

*THE RELATIONSHIP OF CHLORINATION AND  
PROTEIN AND LIPID CONTENT TO  
BAKING QUALITY OF SOFT WHEAT FLOUR*

by

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Dissertation submitted to the Faculty of the  
Virginia Polytechnic Institute and State University  
in partial fulfillment of the requirements for the degree  
of  
DOCTOR OF PHILOSOPHY  
in  
Human Nutrition and Foods

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**(ABSTRACT)**

*The purpose of this research was to examine the relationship of the protein and lipid content of soft wheat flour to baking quality and their changes as a result of chlorination. Coker 916, soft red winter wheat, was grown under different fertilization treatments involving rate and application time to alter protein content. One-half of all flour treatments were subjected to chlorination.*

*The lipid and protein content, gliadin and glutenin composition, microstructure, rheological properties, ash, pH and color were examined. Baked angel food cakes were evaluated by volume, tenderness, color, cohesiveness, crumb structure and microstructure (SEM). Gelatinization temperatures of batters were measured by Differential Scanning Calorimetry (DSC). Sensory analysis was judged by quantitative descriptive analysis (QDA).*

*Increased levels of fertilization of the grain increased the protein content (8-11%) of the flour. Cakes from flours of increased protein content were significantly lower in volume than cakes of flours of lower protein content. Chlorination decreased protein content in flour which affected the*

*glutenin composition in some flours. Gliadin content remained unchanged. Doughs of chlorinated flours were significantly less stable as measured by mixing time stability and mixing time index.*

*Chlorination decreased the unsaturated fatty acid methyl esters (FAME). There was strong correlation between evenness of cell structure and the decrease of fatty acids 18:1, 18:2, and 18:3 with chlorination. Phospholipids which were qualitatively evaluated were implicated with cell evenness.*

*Cakes of chlorinated flours were significantly less in volume and tenderness, but the structure showed complete starch gelatinization, increased whiteness and cell evenness. Sensory data correlated significantly with objective data.*

*Chlorination of flour stabilized the structure of the baked product. Low protein and decreased FAME content improved the baking properties of the flour. Flours of lower protein and a lower lipid content that are chlorinated are recommended for baked products such as cakes and biscuits.*

## ACKNOWLEDGEMENTS

I would like to thank my major professor, Dr. Janet Johnson, for her guidance, patience and encouragement that she has shown me over the years. I want to thank the members of the advisory committee: Dr. Marilyn Schnepf and Dr. William Barbeau for their friendship, inspiration and guidance that they have offered both in and out of the classroom; Prof. Rodney Young for his suggestions and guidance; and Dr. Merle Pierson, for his guidance and suggestions during this course of study.

Special thanks to \_\_\_\_\_ who was always there to lend a hand, to listen to or to help me through any of the many dilemmas that befall a graduate student. Another special thanks to \_\_\_\_\_ for technical support during the study. A special remembrance to \_\_\_\_\_ whose technical support was deeply appreciated during the study.

Also, a special thanks to Dr. Anne Hertzler whose encouragement and friendship helped me through the rough spot after returning to school.

I want to thank the entire sensory panel for their participation in the study and helping to make it a success. The HNF office staff also must be recognized in their assistance for helping in the study and their encouragement.

I would like to recognize \_\_\_\_\_ of Minnel Milling Corporation, Fostoria, Ohio who chlorinated the flour for the study.

Special thanks goes to Dr. Marvin Lentner for assistance with the experimental design and statistical analysis.

Finally, I want to thank my family for their support during these student years.

Also, with deepest appreciation to \_\_\_\_\_ who put this manuscript into print.

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**GLOSSARY FOR FLOUR TREATMENTS**

TREATMENT 3 = 50 + 0

TREATMENT 6 = 0 + 50

TREATMENT 4 = 0 + 100

TREATMENT 7 = 100 + 0

TREATMENT 13 = 50 + 100

TREATMENT 15 = 100 + 50

## *CHAPTER I*

### *INTRODUCTION AND OBJECTIVES*

Wheat has been the staple cereal grain for centuries. Many different varieties of wheat are grown in the United States. New varieties released by breeding programs are at least as good as existing ones. In the U.S., varieties are developed by federal, state and private breeders who use novel methods to improve varieties and speed their release. Some varieties may differ in appearance or performance from that which is typical of their class, and some have more than one genetically distinct biotype (Bietz, 1979).

In recent years, increased demand for cereals promoted more intensive cultivation methods. Sophisticated agronomic practice, including extensive use of nitrogenous fertilizers increased the yield of the grain.

Nitrogen fertilization affects yield and protein content of wheat grain, and the baking qualities of wheat flour. The rate and timing of nitrogen fertilizer applications are crucial factors that determine yield and protein content of wheat. The judicious use of nitrogen fertilizers may improve both the yield and the baking quality, the latter mainly due

to increased grain/flour protein level. In many cases baking quality improves with increased flour protein content but baking quality may not increase in proportion to protein content.

The basic definition of wheat quality varies from one class of wheat to another and depends on the grain suitability for a given product. For example, the quality of soft winter or white wheat variety is defined in terms of suitability for soft wheat milling and production of cakes, cookies and crackers. The quality of any wheat cannot be expressed in a single term because quality depends on several functional properties (milling, baking, processing and physical dough), each important in the production of bread, pastry, or macaroni products.

Chlorination has been used to improve the baking properties of soft wheat flours. Chlorine has two specific functions: (1) improve the color of the flour; and (2) improve the baking quality as to symmetry, crumb size and strength of the crumb thus preventing collapse of the baked product.

Wheat flour which constitutes the framework of a baked product is made up of three components: proteins, lipids and starch. During the last twenty-five years intensive interest and research was conducted concerning the function of these

components and the effect they have on a baked product.

Chlorination has an altering effect on the proteins and lipids in the flour thus affecting the outcome of the baked product.

The objectives of this study were:

1. To study the effects of various levels of nitrogen fertilization on the protein and baking quality of soft red winter wheat (Coker 916) flour.
2. To study the effect of chlorination on protein and lipids and their subsequent effect on the baking quality of soft red winter wheat flour.
3. To evaluate the physical and sensory properties of flour and the effect of chlorination on these properties.

## CHAPTER II

### REVIEW OF LITERATURE

#### 2.01 *Soft and Hard Wheats in General*

Soft wheats, both red and white, are grown in the upper part of the U.S. from the Atlantic to the Pacific coasts. These areas produce the flour used for almost all baked products except yeast-raised bread and rolls. However, not all soft wheats are identical (Minor, 1984). Most soft wheats grown for domestic processing have similar inherent grain and flour properties. The principal bases for wheat selection by a miller are its protein content, test weight, and moisture. Grains with a low protein content are usually sought for their baking ability in cakes and certain pastries. Predominant varieties may differ according to area of growth. Thus soft white wheat accounts for much of the production in Michigan, New York, and the Pacific Northwest, whereas other soft wheat states grow red wheats (Minor, 1984). Between 1979-1982 soft red winter wheat production increased more than 90% in the southeastern U.S.

Finney (1987) stated that a comprehensive soft wheat

flour evaluation program should include the following tests:

1. Dockage: this is defined as being all the material that can be readily removed from the wheat by prescribed mechanical means;
2. Test weight: this is a rough measure of the density of the wheat;
3. Moisture: this factor is important when considering the keeping quality of wheat. Usually the accepted value is 14 percent;
4. Protein: the content in wheat varies from about 6% to up about 20%, depending in part on variety and class but more largely on environmental factors during growth (rainfall and available soil nitrogen);
5. Ash: this is reported on a 14% moisture basis. This criteria is used as an indicator of the procedure degree of milling, but it is also a rough measure of flour color;
6. AWRC (alkaline water retention capacity): the lower the gain in weight of the flour by water the better the flour quality for pastry needs;
7. Alpha-amylase: the Cibacron dye method measures the alpha-amylase activity in cereals and expresses it in dextrizing units per gram (DU/g). In this test a higher value means higher enzyme activity and poorer flour quality;

8. Starch damage: work in this area indicates that starch damage plays an important role in governing the performance of bread flours. Further, it has been established that under a fixed set of milling conditions the level of starch damage is related to the kernel hardness or vitreousness of the parent wheat;
9. Friability: particle size in a finished, conventionally milled flour can be interpreted as a measurement of the friability of the wheat endosperm under the conditions of milling. Some mellow wheats tend to "shell out" their endosperm readily, whereas the friability of the more vitreous wheats requires larger work input; and
10. Appropriate baking or other end product tests.

The difference between hard wheat and soft wheat is under genetic control. A good test to determine hard and soft wheat is by grinding the kernels under set conditions and determining the particle size of the resultant meal (Hoseney, 1981). Both protein quantity and quality are considered to be primary factors in measuring the potential of a flour in relation to its end-use (Pratt, 1971). The average protein in the hard winter wheat crop needs to be above 12% to provide millers with sufficient baking-quality for the wholesale

baking industry. Bakers also correlate increased ash content with a deterioration in baking quality, leading to a darker and coarser interior bread characteristics (Jackel, 1987).

The differences between hard wheat and soft wheat are due to the protein and starch components. Three theories are proposed for the source of the differences:

- a. variation in the ratio of protein to starch,
- b. starch and protein components are intrinsically higher in hard wheats; and
- c. binding forces between the starch and protein differ (Hoseney and Seib, 1973).

A soft wheat variety grown under conditions to produce higher than normal protein content will still be relatively soft (Hoseney and Seib, 1973). Differences in hardness were found to involve the continuity of the protein matrix and the strength with which it physically entrapped starch granules.

The primary determinant of wheat hardness is genetically controlled and appears to relate to factors influencing the degree of compactness of endosperm cell components. Environment and protein content are also of significance in determining the extent to which an ordered structure forms (Stenvert and Kingswood, 1977). There is a possibility for wheats to be softer at higher protein content or to be harder at higher protein content, depending on whether or not the

environmental conditions are conducive to the laying down of an ordered protein matrix. Under the same environmental conditions, however, hardness should increase with increasing protein content until the threshold level for the laying down of a continuous matrix is reached (Simmonds et al., 1973). Wheat hardness appears to be determined by the physical structure of the endosperm-protein matrix.

Environmental conditions can modify the manner in which the available protein is arranged, and if there is insufficient protein to form a continuous protein matrix under prevailing environmental and genetic influences, a softer grain structure results (Simmonds, 1973). The exact description of the molecular basis for the regulation of this grain property has proved elusive. Experiments have been carried out and it appears that protein plays an important role in conferring endosperm softness on wheats. The mechanism by which the protein causes this effect is not yet known (Anon., 1986).

Four methods have been given for evaluating the hardness of wheat:

1. work involved to grind;
2. time to grind;
3. particle size resulting from grind; and
4. near infrared (N/R) reflectance of ground wheat

(Miller et al., 1982).

The greatest differentiation between soft and hard red winter wheat is obtained with the Brabender automatic micro-hardness tester (Miller et al., 1982). Wheat hardness measurements on pearled wheat showed that the bran had some effect on the magnitude of the hardness index, but it essentially had no effect on the ranking of the cultivars within a group covering soft, hard, red spring and durum classes. The measurement of hardness on debranned wheat did not show any significant correlation with protein content (Obuchowski and Bushuk, 1980).

Starch is a major component of wheat flour which comprises 65-70% of flour below 80% extraction at a 14% moisture level. It occurs in discrete granules whose size and shape depend upon the source (Shelton and D'Appolina, 1985; Hosney et al., 1983). Functions of starch were given as (Shelton and D'Appolina, 1985):

1. dilutes the gluten to an appropriate consistency;
2. furnishes maltose by amylase action for fermentation;
3. provides a surface for strong gluten bonding;
4. provides flexibility for expansion during partial gelatinization during baking;
5. sets the loaf structure by providing a rapid network

to prevent loaf collapse upon cooling.

Starch properties are altered by the use of bleaching agents. Evidence suggests that modification of the starch by chlorine is responsible for the improved baking performance of chlorine treated flours. The modification appears to alter the starch's pasting properties and thus allows the use of more sugar and shortening in the formula (Hoseney et al., 1983).

Cake flour is commonly treated with chlorine gas to enhance its cake-baking properties. Cakes baked with chlorine treated flour have higher volume, finer grain and more tender texture than do cakes baked using untreated flours. Chlorinating flour increases the oil binding and water binding capacity of starch (Chung and Pomeranz, 1981).

## *2.02 The Effect of Fertilization on Wheat*

The high yielding soft winter wheats grown in the United States generally have a lower protein content, which is desirable for flours for pastries. Over-fertilization of the soft winter wheats with nitrogen may increase grain protein above 10% resulting in reduced milling performance and baking quality of the flour. Common nitrogen sources that are used for fertilization are: (1) anhydrous  $\text{NH}_3$ ; (2) urea ammonium

nitrate solution (UAN); (3) urea; (4) ammonium nitrate and (5) ammonium sulfate (Halvorson and co-workers, 1987).

The effect of timing of fertilization on winter wheat was recognized by Doll (1962). The study found spring applications better than fall applications for yields of wheat. The yields obtained with split applications (half of the nitrogen applied in the fall and half in the spring) tended to be higher than those obtained when all the nitrogen was applied in the fall, but lower than those when all nitrogen was applied in the spring. Hucklesby (1971) also found that late spring application of nitrogen showed an increase in percent grain protein. The reason for the increase in percent grain protein observed was the availability of soil nitrogen during April and May. Therefore, two factors important are both date of application and the amount of nitrogen applied.

Roth (1987) found that split applications of nitrogen fertilizer had the potential to increase yields in the northeastern United States but only under certain conditions. It was suggested that intensive management systems for winter wheat in the eastern United States need to be flexible so that the nitrogen timing and fungicide applications are based on the characteristics of each wheat crop and environment. Terman (1979) concluded that various environmental factors greatly affect grain yields and protein

concentrations. These factors included: nitrogen and moisture supply, light, temperature and other growth factors.

Smika and Greb (1973) studied the effects of soil and temperature on wheat growth and protein content. The investigators concluded that maximum air temperature above 32 degrees C during the last 2 to 3 weeks before maturity was detrimental to wheat grain protein content. Wheat was also found to use water from deeper soil depths only when water nearer the soil surface was depleted. If this occurred the deeper water was available for increase grain yield, but limited plant uptake of nitrogen.

Harper and co-workers (1987) investigated nitrogen surplus and deficit of nitrogen absorption/desorption in the soil and atmosphere by translocation within the plant. By use of isotope analysis the observation made was that about a third of the amount of nitrogen in the grain was derived from fertilizer nitrogen. The results emphasized the importance of the spring fertilizer top dressing for winter wheat.

The influence of water on wheat and fertilization is another factor studied. The influence of winter rainfall on nitrogen fertilization of winter wheat was recognized by Doll (1962). Doll's research observed the effect of winter rainfall on nitrogen leaching was an important factor in wheat yields. When winter rainfall was over 10-12 inches, fall nitrogen applications were less effective than spring applications.

Terman and associates (1969) studied the amount of protein in the wheat as it was affected by nitrogen and water. The researchers found that the soil content of nitrogen markedly influenced protein relationships. At low available soil nitrogen levels, protein contents were consistently low and response to applied nitrogen high. The effect of moisture on grain protein content was unclear in the study.

Eck (1988) recognized that the relationship between water and fertilizer needs was not well defined. Grain protein concentration increased with increasing increments of applied nitrogen with all treatments. Differences in grain protein between water treatments tended to be greatest at intermediate nitrogen levels, where, with adequate water, most of the applied nitrogen was used in increasing yields without substantially increasing grain protein. It was concluded that stress from irrigation should be prevented during tillering and during heading and grain filling. Stress during tillering and jointing limits yield potential that is not regained when stress is relieved. Therefore, there is still potential for maximum yields if precipitation occurs during heading and grain filling, while if plants are stressed earlier, the yield potential is lost.

Protein content has been studied as a post-harvest indicator of sufficiency of nitrogen for maximum grain yield. Gross and co-workers (1982) studied the relationship between

grain protein and wheat yield. The researchers found there was no apparent correlation that existed between relative yield and protein content among the nitrogen deficient observations. Also regression analysis between yield and grain protein for the nitrogen deficient point were non-significant. According to the author this implied that wheat grain protein content cannot be used as a predictor of yield loss due to nitrogen deficiency at a given protein content.

Walsh and co-workers (1975) studied the effect of nitrate fertilization on flour amino acid and composition and the way it can be used as a predictor for bread baking quality. The researchers found increased amounts of nitrogen fertilizer increased protein content. The increased flour protein content was highly correlated with glutenin, gliadin, albumin, and total gluten levels of the flour. These same three constituents were also correlated with baking absorption and bread loaf volume.

The sulfur/nitrogen balance of the wheat affects grain quality and consequent flour quality. The plant, in its synthesis of protein, derives the required elements from the carbon dioxide of the air and from the water, nitrogen compound and mineral substances from the soil. Since the supply of carbon dioxide is for all practical purposes limitless, it is the soil constituents that form the chief

limiting factors in protein production (Pyler, 1973).

The limiting factor in the production of protein appears to be in the amount of available nitrogen in the soil at different stages of development of the crop in relation to soil moisture, mineral nutrients in the soil, and environmental factors (total rainfall, the seasonal distribution of rainfall, and the temperature). High nitrogen soils characterize in general the hard wheat areas and account for the high protein grains, whereas the soils in soft wheat areas are usually lower in the amount of nitrogen compounds they contain and thus produce grain low in protein content. Rainfall, both in its amount and distribution, exerts a considerable effect upon the protein content of wheat. Generally, wheats originating in low rainfall areas tend toward higher protein contents because of the greater nitrogen supply in the soil (Pyler, 1973).

Sulfur deficiency limits yields of wheat grain and limits the response to nitrogen fertilizer aimed at increasing yields. The grain produced by sulfur deficient plants has a low sulfur content and, therefore, an increase in N/S ratio.

Grain hardness is increased in sulfur deficient wheat. Flours from such wheat yield excessively tough doughs. There is an increase in resistance to extension and lower extensibility (Schofield, 1985).

Experimentation has been conducted to determine lipid

composition change associated with sulfur deficiency in wheat. No significant relationship was found between lipid composition and nitrogen content (MacRitchie, 1986). The findings tended to discount the possibility that a relationship between sulfur and lipid contents might have been obscured by an overriding effect caused by nitrogen fertilizer (MacRitchie, 1986).

After a century of extensive rather than intensive efforts in wheat production in North America, researchers and growers have begun to implement improved management to achieve higher yields. Fertilizer management is an important part of the overall management package aimed toward higher yields. As nutrient rates increase to meet higher yield goals, the importance of a complete management package becomes even more pronounced (Halvorson and co-workers, 1987).

### *2.03 The Role of Protein in Flour and Baking*

Protein content of wheat is one of the principal quality parameters. The protein content is influenced by environmental conditions during growth; also by genotype and interaction of genotype with growth environment (Donovan et al., 1977). The maximum weight of nitrogen (N) per kernel was found to be identical for each wheat crop, whereas maximum kernel dry weights were different. The outcome meant that the

differences in weight per kernel are entirely responsible for difference in percent nitrogen. The total nitrogen of wheat can be divided into protein and non-protein portions (Donovan et al., 1977).

Relative differences in the functionality of the proteins are due to differences in fundamental properties:

1. different proteins that comprise the constituent of the flour;
2. differences in the relative proportions of specific proteins;
3. differences in the way proteins interact with each other, with other flour constituents and with added ingredients (Bushuk, 1985).

Two factors contribute to functionality of protein: protein content and protein quality. Protein content may be determined precisely but quality is extremely complex and very difficult to measure. Protein content depends on soil nitrogen, soil moisture, and temperature during the growing season. The quality is a genotype trait in which each variety inherits the quality of its protein from its parents. Some factors which may affect it are abnormal environmental conditions, such as high temperatures during grain filling period or a wet harvest condition, and improper post-harvest conditions (Bushuk, 1985).

Early work demonstrated there was a simple correlation between some bread-making quality parameters and the proportion of each protein fraction or combination of fractions. This investigation showed that the proportions of both glutenin and residue protein had a direct effect on baking performance (Orth and Bushuk, 1972). Gliadin and glutenin are synthesized in the development of the endosperm of the wheat grain where they are deposited in the protein storage bodies. Glutenin and gliadin have similar amino acid compositions: they are rich in proline, glutamate and amide nitrogen, but are poor, though, in charged residues, particularly the basic amino acids (Schofield, 1985). Glutenin is a mixture of high molecular weight proteins which are made up of polypeptide chains with the links between the chain as disulfide bonds. Two of the half cysteine residues combine with their counterparts on one chain only, while the other two form bonds with a different chain. During mixing the long linear polypeptide chains are extended so that they interlace the dough in all directions. Extension of the individual polypeptide chains that make up the orientated long linear polypeptide chain continues with mixing. Excessive mixing produces weak doughs by drastically reducing the degree of polymerization of glutenin and destroys many of its secondary cross-linkages (Pyler, 1983 (a)).

In order to determine the functionality of glutenin, the

disulfide linkages that associate cystine residues are either inter-chain or intra-chain. Intra-chain disulfide linkages impart a globular tightly folded conformation to individual polypeptide chains. Low molecular proteins are the result. The inter-chain favor the formation of highly branched polymers. Therefore, an optimum balance of inter-and intra-chain disulfide bonds is required for glutenin to have good functionality (Pyler,1983 (b)).

Gliadin interferes with glutenin polymers to form very extended cooperative networks associated through a large number of covalent bonds. There must be an equal balance of gliadin and glutenin to allow for expansion of gas bubbles, especially during the fermentation process in bread making. A decrease in gliadin will cause an undersize loaf of bread while an increase will cause the rupturing of gas cell walls and the collapse of the product (Schofield, 1985).

Gluten protein, a hydrated gliadin-glutenin complex, is usually responsible for the variation in baking quality. A study that was conducted (MacRitchie, 1980) demonstrated that gluten proteins manifest its variations from one flour to another by interacting with the lipid component. The question whether loaf volume can be attributed to the whole gluten protein or to a specific protein fraction remains unanswered. A highly significant correlation was found between gluten protein amide content and loaf volume index for thirty seven

flours covering a wide range of baking quality (MacRitchie, 1979). The percent of glutamine and asparagine in gluten protein varied between 31.2 to 33.7 for the flours examined. Since there was a good correlation between loaf volume index and solubility of gluten protein as well as side chain amide, the author indicated that the degree of amidation was important and warranted further investigation (MacRitchie, 1979).

Proteins of the flour play an important role in baking, especially in establishing structure for the baked product. Extensive work has been conducted with bread and the relation of protein to loaf volume. It is well established, even from early studies, that mixing plays a role in the development of the structure (Tsen, 1967). When the gliadin and glutenin proteins are hydrated separately, gliadin is the more extensible and tacky and glutenin the more elastic and tough. Hydrated together, they form a cohesive, elastic three dimensional gluten network (Campbell, 1972). Cohesiveness and elasticity are such that bubbles of gas are able to expand without an undue amount of coalescence or escape to the atmosphere. Gluten proteins take up twice their weight of water during hydration. The disaggregation occurring during the early stages of mixing probably is largely physical, resulting from tearing and shearing forces. With hydration and disaggregation, followed by protein-protein interaction,

a continuous gluten matrix forms (Campbell, 1972).

#### *2.04 The Role of Lipid in Flour*

Wheat flour lipid, although only constituting about 2% by weight of flour, makes important contributions to dough properties, baking behavior and bread staling (MacRitchie, 1981). The variation in lipid content produces the greatest changes, on a weight basis, in characteristics such as loaf volume and texture.

The lipids of wheat flour form 1-2% of the endosperm, 8-15% germ and about 6% of the bran, with an average value of 2-4% of the whole grain. Flour of 70% extraction contains about 2% of the lipid, but research has suggested that lipids participate in processes which are of great importance in baking:

1. oxygen uptake of doughs;
2. oxidative improvement;
3. aging of the flour;
4. crumb softening;
5. staling phenomena;
6. nutritional: consists of essential fatty acids and vitamin E (Fisher et al., 1964).

Flour lipids may be divided into those inside starch

granules which are true starch lipids, and all lipids outside the starch granule which, for want of a better term, are called non-starch lipids (Morrison, 1978). Flour contains all the endosperm lipids plus a small proportion of germ (and perhaps aleurone) lipids transferred to the flour during milling (Morrison, 1981).

Triacylglycerides compose over 80% of non-polar lipids; digalactosylmonoglycerides over 50% of glycolipids and lysophosphatidyl choline 48% of phospholipid (Chung and Pomeranz, 1981). According to these authors, red wheats contain more lipids than white wheats, but white wheats contain generally more tocopherols than red wheats (spring > winter). White wheat lipids in general were found to be poorer in oleic acid but richer in linoleic acid than red wheat lipids (Chung and Pomeranz, 1981).

Research has shown the effects lipids have on baking. Evidence points to a classification of flour lipids into three groups according to their effects in baking (MacRitchie, 1977). The polar galactolipids and phospholipids increase loaf volume. Compounds of intermediary polarity (free fatty acids, monoacylglycerides, and monogalatosyl diacylglycerides) are strongly surface active, but in certain cases they may act as an anti-foaming agent, and therefore, decrease volume. The non-polar compounds, such as triacylglycerides, have little affect in the absence of other lipids, but have beneficial

effects while other lipids are present.

X ray and electron microscope studies of the structure of wheat proteins were the basis for a lipoprotein model for wheat gluten. A lipoprotein model was postulated which occupies 2 to 5% of the elastic gluten structure. In this model, oriented bimolecular leaflets of phospholipid in the lipoprotein are present in the protein sheets. The phospholipid layers provide a plane along which slippage can occur, thus permitting plastic deformation of dough or gluten. The proposed model was considered to provide for sufficient plasticity to give optimum baking characteristics (Mechan, 1971; Campbell, 1972).

Long chain fatty acids are the major constituents of oil in grain crops. The effect of environment on the fatty acid composition of numerous seed crops has been recognized. Temperature has been shown to be a major factor responsible, and the relationship of temperature to desaturation mechanisms has been demonstrated. The only significant difference between the winter and spring sown wheat samples in the research work (Welch, 1975) was a linoleic acid increase in the wheat of winter sowing. The increase might be the result of low temperature increasing desaturation activity even though the overall degree of unsaturation was not increased significantly.

Although the lipid fraction of wheat is relatively minor,

tocopherols (vitamin E) and polyunsaturated fatty acids, especially linoleic and linolenic acids are of interest nutritionally, functionally, and their effect on storage stability. Research has shown that stearate and linolenate are usually present in the range of 1 to 3% respectively, while linoleate, oleate, and palmitate are the three major fatty acids (Davis et al., 1980). Linoleate was by far the major fatty acid with mean class values of 50% (durum) to 59% (Soft White Winter Wheat, Hard White Winter Wheat). Marked differences in baking quality would presumably be found between wheats with 44% of the fatty acid methyl ester as linoleate and wheats with 74% as linoleate (Davis et al., 1980). Linoleate acid has also been attributed as the main component responsible for the uptake of oxygen by doughs during mixing (Fisher et al., 1964). There are still questions to be answered whether the supply of linoleic acid could ever be a limiting factor in determining differences in flour quality (Pomeranz, 1985).

When lipids are removed from their respective flours the baking results are poor. When they are returned to their respective extracted flours the original baking quality is restored. Cakes made with untreated defatted flours and chlorine treated defatted flours yielded cakes that were of poor grain (Johnson et al., 1979). The baking properties of both chlorine-treated and untreated flours were restored to

their original quality by replacing their extracted lipids.

When extracted lipids from flour of pH = 4.0 were added to the defatted flours of low and intermediate chlorine treatment, the baking performance was inferior to the responses with their own lipid extract. Addition of lipids from the low and intermediate chlorination rate sources improved bakery function of the highly chlorinated flour residue (Kissell et al., 1979).

The interchanging of chlorinated and unchlorinated lipids was conducted in another study to test the functionality of lipids in respect to cake volume (Spies et al., 1978). When free lipids of unchlorinated flour were put into defatted chlorinated flour, cakes had larger volumes. The larger volume cakes were also followed by lower test scores. The low scores resulted in an increase in cell size of the cakes. Another study using high ratio layer cakes confirmed these results (Donelson et al. 1984). The investigators found that chlorinated lipids were the primary component contributing to cake quality potential.

Aging of the flour has been looked at as another alternative other than chlorinating to improve the flour's baking ability. Storing the flour at room temperature for two months improved the volume and grain of cakes baked from the same flour (Johnson and Hosney, 1980). When untreated flour was defatted and stored at room temperature for two months its

cake-baking properties after reconstitution of lipids were comparable to those of chlorine-treated flour. Another study looked at the extent of lipid involvement in the aging process (Clements and Donelson, 1982). Flours stored at 4 degrees C. for nine weeks yielded cakes of increased volumes of the same order as those baked from bleached flours. The results indicated that:

1. expansion depends on the flour;
2. length of exposure to air;
3. lipids in an unbleached flour exposed to air for an extended period may undergo changes that cause them to behave like lipids from a bleached flour.

#### *2.05 Protein-Lipid Interaction*

The product most often used to study protein - lipid interaction is bread. The importance of lipid - protein complexing has been underscored by Ponte and Baldwin (1972) who suggested that polar lipids, particularly the glycolipids, were bound to gliadin by hydrophilic bonds and glutenin by hydrophobic bonds. Glycolipids and phospholipids are good foaming agents and promote the formation of fine uniform gas cells of high stability. Non-polar lipids act as a foam depressant. Since loaf volume and texture depend to a large

extent on the gas cell structure and stability, the relative proportions of the two types of lipids is of decisive importance. Dough mixing results in the binding of the lipid by glutenin components with polar lipids being preferentially bound (Pylar, 1983 (b); Larson, 1986).

The interaction between gluten and flour lipids is a subtle one that is not readily detectable by standard physical dough testing instruments. The nature and stability of the gas cell structure in the dough is a critical factor. Tension active lipids play an important role in providing elasticity to the dough (MacRitchie, 1980). The volume and texture which depend to a large extent on the gas cell structure and stability are very sensitive to the relative proportion of the two types of lipids present. The nature of protein - lipid association and the variations in gluten protein from different flour samples may produce changes in the binding (MacRitchie, 1980).

When flour is wetted and the dough mixed, the size of protein aggregates decrease as reflected by an increase in protein extractability. Lipids that were extractable from flour with non-polar solvents (free lipids) become bound (Chung, 1986). Therefore, the free lipids in the flour are decreased with the addition of water and there is an increase in lipid binding as the extent of dough development increases (Chung, 1986). In most flour based foods, especially baked

bread, multiple interactions occur among wheat components and/or added components. Protein-starch complexes mediated by lipids have important functional roles in baked goods (Chung, 1986).

