-80°C for 30-40 minutes, stirring frequently during the incubation, after which they were cooled and 10 ml alcohol were added to each mixture. Samples were transferred to Mojonnier fat extraction flasks by washing each beaker with 25 ml ethyl ether divided into 3 portions, and the flasks were shaken vigorously for 1 minute after which 25 ml redistilled petroleum ether were added and again the flasks were shaken for 1 minute. Flasks were let stand at room temperature for 1 hour until the upper fat-ether layer was clear. The fat layer was drown off and filtered through glasswool packed on a filter paper in a funnel, into a dried preweighed 125 ml flask.

The liquid remaining in the fat flask was reextracted twice with 10 ml of each ether. The upper layer was filtered into the same flask, then the funnel and the tip of it's stem were washed with a mixture of equal parts of the two ethers. The ether was evaporated slowly on a steam bath, then the fat was dried in a Labline, Inc. fat oven at 70°C for 2.5 hours. Flasks were cooled for 30 minutes

in a dessicator, then reweighed. The weight was corrected by running a blank determination on the reagents used and the fat was expressed as percentage fat by acid hydrolysis.

Ash.

The method 08-01 of the AACC (1961) was followed, where approximately 2 ± 0.01 g well mixed samples were weighed into dried preweighed porcelain ashing dishes. The dishes were put in a Temco muffle furnace equipped with Barber-Colman Thermostat, and the oven temperature was increased gradually to 525°C. The samples were incarcerated until a constant weight was obtained. Ashing dishes were cooled in a dessicator to room temperature, and were weighed. Ash was expressed as percentage of sample weight.

Sulfhydryl Groups.

The procedure developed by Ellman (1959) and modified by Volpe (1976), was followed for the determination of sulfhydryl groups in the two flour samples. The reagent used for these analyses were:

Sodium phosphate buffer 0.01 M pH 8.0; containing 1.1% sodium lauryl sulfate and 0.4% di Na. EDTA.

5,5'dithiobis-(2-nitrobenzoic acid) solution (DTNB): Fourty mg DTNB were dissolved in 10 ml 0.1 M sodium phosphate pH 7.0.

To determine sulfhydryls, 5 ml of 0.01 M sodium phosphate buffer pH 8.0 were added to 10 mg of dry sample. The mixture was boiled for 30 minutes, then cooled, and 0.2 ml

DTNB solution were added. The color was allowed to develop for 45 minutes after which the samples were centrifuged for 10 minutes at 1000 rpm in Sorvall Glc-1 centrifuge with type GSA rotor. The absorbancy was read with Beckman DB-G Grating Spectrophotometer equipped with visible and ultraviolet light sources, and 1 cm pathlength quartz cuvettes, using the wavelength 412 nm and 600 nm to correct for the turbidity in the presence of starch. The extinction coefficient of 13,600 was used for determining the μmoles of sH/g of sample. The correction was made for the cottonseed flour color, and a blank was run along with each determination.

Total Sulfhydryl Groups.

A method based on the reduction of disulfides to sulfhydryls, then the determination of sulfhydryls in the protein sample was developed by Cavallini et al. (1966), and modified by Volpe (1976) was followed. The following reagents were used for this test.

Sodium Phosphate buffer 0.05 M and pH 7.4 was prepared and 10 ml of 0.02 M di Na EDTA were added to the buffer at the ratio of 10 ml di Na EDTA/200 ml buffer.

Urea-Sodium Borohydride Solution: Ten grams urea and 0.25 g sodium borohydride were dissolved in 10 ml deionized water.

Potassium Phosphate-Hydrochloric Acid Solution: It was prepared by dissolving 13.6 g mono potassium phosphate and 1.66 ml of 37% hydrochloric acid in 100 ml deionized water.

DTNB Solution: 40 mg of 5,5'dithiobis-(2-nitrogenzoic acid) were dissolved in 10 ml 0.1 M sodium phosphate buffer pH 7.0.

Anti-Foaming Agent: 1-Octanol

Three mg dry sample were dispersed in 1 ml sodium phosphate buffer pH 7.4, then 2 ml 1-octanol and 1 ml urea-sodium borohydride solutions were added. This mixture was shaken and incubated in 40^{o}C water bath for 30 minutes. The mixtures were cooled, and 0.5 ml potassium phosphate-hydrochloric acid solution was introduced carefully to wet the walls of the test tube in order to destroy the traces of sodium borohydride. Five minutes were allowed for the destruction of sodium borohydride after which 1 ml acetone was added and mixed to complete the destruction.

The determination of sulfhydryl groups was completed according to Ellman's procedure (1959) where 0.2 ml of DTNB solution was added to the mixture and the reaction was allowed to develop for 45 minutes after which the absorbancy was read at 412 and 600 nm and the μmoles SH/g sample were calculated using 13,600 as an extiction coefficient. A blank was run throughout all determinations.

Estimation of Rheologically Active Thiol and Disulfide Groups in Dough.

The quantitative method developed by Jones et al. (1974) for distinguishing the rheologically-important thiol and disulfide groups from those that are unimportant was followed. All experiments were carried out in the 50 g bowl of the Farinograph, at 30°C. A solution of 1 g sodium chloride in a volume of water necessary to center the peak of the Farinograph curve on the 500 BU-line was added, and was kept constant throughout all the testing. Mixing was continued for 30 minutes after which the curves were evaluated for maximum resistance and resistance to mixing after 30 minutes.

The dithiothreitol for the determination of disulfide groups in 0% and 8% cottonseed substituted doughs, was dissolved in 0.1 ml ethanol and added with the salt solution to the flour at zero time. Solid DTT was added (5 to 200 μmoles) in 4-5 minute intervals after the dough reached the maximum development for serial addition.

Determination of the reactive sulfhydryl groups in 0 and 8% cottonseed substituted dough was performed by adding N-ethylmaleimide (0 to 60 μmoles) to the salt solution added to the flour at zero time in 0.1 ml ethanol.

Evaluation of Bonding Systems in Dough.

The mixograph was used for this study where 30 ± 0.01 g on 14% m.b. of 0, 8 and 16% cottonseed substituted flour mixes were weighed and were titrated with water necessary to produce a dough of the consistency determined by a Farinograph curve peak centered on the 500 BU line. The absorption was kept constant throughout testing and all the solid reagents were added dry to the flour, while the liquid reagents were added at zero time with the addition of water, and on a substitution basis of the water volume. The reagents used for this study were:

Urea: It was added at 0.02, 0.04, 0.06, 0.08, 0.1 and 1 M based on the total volume of both flour and water.

Sodium-Dodecyl Sulfate: SDS was added as a solid reagent to the flour at the following amounts: 0.125, 0.25, 0.5 and 1 g.

Succinic Anhydride: It was added as a dry powder to the flour and the quantities were: 0.02, 0.1, 0.2, 1.0 and 2.0 g.

Amino Acid Analysis.

Amino acid analysis of straight grade HRW wheat flour, 85% extraction HRS wheat flour and liquid cyclone processed cottonseed flour were performed on 9 hydrolysates of the

proteins of the three flours. A Beckman Model 120 C Amino Acid Analyzer was used where the amino acids were separated by column chromatography, and the intensity of the color resulted from their reactions with ninhydrin was automatically recorded according to the procedures described in the literature by Moore and Stein, 1948, 1951, 1954; Moore et al., 1958; Spackman et al., 1958. Samples were prepared according to the method described in the Beckman manual for amino acid analysis (Toeffer, 1965). A standard amino acid calibration mixture was used for comparing the chromatograms obtained.

Sulfur Containing Amino Acids. Methionine and cysteine are instable during acid hydrolysis according to Schram et al. (1953). Preliminary oxidation of performic acid was performed to oxidize cysteine to cysteic acid and methionine to methionine sulfone. Samples were then hydrolyzed and amino acids were determined as previously described.

Tryptophan. Tryptophan is labile during hydrolysis so it was not determined but values for tryptophan was supplied from the literature for each flour sample.

Scanning Electron Microscopy Studies

The effects of cottonseed substitution of wheat flour and emulsifier polysorbate 20 were studied on 0 and 8% substituted dough systems. The mixograph was used to mix 0 and 8% substituted dough, with and without 0.5% emulsifier (Tween 20) to optimum development. Immediately after

the mixing, thin strips of dough were cut with a pair of scissors from a smooth freshly exposed surface (Hooper and Volpe). Dough strips were quickly immersed in liquid nitrogen then fractured to pieces 2-3 cm long, then were freeze dried. Some dried samples were fractured.

The effect of sample preparation on the electromicrographs was studied, where 0, 8% substituted doughs were prepared by mixing dough to the maximum development in a mixograph with and without 0.5% emulsifier Tween 20. Thin strips of dough were cut with a pair of scissors from a smooth freshly exposed surface. Dough strips were then divided into small segments about 2-3 mm long then fixed for 24 hours at $10^{o}C$ in 0.1 M phosphate buffered glutaraldehyde (5%), adjusted to pH 6.0. After fixation, samples were dehydrated in graded ethanol series 10 to 100%, after which they were critical point dried (CPD) with CO_2 as the ambient liquid. Some CPD samples were fractured.

All specimens were mounted on aluminum stubs using television tube coat, then were coated with gold, and viewed at 20 KEV in ISI Super Mini scanning electron microscope. All sample preparations were duplicated and the representative areas were carefully examined.

RESULTS AND DISCUSSION

Farinograph Studies of HRW Wheat Flour Supplemented with LCP Cottonseed Flour and Unmodified Starch.

Absorption is the amount of water that flour requires to form a dough of optimum consistency for bread-making. It is usually determined to be the amount of water required to center the Farinograph curve on the 500 BU line. The absorption is actually dependent upon by the amount of water required by the various flour components, particularly by starch and protein (Bushuk and Hlynka, 1964). The increased absorptions of wheat flours substituted with different oilseed proteins have been reported by many researchers. Cottonseed protein concentrates prepared by wet-extraction and spray or freeze dried at different pH were shown to have different water absorptions (Khan et al., 1976). In the LCP cottonseed flour system, the water absorption increased as the level of cottonseed flour increased in the system (Table 1); however, the maximum absorption seems to be obtained at the 8% level of substitution, after which there was no significant increase in absorption.

Table 1. Farinograph data[1] and SD for HRW wheat flour dough substituted with LCP[2] cottonseed flour

Cottonseed Flour %	Absorption[3] %	Peak Time min	M.T.I.[4] B.U.	T.M.D.[5] B.U.
0.0	58.67 ±0.5	3.57 ±0.1	33.0 ±4.8	87.0 ±4.7
4.0	60.94 ±0.6	3.23 ±0.2	117.0 ±4.9	187.0 ±4.8
8.0	62.32 ±0.6	3.60 ±0.3	82.0 ±10.6	110.0 ±0.0
12.0	62.97 ±0.6	3.47 ±0.1	107.0 ±2.8	160.0 ±0.0
16.0	62.90 ±0.7	3.70 ±0.1	135.0 ±10.8	217.0 ±4.7

1) Average of three replications.

2) Liquid Cyclone Processed cottonseed flour.

3) Absorption is expressed on 14% M.B.

4) Mixing Tolerance Index.

5) Twenty Minute Drop.

Wheat flour was substituted with wheat starch at 0, 4, 8, 12, 16% of flour wheat in order to study the effect of dilution per se as compared to LCP cottonseed effects.

The water absorption in the wheat flour/wheat starch system followed an opposite trend from that of wheat flour/ cottonseed system, as can be seen from Figure 1.

Data presented in Table 2 shows a decrease in water absorption with increasing the level of wheat starch in the system, with a mimimum absorption obtained at the 8% level of substitution.

Starch absorbs somewhat less than its own weight of water (27-35%), while gluten, on the other hand, absorbs more than its weight of water (109 - 215%) (Bushuk et al., 1964). This can explain the difference observed in absorption between the two systems. Hagenmaier (1972) studied the water binding of some oilseed isolates and reported that there was an increase of water binding with larger values of hydrophilic groups. This could be another reason for the higher Farinograph water absorption values with higher levels of cottonseed flour. Cottonseed flour has high glutamic and aspartic acids content. Results suggest that there must be a minimum amount of gluten present in the system in order to get the maximum absorption, which can be seen from Tables 1 and 2. The dilution of wheat gluten with either plant protein or starch with up to 8% showed clearly that effect.

Figure 1. Farinograph absorption of dough systems substituted with cottonseed flour, and starch

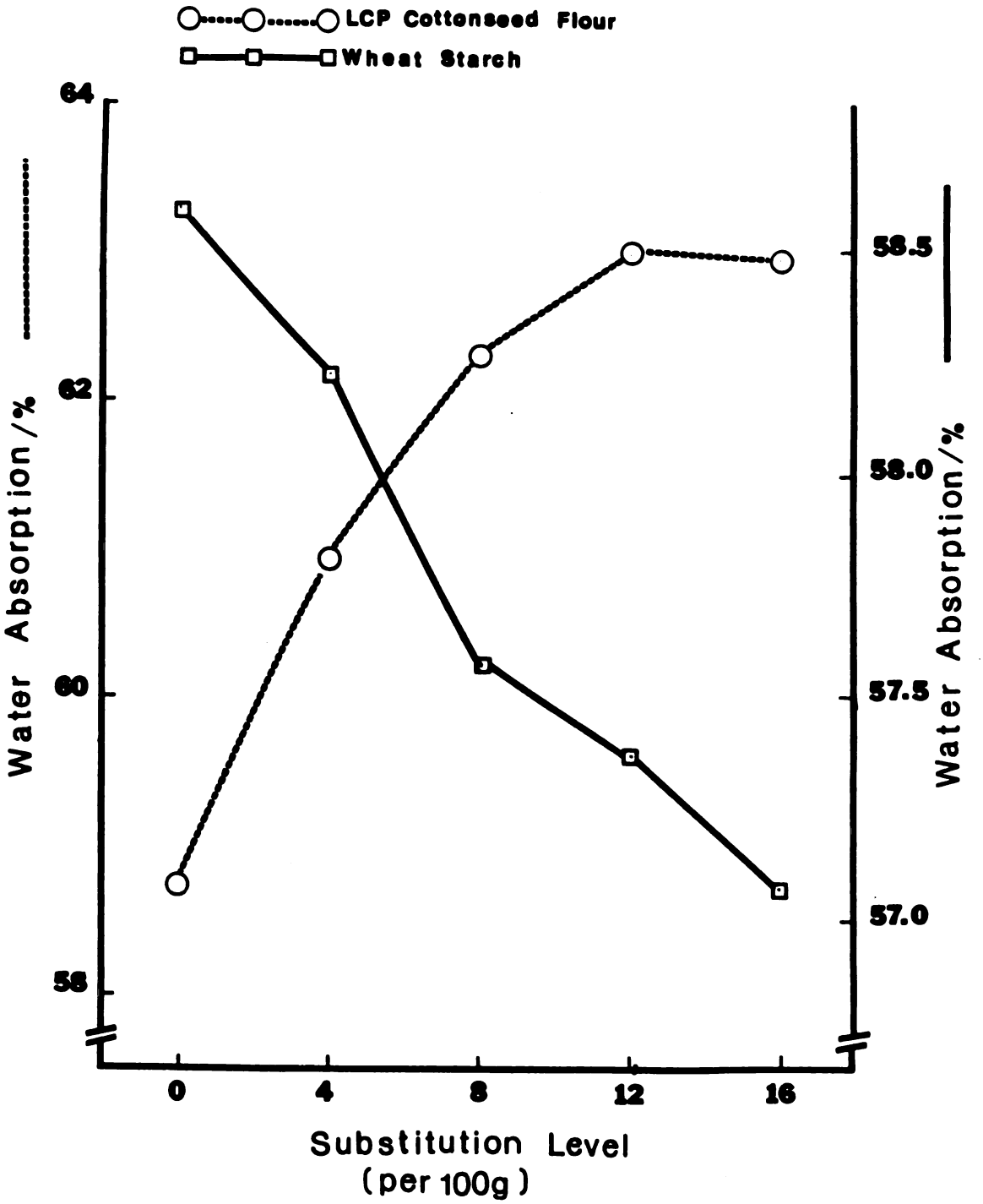

LCP Cottonseed Flour
Wheat Starch
Water Absorption /%
64
62
60
58
58.5
58.0
57.5
57.0
Water Absorption/%
0
4
8
12
16
Substitution Level
(per 100g)

Table 2. Farinograph Data[1] and SD for HRW wheat flour dough substituted with wheat starch[2]

Starch %	Absorption[3] %	Peak Time min	M.T.I.[4] B.U.	T.M.D.[5] B.U.
0.0	58.55	3.67 ±0.9	42.0 ±6.2	37.0 ±2.4
4.0	58.24	3.37 ±0.2	35.0 ±4.1	50.0 ±4.1
8.0	57.62	3.07 ±0.1	33.0 ±6.2	42.0 ±2.4
12.0	57.40	3.45 ±0.2	47.0 ±4.7	55.0 ±4.1
16.0	57.08	3.22 ±0.1	48.0 ±6.2	68.0 ±2.4

1) Average of three replications.

2) Unmodified wheat starch.

3) Absorption is expressed on 14% M.B.

4) Mixing Tolerance Index.

5) Twenty Minute Drop.

Arrival time is a measure of the rate of hydration of flour constituents. Bushuk et al. (1964) reported that there is a decrease in the rate of hydration with the increase in protein content. The substitution with cottonseed flour (Table 1) caused an increase in the arrival time with increasing the level of cottonseed flour in the system, while wheat starch (Table 2) caused a decrease in the arrival time, as can be seen from Figure 2. That could be resulting from diluting the protein with wheat starch.

The peak time is the time the dough takes to reach maximum consistency or minimum mobility. The results of peak time presented in Tables 1 and 2 are consistent with the results obtained on the rate of hydration (Figure 3); wheat/cottonseed systems show an increase in the peak time with increasing the level of cottonseed flour, with an unusual drop at the peak time at the 4% level of substitution. The wheat/starch system show a different trend with a drop in the peak time at 8% level of substitution, which indicate that the peak time is actually related to physical development of gluten, rather than to the protein content.

Mixing Tolerance Index (M.T.I.), which is the difference in BU from the top of the curve at the peak to the top of the curve measured at 5.0 minutes after the peak; and the Twenty Minute Drop (T.M.D.), are both measures of dough breakdown. Table 1 and Figure 4A show the increase in the dough breakdown with the high levels of cottonseed flour

Figure 2. Farinograph arrival time of wheat/cottonseed and wheat/starch doughs

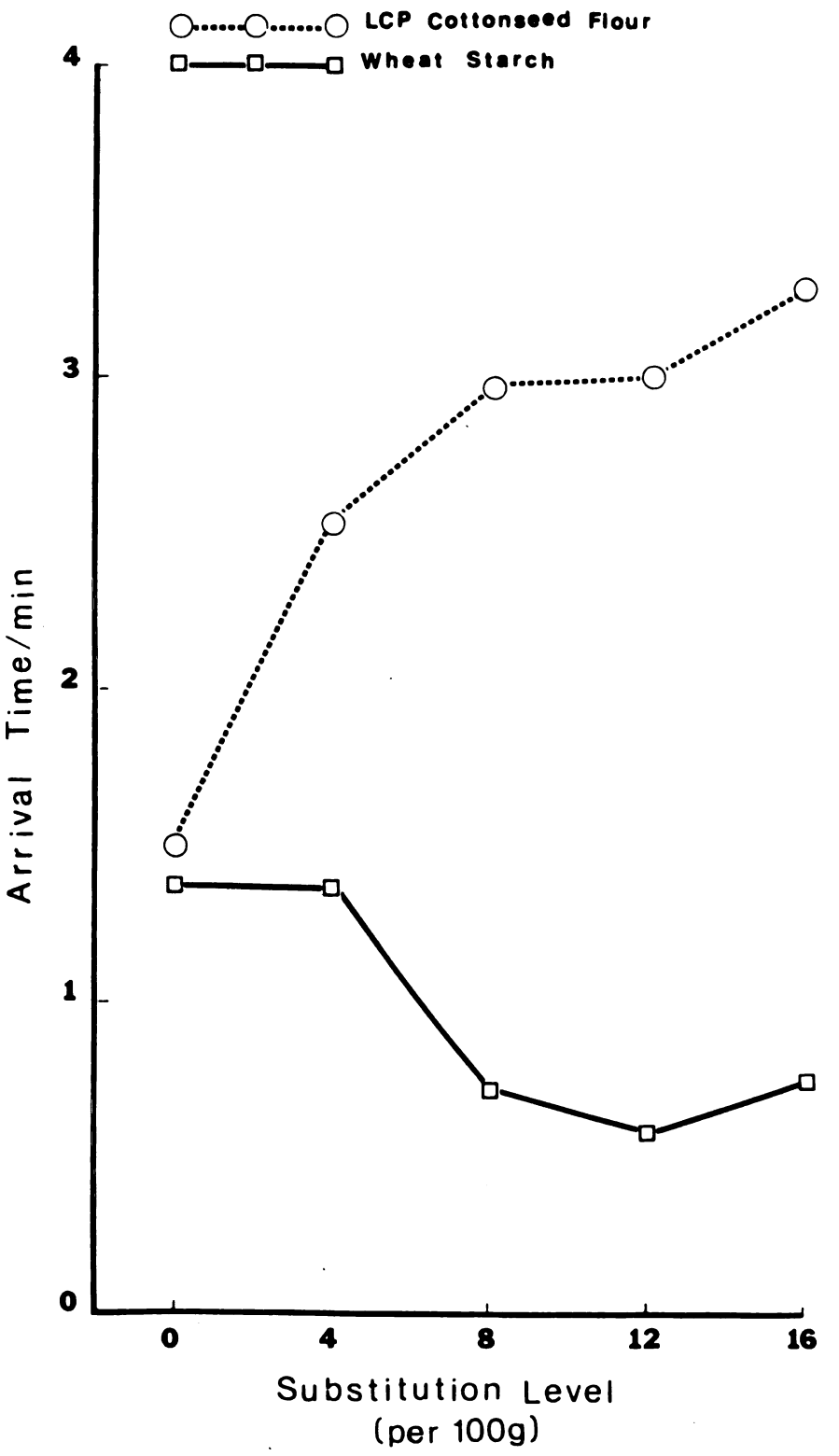

LCP Cottonseed Flour
Wheat Starch
Arrival Time/min
0
1
2
3
4
0
4
8
12
16
Substitution Level
(per 100g)

Figure 3. Farinograph peak time of wheat/cottonseed and wheat/starch doughs

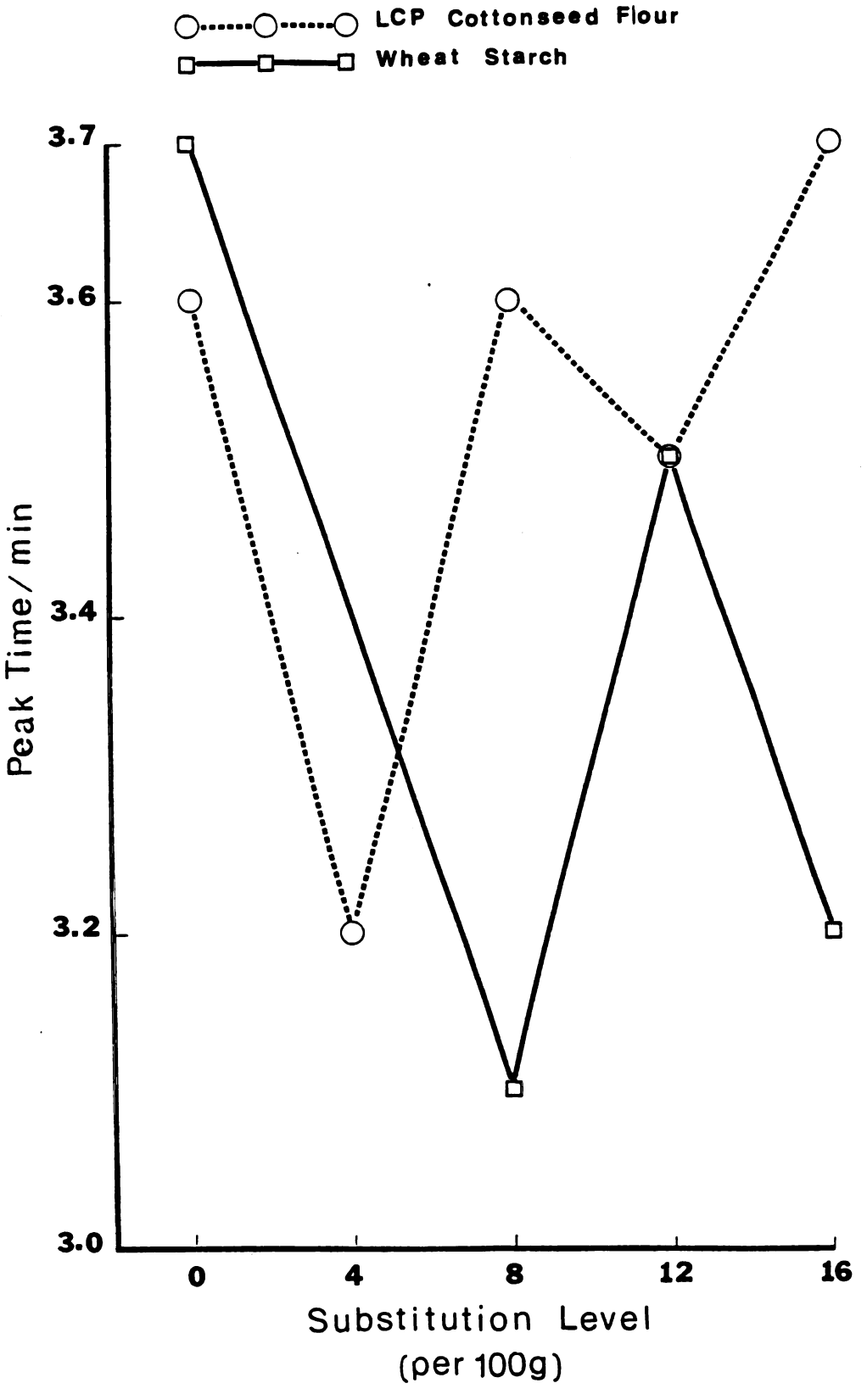
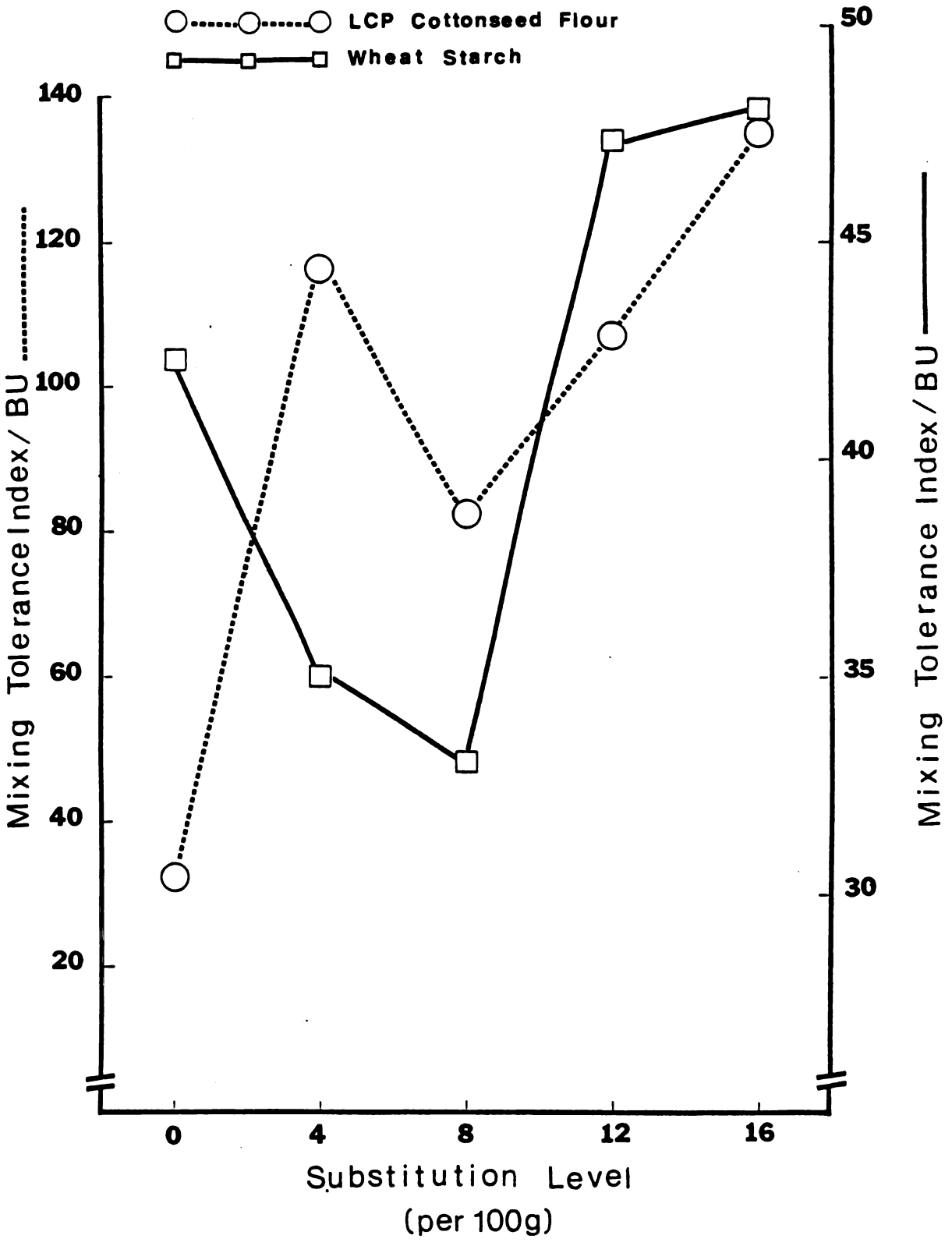

LCP Cottonseed Flour
Wheat Starch
3.7
3.6
3.4
3.2
3.0
Peak Time/min
0
4
8
12
16
Substitution Level
(per 100g)

Figure 4A. Effect of cottonseed flour and starch on Farinograph Mixing Tolerance Index


LCP Cottonseed Flour
Wheat Starch
Mixing Tolerance Index / BU
Mixing Tolerance Index / BU
140
120
100
80
60
40
20
50
45
40
35
30
0
4
8
12
16
Substitution Level
(per 100g)

(12 and 16%) as can be seen from the mixing tolerance index which indicates that the Farinograph curves drop as much as 135 BU. There is also an increase in the dough breakdown with the addition of starch, up to 47-48 BU at 12 and 16% levels. T.M.D. (Figure 4B) whows the same thing.

The data suggest that starch contribute, to the stability of wheat flour dough substituted with cottonseed flour, and that a certain balance between protein and starch must be obtained in order to get the optimum Farinograph properties, which are used as an indication of flour performance in bread-making.

Viscoamylograph Study.

Viscoamylograph was used to determine the effect of alpha-amylase on the viscosity of flour as a function of temperature. The high viscosity of the starch gel is counteracted by the action of alpha-amylase, which liquifies starch granules during heating of slurry. The amylograph value provides information on the possible effect of alpha-amylase or on the starch gelatinization during baking process (AACC, 1960).

The potential of wheat/cottonseed flour blends to produce good loaf volume was studied. The peak viscosity and the viscosity after 60 minutes are represented in Figure 5; a slight decrease in both viscosities with the higher levels of cottonseed flour in the blends is apparent. These results suggest that there is a good potential,

Figure 4B. Effect of cottonseed flour and starch on Farinograph Twenty Minute Drop

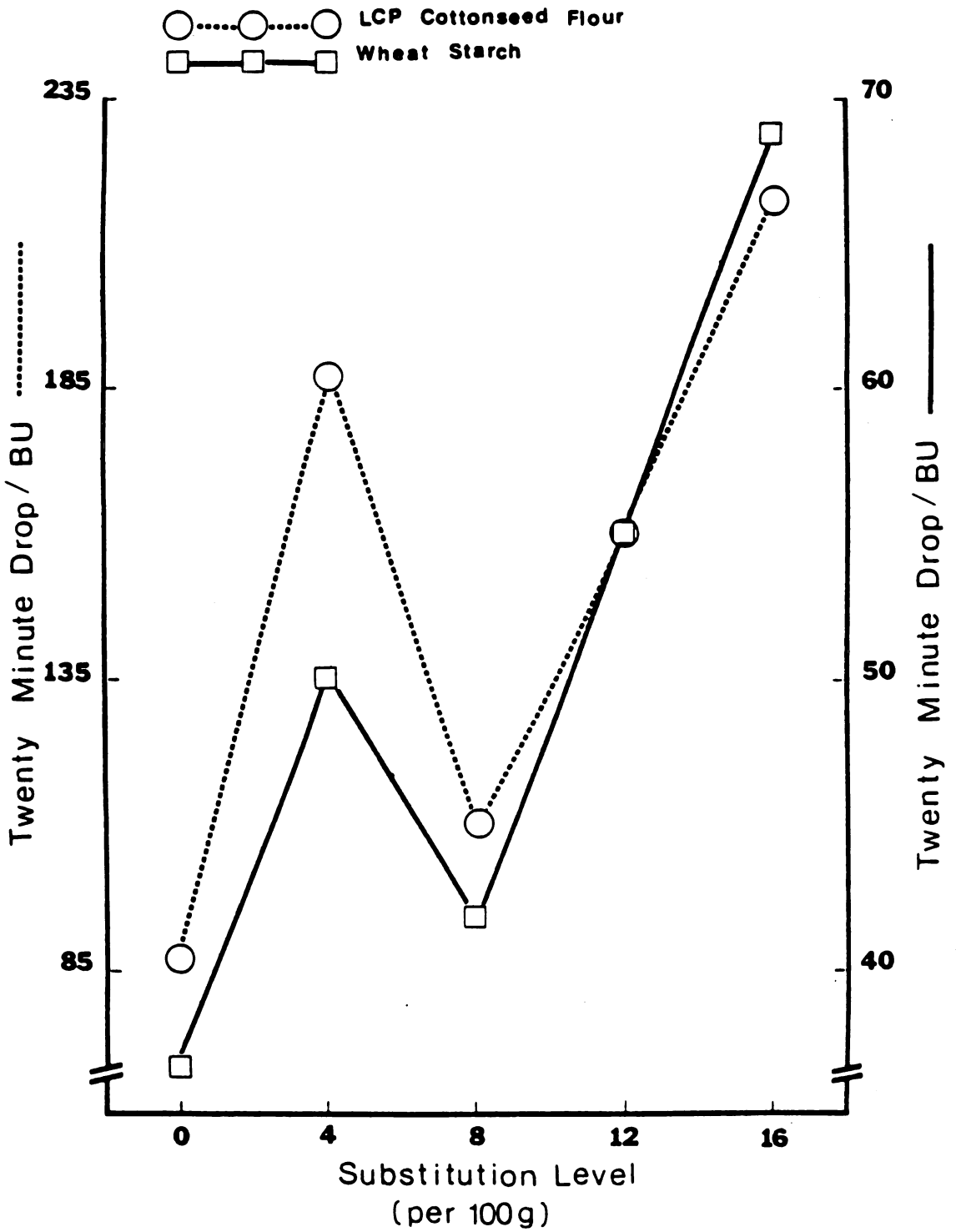
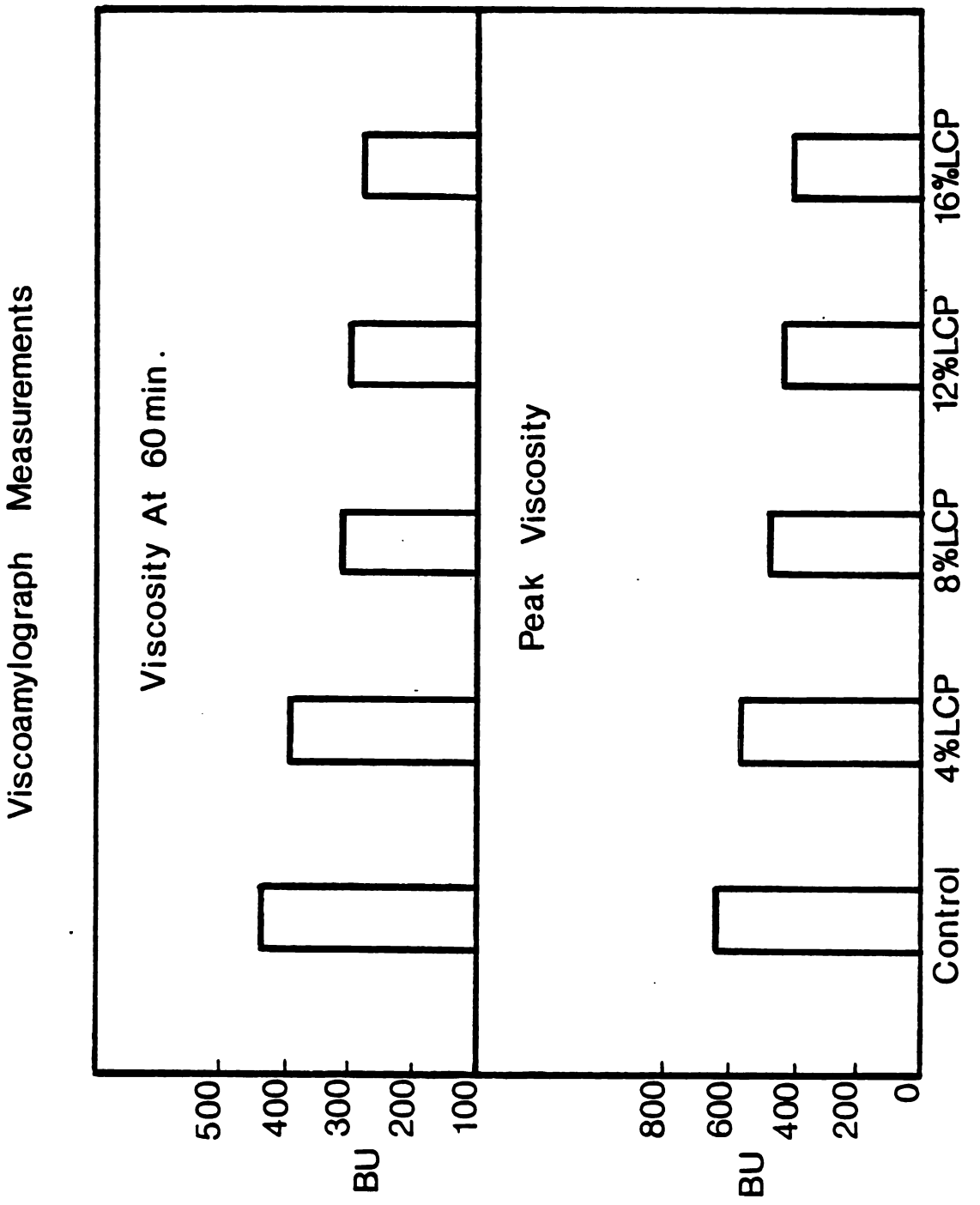

LCP Cottonseed Flour
Wheat Starch
235
185
135
85
70
60
50
40
Twenty Minute Drop / BU
Twenty Minute Drop / BU
0
4
8
12
16
Substitution Level
(per 100g)

Figure 5. Viscoamylograph peak viscosity and viscosity at 50 min for wheat flour substituted with LCP cottonseed flour


Viscoamylograph Measurements
Viscosity At 60 min.
Peak Viscosity
BU
500
400
300
200
100
800
600
400
200
0
Control
4%LCP
8%LCP
12%LCP
16%LCP

even at the high level of cottonseed flour (16%), to give enough fermentable carbohydrate in the flour blend, therefore, a good loaf volume.

Baking Study.

Bread was baked using 100 g flour, 2 g dry yeast, 2 g active dry yeast, 2 g sugar, 2 g salt, and the amount of water added was exactly the Farinograph water absorption, previously determined for each flour blend. The pH of dough prepared with different levels of cottonseed flour increased as the level of cottonseed increased in the system (Figure 6). During fermentation, there was a drop in pH at all levels of substitution with cottonseed but the pH of the substituted dough was much higher than the control dough, as can be seen in Figure 6. Mathason (1978) reported that the optimum pH value of white bread and rolls is 5.2 when bread's pH is higher than 5.8, it would contribute to dark crust, an open, crumbly grain, lack of flavor and sharp corners on pan loaves.

The protein content of bread made with cotton seed increased from 7.82% in the control to 12.68% at the 16% level of substitution, which is equal to 62.15% increase in the protein content (Table 3). Although the higher levels of cottonseed flour in bread did not produce bread that can be defined as high protein bread (15% protein), it increased the protein significantly with each addition of cottonseed flour. Breads made with LCP cottonseed flour

Figure 6. Effect of cottonseed flour on pH of dough during fermentation

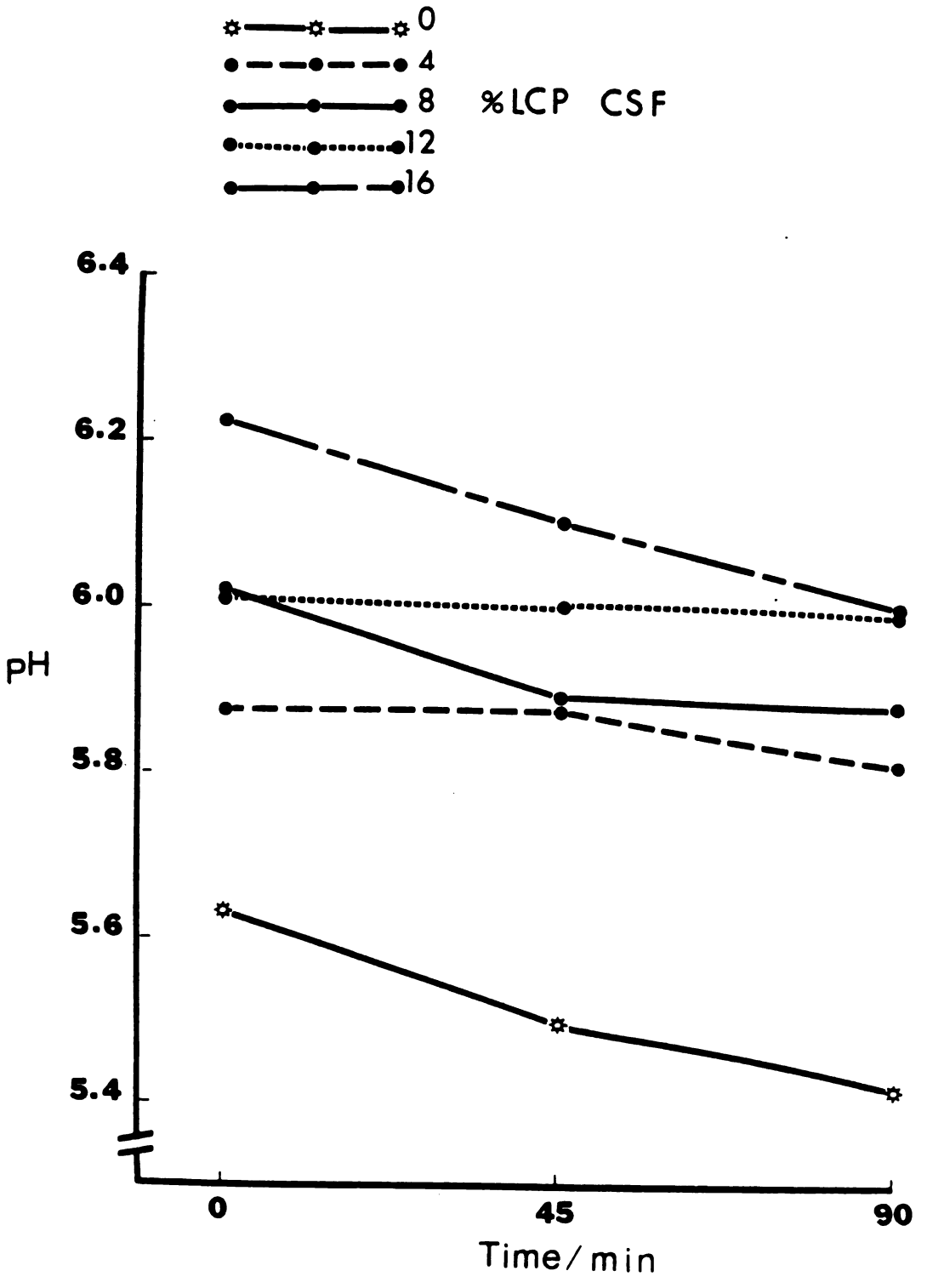

0
4
8
12
16
%LCP CSF
6.4
6.2
6.0
5.8
5.6
5.4
pH
0
45
90
Time/min

Table 3. Proximate analysis[1] of bread prepared with different levels of LCP cottonseed flour

Substitution level %	Moisture %	Protein %	Fat %	Ash %
0	42.34	7.82	1.26	1.30
4	38.14	9.35	1.86	1.70
8	37.95	10.92	1.58	1.74
12	37.95	12.38	1.34	1.79
16	38.26	12.68	1.97	2.06

[1]Values are average of three replications and are expressed on as is moisture basis.

actually met the Federal standard level of moisture in bread of 38%. Ash content also increased with an increasing level of cottonseed flour in bread.

White bread made from untreated HRW wheat flour and bread substituted with LCP cottonseed flour were baked, and were evaluated objectively for volume, specific volume, compressability and color. Breads were evaluated for crust color and character, crumb color, grain texture and for general acceptability by taste panelists. These results (Table 4) show that there is no significant effects of cottonseed flour substitution on loaf volume, specific volume or compressability. In fact there was a slight increase in loaf volume and specific volume with the substitution of wheat flour with 4, 8, and 12% LCP cottonseed flour.

The addition of LCP cottonseed flour significantly influenced bread color. The lightness and the yellowness of bread substituted with cottonseed flour decreased significantly with an increasing level of substitution indicating a darker, more greenish color bread (Figure 7).

Sensory evaluation data (Table 5) indicated the significant effect of cottonseed flour on bread crust and crumb color ($p<0.0005$) which indicate a darker product with higher levels of cottonseed flour; it is consistent with objective color measurement. Grain texture scores increased significantly ($p<0.0005$) with the addition of cottonseed,

Table 4. Mean and standard deviation of objective measurement of wheat/cottonseed flour bread

Substitution level %	Loaf		Comp	Color		
	vol cc	sv cc/g	lb/cm	L***	a***	b*
0	474[a] ±23	3.50[a] ±0.3	37.35[a] ±4.1	42.53[c] ±1.2	-1.23[a] ±0.2	10.83[c] ±0.5
4	484[a] ±49	3.68[a] ±0.4	48.12[a] ±11.9	36.73[b] ±0.6	-0.43[b] ±0.2	10.30[ab] ±0.6
8	483[a] ±42	3.68[a] ±0.4	39.50[a] ±8.2	31.90[a] ±0.2	-0.35[b] ±0.2	9.90[a] ±0.4
12	481[a] ±17	3.56[a] ±0.1	46.08[a] ±10.5	31.08[a] ±0.6	0.70[bc] ±0.2	10.05[ab] ±0.4
16	468[a] ±12	3.42[a] ±0.1	44.83[a] ±5.0	29.73[a] ±0.7	0.83[c] ±0.1	9.82[a] ±0.2

Means followed by the same letters are not significantly different at $p \leq 0.05$ Duncan's Multiple Range Test.

*, **, *** represent significant levels of $p \leq 0.05$, $p \leq 0.005$ and $p \leq 0.0005$ respectively.

Figure 7. Bread substituted with LCP cottonseed flour
a) Control (untreated)
b) 4% cottonseed flour
c) 8% cottonseed flour
d) 12% cottonseed flour
e) 16% cottonseed flour


ا
ب
ج
د
ه

Table 5. Mean and standard deviation of sensory evaluation of wheat/cottonseed flour bread

Cottonseed level %	Crust*** Color 0-10[2]	Crust** Character 0-10	Crumb*** Color 0-10	Grain*** Texture 0-10	General[1] Acceptability 0-10
0	3.33^{a} ±0.52	7.47^{bc} ±1.54	2.00^{a} ±1.04	6.44^{b} ±0.52	7.50^{a} ±2.40
4	6.08^{b} ±0.44	7.86^{c} ±0.65	5.96^{b} ±0.61	4.64^{a} ±0.59	7.72^{a} ±0.65
8	7.99^{c} ±0.60	6.67^{abc} ±0.61	7.71^{c} ±0.44	8.43^{c} ±0.49	7.29^{a} ±0.80
12	8.85^{cd} ±0.42	6.01^{ab} ±0.46	9.16^{cd} ±0.69	6.92^{b} ±0.23	7.34^{a} ±0.45
16	9.42^{d} ±0.59	5.53^{a} ±0.21	9.88^{d} ±0.90	6.87^{b} ±0.51	6.79^{a} ±0.20

1) Not significant.

2) See Appendix 1 for explanation of 0-10 scales.

, * Correspond to significance levels of $p \leq 0.005$ and $p \leq 0.0005$ respectively. Means followed by the same letter are not significantly different at $p<0.05$ Duncan's Multiple Range Test.

indicating a much more open grain with the high levels of cottonseed in bread, however the 8% level of substitution had the coarsest grain of all the levels. Similar changes in crumb grain have been reported by Fleming and Sosulski (1978) on bread containing soy flour, sunflower, faba bean and field pea. Rooney et al. (1972) reported a similar effect of solvent extracted cottonseed flour, on bread texture.

Brean was generally scored as hence slightly unacceptable (between 6-8 on the sensory scale).

Mixograph Studies of Effect of Sodium Chloride, Potassium Bromate, and Polysorbate (20) on HRW Wheat/LCP Cottonseed Flour Dough

One approach to improve bread characters is to use different additives. The effect of additives on mixograms was evaluated. Each additive was evaluated separately and in combination with the others. All the possible interactions, at all levels of additives were studied. Mixograms were evaluated for peak time, peak height, curve height at 9 minute point, and the area under the curve.

Single Additive Effects

From Table 6 it can be seen that cottonseed, salt and conditioners significantly affected all the mixograph characters studied ($p<0.0005$), potassium bromate's effect was less significant on peak height ($p<0.05$) and height of

Table 6. Mean squares and F statistics significance for mixograph parameters of HRW wheat/ LCP cottonseed dough

Source of variance	Degrees of Freedom	Peak Time	Peak Height	Ht at 9 min point	Area under curve
Cottonseed	2	21.12***	18.03***	33.99***	8611.99***
Salt	2	171.74***	0.84***	21.45***	1973.03***
Tween 20	2	10.02***	1.00***	0.34***	509.90***
Bromate	2	17.79***	0.16*	0.18**	566.46***
Cottonseed*Salt	4	12.87***	0.1NS	0.34***	290.28***
Salt*Tween 20	4	0.62***	0.13*	0.31***	40.20NS
Tween 20*Bromate	4	0.70***	0.22**	0.55***	59.66*
Cottonseed*Tween 20	4	1.06***	0.03NS	0.10**	115.91***
Salt*Bromate	4	1.72***	0.22**	0.12**	36.87NS
Cottonseed*Bromate	4	0.47***	0.09NS	0.09*	172.72***
Cottonseed*Salt*Tween 20	8	0.32***	0.06NS	0.06*	71.83***
Cottonseed*Salt*Bromate	8	0.26***	0.22***	0.20***	82.24***
Cottonseed*Tween 20*Bromate	8	0.31***	0.05NS	0.05NS	56.74**
Salt*Tween 20*Bromate	8	0.65***	0.13***	0.10***	41.40*
Cottonseed*Salt*Tween 20*Bromate	16	0.26***	0.13***	0.11***	81.53***
Residual error	81	0.06***	0.49	0.02	16.94

* $p<0.05$
** $p<0.005$
***$p<0.0005$
NS $p<0.05$

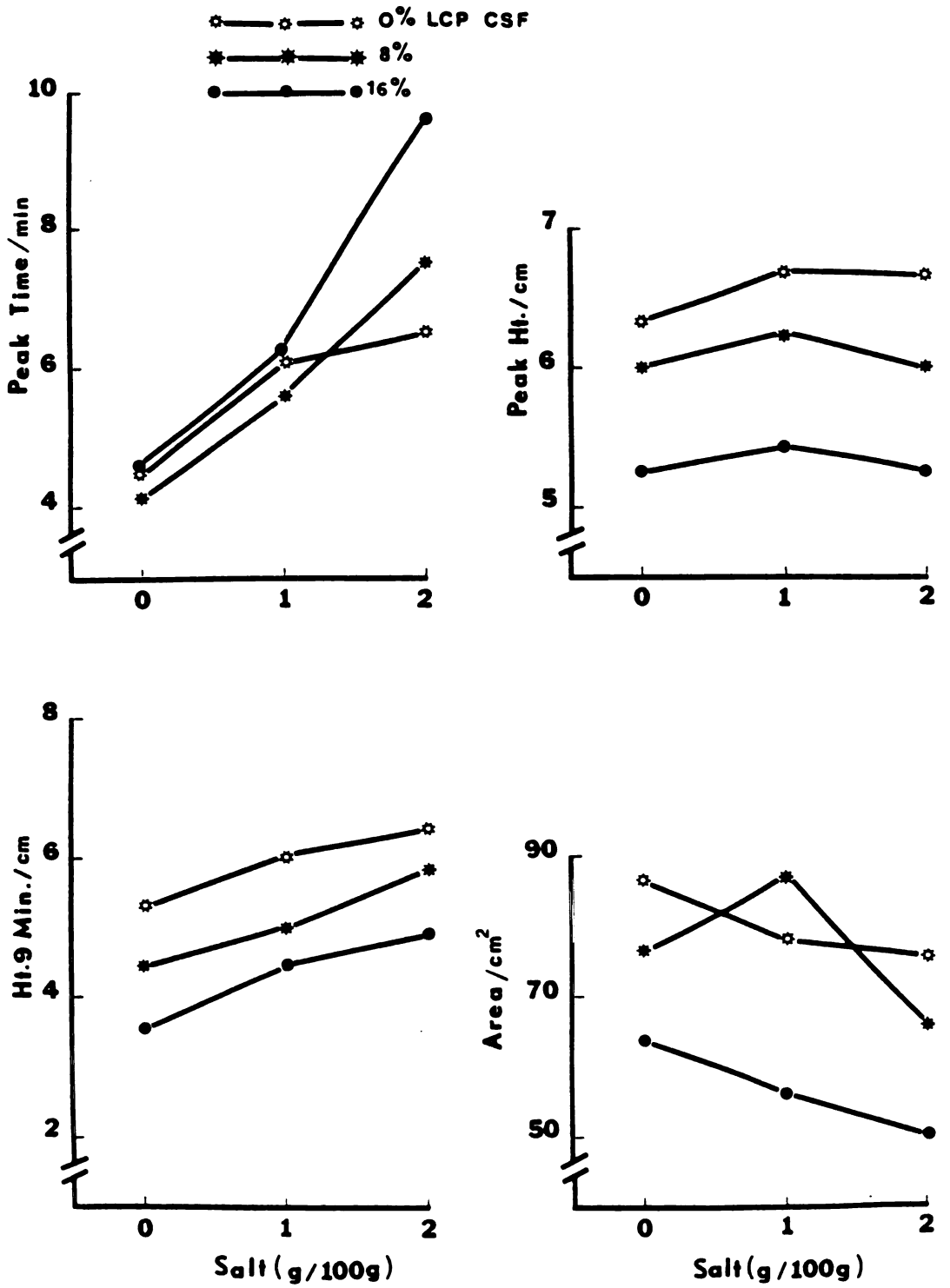
curve at 9 min point ($p<0.0005$). Figures 8A through C represent those effects.

Salt.

Adding salt to the flour markedly affected the physical characteristics of the dough, especially when 2% salt was added. Figure 8A shows that increasing the salt level from 1 to 2% increased the peak time, and this effect was noticable with the higher levels of cottonseed flour in dough. Salt increased the peak height especially at 1% level, and the curve height at 9 min point. The area under the curve decreased with the addition of salt. This effect was very pronounced for the 8% level of substitution with LCP cottonseed flour, and 2% level of salt.

The effect of salt is thought to be primarily due to the changes in gluten hydration, a phenomenon Bushuk and Hlynka (1964) explained as free and bound water. The presence of salt in dough system increased the amount of mobile or free water in dough by occupying the sites once occupied by bound water, thus altering the gluten structure, which results in longer peak time. The increase in gluten strength is represented by the increase in peak height as well as the increase in the curve height at 9 min point (Figure 8A). The effect of salt on strengthening or toughening wheat flour dough has been reported in the literature (Galal et al., 1978).