Lipids comprise a minor component of wheat flour. Many scientists have tried to correlate lipid content or composition with genetic difference in bread making quality of wheats, but no significant relationship has been established (Chung, 1986). Free lipids were extracted from poor quality gluten as well as from good quality gluten regardless of whether the lipids were solubilized or precipitated. The investigators resolved that while lipid binding is enhanced during dough mixing, lipid protein interaction is weaker in poor rather than in good quality gluten (Chung, 1986).

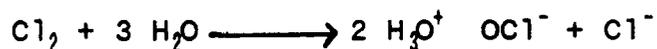
The amount for the lipid content of gliadin and glutenin protein fractions of flour is highly variable. Most of the flour and gluten lipid is associated with the glutenin (Zawistowska, 1985). Generally, it was found that protein-lipid complexes which depend on a variety of secondary molecular forces are not very stable. Actual stability strongly depends on solvent type and concentration, presence of salt ions, pH and temperature. Recent evidence suggests that the interactions depend on the specific nature of the interacting lipids and proteins. Most polar lipids would be

expected to interact strongly with protein having amino acids with polarizable or charged amino acids on their surfaces (Zawistowska, 1985).

### 2.06 Treatment of Flour with Chlorine

Chlorination is a process commonly used to improve cake baking qualities of soft wheat flours. Improvement is defined as cakes having higher volume, superior grain and tender texture. According to Kulp (1972) the chlorination treatment also increases the sugar and shortening carrying capacity of the flour thus permitting the use of high ratio formulas (often 130% sugar on a flour weight basis).

Chlorination is done after milling by the Nuchlor process (Blanshard, and co-workers, 1986) at levels between 900-2000 ppm. chlorine aiming for a flour pH 4.8-5.2. The exact chlorine requirements depend on protein and moisture content of the flour. During chlorination only half the chlorine reacts with the flour solids. The residual chlorine reacts with moisture to form hypochlorite and chloride (Gough et al., 1978). The reaction is as follows:



The hypochlorite ion ( $\text{OCl}^-$ ) is a powerful oxidizing agent

and contributes to the bleaching effect of the flour. The presence of the hydronium ion accounts for the marked drop in pH of the flour. Ash and Colmey (1973) listed the effects of pH on the baked product: (1) flavor; (2) crumb and crust color; and (3) volume, grain and texture.

Estimates by researchers of the distribution of chlorine among carbohydrate, protein and lipid vary, but there is agreement that the majority of the chlorine absorbed by the flour reacts with the lipids and proteins, and 5% or less reacts with the carbohydrate fraction (Blanshard et al., 1986).

The principal action of chlorine on wheat flour is believed to involve changes in the starch component (Sollars, 1961). Kulp and Tsen (1972) found that improvement of cake baking quality was achieved at chlorine treatment levels up to 2000 ppm, leading to higher water absorption and protein dispersion. The evidence indicated that chlorination produced changes in the characteristics of the starch component. Above 2000 ppm, the researchers detected a decrease in baking quality associated with extensive depolymerization of the starch molecules and enormous swelling of the starch granules. When water is limiting, the action of high levels of chlorine depolymerized starch without the appearance of reducing sugars, owing to formation of a second ring structure (Fig. 1.) (Whistler, 1966; Whistler, 1968):

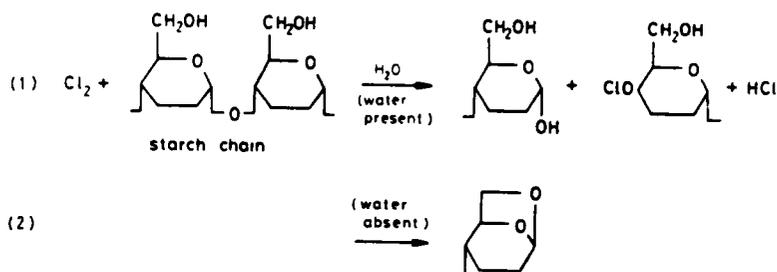


Fig.1 Effect of chlorination on starch.

Varriano-Marston (1985) summarized evidence that the starch fraction was the major factor that determined the efficiency of flour chlorination. The investigator presented evidence that the starch granules consist of crystallites interconnected by chains in the amorphous regions or tie molecules. If the tie molecules are broken by oxidative depolymerization, mechanical stress, or enzyme action the structure is relaxed and opened with relieved strain between crystallites. Water can enter more readily into the interstitial spaces of the relaxed molecule and become more tightly bound. Subsequent increases in starch swelling rate and solubility account for the higher viscosity of cake

batters made with chlorinated flours and their greater stability after baking. Johnson and co-workers (1980) indicated that oxidative changes in the starch molecules were responsible for the improving action of chlorine. The change was also accelerated by heating and defatting flour.

Chlorination effects of other flour components have been suggested, notably the effect of chlorine on flour lipids. The reaction of chlorine with wheat lipids was reported by Daniels(1963) and Gilles (1964). These researchers indicated that oxidation reactions are responsible for the improved baking quality of chlorinated cake flour. Wei and co-workers (1984) demonstrated with laboratory bleached flour that 27-33.7% of the total  $^{36}\text{Cl}$  was incorporated into flour lipids, of which 31.2-39.1% of the radioactivity went to non-polar lipids and 60.9-68.8% to polar lipids. Johnson (1979) found no effect of chlorination on bound starch lipids and only detected chlorination and hydrolysis of free non-starch lipid components.

Tsen and Kulp (1971) studied the effect of chlorination on flour proteins. The flour proteins were progressively cleaved by each increment of chlorine evidenced by increasing extractability of proteins in water and acetic acid. The increased extractability was attributed to the dispersing hydrolytic and oxidative action of chlorine. The oxidative effect of chlorine was also responsible for the oxidation of

SH to SS and the degradation of aromatic amino acids. The inter- and intra-molecular hydrogen bonds of the protein molecules were gradually broken by the action of chlorine, which caused an increase of dispersibility of proteins (Tsen and Kulp, 1971). Concurrent with these changes, chlorine cleaved peptide bonds and lowered the pH of the flour. Tsen and Kulp (1971) speculated that chloro-derivatives of tyrosine and possibly of other aromatic amino acids may interact with other flour and/or batter components, contributing to the structural stability of the cake.

There is circumstantial evidence that proteins bound to starch granules may be affected by heat and chlorination to improve baking qualities of cake flours. Both treatments enhance the hydrophobicity (oil binding ability) of wheat starch which is reversed by treatments designed to remove protein from the surface of the granules (Seguchi, 1984). Chlorination modifies the surface proteins, which in some way increases the hydrophobicity of the starch granule surface.

Examination of the surface features of native and chlorinated starches by scanning electron microscopy after treatment with amyloglucosidase has shown that the native granules were heavily pitted. Chlorine treated granules had smooth surfaces even though amyloglucosidase digestion removed equal quantities of carbohydrate from each type of starch (Greenwell and co-workers, 1985). Removal of protein from

native starch granule surface, either by proteinase or alkali treatment abolished the pitting produced by subsequent amyloglucosidase treatment. Chlorination thus altered the pattern of amyloglucosidase-catalyzed degradation of the granule surface. The granule surface of the starch is affected by the chlorine by altering a particular subset of starch granule surface proteins (Greenwell and co-workers, 1985). The observations suggest that chlorine treatment does change the proteins at or near the starch granule surface but the significance of these effects in relation to improved baking quality on "high-ratio" cake flours remains to be elucidated.

Allen and co-workers (1982) studied the effect of chlorine treatment of wheat flour on the heat gelatinization of wheat flour and starch by differential scanning calorimetry (DSC). An endothermic transition occurred in both flours and starches at a temperature commonly associated with starch gelatinization. The chlorine treatment did not significantly affect the transition temperatures nor enthalpies of either the flour or the starch isolated from it.

The role of starch in the improvement of cake flour by chlorine is well established and further evidence of the involvement of lipids as been verified by Gaines and Donelson (1982). Active research is still continuing in these areas cited in order to clarify these findings.

## 2.07 *Cake Baking*

A complete cake batter system contains a large number of highly reactive components which can become modified or interact with other components as the temperature of the batter increases during baking. Ingredients, mixing (or manipulation), and temperature all have an effect on the final outcome of the baked product. This section will review the research regarding the achievement of a successfully baked cake.

Three ingredients are needed for batter stabilization in the early stages of baking: (1) soluble proteins; (2) polyvalent cations; and (3) surface active lipids (Howard, 1972). The thermal stability of the foam structure in a fluid cake batter while being heated is dependent on soluble protein and more specifically unhydrolyzed proteins. In addition, Howard (1972) reported that the absence of calcium ions in the batter caused a very unstable batter which leads to a rapid separation of the aqueous phase and shortening.

The effects of some shortening ingredients important to producing a final cake structure were used to measure the cohesive or resistive forces that are produced within the baking cake. Cohesion began to develop around twelve minutes from the start of the baking and rose slowly for the next

three minutes; a rapid rise in the force followed and tended to level off in the last five minutes of baking. Cohesive forces appeared to result from the opposing action of toughening (flour, egg whites) and tenderizing (sugar and shortening) agents (Patton et al., 1981).

When a batter is mixed, hydrated particles are rubbed against each other, the mixer bowl or the mixer blades. The hydrated surface is removed exposing a new layer of particles to the excess water in the system. The mixing required could be viewed as the energy necessary to develop the dough, and the optimum time should be defined as when all the protein and starch are hydrated (Hoseney, 1985). The protein content of the flour can affect mixing time with low protein samples (less than 12% protein) requiring more mixing time simply because they contain less protein (Hoseney, 1985).

The effect of flour granulation and particle size on cake volume has been investigated. A fine granulation is generally considered to be desirable in cake flour. Miller et al. (1967) found that flour is improved for cake making when the coarser flour particles are reduced. They also found that starch damage should be kept below five percent. Cake volume of high standards have resulted from softer textured wheats with lower protein content which produced smaller flour particle sized on milling (Gaines, 1985). Wheat class also can have an effect on the particle size distribution. The effect of pin

milling on the particle size distribution of patent flours differed with wheat class (Chaudhary, 1981). This characteristic appeared to be related to flour granulation. The component governing flour granularity was also the determinant in cake volume (Chaudhary, 1981; Gaines and Donelson, 1985).

Cakes prepared from flours without chlorination are of such poor quality (i.e., low volume and dense structure) that differences among flours may appear negligible. Batters from chlorinated flours exhibited excellent expansion in the oven whereas batters from typical untreated flours exhibited severely restricted expansion (Donelson and Clements, 1986). Also, omission of emulsifiers or removal of free lipids from the flour virtually eliminated batter expansion resulting in sharply reduced volumes and sunken contours (Donelson and Clements, 1986). A correlation of 0.98 was obtained between maximum batter expansion and cake volume for the reconstituted flours. The extracted flour residues were controlling the degree of expansion and, therefore, influencing cake volume (Donelson and Clements, 1986). One earlier study (Johnson et al., 1979) demonstrated that both chlorine treated and untreated defatted flours yielded cakes with a much poorer grain than did their respective non-defatted flours. Microscopic studies showed that air cells made with defatted flours were aggregated compared with the more evenly dispersed

air cells in batters made with non-defatted flours (Johnson et al., 1979).

A series of studies was conducted to test the effectiveness of chlorinated and untreated flours with respect to cake outcome. One study investigated the effects of certain ingredients on the aforementioned flour types. The untreated flour gave higher volume at shortening levels below 28%, but the grain was coarse. With the chlorine-treated flour, there was an increase in gumminess as the shortening level was increased (Johnson and Hosney, 1979).

Eggs were found to enhance the structural properties of cakes baked from untreated flour. Cakes baked from the chlorine -treated flour had good volume, fine grain and good overall quality (Johnson and Hosney, 1979). Another study investigated the changes in starch caused by chlorine treatment. Starches isolated from untreated flour and from chlorine - treated flour performed equally well in cakes baked from reconstituted flours when the untreated starch was air dried (Johnson et al., 1980).

A study was conducted on the mechanism of bubble expansion and its relation to starch gelatinization and protein coagulation. In the early stage of baking, as the temperature increased, batter volume increased by expansion of bubbles from the increase in vapor pressure of water and air in the bubbles (Mizukoski et al., 1980). Further increase

in temperature caused starch swelling which was observed as the increase of light transmission in the model system (Mizukoski et al., 1980). At the temperature of maximum light transmission, the starch was almost gelatinized and protein coagulation was accelerated. At the same time, the sol of the cake batter began to change to the gel-like structure of cake. Formation of a continuous gel phase depressed the expansion of bubbles and further increased in pressure in the bubbles caused the gas in the bubble to be released (Mizukoski et al., 1980).

Batter viscosity which was influenced by batter moisture content and flour chlorination was compared with cake expansion during baking (Gaines and Donelson, 1982). Variation in the cake expansion was attributed to the differences in apparent viscosity of the batter. Bleached flour at optimum batter moisture content achieved higher pasting viscosity more rapidly than the unbleached flour. Also, a varietal controlled mechanism (perhaps protein quality) could also have influenced batter rheology during baking and may have been responsible for the difference in the batter expansion of the two flours (Gaines and Donelson, 1982). Cakes were also made from defatted bleached and unbleached flours reconstituted with the heat-treated lipids. Expansion was retained as volume when heat-treated lipids were added to defatted bleached flours, but cakes usually collapsed when lipids were added to defatted

unbleached flours (Clements and Donelson, 1982.)

In order to prevent shrinkage of cakes from untreated flours, one research team explored the idea that this can be prevented by giving a mechanical shock to the cake immediately after baking (Ohtsubo et al., 1978). The cake is dropped from a height 6 to 20 cm. immediately after being withdrawn from the oven. The grain and texture was found to be finer than those of the cakes baked from chlorinated flour (Ohtsubo et al. 1978). The research team explained that if the airtight structure of the cell wall is still maintained after a cake is removed from the oven, a sudden decrease in the inner pressure of each cell caused by cooling in the atmosphere might cause the shrinkage of the cell wall which possibly results in the shrinkage of the crust. A shock given to a baked cake immediately out of the oven might cause cracks in the cell wall, breaking the airtight structure so that the pressure gap can be avoided (Ohtsubo et al., 1978).

## *2.08 Review of Methodology*

### *2.08a Examination of the Flour*

The pH is an important factor for high ratio cakes and determines the degree of chlorination. The best baking results have been achieved between pH 4.6-4.8. Unbleached

flour is approximately pH 6. Preliminary results using unbleached flour with pH 6 resulted in cakes with no volume, and collapsed cell structure.

The ash content offers a convenient means for checking on the efficiency of the milling process. Since the ash content of flour over and above the natural mineral content of the endosperm is largely derived from the bran, an excessively high ash content is indicative of relatively high admixtures of branny material to the flour, such as occur with low grade flours (Pyler, 1973). In an average soft wheat the ash content of the endosperm is about 0.32 percent and of the bran about 5.8 percent.

For some time the ash content of flour has been considered an important measure of flour quality. Mineral content of flour per se is not related to final performance but gives an indication of the miller's skill and the degree of refinement in processing. Flour products which contain higher levels of ash are darker in color and are assumed to contain greater quantities of fine bran particles or that portion of the endosperm adjacent to the bran (Pomeranz, 1989).

Examination of the flours (chlorinated and unchlorinated) under the scanning electron microscope and light microscope will give some insight into the changes that chlorination has caused to the protein, lipid and starch constituents in the

flour. Flours are very simply prepared for SEM, usually by being dusted dry onto a stub which has been coated with adhesive (Angold, 1979). Selective staining, however, cannot be undertaken in the scanning electron microscope so that all interpretation of structure is based on morphology. Therefore, the light microscope should be used hand-in-hand with the scanning electron microscope.

In the light microscope, fixation is carried out in order to preserve material against decay and undesirable changes which may otherwise result from contact with reagents used in preparing specimens for observation. Fixation is applicable for the most part, only to protein, since starch neither requires nor responds to fixatives, owing to its natural impermeability (Angold, 1979). For the permanent mounts used in light microscopy, fixation, followed by infiltration, embedding, thin sectioning on a microtome or ultramicrotome, staining and mounting is necessary (Evers, 1979).

A selection of frequently used preparations is listed below for staining:

(a) for temporary mounts an extremely useful dye is a 0.3% solution of iodine in 6% potassium iodide. In this form or as a component of chlor-zinc iodide, iodine stains normal starch blue violet and protein a straw color. Starches consisting of a higher than normal amylopectin content can be identified by their red coloration. Regions of different

composition within the same granule can be distinguished by this differential staining effect (Evers, 1979).

(b) A permanent method of staining starch granules which does not distinguish between damaged and undamaged granules is the histochemical periodic acid-Schiff (PAS) reaction. In this reaction cleavage of alpha-glycols by periodic acid oxidation produce aldehyde groups: these react under acid conditions and in the presence of sulfur dioxide with the dye basic fuchin to form a chromophore in situ. This method is frequently used in preparations made with glycol methacrylate as an embedding medium. Good results are achieved with PAS used with polychromatic toluidine blue or the similar azur A. In addition to their polychromatic reaction with nucleic acids and cell wall components, these dyes stain proteins blue. Pomeranz and Schallenberger (Evers, 1979) tested twenty-nine of the basic and acid dyes cited for protein staining in the literature. In their opinion the most satisfactory one was ponceau 2R. Bromophenol blue, used with mercuric chloride, is another frequent choice for proteins which are not water soluble.

Aranyi and Hawrylewicz (1968, 1969) published the advantages of using SEM for the study of cereals and flours. The SEM provides a very large depth of focus (generally as large as the field of view), the possibility of viewing much larger samples than in the conventional electron microscope,

and easier sample preparation. The surface of the sample can be examined directly without any special treatment, thus ensuring the absence of artifacts. The sample is viewed at an angle and therefore shadowing of the surface is inherent in the technique.

Rooney and coworkers (1983) pointed out the limitations of the SEM and emphasized the use of another microscope in conjunction with SEM when studying cereal grains. The SEM views small areas of a much larger specimen. The SEM by itself can provide only qualitative structural information. Unlike light microscopy, there are no colored or electron opaque stains available to specifically identify chemical or biological constituents in a SEM specimen. If more than structural questions are to be addressed, SEM data must be complimented by techniques capable of providing compositional or chemical information.

In the Hunter Colorimeter when using L, a, b it is almost always shown that the b dimension measuring yellowness is the most critical of the three. In fact, the use of bluing which decreases L but increases blueness, produces a visually "whiter" product. In studies, b is typically found to be three or four times as important (Hunter and Harold, 1987). The a dimension is the least important in normal practice in the U. S. marketplace. Three numbers are necessary to completely identify either a color or a color difference.

However, two dimension, L and b, are sometimes adequate for the identification of a white color (Hunter and Harold, 1987).

#### ***2.08b Batter Measurement: Differential Scanning Calorimetry***

The denaturation of the components of a baked product such as cake or bread can be observed under simulated baking conditions by differential scanning calorimetry. The amounts of the components of the cake mixture, the way they are arranged in the dispersion and the temperature ranges over which they are denatured all determine the acceptability of the baked product (Donovan, 1977).

Differential scanning calorimetry (DSC) is particularly well suited for the study of the intermeshing of starch and protein in a baked product. It measures the heat flow into a sample as a function of temperature as the components of the sample are heat denatured. DSC is not a measure of the physical properties of the resulting baked product, but it provides fundamental data such as the temperature ranges of gelatinization of starch and denaturation of protein (Donovan, 1977). The way temperature ranges are affected by a low molecular weight ingredient like sugar can reveal why one cake proves acceptable and another is a failure.

Sugar raises the denaturation temperature of the egg

white proteins. The effect of sucrose concentration on the gelatinization temperature of the starch is more marked. The gelatinization of the starch occurs roughly simultaneously with denaturation of the major portion of the protein. The batter has attained maximum volume when the protein and starch intermesh simultaneously to form the "structural framework" of the cake. The cake composite, permeated by gas bubble, is similar to brick and mortar. The starch grains form the bricks (rather rounded, flexible or rubbery ones, depending on water absorption), and the protein, the mortar (Donovan, 1977).

## **CHAPTER III**

### **MATERIALS AND METHODS**

#### **3.01 *Wheat***

Coker 916, (Walker) a soft red winter wheat, is one of the predominant varieties of wheat grown in the southeastern United States. Coker 916 was grown by the Agronomy Department of Virginia Polytechnic Institute and State University during the 1988 growing season for experimental purposes. Various levels of nitrogen fertilizer were applied to the wheat at growth stages 25 and 30. (Table 1).

#### **3.02 *Experimental Milling***

Cleaned wheat was weighed and milled on a Buhler Laboratory Mill, bucket type (Buhler Bros., Inc., Uzwil, Switzerland) according to AACC Method 26-20 (AACC, 1983). The milling was conducted at Midstate Mills, Newton, NC. Three breaks and three reduction steps were used. Break roll settings were: left break roll 8-10; right break roll 3-5. Reduction rolling settings were: left reduction roll 4-6;

TABLE 1		
Fertilization Regimens of Coker 916 Flour		
Wheat	Nitrogen Treatment lb/ac	
	Growth <u>25</u>	Stage <u>30</u>
TRT3 (NC) <sup>1</sup>	50	---
TRT3 (C) <sup>2</sup>	50	---
TRT6 (NC)	---	50
TRT6 (C)	---	50
TRT4 (NC)	---	100
TRT4 (C)	---	100
TRT7 (NC)	100	---
TRT7 (C)	100	---
TRT13 (NC)	50	100
TRT13 (C)	50	100
TRT15 (NC)	100	50
TRT15 (C)	100	50
Commercial Flour	---	---
<sup>1</sup> NC - Not chlorinated <sup>2</sup> C - Chlorinated		

right reduction roll 1-2. The flour was rebolted over a 40 mesh silk screen.

### *3.03 Chlorination of Flour*

Twelve pounds of flour from wheat of each treatment were chlorinated at Minnel Milling Co., Fostoria, Ohio. The final pH of the flour was specified to be 4.6-4.8 (Ash and Colmey, 1973).

The experimental flour was placed into a chlorination box containing at least 5 lb. but no more than 10 lb. The box was rotated as the chlorine gas was added in order to insure even chlorine distribution onto the flour particles. Chlorine gas was of high purity, and packed in cylinders under pressure. The pH of the flour was taken before the chlorination process, and then read during processing at certain points until the desired pH was reached.

### *3.04 General Aspects: pH, Ash and Color Determinations*

The pH of both chlorinated and unchlorinated flours was determined by AACC Method 02-52, Appendix D (AACC, 1983). With a Fisher Accumet pH Meter, (Fairlawn, NJ, Model 600) with pencil combination electrode. All analyses were done in triplicate.

The ash content of both chlorinated and unchlorinated flours was determined by AACC Method 08-01, Appendix C (AACC, 1983). The instrument used was a Fisher Isotemp Programmable Ashing Furnace (Model 495). The initial rate of heating was 5 degrees C/minute with the transition temperature for flaming the flour at 250 degrees C. This temperature was maintained for one and a half hours. The final rate was set at 5 degrees C/min for a final temperature of 550 degrees C.

The Hunter Colorimeter, (Model D25; Hunter Labs, Fairfax, VA,) was used to measure the color of the chlorinated and unchlorinated flours (Appendix E). The instrument was standardized using the white standardization tile for L, a, and b values:

L = visual lightness

a = red (+), green (-)

b = yellow (+), blue (-)

The standardization of the instrument was checked between each sample with the white standard tile with L = 91.97, a = -.80, and b = - 1.00. Delta E, a single numerical value was calculated for each sample as follows:

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$$

### *3.05 Protein Content of Flour - Crude Protein*

Protein content of the chlorinated and unchlorinated flours was analyzed by Kjeldahl nitrogen analysis, AACC 46-12, Appendix B (AACC, 1983). This procedure assumes a constant relation between total nitrogen and the polymers of amino acids which link together to form proteins. In wheat flour this relation is expressed by multiplying the nitrogen content by 5.7. For many years, protein quantity was determined by this measurement and has been designated as "crude protein".

### *3.06 Separation of Protein Subunits*

#### *3.06a Gliadin Fraction*

Wheat flour proteins were extracted with 70% ethanol and were examined by polyacrylamide gel by a modified procedure of Lookhart and co-workers (1988, Appendix G ). The concentration of the sample was doubled because of poor resolution in the gel. The loading in the gel was increased from 10 ul. to 15 ul. with a Hamilton syringe. Finally, the gel was allowed to run an additional 10 minutes after the samples ran off the gel in order to maximize resolution.

The electrophoresis unit was connected to a circulating

water bath (Brinkman RM6 Refrigerating Circulator, Sybron Corp, Westbury, NY) which was maintained at 12 degrees C. The running buffer was prepared a day ahead and chilled (purified aluminum lactate, pH = 3.1).

The gel ran for 2 1/2 hours. The gel with a concentration of 6 % was difficult to handle. The gel was floated off the glass plate and never handled by the researcher. The gel was stained with 50% trichloroacetic acid plus 1% Coomassie blue solution for six hours and destained overnight in 50% trichloroacetic acid plus water (75 ml. + 225 ml.). After destaining, the gel was placed in a clean destaining solution and refrigerated to allow for sharpening of the bands.

### *3.06b Molecular Markers*

Reference molecular weight markers (Sigma Chemical , St. Louis, MO) represented a range in molecular weight from 14,300 to 97,400 daltons. The following markers were used: lysozyme trypsinogen, glyceraldehyde 3-P dehydrogenase, egg albumin, bovine albumin, and phosphorylase b. Molecular weights of the markers are listed in Table 2.

A mixture of the markers was prepared by adding 1 mg of each and dissolving in 1 ml of running buffer (pH = 3.1). Each marker was also ran separately to determine their

TABLE 2  
MOLECULAR WEIGHT MARKERS FOR POLYACRYLAMIDE GEL  
(GLIADIN)

<u>Reference Protein</u>	<u>Approx. Molecular Wt., Daltons</u>
Lysozyme	14,3000
Trypsinogen	24,000
Glyceraldehyde 3P dehydrogenase	36,000
Albumin, egg	45,000
Albumin, bovine	66,000
Phosphorylase b	97,4000

resolution in the gel.

### *3.06c Glutenin Fractionation of Flour:*

#### *Sodium Dodecyl Sulfate Polyacrylamide Gel*

Following fractionation, the composition of glutenin was analyzed by sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE) by the method suggested of Lookhart and Albers (1988; Appendix F). An 8% to 18% linear polyacrylamide concentration gradient was used. A 4% acrylamide solution was prepared for the stacking gel.

SDS is a highly charged anionic detergent which binds strongly to protein molecules and effectively swamps the charge groups on the protein itself. This results in all the proteins present forming complexes with the detergent, all of which have almost identical charge to mass ratio (Weber and Osborne, 1969). Then when proteins are subjected to electrophoresis in the presence of the detergent (SDS) they are separated by virtue of any charged differences, but purely on the basis of difference in the size of the protein detergent complex.

The larger the complex the harder it is to pass through the pores of the gel and the more it is retarded. The size of the complex is largely determined by the length of the polypeptide chains and hence, approximately the protein's

molecular weight. Since the protein polypeptide chains are completely unfolded (denatured) it is the subunit molecular weight that is obtained and not the molecular weight of the native molecule.

A gradient gel was also used in separating the glutenin subunits. The advantage of its use is that smaller components are slowed down relative to larger ones and a better spread of zones across the gel surface is obtained. For complex mixtures of proteins a gradient gel often gives a resolution better than a gel of constant composition, because while it never provides the optimum pore size for separating any 2 particular components all the time it does provide a compromise in which any 2 components are subjected to optimal separation conditions for some of the time (Andrews, 1988).

#### *3.06d Molecular Markers*

Reference molecular weight markers represented a range of 14,300 to 205,000 daltons. A vial of the high molecular weight standard mixture (MW-SDS-200, Sigma Chemical Company, St. Louis, MO) plus 2 extra markers: trypsinogen and lysozyme were prepared with 1.0 ml of running buffer. Molecular weights of the molecular markers are listed in Table 3.

TABLE 3  
MOLECULAR WEIGHT MARKERS FOR SDS-PAGE GEL  
(GLUTENIN)

<u>Reference Protein</u>	<u>Approx. Molecular Wt., Daltons</u>
Lysozyme	14,300
Trypsinogen	24,000
Carbonic anhydrase	29,000
Albumin, egg	45,000
Albumin, bovine	66,000
Phosphorylase b	97,000
Beta-Galactosidase	116,000
Myosin	205,000

### 3.06e Electrophoresis

Acrylamide solutions for the separating gels (8% and 18%) were prepared with initiator and catalyst for polymerization (ammonium persulfate and TEMED [N, N, N<sup>1</sup>, N<sup>1</sup> tetramethylethylene diamine]) by the procedure of Lookhart and Albers, (1988), (Appendix F) and degassed with a tap aspirator until no bubbles were visible. The initiator and catalyst were added to the acrylamide solutions in their respective gradient wells, (Bio-Rad model 385 Gradient Former, BioRad Laboratories, Richmond, CA). The acrylamide was delivered to the prepared gel sandwich (Hoefer, Vertical Slab Gel SE 600, Hoefer Scientific Instruments, San Francisco, CA) with the aid of a peristaltic pump calibrated to deliver 10 ml./minute (Isco Wiz Pump/Diluter/Dispenser, Lincoln, NE). The acrylamide was overlaid with 1 ml water covered and allowed to polymerize overnight without disturbance.

A 4% stacking gel was prepared the following day according to the procedure of Lookhart and Albers, 1988 (Appendix F). It was degassed until no visible bubbles were present. The initiator and catalyst, ammonium persulfate and TEMED, were added, the solution was gently swirled for twenty strokes. The stacking gel was delivered on top of the gradient gel with the aid of a 50 ml. syringe, and a well forming comb was added. The comb contained 15 lanes and the

gel was allowed to polymerize for 2 hours.

The soft wheat flour contained a relatively low amount of protein, therefore, the sample amounts were doubled. The samples were weighed into 1.5 ml. sample vials fitted with 7.7 mm. PTFE-FACED, SEPTA liners (Altech Assoc. Inc., Deerfield, IL). The samples were placed in a boiling water bath to extract the glutenin.

The lower buffer reservoir was filled with running buffer which had been connected to a circulating water bath (Brinkman RM6 Refrigerating Circulator, Sybron Corp., Westbury, NY) and maintained at 20 degrees C.