Figure 8A. Effect of salt on mixograph properties of 0, 8, 16% dough substituted with cottonseed flour


0% LCP CSF
8%
16%
Peak Time/min
10
8
6
4
0
1
2
Peak Ht./cm
7
6
5
Ht.9 Min./cm
8
6
4
2
Area/cm²
90
70
50
Salt (g/100g)
Salt (g/100g)

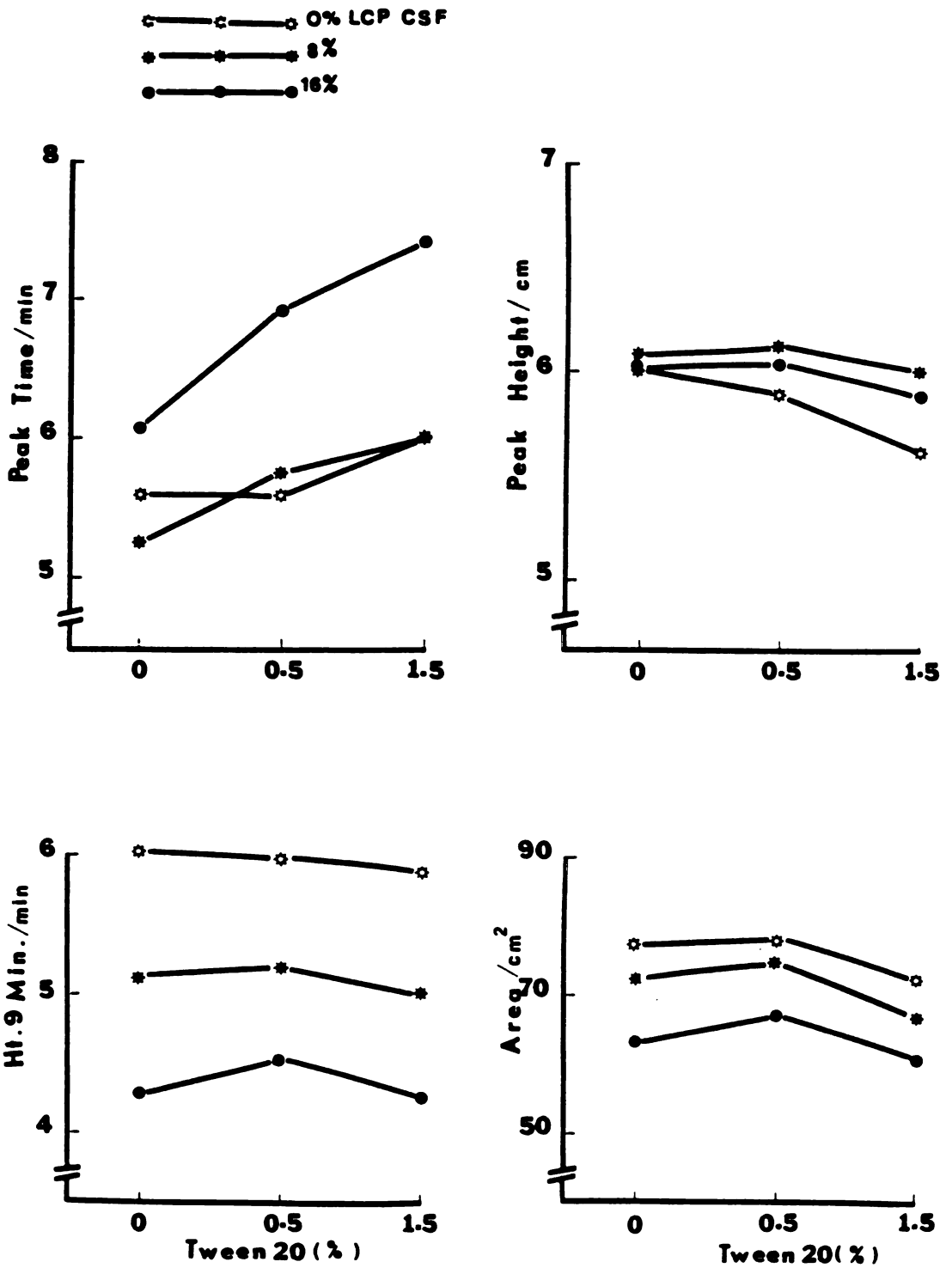
Gluten proteins precipitate at ionic strength above 0.04 corresponding to a level of addition of 7-8 mmoles of sodium chloride. Since gluten proteins are insoluble in the presence of salt, they will favor compact forms minimizing protein-solvent contacts. The salt-soluble proteins, on the other hand, favor elongated configuration giving a maximum number of protein-solvent contacts, thus enabling maximum inter-chain bonding. As the gluten protein content in dough increases, the ratio of salt-soluble to salt-insoluble proteins will decrease thus the strengthening effect of salt is more noticable.

Substituting 8 and 16% of LCP cottonseed flour for wheat flour increased the protein content of dough, but decreased its gluten content. According to the effect of salt on protein explained before (Bennett et al., 1965), the dough strength would decrease with increasing the level of cottonseed flour. This effect can be seen clearly (Figure 8A); all the doughs with cottonseed showed weaker gluten strength represented by lower peak height, lower height at 9 min. point and smaller area than the control flour.

Polyoxyethylene Sorbitan (20) Mono Laureate (Tween 20).

Tween 20 significantly affected all mixograph properties ($p<0.0005$ (Table 6). As a dough conditioner, Tween 20 strengthened the dough as can be seen from Figure 8B, by the increase in peak time, peak height, height at 9 min point and the area under the curve. The conditioner was

Figure 8B. Effect of conditioner "Tween 20" on mixograph parameters of 0, 8, 16% doughs substituted with cottonseed flour


0% LCP CSF
8%
16%
Peak Time/min
8
7
6
5
0
0.5
1.5
Peak Height/cm
7
6
5
0
0.5
1.5
Ht.9 Min./min
6
5
4
0
0.5
1.5
Tween 20 (%)
Area/cm²
90
70
50
0
0.5
1.5
Tween 20(%)

very effective in strengthening the 8 and 16% cottonseed protein substituted dough compared to the control. The maximum dough conditioning was observed at 0.5% levels of conditioner, while the higher level, 1.5%, seems to have an adverse effect on dough physical characters.

Sullivan (1952) attributed the action of dough conditioner to its reaction with protein. Greene (1975) explained the mechanism of action of dough conditioners; he divided the hydration of flour to form a dough into four reactions that tend to progress spontaneously. He hypothesized four different levels of free energy at each reaction. In the explanation of the action of dough conditioners on increasing mixing tolerance, Greene, thought that the reaction of the dough conditioner would take place at the fourth stage of dough development. The increases of the activation energy leading to the fourth stage of reaction causes the formation of ruptured aggregates (breakdown). The action of conditioners which serves to reduce the energy requirement for dough development would fit in his model. Lowering energy requirement means increasing the number of molecular allignments, which are capable of absorbing the energy and lead to the formation of "critical aggregates" which is characteristic of an optimum developed dough. These two cinditioning effects take place only when dough is under shear.

Potassium Bromate:

The improver action appears to be as follows: the structure of dough is due to the properties of gluten proteins. The mobility of the dough system is facilitated by the sulfhydryl-disulfide interchange mechanism. The following is a schematic representation of the interchange that takes place:

$$P_1S\text{-}SP_2+RSH \rightarrow P_1S\text{-}SR+P_2SH$$

Protein chains P_1 and P_2 crosslinked through a disulfide link, react with a free sulfhydryl compound RSH to liberate the protein chain P_2 as a sulfhydryl compound P_2SH_2 which can in turn react with another disulfide link

Bromate reacts with SH groups, thus controling the sulfhydryl-disulfide chain reaction. Flour improving agents enhance the elastic properties and general stability of dough. Bromate is a slow acting improver in contrast to all the other oxidants, thus it reacts at all stages of mixing with the final reaction taking place during baking. It should be obvious that while other improvers would influence dough properties, bromate would be of special interest in relation to the behaviour of dough in the pan and in the oven (Hlynka, 1970).

The data presented in Figure 8C and Table 6 indicated that the maximum effective level of the improver reaction

was 30 ppm $KBrO_3$, after which there was no significant effect on the dough rheology. The improver was effective in strengthening dough containing cottonseed as can be seen from increasing peak time and the height of curve at 9 min point. Comparing this effect with the effect of salt and conditioner on both parameters, it is less significant ($p<0.005$ vs $p<0.0005$).

Mixograph Studies of the Interaction Between Salt, Tween 20 and Potassium Bromate

Double Combination Effects

Double combinations of additives were tested on the 8% LCP cottonseed flour substituted doughs. The mixograph parameters studied were subjected to 3^3 factorial analysis and are presented in Table 6. The significant effects were subjected to Duncan's multiple range test (Table 7). Double combination of additives including salt as one of the additives showed increased peak time ($p\leq0.0005$), with maximum peak times 7.19 and 7.20 minutes obtained with the combinations (2% salt, 1.5% conditioner) and (2% salt, 50 ppm $KBrO_3$) respectively in the 0% LCP dough system and the combinations (2% salt, 1.5% conditioner), (30 ppm $KBrO_3$, 1.5% conditioner) in the 8% LCP dough systems gave the longest peak times 7.8 min. For the economic production of bread in industry the strengthening effects of salt and conditioners are required for the continuous process method but too long mixing would require more energy and time. The combination of 30 ppm $KBrO_3$, 1.5% Tween 20, increased

Table 7. Effect of the interaction of double combinations of additives on mixograph characters at the 0% LCP cottonseed flour dough

		Peak Time[1] min			Peak Height[1] cm			Ht 9 min[1] cm			Area[1] cm^2		
		Tween 20 (level %)											
		0.0	0.5	1.5	0.0	0.5	1.5	0.0	0.5	1.5	0.0	0.5	1.5
	0.0	4.27^{a}	4.49^{a}	4.73^{a}	6.50^{c}	6.33^{ab}	5.98^{a}	5.73^{b}	5.28^{a}	5.12^{a}	93.75^{a}	90.75^{a}	81.08^{a}
Salt %	1.0	6.19^{c}	5.71^{b}	6.32^{c}	6.58^{c}	6.73^{c}	6.50^{c}	6.02^{b}	6.15^{c}	6.00^{b}	79.60^{a}	82.25^{a}	74.38^{a}
	2.0	6.30^{c}	6.57^{c}	7.19^{d}	6.67^{c}	6.70^{c}	6.42^{c}	6.35^{c}	6.45^{c}	6.38^{c}	78.65^{a}	78.52^{a}	69.60^{a}
		Tween 20 (level %)											
		0.0	0.5	1.5	0.0	0.5	1.5	0.0	0.5	1.5	0.0	0.5	1.5
	0.0	4.89^{a}	5.19^{a}	5.49^{b}	6.65^{b}	6.77^{b}	6.42^{b}	4.89^{a}	5.27^{a}	5.04^{a}	90.13^{bc}	92.95^{c}	81.52^{b}
$KBrO_3$ ppm	30.0	5.99^{c}	5.78^{b}	6.42^{c}	6.55^{b}	6.63^{b}	6.17^{a}	5.21^{a}	5.33^{a}	5.01^{ab}	80.38^{b}	80.37^{b}	72.13^{a}
	50.0	5.81^{b}	5.81^{b}	6.33^{c}	6.55^{b}	6.37^{b}	6.32^{ab}	5.28^{a}	5.03^{a}	5.11^{a}	81.48^{b}	78.20^{a}	71.42^{a}
		Salt (level %)											
		0.0	1.0	2.0	0.0	1.0	2.0	0.0	1.0	2.0	0.0	1.0	2.0
	0.0	4.37^{a}	5.46^{b}	5.75^{b}	6.45^{b}	6.65^{b}	6.73^{b}	5.48^{a}	5.90^{b}	6.33^{c}	95.95^{a}	83.60^{a}	85.05^{a}
$KBrO_3$ ppm	30.0	4.58^{a}	6.49^{c}	7.12^{d}	6.18^{a}	6.63^{b}	6.53^{b}	5.40^{a}	6.20^{c}	6.38^{c}	85.53^{a}	75.72^{a}	71.63^{a}
	50.0	4.55^{a}	6.28^{c}	7.20^{d}	6.18^{a}	6.53^{b}	6.52^{b}	5.25^{a}	6.07^{b}	6.47^{c}	84.10^{a}	76.92^{a}	70.08^{a}

[1] Means followed by the same letter are not significantly different at $p \leq 0.05$ Duncan's Multiple Range Test.

peak time significantly $p \leq 0.0005$ from 4.89 minutes in the control system to 6.42 minutes in the double additives system.

Peak height was significantly affected by the interaction between double combinations of additives (Table 6). The maximum peak height was obtained at the 0.5% level of conditioner (Tween 20) with 1% salt both the 0 and 8% and 30 ppm $KBrO_3$, for 8% LCP systems. Peak height generally decreased when potassium bromate and salt were added together; this effect can be seen from Tables 7 and 8. The peak height increased as salt and bromate were added at all levels of combinations, with maximum effect (6.63 cm) obtained at the combination of 30 ppm $KBrO_3$ with 0.5% conditioner.

The salt increased the stability of both 0 and 8% LCP dough systems through its interaction with conditioner and potassium bromate as can be seen from curve height at 9 minute point. Table 7 shows that the maximum stability was reached with the combination levels of 2% salt, 0.5% conditioner and 50 ppm $KBrO_3$, 2.0% salt. The 8% LCP dough system stability also increased when the double combinations that included salt were added (Table 8). The maximum stability was obtained when salt was added at 2% level with 30 ppm $KBrO_3$ (5.98 cm) and 2% salt with 0.5% conditioner (5.95 cm). The interaction between potassium bromate and conditioner in both dough systems (Tables 7 and 8) indicated

Table 8. Effect of the interaction of double combinations of additives on mixograph characteristics at the 8% level of LCP cottonseed flour in dough

		Peak Time[1] min			Peak Height[1] cm			Ht 9 min[1] cm			Area[1] cm^2		
		Tween 20 (level %)											
		0.0	0.5	1.5	0.0	0.5	1.5	0.0	0.5	1.5	0.0	0.5	1.5
	0.0	3.50^{a}	4.46^{b}	4.59^{b}	6.00^{a}	6.05^{a}	5.85^{a}	4.42^{a}	4.50^{a}	4.25^{a}	73.68^{a}	81.85^{a}	74.35^{a}
salt %	1.0	4.29^{b}	5.40^{c}	5.65^{c}	6.28^{a}	6.27^{a}	6.05^{a}	5.02^{b}	5.15^{b}	4.97^{b}	79.93^{a}	83.45^{a}	79.22^{a}
	2.0	7.34^{e}	7.20^{d}	7.80^{e}	6.03^{a}	6.22^{a}	5.87^{a}	5.75^{c}	5.95^{c}	5.82^{c}	65.82^{a}	70.88^{a}	62.23^{a}
		Tween 20 (level %)											
		0.0	0.5	1.5	0.0	0.5	1.5	0.0	0.5	1.5	0.0	0.5	1.5
	0.0	4.20^{a}	5.36^{b}	5.74^{c}	6.08^{a}	6.18^{a}	5.90^{a}	4.67^{a}	5.18^{b}	4.98^{ab}	77.00^{b}	82.15^{b}	72.37^{b}
$KBrO_3$ ppm	30.0	5.72^{c}	6.11^{c}	6.05^{c}	6.08^{a}	6.25^{a}	5.95^{a}	5.23^{b}	5.35^{b}	5.02^{b}	70.47^{a}	77.32^{b}	73.13^{b}
	50.0	5.83^{c}	5.59^{b}	6.26^{c}	6.15^{a}	6.10^{a}	5.92^{a}	5.28^{b}	5.07^{b}	5.03^{b}	71.97^{a}	76.72^{b}	70.30^{a}
		Salt (level %)											
		0.0	1.0	2.0	0.0	1.0	2.0	0.0	1.0	2.0	0.0	1.0	2.0
	0.0	3.83^{a}	4.74^{b}	6.73^{d}	5.82^{a}	6.32^{a}	6.03^{a}	4.20^{a}	4.98^{b}	5.65^{c}	78.65^{a}	84.38^{a}	68.38^{a}
$KBrO_3$ ppm	30.0	4.42^{b}	5.64^{c}	7.82^{e}	6.07^{a}	6.13^{a}	6.08^{a}	4.55^{a}	5.07^{b}	5.98^{c}	76.22^{a}	78.82^{a}	65.88^{a}
	50.0	4.29^{ab}	5.59^{c}	7.80^{e}	6.02^{a}	6.15^{a}	6.00^{a}	4.42^{a}	5.08^{b}	5.84^{c}	75.02^{a}	79.30^{a}	64.67^{a}

[1] Means followed by the same letter are not significantly different at $p \leq 0.05$ Duncan's Multiple Range Test.

that the interaction between 30 ppm $KBrO_3$ and 0.5% conditioner had the maximum effect on stability (5.33, 5.35 cm) in the 0 and 8% LCP cottonseed dough systems, respectively.

The area under the curve decreased significantly ($p<0.05$) when all levels of combinations of $KBrO_3$ and conditioner were added to the dough. The combination including salt as a factor did not have any significant effect on reducing the area.

Triple Combinations Effects

The effects of the three additive combinations were tested on the mixograph characters of 0 and 8% LCP dough systems. Table 6 shows that the interaction between the three additives significantly ($p<0.0005$) increased the mixograph peak time. In the 0% LCP dough, the interaction between the additives salt (2%), $KBrO_3$ (50 ppm), and conditioner (1.5%) produced the longest peak time of all interactions (Table 9). In the 16% LCP dough system the combination of additives that increased the peak time to the maximum were conditioner (1.5%), $KBrO_3$ (30 ppm) and salt (2%) (Table 10).

Peak height was significantly reduced ($p<0.005$) when the three additives were added together. The triple combinations that contained salt had slightly higher peak height as can be seen from Tables 9 and 10. The combination of 1% salt, 30 ppm $KBrO_3$ and 0.5% conditioner in 0% LCP dough

Table 9. The effect of the interaction between the three additives on the mixograph characters of a control wheat dough

		Peak Time[1] min			Peak Ht[1] cm			Ht 9 min[1] cm			Area[1] cm^2		
		KBrO3 level (ppm)											
		0	30	50	0	30	50	0	30	50	0	30	50
		Salt level (%)											
	0.0	4.13^{a}	4.40^{b}	4.23^{a}	6.55^{cd}	6.50^{bcd}	6.45^{bcd}	5.55^{cd}	5.90^{d}	5.75^{cd}	94.9^{b}	94.6^{b}	91.9^{b}
Tween 20%	0.5	4.60^{b}	4.50^{b}	4.41^{b}	6.70^{d}	6.30^{bcd}	6.00^{ab}	$5,50^{c}$	5.40^{bc}	4.95^{a}	106.7^{c}	85.4^{ab}	80.3^{a}
	1.5	4.38^{b}	4.88^{b}	4.95^{b}	6.10^{abc}	5.75^{a}	6.10^{abc}	5.40^{c}	4.90^{a}	5.05^{ab}	86.4^{ab}	76.7^{a}	80.2^{a}
		Salt level (1%)											
	0.0	5.30^{ab}	6.75^{e}	6.52^{de}	6.65^{a}	6.60^{a}	6.50^{a}	5.75^{a}	6.15^{bc}	6.15^{bc}	87.1^{c}	73.6^{a}	78.1^{abc}
Tween 20%	0.5	5.25^{a}	5.87^{bc}	6.03^{cd}	6.80^{a}	6.90^{a}	6.50^{a}	6.10^{abc}	6.35^{c}	6.00^{abc}	86.9^{bc}	80.5^{abc}	79.4^{abc}
	1.5	5.81^{abc}	6.84^{e}	6.29^{cde}	6.50^{a}	6.40^{a}	6.60^{a}	5.85^{ab}	6.10^{abc}	6.05^{abc}	76.9^{ab}	73.1^{a}	73.3^{a}
		Salt level (2%)								a			
	0.0	5.25^{a}	6.82^{bc}	6.84^{bc}	6.75^{a}	6.55^{a}	6.70^{a}	6.20^{a}	6.40^{a}	6.45^{a}	88.5^{d}	73.0^{bc}	74.5^{bc}
Tween 20%	0.5	5.71^{a}	7.00^{cd}	7.00^{cd}	6.80^{a}	6.70^{a}	6.66^{a}	6.40^{a}	6.45^{a}	6.50^{a}	85.4^{d}	75.3^{bc}	74.9^{bc}
	1.5	6.29^{b}	7.53^{de}	7.75^{e}	6.65^{a}	6.35^{a}	6.25^{a}	6.40^{a}	6.30^{a}	6.45	81.4^{cd}	66.7^{ab}	60.8^{a}

[1]Means followed by the same letter are not significantly different at $p \leq 0.05$ using Duncan's Multiple Range Test.

Table 10. The effect of the interaction between the three additives on the mixograph characters of 8% LCP cottonseed flour dough.

		Peak Time[1] min			Peak Ht[1] cm			Ht 9 min[1] cm			Area[1] cm^2		
		KBrO3 level (ppm)											
		0	30	50	0	30	50	0	30	50	0	30	50
		Salt level (0%)											
	0.0	3.40^{a}	3.59^{abc}	3.50^{ab}	6.40^{d}	5.80^{abc}	5.80^{abc}	4.35^{b}	4.45^{bc}	4.45^{bc}	82.8^{bc}	87.7^{c}	70.7^{a}
Tween 20%	0.5	4.00^{bcd}	5.15^{e}	4.24^{d}	5.60^{ab}	6.30^{cd}	6.25^{cd}	4.25^{ab}	4.80^{c}	4.45^{bc}	82.5^{bc}	81.4^{bc}	81.7^{bc}
	1.5	4.10^{cd}	4.53^{d}	5.14^{e}	5.45^{a}	6.10^{bcd}	6.00^{bcd}	4.00^{a}	4.40	4.35^{b}	70.7^{a}	79.7^{abc}	72.7^{ab}
		Salt level (1%)											
	0.0	3.70^{a}	5.42^{bc}	5.63^{bc}	6.15^{ab}	6.35^{ab}	6.35^{ab}	4.70^{a}	5.15^{bc}	5.20^{c}	80.4^{ab}	77.3^{a}	82.2^{ab}
Tween 20%	0.5	5.16^{b}	5.76^{bc}	5.27^{bc}	6.60^{b}	6.20^{ab}	6.00^{a}	5.25^{c}	5.25^{c}	4.95^{abc}	88.1^{b}	83.7^{ab}	78.6^{ab}
	1.5	5.35^{bc}	5.74^{bc}	5.87^{c}	6.20^{ab}	6.85^{a}	6.10^{ab}	5.00^{abc}	4.80^{ab}	5.10^{bc}	85.1^{ab}	75.5^{a}	77.2^{a}
		Salt level (2%)											
	0.0	5.50^{a}	8.16^{e}	8.37^{e}	5.70^{a}	6.10^{ab}	6.30^{b}	4.95^{a}	6.10^{cd}	6.20^{d}	67.9^{ab}	66.5^{ab}	63.1^{a}
Tween 20%	0.5	6.92^{b}	7.44^{bcd}	7.25^{bc}	6.35^{b}	6.25^{b}	6.05^{ab}	6.05^{cd}	6.00^{bcd}	5.80^{bc}	75.9^{b}	66.9^{ab}	69.9^{ab}
	1.5	7.77^{cde}	7.87^{de}	7.77^{cde}	6.05^{ab}	5.96^{ab}	5.65^{a}	5.95^{bcd}	5.85^{bcd}	5.65^{b}	61.4^{a}	64.3^{a}	61.1^{a}

[1]Means followed by the same letter are not significantly different at $p \leq 0.05$ level using Duncan's Multiple Range Test.

system produced the highest peak (6.90 cm) vs (5.75 cm) peak height when the combination 0% salt, 30 ppm $KBrO_3$ and 1.5% conditioner (Table 9). Similar effects were observed in the 8% LCP dough systems as shown in Table 10.

Dough stability as measured by the mixograph curve at the 9-minute point followed the same trend as did the data for area.

These results show clearly the effect of salt on strengthening the dough structure. The toughening effect of salt increased as the level of salt increased in both the double and triple additive combinations, and it was measured by increasing the peak time and decreasing peak height, 9 min ht and area under the curve. These decreases resulted from a very high resistance of dough to the mixer. When bromate was combined with salt at different levels, the bromate did not show a strong effect. This could be due to the fact that potassium bromate is a slow acting oxidant, and does not exert all its oxidative reaction during dough developing. When bromate was used at its highest level the action of bromate as an oxidant was clear, since it also toughened the dough in the presence of salt.

Baking Study.

Bread substituted with 8% LCP cottonseed flour was baked with all the levels of combinations according to a 3^3 factorial design (Table 11).

Table 11. All the possible combinations of salt, conditioner and bromate each at 3 levels

Single Additive	Double Combination	Triple Combination
Salt 1%	0.5% Tween 20, 30 ppm $KBro_3$	1% salt, 0.5% Tween 20, 30 ppm $KBro_3$
2%	0.5% Tween 20, 50 ppm $KBro_3$	1% salt, 1.5% Tween 20, 50 ppm $KBro_3$
	1.5% Tween 20, 30 ppm $KBro_3$	1% salt, 0.5% Tween 20, 50 ppm $KBro_3$
$KBro_3$ 30 ppm	1.5% Tween 20, 50 ppm $KBro_3$	1% salt, 1.5% Tween 20, 30 ppm $KBro_3$
50 ppm		
	1% salt, 0.5% Tween 20	2% salt, 0.5% Tween 20, 30 ppm $KBro_3$
Tween 20 0.5%	1% salt, 1.5% Tween 20	2% salt, 1.5% Tween 20, 50 ppm $KBro_3$
1.5%	1% salt, 30 ppm $KBro_3$	2% salt, 0.5% Tween 20, 50 ppm $KBro_3$
	1% salt, 50 ppm $KBro_3$	2% salt, 1.5% Tween 20, 30 ppm $KBro_3$
	2% salt, 0.5% Tween 20	
	2% salt, 1.5% Tween 20	
	2% salt, 30 ppm $KBro_3$	
	2% salt, 50 ppm $KBro_3$	

[1]No additive breads were baked as the zero level for each additive.

[2]Polyoxyethylene (20) Sorbitan Mono Laurate in clear liquid form.

The baked bread was subjected to factorial analysis (Table 12) where all the main effects as well as all the possible interactions were tested. Salt significantly ($p \leq 0.005$) affected the bread volume, 1% salt increased loaf volume while 2% decreased it (Table 13A). The very pronounced effect of additives was observed on the compressability where all single additives as well as their interactions reduced the force needed to compress the bread sample to a constant height indicating these breads had a softer crumb. Results for volume and compressability are presented in Tables 13A, B and C. When single additives were added to bread, salt had a significant effect on volume (Table 13A) especially at 1% level (552 cc vs 512 cc untreated bread). The effect of salt on volume is obvious even when combinations of additives were added (Table 13A and B). The 2% salt in single or combined additives reduced loaf volume, and crumb softness (Table 13A).

The double combinations of additives reduced bread volume and produced breads that have harder crumb than the triple combinations (Tables 13B, C). The highest volume (637 cc) and the softest crumb (17.8 g/cm) were obtained at the combination of 1% salt, 1.5% Tween 20, 50 ppm $KBrO_3$.

Sensory Evaluation.

Sensory evaluation data (Table 14) indicate the significant effect of additives and their interactions on grain texture. Salt and any additive combination that included

Table 12. Mean square of analysis of variance of 8% substituted bread, and the effect of additives and their interaction on objective measurements

Source of Variation	Degrees of Freedom	Volume cc.	Sp. Volume cc/g	pH	Compressability g/cm
S[1]	2	38538.89**	2.68**	0.28***	1509.67***
C[2]	2	6955.56	0.47	0.13**	1215.33***
B[3]	2	10376.39	0.60	0.04*	1021.48**
SXC	4	1196.53	0.12	0.04*	1383.63***
SXB	4	3652.78	0.21	0.07**	2407.68***
CXB	4	2254.86	0.13	0.00	476.34**
SXCXB	8	3043.75	0.18	0.39*	1001.53***
Residual Error	27	4304.17	0.27	0.01	110.51
Total	53				

* p 0.05
** p 0.005
***p 0.0005
[1]salt
[2]conditioner (Tween 20)
[3]Potassium bromate

Table 13A. The mean[1] effect of single additives on bread volume and compressability

Additives	Level of Additive	Volume cc ±SD	***Compressability g/cm ±SD
No additive	0.0	513±50.3	65.6±24.8[a]
Salt %	1.0	**552±74.7	46.7±25.9[a]
	2.0	460±62.2	64.6±25.5[a]
$KBrO_3$ ppm	30	514±60.8	52.6±22.9[a]
	50	529±80.0	52.4±28.8[a]
Tween 20%	0.5	505±63.0	63.4±29.8[a]
	1.5	529±73.6	47.6±21.7[a]

** $p \leq 0.005$
***$p \leq 0.0005$
[1]Means followed by the same letter are not significantly different $p \leq 0.05$ Duncan's Multipls Range Test.

Table 13B. The mean[1] effect of double combinations of additives on 8% LCP cottonseed flour bread volume and compressability

Additive Combinations	Volume cc	***Compressability g/cm
0.5% Tween 20, 30 ppm $KBrO_3$	490	60.13[a]
0.5% Tween 20, 50 ppm $KBrO_3$	541	49.8[a]
1.5% Tween 20, 30 ppm $KBrO_3$	490	44.7[a]
1.5% Tween 20, 50 ppm $KBrO_3$	555	42.3[a]
1% salt, 0.5% Tween 20	541	57.1[a]
1% salt, 1.5% Tween 20	572	36.5[a]
1% salt, 30 ppm $KBrO_3$	556	35.4[a]
1% salt, 50 ppm $KBrO_3$	600	29.2[b]
2% salt, 0.5% Tween 20	464	59.2[a]
2% salt, 1.5% Tween 20	468	48.8[a]
2% salt, 30 ppm $KBrO_3$	476	58.2[a]
2% salt, 50 ppm $KBrO_3$	467	57.6[a]

***$p \leq 0.0005$

[1]Means followed by the same letter are not significantly different at $p \leq 0.05$ Duncan's Multiple Range Test.

Table 13C. The mean[1] effect of triple combinations of additives on 8% LCP cottonseed flour bread volume and compressability

Additive Combinations	Volume cc	***Compressability g/cm
1% salt, 0.5% Tween 20, 30 ppm $KBrO_3$	532	37.2[a]
1% salt, 1.5% Tween 20, 50 ppm $KBrO_3$	637	17.8[b]
1% salt, 0.5% Tween 20, 50 ppm $KBrO_3$	605	29.1[a]
1% salt, 1.5% Tween 20, 30 ppm $KBrO_3$	587	24.1[a]
2% salt, 0.5% Tween 20, 30 ppm $KBrO_3$	470	52.2[a]
2% salt, 1.5% Tween 20, 50 ppm $KBrO_3$	487	35.2[a]
2% salt, 0.5% Tween 20, 50 ppm $KBrO_3$	495	33.3[a]
2% salt, 1.5% Tween 20, 30 ppm $KBrO_3$	497	42.1[a]

***$p \leq 0.0005$

[1]Means followed by the same letters are not significantly different at $p \leq 0.05$ Duncan's Multiple Range Test.

Table 14. Mean squares and F statistics significance for sensory evaluation parameters of 8% LCP cottonseed flour bread baked with additives

Source of Variance	Degrees of Freedom	Crust Color	Crust Character	Grain Texture	Overall Acceptability
Salt	2	6.53***	0.64	30.39***	9.85***
Conditioner	2	0.75	0.09	5.02***	3.80***
Oxidant	2	1.19	0.72	1.96*	1.12
Salt X Conditioner	4	0.24	0.74	4.05***	0.90
Salt X Oxidant	4	1.15	0.82*	3.02***	0.65
Conditioner X Oxidant	4	0.82	0.06	1.61**	0.83
Salt X Conditioner X Oxidant	8	0.25	1.51***	4.98***	2.93
Error	27	0.53	0.29	0.38	0.36
Total	53				

* $p \leq 0.05$
** $p \leq 0.005$
*** $p \leq 0.0005$

salt significantly improved the grain texture ($p<0.0005$). However at the high levels of salt, some panelists indicated that grain texture was compact. The bread was generally acceptable. Crust color and character were not significantly affected by the additives.

The double combination results (Table 15) show that the grain texture tended to be more compact as the level of salt and conditioner increased. When 2% salt was added, the grain texture was scored 5.94 by the panelists on 0-10 scale, when 2% salt was combined with 0.5% conditioner the grain texture was more compact and scored 4.40, the combination of 2% salt, 1.5% conditioner produced a very compact bread that was scored 3.92 by the panelists. The high level of salt (2%) had the same effect on grain texture when it was combined with potassium bromate (Table 15). As the level of $KBrO_3$ increased in the combination, the bread grain tended to be more compact.

The salt at the lower level (1%) acted differently. The grain texture was very acceptable when 1% salt was added with either conditions (0.5% level) or bromate (30 or 50 ppm levels).

The interaction between conditioner and potassium bromate was somewhat different. The grain texture was scored more open as the level of the conditioner increased in the additive combination (Table 15). The best bread score for grain texture was obtained with 0.5% conditioner,

Table 15. The effect of interactions between two additives on the sensory evaluation characters[1] of 8% LCP cottonseed flour bread

		Crust Color 0-10[1] (NS)			Crust Character 0-10[2] (NS)			Grain Texture 0-10[3]			Overall Acceptability 0-10[4] (NS)		
							Tween 20 (level %)						
		0.0	0.5	1.5	0.0	0.5	1.5	0.0	0.5	1.5	0.0	0.5	1.5
	0.0	5.26	5.21	4.91	5.07	5.53	5.78	6.77^{bcd}	6.33^{bc}	7.97^{d}	4.36	5.19	3.87
salt %	1.0	5.88	6.25	6.00	5.59	4.88	5.19	7.53^{cd}	6.36^{bc}	7.07^{bcd}	5.32	4.73	4.16
	2.0	6.46	6.47	5.84	5.13	5.14	5.00	5.94^{b}	4.40^{a}	3.92^{a}	6.23	5.90	5.46
							Potassium Bromate (ppm)						
		0	30	50	0	30	50	0	30	50	0	30	50
	0.0	5.52	4.70	5.15	5.52	5.59	5.27	7.40^{cd}	7.64^{d}	6.02^{bc}	4.41	4.00	5.01
salt %	1.0	6.19	5.37	6.57	5.41	5.64	4.61	6.19^{bcd}	7.35^{cd}	7.42^{cd}	4.64	4.49	5.08
	2.0	6.07	6.46	6.24	4.79	5.16	5.32	5.00^{ab}	4.83^{ab}	4.43^{a}	6.11	5.80	5.69
							Potassium Bromate (ppm)						
		0	30	50	0	30	50	0	30	50 ^{ab}	0	30	50
	0.0	6.38	5.29	5.92	5.26	5.54	5.00	7.07^{ab}	7.15^{b}	6.02^{ab}	5.12	5.10	5.70
Tween 20%	0.5	6.00	5.58	6.35	5.21	5.26	5.07	5.79^{ab}	5.62^{a}	5.67^{ab}	5.14	4.88	5.80
	1.5	5.40	5.66	5.68	5.25	5.59	5.13	5.73^{ab}	7.05^{ab}	6.18^{ab}	4.90	4.31	4.28

[1]Means followed by the same letter are not significantly different at $p \leq 0.05$ Duncan's Multiple Range Test.
[2]
[3]pale-dark
[4]thick-thin
[5]compact-open
unacceptable-acceptable
NS not significant

30 ppm potassium bromate combination.

Bread baked with three combinations of additives showed their significant ($p \leq 0.0005$) effect on crust character and grain texture (Table 14). The best crust character was obtained with the combination of 0.5% conditioner, 1% salt, 50 ppm potassium bromate and the combination, 1.5% conditioner, 1% salt, 50 ppm potassium bromate (Table 16).

Comparing the effect of single additives and the effect of double or triple combinations of additives on bread, it is obvious that there was an interaction between the additives when added together, and it caused improvement to the physical characters of bread (Figure 9A, B, C).

Wheat/cottonseed flour breads have not been yet commercially produced in spite of the extensive research that has been done in this area. Some of the reasons that hinder their commercial production are: the physical bread character (volume, grain texture), flavor and color. In this part of the study the optimum levels of each additive (oxidant, conditioner, salt) were determined to produce optimum 8% cottonseed bread characters.

The data were subjected to the multiple regression analysis where the regression equation was used:

$$\psi = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 +$$

$$\beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3$$

Table 16. The mean effect of interactions between three additives on the sensory evaluation characters of 8% LCP cottonseed flour bread

		Crust[1] Character 0-10			Grain[2] Texture 0-10		
		Potassium Bromate (ppm)					
		0	30	50	0	30	50
		Salt level (0%)					
Tween 20 (%)	0.0	4.12^{a}	5.60^{bc}	5.51^{bc}	6.37^{bc}	9.43^{e}	4.51^{a}
	0.5	6.56^{bc}	5.16^{ab}	4.88^{ab}	7.70^{cd}	5.57^{ab}	5.73^{ab}
	1.5	5.88^{bc}	6.03^{bc}	5.43^{bc}	8.15^{de}	7.94^{d}	7.81^{d}
		Salt level (1%)					
Tween 20 (%)	0.0	6.31^{bc}	6.31^{bc}	4.16^{a}	8.51^{de}	5.85^{ab}	8.24^{cde}
	0.5	4.32^{ab}	5.30^{abc}	5.02^{abc}	4.86^{a}	7.22^{bcd}	7.00^{bc}
	1.5	5.59^{bc}	5.32^{abc}	4.67^{ab}	52.1^{a}	8.99^{e}	7.02^{bc}
		Salt level (2%)					
Tween 20 (%)	0.0	5.35^{a}	4.71^{a}	5.33^{a}	6.35^{c}	6.17^{c}	5.31^{bc}
	0.5	4.75^{a}	5.34^{a}	5.33^{a}	4.82^{ab}	4.09^{ab}	4.30^{ab}
	1.5	4.29^{a}	5.43^{a}	5.29^{a}	3.83^{ab}	4.23^{ab}	3.70^{a}

Means followed by the same letters are not significantly different at $p \leq 0.05$ Duncan's Multiple Range Test.
[1]Scale thick-thin.
[2]Scale compact-open.

Figure 9A. Effect of additives on 8% LCP cottonseed bread
Single Additives
a) Control Untreated Bread
b) Potassium Bromate 30 ppm
c) Potassium Bromate 50 ppm
d) Salt 1%
e) Salt 2%

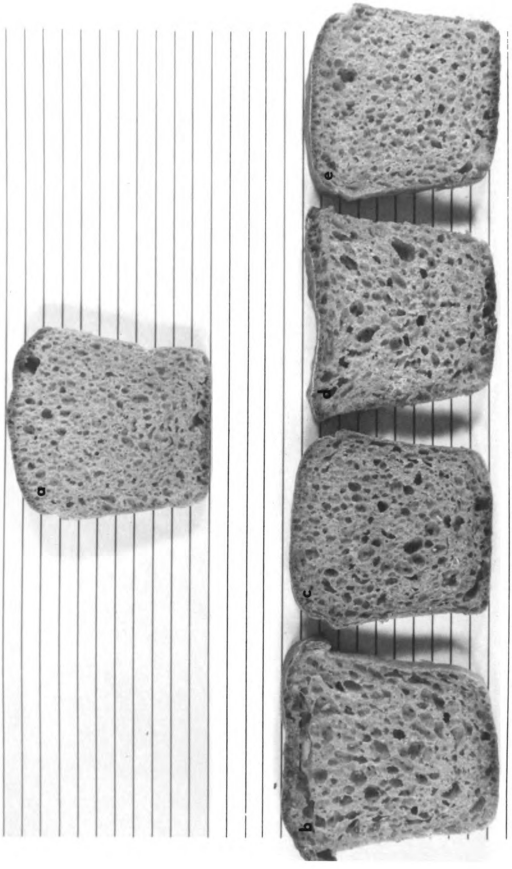

a
b
c
d
e

Figure 9B. Effect of additive interaction on 8% LCP cottonseed bread

Double Combinations

a) Control Untreated Bread
b) Conditioner 0.5% + $KBrO_3$ 30 ppm
c) Conditioner 1.5% + $KBrO_3$ 50 ppm
d) Conditioner 1.5% + $KBrO_3$ 30 ppm
e) Conditioner 0.5% + $KBrO_3$ 50 ppm
f) Salt 1% + $KBrO_3$ 30 ppm
g) Salt 1% + Conditioner 0.5%

h) Salt 1% + $KBrO_3$ 50 ppm
i) Salt 1% + Conditioner 1.5%
j) Salt 2% + $KBrO_3$ 30 ppm
k) Salt 2% + Conditioner 0.5%
l) Salt 2% + $KBrO_3$ 50 ppm
m) Salt 2% + Conditioner 1.5%


a
b
c
d
e
f
g

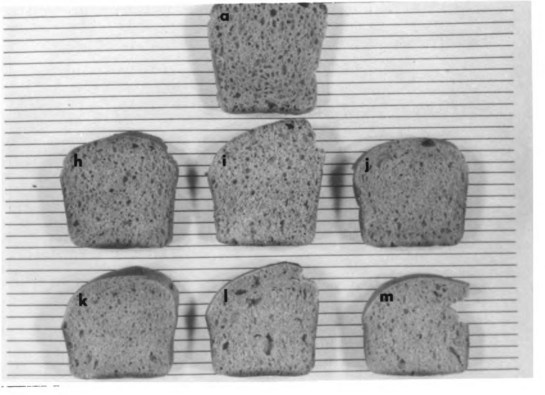
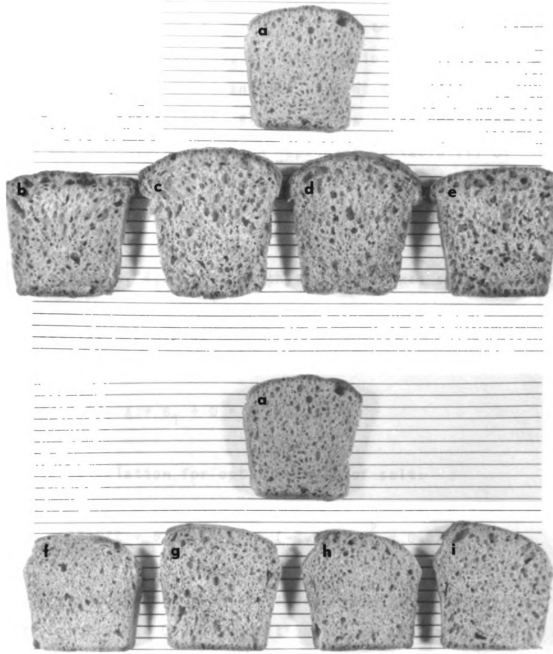

a
h
i
j
k
l
m

Figure 9C. Effect of additive interaction on 8% LCP cottonseed bread
Triple Combinations
a) Control Untreated Bread
b) Salt 1% + Conditioner 0.5% + $KBrO_3$ 30 ppm
c) Salt 1% + Conditioner 1.5% + $KBrO_3$ 50 ppm
d) Salt 1% + Conditioner 0.5% + $KBrO_3$ 50 ppm
e) Salt 1% + Conditioner 1.5% + $KBrO_3$ 30 ppm
f) Salt 2% + Conditioner 0.5% + $KBrO_3$ 30 ppm
g) Salt 2% + Conditioner 1.5% + $KBrO_3$ 50 ppm
h) Salt 2% + Conditioner 0.5% + $KBrO_3$ 50 ppm
i) Salt 2% + Conditioner 1.5% + $KBrO_3$ 30 ppm


a
b
c
d
e
a
f
g
h
i

where X_1, X_2 and X_3 are salt, conditioner and potassium bromate respectively and X_1^2, X_2^2, X_3^2 are the second order terms and X_1X_2, X_1X_3, X_2X_3 are interactions between salt X conditioners, salt X oxidant and oxidant X conditioner. β_0 - β_{23} are coefficient of regressions. The predicted value was calculated for each dependent variable (Y) of interest in this case bread texture and specific volume. The optimum values were calculated using the equation

$\psi = \beta_0 + \beta_1 X_1 + \beta_2 X_2^2$ this equation was solved to find the 1st derivative

$$\frac{dY}{dx} = 2\beta_2 X + \beta_1 + 0 = 0$$

Example calculation for optimum level of salt:

$$\psi = 38.972 + 6.579 X_1 + 2 (-4.918) X^2 = 0.67\%$$

The three independent variables were calculated and were found to be: salt 0.67%, conditioner 0.4% and oxidant to be 37 ppm.

An optimum bread was then baked using the optimum levels of additives. Bread was evaluated by 16 panelists for crust color, crust character, grain tenderness, flavor, overall acceptability and volume. The data presented in Table 17

Table 17. Sensory evaluation data of optimum 8% LCP cottonseed bread and untreated ones

Treatment of Bread	Volume cc	Specific Volume cc/g	Crust[1] Color 0-10	Crust[2] Character 0-10	Grain[3] Texture 0-10	Tenderness[4]	Flavor[5]	O.A.[6]
Optimum	538	4.03	6.48	5.40	5.63	5.63	4.75	4.00
Untreated	436	3.17	6.02	4.09	5.71	3.82	3.18	3.46
Control*	495	3.69	5.10	5.16	6.73	7.60	5.25	5.49

[1]pale-dark
[2]thick rubbery-soft tender
[3]coarse uneven-uniform cells
[4]tough-tender
[5]bad-excellent
[6]Overall acceptability, unacceptable -- acceptable
*Made of 85% extracted HRW wheat flour plus all the optimum levels of additives

are means of 64 taste panel scores. Optimum bread was served to the panelists along with a sample of 8% LCP cottonseed untreated bread and a control of HRW wheat bread made of extracted flour and, all with the optimum levels of additives to minimize the color factor in scoring the cottonseed flour breads that always underscored any plant protein substituted bread if tested against white bread. Volume and specific volume were higher than untreated bread. Sensory evaluation data indicate that optimum bread was scored better than the untreated bread. The tenderness, flavor and overall acceptability effectively improved with optimum combination of additives over the untreated system. The optimum cottonseed flour scored better than the control bread in volume, specific volume, crust color and grain texture (Figure 10A, B).

Egyptian "Baladi" Bread

Many attempts have been made to improve the protein quality of various types of Western bread by substituting with different protein sources and synthetic amino acids. However the studies on the substitution of Arabic bread with protein sources are few (Shakir et al., 1960; Maleki et al., 1968; Dalby, 1970; Shehata, 1970).

Egyptian bread, which is a typical type of bread consumed in the Middle East was substituted with 0, 4, 8, 12 and 16% LCP cottonseed flour. The data in Table 18 show

Figure 10A: Effect of optimum levels of additives on LCP cottonseed bread
a) Untreated 8% LCP cottonseed bread
b) Salt 0.7% + Conditioner 0.4% + $KBrO_3$ 37 ppm

10B: Comparison between optimum bread made of white flour, dark flour, and 8% LCP cottonseed flour
a) Bread made of 85% extracted HRW wheat flour
b) Bread made of 8% LCP cottonseed substituted flour
c) Bread made of straight grade HRW wheat flour.

A

B

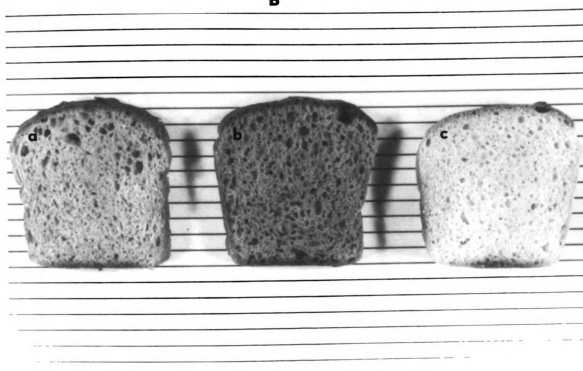


Table 18. Effect LCP cottonseed flour on Egyptian bread volume and specific volume

Cottonseed level %	Loaf Volume** cc	Loaf Specific Volume* cc/g
0	581.00±105.01[c]	4.57±0.97[b]
4	640.13±110.71[e]	4.99±0.90[a]
8	594.67±105.39[d]	4.65±0.80[b]
12	550.00±101.41[a]	4.34±0.82[b]
16	556.44±105.69[b]	4.41±0.80[b]

Value followed by the same letter are not significantly different at $p \leq 0.05$ D-ncan's Multiple Range Test

* $p<0.05$

** $p<0.001$

a decrease in loaf volume and specific volume at the high levels of cottonseed (12 and 16%). At the levels of 4 and 8% cottonseed, the volume and specific volume increased indicating a better product than the control loaf.

The bread was tasted by 6 Middle Eastern panelists for crust color, crumb color, flavor, texture, aroma and acceptability. The results indicated (Table 19) a significant decrease in crust and crumb color ratings indicating that a darker product was produced when the flour was substituted with higher levels of cottonseed flour. All the sensory data show a significant decrease in flavor, texture, aroma and general acceptability scores with increasing level of cottonseed bread. Nevertheless, the bread prepared with 4 and 8% LCP cottonseed flour was scored in the acceptable range for all the sensory characters tested except color (Figure 11).

Sensory data suggest that the adverse color scored may have influenced the judges scoring on the other attributes even though some of these attributes received acceptable scores.

Effect of Four Dough Conditioners on the Mixograph Properties.

The mechanism of action of dough conditioners was discussed in a previous section. The comparison between the effect of dough conditioners: Tween 60, Polyoxyethylene 10-stearyl ether, Tween 20, and Tendem 552 on peak time,

Table 19. Effect of LCP cottonseed flour on breadmaking properties

Cottonseed Level %	Crust Color [1]	Crumb Color [2]	Flavor [3]	Texture [3]	Aroma [3]	General Acceptability [3]
0	6.80±0.1^{a}	5.96±0.4^{a}	5.48±0.1^{a}	5.32±0.1^{a}	5.40±0.3^{a}	5.70±0.1^{a}
4	4.88±0.7^{b}	3.76±2.5^{b}	4.40±1.0^{b}	3.92±1.2^{c}	4.28±0.9^{b}	4.44±0.8^{b}
8	3.96±0.5^{bc}	3.26±0.4^{ab}	4.26±1.1^{b}	4.36±1.1^{b}	4.12±0.9^{b}	4.24±0.6^{bc}
12	3.72±0.2^{cd}	3.32±1.5^{bc}	3.98±0.7^{bc}	4.42±1.4^{b}	3.98±1.1^{b}	3.98±1.5^{bc}
16	2.84±1.5^{d}	2.76±1.1^{c}	3.50±0.8^{c}	3.70±0.8^{c}	3.26±1.2^{c}	3.38±0.9^{c}

[1]Crust color scale is from 1-7 where 1 is very dark brown and 7 is light tan.
[2]Crumb color scale is 1 greenish dark yellow to 7 oatmeal color.
[3]The scale is 1 to 7 where 1 is very poor and 7 is excellent.

Figure 11A: A typical loaf of Egyptian bread.

Figure 11B. Effect of LCP cottonseed flour on Egyptian bread characters
0, 4, 8, 12 and 16 represent percent levels of substitution with cottonseed flour


12
16
8
4
0

peak height, height at 9 minute point and the area under the curve were studied. From the results shown in Table 20, it can be seen that the conditioner and its level affected significantly all the mixograph characters ($p<0.0005$). Peak time increased with the addition of these dough conditioners, ranging from 3.72 minutes for the control to 4.87 minutes with the addition of the higher level of mono and diglycerides of Polysorbate (60) (Tandem 552) (Table 21). This dough conditioner used at the 0.5% level also significantly increased the mixograph height at 9-minute point, which indicates its strengthening effect of flour proteins. The area under the curve also was significantly affected by dough conditioners (Table 21): polyoxyethylene (60) sorbitan monostearate (Tween 60) produced the largest area when it was added at 0.5% level; while polyoxyethylene 10-stearyl ether at 0.5% level and polyoxyithylene (20) sorbitan mono laurate, at 0.5% level had the second largest influence on the area. These data show that the maximum effect of dough conditioner on the mixograph characters was reached when the conditioner level was 0.5% of flour weight. The term dough conditioner refers to those adjuncts, usually surface active, that possess the ability to strengthen the gluten structure of dough and thus improve its gas retaining ability. Langhans et al. (1971) found that the addition of 0.2% polysorbate 60 (polyoxyethylene (60) sorbitan monostearate) imparted a dry consistency to dough

Table 20. Mean square of mixograph characters as affected by cottonseed level conditioners and conditioner level

Source of Variation	Degrees of Freedom	Peak Time min	Peak Height cm	Ht 9 min cm	Area cm^2
Cottonseed level	4	1.3148***	5.4269***	11.1444***	1871.5352***
Conditioner	8	1.7698***	0.6781***	0.1741***	78.0494***
Cottonseed X Conditioner	32	0.1181***	0.0579	0.0453***	34.3679***
Residual Error	45	0.0104	0.0355	0.0155	7.9066
Total	89				

***p<0.0005

Table 21. The average* effect of dough conditioners on mixograph properties of HRW wheat/LCP cottonseed flour doughs

Dough Conditioners	Level of Conditioner %	Peak Time min	Peak Height cm	Ht 9 min cm	Area cm^2
No conditioner	0.0	3.72^{e}	6.08^{a}	4.42^{ab}	81.86^{a}
Tween 60	0.5	4.14^{bc}	5.77^{ab}	4.29^{abc}	82.52^{a}
	1.5	4.27^{b}	5.51^{bc}	4.17^{bc}	74.41^{b}
Polyoxyethylene	0.5	4.00^{cd}	5.89^{ab}	4.38^{abc}	80.77^{ab}
10 stearyl ether	1.5	3.90^{cde}	5.64^{abc}	4.13^{c}	78.94^{ab}
Tween 20	0.5	3.71^{e}	5.94^{ab}	4.28^{abc}	80.87^{ab}
	1.5	3.86^{de}	5.70^{ab}	4.20^{bc}	76.51^{ab}
Tandem 557	0.5	4.73^{a}	5.48^{bc}	4.55^{a}	77.65^{ab}
	1.5	4.87^{a}	5.24^{c}	4.32^{abc}	76.50^{ab}

*Values followed by the same letter are not significantly different at $p \leq 0.05$ Duncan's Multiple Range Test

as measured by Farinograph; Knightly (1962) reported a similar effect using polyoxyethylene sorbitan.

Surfactants contain two distinct types of functional groups, a hydrophilic and a hydrophobic group. The hydrophobic group is usually a hydrocarbon in nature while the hydrophilic one may be ionic or polar non-ionic group (such as polyhydrol or polyoxyethylene). Individual surfactant molecules tend to orient themselves in air-water or oil-water interfaces so that hydrophilic group has maximum and hydrophobic group has minimum contact with water. In this mode they lower the surface tension between immisible phases. The surfactants that work as dough conditioners must be anionic compounds or if not anionic, they should contain polyoxyethylene compounds because of their actions to insolubilize gluten proteins. The effectiveness of these surfactants is related to the hydrocarbon chain length since the longer the chain, the more effective the surfactant becomes.

The introduction of the continuous-process breadmaking in Egypt necessitates the use of dough strengthener to overcome the weakening effect of continuous mixing on the gluten. These dough conditioners also serve as an anti-staling agents to extend the bread shelf life.

The effect of dough conditioners: Polyoxyethylene (60) Sorbitan Mono Stearate (Tween 60), Polyoxyethylene (10) Stearyl Ether, Polyoxyethylene (20) Sorbitan Mono Laurate

(Tween 20) and Mono and Diglycerides of Polysorbate (60) (Tandem 552), on bread volume and specific volume, is presented in Table 22. There is a significant effect of conditioner, level of conditioner and level of cottonseed on bread volume ($p<0.0005$). The interaction between the three factors on volume was also significant ($p<0.01$). Conditioners Tween 60, Tween 20 and Tandem 552 were the most effective in increasing loaf volume, especially with high levels of substitution with cottonseed (Table 23). In 0% cottonseed flour bread both levels of Tween 20 increased loaf volume slightly over the untreated bread. At 8% level of cottonseed both levels of conditioner Tween 60 increased both volume and specific volume significantly over the control. Tween 20 and Tandem 552 significantly increased loaf volume over bread made with POESE and the control bread. At the 12% level of cottonseed, Tween 20 at 0.5% was very effective in increasing bread volume, while at tht 16% cottonseed level, all three conditioners except POESE were effective in increasing volume, as well as specific volume.

Effect of four dough conditioners on retaining bread softness after 3 and 6 days of storage is presented in Table 24. The bread tenderness (Figure 12) decreased significantly after 3 and 6 days of storage ($p<0.005$). The rate of bread staling was higher at the 0% cottonseed flour level. The effect of cottonseed on dilution of starch contents can explain that effect.