Ten ul. aliquots of each sample and 5 ul. of the molecular weight reference markers were loaded into sample wells with a Hamilton syringe. The electrophoresis was started at 20 ma, once the dye front entered the gradient gel. The dye front was allowed to migrate off the acrylamide and electrophoresis was continued for 5 minutes after the leading edge of the dye front migrated off the acrylamide.

The gel was stained overnight in Staining Solution containing Coomassie blue (Appendix F) and placed in destain I (See Appendix F) for 6 hours. A charcoal sponge was used to absorb the dye. This destaining solution was used three times before it was changed. The gel was then kept in destain II (Appendix F) for permanent preservation.

There was difficulty in staining the gels. Gels were

stained three times in order to get a satisfactory color that would be able to be scanned. Gels have a little more difficulty when SDS is used than in detergent free PAGE systems. SDS may interfere with dye binding. A procedure recommended by Andrews (1988) is to pre-fix the zones in the gel (and wash out some of the SDS) by immersing it in 10% sulfosalicylic acid solution for 30-60 minutes, followed by staining in 1% Comassie Blue R-250 solution.

### *3.06f Alternative Extraction Procedure*

Another extraction method was used to determine the strength of the disulfide bonds in the soft red winter wheat and also, to test the effect of chlorination on the bond. A 2 X Treatment Buffer (Appendix F) was made up with SDS and contained no mercaptoethanol. Treatment with SDS and a disulfide bond-breaking reagent caused the proteins to be reduced to random coil polypeptides, with charge differences eliminated by the presence of bound SDS. All procedures were kept the same as described previously and the testing was done in duplicate.

### *3.06g Densitometer Scans*

Densitometer scans were done on both polyacrylamide gels

(gliadin and glutenin). An ISCO Gel Scanner Model 1312 (ISCO Model 228 Absorbance Monitor, Lincoln, NE). was used for all scans. Band densities were calculated with a Hewlett Packard 3390 Electronic Integrator (Hewlett Packard, Palo Alto, CA). Gel scans were run at 60 cm/hr.

### *3.07 Lipid Analysis*

#### *3.07a Crude Fat Content: Soxhlet Analysis*

The Soxhlet method was used to determine the percent of crude fat content of the chlorinated and unchlorinated flours. Petroleum ether, a non polar solvent, was used as the extracting solvent (Appendix H). Those lipids that are extractable from a dry material (usually flours or freeze-dried dough) with a non-polar solvent are free lipids, and the remaining lipids are bound lipids.

The refluxing of the Soxhlet ran for 48 hours to insure complete extraction of the lipids from the flour. The petroleum ether was heated just to the boiling point to enhance the extraction's effectiveness.

The extraction temperature was held near the boiling point of the solvent which was 100 degrees C. Morrison (1989) implies that such a procedure is extravagant and exposes the lipids to the risk of serious oxidation so that they are

unsuitable for detailed analysis.

The packages (sample bundles) were refluxed without the thimble in the percolation column. The thimble deteriorated under the condition of analyses causing sample contamination and gain in weight and error in the final weighings. All samples were done in triplicate. Adjustments were made on the final % crude fat according to 14% flour moisture.

### 3.07b Fatty Acid Analysis: Extraction and Methylation

Lipids in chlorinated and unchlorinated flours were quantified as fatty acid methyl esters (FAME) by gas chromatography by the method suggested by Morrison and co-workers (1980; Appendix H). Heptadecanoic (17:0) acid methyl ester (Sigma Chemical, St. Louis, MO) was used as an internal standard.

Hydrolysate lipid was used as the measure of total acyl lipid as FAME. Flour samples were subjected to concentrated hydrochloric acid and methanol and heated for an extended period of time. The remaining lipid was extracted with chloroform and evaporated under nitrogen.

The hydrolysate was converted to FAME for quantification by gas chromatography. Methylation took place by adding boron trifluoride (Supelco, Bellefonte, PA) and benzene to the lipid and heated for 10 minutes. Samples were evaporated under

nitrogen to about half. Vials were sealed and stored at 0 degrees C before quantification.

A Shimadzu gas chromatograph GC-9A (Shimadzu Corp., Kyoto, Japan) and a Shimadzu GR-4A Chromotopac (Shimadzu Corp., Kyoto, Japan) were used for quantification of fatty acid methyl esters. Soft red winter wheat contains small percentages of lipid, therefore, a sample size of 0.75  $\mu$ l. was used. The following criterion was used for detecting fatty acids on the chromatograph: SP 2300 fused silica capillary column 30 meters, 0.32 mm. ID, 0.20  $\mu$ m. film. Column temperature was 180 degrees C then programmed to 200 degrees C at 5 degrees C/minute and held 5 minutes. Linear velocity was 20 cm/sec. Helium was the carrier gas. Detection was done with a flame ionization detector (FID  $2 \times 10^{-11}$  AFS). Sample size was 0.75  $\mu$ l. delivered with a Hamilton syringe, which split : 35:1. After every three samples tested on the chromatograph, the instrument was purged by bringing the chromatograph to 200 degrees C and holding until the column was clear of the interfering residue that masked peak formation.

Calculations were made on 16:0, 16:1, 18:0, 18:1, 18:2, and 18:3. The internal standard was used as the reference in the calculation. All calculations are based on 100 g of flour, and the entire procedure was carried out in triplicate.

### *3.07c Thin Layer Chromatography (TLC): Qualitative Method*

Thin layer chromatography (TLC) was used for the separation and determination of principle and trace natural components, additive, adulterants, contaminants, and decomposition products of foods. The stationary phase was a layer of fine particle powder bound to a glass plate. The sample solution was applied as a spot and, after evaporation of the solvent, ascending development was carried out by the flow of a mobile phase through the stationary phase. After the mobile phase has traveled the necessary distance, the chromatograph was dried, stained, and the separated components located. The degree of movement and the size of spots relative to standards are characteristic of the identity and amount of each substance (Sherma, 1984).

Thin layer chromatography was used as a qualitative tool to identify the major polar lipids present in the flours. Polar lipids are primarily bound lipids and, therefore, require a stronger solvent for extraction. The procedure of Morrison and co-workers (1980) was used (Appendix N). Water saturated n-butanol (WSB, 35:65 (v/v)) was used as the extraction solvent. According to Morrison (1980), flour lipids extracted with WSB contain much more lysophosphatidylcholine (LPC), the principle lipid in wheat starch.

The starch lipids (or polar lipids) were extracted in 3 hours at 100 degrees C. There were three extractions done on an hourly basis. The flour was subjected to the WSB and then aliquots of the extract were removed.

The important factor with the extraction process is timing. Efficient extraction with water saturated n-butanol took 3 hours. Morrison and co-workers (1975) found that time and temperature played an important role in complete extraction of starch lipids. Non-starch lipids are removed within 30 minutes, while starch lipids are extracted very slowly.

The extract was evaporated under nitrogen. Chloroform was used to remove the lipids from the flask. Samples were placed in vials lined with teflon caps and frozen to preserve the lipid product.

Precoated plates of silica gel G with a pore size of 150 um in thickness were used for separation and analyses (Analtech, Newark, DE). The binder property G (Gypsum) was used on the plate. Activation of the plates was carried out at 110 degrees C for one hour before use as required for the chromatography of more polar substances.

### **3.07c (i)      *Sample Analysis***

Samples and standards were applied manually with the use

of glass capillary tubes (microcaps). The disposable microcaps, 15  $\mu$ l., are filled by capillary force and dispensed when touched gently to the layer. Sample application was made 2 cm from the bottom of the layer and a spotting guide (Analtech, Newark, DE) was used for the application. The standards used were the following:

1. L-alpha-Phosphatidyl DL-Glycerol, from egg yolk lecithin (Sigma Chemical Co., St. Louis, MO)
2. L-alpha Phosphatidylcholine from soybean, (Sigma Chemical Co., St. Louis, MO)
3. L-alpha Phosphatidylethanolamine (L-alpha Cephalin) from soybean (Sigma Chemical Co, St. Louis, MO).

### *3.07c (ii) Development of Chromatograms*

Layers were developed in the ascending direction in a glass tank (Analtech, Newark, DE). The mobile phase (Appendix N) was poured into the bottom of the tank and the plate was placed with the bottom end immersed in the solvent and the sample origins about 1 cm. above the solvent level. The solvent competes with the chromatographed substances for the absorbent sites. The more polar substances require a more polar solvent to cause migration. A blend of solvents was used. The advantage of solvent mixtures is that resolution can be improved (Sherma, 1984). The layer was developed to

within 2 cm from the top. The plate was removed and allowed to air dry for 10 minutes.

### *3.07c (iii) Detection of Zones*

Compounds on the layer can be reacted with a reagent giving a colored or fluorescent product. Zinzadze reagent (Supleco, Bellefonte, PA) was applied by spraying with visualization reagent sprayers (Analtech, Newark, DE). The whole area was sprayed under a high-draft hood. The appearance of blue spots were an indication of the presence of polar lipids. The results were preserved by photocopies.

### *3.08 Dough Rheological Properties: Farinograph*

Dough rheological properties were examined with the Farinograph (C.W. Brabender Instruments, South Hackensack, NJ) using the (AACC Method 54-21; AACC, 1983) constant flour weight Procedure for 50 g. sample (Appendix O). All samples were run in duplicate. The resistance to dough to mixing was evaluated by arrival time, peak time, departure time, mixing stability, and mixing time index. The methods for calculating these measurements are defined as follows:

1. Arrival time: the time required for the top of the curve to reach the 500 BU line after the mixer has been started and the water introduced.
2. Peak time (or dough development time): time to the nearest half-minute between the first addition of water and the development of the dough's maximum consistency.
3. Departure time: the time to the nearest half-minute, from the first addition of water until the top of the curve leaves the 500 BU line.
4. Mixing stability: the difference in time, to the nearest half-minute, between the point at which the top of the curve first intercepts the 500 BU line (arrival time) and the point at which the top of the curve leaves the 500 BU line (departure time). The value gives tolerance to mixing.
5. Mixing time index: the difference in Brabender units between the top of the curve at the peak and the top of the curve measured 5 minutes after the peak.

### 3.09 *Microscopy of Flour*

#### 3.09a *Light Microscope*

The light microscope is of great use in the study of

13507 552-7607  
7097-2555  
CASH

flours. The size and shape of the starch granules may indicate the species of cereal used in the preparation of the flour. Appropriate stains were used along with the light microscope to identify the constituents of flours.

A light microscope (Vanox Brightfield Microscope, Model AH-51, Olympus Optical Co., Tokyo, Japan) was used to study the chlorinated and unchlorinated flours. The microscope was equipped with a 35mm camera. The magnifications used were 40X and 100X. Under 100X magnification the sample was immersed under oil for viewing.

Permanent mounts were made of the flours studied. The procedure suggested by Flint and Moss (1970) was used for mounting and staining (Appendix P).

Three stains were used:

1. Ponceau 2R, No. P-7632 (Sigma Chemical, St. Louis, MO) to stain protein in the flour red.
2. Chlorazole black E, No. C-1144 (Sigma Chemical, St. Louis, MO) to stain the starch in the flour black.
3. a. Pararosaniline No. P-7632 (Sigma Chemical, St. Louis, MO).  
b. Toluidine Blue No. t-3260 (Sigma Chemical, St. Louis, MO). Pararosaniline and Toluidine are used together and are collectively known as Paragon Stain (Fuchin). This stain dyed the protein blue and the starch pink.

A smear was made on a slide, dried and then stained for a certain time period. The slide washed, placed in alcohol (Reagent alcohol, A962-4, Fisher Scientific, Fair Lawn, NJ) for dehydration; fixed in xylene (Fisher Scientific, Fairlawn, NJ) and mounted (Permount SP-15-100, Fisher Scientific, Fairlawn, NJ). The alcohol, xylene and permount were of histological grade.

### *3.09b Scanning Electron Microscope (SEM)*

The scanning electron microscope (SEM) was used in examining the chlorinated and unchlorinated flours (JEOL JSM-35C, Medford, MA). This instrument was used in conjunction with the light microscope to help in interpreting results. Selective staining cannot be undertaken in the SEM. Therefore, all interpretation of structure is based on morphology. The resolution of the instrument was 60 Å with a magnification range of 10 to 180,000X.

Flour samples were dusted dry onto a stub which was coated with adhesive. The adhesive used was conducting paint (Ludd Research Industries, Burlington, VT). Prior to observation under SEM, the stubs were sputtered coated (API Sputter Coater, Structure Probe, Inc., West Chester, PA) with gold for 40 seconds under argon. Gold, the conducting metal, served as a source of secondary electrons. Samples were

photographed under 660X magnification.

### ***3.10 Baking Characteristics of Experimental Flour***

#### ***3.10a Experimental Design***

Experimental design is defined as the process of planning the experiment so that appropriate data will be collected, which may be analyzed by statistical methods resulting in valid and objective conclusion. (Montgomery, 1976). The two basic principles of experimental design are replication and randomization.

In certain experiments randomized block designs may not be able to run all the treatment combinations in each block. Situations usually occur due to shortages of experimental apparatus or facilities, or the physical size of the block. For this problem a balanced incomplete block design in which every treatment was not present in every block was used. A balanced incomplete block design is a design in which any two treatments appear together an equal number of times (Montgomery, 1976).

A possible design in balanced incomplete blocks is that in which all possible sets of "u" treatments, taken  $k$  at a time are used. In addition, if there are "a" treatments and  $b$  blocks, the assumption is made that each block contains  $k$

treatments, and that each treatment occurs  $r$  times in the design (or is replicated  $r$  times). Furthermore, the number of times each pair of treatments appears in the same block is

$$\lambda = \frac{r(k-1)}{a-1}$$

If  $a = b$ , the design is said to be symmetric. See Table 4 for the design used in testing the flours for baking and sensory.

The balanced incomplete block design was used for baking and sensory analysis. Two blocks were used each week, with baking done on Monday and Wednesday, and sensory and baking quantification performed on Tuesday and Thursday for a total of 6 1/2 weeks. Each flour was assigned a number in chronological order: Softasilk, No. 1; TRT3-UN-No.2; TRT3-B1-No.3, etc. See Table 5 for assignments of flour to numbers and respective blocks.

### *3.10b Baking of Angel Food Cakes*

Angel food cakes were baked by the AACC Method 10-15 (AACC, 1983; Appendix I). Dried egg albumen (Henningsen Foods, Inc., Omaha, NE) was reconstituted 18 hours before the bake. The dried egg albumen contained ca 0.1% sodium lauryl sulfate which served as a whipping agent. The dried egg

TABLE 4

Balanced Incomplete Block Design  
For Testing Experimental Flours

$a=13$ ,  $k=4$ ,  $r=4$ ,  $b=13$ ,  $\lambda=1$

Block

(1)	1	2	4	10
(2)	2	3	5	11
(3)	3	4	6	12
(4)	4	5	7	13
(5)	5	6	8	1
(6)	6	7	9	2
(7)	7	8	10	3
(8)	8	9	11	4
(9)	9	10	12	5
(10)	10	11	13	6
(11)	11	12	1	7
(12)	12	13	2	8
(13)	13	1	3	9

TABLE 5														
Assignment of Flours to Blocks For Baking and Sensory Evaluation														
Flour	Assigned No.	Block												
		1	2	3	4	5	6	7	8	9	10	11	12	13
Softasilk	1	X				X						X		X
TRT3 (NC)	2	X	X				X						X	
TRT3 (C)	3		X	X				X						X
TRT4 (NC)	4	X		X	X				X					
TRT4 (C)	5		X		X	X				X				
TRT6 (NC)	6			X		X	X				X			
TRT6 (C)	7				X		X	X				X		
TRT7 (NC)	8					X		X	X				X	
TRT7 (C)	9						X		X	X				X
TRT13 (NC)	10	X						X		X	X			
TRT13 (C)	11		X						X		X	X		
TRT15 (NC)	12			X						X		X	X	
TRT15 (C)	13				X						X		X	X

albumen was covered with plastic wrap and stored overnight at ca 2 degrees C. Stock egg solution was refrigerated during bake to minimize variation in whipping performance.

A household electric oven (P/7, General Electric, Schnectady, NY) was used for baking. The oven was preheated to 200 degrees C/400 degrees F. The procedure was modified from 190 degrees C/375 degrees F to yield consistent results. Baking time was reduced from 30 minutes to 25 minutes.

Tube pan, 10 inches in diameter of aluminum (Mirro Corp. Minitowac, WI) was used for baking. Pans were washed thoroughly prior to baking to remove all traces of grease. Small amounts of fat would cause the cake to "fall" out of the pan while cooling. The tube pan was tared on a large capacity balance (Toledo Scale, Toledo Scale Co, Toledo, OH). Six hundred and fifty grams of batter was transferred with rubber spatula. The pan was rotated briskly and a blunt type knife was drawn through the batter to break up any large bubbles.

A Hobart electric mixer (K-5A; Hobart Corp, Troy, OH) was connected to a timer control (Universal Timer, Model 171, DIMCO Gray Corp., Dayton, OH) was used. All mixing was done in a 10-qt. bowl with a wire whip furnished with the mixer.

Modifications in the formulation were made. Cream of tartar was substituted for monocalcium phosphate, monohydrate (MCP) in the formula. MCP has a neutralizing value of 80

(Pyler, 1973). The acidifying agent used was potassium acid tartrate, commonly referred to as cream of tartar. Cream of tartar has a neutralizing value of 45. After preliminary tests, the acid salt was increased from 1.5 to 2.5 g to stabilize egg white foam and prevent shrinkage of the cake during cooling.

Another modification made in the baking formula was the specific gravity of the egg white foam. The AACC-10-15 method (AACC, 1983) called for whipping the foam to end point specific gravity of 0.14 - 0.13. Preliminary tests indicated undersized cakes. According to Pyler (1973), maximum cake volume was obtained when the egg foam specific gravity was between 0.150 and 0.170. Above 0.170 the foam contained insufficient aeration to produce a light cake, while below 0.150 the stability was reduced to a point where considerable shrinkage occurred. Therefore, 0.150-0.170 was the standard set. The egg whites were whipped at No. 10 speed for 2 minutes and the specific gravity was determined (Appendix I). Samples were weighed on a Mettler PE 600 (Mettler Instrument Corp., Heightstown, NJ).

After removal from the oven, cakes were inverted and allowed to cool for 2 hours in pan. Other tests will be discussed in later sections.

### *3.10c Batter Measurements*

#### *3.10c (i) pH and Specific Gravity of Batter*

The pH and specific gravity were taken on remaining batter samples after cakes were placed in the oven. This was done so that it would not prolong the time before baking of the batter, and thus, air would be lost leading to loss of volume of the baked product.

A Corning Scientific Instrument pH meter (Model 5, Scientific Instruments, Medford, MA) with pencil combination electrode was used to make the pH measurements. The pH electrode was cleaned daily in a solution of pepsin in 0.1 N HCl to remove any protein, which would interfere with pH measurements.

The specific gravity of the batter followed the same method that was used in measuring foam specific gravity. A 1/4 cup dry measure was used and the cup was filled. Care was taken to prevent air voids. The batter was scraped off and weighed.

*3.10c (ii) Differential Scanning Calorimetry (DSC) Measurement  
of Cake Batter*

A fundamental requirement for a good cake is the formation of a structural framework of starch granules and protein viscous enough to trap gas bubbles formed on mixing by heating. The structure should be strong enough to be self-supporting when the cake is removed from the oven. A differential scanning calorimeter (DSC) with micro-processor controller and data handling (Perkin-Elmer System-4, Norwalk, CT) was used for the study of the gelatinization of starch and denaturation of protein in the baked product.

In DSC the sample and reference were each provided with individual heaters allowing the determination to be conducted with no temperature difference between sample and reference. The sample and reference pan were maintained at the same temperature.

The batter was made up at 1/10 the amount. All ingredients were mixed gently until combined. Originally, the batter with folded egg white was used for the DSC. The batter was highly aerated and made measuring difficult in the sample pans. Samples of batter between 25-35 mg. were weighed on a Perkin AD-6 computerized micro-balance (Perkin-Elmer, Norwalk, CT). The samples were encapsulated in stainless

steel capsules with lids that were crimped into position (Perkin Elmer, Norwalk, CT) and hermetically sealed to prevent evaporation of water.

A calibration procedure was used to obtain the calibration constant and fix the temperature scale accurately. Calibration of the instrument was carried out each time with indium standard ( $\Delta H$  fusion = 6.8 cal/g.; melting point 156.4 degrees C). The sample was heated from 30 to 140 degrees C at a scan rate of 10 degrees C/minute. The sensitivity was set at 5 mcal/sec. The reference material was a sealed empty aluminum pan.

A thermogram which is a plot of heat flow against temperature was derived from each sample. The thermogram gave the temperature ranges of starch gelatinization and protein denaturation.

### *3.10d Volume of Angel Food Cake*

After 2 hours of cooling in the pan, the cake was removed and the height was measured ( $h_d$ ). After an additional 2 hr. cooling, the height ( $h_c$ ) was taken again as an index of shrinkage (Appendix J). The  $h_c$  was used in the formula for calculating volume:

$$\text{Vol (v)} = \pi hc \frac{(D^2 - d^2)}{4}$$

The cake was cut on diameter with a serrated knife to cause minimum deformation. The maximum and minimum outside (D) and inside (d) diameters were measured (Appendix J).

### *3.10e Penetrometer*

The penetrometer (Lab Line, Inc., Melrose Park, IL) with a circular plunger of 5 cm in diameter and automatic timer was used to measure deformation of the baked product (Appendix K). The cake was cut with a serrated knife to avoid deformation.

Uniform cake slices of 4 X 4 cm were placed on the stand and the plunger was adjusted so that it touched the top of the cake piece. After 30 seconds of depression by the cylinder, a reading was taken in mm.

### *3.10f Cohesiveness Index*

A penetrometer (Lab Line, Inc., Melrose Park, IL) with a circular plunger (5 cm.) was used to measure the cohesive index. The pointer on the penetrometer scale was set to zero

when the plunger was brought to contact with the surface of the test slice without causing any of its deformation.

The plunger was placed on the surface of the cake slice. The depth of the penetration was recorded and returned immediately to its zero position. After a 15 second rest, the compression was repeated. Three compression cycles were applied to the same spot of each slice.

Cohesiveness indices were calculated from the data. The depths of penetration after the second and third compression cycle were each expressed as the percentage of the penetration depth after the first compression and the mean value of these two percentages was taken as a "cohesiveness index" (Kamel and Rasper, 1986).

### *3.10g Compressimeter*

The Baker Compressimeter (F. Watkins Corp, Wallace Tiernan, Belleville, NJ) was used to evaluate compression as an index to tenderness (Appendix L). A sample of 1 X 1 X 1 cm. was cut with a serrated knife from the center of the cake.

After several trial runs, the following criteria were used in deriving data:c4

1. compression of sample was run up to a distance of 2.5 cm.

2. plunger position 2 was used. The mechanical advantage is 6.06. This value is used when calculating the compression.

### *3.10h Color of Internal Crumb*

Interior color of the angel food cake was measured by a Hunter Lab Lab-Scan Spectrocolorimeter (Reston, VA; Appendix Q). Cake slices of 6X6X4 cm. were sufficient to completely fit over the aperture that supplied the light source.

### *3.10i Cell Uniformity*

Cell uniformity was defined as the number of cells being even or uneven in shape. The shape of the cell can be rounded or not fully formed.

Prints were made to record the cell uniformity. Pieces of cake measuring 6 X 7 X 2 cm. were cut with a serrated knife to prevent deformation. The pieces were placed on waxed paper and allowed to stand for three hours. An ink pad (Dennison Carter's Felt Stamp Pad, Black Ink, Size 1, Framingham, MA) was soaked with black ink (Sanford's Roll On Stamp-Pad Inker (Black) 358701, Sanford Corp., Bellwood, IL). The cake was removed from the waxed paper and the downward side was placed

on the ink pad. The piece was brought to the white paper and carefully pressed. The cake piece was replaced on the pad and again another print was made. This was repeated 4 times to allow the cake to soak up more ink and the print became darker.

A template 2.5 X 2.5 cm. was placed in the lower left hand corner of the print and marked off by pencil. The number of even and uneven cells were counted and recorded for evaluation. Even cake cell was defined as a round, small closed cell, an uneven cake cell was defined as a large, elongated, half-open cell.

### *3.10j Cell Size*

Cell size was examined by photocopying pieces of angel food cake (Savin # 7350 Copier, Stamford, CT). After the cakes were cut for volume measurement one half was used for the photocopying. Care was taken in cutting and handling to prevent deformation of the sample.

A piece of transparent plastic wrap was placed over the glass plate of the photocopier. The plastic was made as smooth as possible to prevent wrinkles, and thus, interference with the image produced. The settings used were: High contrast; darker copy; number 2.

*3.10k Scanning Electron Microscope (SEM) Examination of  
Baked Angel Food Cake*

The scanning electron microscope (JEOL, JSM-35C SEM, JEOL USA, Medford, MA) was used to observe the flour's performance in the baked product. Pieces of angel food cake, 4 X 8 X 4 cm. were freeze dried (VIRTIS Research Equip, Gardner, NY) for 48 hr. Angel food cake contains a high percentage of moisture and must be freeze-dried. Freeze-drying minimizes the generation of volatile vapors in vacuo inside the SEM (Kalab, 1983).

Samples were taken from a depth of 1 to 2 cm. below the surface of the cake sample in order to avoid areas affected by external effects. This was performed with a thin-blade spatula. The powder was sprinkled onto a stub with a silver adhesive (Conducting paint, Ladd Research Industries, Burlington, VT). The powder was distributed evenly over the stub, and with the silver cement the fragments were bonded to the stub more firmly.

The stubs with powdered particles were were sputter coated (SPI Sputter Coater, Structure Probe, Inc., West Chester, PA) with gold under an argon atmosphere for 40 sec. In sputter coating, the coating material (gold) is the cathode and the specimen is place on the anode.

The metal coated samples were examined by SEM at 600X and 1300X immediately after coating. Samples were kept in a desiccator before and after viewing.

### *3.11 Sensory Evaluation*

Sensory evaluation was based upon a modification of Quantitative Descriptive Analysis (QDA) (Stone and Sidel, 1985). A laboratory panel of 20 judges was selected from the graduate students of the Department of Human Nutrition and Foods. Panelists selected were not taking any medication which would interfere with taste acuity, and were available for all scheduled training and testing sessions. There were 3 men and 17 women panelists. Each panelist was assigned a number between 1 to 20 which was kept during the entire testing period.

A group language development session was conducted with all sensory panelists present. The investigator acted as a non-participatory leader in order to not bias the group. Each panel member was presented two slices of angel food cake which was baked from an unchlorinated and chlorinated flour. During the group discussion, the panelists identified the sensory attributes of angel food cake. Anchor words were decided upon by the group with increasing intensity of each attribute. The

leader recorded the attributes and anchor words.

The sensory leader is responsible for the preparation and presentation of test samples known to differ, but the assessors develop the language by themselves. If the panel members were being indoctrinated to QDA for the first time, the sensory leader almost certainly would be involved in the development of the vocabulary (Powers, 1984). Normally a vocabulary has to be originated by sampling food and discussing the value and meaning of the terms among the assessors and with the sensory leader. Practice than has to be acquired in scaling the sensory different attributes agreed upon.

The vocabulary developed by the panelists in the session was used for the preliminary scorecard. Each attribute was used to label a 15 cm. horizontal line, which was marked with appropriate anchor words. See Figure 2.

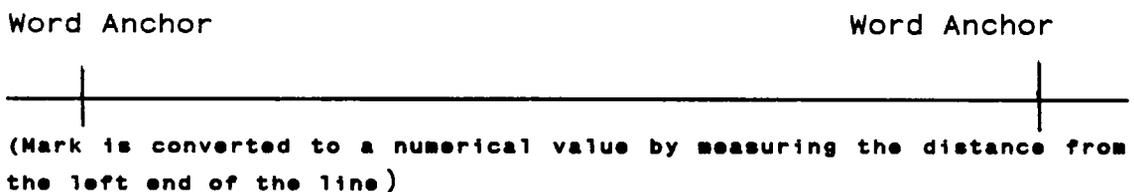


Figure 2: An example of a graphic rating scale (or line scale used to obtain relative intensity measures from the subjects).

The panelists were instructed to mark a vertical line across the line which best expressed the quantity of the attribute in relationship with the anchor word. The mark was converted to a numerical value by measuring the distance from the left end of the line.

The panelists agreed upon six attributes to be evaluated. The scorecard is shown in Fig. 3. The six attributes were color, cell size, cell uniformity, chewiness, cohesiveness, and texture. The data derived from these characteristics were also correlated with the data derived from the angel food cake analyses.

### *3.11a Operation and Environment of Sensory Analysis*

The sensory analysis sessions were conducted in a laboratory with individual booths of neutral background and fluorescent lights to prevent interaction among the panelists during the sessions.

All samples were prepared on the day of testing. The panelists analyzed 4 cakes per session. There were two sessions per week for 6 weeks. In keeping with the balanced incomplete block design (Table 4). Slices of cake 2 1/2 X 2 1/2 cm, were individually wrapped in plastic wrap. A random three digit number was assigned to each cake every session.



## Sensory Scorecard



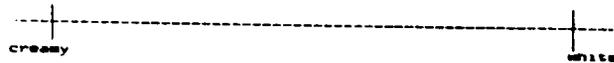
PANELIST # \_\_\_\_\_

### INSTRUCTIONS:

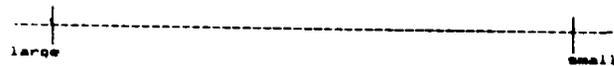
Please examine the following cake samples and rate them according to the characteristics discussed. Place a vertical line that best describes the property of the sample (e.g. 4/7 3/3).

Take sufficient time and sample to evaluate each characteristic.

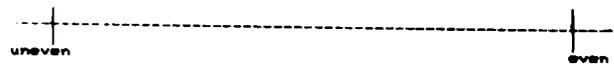
1. CRUMB COLOR:



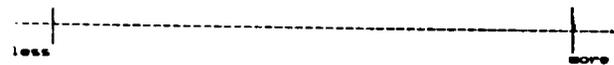
2. CELL SIZE:



3. CELL UNIFORMITY:



4. CHEWINESS:



5. COHESIVENESS:



6. TEXTURE:



**Fig. 3: Sensory scorecard for cake samples.**

That number appeared on the cake piece and also on the plate next to the cake. The color of the plate was royal blue (Party Plates, 9 in. Solo Cup Co., Urbana, IL) to contrast the color of the cake.