Table 22. Mean squares of analysis of variance of effect of dough conditioners on volume or specific volume of Egyptian bread

Source of Variance	Degree of Variance	Volume cc	Specific Volume cc/g
Conditioner	3	17.4712***	30.0864
Level of conditioner	2	4.3852***	474.9726***
Level of cottonseed	4	18.9268***	30.9828*
Conditioner X level of conditioner	6	4.9891***	36.2326*
Conditioner X level of cottonseed	12	1.3008**	19.1126
Conditioner X level of cottonseed	8	4.5384***	52.0658***
Conditioner X level of conditioner X level of cottonseed	24	1.0630**	11.0809
Residual Error (0+within)	840	0.5406	13.6181
Total	899		

* $p<0.05$
** $p<0.005$
*** $p<0.0005$

Table 23. Effect of conditioners on loaf volume and specific volume of Egyptian bread made with 0, 4, 8, 12 and 16% LCP cottonseed flour

Dough Conditioner	Level of Conditioner %	Loaf vol[1] cc	Loaf[2] s.v. cc/g	Loaf vol[1] cc	Loaf[2] s.v. cc/g	Loaf vol[1] cc	Loaf[2] s.v. cc/g	Loaf vol[1] cc	Loaf[2] s.v. cc/g	Loaf vol[1] cc	Loaf[2] s.v. cc/g
		Cottonseed Flour Level %									
		0		4		8		12		16	
No conditioner	0.0	635	5.27^{ab}	733^{a}	5.69^{a}	550^{d}	4.40^{c}	585^{ab}	4.59^{ab}	539^{cd}	4.16^{cd}
Tween 60	0.5	578^{b}	4.47^{cd}	692^{a}	5.40^{ab}	604^{bcd}	4.73^{bc}	560^{b}	4.45^{bc}	553^{bcd}	4.34^{bcd}
	1.5	612^{ab}	4.88^{bc}	672^{b}	5.25^{ab}	584^{bcd}	4.56^{bc}	579^{ab}	4.58^{ab}	612^{ab}	4.78^{ab}
Polyoxyethylene 10 stearyl ether	0.5	538^{c}	4.11^{de}	522^{c}	4.06^{d}	472^{e}	3.72^{d}	464^{c}	3.65^{d}	442^{e}	3.56^{e}
	1.5	510^{d}	3.89^{e}	506^{c}	3.91^{d}	564^{cd}	4.50^{bc}	541^{b}	4.29^{bc}	514^{d}	4.06^{d}
Tween 20	0.5	656^{a}	5.06^{ab}	652^{b}	5.10^{bc}	680^{a}	5.28^{a}	628^{a}	4.98^{a}	579^{abc}	4.62^{abc}
	1.5	660^{a}	5.48^{a}	698^{ab}	5.41^{ab}	618^{bc}	4.78^{bc}	542^{b}	4.29^{bc}	596^{abc}	4.78^{ab}
Tandem 552	0.5	520^{c}	3.96^{de}	612^{c}	4.76^{c}	640^{ab}	4.95^{ab}	542^{b}	4.17^{bc}	617^{a}	4.88^{a}
	1.5	520^{c}	4.05^{de}	674^{b}	5.30^{ab}	640^{ab}	4.94^{ab}	527^{b}	4.08^{cd}	565^{abcd}	4.47^{bcd}

[1]volume cc

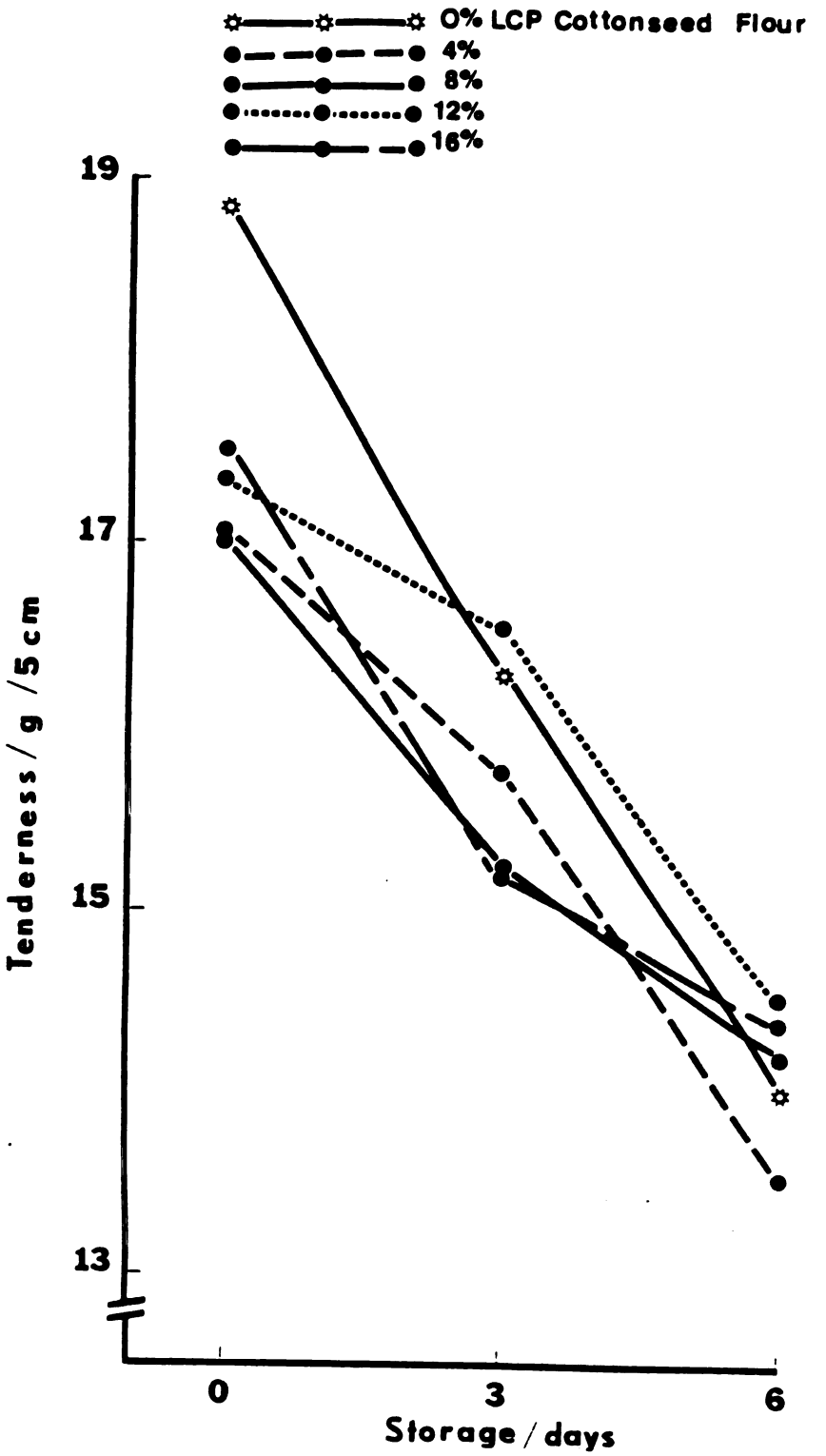
[2]specific volume

Table 24. Effect of conditioners on bread tenderness after 3 storage time

Cottonseed level %	Storage period (days) 0	3	6
		Tween 60	
0	20.36	15.64	14.87
4	16.69	15.60	14.12
8	16.18	13.69	13.74
12	16.29	15.22	14.35
16	16.15	14.72	14.36
		Polyoxyethylene 10 Stearyl Ester	
0	18.05	15.19	14.28
4	17.11	14.97	14.07
8	18.09	15.43	15.77
12	19.21	16.79	15.03
16	17.23	13.34	13.99
		Tween 20	
0	18.71	17.43	14.39
4	17.21	15.99	13.04
8	17.23	16.50	14.27
12	13.39	17.25	15.04
16	17.28	16.65	15.96
		Tandem 552	
0	18.10	16.94	12.88
4	17.47	16.54	12.83
8	16.77	15.73	12.98
12	16.61	16.55	13.49
16	17.34	16.53	13.31

Tenderness was measured as the resistance of a round piece of bread to shear by a single blade. The softer and elastic bread the more the resistance to shear. Expressed as g/cm (diameter of test piece of bread).

Figure 12A. Tenderness of Egyptian bread substituted with cottonseed flour after 0, 3 and 6 days of storage


0% LCP Cottonseed Flour
4%
8%
12%
16%
19
17
15
13
Tenderness / g /5cm
0
3
6
Storage / days

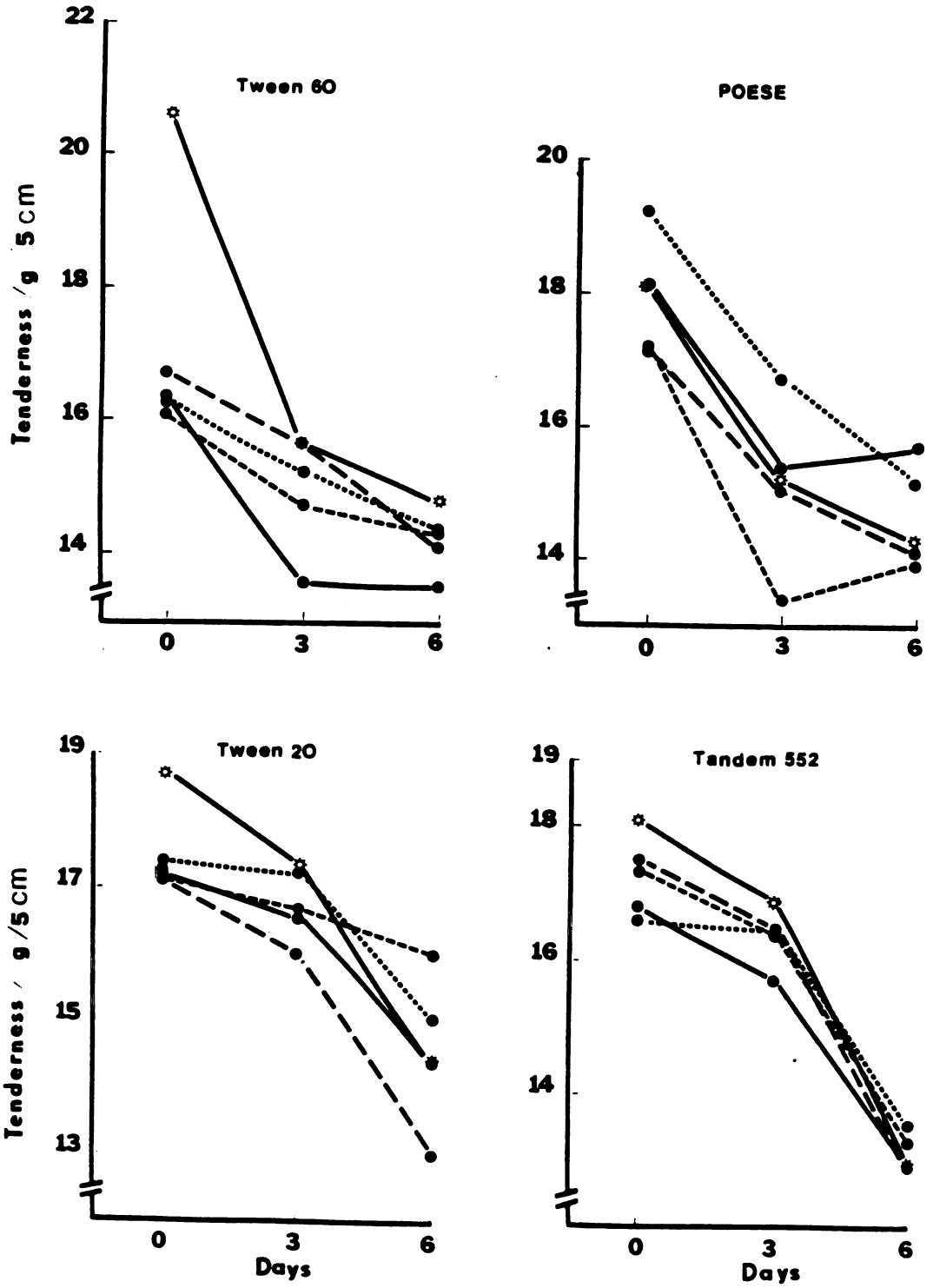
When dough conditioners were added to bread, the rate of staling significantly decreased. Tween 20 and Tandem 552 were the most effective as anti-staling factors, (Figure 12B). Tween 20
retained the softness after 6 days of storage better than any other dough conditioner studied, with higher reading for softness.

Bread staling starts directly after the bread is taken out of the oven. It was reported that softness can be affected by the moisture content of loaf but loss of moisture of bread has no significant relationship with the rate of crumb firming. The staling phenomenon according to Lindet (1902) due to starch retrogradation during which moisture migrates from the swelling starch granules to the gluten (Knightly et al., 1973). The crystallization of amylopectin was profoundly affected by long chain fatty acids.

Mono and diglycerides specially fully saturated monoglycerides at 0.3% of flour weight produced the optimum results on retardation of bread staling and increasing loaf volume. Polyoxyethylene mono stearate reduced bread firmness during aging.

Surfactants (dough conditioners) were reported to slow the rate of firmness by forming a complex with amylopectin fraction within the starch granules. The ability of saturated fatty acids to complex with amylopectin increases with increasing carbon chain length up to C-16 (palmitate)

Figure 12B. Effect of dough conditioners on substituted bread after 0, 3 and 6 days of storage


Tween 60
POESE
Tween 20
Tandem 552
Tenderness /g 5cm
Tenderness / g /5cm
Days
22
20
18
16
14
19
17
15
13
0
3
6

(Knightly et al., 1973).

Supplementation value of cottonseed flour on Egyptian "Baladi" bread.

In the Middle East, the caloric contribution of bread to the diet may be as high as 80-90% (Dalby, 1969). In 1969, Abbott stated that as much as 64% of the daily protein intake in this area is derived from cereal. The present food resources of Egypt do not satisfy domestic demand and additional quantities of certain foods, principally wheat, are imported.

The protein content of bread baked with 0, 4, 8, 12 and 16% LCP cottonseed protein increased significantly with increasing the LCP cottonseed flour in bread (Table 25).

The total amino acids of LCP cottonseed flour, 85% extracted HRW wheat flour, and cottonseed/wheat flour combinations are presented in Table 26.

Lysine is the first limiting amino acid in wheat flour, the substitution with LCP cottonseed flour increased the lysine content from 0.28% to 0.54% with 16% substitution with cottonseed flour. Lysine also is the first limiting amino acid and methionine is the second limiting amino acid in cottonseed protein for the supporting of growth in chicks (Fisher, 1965). Comparing the essential amino acid composition of all wheat/cottonseed flour mixes (Table 27) to the FAO/WHO suggested pattern of amino acid requirements of infant, school child and adult indicated that all the

Table 25. Proximate composition[a] of bread prepared with different levels of cottonseed protein

Level of cottonseed in bread %	Protein[b] %	Fat %	Ash %	Moisture %
0	12.52	2.02	1.81	12.09
4	14.70	2.49	2.19	12.00
8	17.72	2.50	2.33	12.68
12	19.03	2.65	2.43	14.27
16	20.58	2.71	2.73	15.29

[a]All values are expressed on 14% moisture basis and are average of duplicates.
[b]Nx6.25 for all loaves containing cottonseed flour and Nx5.7 for control loaves.

Table 26. Protein and amino acid composition of wheat flour (WF), LCP cottonseed flour and their mixtures

Amino Acids	Parameters	100% WF %	100% LCP %	96% WF 4% LCP %	92% WF 8% LCP %	88% WF 12% LCP %	84% WF 16% LCP %
	Moisture	10.05	2.03	9.73	9.41	9.09	8.77
	Protein	11.87[a]	59.41[b]	13.77	15.76	17.58	19.48
Lysine		0.30	2.92	0.41	0.52	0.61	0.76
Histidine		0.28	1.93	0.35	0.41	0.48	0.54
Arginine		0.57	1.44	0.60	0.64	0.67	0.71
Tryptophan		0.20	8.48	0.53	0.86	1.19	1.52
Aspartic Acid		0.55	2.09	0.61	0.67	0.73	0.80
Threonine		0.33	6.13	0.56	0.79	1.03	1.26
Serine		0.56	2.08	0.62	0.68	0.74	0.80
Glutamic Acid		3.58	2.94	3.55	3.53	3.50	3.48
Proline		1.32	13.36	1.80	2.28	2.76	3.25
Glycine		0.41	2.58	0.50	0.58	0.67	0.76
Alanine		0.36	2.48	0.45	0.53	0.61	0.70
Cystine		0.30	2.43	0.39	0.47	0.56	0.64
Valine		0.74	2.32	0.80	0.87	0.93	0.99
Methionine		0.19	3.76	0.33	0.48	0.62	0.76
Isoleucine		0.45	0.74	0.46	0.47	0.48	0.50
Leucine		0.85	3.96	0.97	1.10	1.22	1.35
Tyrosine		0.37	2.11	0.44	0.51	0.58	0.65
Phenylalanine		0.61	3.87	0.74	0.87	1.00	1.13

[a]NX5.7 wheat flour

[b]NX6.25 for cottonseed

Table 27. Essential amino acid content[a] of wheat/LCP cottonseed flour mixtures and FAO/WHO suggested pattern of amino acid requirement[b]

Amino Acids	Ratio of wheat:LCP cottonseed in mixes					Suggested FAO/WHO Patterns		
	100:0	96:4	92:8	88:12	84:16	Infant	School Children	Adult
Histidine	2.36	2.54	2.62	2.73	2.77	1.4		
Isoleucine	3.79	3.34	3.00	2.73	2.57	3.5	3.7	1.8
Leucine	7.16	7.04	7.02	6.94	6.93	8.0	5.6	2.5
Lysine	2.53	2.98	3.32	3.47	3.90	5.2	7.5	2.2
Methionine	1.60	2.40	3.06	3.53	3.90			
½ Cystine	2.53	2.83	3.00	3.19	3.29			
Total sulfur amino acids						2.9	3.4	2.4
Phenylalanine	5.14	5.37	5.55	5.69	5.80			
Tyrosine	3.12	3.20	3.25	3.30	3.34			
Total aromatic amino acids						6.3	3.4	2.5
Threonine	2.78	4.07	5.04	5.86	6.47	4.4	4.4	1.3
Valine	6.23	5.81	5.55	5.29	5.08	4.7	4.1	1.8

[a] g/100 g protein
[b] Castro et al. (1976)

mixtures contained adequate quantities of essential amino acids to meet the requirements of adults. The lysine, leucine and isoleucine were lower than the amounts required to support the normal growth of infant.

Cottonseed flour was high in sulfur containing amino acids, aromatic amino acids, threonine and valine. The supplementation of wheat flour with LCP cottonseed flour substantially increased its content of lysine, the first limiting amino acid in wheat.

Harden and Yang (1975) studied the protein quality and substitution value of cottonseed flour, and reported that the lysine value of cottonseed flour samples (LCP, glanded and glandless flours), were higher than FAO pattern (1965) which was much lower than the pattern used for our study (4.2 vs 5.2, 7.5). They reported a decrease in lysine content during baking of 9%.

Chemical Modification Study

Protein modification usually refers to the intentional alteration of protein structure by physical, chemical or enzymatic factors to improve functional properties. The modification of food protein may involve alteration in structure or conformation at all levels of organization: primary, secondary, and tertiary structures. It also may disrupt or reform covalent bonds and secondary forces using chemical treatment (Kinsella, 1976).

Urea.

Urea solution is a highly polar solvent. Although there is some evidence that urea disrupts the hydrophobic bonds, generally its major action is to break hydrogen bonds.

The 0, 8 and 16% cottonseed substituted dough systems were treated with urea to study the effect of hydrogen bonding on dough structure .

Doughs were mixed in a mixograph the curves were evaluated for peak time, peak height and the height after 9 minutes of mixing as a measure of dough strength (Figure 13A, B, C). In all dough systems, the 1 M urea showed extremely low curve height at 9 minute point, with a very short peak time (0.74 vs 4.13 min for 0% substituted dough). The 8 and 16% LCP doughs had a better consistency than the 0% LCP system as indicated by the 9 minute height of the curve indicating less dependence on hydrogen bonds for dough development in the cottonseed substituted system.

Since urea is an amide compound, it may associate with polar groups on proteins and thereby eliminate sites for protein, hydrogen bonding. It was reported that 3 M urea caused disassociation of gliadin proteins (Krull & Wall, 1969). The low levels of urea (0.2-0.08 M) had a slight strengthening effect on the 0% LCP system which could result from unfolding some of protein molecules thus exposing more sites to react and increase the dough strength. That

effect was not noticable at 8 and 16% LCP systems.

Sodium-dodecyl-sulfate.

(SDS) was added to control and substituted dough systems, and the mixograph curves were studied for peak time, height, and curve height at 9 minute point. The graphs in Figure 14A, B, C show a pronounced strengthening effect of (SDS) over all dough systems; when 0.125, 0.25 g were added. The higher levels 0.50 and 1.0 g, caused more denaturation of the proteins, thus reduced the gluten protein functionality, and the final viscosity (as measured by the 9-minute drop) was drastically decreased. The denaturation effect of (SDS) was more pronounced on the LCP cottonseed substituted dough systems.

Similar effect of (SDS) was reported by Volpe (1976), on dough systems substituted with single cell proteins. Sodium-dodecyl-sulfate is a detergent, its action is to disrupt most protein-protein and protein-lipid interactions. The effect of (SDS) on unfolding protein molecules was more noticable when it was added at high level (1 g/30 g flour).

Succinic Anhydride.

The effect of amide group in dough structure was studied by applying succinic anhydride at different levels (0.02, 0.1, 0.2, 1.0 and 2.0 g). Habeeb et al. (1958) reported that succinylated proteins showed increased intrinsic viscosities with a decrease in sedimentation coefficient at neutral and alkaline pH values caused by

Figure 13. Effect of urea on 0, 8 and 16% substituted dough rheology

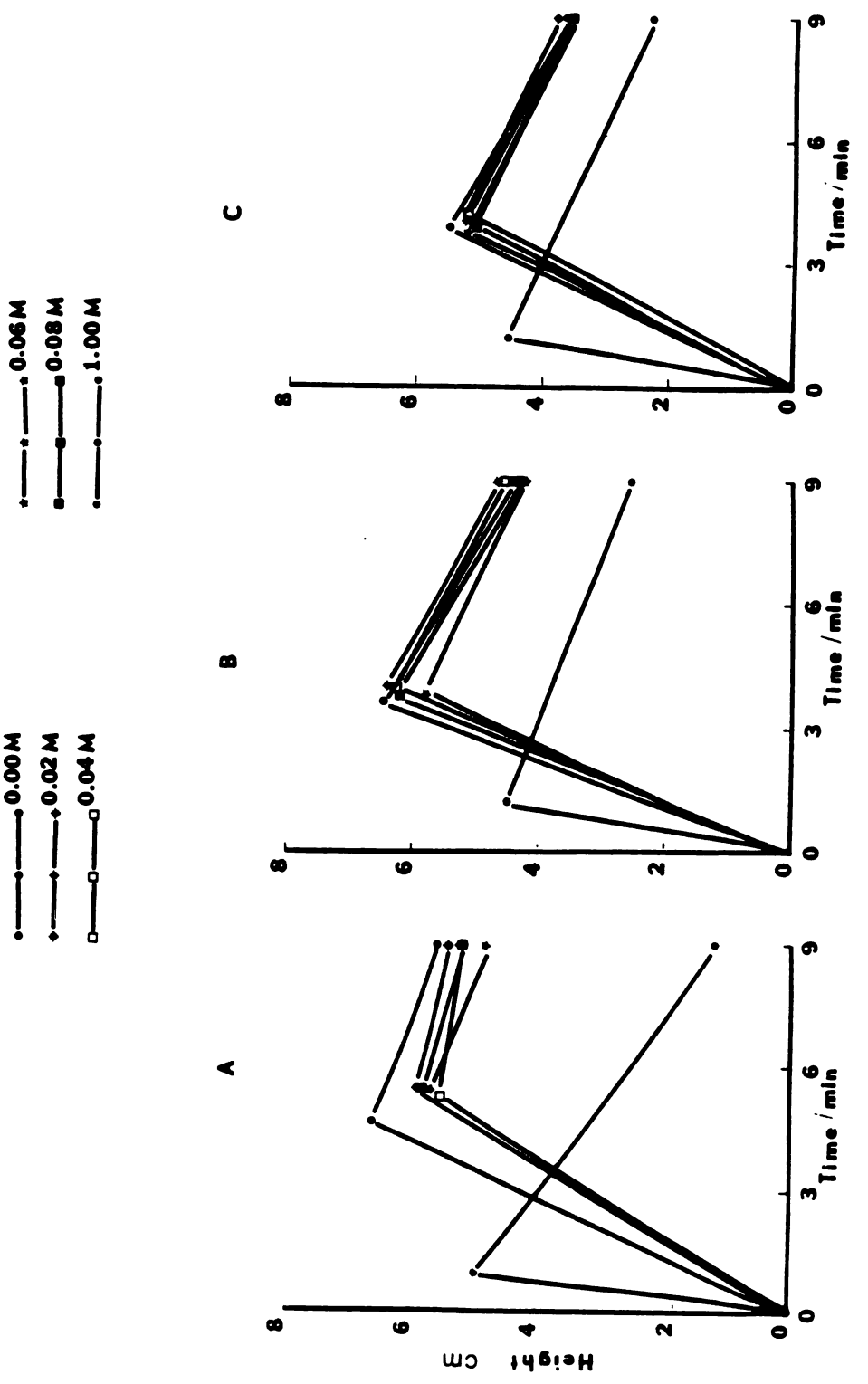

0.00M
0.02M
0.04M
0.06M
0.08M
1.00M
A
B
C
Height cm
Time / min
0
2
4
6
8
3
9

Figure 14A. Effect of SDA on 0% substituted dough rheology

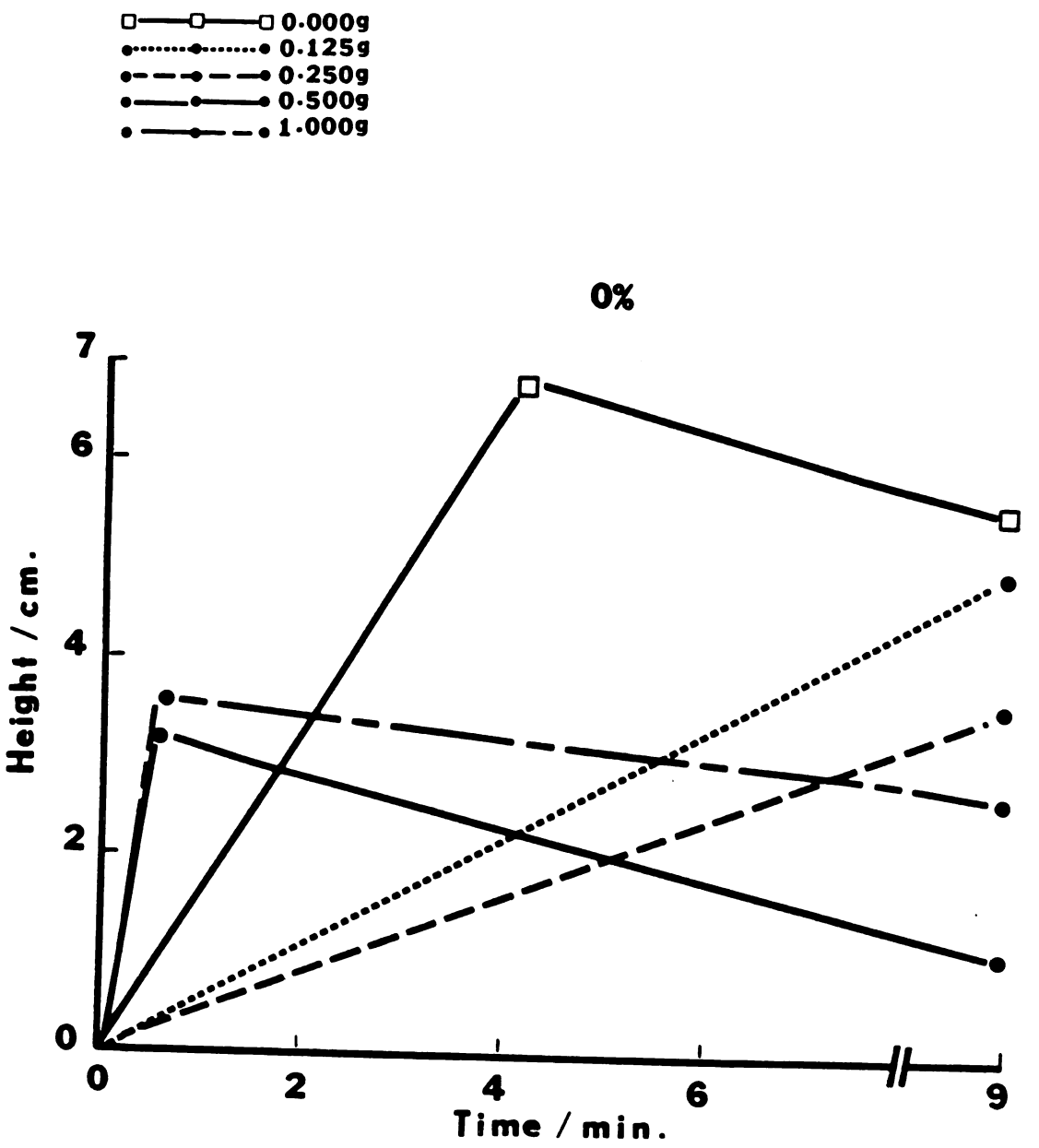
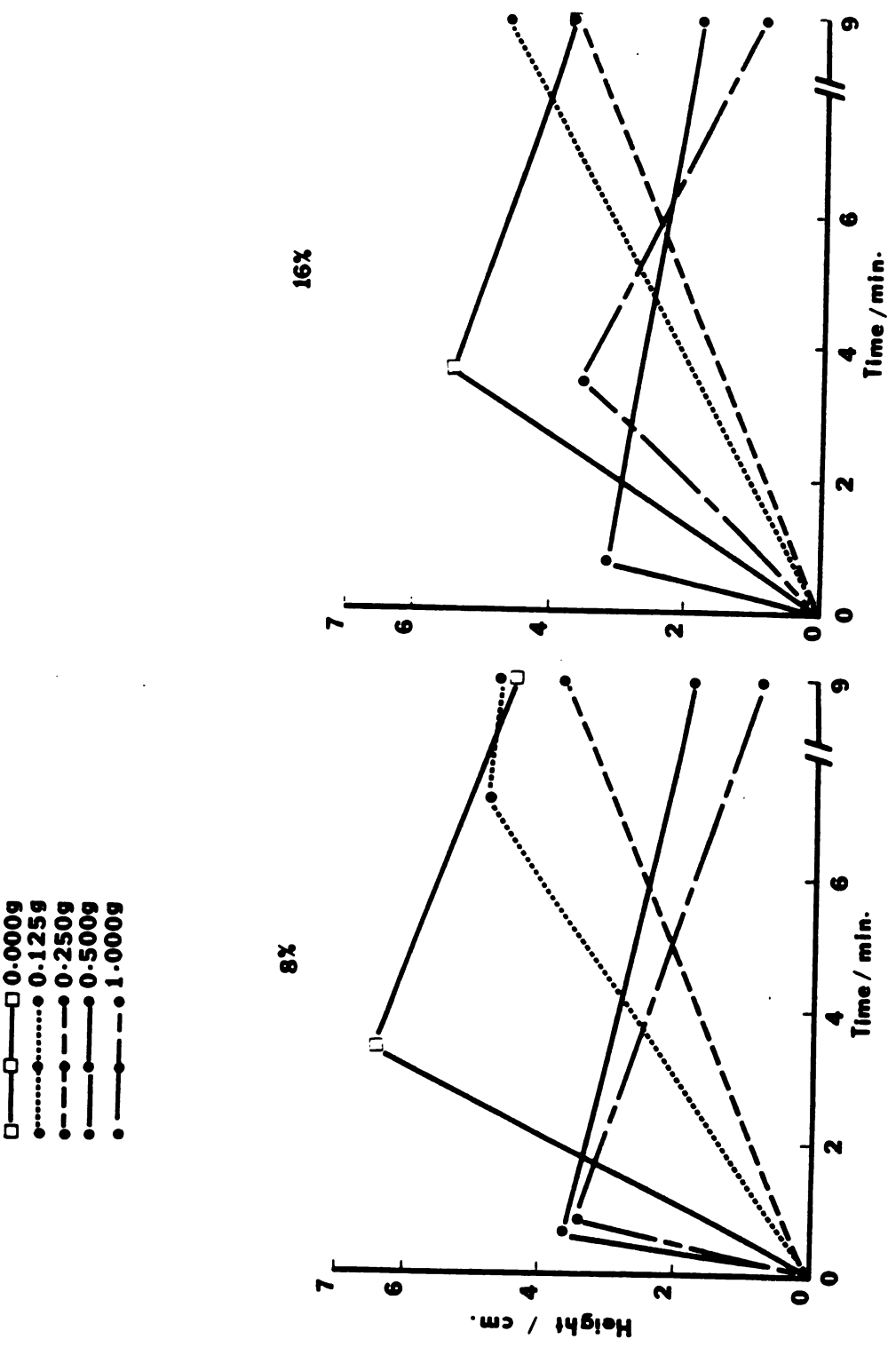

0.000g
0.125g
0·250g
0·500g
1·000g
0%
Height / cm.
Time / min.
0
2
4
6
7
9

Figure 14B. Effect of SDS on 8 and 16% substituted dough rheology

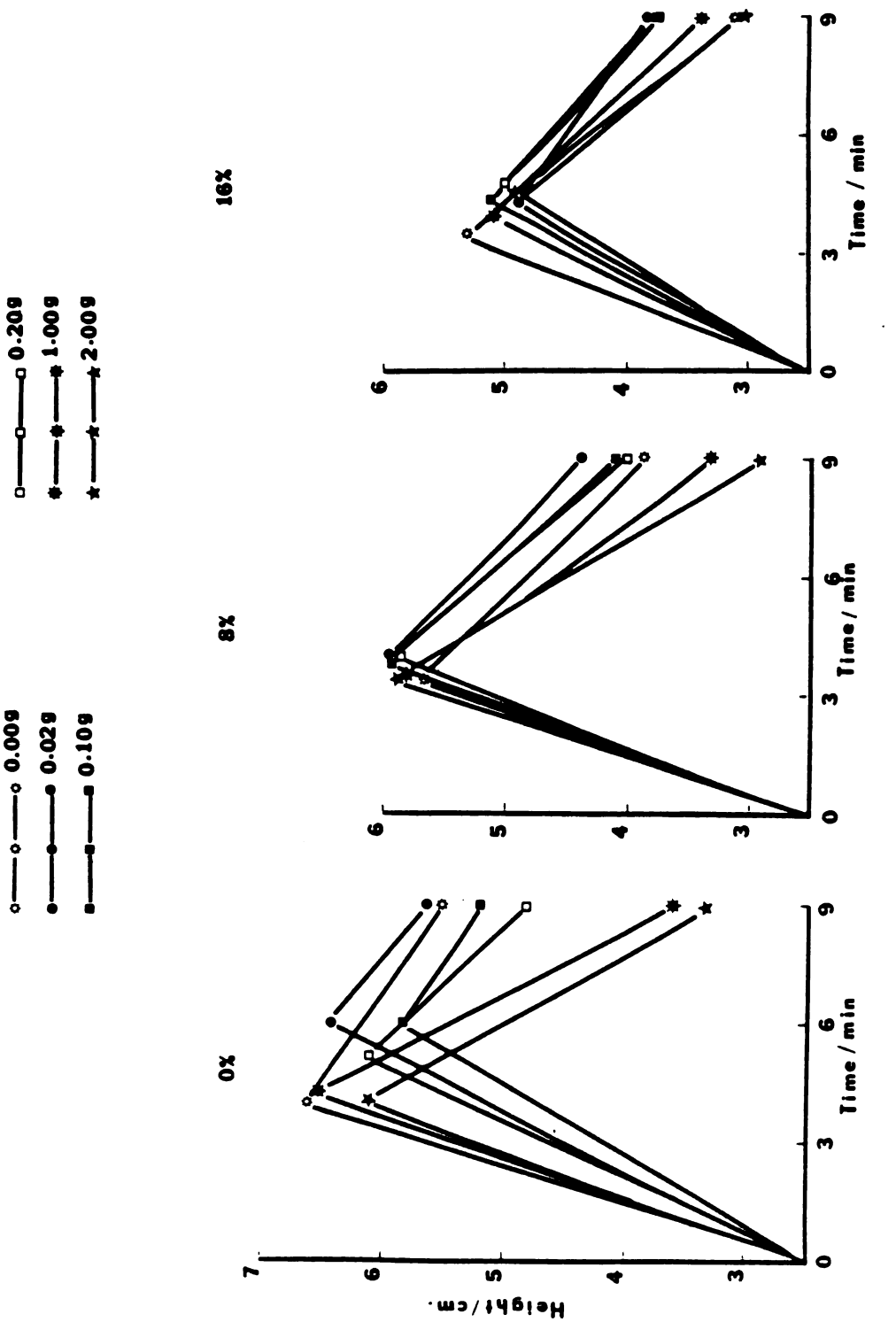

0.000g
0.125g
0.250g
0.500g
1.000g
8%
16%
Height / cm.
Time / min.
Time / min.

unfolding and molecular expansion.

Addition of succinic anhydride to the control dough system (Figure 15A) at levels (0.1 - 2.0 g/30 g flour), reduced the dough viscosity as measured by the drastic drop in the mixograph curve at 9-minute point, while 0.02 g succinic anhydride improved the dough consistency. Comparing the effect of the additive on control and substituted dough systems (Figure 15B, C) it can be seen clearly that addition 0.02, 0.1 and 0.2 g succinic anhydride improved the dough consistency over that of the control system. The improving effect was even more pronounced with the 16% LCP cottonseed flour dough system, where even the highest level of succinic anhydride added (2.0 g) improved the dough consistency (Figure 15C).

Extensive succinylation increased the susceptibility of bovine serum albumin's disulfide bonds to reduction, and decreased its ability to precipitate. The reaction of succinic anhydride is mostly with ε-amino groups of lysine, and an excess of the succinic anhydride was necessary to succinylate most ε-amino groups of lysine. This mechanism explains the reason why succinic anhydride worked better on the substituted dough systems. Cottonseed flour as was shown before is higher in amino acid lysine content than wheat flour. The substitution of wheat flour with cottonseed flour increased the lysine content of wheat/cottonseed mixtures, thus promoting more ε-amino

Figure 15. Effect of succinic anhydride on 0, 8 and 16% substituted dough rheology


0.00g
0.02g
0.10g
0.20g
1.00g
2.00g
0%
8%
16%
Height/cm.
Time/min

lysine for succinic reaction. This explains the effectiveness of high succinic anhydride levels 1 and 2 g on strengthening rather than weakening the substituted dough consistency.

Disulfide and Sulfhydryl groups.

The only covalent bonds that are known to be significant in dough structure are the disulfide linkages between proteins. The importance of thiol groups and disulfide bonds in determining the rheological properties of dough has been recognized by many workers. It has been noted that the modification of only a portion of the thiol groups produces the maximum effect on the rheological properties of dough and several attempts have been made to classify the sulfur groups of wheat flour into "reactive" and "non-reactive" on the basis of their chemical reactivity.

N-ethylmaleimide was used for thiol group determination, and dithiothreitol for disulfide bonds, both reagents react stoichiometrically.

The estimation of rheologically important thiol and disulfide groups in dough was done in the small (50 g) bowl of the Farinograph with development time and the resistance to mixing being used as rheological parameters. The total thiol and disulfide groups in wheat flour and cottonseed flour were determined according to the method described in the methods (Ellman's).

N-ethylmaleimide.

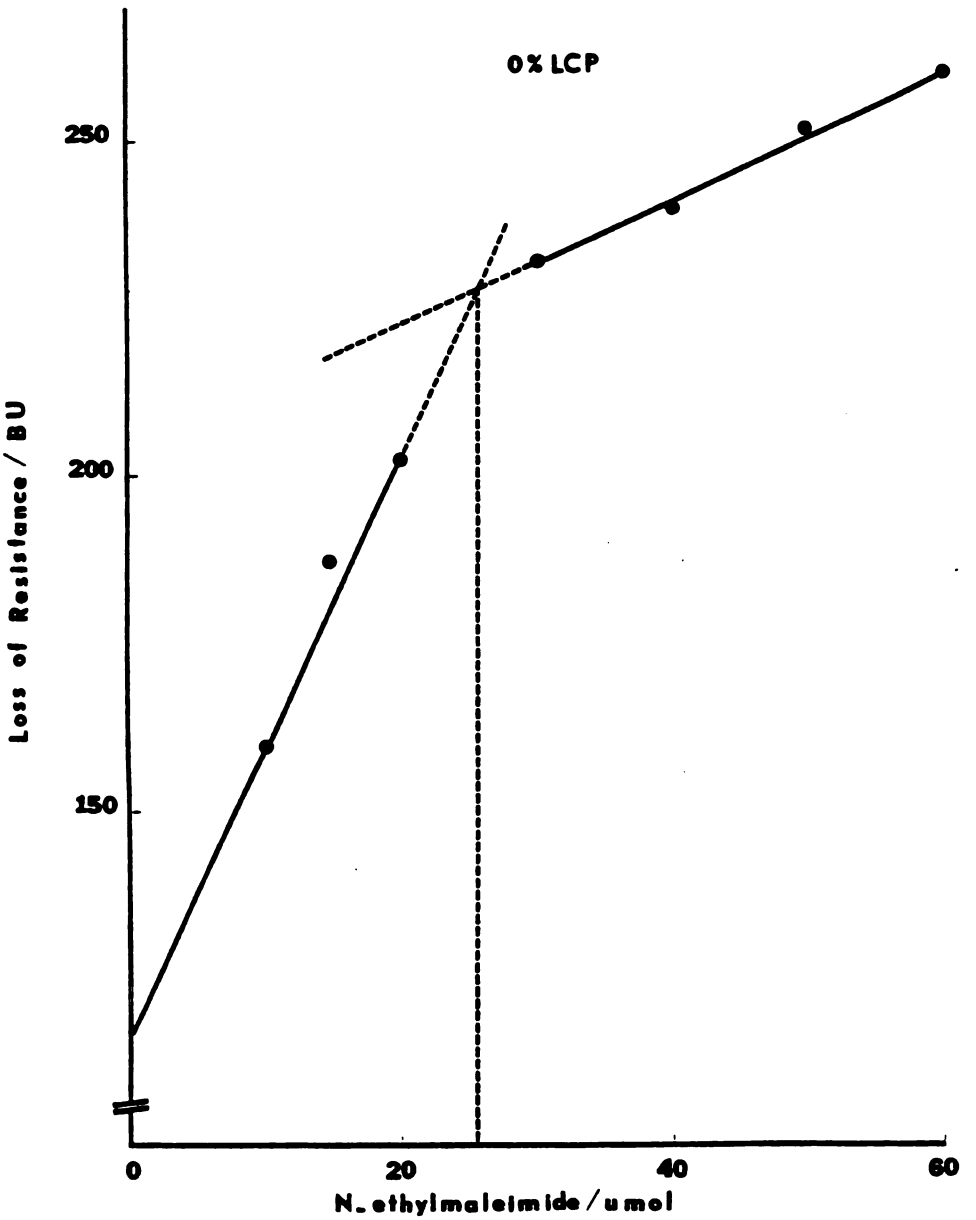
N-ethylmaleimide (NEMI) as a SH blocking reagent was added to 0 and 8% cottonseed flour doughs (0 to 60 µmol) at zero time. The doughs were mixed in the Farinograph for 30 minutes at which time the resistance to mixing was measured. The dough to which (NEMI) was added developed more rapidly and has less resistance to mixing after long mixing time (30 min). Meredith and Bushuk (1962) reported similar effects on Farinograph curves.

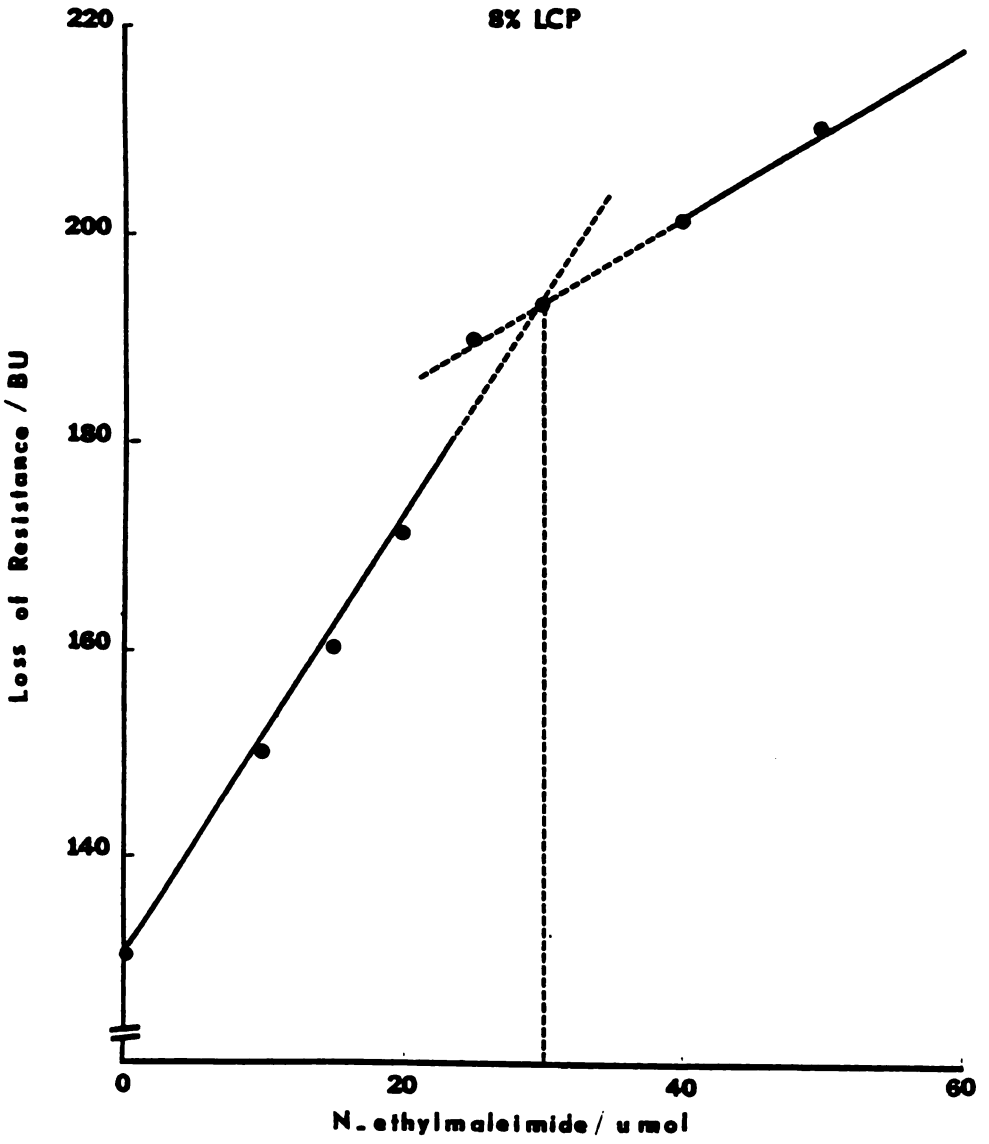
Figure 16A shows the effect of (NEMI) on the mixing tolerance. The loss of resistance was directly proportional to the amount of reagent in the range 0 - 30 µmol. The amount of (NEMI) consumed in this area is considered to be the amount of thiol that are important to the mixing tolerance, with the maximum amount of 26 µmol for the control dough system. The 8% substituted system (Figure 16B), the range 0 - 30 µmol NEMI was directly proportional to the loss of resistance with 30 µmol as the maximum amount of thiol involved in mixing tolerance of dough.

Dithiothreitol (DTT).

For determination of sulfhydryls important to mixing resistance, reagent DTT was added serialy to dough after it reached the full development. The reagent was added (5 - 500 µmol) at intervals of 5 to 15 minutes. The resistance to mixing (the height of the Farinograph curve after 30 minutes of mixing in Brabender units) was measured.

Figure 16. Effect of NEMI on loss of resistance in A - wheat dough and B - 8% LCP cottonseed substituted dough systems


0% LCP
250
200
150
Loss of Resistance / BU
0
20
40
60
N-ethylmaleimide / umol


8% LCP
220
200
180
160
140
Loss of Resistance / BU
0
20
40
60
N-ethylmaleimide / u mol

Dithiothreitol was also added to dough at zero time (0 - 100 μmol) after which the dough was mixed for 30 minutes. The SS involved in dough development were estimated.

Figure 17A and B shows the resistance of control and substituted dough systems to mixing after DTT was added. The control system showed the range of SS to be 0 - 67 μmol with maximum of 55 μmol DTT, while in the 8% substituted system (Figure 17B) it was found to be in the range 0 - 90 with maximum of 42 μmol of reagent.

The disulfide groups involved in dough development (Figure 18A, B) show that the maximum effect on development time was achieved with 30 μmol DTT for the control system and only 12 μmol in the 8% substituted dough system.

Jones et al. (1975) converted the contents of disulfide and thiol that are involved in mixing to a ratio mixing ss/mixing sH. Table 28 summarizes all the results for this section. It is obvious that the number of disulfides involved in developing dough was much less than that involved in resistance to mixing 30 vs 55 in control system, 12 vs 42 in 8% substituted system. Thiol groups involved in mixing were only 31% of the total sH groups in control system and only 11.7% in the 8% substituted system. Thiol groups involved in dough development were only 3% of the total sH groups in control system and only 1% in the 8% substituted system.

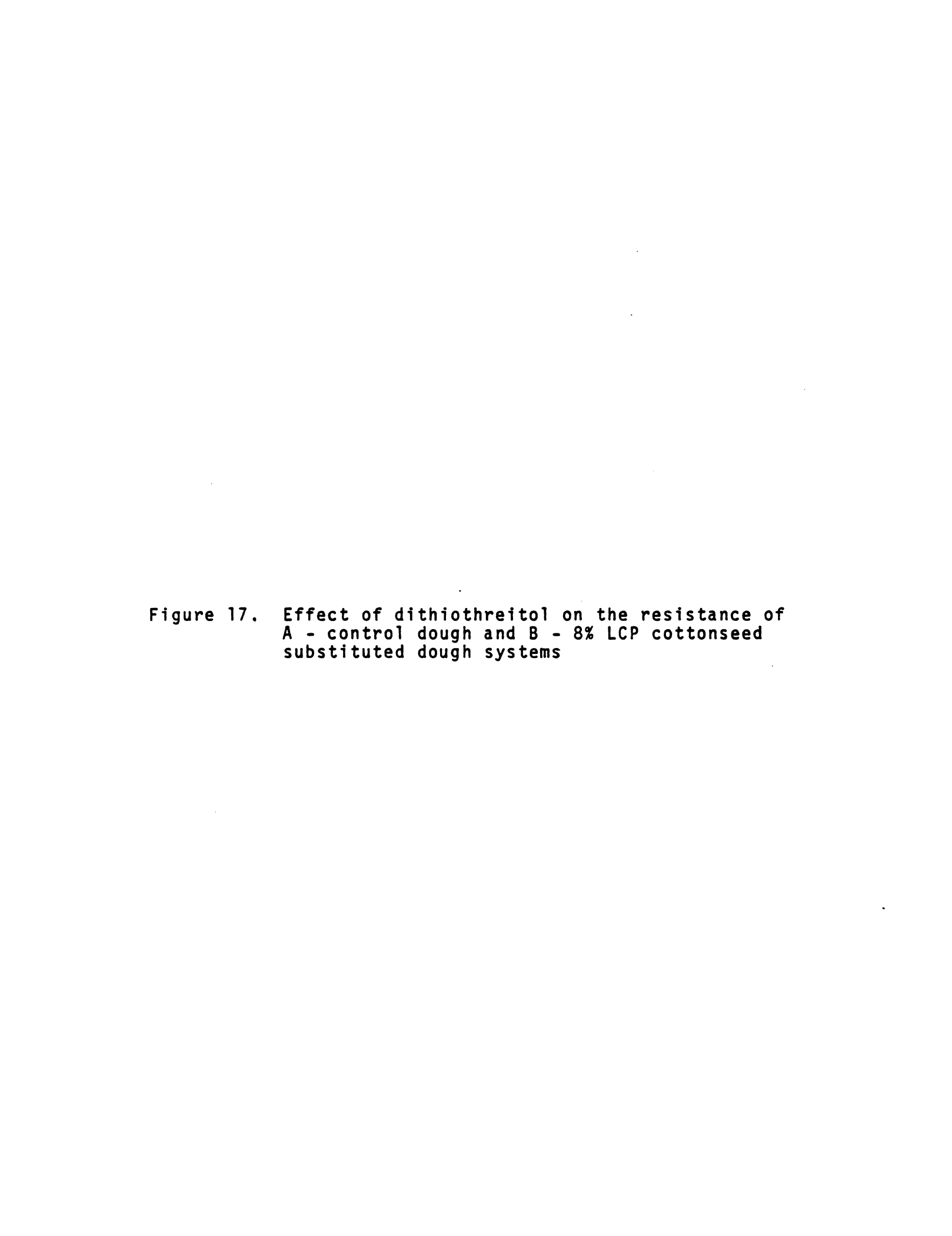
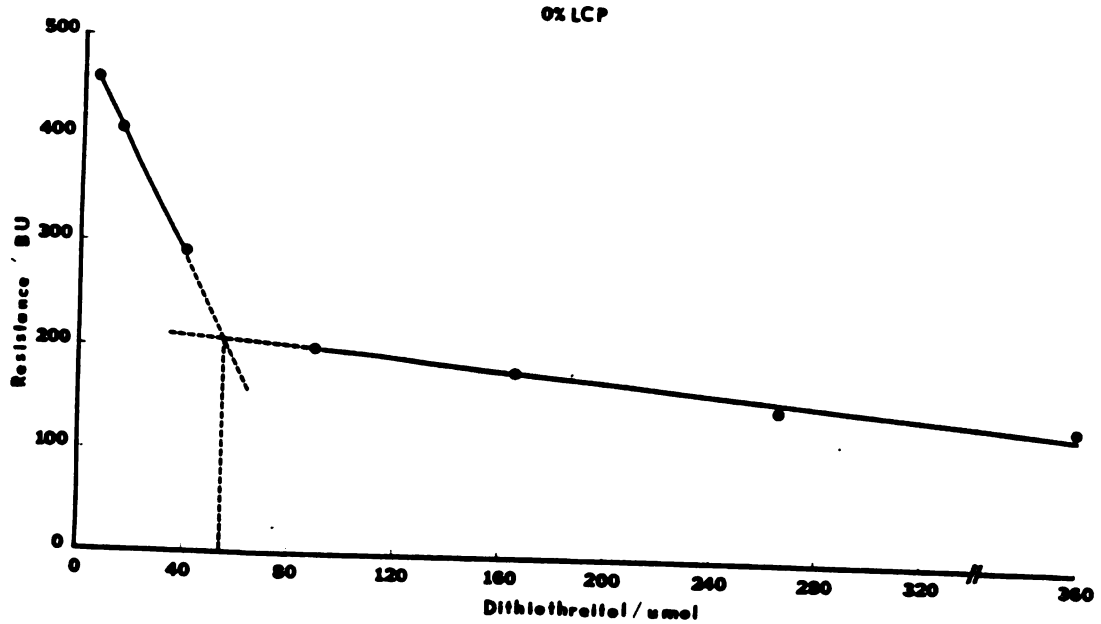


Figure 17. Effect of dithiothreitol on the resistance of A - control dough and B - 8% LCP cottonseed substituted dough systems


0% LCP
Resistance / BU
500
400
300
200
100
0
0
40
80
120
160
200
240
280
320
360
Dithiothreitol / umol

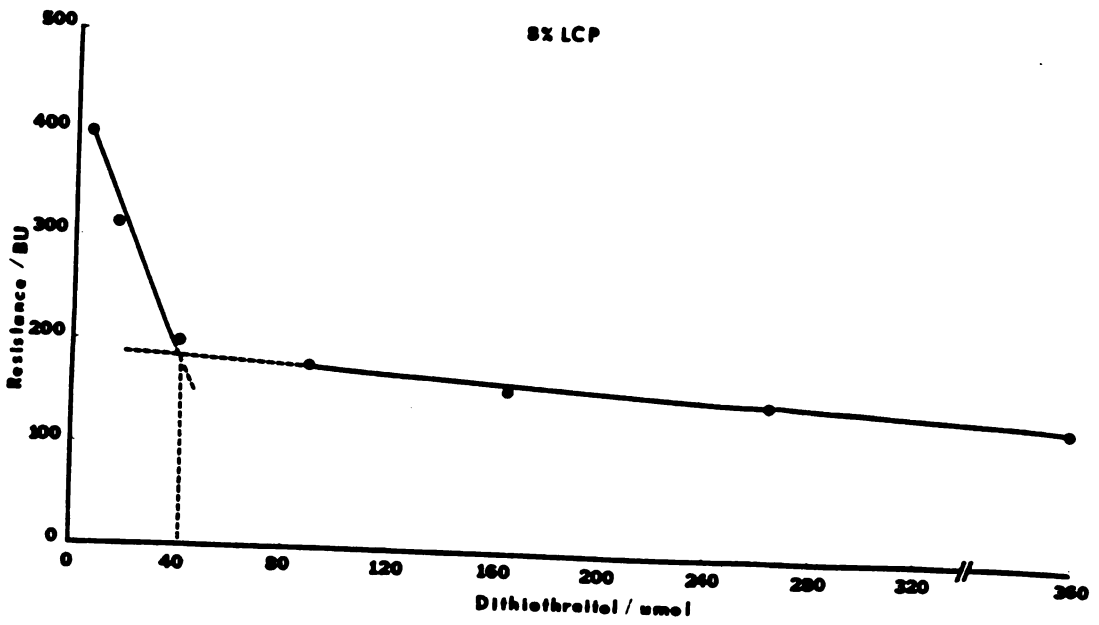
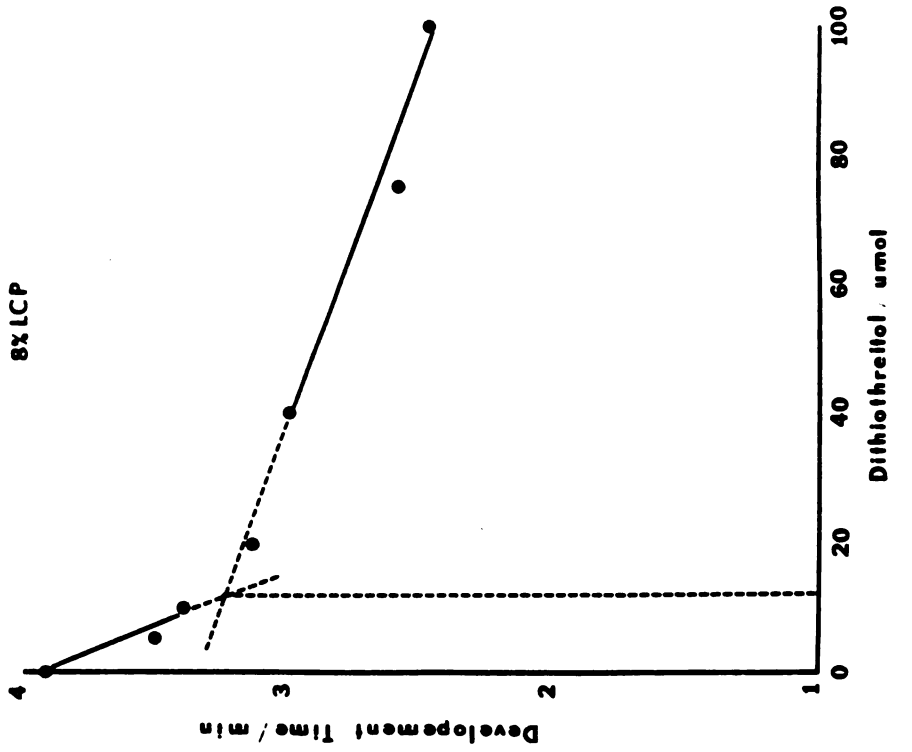