Individual trays were made up for each panelist. The tray contained plastic plate with 4 samples, napkin, scorecard, pencil and paper-cup with water. The paper cup was plain and odor free.

### *3.12 Statistical Analysis*

A randomized incomplete block design was used in the study. Randomized means the treatments are randomly applied to the units in the blocks. Assumptions are made that there is no interaction between blocks and treatments.

Statistical analysis was conducted on all main effects. The main effects were: flour, baked product data and sensory attributes. The flour data was analyzed first to determine if any significant differences existed. Once differences were established, all data were used in analyzing the balance of the results derived. The analyses included (1) Two way-ANOVA; (2) orthogonal contrasts; (3) multiple contrasts: Tukey test; (4) correlations. The multiple comparisons were developed to permit data snooping.

Orthogonal contrasts were used as part of the statistical analysis. In an ANOVA framework, it is possible by use of orthogonal contrasts to partition SST (treatment sum of square) into meaningful components. These components are associated with certain specific comparisons of particular interest. Orthogonal contrast provided a breakdown of the various components reflecting the relative contributions of the various terms.

Sample means were plotted to assess a trend in the data. The graphs yielded a general idea of patterns that were present. The goal was to evaluate a possible trend in the means in a statistical manner. The graphs were also used to study if interaction was occurring within the data. Two factors interact if the trend between treatments in one factor varies depending on which treatment is present in the other factor (O'Mahony, 1986a). Parallelism indicates no interaction. When the graphs are no longer parallel, there is no consistency between the factors, and there is interaction.

Correlations were also derived after all the data was analyzed for its significance. Correlation is a test that looks for similarities between two sets of data. The correlation examines how well the data relate to each other (O'Mahony, 1986b).

**CHAPTER IV**  
**RESULTS AND DISCUSSION**

**4.01 *Microscopy of Experimental Flours***

Light and scanning electron microscopy examination of flours complements each other. Scanning electron microscopy (SEM) provided information based on the shape, or location of the individual components of the system and light microscopy provided more information about the chemical composition of the components.

**4.01a *Scanning Electron Microscopy (SEM)***

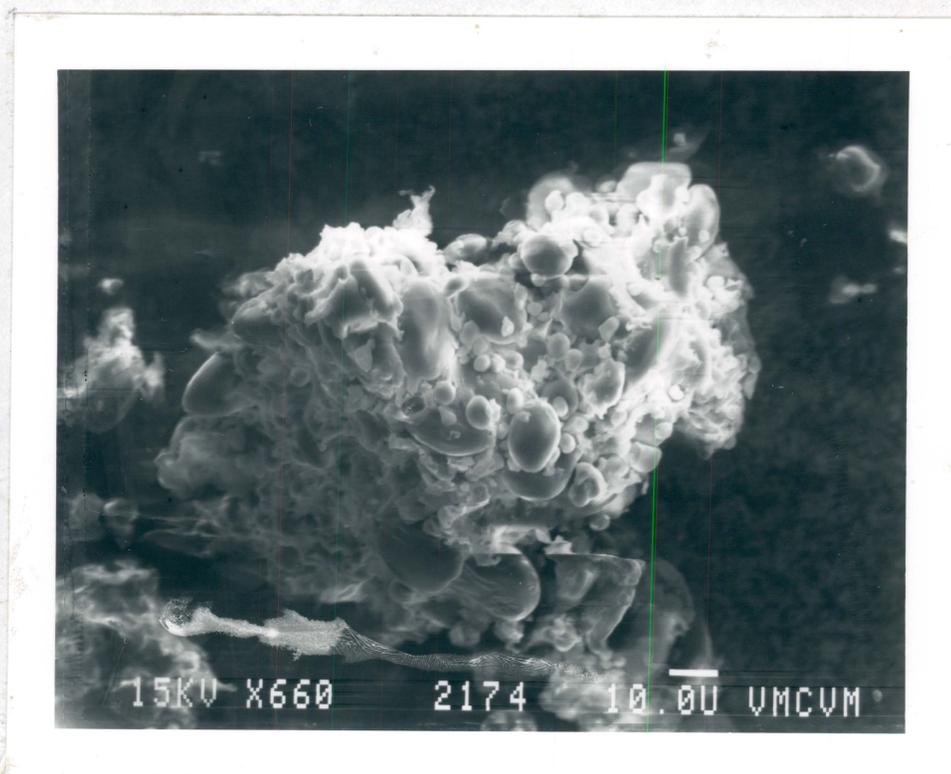
Samples of flour were attached to specimen stubs (see Methodology Chapter 3) and coated with gold. The absence of fixation, embedding and thin-sectioning techniques permitted the samples to be viewed in their most natural state (Aranyi and Hawrylewicz, 1969).

Wheat starch granules were present as two distinct types or populations. Although different in size and shape, they appeared equally distributed through the endosperm. Hall and Sayre (1970) described the two types of granules found in hard and soft wheats as:

- A. Type "A" granules in hard wheat starch are lenticular in shape with smooth surfaces. They range in size from 15-20 micrometers with most in the 20-35 micrometer range.
- B. Type "B" granules in soft wheat starch are spherical and smaller, ranging in diameter from 1-10 micrometers.

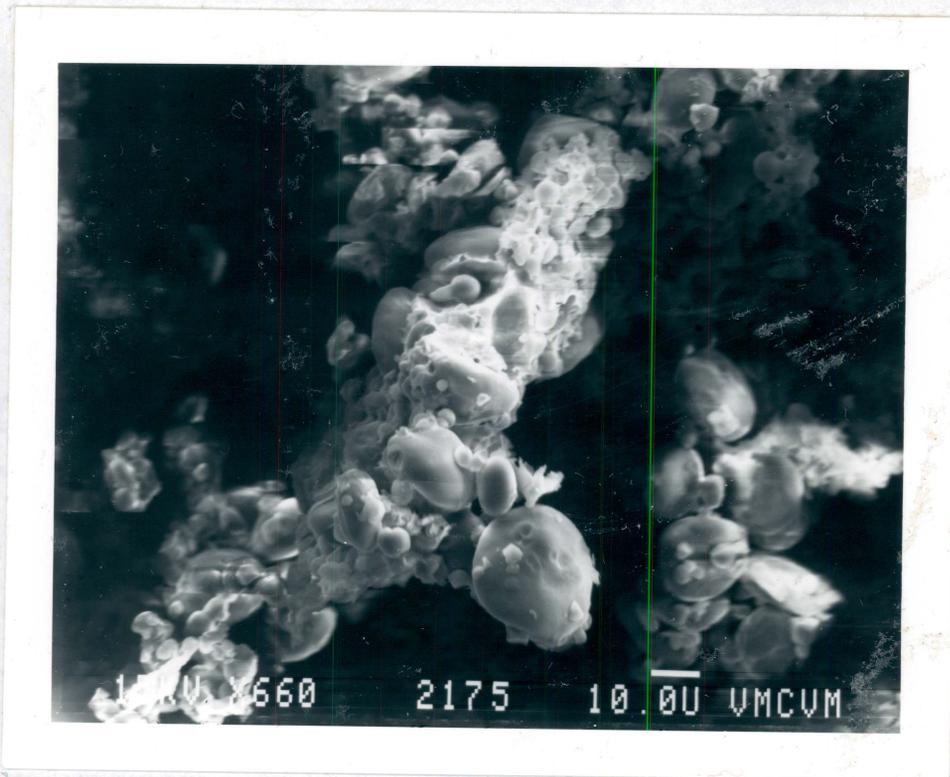
The flours used in the study were soft red winter wheats which were grown under different fertilization conditions. Type "B" granules were observed under the SEM. Figures 4 and 5 illustrate the soft wheat flour, chlorinated and unchlorinated, as it appeared under the SEM. The spherical "B" type granules show variations in shape from round to oval. The endosperm of wheat can be characterized as consisting of starch embedded in a protein matrix.

The flours were divided into 2 portions with 1 portion subjected to chlorination. The unchlorinated flour granule had the protein matrix attached to it (Figure 4). After bleaching (Figure 5) the granules appeared smoother. This is in agreement with Pomeranz (1976) who discussed the effect of various levels of chlorine treatment on the starch granule. At high treatment levels, SEM micrographs indicated that the



TRT 6 Unchlorinated flour: 660X magnification.

Fig.4: SEM observation of Coker 916 flour.



TRT 6 Chlorinated flour: 660X magnification.

Fig.5: SEM observation of Coker 916 flour.

surfaces of the starch were somewhat smoother than those at the lower treatments.

The protein of the mature wheat endosperm was present as an amorphous matrix that appears by SEM to coat or engulf the starch. Figure 4 illustrates this in the unchlorinated state. SEM does not reveal the presence of any discrete protein bodies. Rooney and coworkers (1983) have hypothesized that protein bodies are synthesized during development but apparently fuse and are deformed so that the mature matrix is an amorphous continuum. The SEM demonstrates this principle in a general way and it will be illustrated fully in the next section dealing with the light microscope and corresponding stains.

Air spaces exist between starch granules (Figures 4 and 5). The spaces are more evident in the chlorinated sample than in the unchlorinated sample. The SEM illustrates this point in a limited way and this factor will be more in evidence when viewed under the light microscope. The presence of air spaces explains that although the soft wheat possesses matrix protein it is not as tightly packed as hard wheat endosperms.

A summation of observations made under SEM is shown in Table 6. Chlorination had an effect on the starch granule.

TABLE 6

## Flour Differences Observed Under SEM

<u>Unchlorinated Flour</u>	<u>Chlorinated Flour</u>
1. Small to large type "B" granules	1. Small to large type "B" granules.
2. Starch granules are veiled by a protein matrix.	2. Starch granules are exposed.
3. Starch granules appear tightly packed.	3. Air spaces between starch granules are exposed.

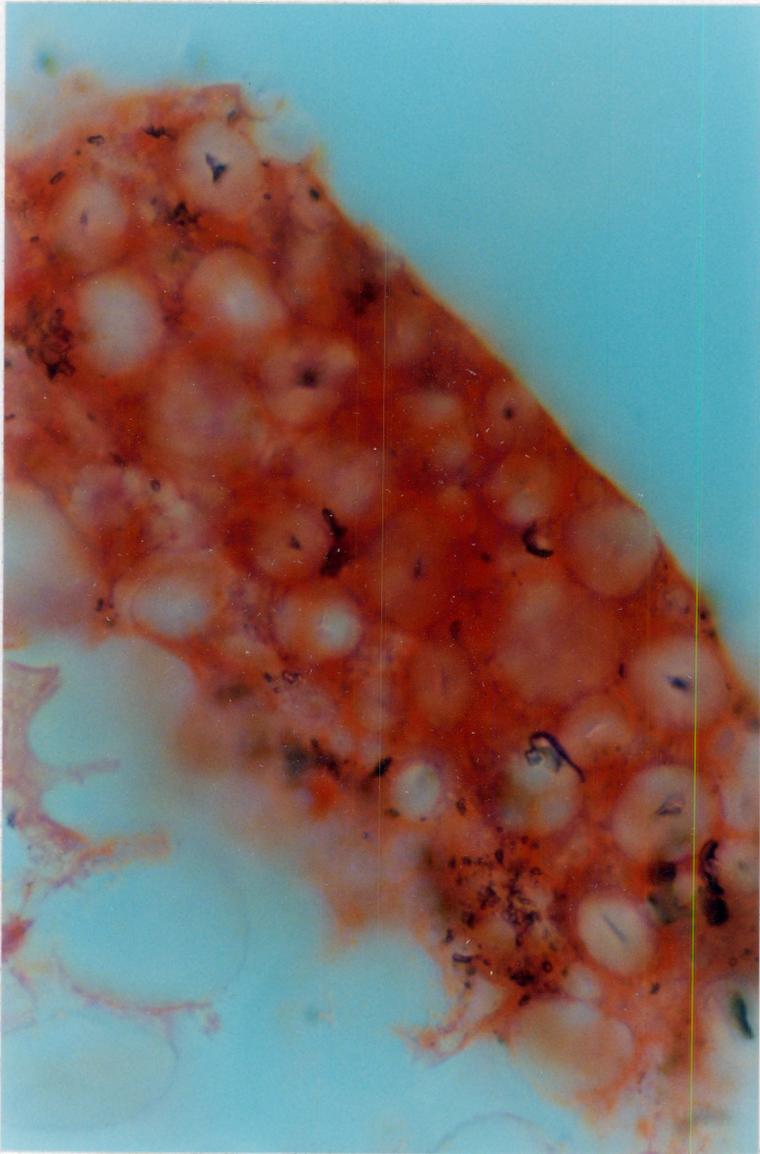
The starch granule was more exposed due to chlorination permitting greater absorption of fluid.

#### *4.01b Light Microscope*

The light microscope was used in conjunction with SEM to observe the biochemical nature of the flour. The dyes used with the light microscope serve as a complement to the observations made with SEM. The assumptions made with SEM were confirmed with the selected histochemical reagents (Moss, 1985).

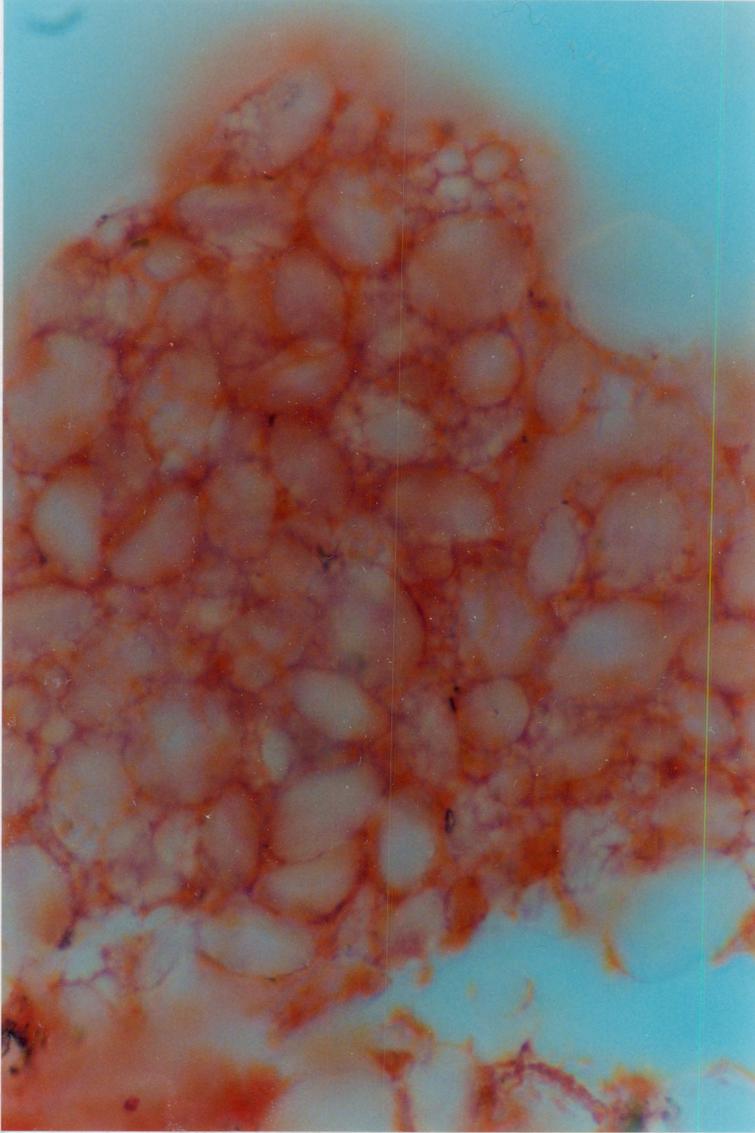
Figures 6 and 7 illustrate the effect of Ponceau 2R for staining protein in wheat flour. Figure 6 shows the unchlorinated flour where there is a higher concentration of protein adhering to the starch granules. Figure 7 shows the chlorinated flour of the same treatment with a lower protein concentration. Starch granules are more exposed in the chlorinated flour. The protein matrix was not as continuous as in hard red winter wheat (Pomeranz, 1986).

Figures 8 and 9 show two unchlorinated flours using chlorazol Black E stain. The blocking agent used to prevent protein staining was unavailable from the manufacture. The stain does show starch damaged due to milling. The amount of damaged starch is an important factor when considering the



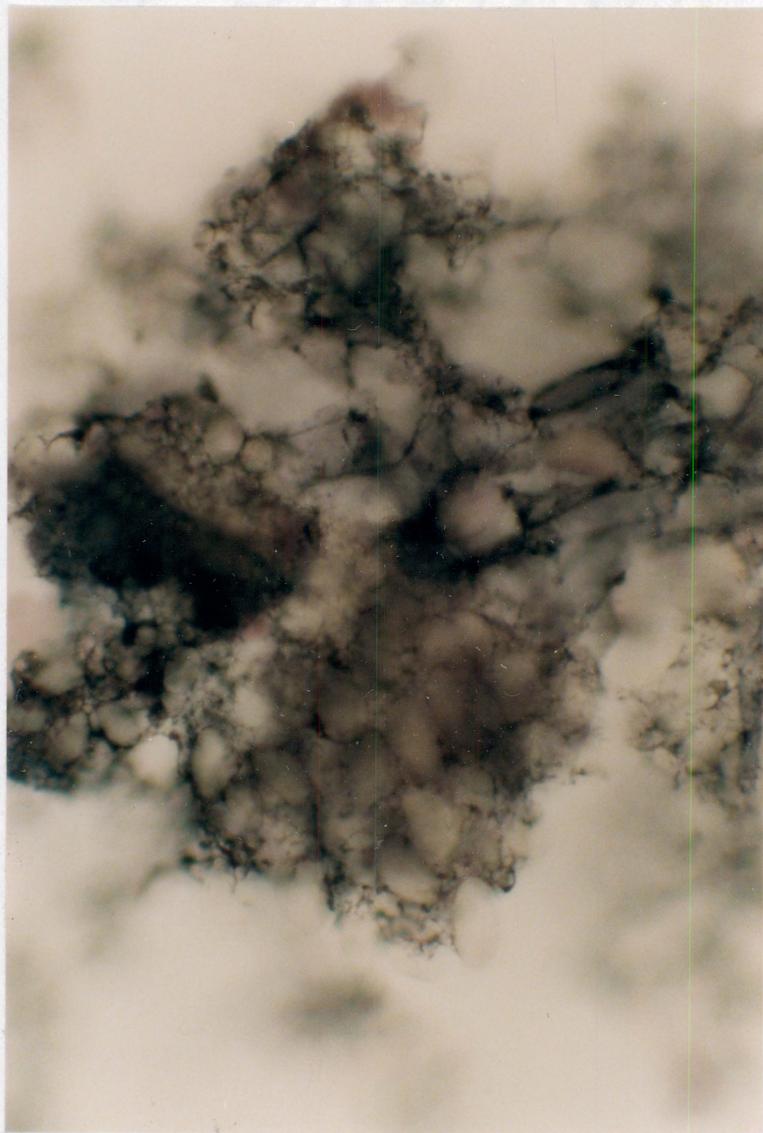
TRT. 4: Unchlorinated, dyed with Ponceau 2R (100X)

Fig. 6: Light microscope observation of Coker 916 flour.



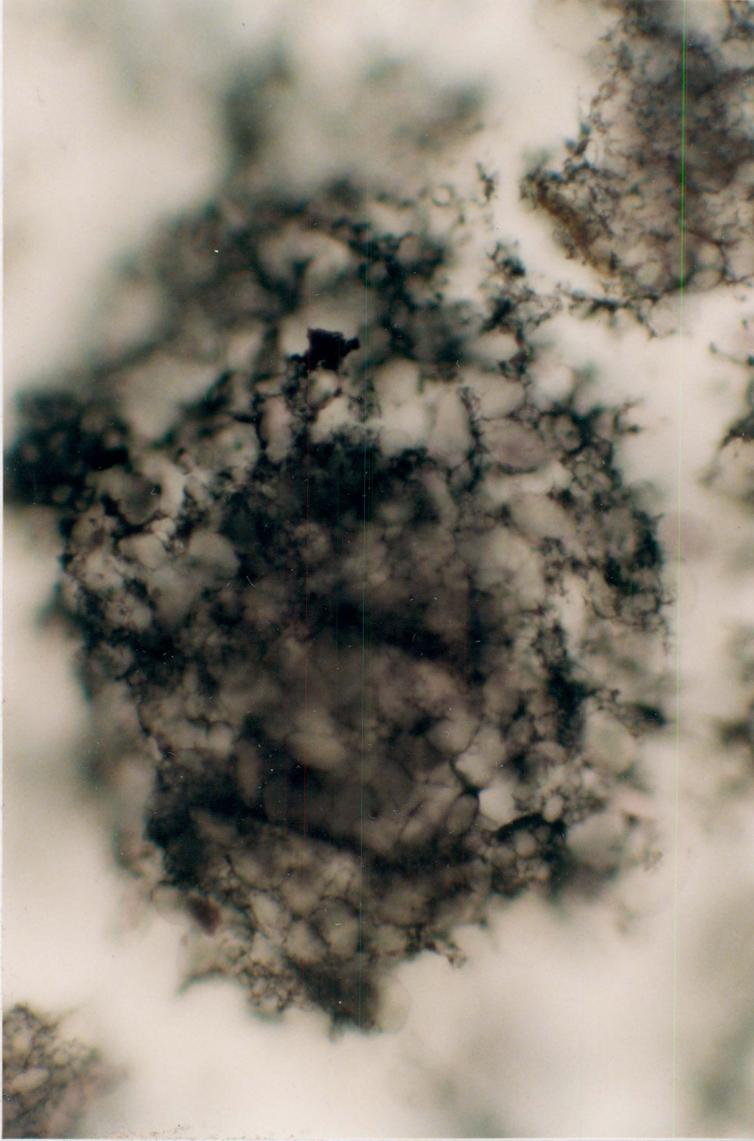
TRT.4: Chlorinated, dyed with Ponceau 2R (100X)

Fig. 7: Light microscope observation of Coker 916 flour.



TRT. 7: Unchlorinated, dyed with chbrazol blaçk E (40X)

Fig.8: Light microscope observation of Coker 916 flour.



TRT.6: Unchlorinated, dyed with chlorazol black E (40X)

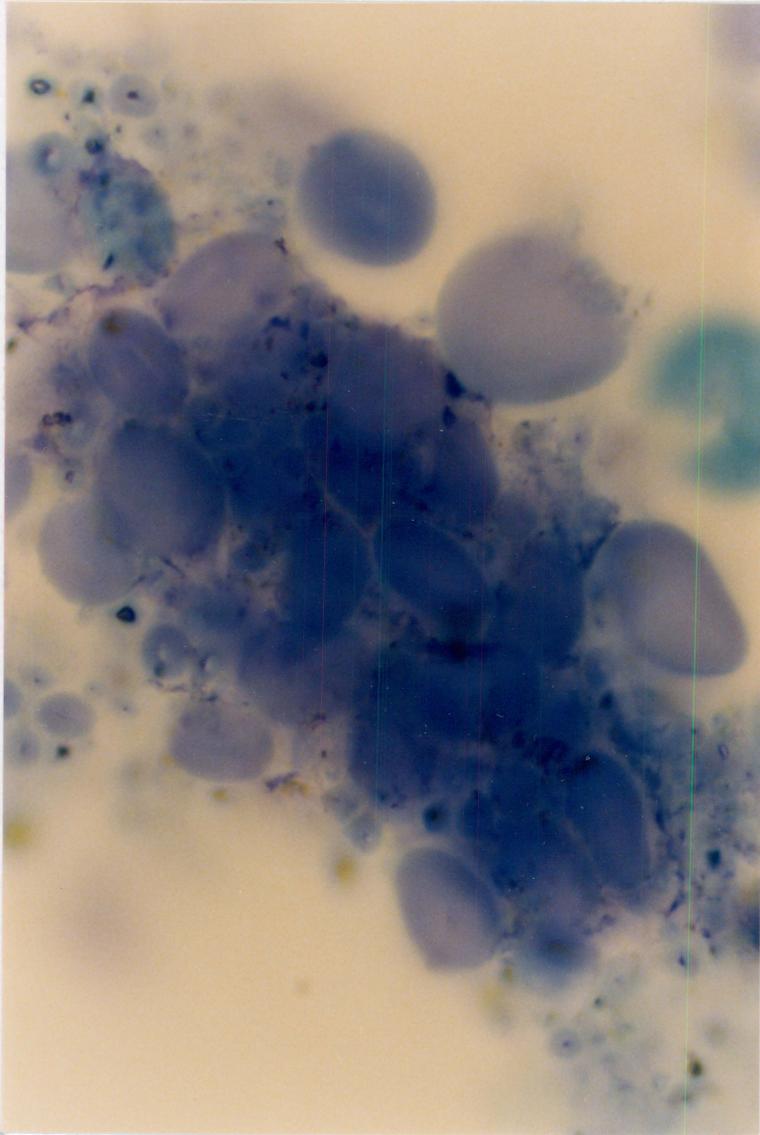
Fig. 9: Light microscope observation of Coker 916 flour.

baking quality of flour. This also suggests the value pin milling may have on cake flour in releasing starch granules from the protein matrix so that water can contact the starch granule causing structural changes in the baked product.

Figures 10, 11 illustrate the use of Fuchin in staining of chlorinated and unchlorinated treatment 6. Fuchin stained both protein and starch and allowed the examiner to make a check on the entire components of the flour. The shapes of the starch granules are more in evidence along with the spaces between them. Protein was dyed blue and was viewed as a veil over the granules. This is noted in Figures 10 and 11 (TRT 6- Unchlorinated and Chlorinated).

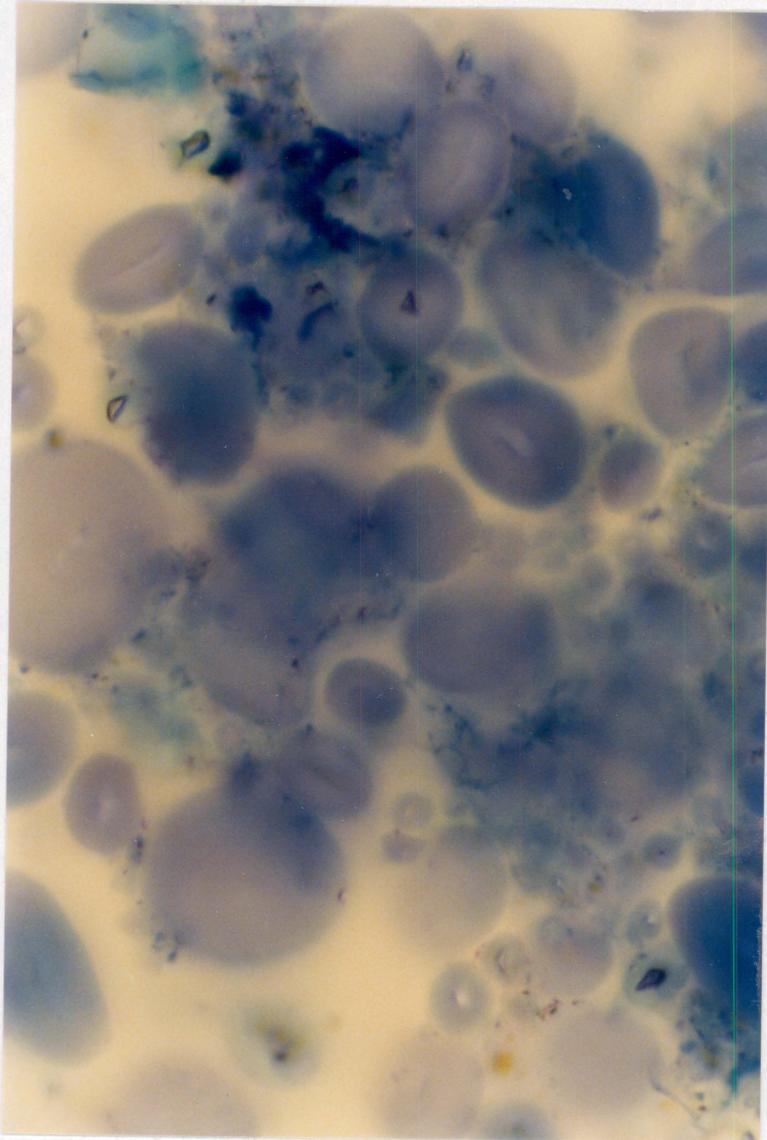
Some circular indentations are also noted in Figure 11 (TRT-6 Chlorinated). According to Hall and Sayre (1970), these indentations may be due to the packing effect of the smaller oval granules in the intact endosperm cell.

Each dye was very specific in staining and identifying the main biochemical constituents of the flour, namely protein and starch. The dyes were effective in observing the effects that chlorination had on these compounds. Fuchin dye was particularly effective in staining both the starch and protein matrix. This gave the observer a clearer insight and appreciation of the functional properties of the starch granule.



TRT6: Unchlorinated, dyed with fuchsin(100X)

Fig.10: Light microscope observation of Coker 916 flour.



TRT6: Chlorinated, dyed with fuchin (100X)

Fig.11: Light microscope observation of Coker 916 flour:

By SEM examination, the starch granules with evidence of damage to milling are packed, and appear smoother in chlorinated than in unchlorinated flour. The spherical shape of the starch granule is type "B" which is typical of soft wheat. The protein of both flours is an amorphous matrix that coats the starch granule.

Light microscopy stains are required for viewing and biochemical analysis. Air spaces are viewed between the starch granules, and protein coats the starch granule as observed with fuchin dye and Ponceau 2R. The fuchin dye also stained the starch granule to give the observer the size and shape of the starch granule. The smoothness of the starch granule could not be differentiated with the dyes used.

The SEM and light microscopy were used jointly for the examination of the wheat flour. Other analyses are also needed along with this mode of study.

#### ***4.02 Dough Rheological Properties: Farinograph***

Dough resistance is determined by the rheological properties of the dough, particularly viscosity. Surface properties of the dough, particularly sticking to the bowl walls and blades, contribute to the resistance measured. By

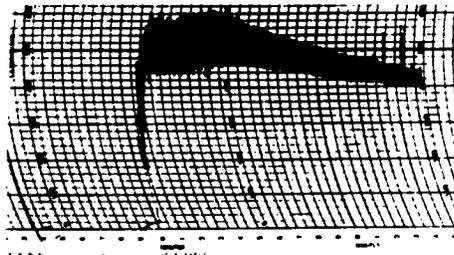
definition the viscosity of a material is the ratio of shear stress and the corresponding rate of shear.