8% LCP
Resistance / BU
500
400
300
200
100
0
0
40
80
120
160
200
240
280
320
360
Dithiothreitol / umol

Figure 18. Effect of dithiothreitol on development times of A - Control dough and B - 8% LCP cottonseed substituted dough systems


8% LCP
Development Time / min
Dithiothreitol / umol
4
3
2
1
0
20
40
60
80
100

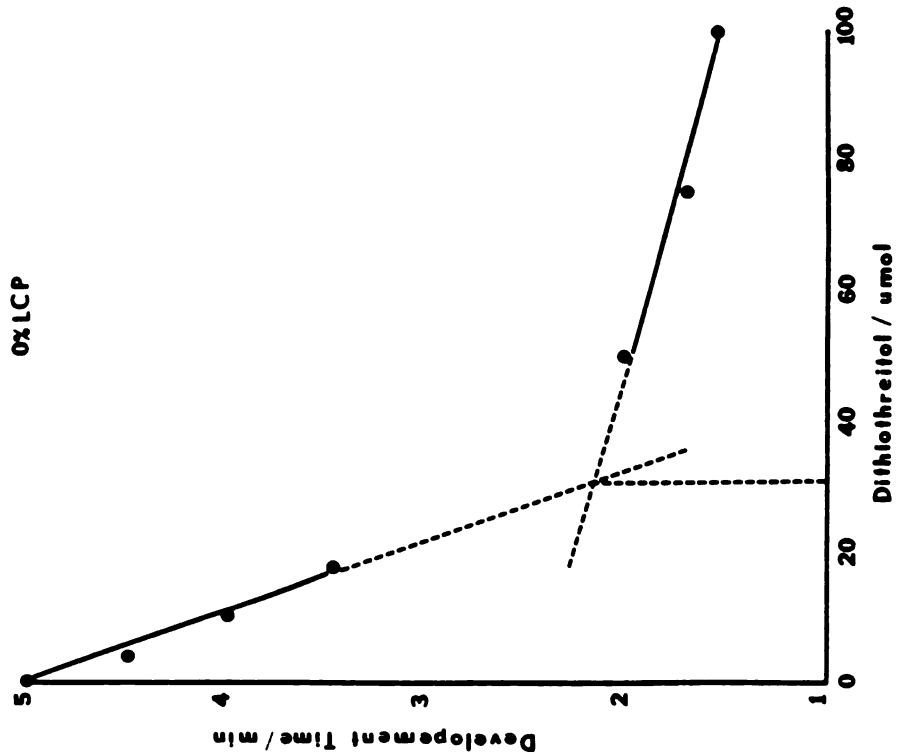

0% LCP
Development Time / min
Dithiothreitol / umol
5
4
3
2
1
0
20
40
60
80
100

Table 28. Some chemical and physical properties of wheat flour and 8% LCP substituted flours and doughs

	Flour	
	HRW wheat	8% LCP cottonseed
Protein %	11.9	59.6
Water absorption %	62.0	64.0
Development Time (min)	5.0	3.8
Total thiol (μmol/50 g flour)	183.3	256.0
Thiol involved in mixing tolerance	26.0	30.0
Total disulfide (μmol/ 50 g flour)	1010.0	4600.0
Disulfide involved in development (μmol/50 g of flour)	30.0	12.0
Disulfide involved in resistance to mixing (μmol/50 g flour)	55.0	42.0
*Mixing SS/mixing SH	2.1	1.4
Total SS/total SH	12.1	18.0

**SS involved in resistance to mixing/SH involved in mixing tolerance (μmol/50 g flour) 55/26 and 42/30

The relationship between the ratio of total disulfide to total thiol of a flour and the volume of a loaf baked from that flour was reported in the literature. Using the ratio involving only those groups that are rheologically important (Table 28) a better correlation with volume would be expected. The 8% substituted dough system show lower mixing SS/SH and total SS/SH ratios which would explain the smaller loaf volume with dough substituted with plant proteins.

Scanning Electron Microscopic (SEM) Studies

Morphological structure is often a clue to the functionality of a food material. The complex interaction between starch grains, proteins, lipid and water during the conversion of flour to dough involved changes in morphology of protein bodies from spherical structures in the seed to elonged strands of protein after hydration. Mechanical dough development, with stretching of gluten strands over starch produces a continuous film of protein (Chabot, 1979). In this part of the study, hard red winter (HRW) wheat flour and water were mixed to the optimum developed dough, and the protein matrix formed was studied with the addition of 8% LCP cottonseed flour and with 0.5% of the dough conditioners (Tween 20).

Previously, Khoo (1975) and Aranyi (1969) described the hydrated and mixed dough as having an even distributed starch grains over the entire area viewed under the SEM.

and the protein matrix as a smooth veil-like network that is stretched over starch grains. Figure 19A, B, and C show a continuous veil-like film of gluten proteins in wheat flour/water system where the starch grains are almost all coated with the protein film. The lower magnification shows the protein film to be continuous, while higher magnification shows (3000X) that there are some holes or pockets at the area of protein/starch interface. In Figure 19C, the protein film appears to be slightly thick in the areas between the starch granules, while it appears thinner in the areas where the film covers the starch grains which is due to the stretching of the protein matrix to cover starch grains.

The concentrated plant proteins incorporated in bread to increase the quality and quantity of protein at a low cost, caused deterioration in bread quality characteristics such as volume, crumb texture, and crumb compressibility. These characteristics were not due to the lack of fermentable components, as has been explained previously. Therefore the dough structure seemed unable to retain the gas during baking.

In this part, the effect of liquid cyclone processed cottonseed flour on the microstructure of wheat flour dough was studied. Figure 20A, B and C represent HRW wheat flour dough substituted with 8% LCP cottonseed flour. At relatively lower magnification, the veil-like protein film seems to be formed; higher magnification, however, shows a

Figure 19. Scanning Electron Micrograph of HRW wheat flour dough
a) 1000 X
b) 3000 X
c) 5000 X

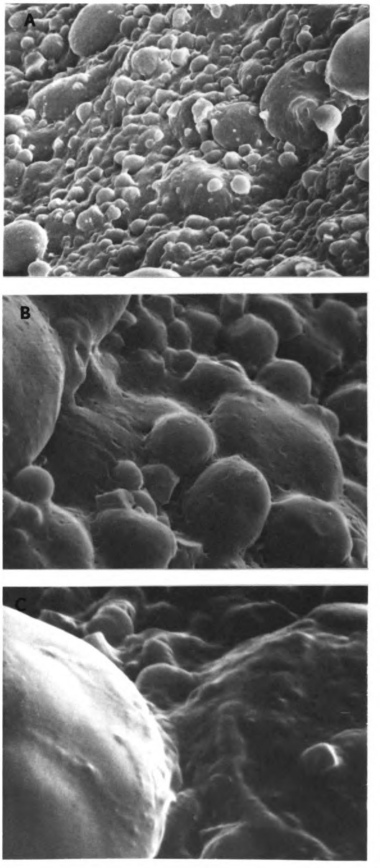
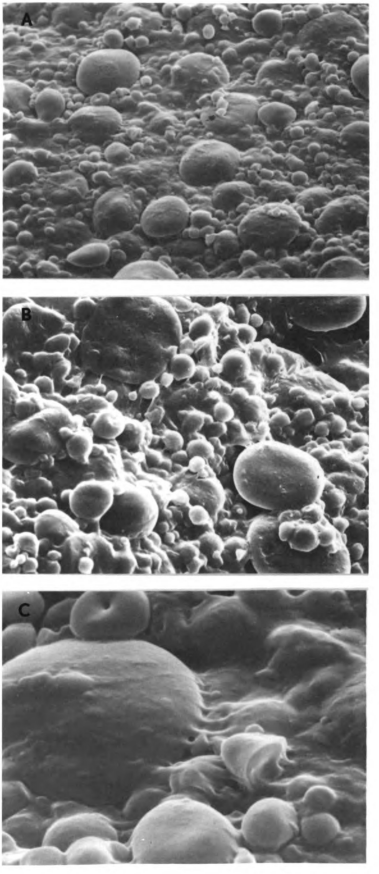

A
B
C

Figure 20. Scanning Electron Micrographs of 8% LCP cottonseed/HRW wheat flour dough
a) 700 X
b) 1000 X
c) 3000 X

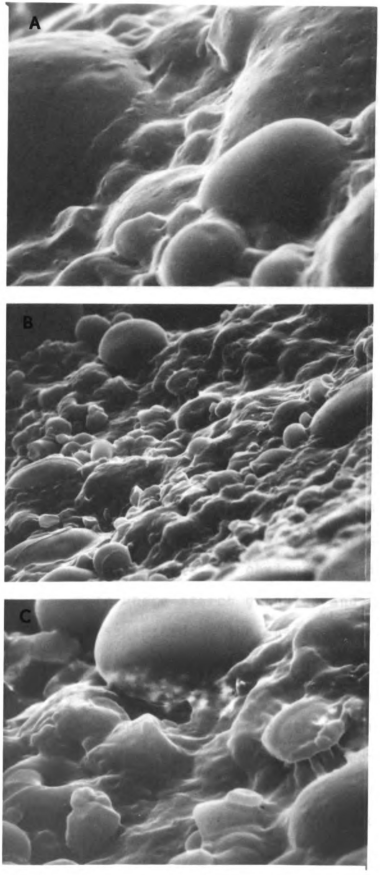

A
B
C

severe disruption of the protein matrix, especially at the thin-covered areas over the starch grains. The protein matrix generally appeared to be thick and less flexible as can be seen from Figure 20C.

Compared with control flour (Figures 19 and 20B and C), the LCP cottonseed flour blend was shown to disrupt the well defined protein-starch complex observed in wheat flour dough alone. This caused the protein matrix to be weaker, and unable to withstand the expansion and pressure that occur during baking. The tearing of the protein film in LCP cottonseed flour dough system, would permit the gases that cause oven-rise to escape and result in smaller loaf volume Fleming and Susulski, 1978 and Evans et al. (1977) have reported similar results with the incorporation of soy flour, sunflower, faba bean, field peas, and single cell protein concentrates.

The incorporation of dough conditioners have been one of the approaches researchers used to overcome the deleterious effect of plant protein concentrates on dough and bread systems. The effect of conditioner (Tween 20) at the 0.5% level was studied. The addition of the dough conditioner to HRW dough (Figure 21A, B, C) resulted in a much thinner protein sheets that are more fluid and continuous, and which appear to be very extensible. The protein matrix seems to be much more continuous with fewer pockets than the control dough (Figure 19B and C). The

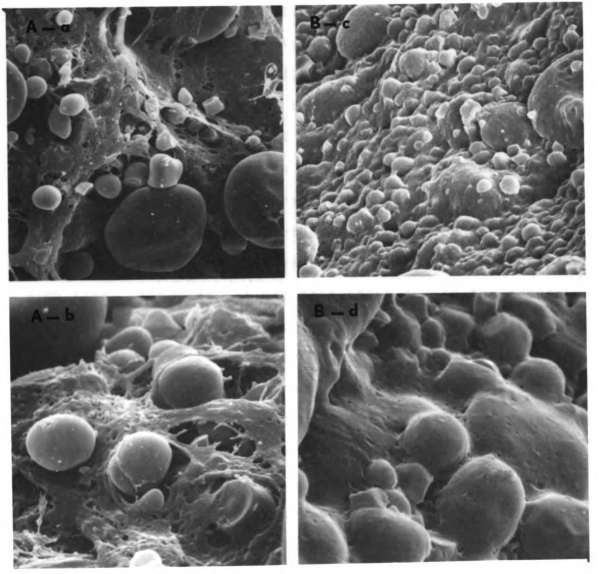
Figure 21. Scanning Electron Micrographs of Dough and 0.5% Conditioner Tween 20
a) HRW wheat flour dough at 8000 X
b) HRW/cottonseed flour dough at 1600 X
c) HRW/cottonseed flour dough at 8000 X


A
B
C

pronounced effect of the dough conditioner was observed when the conditioner was added to dough systems substituted with LCP cottonseed flour. Comparing Figure 21B and C with Figure 19B and C, it is obvious that the conditioners improved the protein matrix and made it more flexible and continuous with minimum tearing of the protein matrix.

The interpretation of microscopical data may present difficulties when samples are chemically treated with fixatives and/or dehydration prior to examination as it is the case in the electron microscopy. As an example of the effects of different preparation methods on structural characteristics, flour/water mixtures was prepared with two different procedures: fixed, dehydrated, critical point dried samples and nitrogen freezing, freeze fractioned, and freeze dried samples. Figure 22A and B show the extremely different structural characteristics of these wheat flour doughs from the unfixed samples. Comparing micrographs A, B with C and D in Figure 22, it was obvious that there were voids in the structure where starch granules and proteins aceous material have separated in the chemically treated samples (Figure 22A, B). The continuous protein film was drastically disrupted; however, fine strands of protein were present between starch granules. The protein matrix appeared thick, spongy, unflexible and torn especially at the thin-covered areas covering the starch grains. The same effect was found in dough systems substituted with 8%

Figure 22. Scanning Electron Micrographs of HRW Wheat Dough Prepared with Different Methods
A. Fixed in glutaryldhyde, ethanol dehydrated and CPD
a) 1,600 X
B) 4,000 X
B. Nitrogen frozen, freeze fractured, and freeze dried
c) 1,000 X
d) 3,000 X


A — a
B — c
A — b
B — d

LCP cottonseed flour (Figure 23A, B, C D and E). In a previous part of this SEM study, it has been shown that LCP cottonseed/wheat dough had less flexible proteinous matrix. When those doughs were fixed, dehydrated and critical point dried, the unflexability of the protein matrix was drastically increased causing most of the starch granules to completely separate from the protein fiber as can be seen from Figure 23A, B as compared to Figure 223, D and E.

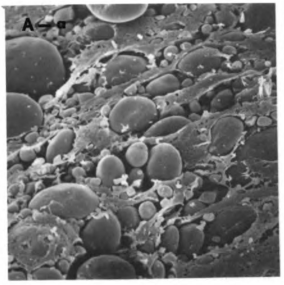
Fixation and dehydration procedures prior to critical point drying have the disadvantage of long sample preparation time. Moreover, it has been shown during the course of this study that these procedures drastically altered the ultrastructure of flour/water dough. Similar effects of these procedures have been recognized in biological science as causing morphological and chemical changes in samples (Wolman, 1955; Hopwood, 1969; Hyatt, 1970). Diffusion, loss of chemical constituents, protein denaturation, and cross-linking of molecules are some of the alterations that occur as a result of fixation and dehydration, and they result in drastic distortions in the structure.

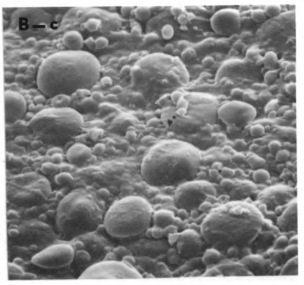
Chabot et al. (1979) reported similar results with bread samples studied under SEM, and he concluded that equeous fixatives washed starch out of bread samples causing them to separate from the protein matrix.

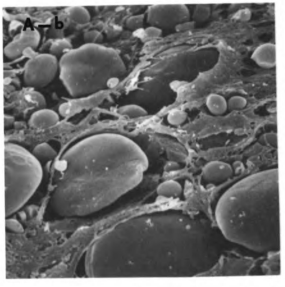
Figure 23: Scanning Electron Micrographs of HRW Wheat Dough Substituted with 8% LCP Cottonseed Flour Prepared with Different Methods

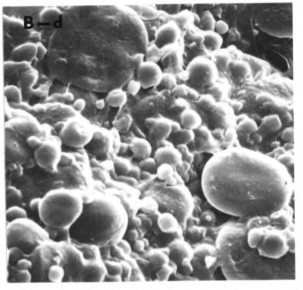
A. Fixed in glutarldhyde, ethanol dehydrated and CPD
   a) 800 X
   b) 1,600 X

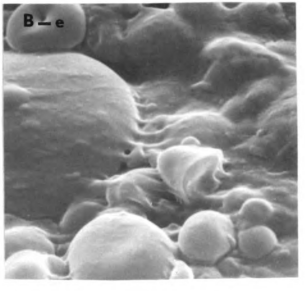
B. Nitrogen frozen, freeze fractured, and freeze dried
   c) 700 X
   d) 1,000 X
   e) 3,000 X


A—a


B—c


A—b


B—d


B—e

APPENDIX

BREAD SCORE SHEET

Sample number: Name:

Date:

Instructions:

1. Please do not taste. This test is made for judging the overall appearance of bread only.

2. Place an "X" along the horizontal line indicating your judgment.

Crust Color

Pale — Dark

Crust Character

Thick — Thin

Crumb Color

White — Brown

Grain & Texture

Compact — Open

Overall Acceptability

Unacceptable — Acceptable

Comments:

Figure 24. Score card used for sensory evaluation of white bread

BREAD SCORE SHEET

Date:

Instructions: Name:

1) Test your reference sample first then the other samples.

2) The reference sample should be re-tested as often as necessary to be able to grade the other samples.

CRUST COLOR	CRUMB COLOR	FLAVOR, ETC.[1]
1. Very dark brown	1. Greenish dark yellow	1. Very poor
2. Dark brown	2. Dark yellow	2. Poor
3. Sl. dark brown	3. Sl. dark yellow	3. Just below fair
4. Light brown	4. Yellowish oatmeal	4. Fair
5. Dark tan	5. Dark oatmeal color	5. Good
6. Tan	6. Sl. dark oatmeal	6. Very good
7. Light tan	7. Oatmeal color	7. Excellent

Sample #	Crust Color	Crumb Color	Flavor	Texture	Aroma	General Accept.	Comments

[1]Use the same scale for flavor, texture, aroma, and general acceptability

Figure 25. Score card used for sensory evaluation of Egyptian bread.

BREAD SCORE SHEET

Sample Number: Name:

Date:

Instructions:

Please place an "X" along the vertical line indicating your judgment.

Crust Color

Pale — Dark

Crust Character

Thick Rubbery — Soft Tender

Grain Texture

Coarse Uneven cells — Uniform cells

Tenderness

Tough — Tender

Flavor

Bad — Excellent

Overall Acceptability

Unacceptable — Acceptable

Comments:

Figure 26. Score card used for sensory evaluation of optimum bread

SUMMARY AND CONCLUSIONS

The objectives of this study were: to observe the effects of substitution with varying levels (0, 4, 8, 12 and 16%) of Liquid Cyclone Processed (LCP) cottonseed flour on dough rheology and bread physical characters and to compare the effects of LCP cottonseed flour and starch on dough; to study the rheology effect of salt, conditioner and oxidant, each at three levels and their combinations on dough rheology and baked bread; to optimize the levels of additives to produce optimum bread; to evaluate the functionality of LCP cottonseed flour in Egyptian bread system and the effect of four dough conditioners on dough rheology and on baked bread shelf life; to evaluate the substituted Egyptian bread protein and amino acid contents; to study the effect of LCP cottonseed flour, conditioner, and sample preparation method on scanning electromicrographs.

Farinograph studies of 0, 4, 8, 12 and 16% LCP cottonseed flour dough showed an increase in water absorption, arrival time, and dough breakdown as the level of cottonseed flour increased in the dough system from 0 to 16%, with the maximum effect obtained between the substitution levels of 4 and 8% LCP cottonseed flour. Wheat flour dough

substituted with wheat starch at levels 0, 4, 8, 12 and 16% showed a different trend. Farinograph water absorption, arrival time and dough breakdown decreased as the level of substitution increased, and the maximum effect was obtained between 4 and 8% level of substitution, which shows that there is a minimum amount of gluten that must be present in dough system in order to get the optimum dough and bread characters.

Viscoamylograph studies showed that in spite of the decrease in the viscosity of flour blend slurry after 60 minutes and the peak viscosity, with the higher levels of substitution with cottonseed flour, there was enough fermentable carbohydrate which suggested a potential for good loaf volume.

The bread baked with 0, 4, 8, 12 and 16% LCP cottonseed flour showed an increase in protein, lipid, ash and moisture as the levels of cottonseed flour increased. Sensory evaluation of bread indicated that higher levels of cottonseed flour produced bread with very coarse and open grain and darker crust and crumb colors.

Mixograph studies on the 0, 8 and 16% substituted doughs indicated that salt, conditioner and oxidant when they were added separately to doughs increased peak time, peak height, and the curve height at the 9-minute point as measures of dough strength. This effect reached its maximum with 0.5% conditioner and 30 ppm potassium bromate, while

it increased as the salt level increased from 1 to 2%. The additive interaction studies showed the effectiveness of all the additive combinations that included salt at any level, on stabilizing the cottonseed substituted doughs, especially at 12 and 16% levels of substitution. Potassium bromate, when it was added with conditioner, did not significantly influence dough rheology. The effect of the additives and their interaction on the 8% substituted bread showed the significant effect of salt on volume. The 1% salt level increased the volume while the 2% level caused a decrease in bread volume. Double combination of additives reduced the volume, and produced bread with a harder crumb than the triple additive combinations. The sensory evaluation of bread showed that use of 1% level of salt improved grain texture, when it was added with 0.5% conditioner and 30 ppm $KBrO_3$.

Multiple regression analysis of 8% substituted bread showed that the optimum levels of additives to produce optimum grain texture, volume, and compressability were 1% salt, 0.4% conditioner and 37 ppm potassium bromate. Optimum bread was baked and evaluated objectively and for sensory characters. Loaf volume and specific volume were higher than the control untreated system. The optimized bread also was given better sensory scores for tenderness, flavor and overall acceptability.

Egyptian bread baked with 0, 4, 8, 12 and 16% LCP cottonseed flour showed a decrease in loaf volume at the high level of cottonseed (12 and 16%). Protein content of bread increased as well as the fat, ash and water as the level of substitution with cottonseed increased. The amino acid lysine content also increased with the higher levels of cottonseed flour in bread. Sensory evaluation of bread showed that 12 and 16% levels were objectionable but below these levels of substitution the bread was acceptable.

The effect of four dough conditioners on mixograph characters showed that mono and diglycerides of Polysorbate (60) (Tandem 552) at 0.5% level increased mixogram height at 9-minute point indicating strengthening of dough structure. Egyptian bread baked with the four dough conditioners retained its softness after 3 days of storage with Tween 20 and Tandem 552. Loaf volume increased when dough conditioners were added.

Chemical modification of control and 8% substituted dough showed that the reagents were more detrimental to the substituted dough systems than to the control. Urea caused dough strengthening when added at low levels (0.02-0.08 M) while higher levels substantially weakened the dough structure. Succinic anhydride increasingly weakened the dough structure as the level increased, in both 0 and 8% substituted dough systems, but it was more noticable in the 8% substituted system. Sodium-dodecyl-sulfate at low levels

(0.125-0.25 g/30 g flour) partially denatured the proteins and increased the dough strength in both systems as measured by significantly increased peak time, while higher levels drastically reduced arrival time, peak height and the mixogram height at 9-minute point indicating weakening dough structure.

From the determination of reactive sulfhydryl-disulfide levels in both dough systems, it was found that the number of disulfides involved in dough development was less than that involved in resistance (30 vs 55) in control system and (12 vs 42) in substituted systems. Thiol groups involved in mixing were only 31% of the total thiol groups in control system and only 11.7% in the 8% substituted system.

Scanning electron microscopic studies showed that the incorporation of LCP cottonseed flour into wheat flour dough caused deterioration to the protein matrix, causing it to be thicker, less flexible, with some holes indicating its breakage especially in the interface between starch and protein. Conditioner (Tween 20) at 0.5% level was shown to partially overcome this deletinous effect by causing the protein matrix to be more fluid, flexible, and continuous, covering all the protein bodies and starch granules. The study suggested a better loaf volume potential due to the ability of the continuous flexible protein matrix to hold the gases during baking.

Fixation, dehydration and critical point drying were shown to alter the ultrastructure of dough drastically which might have lead to misinterpretation of the electron-micrographs of samples prepared using these chemicals.

PROPOSAL FOR FUTURE RESEARCH

The area of producing high protein bread is of growing interest all over the world and especially in the area of the Third World countries. The technology of producing different protein concentrates is still beyond the economical capacity of these countries. The area of processing of oilseeds for the production of an acceptable flour or concentrate still needs research to overcome the strong flavor and color that exist with all cottonseed flours produced by various methods. These strong flavor and off color are considered to be the main factors for not producing cottonseed flour bread commercially.

The instruments used for rheological studies and for objective measurements of bread such as volume, compressability or tenderness need further standardization. Optimizing baking methods to specific set of conditions makes comparative studies not valid. Subjective evaluation of product quality in spite of their importance, lack consistency and need further statistical study in order to get reliable evaluation of products.

The area of developing new additives is growing; the use of all the newly developed additives that are very

specific is one area of growing interest, that can be used to optimize the bread characters.

The mechanical production of Egyptian bread will increase the production which will necessitate the use of anti-staling factors in its production. Packaging is an area of growing importance in Egyptian bread production for increasing the shelf life. Liquid Cyclone Processed cottonseed flour as a source of protein can be added to cookies or cakes and introduced to school children through lunch programs in Egypt.

Further studies of the nature of molecular bonding of gluten should be developed. This will help the determination of the bonds that are more significance in dough strength and development.

The scanning electron microscopy techniques for bread and dough studying should be more developed. The increased resolution of newly developed electron microscopes will give even more information about the dough structure, and enable researchers to study further each component (starch, protein, lipid) of that complex system (dough).

REFERENCES

REFERENCES

Abbott, J.C. Economic factor affecting the distribution of world protein sources, p. 13. In: Milner, M. (ed.). Protein. Enriched Cereal Foods for World Needs. American Association Cereal Chemists: St. Paul, MN (1969).

Altschul, A.M., Lyman, C.M., Thurber, F.H. "Processed Plant Protein Feedstuffs", A.M. Altschul, Ed., p. 469, Academic Press, New York (1958).

American Association of Cereal Chemists. AACC Approved Methods. 7th Ed. The Association, St. Paul, MN (1962).

Aranyi, C. and Hawrylewicz, E.J. Application of scanning electron microscopy to cereal specimens. Cereal Science Today 14:230 and 253, (1969).

Aranyi, C. and Hawrylewicz, E.J. Ultrastructure of developing wheat endosperm. Cereal Chem. 45:500 (1968).

Bacigalupo, A., Aguilar, T.S., de la Fuente, R.L. and Riestra, J.V. Bread enrichment with prolatperuvian cottonseed flour. Cereal Science Today 12:431 (1967).

Bailey, A.E. Cottonseed and Cottonseed Products. Wiley (Interscience), New York (1948).

Balls, A.K. and Hale, W.S. Further studies on the activity of proteinase in flour. Cereal Chem. 13:656 (1936).

Baker, J.C. and Mize, M.D. Mixing doughs in vacuum and in the presence of various gases. Cereal Chem. 14:721 (1937).

Beckwith, A.C., Wall, J.S. and Dimler, R.J. Amide group as interaction sites in wheat gluten proteins. Effects of amide-esters conversion. Arch. Biochem. Biophys. 103:319 (1963).

Belderok, B. Bedeutumg der thiol-und disulfedgruppen fur die Zuchtung des weizens auf backqualitat. Getreide Mehl 17:20 (1967).

Bennet, R. and Coxpoch, J.B.M. I. Dough consistency and measurement of water absorption on the Brabender Farinograph and Simon "Research" water absorption. Trans. Am. Assoc. Cereal Chem. 11:72 (1953).

Bennet, R. and Ewart, J.A.D. The effects of certain salts on doughs. J. Sci. Fd. Agric. 16:199 (1965).

Berardi, L.C., Martinez, W.H., Fernandez, C.J. Cottonseed protein isolates: two step extraction procedure. Food Technol. 23(10):75 (1969a).

Berardi, L.C. and Goldblatt, L.A. Gossypol. In: Toxic Constituents of Plant Food Stuffs. Liener, I.E., Ed., p. 211. Academic Press. New York (1969b).

Berardi, L.C., Martinez, W.H., Fernandez, C.J., and Gajee, B.B. A low molecular weight protein isolate from cottonseed: Purification and functional properties. Food Technol. 23(4):514 (1969c).

Bernardin, J.E. Gluten protein interaction with small molecules and ions - the control of flour properties. Baker's Digest 52(4):20 (1978).

Bietz, J.A. and Wall, J.S. Isolation and characterization of gliadin like subunits from glutenin. Cereal Chem. 50:537 (1973).

Bloksma, A.H. Effect of potassium iodate on creep and recovery and on thiol and disulfide contents of wheat flour doughs. Symposium: Rheology and texture of food stuffs. Soc. Chem. Ind. Monograph No. 27 p. 153. London (1968).

Bloksma, A.H. Rheology and Chemistry of Dough. In: "Wheat Chemistry and Technology". Ed. by Pomeranz, Y., Chapter II, American Association of Cereal Chemistry, St. Paul, MN (1978).

Blouin, F.H., and Cherry, J.P. Identification of color-causing pigments in biscuits containing cottonseed flour. Presented at the 38th Annual Meeting of the Institute of Food Technologists, Dallas, TX, June 4-7 (1978).

Boatner, C.H. In: Cottonseed and Cottonseed Products. Bailey, A.E., Ed., Chapter VI, P. 213. Wiley (Interscience). New York (1948).

Boloorforooshan, M. Bean Protein Evaluation and Supplementation. Ph.D. Thesis. Michigan State University (1977).

Brahams, J.E., Jarquin, R., Elias, L.G., Gonzalez, M. and Bressani, R. Effect of calcium and gossypol on the performance of swine and on certain enzymes and other blood constituents. J. Nutr. 91:47 (1967).

Bressani, R. and Elias, L.G. Cambios en la composicion quimica yen et valori nutritivo del la proteina de semilla de olgodon durante su elaboracion. Arch. Lat. Nutricion 17(4):319 (1968).

Bressani, R. The use of cottonseed proteins in human foods. Food Technol. 19(11):51 (1965).

Bressani, R., Elias, L.G. and Braham, E. Cottonseed protein in human foods. In: World Protein Resources. Advances in Chemistry Series. Ed. by Gould, R.F. Amer. Chem. Soc. Washington, D.C. (1966).

Bressani, R., Elias, L.G., Braham, E. and Erales, M. Long term feeding studies with vegetable mixtures containing cottonseed flour produced by different methods. J. Agr. Food Chem. 17:1135 (1969).

Bressani, R., Elias, L.G., Jarquin, R. and Braham, J.E. All vegetable protein mixtures for human feeding. Effect of cooking mixtures containing cottonseed flour on free gossypol content. Food Technol. 18:98 (1964).

Bushuk, W. and Hlynka, I. Water as a constituent of flour, dough and bread. Baker's Digest 38(6):43 (1964).

Buttrose, M.S. Ultrastructure of developing wheat endosperm. J. Biol. Sci. 16:305 (1963).

Cabrera, J., Zapata, L.E., de Buckle, T.S., Ben-Gera, I., de Sandoval, A.M. and Shomer, I. Production of textured vegetable protein. J. Food Sci. 44:826 (1979).

Calvel, R. L'action dur sel sur les pates fermenters et sur les caracheristiques dur product fabrique. Bull. Arciehs Eleves Ecole France. Meunerie 40:241 (1969).

Carpenter, K.J. and Booth, V.H. Section 1. Chemical Procedures. Nutr. Abstr. Rev. 43:424 (1973).

Castro, C.E., Yang, S.P. and Harden, M.L. Supplemental value of Liquid Cyclone Processed cottonseed flour on the proteins of soybean products and cereals. Cereal Chem. 53:291 (1976).

Cavallini, D., Graziani, M.T., and Dupre, S. Determination of disulfide groups in proteins. Nature 212:294 (1966).

Chabot, J.R., Hood, L.F., and Liboff, M. Effect of scanning electron microscopy preparation methods on the ultrastructure of white bread. Cereal Chem. 56:462 (1969).

Chabot, J.R. Preparation of food science sample for SEM-scanning electron microscopy. SEM Inc. AMF O'Hare, IL, USA, p. 279 (1979).

Conkerton, E.J. and Frampton, . Reaction of gossypol with free ε-amino group of lysine in proteins. Arch. Biochem. Biophys. 81:130 (1959).

Dalby, G. Protein Fortification of Bread: Baladi Bread as an Example, p. 174. In: "Protein-Enriched Cereal Foods for World Needs", Milner, M. (ed.). Am. Assoc. Cereal Chem., St. Paul, MN (1969).

D'Appolonia, B.L. Rheological and baking studies of legume-wheat flour blends. Cereal Chem. 54:53 (1977).

Dempster, C.J., Cunningham, D.K., Fisher, M.H., Hlynka, I., and Anderson, J.A. Comparative study of the improving action of bromate and iodate by baking data, rheological measurements and chemical analysis. Cereal Chem. 33:221 (1956).

DeMuelenaere, H., and Feldman, R. Availability of amino acids in maize. J. Nutr. 72:447 (1960).

Dendy, D.A.V., Clarke, P.A. and James, N. The use of blends of wheat and nonwheat flours in breadmaking. Tropical Science 12:131 (1970).

Ellman, G.L. Tissue sulfhydryl groups. Arch. Biochem. Biophys. 82:70 (1959).

El-Tinay, A., Chandrasekhar, H., and Ramanatham, G. Protein and gossypol extractability from cottonseed flour. J. Sci. Fd. Agric. 31:38 (1980).

Engleman, E.M. Ontogony of oleurone grams in cotton embryo. Amer. J. Bot. 53:231 (1966).

Evans, L.G., Volpe, T. and Zabik, M.E. Ultrastructure of bread dough with yeast single cell protein and/or emulsifier. J. Food Sci. 42:70 (1977).

Evans, R.J. and Bandemer, Selma L. Nutritive value of legume seed proteins. J. Agr. Food Chem. 15:439 (1967).

Ewart, J.A.D. A modified hypothesis for the structure and rheology of glutenin. J. Sci. Fd. Agric. 23:687 (1972).

FAO-PAG. Protein-Calories Advisory Group of the United Nations System 2,1 (1975).

Fisher, M.H., Aitken, T.K. and Anderson, J.A. Effects of mixing, salt and consistency on extensigrams. Cereal Chem. 26:81 (1949).

Fisher, H. Unrecognized amino acid deficiencies of cottonseed protein for the chick. J. Nutr. 87:9 (1965).

Fleming, S.E. and Sosulski, F.W. Breadmaking properties of four concentrated plant proteins. Cereal Chem. 54: 1124 (1977).

Fleming, S.E., Sosulski, F.W. Microscopic evaluation of bread fortified with concentrated plant proteins. Cereal Chem. 55:373 (1978).

Fleming, S.E. and Sosulski, F.W. Nutritive value of bread fortified with concentrated plant proteins and lysine. Cereal Chem. 54:1238 (1977).

Food & Drug Administration. 1974. Food additives permitted for human consumption - modified cottonseed products. Fed. Regist. 39 (No. 177): 32735.

Fullington, J.G. Interaction of phospholipid-metal complexes with wheat protein. J. Lipid Res (8):609 (1967).

Galal, A.M., Varriano-Marston, E. and Johnson, J.A. Rheological dough properties as affected by organic acid and salt. Cereal Chem. 55:683 (1978).

Gardner, H.K., Jr., Hron, Sr., R.H. and Vix, H.L.E. Removal of pigment glands (Gossypol) from cottonseed. Cereal Chem. 53:549 (1976).

Gardner, H.K., Jr., Hron, Sr., R.H. and Vix, H.L.E. Liquid cyclone process for edible cottonseed flour production. Oil Mill. Gaz. 78:17 (1973).

Gastrock, E.A., D'Agnin, E.L., Eaves, PH., Cross, D.E. Edible flour from cottonseed by liquid classification using hexane. Cereal Sci. Today 14(1):8 (1969).

Goldstein, S. Sulfhydryl und disulfidgruppen der Kebersiweisse und ihre beziehung zur backfahigkeit der bromtmehde. Mitt. Gabiete Lebensm. Hyg. (Bern) 48:87 (1957).

Graza, R. The subsieve-size fractions of a soft wheat flour produced by air classification. Cereal Chem. 36:465 (1959).

Greene, F.C. On the mechanism of the functionality of surfactant dough conditioners. Baker's Digest 49(3): 16 (1975).

Grogg, B. and Melms, D. A method of analyzing extensigrams of dough. Cereal Chem. 33:310 (1956).

Habeeb, A.F., Cassidy, M.G. and Singer, H. Molecular structural effects produced in proteins by reaction with succinic anhydride. Biochem. Biophys. Acta 29: 587 (1958).

Hale, F., Lyman, C.M. Effect of protein level in the ration on gossypol tolerance in growing fattening pigs. J. Animal Sci. 16:364 (1957).

Harden, M.L. and Yang, S.P. Protein quality and supplementary value of cottonseed flour. J. Food Sci. 40:75 (1975).

Harper, G.A. and Smith, K.J. Status of cottonseed protein. Eco. Bot. 22:63 (1968).

Hayes, T.L., Pease, R.F.W., and McDonald, L.W. Application of the scanning electron microscope to biologic investigations. Lab. Invest. 15(8):1320 (1966).

Hedin, P.A., Thompson, A.C. and Gueldner, R.C. Recent Advances in Phytochemistry:. Vol. 10, Edited by J.W. Wallace and R.L. Mansell, Plenum, New York (1976).

Hlynka, J. Rheological properties of dough and their significance in the bread-making process. Baker's Digest 44(2):40 (1970).

Hopkins, D.T. Central Research Ralston Purina Co., St. Louis, MO. As cited by Martinez, W.H., Berardi, L.C. and Goldblatt, L.A. In: Cottonseed Products composition and functionality. J. Agric. Fd. Chem. 18:161 (1970).

Hopwood, D. Fixatives and Fixation: A review. Histochem. J. 1:333 (1969).

Hoseney, R.C., Finney, K.F., Pomeranz, Y. and Shogren, M.D. Functional (breadmaking) and biochemical properties of wheat flour components. IV. Gluten proteins fractionation by solubilizing in 70% ethyl alcohol and in dilute lactic acid. Cereal Chem. 46:495 (1969).

Hoseney, R.C., Hsu, K.H. and Ling, R.S. Use of diacetyltartaric acid esters of monoglycine in breadmaking. Baker's Digest 50(2):28 (1976).

Hoseney, R.C., Seib, P.A. and Deyoe, C.W. Use of salts of 6-Acyl esters of L-ascorbic and D-isoascorbic acids in breadmaking. Cereal Chem. 54:1062 (1977).

Heubner, F.R. and Wall, J.S. Wheat glutenin subunits. I. Preparative separation by gel-filtration and ion-exchange chromatography. Cereal Chem. 51:228 (1974).

Hyatt, M.A. Principles and Techniques of Electron Microscopy. Vol. 2, p. 44, Van Nostrand Reinhold Co., New York (1970).

Jarquin, R., Bressani, R., Elias, L.G., Tejada, C., Gonzalez, M. and Braham, J.E. Effect of cooking and calcium and iron supplementation on gossypol toxicity in swine. J. Agr. Food Chem. 14:275 (1966).

Jeffers, H.C., Rubenthaler, G.L., Finney, P.L., Anderson, P.D. and Bruinsma, B.L. PEA: a highly functional fortifier in wheat flour blends. Baker's Digest 52(3): 36 (1978).

Jonassen, H.B. Interaction of gossypol with ferrous iron. J. Am. Oil Chem. Soc. 32:424 (1955).

Jones, D.B. and Divine, J.P. The protein nutritional value of soybean, peanut and cottonseed flours and their value as supplements to wheat flour. J. Nutr. 28:41 (1944).

Jones, I.K., Phillips, J.W., Francis, J.R. and Hird, The estimation of rheologically important thiol and disulfide groups in dough. J. Sci. Fd. Agric. 25:1 (1974).

Jones, L.A. Gossypol and some other terpenoids, flavonoids and phenols that affect quality of cottonseed protein. J. Am. Oil Chem. Soc. 56:727 (1979).

Jones, R.W., Babcock, G.E., Taylor, N.W., and Senti, F.R. Molecular weight of wheat gluten fractions. Arch. Biochem. Biophys. 94:483 (1961).

Kadan, R.S., Freeman, D.W., Ziegler, Jr., G.M. and Spadaro, J.J. Air classification of defatted glanded cottonseed flours to produce edible protein products. J. Food Sci. 44:1522 (1979).

Kasarda, D.D., Bernardin, J.E. and Nimmo, C.C. Wheat proteins in "Advances in Cereal Science and Technology", Y. Pomeranz, ed. p. 158, American Association of Cereal Chemists, St. Paul, MN (1976).

Khan, K. and Bushuk, W. Glutenin: structure and functionality in breadmaking. Baker's Digest 52(2):14 (1978).

Khan, K. and Bushuk, W. Studies of glutenin. IX. Subunit composition by sodium dodecyl sulfate-Polyacrylamide gel electrophoresis at pH 7.3 and 8.9. Cereal Chem. 54:588 (1977).

Khan, M.V., Lawhon, J.T., Rooney, L.W. and Cater, C.M. Bread-baking properties of cottonseed protein concentrates prepared from solvent-extracted glandless cottonseed flour. Cereal Chem. 53:388 (1976).

Khoo, U., Christianson, D.D. and Inglett, G.E. Scanning and transmission microscopy of dough and bread. Baker's Digest 49(4):24 (1975).

Kinsella, J.E. Functional properties of proteins in foods: A survey, Critical Reviews in Food Science and Nutrition:219, April (1976).

Knightly, W.H. Monoglyceride performance in bread as a function of chemical composition. Abstr., AACC Annual Meeting (1962).

Krull, L.H. and Wall, J.S. Relationship of amino acid composition and wheat protein properties. Baker's Digest 43(4):30 (1969).

Langhans, R.K., Thalheimer, W.G. Polysorbate 60: Effects in bread. Cereal Chem. 48:283 (1971).

Lawhon, J.T., Rooney, L.W., Cater, C.M., Mattil, K.F. Evaluation of protein concentrate produced from glandless cottonseed flour by a wet extraction process. J. Food Sci. 37:778 (1972).

Lea, C.H., Hannan, R.S. Biochemical and nutritional significance of the reaction between proteins and reducing sugars. Nature (London) 165:438 (1950).

Lindet, L. Compt. rend. 134:908 (1902).

Maleki, M., and Djazayeri, A. Effect of baking and amino acid supplementation on the protein quality of Arabic bread. J. Sci. Fd. Agric. 19:449 (1968).

Margulis, H., and Campagne, Y. Influence des acides et des sels mineraux sen les indices olueographics des farines. Ind. Alunent. Agr. (Paris) 72:485 (1955).

Martinez, W.H., Berardi, L.C. and Goldblatt, L.A. Cottonseed protein products - composition and functionality. J. Agric. Fd. Chem. 18:961 (1970).

Martinez, W.H., Berardi, L.C., Pfeifer, V.F. and Crovetto, A.J. The production of protein rich concentrates by air classification of defatted cottonseed flour. J. Amer. Oil Chem. Soc. 44:139A (1967).

Martinez, W.H. "Conference on Protein Rich Food. Products from Oilseed. New Orleans, U.S. Dept. Agr. ARS 72-7, p. 33 (1969).

Martinez, W.H. "Proc. of Seventeenth Cottonseed Processing Clinic, New Orleans, 1968", U.S. Dept. Agr. ARS 72-69, p. 18 (1969).

Martinez, W.H. Southern Utilization Research and Development Division, ARS, USDA, New Orleans, LA, unpublished data (1969).

Mathason, I.J. pH and T.T.A. determination for fermentation control. Baker's Digest 52(3):10 (1978).

Matthews, R.H., Elinora, J., Sharpe, and Clark, W.M. The use of some oilseed flours in bread. Cereal Chem. 47: 121 (1970).

Mauritzen, C.A.M. and Stewart, P. Disulphide-sulphhydryl exchange in dough. Nature 197:48 (1963).

Mayorga, H., Gonzalez, J., Menchu, J.F. and Rolz, C. Preparation of a low gossypol cottonseed flour by dry and continuous processing. J. Food Sci. 40:1270 (1975).

McConnell, L.M., Simmonds, D.H., and Bushuk, W. High protein bread from wheat-faba bean composite flours. Cereal Sci. Today 19:517 (1974).

McDonald, L.W., Pease, R.F.W., and Hayes, T.L. Scanning electron microscopy of sectioned tissues. Lab. Invest. 16(4):532 (1967).

McMichael, S.C. Hopi cotton, a source of cottonseed free of gossypol pigments. Agron. J. 51:630 (1959).

Mecham, D.K. The sulfhydryl and disulfide contents of wheat flour: dough and proteins. Baker's Digest 42(1):26 (1968).

Meinke, W.W. and Reiser, R.R. Air classification and chlorine treatment process for the production of low gossypol content cottonseed flours. (1962). In "Proceeding of a Conference on Cottonseed Protein for Animal and Man", New Orleans, 1960. U.S. Agric. Res. Serv. ARS 72-24:60.

Meredith, P. and Bushuk, W. The effect of lactate, N-ethylmaleimide and oxygen on the mixing tolerance of doughs. Cereal Chem. 39:411 (1962).

Milic, B., Stojanovic, S., Vucurevic, N. and Turcic, M. Chlorogenic acid guinic acids in sunflower meal. J. Sci. Fd. Agric. 19:108 (1968).

Miller, B.S. and Johnson, J.A. High levels of alpha amylase in baking. II. Proteolysis in straight and sponge doughs. Baker's Dig. 21(4):111 (1947).

Miravalle, R.J. Conference on Protein-Rich Food Products from Oilseeds, New Orleans, 1968", U.S. Dept. Agr. ARS 72-71 p. 76, 1969.

Moore, A.T. and Rollins, M.L. New information on the morphology of the pigment gland of cottonseed. J. Amer. Oil Chem. Soc. 38:150 (1961).

Muszynska, G., and Feifer, I. The arginase inhibitor from sunflower seeds: Purification and inhibitory properties. Acta. Biochem. Pol. 17 (1970).

Neukom, H., Geissmann, T., and Painter, T.J. New aspects of the function and properties of the soluble wheat flour pentosans. Baker's Digest 41(5):52 (1967).

Newbold, M.W. Crumb softeners and dough conditioners. Baker's Digest 50(4):37 (1976).

Neilsen, H.C., Babcock, G.E. and Senti, F.R. Molecular weight studies on glutenin before and after disulfide-bond splitting. Arch. Biochem. Biophys. 96:252 (1962).

Oflett, C.W., Mehltretter, C.L., MacMasters, M.M., Otey, F.H. and Senti, F. Effect on crumb firmness. II. Action of additives in relation to their chemical structures. Cereal Chem. 35:142 (1958).

Olsen, R.L. Evaluation of LCP cottonseed flour. Oil Mill Gaz. 66:7 (1973).

Orth, R.A. and Bushuk, W. A comparative study of proteins of wheats of diverse baking qualities. Cereal Chem. 49:268 (1972).

Orth, R.A., Dronzek, B.L. and Bushuk, W. Studies of glutenin. IV. Microscopic structure and its relations to breadmaking quality. Cereal Chem. 50:688 (1973).

Osborne, T.B. and Mendel, L.B. The nutritive value of proteins. J. Biol. Chem. 37:223 (1919).

Pease, R.F. Scanning electron microscopy. Lab Management 6:13 (1968).

Pence, J.W., and Olcott, H.S. Effect of reducing agents on gluten proteins. Cereal Chem. 29:292 (1952).

Pomeranz, Y., Finney, K.F. and Hoseney, R.C. Molecular approach to breadmaking. Science 167:944 (1970).

Pomeranz, Y. Molecular approach to breadmaking: An Update and New Perspectives. Baker's Digest 54(1):20 (1980).

Pomeranz, Y. Scanning electron microscopy in food science and technology. Adv. Food Res. 22:205 (1976).

Pomeranz, Y. J. Sci. Fd. Agric. 16:586 (1965).

Pomeranz, Y., Shogren, M.D., Finney, K.F. et al. Fiber in breadmaking effects on functional properties. Cereal Chem. 54:25 (1977).

Pomeranz, Y. Wheat Chemistry and Technology. American Association of Cereal Chemistry. St. Paul, MN. p. 543 (1978).

Pringle, W., Williams, A. and Hulse, J.H. Mechanically developed doughs from composite flours. Cereal Sci. Today 14:114 (1969).

Pyler, E. Protein supplementation. Baker's Digest 53(1): 6(1979).

Richardson, A.E., Texas Univ. Bulletin No. 1727, pp. 4-20 (1917).

Ridlehuber, J.M. and Gardner, Jr., H.K. Production of food-grade cottonseed protein by the liquid cyclone process. J. Am. Oil Chem. Soc. 51:153 (1974).

Robin, R.S. and Klein, R.M. Chlorogenic acid as a competitive inhibitor of indolactic acid oxidase. Arch. Biochem. Biophys. 70:11 (1957).

Rooney, L.W., Gustafson, C.B., Clark, S.P., Cater, C.M. Comparison of the baking properties of several oilseed flours. J. Food Sci. 37:14 (1972).

Sabir, M.A., Sosulski, F.W. and Finlayson, A.J. Chlorogenic acid-protein interactions in sunflower. J. Agric. Fd. Chem. 22:575 (1974).

Schaller, R.E. and Lapple, C.E. Particle size classification of plastic powders. Presented at 162nd ACS Meeting. Washington, D.C. Sept. (1971).

Schnickels, R.A., Warmbier, H.C., Labuza, T.P. Effects of protein substitution on nonenzymatic browning in an intermediate moisture food system. J. Agric. Fd. Chem. 24:901 (1976).

Seckinger, H.L., Wall, M.J. Lipid distribution in the protein matrix of wheat endosperm as observed by electron microscopy. Cereal Chem. 44:669 (1967).

Shakir, S.R., Sedky, A. and Mohammad, M.S. Studies on Egyptian bread. II. Chemical composition of some types of bread consumed in various localities of Egypt. Alexandria J. Agr. Res. 3:89 (1960).

Sharma, M.P., Smith, F.H., Clawson, A.J. Effects of levels of protein and gossypol, and length of feeding period on the accumulation of gossypol in tissue of swine. J. Nutr. 88:434 (1966).

Shehata, N.A. and Fryer, B.A. Effect of protein quality of supplementing wheat flour with chickpea flour. Cereal Chem. 47:663 (1970).

Shorgon, M.D., Finney, K.F. and Hoseney, R.C. Functional (breadmaking) and biochemical properties of wheat flour components. 1. Solubilizing gluten and flour protein. Cereal Chem. 46:93 (1969).

Sidwell, V.D. and Hammerle, O.A. Changes in physical and sensory characteristics of doughs and of bread containing various amounts of fish protein concentrate and lysine. Cereal Chem. 47:739 (1970).

Simmonds, D.H. The ultrastructure of the mature wheat endosperm. Cereal Chem. 49:212 (1972).

Smith, F.H. and Clawson, A.J. The effects of dietary gossypol on animals. J. Am. Oil Chem. Soc 47:443 (1970).

Smith, K.J. Practical significance of gossypol in feed formulation. J. Am. Oil Chem. Soc. 47:448 (1970).

Sosulski, F.W. and Bakal, A. Isolated proteins from rapeseed, flax and sunflower meals. Can. Inst. Food Technol. 2:28 (1969).

Sosulski, F. Organoleptic and nutritional effects of phenolic compounds on oilseed protein products. A Review. J. Amer. Oil Chem. Soc. 56:711 (1979).

Squibb, R.L., Wyld, M.K., Scrimshaw, N.S. and Bressani, R. All vegetable protein mixtures for human feeding. I. Use of rats and baby chicks for evaluating corn based vegetable mixtures. J. Nutr. 69:343 (1959).

Sullivan, B. Proteins in flour. Review of the physical characteristics of gluten and reactive groups involved in change in oxidation. J. Agr. Fd. Chem. 2(24)1231 (1954).

Tanaka, K., Furuhawa, K., Matsumoto, H. The effect of acid and salt on the farinograms and extensigrams of dough. Cereal Chem. 44:675 (1967).

Taylor, N.W. and Cluskey, J.E. Wheat gluten and its glutenin component: viscosity, diffusion and sedimentation studies. Arch. Biochem. Biophys. 97:399 (1962).

Terrell, R.N., Brown, J.A. and Carpenter, Z.L. Effects of oilseed proteins at two replacement levels on chemical, sensory and physical properties of frankfurters. J. Food Sci. 44:865 (1979).

Tenney, R.J. Dough Conditioners/Bread Softeners. The surfactants used in breadmaking. The Baker's Digest 52(4):24 (1978).

Tkachuk, R. and Hlynka, I. Some properties of dough and gluten in D_2O. Cereal Chem. 45:80 (1968).

Tsen, C.C. and Bushuk, W. Reactive and total sulfhydryl and disulfide contents of mixing properties. Cereal Chem. 45:58 (1968).

Tsen, C.C., Hoover, W.J. and Phillips, D. High protein breads: Use of sodium stearoyl-2-lactylate and calcium stearoyl-2-l ocylate in their production. Baker's Digest 45(2):20 (1971).

VanSumere, C.F., Albrecht, J., Dedonder, A., dePooter, H. and Pe, I. In "The Chemistry and Biochemistry of Plant Proteins". Edited by J.B. Harborne and C.F. VanSumere, p. 211, Academic Press, London (1975).

Varriano-Marston, E. A comparison of dough preparation procedure for scanning electron microscopy. Food Tech. 31(10):32 (1977).

Vix, H.L.E., Gardner, H.K., Jr., Lambou, M.G. and Rollins, M.L. Ultrastructure related to cottonseed and peanut processing and products. In "Seed Proteins", Ed. Inglett, G.G., p. 212. AVI Publishing Co., Westport, CT (1972).

Volpe, T.A. Physical and Rheological Characteristics of a Substituted Dough System Using a Yeast Protein Isolate. Ph.D. Dissertation. Michigan State University (1976).

Wall, J.S., Proc. of Conference on Cottonseed Protein Concentrates. New Orleans, 1964; U.S. Dept. Agr. ARS 72-38, p. 43 (1965).

Wall, J.S. and Beckwith, A.C. Relationship between structure and rheological properties of gluten proteins. Cereal Sci. Today 14:16 (1969).

Wall, J.S. Disulfide Bonds: determination, location and influence on molecular properties of proteins. J. Agr. Fd. Chem. 19:619 (1971).

Wall, J.S. Cereal proteins. In: "Proteins and their Reactions", pp. 315-341, Connecticut (1964).

Wehrli, H.P. and Pomeranz, Y. The role of chemical bonds in dough. Baker's Digest 43(6):22 (1969).

Wolf, W.J. Lipoxygenase and flavor of soybean protein products. J. Agr. Fd. Chem. 23:136 (1975).

Wolman, M. 1955. Problems of fixation in cytology, histology and histochemistry. In "International Review of Catology" Vol. IV, ed. Bourne, B.H. and Danielle, J.F. p. 79. Academic Press, Inc., New York.

Woychik, J.H., Huebner, F.R. and Dimler, R.J. Reduction and starch-gel electrophoresis of wheat gliadin and glutenin. Arch. Biochem. Biophys. 105:151 (1964).

Yatsu, L.Y. The ultrastructure of cyloledonary tissue from gossypium hirsutum L. seeds. J. Cell Biology 25:193 (1965).

Ziemba, J.V. Cottonseed proteins enter new era. Food Engr. 44(6):70 (1972).