The farinograph mixer acts as a sensing element that measures the dough's resistance to mixing during successive stages of its development (Bloksma, 1984).

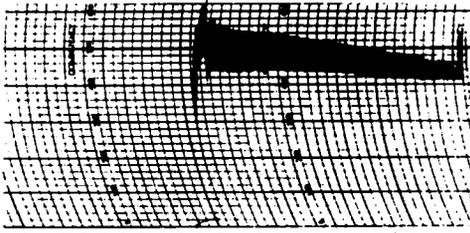
Figures 12 and 13 illustrate the farinograms of experimental soft red winter wheat flours both unchlorinated and chlorinated. The curves in each figure are a reflection of three basic processes: absorption of water (ABS), dough development, and dough breakdown.

Although these stages are simultaneously occurring, there is a period of dominance for each stage. During the early stages (up to approximately 2 minutes) the mixing curve is largely a reflection of the absorption of water by the various flour components (protein, starch, pentosans). There is an increase in viscosity of the major flour components as they imbibe water. There is also an interaction of protein fibrils that lead to the development of the gluten complex upon further mixing.

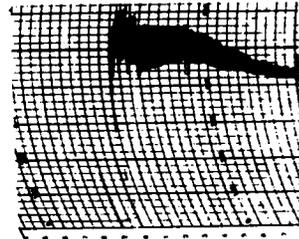
The farinograph absorption of water is dependent upon protein content, starch (including damaged starch), gluten strength and pentosans. Table 7 lists the percent absorbance of the experimental flours. Protein content increased with fertilization treatment for the non-chlorinated flours. This was true for the majority of flour except TRT 7. Chlorination



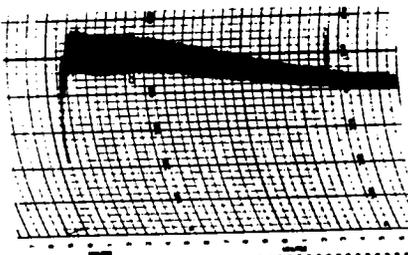
CONTROL; ABS: 57.7%



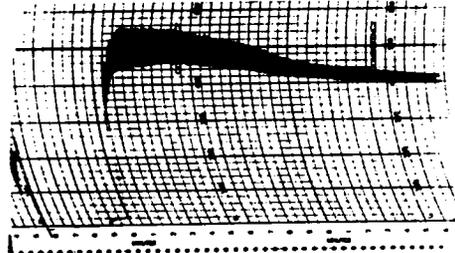
TRT.3;(NC) ABS:58.8%  
TRT.3-50+0



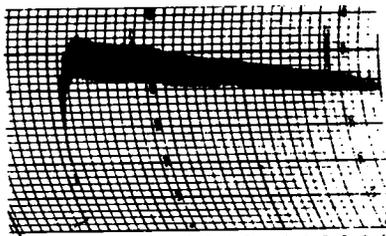
TRT.3;(C) ABS:58.6%  
TRT.3-50+0



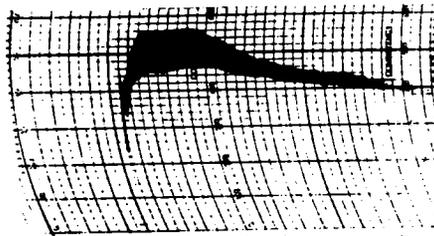
TRT.6;(NC) ABS:60.8%  
TRT.6-0+50



TRT.6;(C) ABS:59.8%  
TRT.6-0+50



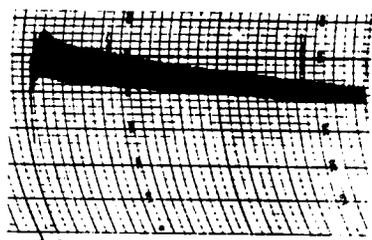
TRT.4;(NC) ABS:58.2%  
TRT.4-0+100



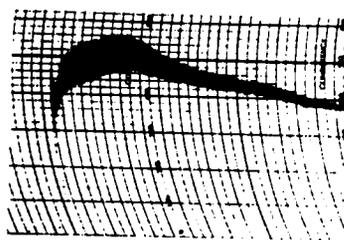
TRT.4;(C) ABS:61.2%  
TRT.4-0+100

NC - Not chlorinated  
C - Chlorinated

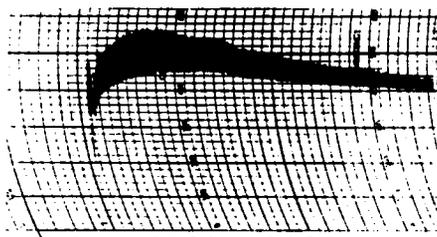
**Fig.12:Farinograms of flours.**



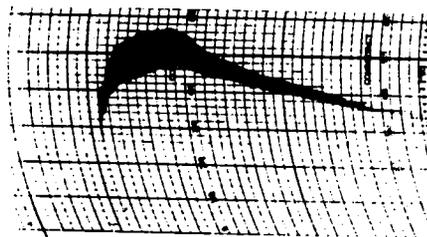
TRT.7;(NC) ABS:56.1%  
TRT.7-100+0



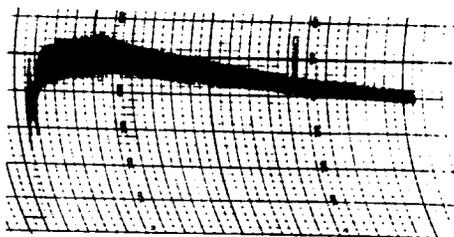
TRT.7;(C) ABS:58.0%  
TRT.7-100+0



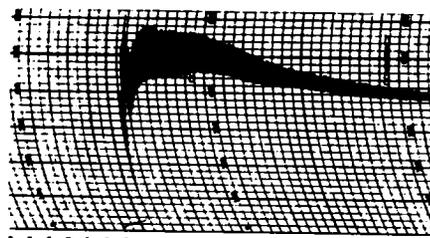
TRT.13(NC) ABS:60.4%  
TRT.13-50+100



TRT.13;(C) ABS:63.4%  
TRT.13-50+100



TRT.15(NC) ABS:61.5%  
TRT.15-100+50



TRT.15;(C) ABS:57.6%  
TRT.15-100+50

NC - Not chlorinated  
C - Chlorinated

**Fig.13: Farinograms of flours.**

TABLE 7  
Farinogram Data For Coker 916 Flours  
Unchlorinated & Chlorinated <sup>1</sup>

Flour	pH	Flour Protein %	Flour Absorb %	Peak Time (Min)	Mix Time Stab (Min)	Mix Time Index (BU)
TRT3 (NC) <sup>2</sup>	5.4a	8.63c	58.8	1.5a	9.0a	30a
TRT3 (C) <sup>3</sup>	4.1b	8.62c	58.6	1.0a	6.5b	50b
TRT6 (NC)	5.7a	8.81c	58.2	1.75a	9.0a	40a
TRT6 (C)	4.3b	7.95d	61.2	2.0a	7.0b	40a
TRT4 (NC)	5.6a	8.96b	60.8	1.75a	5.5a	50a
TRT4 (C)	4.2b	9.01b	59.8	3.0b	4.5b	90b
TRT7 (NC)	5.9a	9.54b	56.1	1.25a	4.5a	70a
TRT7 (C)	4.2b	9.23b	58.0	4.0b	3.5b	100b
TRT13 (NC)	5.4a	10.75a	60.4	3.5a	6.5a	60a
TRT13 (C)	3.9b	10.02a	63.4	3.5a	3.0b	110b
TRT15 (NC)	5.6a	10.10a	61.5	4.0a	7.5a	50a
TRT15 (C)	4.1b	9.06b	57.6	2.75b	4.5b	80b
Control	4.2	8.22	57.7	4.5	10.5	50

<sup>1</sup>All data on 14% moisture basis

<sup>2</sup>NC - Not Chlorinated

<sup>3</sup>C - Chlorinated

Unlike letters within the same treatment are significantly different,  $p < 0.05$ .

caused an increase in absorption for some of the flours except TRT 3, TRT 7, and TRT 15. For TRT 7 and TRT 15, there was a decrease in absorbency.

The absorbency is one indicator of baking quality which indicated the presence of damaged starch. The increase in absorbency upon flour chlorination without a corresponding increase in protein is indicative of damaged starch. These results are in agreement with Tipple, Meredith and Holas (1978) who also found protein content as well as damaged starch as excellent predictors of absorption.

The curves that appear in Figures 12 and 13 and reported in Table 7 had a short peak time and reached an unstable viscosity very quickly. The curves are characteristic of soft red winter wheat flour (D'Appolonia, 1984). The flours contained a percent flour protein ranging from 7.95 to 10.75%. These percentages were typical of soft wheat flour for cakes, cookies and crackers.

The peak time is a measure of the time (minutes) needed for the curve to reach its peak or point of maximum dough consistency. The value usually varies between flours. The peak time is indicative of gluten quality, with strong flours yielding a longer developing time than weak flours. Table 7 lists the peak time of the flours which ranged between 1 - 4.0

minutes. Statistical analysis indicated fertilization had a significant effect on increasing peak time. The correlation coefficient between protein content and peak time for unchlorinated and chlorinated flour was 0.78 and 0.70, respectively.

There were a few instances whereby the protein content and peak time relationship were not consistent. Treatment 7 (100 + 0) had a moderate protein content and the lowest peak time for an unchlorinated flour. The control (commercial flour) had the lowest protein content, but had the highest peak time. This may indicate that not only protein content but protein quality may also have an effect on gluten development. The commercial flour had two peaks in the farinogram. There is no explanation for this occurrence, but when this happens the second peak is considered peak time.

The mixing time stability indicated the flour's tolerance to mixing. The difference in time was measured between the point at which the top of the curve first intercepted the 500 BU line (arrival time) and the point at which the top of the curve left the 500 BU (departure time).

Table 7 lists the mixing time stability for each flour. The higher number is an indication of the flour's tolerance to mixing. The chlorinated flours had a lower mixing time stability than the corresponding non-chlorinated flour. Statistical analysis indicated significant difference between

chlorinated and unchlorinated flours. Flours with lower protein content had a higher mixing time stability ( $r = -0.44$ ) in the unchlorinated state.

The Mixing Time Index (MTI) was calculated as the difference in BU between the top of the curve measured 5 minutes after the peak was reached. Generally, flours with good tolerance to mixing have a low MTI value while the higher MTI indicates a weaker flour. Table 7 lists the MTI values. The chlorinated flours had a higher MTI value than the unchlorinated with the exception of TRT 6 (0+50). Statistical analysis indicated that chlorination had a significant effect on MTI. TRT 13 had the highest MTI value, but the flour also had the lowest pH value (3.9). This indicated over-chlorination and breakdown of flour functionality. A strong correlation ( $-0.966$ ) existed between the mixing time stability and mixing time intolerance for chlorinated flours.

Chlorination appeared to have an effect on the rheological properties of the dough. Mixing time stability of the flour was decreased due to chlorination. This indicated that overmixing of the batter will have a negative effect on the baked product and that short mixing times are required for the experimental flours.

#### *4.03 Proteins*

Wheat grain proteins have a prominent position in determining dough properties and overall suitability of grain for processing (Wrigley and Bietz, 1989). The formation of protein structure of doughs at all levels is dependent on interactions by means of secondary forces, of the polypeptide backbone and the side chains with one another, with water, with ions, and with other molecules. The side chains range from non-polar groups, both aliphatic and aromatic, to the polar acidic and basic groups, which may be negatively or positively charged, respectively. Hydrogen bonding is one of the common secondary interactions in proteins and, along with stabilizing secondary and tertiary structures, is frequently important in protein aggregation during mixing of doughs and batters (Wrigley and Bietz, 1989).

##### *4.03a Effect of Fertilization*

Soft red winter wheat was fertilized under different levels of nitrogen at growth stages 25 and 30 (Table 8). Growth stages 25 and 30 are a system that was set up by Zadoks and coworkers (1974) as a scale for recording the growth stages of cereal. Growth stage 25 is defined as tillering of

TABLE 8

## Protein Content of Soft Red Winter Wheat

Wheat	Protein %
TRT3 (NC)	8.63c
TRT3 (C)	8.62c
TRT6 (NC)	8.81c*
TRT6 (C)	7.95d
TRT4 (NC)	8.96b
TRT4 (C)	9.01b
TRT7 (NC)	9.54b
TRT7 (C)	9.23b
TRT13 (NC)	10.75a
TRT13 (C)	10.02a
TRT15 (NC)	10.10a*
TRT15 (C)	9.06b
Commercial Flour	8.22

NC - Not chlorinated  
C - Chlorinated  
Unlike letters in the column are significantly different,  $p < 0.05$ .  
\*Indicates significant differences due to chlorination.

the plant whereby the main shoot and 5 tillers (sprout) appear. Growth stage 30 is defined when the stem erects itself.

Table 8 lists the protein contents for each fertilization regimen before and after chlorination. There was an increase in percent protein as total fertilization increased. There was a significant difference in protein content of flour as a result of fertilization.

Chlorination decreased the protein content of all treatments with the exception of TRT 3 and 4. The effect of chlorination was significant for treatments 6 (0 + 50) and 15 (100 + 50). The amount of protein in treatment 15 was lowered in value similar to the protein content of treatments 4 (0 + 100) and 7 (100 + 0). This change will be examined throughout the study as related to functionality and performance.

Some mention should be on the procedure used for protein content determination. The Kjeldahl method involves wet digestion of the sample by heating with concentrated sulfuric acid in the presence of a catalyst. This caused a conversion of organic nitrogen to ammonia which is retained as ammonium sulfate until released by distillation in the presence of sodium hydroxide.

The distillate is trapped in the sulfuric acid solution and the amount of ammonia, hence nitrogen is determined by

titration with 0.1N NaOH. There is one point that error may enter into the procedure. This may be the time during carbonization. It is recommended that an additional 30 minutes boil after the digest has become clear (Osborne, 1982). The purpose is to ensure the decomposition of more resistant intermediate compounds in that the boiling temperature may be insufficient to bring about their complete decomposition during carbonization.

#### *4.03b Glutenin of Wheat Flour*

Glutenin molecules are polymers comprising polypeptide subunits linked by interchain disulfide bonds. Glutenin extracted from flour or dough has been shown to comprise extremely large structures with estimated molecular weights of up to several million (Schofield, 1987).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was used to analyze for the presence of glutenin in flour. The technique separates a highly complex mixture of proteins according to molecular weight.

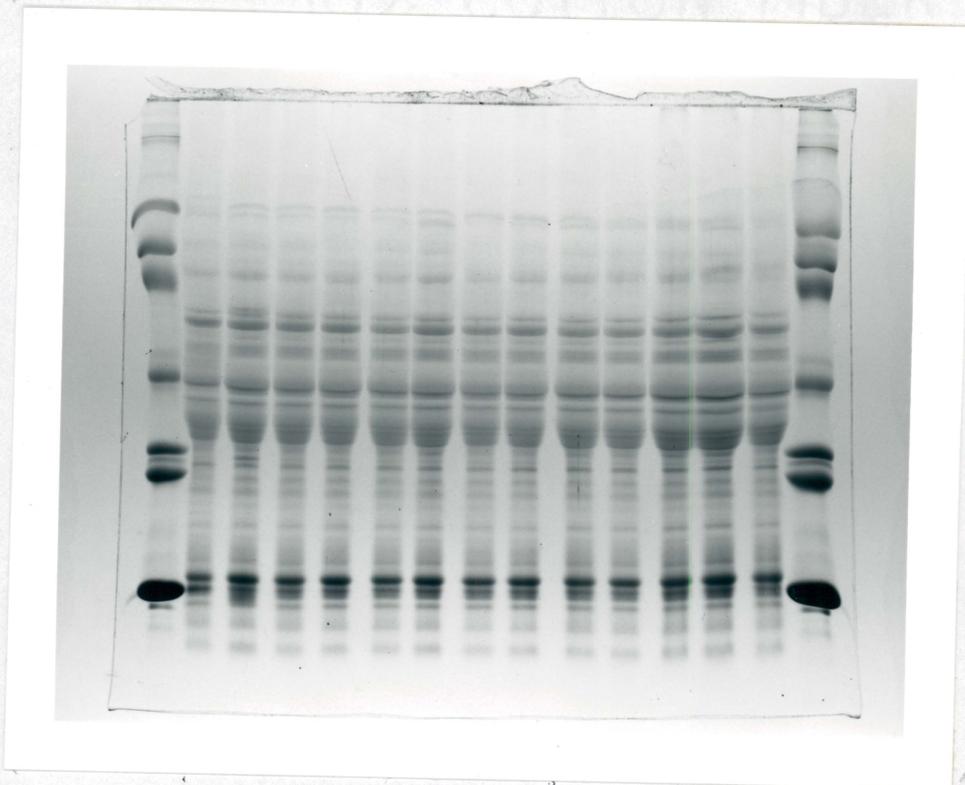
Glutenin molecules are considered as linear chains of polypeptide subunits called "concatenations". These subunits are joined head-to-tail by inter-chain disulfide bonds (Schofield, 1987). The mixture of polypeptide subunits are

cross-linked by intermolecular (intersubunit) disulfide bonds in such a way to yield a broad spectrum of molecular weights ranging up to higher polymers with molecular weights in the millions (Lasztity, 1983). These disulfide bonds are composed of cysteine in which thiol group of two molecules of cysteine have been oxidized to a disulfide group to provide a covalent cross-linkage between them. Due to these cross-linkages present, the bonds were broken before electrophoresis by heating the preparation in the presence of SDS and beta-mercaptoethanol, which reduces them to sulfhydryl groups.

#### *4.03b (i) Results of Electrophoresis*

Figure 14 presents electrophoresis patterns of the gel. The gel was able to accommodate all the samples plus molecular weight markers. The gels showed all samples were similar in protein molecular content. The bands in the upper molecular weight regions appeared very faint. This is one indication of the characteristic of soft red winter wheat with its low protein content.

Other evidence for the presence of these weak stains was the percent of protein content which indicated that the high molecular weight units would be low. The soft red winter wheat studied had a percent protein range of 8-11%. The farinograph results (Chapter 4, Section 4.02) indicated the



From left to right:

Lane 1: Marker (Lysozyme; Trypsinogen; Carbonic Anhydrase; Albumin, egg;  
Albumin, bovine; Phosphorylase b;  $\beta$ -galactosidase; Myosin)

Lane 2: Control

Lane 3: TRT3 (50 + 0): Not chlorinated

Lane 4: TRT3 (50 + 0): Chlorinated

Lane 5: TRT4 (0 + 100): Not chlorinated

Lane 6: TRT4 (0 + 100): Chlorinated

Lane 7: TRT6 (0 + 50): Not chlorinated

Lane 8: TRT6: (0 + 50): Chlorinated

Lane 9: TRT7: (100 + 0): Not chlorinated

Lane 10: TRT7: (100 + 0): Chlorinated

Lane 11: TRT13: (50 + 100): Not chlorinated

Lane 12: TRT13: (50 + 100): Chlorinated

Lane 13: TRT15: (100 + 50): Not chlorinated

Lane 14: TRT15: (100 + 50): Chlorinated

Lane 15: Marker

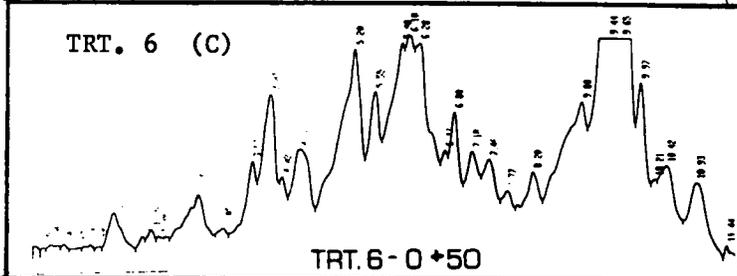
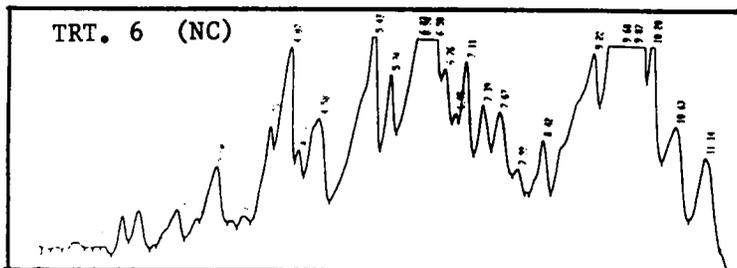
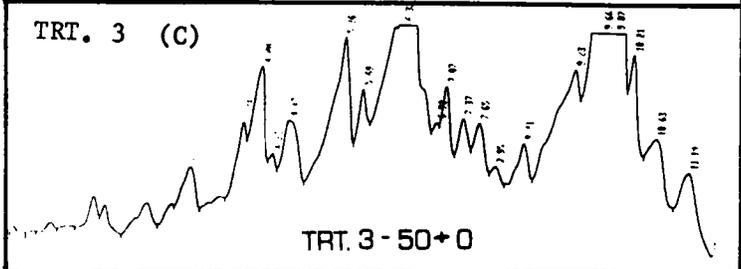
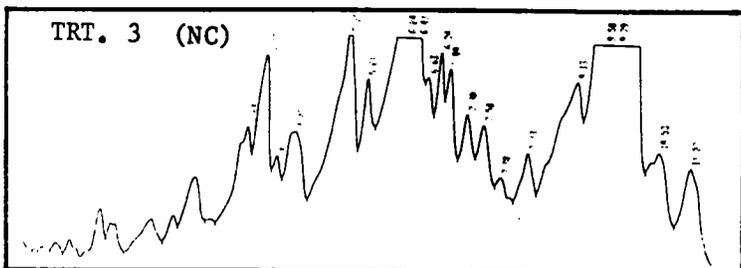
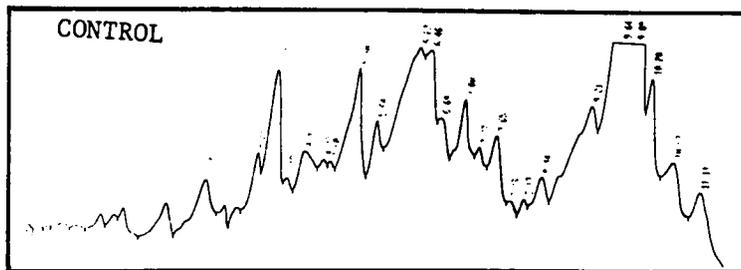
Fig.14: SDS-PAGE of Çoker 916 glutenin.

dough's structure and stability. The mixing tolerance (Bushuk, 1974) was low for the flours tested. Accordingly, this is another indication of the absence of high molecular weight subunits. These high molecular weight subunits are present in harder flours necessary for bread-baking. They take longer to develop, but produce a stronger, more stable dough.

Bushuk (1974) also found that flours that show a long development time in the farinograph contain a much higher proportion of insoluble glutenin than flours with a short development time. Studies with gel filtration suggest that glutenin of wheats with longer dough development times have a higher average molecular weight.

The gels were scanned with a densitomer. Comparison of unchlorinated and chlorinated flour are represented in Figures 15, 16, 17. The scans provided quantitative data of the content of the gel. It was discussed in the previous section, that the protein contents of some of the flours were affected by chlorination (Treatments 6 and 15 having significant change). The effect of chlorination on particular bands is the focus. The log molecular weights of the markers versus scanning times were plotted (Appendix M, Figure 18). This plot was made to determine the change in molecular weight of the various bands.

Three bands were selected : 2:82 (80,000 daltons), 4:00



NC=NOT CHLORINATED

C=CHLORINATED

**Fig.15: Glutenin gel scans.**





(50,000 daltons), and 5:45 (40,000 daltons). The areas under the curve are  $10^6$ . The graph was used to determine the molecular weights from these times.

The molecular weights affected by chlorination were between 40,000 and 80,000 daltons. Table 9 lists the scan rates that were changed. Significant changes as a result of chlorination occurred in band 5:45, for Treatments 6, 4, 13, and 15. The band occurring at 4:0 showed significant changes for Treatments 6, 13, and 15. The band occurring at 2:85 had changes occurring but with no significance in one flour (Treatment 13).

Chlorine has been investigated with its effect upon the protein content of the flour. Kulp (1972) found changes in the proteins by measuring its effect on : a) solubility, b) aromatic amino acids (possible chlorination of tyrosine) and sulfhydryl groups. The protein solubility increased with addition of chlorine. This trend indicated that the chlorine cleaved high molecular weight proteins into smaller water soluble fragments by hydrolytic and oxidative actions.

Tsen (1972) found that sulfhydryl groups were oxidized by chlorination. Farinograph studies indicated that the dough reached an increased dough stability up to 2 oz. chlorine, but a rapid weakening was evident at higher levels. The farinograph results (Table 7) show this trend with a decrease in the Mixing Time Index for chlorinated flour. The trend

TABLE 9

Densitometer Scan Readings, Glutenin  
(AREA x 10<sup>6</sup>)

Flour	Band@ 2:82	Band@ 4:00	Band@ 5:45
TRT3 (NC) <sup>1</sup>	5.85a	10.99a	23.73a
TRT3 (C) <sup>2</sup>	5.21a	10.27a	22.53a
TRT6 (NC)	3.83a	12.23a	21.37a
TRT6 (C)	2.76a	3.54b	11.16b
TRT4 (NC)	5.76a	11.47a	22.23a
TRT4 (C)	5.22a	8.87a	9.60b
TRT7 (NC)	5.99a	11.47a	23.60a
TRT7 (C)	5.27a	10.67a	22.07a
TRT13 (NC)	7.17a	13.26a	21.47a
TRT13 (C)	1.53b	5.99b	12.86b
TRT15 (NC)	4.24a	16.90a	25.17a
TRT15 (C)	2.59a	2.49b	9.04b
Control	4.30	9.92	15.40

<sup>1</sup>NC - Not Chlorinated  
<sup>2</sup>C - Chlorinated  
 Unlike letters within the same treatment are significantly different, p < 0.05.

reflects the oxidative and hydrolytic changes of proteins.

Gough and coworkers (1978) in their review of flour chlorination state that 60% of the cyst(e)ine in the flour is affected. Disulfide linkages due to cystine residues in glutenin can occur either within (intra-) or between (inter-) protein chains and determine the functional properties of the native molecules. If all bonds were within single chains, glutenin would be low in molecular weight and tightly folded, like gliadin. If all disulfides were interchain, the resulting highly branched polymer undoubtedly would not allow suitable alignment of proteins to form a dough. An optimum balance of inter- and intra- chain disulfide bonds is essential for good glutenin performance (Bietz and co-workers, 1973).

Another factor that could contribute to the changes observed in the scans is the change in amino acid content of glutamic acid. The most prominent amino acid, glutamic acid, occurs mostly as the amide, glutamine, which promotes hydrogen bonding between glutenin molecules. The presence of very large amounts of glutamine means there is a potential for very extensive interaction through hydrogen bonds. Although these bonds are relatively weak, the occurrence of large numbers of them in wheat protein enhance their importance and contribute to viscoelasticity (Schofield and Booth, 1983).

Leucine is another amino acid which plays a crucial role

with glutenin. This non-polar side group amino acid can interact with each other, especially in an aqueous environment, to form hydrophobic bonds. This is another important interaction that helps in stabilizing the native conformation.

The bonds illustrate changes that have occurred, but it appears that a particular area was more measurable than other areas (bands at 2:82, 4:00. 5:45). The effect of chlorine was evident in the particular areas noted. The next section will place more emphasis on the strength of the disulfide bond and the effect of chlorine, if any, on the bond.

#### *4.03b (ii) Determination of Glutenin Content Without Beta-Mercaptoethanol*

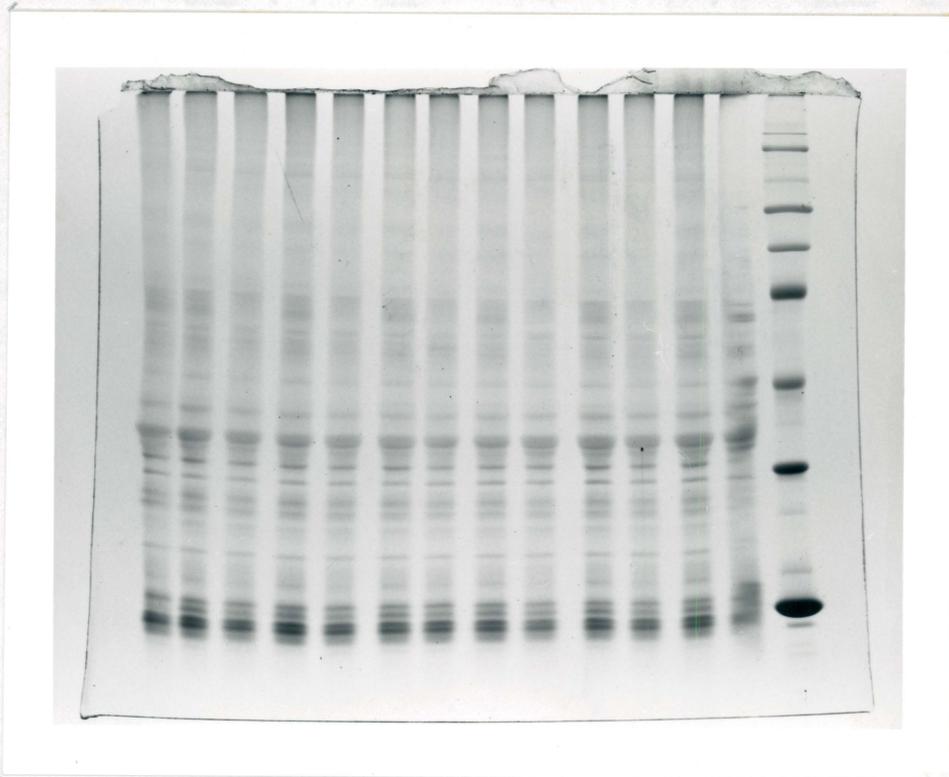
Duplicate SDS-PAGE gels were run, whereby, beta-mercaptoethanol was eliminated from the procedure. Beta-mercaptoethanol was used to break up the disulfide bonds in the glutenin and cause separation of the subunits. SDS gel electrophoresis of an SDS extract of flour without disulfide bond rupture produced a rather different molecular size profile, with much more protein in the high molecular weight range as a continuous streak from the origin. On reduction this material breaks down by disulfide bond rupture to discrete zones throughout much of the molecular-weight range

(low molecular weight and high molecular weight polypeptides). These results suggested that native glutenin is an association of polypeptides of many sizes in many combinations, producing a wide spectrum of oligomeric glutenin proteins (Wrigley and Bietz, 1984).

Figure 20 illustrates the electrophoretic gel produced by the flours without beta-mercaptoethanol. The gel shows that there are streaks at the top of the gel in the high molecular weight region, but as the electrophoresis developed there was separation in the low molecular region.

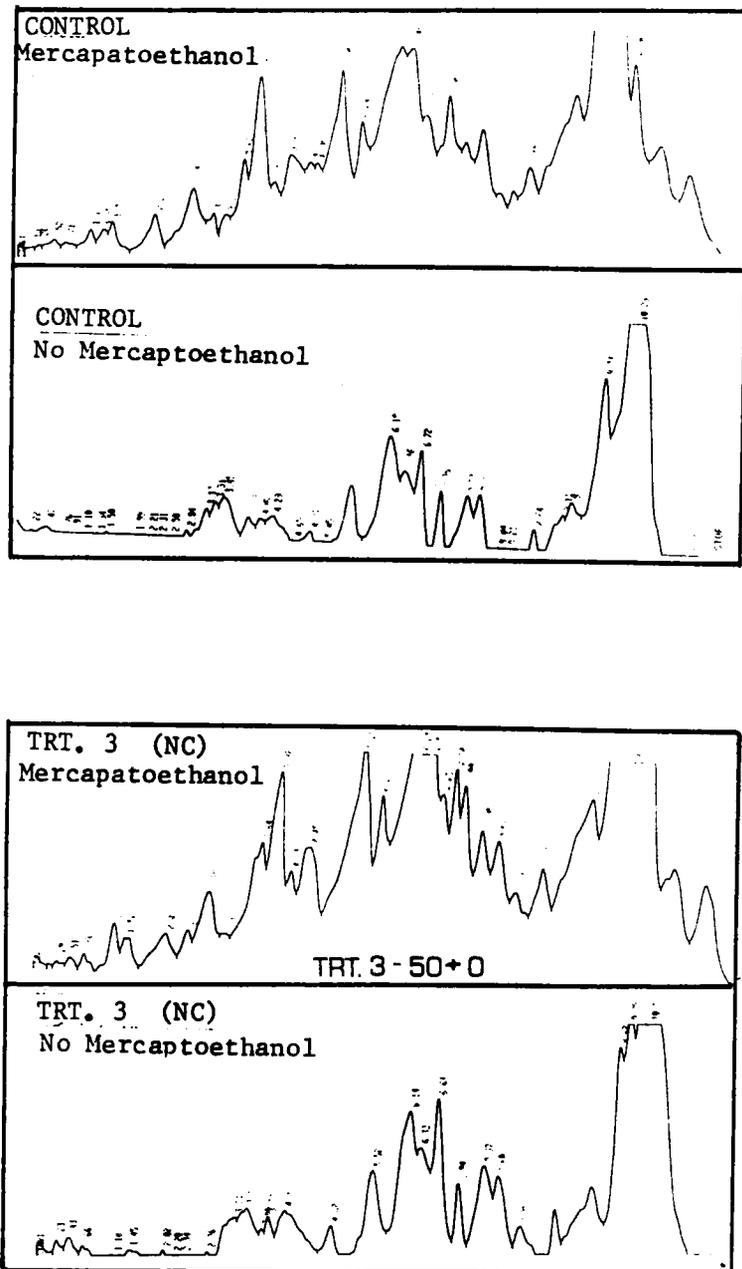
The results of the densitometer scans showed the regions of separation. The log molecular weights of the markers versus scanning times were plotted (Appendix M, Figure 19). Without beta-mercaptoethanol, the separations were at different regions, but there was some separation taking place in the low molecular regions. Figures 21, 22, 23, 24, 25 and 26 show an overlay of densitometer scans with and without beta-mercaptoethanol. There was very little separation that occurred in the high molecular weight regions, but separation occurred in the low molecular weight region.

There is a strong indication directed toward the strength of the bonds and the effect of chlorination on the bonds. Chlorinated flours have similar scans to the unchlorinated flour, therefore chlorination would appear to have a weak influence on the bonds, especially in the high molecular



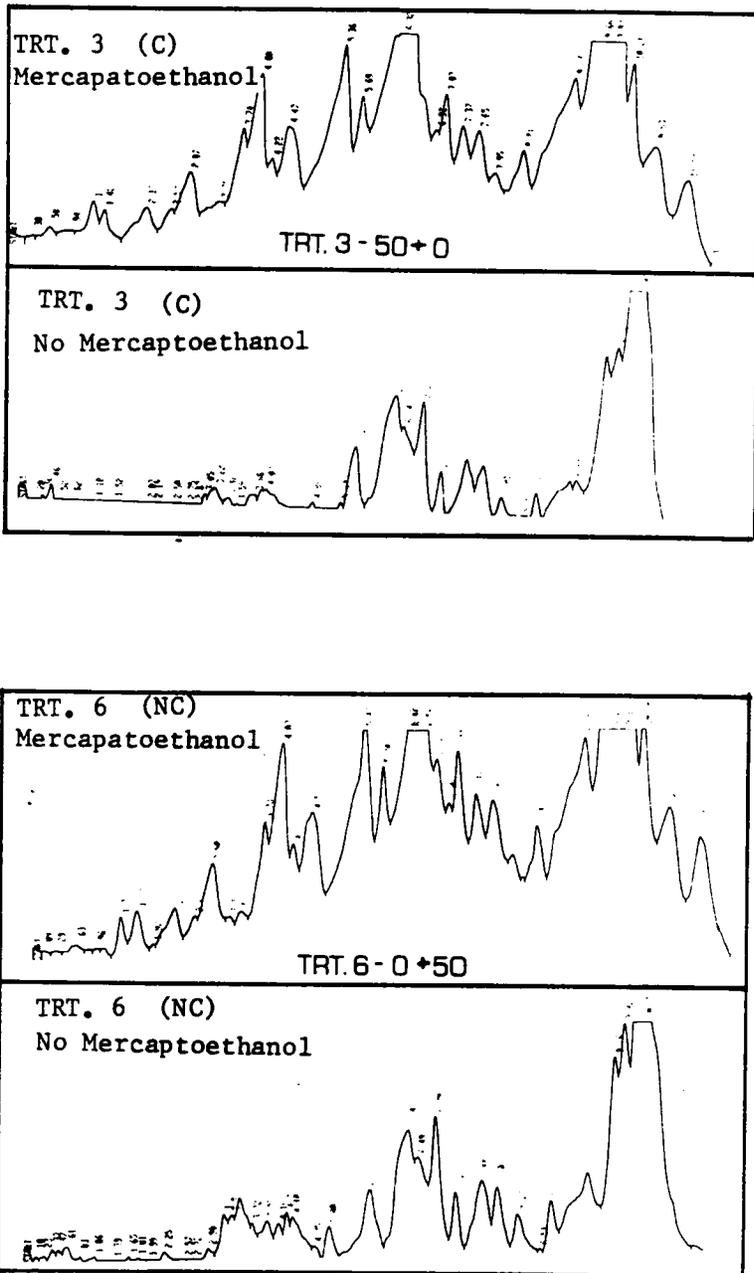
From left to right  
 Lane 1: Control  
 Lane 2: TRT3 (50 + 0): Not chlorinated  
 Lane 3: TRT3 (50 + 0): Chlorinated  
 Lane 4: TRT4 (0 + 100): Not chlorinated  
 Lane 5: TRT4 (0 + 100): Chlorinated  
 Lane 6: TRT6 (0 + 50): Not chlorinated  
 Lane 7: TRT6 (0 + 50): Chlorinated  
 Lane 8: TRT7 (100 + 0): Not chlorinated  
 Lane 9: TRT7 (100 + 0): Chlorinated  
 Lane 10: TRT13 (50 + 100): Not chlorinated  
 Lane 11: TRT13 (50 + 100): Chlorinated  
 Lane 12: TRT15 (100 + 50): Not chlorinated  
 Lane 13: TRT15 (100 + 50): Chlorinated  
 Lane 14: Marker (Lysozyme; Trypsinogen; Carbonic Anhydrase; Albumin, egg;  
 Albumin, bovine; Phosphorylase b;  $\beta$ -galactosidase; Myosin)

Fig. 20: SDS - PAGE of Coker 916 glutenin.  
 w/o  $\beta$ -mercaptoethanol.



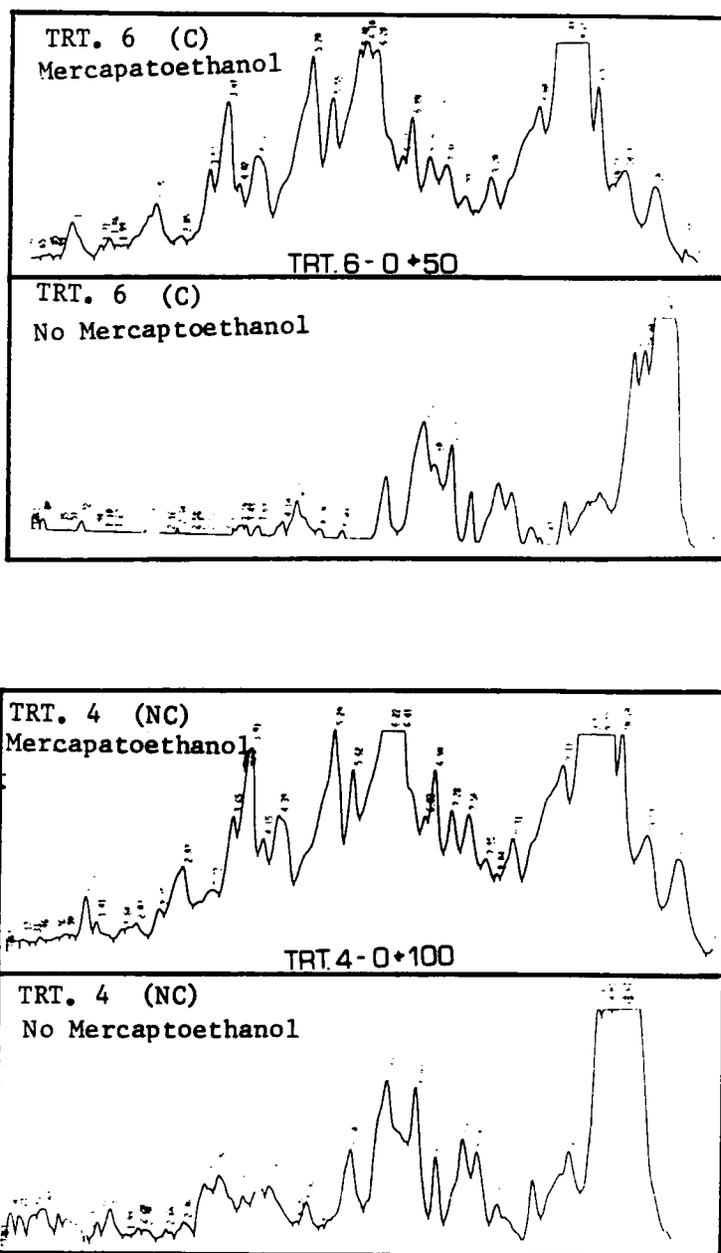
NC=NOT CHLORINATED

Fig.21:Glutenin gel scans w/o  $\beta$ -mercaptoethanol.



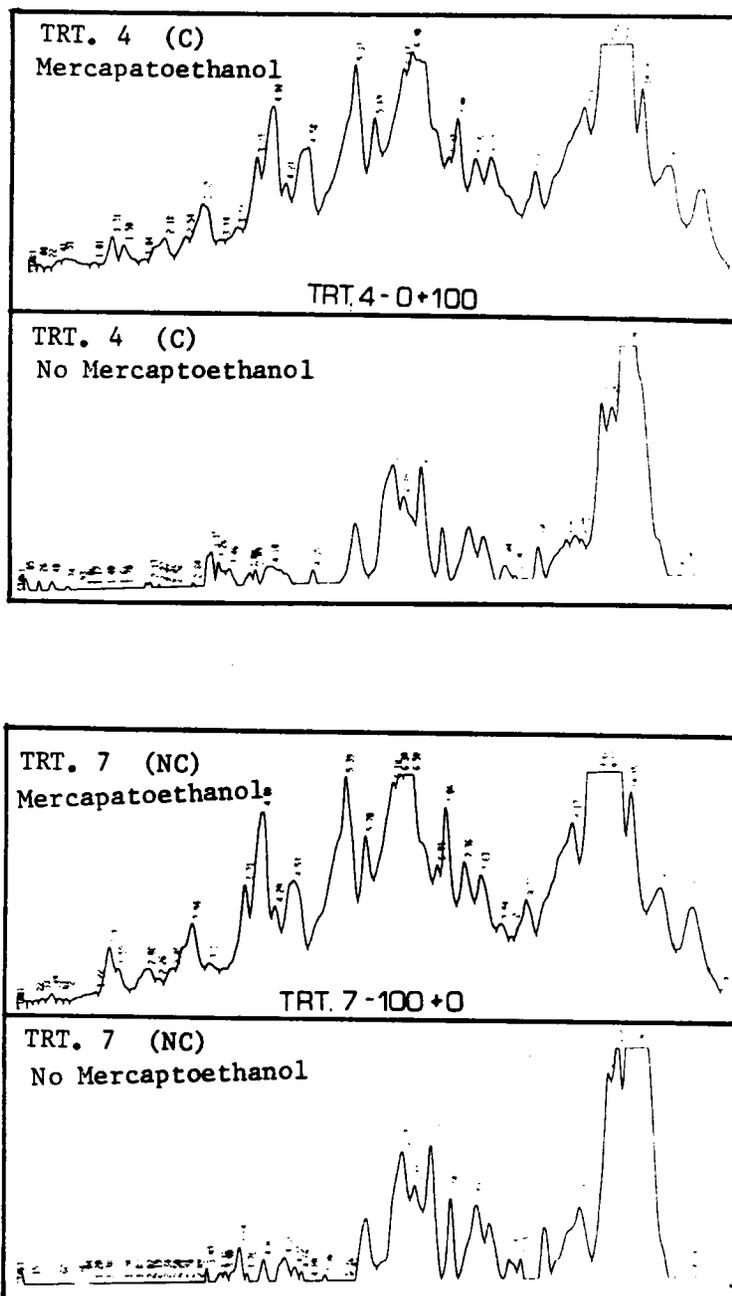
NC=NOT CHLORINATED  
C=CHLORINATED

Fig. 22 Glutenin gel scans w/o  $\beta$ -mercaptoethanol.



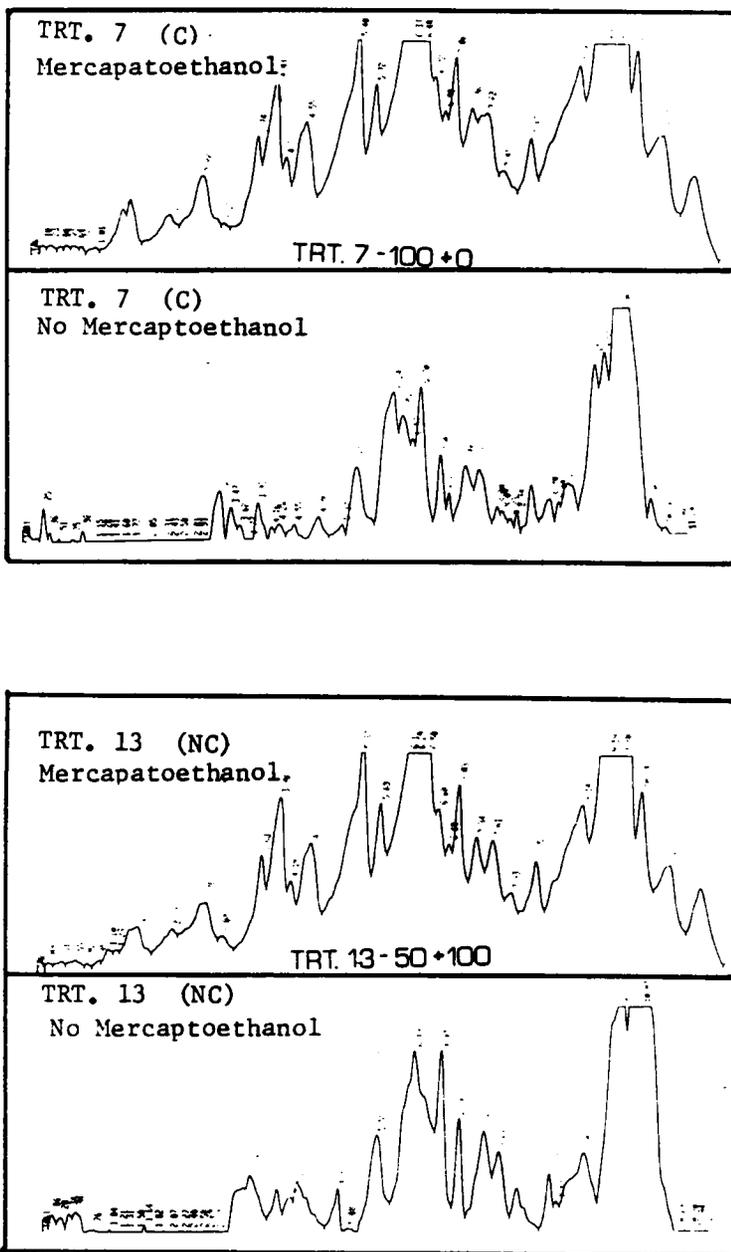
NC=NOT CHLORINATED  
C=CHLORINATED

Fig. 23. Glutenin gel scans w/o  $\beta$ -mercaptoethanol.



NC=NOT CHLORINATED  
C=CHLORINATED

Fig.24:Glutenin gel scans w/o  $\beta$ -mercaptoethanol.



NC=NOT CHLORINATED  
C=CHLORINATED

Fig.25:Glutenin gel scans w/o  $\beta$ -mercaptoethanol.

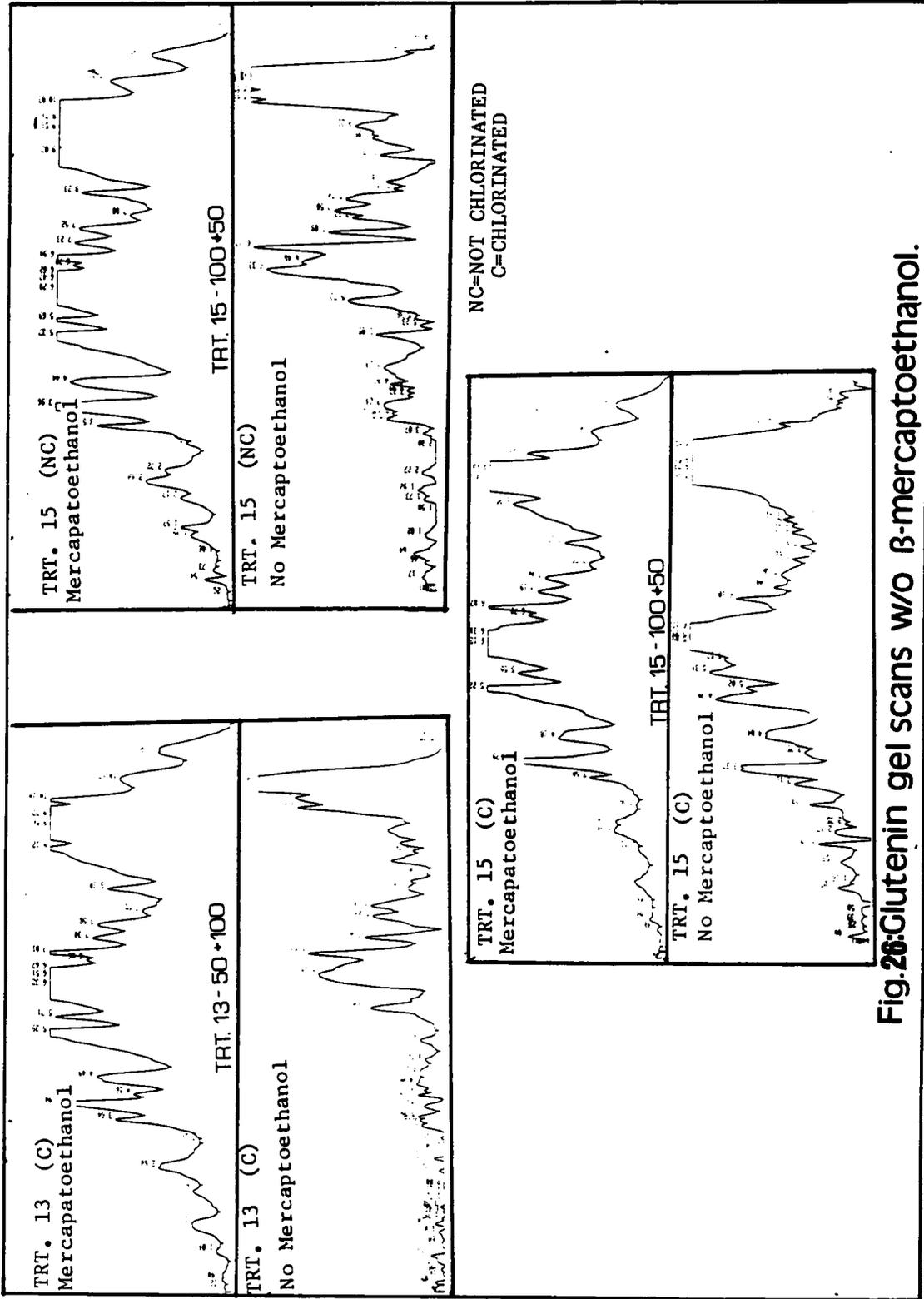


Fig.26:Glutenin gel scans w/o  $\beta$ -mercaptoethanol.

weight region.

The elution that developed was not that clear, but the disulfide bonds were broken due to the bands that were developed. Two indications are given about the strength of the disulfide bonds and the position of the bonds. The order by which the chains are linked will have an effect on the strength of the bonds. The disulfide bond linking the units is a covalent bond therefore, it would appear to have the same type of bonding throughout. The position of these bonds is the next step to observe, since the high molecular weight did not enter the gel.

There is a strong possibility because of the high molecular weight subunits present in glutenin, there must be more of an intra-molecular bonding holding the units in place. Studying the over-lays, the low molecular weight region contained more intermolecular bonds whereby they were linked across to other regions which were easily accessible to chemical action.

The scans showed the distribution of the disulfide bonds among the flours as about the same. The peaks were higher in the higher protein flours, but the distribution of the peaks were the same. Peaks appeared on the densitometer at about 50,000 daltons. This was derived by plotting the log molecular weight of standards versus their mobility (time) (Appendix M, Figure 19). This is in agreement with Kohbrehel

and Bushuk (1978) who found molecular mass estimates of 40-50,000 suggesting that glutenin had been disassociated completely to relatively low molecular weight subunits without cleavage of disulfide bonds.

Evidence has shown (Shofield, 1988) that in amino acid sequence data for some high molecular weight subunits of glutenin, cysteine residues are located near to the N- and C-terminal ends of the polypeptide chains but not in the central regions. When cleavage takes place there is a small decrease in molecular size. According to Schofield (1988) this indicated that these subunits were located near the end(s) of the polypeptide chains. Not all glutenin subunits are alike, however, for some high molecular weight subunits. Cleavage of cysteine residues resulting in a marked increase in molecular weight indicating that in these subunits one or more cysteine residues must be located toward the middle of the polypeptide chain.

The conclusions about the glutenin composition were:

1. intramolecular disulfide bonds were reduced only after the unfolding of the polypeptide chains.
2. there was easy accessibility to the intermolecular disulfide bonds.
3. chlorination showed very little effect in rupturing of the bonds.

#### 4.03c *Gliadin Proteins*

As was established in the previous section, glutenin is the major contributor to the elasticity and cohesive strength of wheat gluten protein. Doughs that are too elastic and inextensible will give a poor baking performance, especially with breads. Gliadins have been proposed as acting as "plasticizers" which interact both among themselves and with glutenins. However, being relatively small molecules, they interfere with the ability of glutenin polymers to form very extended cooperative networks associated through large numbers of covalent bonds. Gliadin does appear to contribute importantly to gluten's rheological characteristics.

Gliadin is a heterogenous mixture of proteins soluble in 70 percent ethanol. Most gliadins have a very similar amino acid composition rich in glutamine and proline. The concentrations of glutamine residues account for the association of gliadins through hydrogen bonding.

##### 4.03c (i) *Electrophoresis*

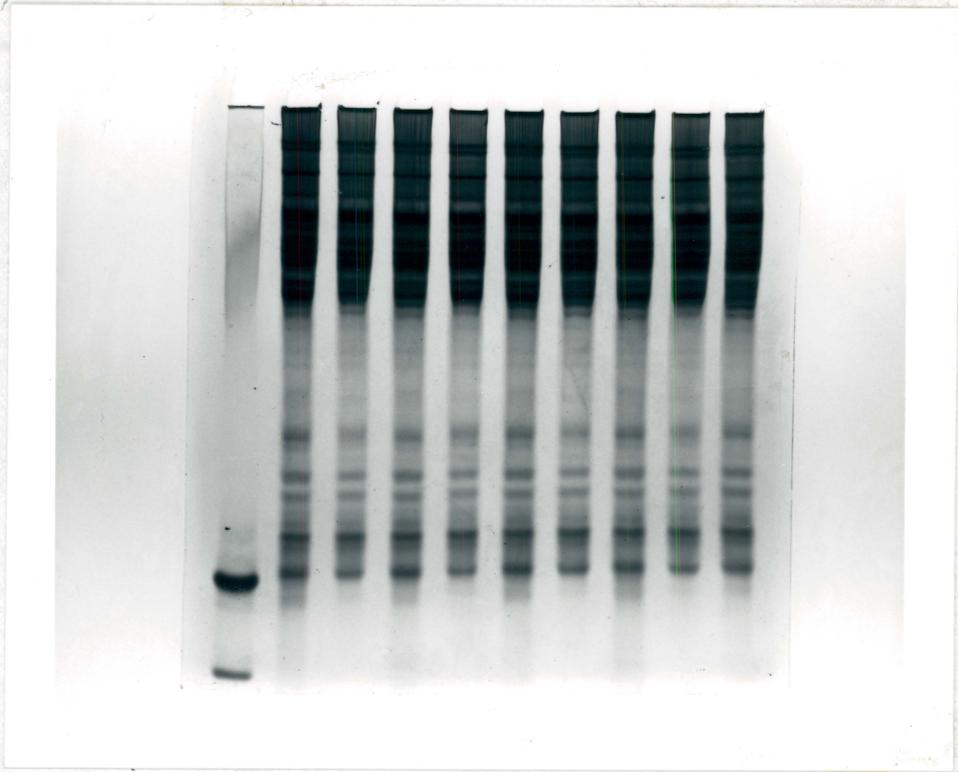
Gliadin proteins are more commonly used in PAGE because of their ready solubility and simplicity of extraction. The aqueous ethanolic extract (70%) which was used has been found to give the best results with PAGE (Khan, 1982). Khan (1982)

also found that a uniform gel (6%) gave satisfactory separation of gliadin proteins. Figures 27 and 28 show the electrophoresis runs of the gliadin proteins of the soft red winter wheat.

The choice of a buffer system affects the PAGE separation of gliadins. The aluminum lactate pH 3.1 buffer system has been used by most workers. This system was used in this study for separating gliadins. However, there was a problem using the standard molecular weights in electrophoresis. Phosphorylase b and glyceraldehyde 3P dehydrogenase were not able to run the entire gel and were streaked when finally resolved. One factor that could account for the poor resolution was the buffer, aluminum lactate. A large number of chemical changes may depress the action of the enzyme. Enzymes are usually inactivated by salts of heavy metals such as silver and mercury, in very small concentrations. Aluminum has been found to be less toxic than other metals.

The hypothesis as to why the enzymes did not penetrate the gel is based on Haldane (1965) who showed that the metal ions (Al) combine with anions of enzyme, but not with the neutral molecules or cations. More precisely the aluminum combines with that particular acidic group of the enzyme whose dissociation determines its efficiency as a catalyst.

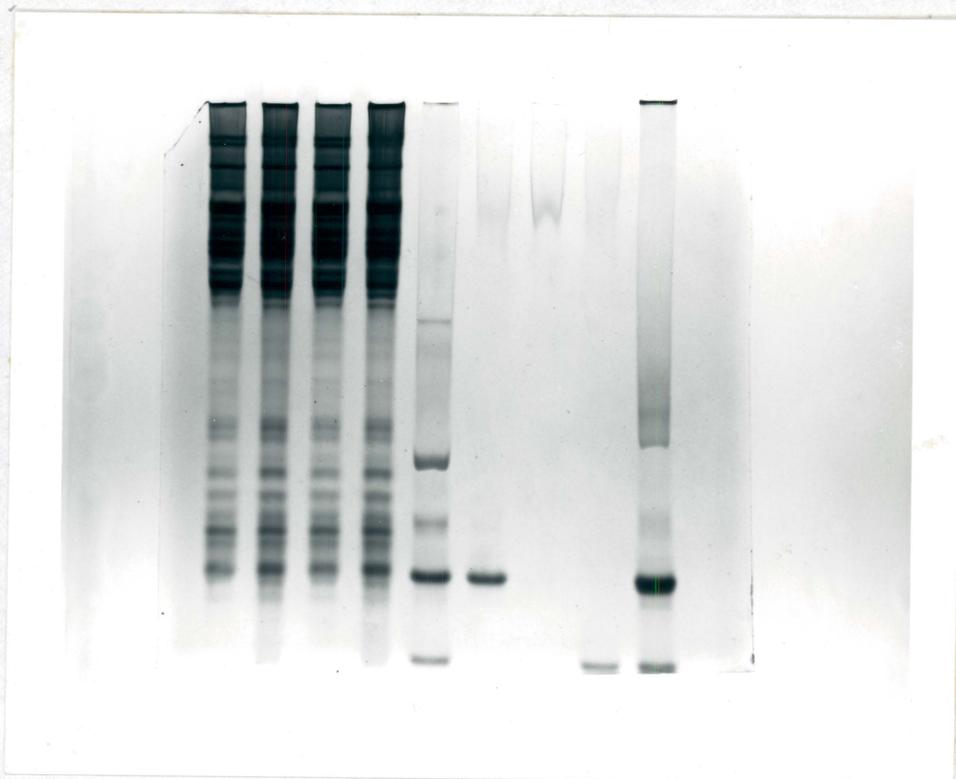
The pH is another factor to consider when measuring the activity of the enzymes. An unfavorable pH may cause



From left to right:

- Lane 1: Marker (Lysozyme; Trypsinogen; Glycerolaldehyde 3P; Albumin, egg; Albumin, Bovine; and Phosphorylase b)
- Lane 2: TRT7 (100 + 0): Chlorinated
- Lane 3: TRT7 (100 + 0): Not chlorinated
- Lane 4: TRT6 (0 + 50): Chlorinated
- Lane 5: TRT6 (0 + 50): Not chlorinated
- Lane 6: TRT4 (0 + 100): Chlorinated
- Lane 7: TRT4 (0 + 100): Not chlorinated
- Lane 8: TRT3 (50 + 0): Chlorinated
- Lane 9: TRT3 (50 + 0): Not chlorinated
- Lane 10: Control

Fig. 27: PAGE of Coker 916 gliadin.



From left to right:

Lane 1: TRT13 (50 + 100): Not chlorinated

Lane 2: TRT13 (50 + 100): Chlorinated

Lane 3: TRT15 (100 + 50): Not chlorinated

Lane 4: TRT15 (100 + 50): Chlorinated

Lane 5: Marker (Lysozyme; Trypsinogen; Glyceraldehyde 3P; Albumin, Bovine;  
and Phosphorylase b)

Lane 6: Trypsinogen

Lane 7: Glyceraldehyde 3P

lane 8: Lysozyme

Lane 9: Marker repeated

**Fig. 28:** PAGE of Coker 916 gliadin.

destruction of an enzyme. The pH of the buffer was 3.1. The relationship between pH and activity can be influenced by a variety of factors.

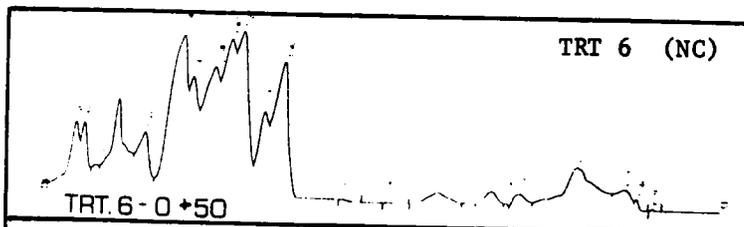
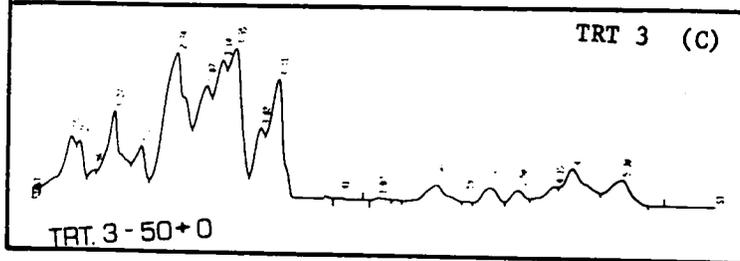
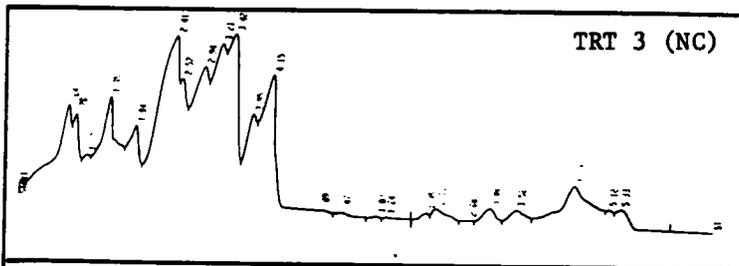
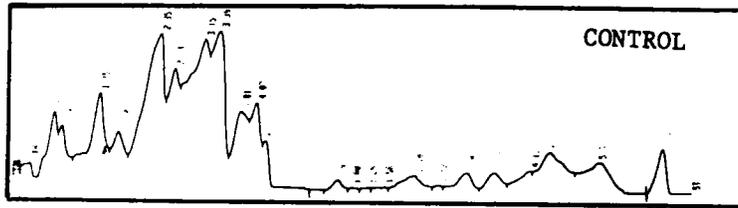
The relation between pH and activity may be dependent on temperature (Haldane, 1965). The temperature of the circulating coolant water had an effect on the resolution and separation of gliadin components. A low coolant temperature 12 degrees C was used for better resolution. The temperature coupled with a low pH could also have had an effect on the two enzymes.

The third possibility would be the size of the molecules. The molecules may be too large to enter the system and streaking would be the result.

The use of the standard molecular weight markers for this section was not feasible. Comparison of the peaks obtained from the densitometer was used to determine the protein changes, if any, in the gliadin content of the flour.

Figures 27 and 28 show the electrophoresis gels of the gliadins. In the non-SDS acrylamide gels, the proteins are primarily separated in reference to their charges, although size does have some influence on the results. Observing the gels, they appeared similar for the unchlorinated and chlorinated flours.

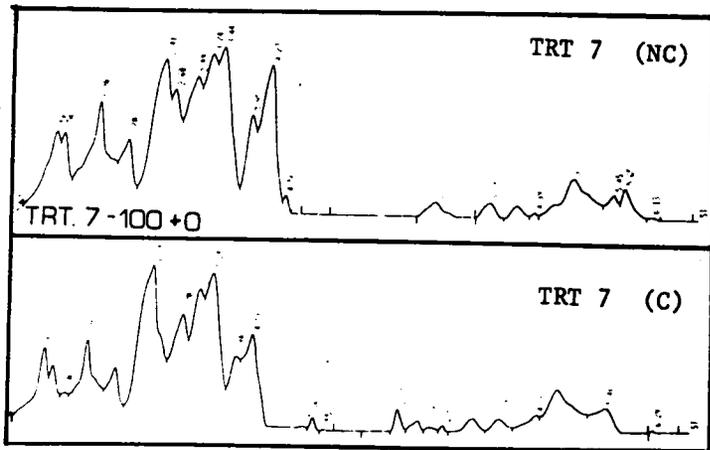
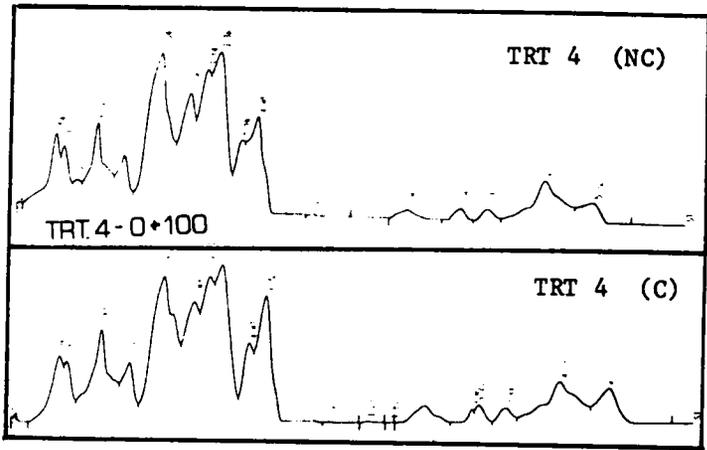
Figures 29, 30 and 31 illustrate the densitometer scans of the gels. The unchlorinated and chlorinated flours are



NC=NOT CHLORINATED

C=CHLORINATED

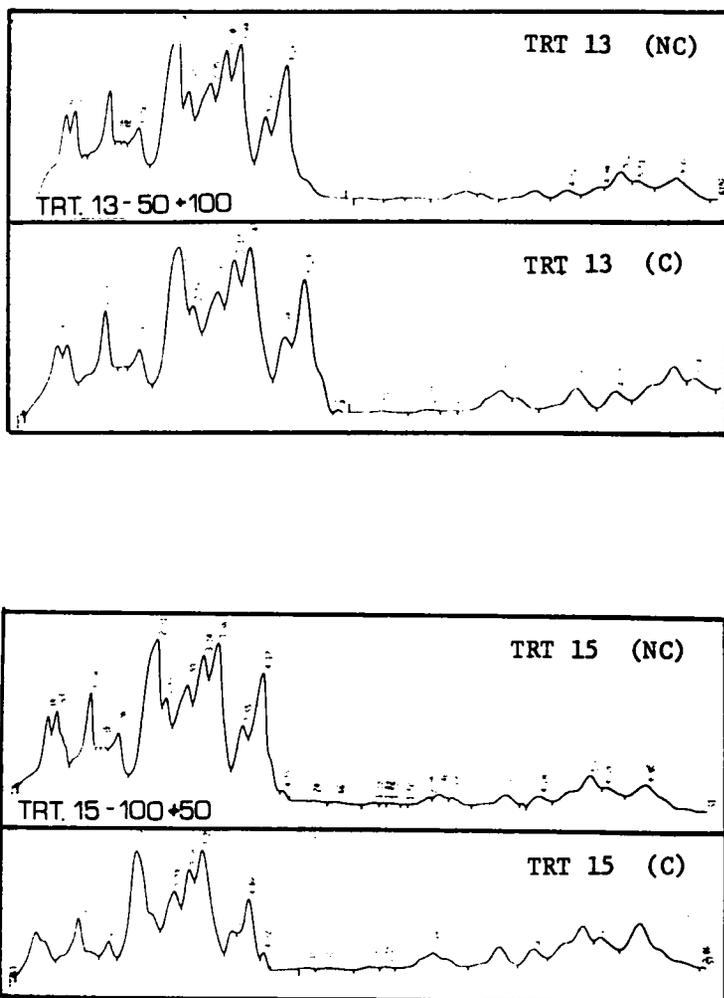
**Fig. 29** Gliadin gel scans.



NC=NOT CHLORINATED

C=CHLORINATED

**Fig.30:Gliadin gel scans.**



NC=NOT CHLORINATED

C=CHLORINATED

**Fig.31: Gliadin gel scans.**

overlayed to determine any differences. Acrylamide is relatively clear when destained, permitting the use of transmission optics for scanning. A measure of the stain uptake in the region is taken where the stain concentration is proportional to the amount of protein present.

Data derived from the scans was not significant. To further determine if there were any differences, the relative mobilities of the bands were determined. The Rf values were calculated for each band by determining the ratio of actual peak position from the origin to the position of the front. Twenty-one bands were determined from the reverse negatives that were made when the pictures of the gels were taken.

These results indicated that gliadins of the same variety have similar or identical electrophoregrams. Lookhart (1981) suggested that relative mobilities can be calculated more precisely when the bands are separated over a longer distance.

The amount of bands present were found to be twenty-one. This is in agreement with Schofield and Booth (1982). Approximately 20-25 bands are usually discernible although the band patterns for gliadins from different varieties of wheat show considerable variation and in all as many as about 45 different bands have been detected in the commonly occurring wheats.

Chlorination had no effect on gliadins. Gliadins are small molecular substances. Their molecular weights range

within 30,000 to 45,000 daltons. The system used was not able to establish this, but it did show the homogeneity of the proteins present. Also, the location and compact structure makes the gliadin impermeable to the effect of chlorination.

In summary, the glutenin fraction was affected by chlorination. Gliadins remain unaffected. The glutenin's functional role is elasticity while gliadin contributes to the extensibility of the dough. It has been found by investigation that too much glutenin will depress loaf volume in bread (Schoefield, 1986). For cake baking, this would also be detrimental.

#### *4.04 Lipids*

The role of lipids in baked goods is a challenging problem to many investigators. The lipid of cereal grains is a chemically complex system. More than 20 distinct chemical species can be separated from wheat flour lipid extract (MacRitchie, 1983).

In general, lipid is a minor component of wheat flour comprising 2-4% by weight of the whole wheat grain. Flour lipids may be divided into those inside starch granules which are true starch lipids, and all other lipids outside the starch granules or non-starch lipids.

#### **4.04a      *Fat Content of Flour: Free Fat and Fatty Acid Methyl Ester***

##### **4.04a (i)    *Free Fat Content***

Analysis of the free fat indicated that the flours contained 1-2% fat (Table 10). Two flours contained less than 1% (commercial flour and TRT 15-chlorinated).

The effect of fertilization on the % free fat indicated the lower fertilization had a higher fat content, while heavy fertilization indicated low fat content (Table 10). Treatments 4 and 7 were different. Treat 15, chlorinated, indicated a change in its lipid content which was lowered considerably.

The type of solvent used could also affect the extraction of the lipids from the flour. Morrison (1989) reported that with hexane as a solvent soft red flours yielded highest lipids (1.01-1.05%). Acetone and blends of chloroform and hexane also attained better results than petroleum ether.

TABLE 10

## Percent Free Fat in Coker 916 Flour

Wheat	% Free-Fat
TRT3 (NC) <sup>1</sup>	1.58
TRT3 (C) <sup>2</sup>	1.59
TRT6 (NC)	1.57
TRT6 (C)	1.42
TRT4 (NC)	1.33
TRT4 (C)	1.30
TRT7 (NC)	1.59
TRT7 (C)	1.73
TRT13 (NC)	1.29
TRT13 (C)	1.43
TRT15 (NC)	1.31
TRT15 (C)	0.880
Commerical Flour	0.662
<sup>1</sup> NC - Not chlorinated <sup>2</sup> C - Chlorinated	

*4.04a (ii) Fatty Acid Methyl Esters (FAME)*

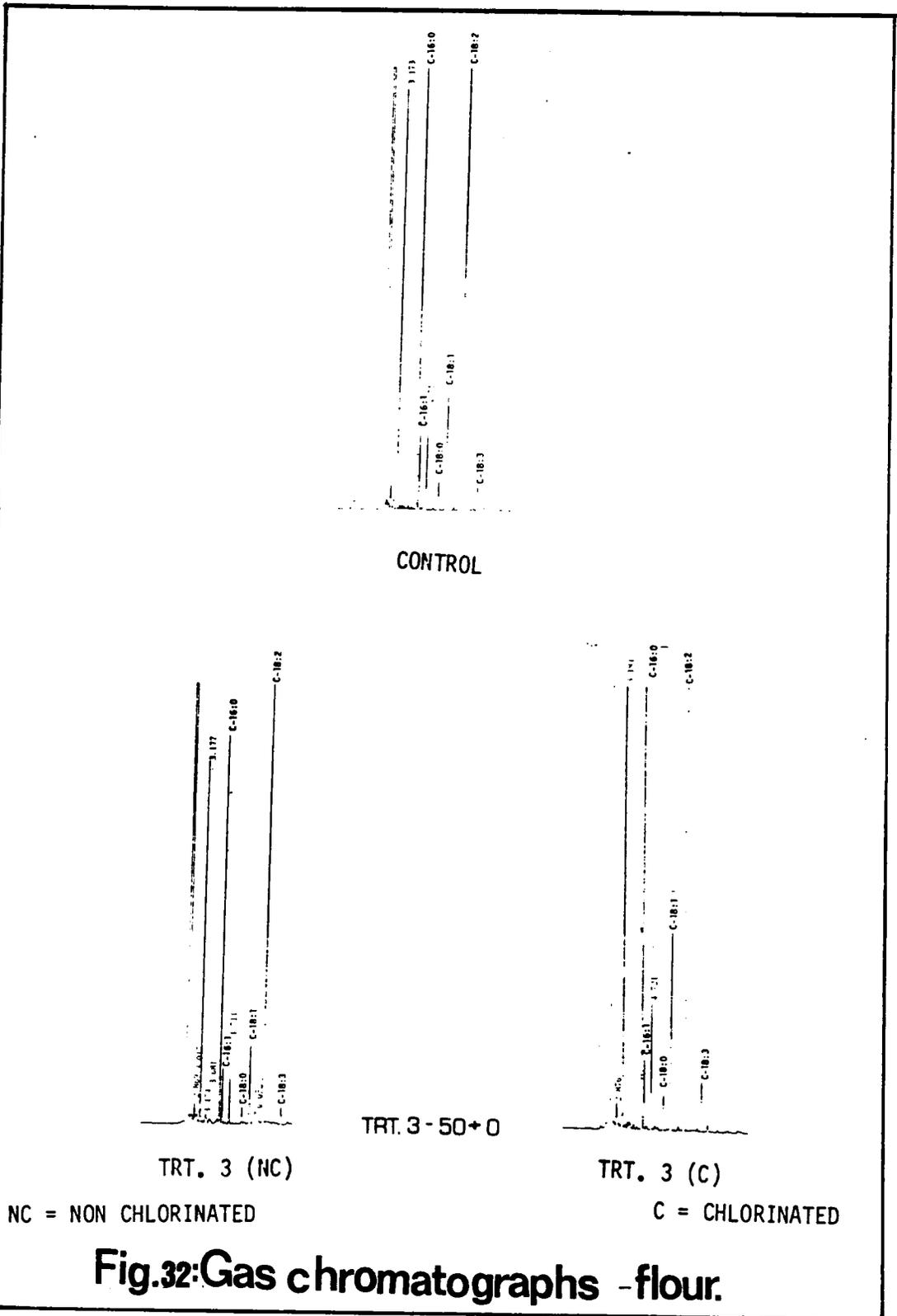
The major fatty acids content determined by gas chromatography were:

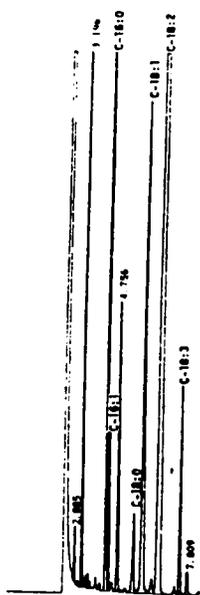
- a. C-16:0 - palmitic acid
- b. C-16:1 - palmitoleic acid
- c. C-18:0 - stearic acid
- d. C-18:1 - oleic acid
- e. C-18:2 - linoleic acid
- f. C-18:3 - linolenic acid

The fatty acid methyl ester content for each flour is found in Table 11 and Figures 32, 33, 34, and 35. The saturated acids, 16:0 and 18:0 were not significantly different among the various flours. Palmitic and oleic showed no effect due to chlorination. The effect of fertilization as analyzed by orthogonal contrast showed no effect on the amount of these acids. The values found for chlorinated and unchlorinated are in agreement with Morrison (1977).

The remaining fatty acid methyl esters, all unsaturated, were significantly different within their own group. The effect of chlorination was observed which had a lowering effect on the FAME value.

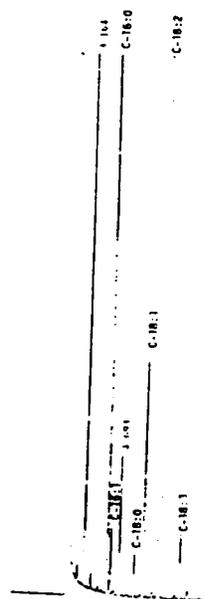
TABLE 11						
Fatty Acid Content in Coker 916 Flour, mg FAME/100 g Flour						
	16:0	16:1	18:0	18:1	18:2	18:3
Control	258.8	27.3	10:4	66.7	366	13.8
TRT3 (NC) <sup>1</sup>	296.2a	33.8a	16.3a	101.7a	589.0a	24.9a
TRT3 (C) <sup>2</sup>	296.6a	24.6b	15.1a	89.6a	498.8b	21.3a
TRT4 (NC)	291.3a	28.1a	15.1a	108.9a	618.20a	27.8a
TRT4 (C)	297.2a	17.4b	17.8a	93.5a	546.07b	22.7b
TRT6 (NC)	299.5a	19.4a	16.9a	123.9a	680.0a	28.2a
TRT6 (C)	307.9a	13.6b	15.2a	91.7b	508.9b	22.7b
TRT7 (NC)	309.6a	23.6a	17.8a	111.1a	626.5a	30.9a
TRT7 (C)	304.4a	23.9a	15.3a	92.4b	548.9b	22.6b
TRT13 (NC)	309.4a	28.7a	16.1a	101.8a	620.3a	27.6a
TRT13 (C)	305.0a	20.3b	15.6a	93.5a	530b	22.5b
TRT15 (NC)	293.2a	33.7a	16.60a	111.07a	623.6a	29.07a
TRT15 (C)	282.7a	24.8b	15.01a	80.0b	467.9b	21.7b
<sup>1</sup> NC - Not Chlorinated <sup>2</sup> C - Chlorinated Unlike letters within the same treatment are significantly different, p < 0.05.						



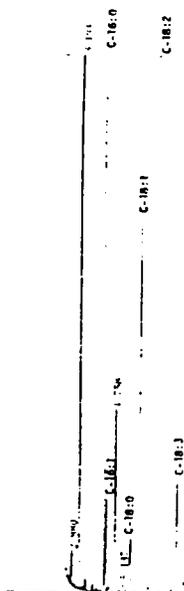


TRT. 4 (NC)

TRT.4 - 0 +100

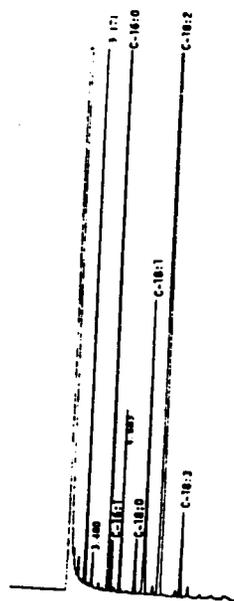


TRT. 4 (C)



TRT. 6 (NC)

TRT.6 - 0 +50

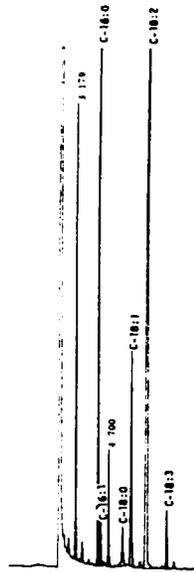


TRT. 6 (C)

NC = NON CHLORINATED

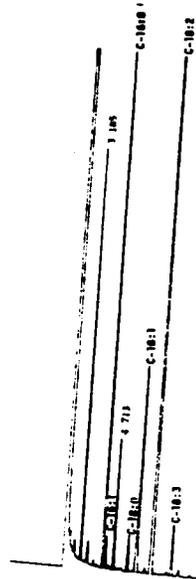
C = CHLORINATED

**Fig. 33: Gas chromatographs - flour.**

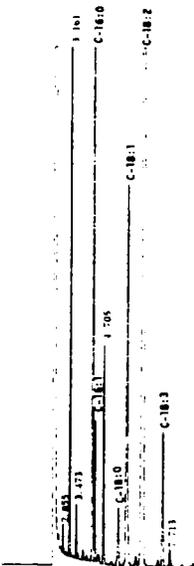


TRT. 7 (NC)

TRT. 7 -100 +0

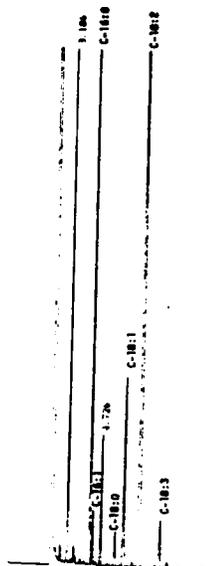


TRT. 7 (C)



TRT. 13 (NC)

TRT. 13 -50 +100

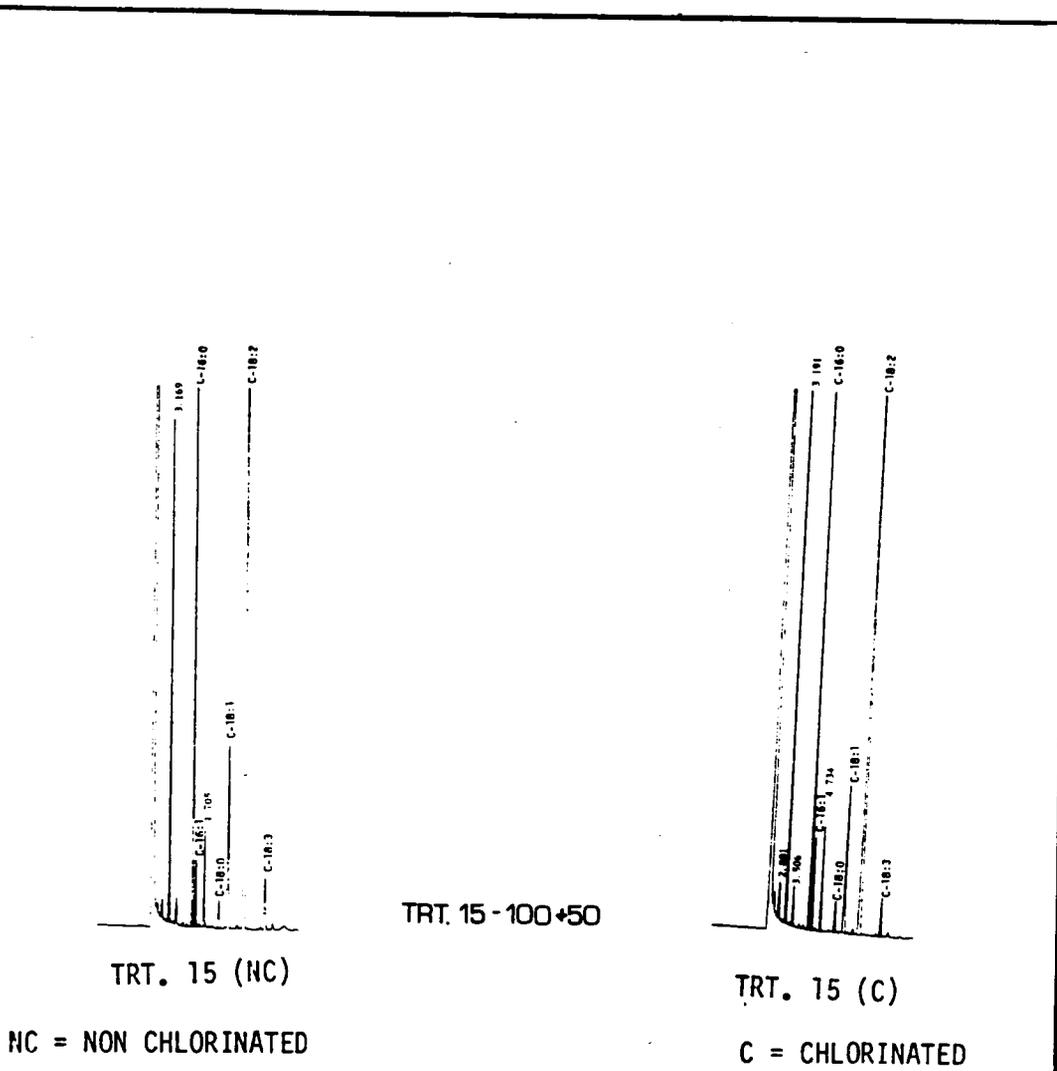


TRT. 13 (C)

NC = NON CHLORINATED

C = CHLORINATED

**Fig. 34: Gas chromatographs - flour.**



**Fig. 35 Gas chromatographs - flour.**

Palmitoleic acid (16:1) showed significant changes in its value due to chlorination. There was a decrease in the value of 16:1 found in each treatment. TRT 3 had the largest change in its value because of chlorination. TRT 7. had the minimal change taking place which was non-significant. A majority of the values were in accordance with Morrison (1977).

Oleic acid (18:1) was significantly different when analyzed by orthogonal contrast. Values for FAME for unchlorinated flours ranged from 101.8 - 123.9 mg. Again, as seen previously, chlorination had a decreasing effect upon the FAME values. Three flours which showed significant decreases in FAME were TRT 6, TRT 7 and TRT 15. One important factor to note, that all values of FAME after chlorination were lowered to an approximate constant value. If these values were plotted a linear relationship would be the result.

This same relationship is again observed with linolenic acid (18:3). Chlorination had an effect by lowering the FAME values. TRT 3 was not significantly different between the unchlorinated and chlorinated counterparts, but the balance of the flours were significantly different as shown by orthogonal contrast. The chlorinated values again were lowered to a constant value.

Linoleic acid (18:2) demonstrated the greatest change due to chlorination. Using orthogonal contrast there was a

significant decrease due to chlorination. All treatments were significantly different from each other due to chlorination. Chlorination had a lowering effect on the amount of linoleic acid. The values are in agreement with that of Morrison (1977).

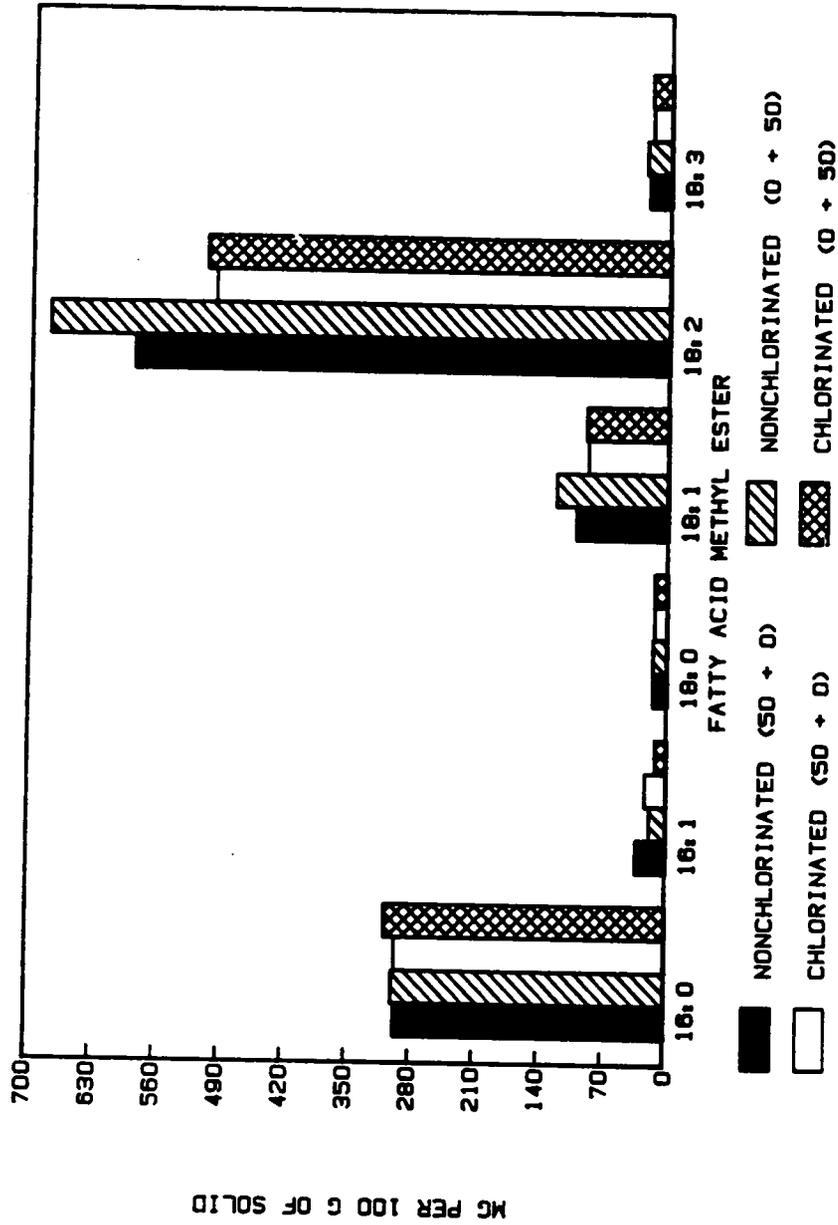
The unsaturated fatty acids were somehow attacked by chlorine during the process. Table 12 illustrates the % decrease of the unsaturated fatty acid due to chlorination. Palmitoleic had the highest loss followed by linolenic acid. These acids were in low amounts in the unchlorinated form, so a small change taking place would account in a high percentage. The other acids are in high amounts, therefore a loss would not be as high. Some flours lost fatty acids at a high rate. Flours 6 and 15 lost the greatest amount of fatty acids during the chlorination process. Treatment 7 did not show a decrease of 16:1 fatty acid which was the only exception found.

The decrease of free fatty acid after chlorination was greater for unsaturated fatty acids than for saturated fatty acids (Figures 36, 37, 38). The flour fatty acids involved in the reduction were: 16:1, 18:1, 18:2 and 18:3. Table 13

TABLE 12

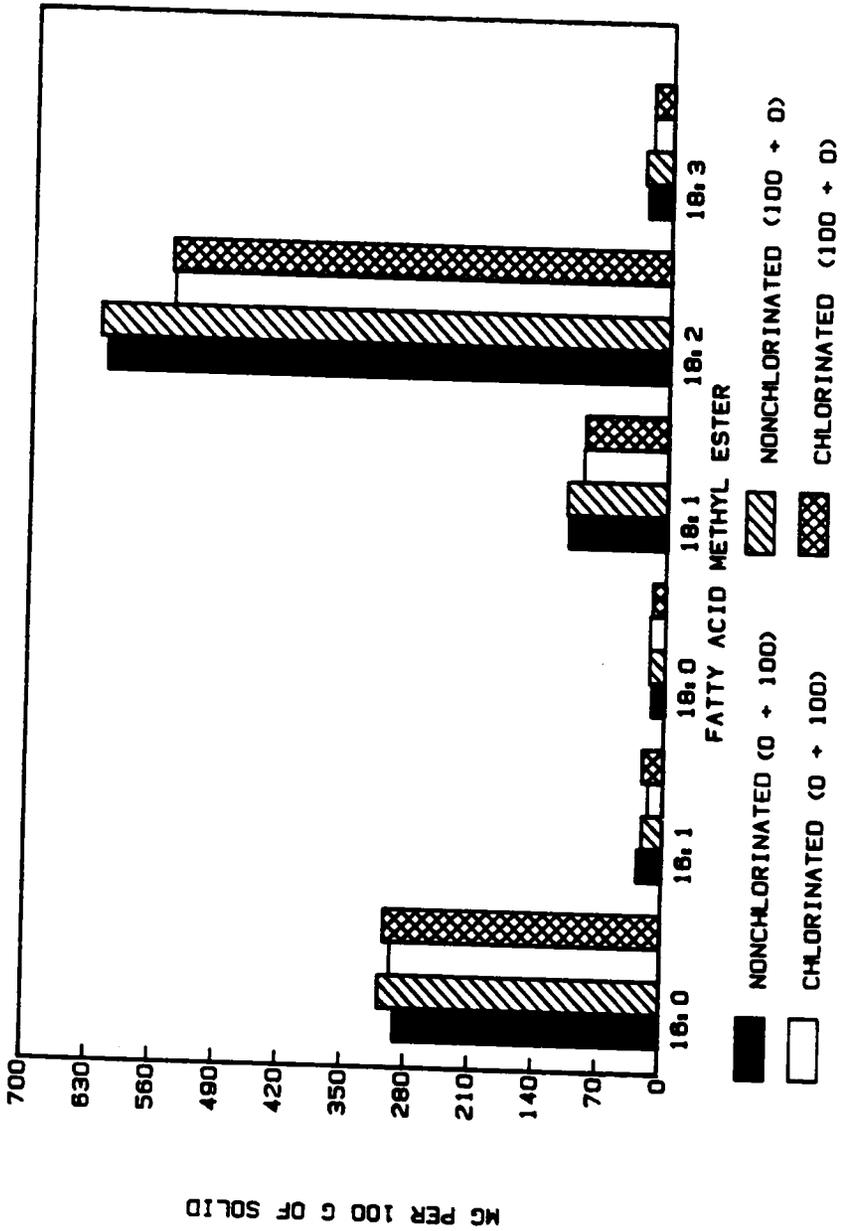
Percentage Decrease of Unsaturated Fatty Acids in the  
Non-Starch Lipids of Chlorine-Treated Coker 916 Flour

	16:1	18:1	18:2	18:3
TRT3	27.0	11.90	15.31	14.45
TRT4	38.10	14.14	11.67	18.35
TRT6	29.90	25.98	25.16	19.50
TRT7	-----	16.83	12.39	26.86
TRT13	29.26	8.15	14.56	18.48
TRT15	26.40	27.97	24.97	25.35



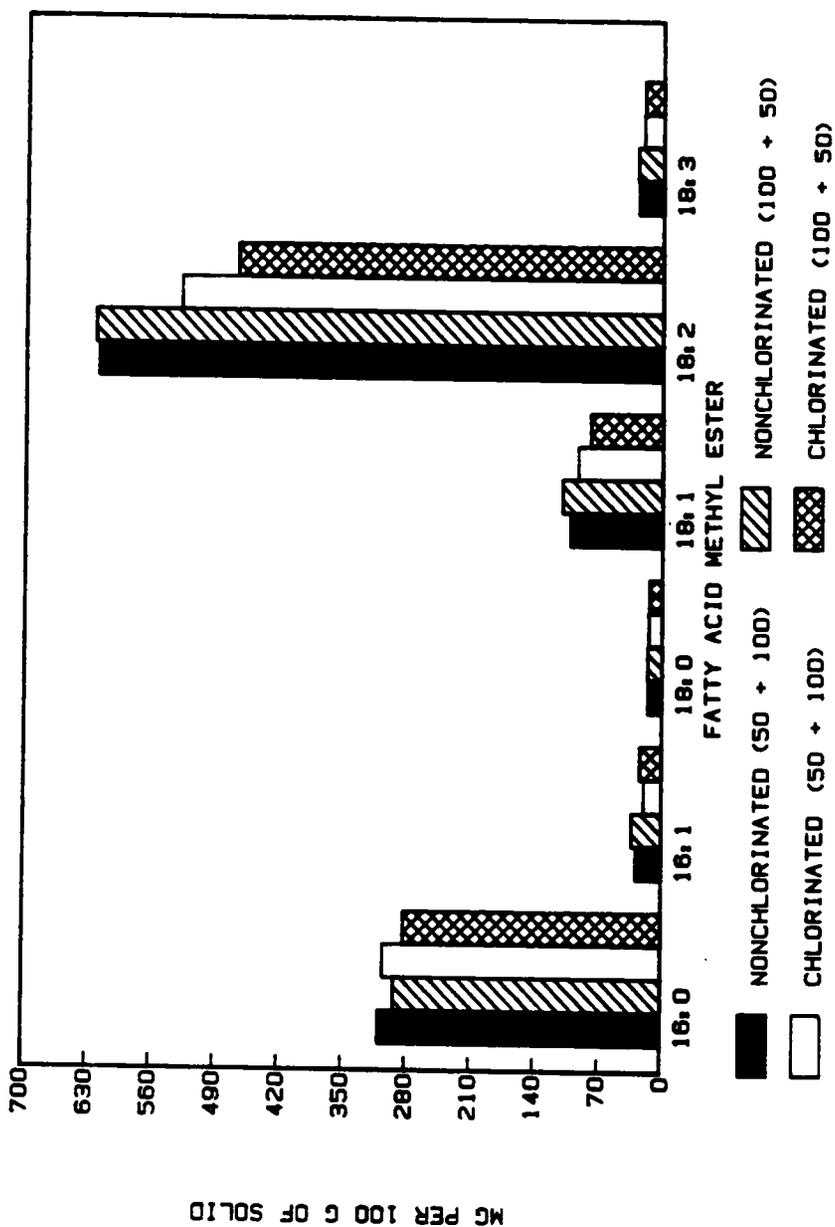
TRT. 3 - 50 + 0  
 TRT. 6 - 0 + 50

**Fig.36:Fatty acid content of flours.**



TRT. 4-0+100  
 TRT. 7-100+0

**Fig. 37: Fatty acid content of flours.**



TRT. 13-50+100  
TRT. 15-100+50

**Fig. 38:Fatty acid content of flours.**

TABLE 13

Percent of Free Fatty Acids in Coker 916 Flour,  
Percent per/100 g flour

	16:0	16:1	18:0	18:1	18:2	18:3
Control	34.80%	3.70%	1.40%	8.90%	49.30%	1.85%
TRT3 (NC) <sup>1</sup>	27.90%	3.18%	1.53%	9.50%	55.47%	2.30%
TRT3 (C) <sup>2</sup>	31.20%	2.60%	1.59%	9.47%	52.70%	2.25%
TRT4 (NC)	26.74%	2.57%	1.39%	10.00%	56.75%	2.55%
TRT4 (C)	29.88%	1.75%	1.79%	9.40%	54.90%	2.28%
TRT6 (NC)	25.64%	1.67%	1.44%	10.61%	58.22%	2.41%
TRT6 (C)	32.07%	1.42%	1.58%	9.55%	53.01%	2.36%
TRT7 (NC)	27.66%	2.11%	1.59%	9.92%	55.96%	2.76%
TRT7 (C)	30.21%	2.37%	1.52%	9.17%	54.48%	2.24%
TRT13 (NC)	28.03%	2.60%	1.46%	9.22%	56.19%	2.50%
TRT13 (C)	30.90%	2.06%	1.58%	9.47%	53.70%	2.28%
TRT15 (NC)	26.48%	3.04%	1.50%	10.03%	56.32%	2.63%
TRT15 (C)	31.69%	2.78%	1.68%	8.96%	52.45%	2.43%
<sup>1</sup> NC - Not Chlorinated <sup>2</sup> C - Chlorinated						

lists the percent of fatty acids present in the flours. The figures are in agreement with Chung (1989). These data confirm previous data reported concerning the reduction of fatty acids by chlorination. Linoleic acid was heavily involved in its reduction during the chlorination process. A theory that has been put forth (Morrison, 1989) explained the effects of chlorine could be related to the accessibility of the unsaturated fatty acid on the starch granule. The position makes for easy access to the chlorine ion and thus attacks the unsaturated bonds of the fatty acid.

According to Morrison (1979), chlorine gas which is used to improve the baking performance of cake flours, attacks about 60% of all the unsaturated fatty acids in the non-starch lipids. It is possible that the chlorine is entirely consumed in fast reactions with non-starch lipids before penetrating into the starch surface.

#### *4.04b Phospholipids*

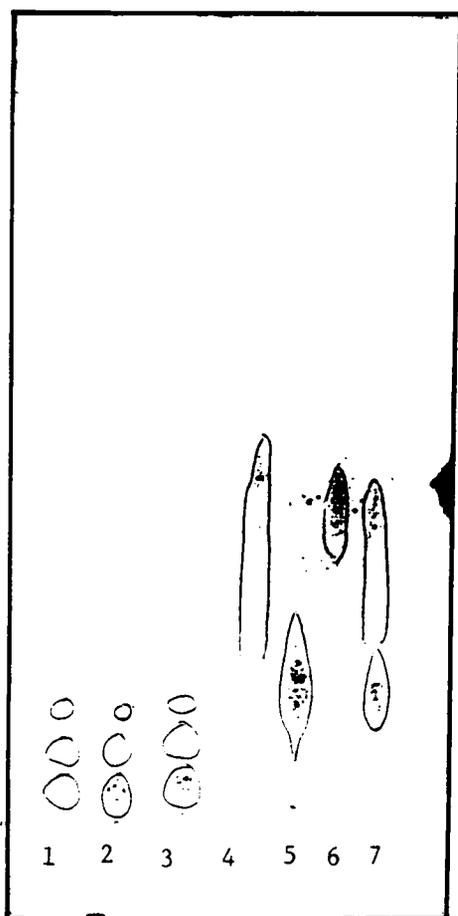
Cereals contain the ubiquitous diacylphosphoglycerides: the major polar lipids are phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylinositol (PI); the major polar lipids are phosphatidylglycerol (PG), phosphatidylserine (PS) and diphosphatidylglycerol (DPG). Phosphatidic acid (PA) is sometimes reported, but it is

unimportant except in actively metabolizing tissue (Morrison, 1983).

Phospholipids were isolated by TLC for qualitative evaluation. Figures 39 and 40 shows the photocopy of plates developed for phospholipids. The three polar lipids identified were: lysophosphatidylcholine (LPC), lysophosphatidylethanolamine (LPE), and lysophosphatidylglycerol (LPG). As mentioned in the literature, these three phospholipids are the main constituents of the starch lipids: Standards were used for identification purposes.

The position of the three phospholipids indicated the polarity by separation on the plate. Lysophosphatidylcholine had the least mobility, thus being less polar. Lysophosphatidylethanolamine had the greatest mobility thus making it the most polar of the three. An unsaturated tank was used because of better resolution of components, but a curved solvent front was formed with the spots near the edges traveling further than those near the center (Figs. 39 & 40). The quality of the separation, the spot shape and the development time are all affected by the type of tank and its degree of saturation (Sherma, 1984).

Table 14 lists the presence of the phospholipids which were extracted from the flours. Treatment 3 (NC, and C) was



Lane 1, Control  
 Lane 2, TRT. 3 NC  
 Lane 3, TRT. 3 C  
 Lane 4, LPG  
 Lane 5, LPC  
 Lane 6, LPE  
 Lane 7, MIX. STANDARDS

TRT. 3 - 50+0

TRT. 6 - 0+50

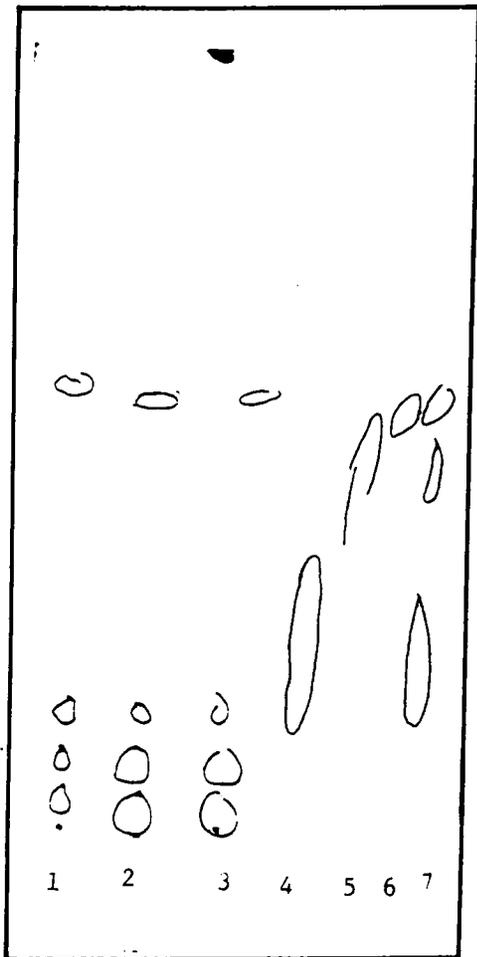
TRT. 4 - 0+100



Lane 1, TRT. 4 NC  
 Lane 2, TRT. 4 C  
 Lane 3, TRT. 6 NC  
 Lane 4, TRT. 6 C  
 Lane 5, LPC  
 Lane 6, LPG  
 Lane 7, LPE  
 Lane 8, MIX. STANDARDS

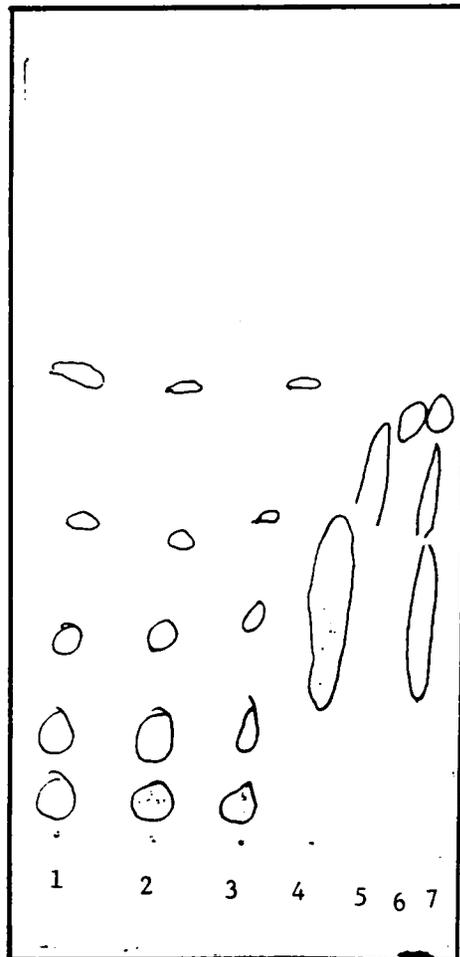
NC=NOT CHLORINATED  
 C=CHLORINATED

**Fig.39:TLC of polar lipids.**



Lane 1, TRT. 7 NC  
 Lane 2, TRT. 7 C  
 Lane 3, TRT. 13 NC  
 Lane 4, LPC  
 Lane 5, LPG  
 Lane 6, LPE  
 Lane 7, MIX. STANDARDS

TRT. 7 - 100 + 0  
 TRT. 13 - 50 + 100  
 TRT. 15 - 100 + 50



Lane 1, TRT. 13 C  
 Lane 2, TRT. 15 NC  
 Lane 3, TRT. 15 C  
 Lane 4, LPC  
 Lane 5, LPG  
 Lane 6, LPE  
 Lane 7, MIX. STANDARDS

NC=NOT CHLORINATED  
 C=CHLORINATED

**Fig.40:TLC of polar lipids.**

TABLE 14

## Presence of Phospholipids in Coker 916 Flour

	Lysophosphatidyl- choline	Lysophosphatidyl- glycerol	Lysophosphatidyl- ethanolamine
Control	X		
TRT3 (NC) <sup>1</sup>	X		
TRT3 (C) <sup>2</sup>	X		
TRT6 (NC)	X	X	X
TRT6 (C)	X	X	X
TRT4 (NC)	X	X	X
TRT4 (C)	X	X	X
TRT7 (NC)	X		X
TRT7 (C)	X		X
TRT13 (NC)	X		X
TRT13 (C)	X	X	X
TRT15 (NC)	X	X	X
TRT15 (C)	X	X	X

<sup>1</sup>NC - Not Chlorinated  
<sup>2</sup>C - Chlorinated

the only experimental flour where only lysophosphatidylcholine was recovered. Lysophosphatidylcholine and lysophosphatidylethanolamine were the only two phospholipids recovered in flours of treatments 7 (NC,C) and 13(NC).

Morrison (1989) suggests that wheat starch lysophospholipids have a fairly uniform composition; average values are lysophosphatidylcholine = 86%, lysophosphatidylethanolamine = 10% and lysophosphatidylglycerol = 4%. This could explain the low recovery rates for lysophosphatidylethanolamine and lysophosphatidylglycerol. Phosphatidylcholine which is present in high amounts is easily recovered.

Another point to emphasize is the location on the starch granule of the compounds. The location may make it more difficult for complete recovery, especially for the compounds which are in smaller quantities. It is commonly supposed that the starch lysophospholipids exist as amylose inclusion complexes in the native granules (Morrison, 1989). The results shown for the chlorinated treated flour do not fully illustrate chlorine's effect, if any, on these lipids. The effect was definite with the non-polar lipids. There is evidence (Morrison, 1989) that the lipid complexation here would have a total resistance to attack by chlorine. Another alternative explanation there is efficient scavenging of chlorine by nonstarch lipids so that it does not reach the starch lipids (Morrison, 1989).

#### *4.04c Summary*

Flour contains all the germ lipids transferred to the flour during milling. Flour lipids also may be regarded as consisting of true starch lipids and all other lipids collectively termed non-starch lipids.

Non-starch lipids in flour are readily extracted at ambient temperatures with the commonly used polar solvent mixtures (hexane, petroleum ether, etc.) but starch lipids are not extracted unless the structure of the starch granules has been disturbed by swelling with water or freeze-drying. Starch lipids can be extracted quantitatively with hot water-saturated n-butanol.

Non-starch lipids are comparatively accessible, while starch lipids are in a highly protected environment and probably are not affected by normal chlorination treatment. There is evidence that chlorine gas alters the unsaturated fatty acids in non-starch lipids. The same effect is not seen with starch lipids after chlorination.

#### *4.05 Ash Content, pH and Color of Flour*

##### *4.05a Ash Content*

Ash is defined as the mineral residue left when a sample of flour is heated in a silica dish under prescribed conditions until all organic material is destroyed, but without causing the volatilization of non-combustible constituents. The most suitable temperature for this purpose is within the range of 550 to 590 degrees C at which a dull red heat is produced. The sample is reduced to a gray white ash and the residue is weighed and weight converted to a % basis (Pylar, 1973).

Since the natural mineral content of the endosperm is largely derived from the bran, an excessively high ash content is indicative of relatively high mixtures of branny material to the flour, such as occurs with low grade flours.

The ash content of the flours are presented in Table 15. There were differences between chlorinated and unchlorinated for some treatments (# 6, and 4). The unchlorinated flours had a lower value than the chlorinated except for treatment 15. The ash content increased significantly with increased fertilization.

The validity of the ash content is in question here. There are two different values between the unchlorinated and

TABLE 15

## Ash Content, pH, and Color of Coker 916 Flour

Flour	Ash Content	pH	Color		
			L <sup>3</sup>	b <sup>4</sup>	$\Delta E$ <sup>5</sup>
TRT3 (NC) <sup>1</sup>	0.303b	5.50a	92.3a	6.53a	92.52a
TRT3 (C) <sup>2</sup>	0.451b	4.06b	93.9b	4.82b	94.12b
TRT6 (NC)	0.369a	5.72a	92.7a	6.70a	92.95a
TRT6 (C)	0.461b	4.28b	95.6b	5.69b	95.61b
TRT4 (NC)	0.365a	5.58a	91.7a	6.30a	91.95a
TRT4 (C)	0.444b	4.23b	93.4b	6.13a	93.89b
TRT7 (NC)	0.390a	5.88a	92.8a	6.21a	92.97a
TRT7 (C)	0.435a	4.17b	94.8b	5.95a	93.97a
TRT13 (NC)	0.375a	5.42a	91.9a	7.96a	92.31a
TRT13 (C)	0.408a	3.88b	93.6a	4.87b	93.57a
TRT15 (NC)	0.485a	5.75a	93.6a	6.20a	93.81a
TRT15 (C)	0.431a	4.06b	95.1a	5.49b	95.23b
Control	0.437	4.21	93.2	4.17	93.75

<sup>1</sup> NC - Not Chlorinated

<sup>2</sup> C - Chlorinated

<sup>3</sup> L: 0 = Black; 100 = White

<sup>4</sup> b: + Yellow; - Blue

<sup>5</sup>  $\Delta E: \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$

Unlike letters within the same treatment are significantly different,  $p < 0.05$ .

chlorinated flours. Actually, the ash content should be the same. The milling process is being examined by this method, and chlorination has nothing to do with milling. Therefore, observing the recorded values and according to Pylar (1973) the objective measurement of color is judged by many to be a better indicator of flour quality than ash.

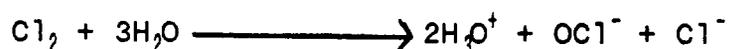
#### *4.05b Flour pH*

One very important factor often overlooked in cake formulations is pH and its pronounced effect on cake quality.

The pH of the flours are listed in Table 15. There are significant differences between the unchlorinated and chlorinated flours. The unchlorinated flours have a pH range from 5.42 to 5.88 and the chlorinated flours 3.88 to 4.28.

Chlorine was used to treat the experimental flours. The purpose was two-fold: a) to improve the baking performance for such factors as cake symmetry, volume, grain and texture and b) to improve the color of the flour and hence, the baked product.

The reaction occurring during chlorination is expressed by Gough (1978):



The rationale used is that flour has a moisture content of 13-15.5% and the chlorine may readily dissolve this flour water giving the above reaction. The presence of the hydrogen ion explains the marked drop in pH that results upon chlorination.

It has been observed, however, that flours of high ash content require more chlorine treatment to mellow the gluten and produce a given pH than do flours of lower ash content (Pyler, 1978). Practical experience has shown that best flour performance is obtained when it is carefully bleached to a pH within the range of 4.6-5. The chlorinated experimental flours fall into a lower range and the ash content was within the normal range of .3 - .4 (Table 15).

Pomeranz (1973) suggested that for angel food cakes soft wheat flour should be bleached to a pH 4.3. This is somewhat lower than desirable for other cakes. The heavy application of chlorine causes some protein breakdown. This was observed to a certain extent with the glutenins in the previous section (4.03).

#### *4.05c Color of Flour*

The color of flour is affected by numerous variables. The most important are wheat variety, milling, storage of flour and the effect of bleaching treatments (Pomeranz, 1989).

The brightness and brilliance of flour are primarily due

to the manner in which the wheat is milled. The uniformity of the whiteness may be maintained with removal of the yellow pigment by bleaching (Shuey, 1975).

In expressing the visual impression of whiteness, however the lightness alone is inadequate. Croes (1961) believes that lightness and the degree of yellowness should be taken into account. In general the whiteness increases if the lightness increases or if the degree of yellowness decreases.

Table 15 lists the Hunter color values of the flour. Chlorination had an effect, in that, there was an increase in the whiteness (L) and a decrease in the b (yellowness). Fertilization had no effect on the color.

According to Hunter and Harold (1987a) when using L, a, b, the b dimension measuring yellowness is the most critical of the three. The use of bluing, which decreases L, but increases blueness, produces a visually "whiter" product. In studies b is typically found to be three or four times as important as L, which is the next most important.

Some factors should be pointed out concerning the b value. Treatments 4, 7 showed no significant change in their b value after chlorination. There are some discrepancies when considering the  $\Delta E$  value:

1. Treatment 3 had a significant decrease in its b value, but L and  $\Delta E$  did not change after chlorination.

2. Treatments 4 and 7 had a very little change in their b value and it was expressed in the L and  $\Delta E$ .
3. These values will be important in considering the color of the internal baked crumb.

#### **4.06 *Baking Properties of Flour: Objective Data***

##### **4.06a *Specific Gravity***

Specific gravity of the batter was measured (Table 16). There was no significant difference found for each flour. The specific gravity of the egg white/sugar ratio was adjusted to 0.15-0.17 before the addition of flour/sugar.

This adjustment was made due to preliminary studies. The AACC 10-15 method suggested 0.13-0.14 as the specific gravity. These figures were too low. Further research provided that the specific gravity of the meringue to be at 0.15-0.17. Anything above 0.17 would yield a compact structure, whereby below 0.15 would provide a collapsed or undersized cake (Pylar, 1973).

Chlorination had no effect on the specific gravity values. The whipping of the egg whites is important to achieve enough air before the final incorporation of flour. Specific gravity is not a good tool to gain any further information about the flour itself.

TABLE 16  
Specific Gravity of Angel Food Cake Batter

Flour	Specific Gravity
Control	0.2904
TRT6 (NC) <sup>1</sup>	0.2959a
TRT6 (C) <sup>2</sup>	0.2963a
TRT3 (NC)	0.2767a
TRT3 (C)	0.2985a
TRT4 (NC)	0.2893a
TRT4 (C)	0.2924a
TRT7 (NC)	0.3021a
TRT7 (C)	0.2963a
TRT13 (NC)	0.2923a
TRT13 (C)	0.2839a
TRT15 (NC)	0.2892a
TRT15 (C)	0.2796a

<sup>1</sup>NC - Not chlorinated  
<sup>2</sup>C - Chlorinated  
a - No significant difference, p > 0.05

#### *4.06b Batter pH and Cake Volume*

The bleaching process generally involves two steps: (a) treatment with chlorine, generally at a rate of 0.5 to 2.5 oz. chlorine per cwt of flour which lowers the flour pH and improves the baking performance with respect to such factors as cake symmetry, grain and texture, and (b) improved color of the cake.

Table 17 lists the pH for each batter. There is significant difference between the batters using unchlorinated and chlorinated flours. For unchlorinated batters, the range was 5.3-5.38 and for chlorinated batters, the range was 5.15-5.20. The chlorinated batters are at the border or below what is considered to be necessary to yield a properly baked cake.

Ash and Colmey (1973) pointed out that cake volume, grain and texture are affected by cake pH. As the pH increases the grain tends to become coarser with heavier cell walls while the volume increases. The authors also emphasize that volume and quality of angel food cakes are especially sensitive to batter pH. Typical pH values for angel food cake batter is 5.2-6.0 (Ash and Colmey, 1973). Angel food cakes need a low pH for egg white functionality.

Volumes (Table 17) of baked angel food cakes were calculated according to AACC 10-15 formula (Appendix J). To fully discuss the volume, the heights of the cake are another factor to consider. Cakes were removed from the oven and

TABLE 17

## Batter pH and Cake Volume

Flour	pH	Height Depanning (cm)	Height Cooling (cm)	Depanning Cooling (cm)	Volume (cc)
Control	5.38	8.70	8.34	0.360	2714
TRT6 (NC) <sup>1</sup>	5.30a	8.50a	8.21a	0.290	2696a
TRT6 (C) <sup>2</sup>	5.20b	8.30a	8.03a	0.270	2608a
TRT3 (NC)	5.38a	8.60a	8.24a	0.360	2662a
TRT3 (C)	5.15b	8.10b	7.89a	0.210	2581a
TRT4 (NC)	5.35a	8.70a	8.48a	0.220	2814a
TRT4 (C)	5.20b	8.30a	8.04b	0.260	2599b
TRT7 (NC)	5.35a	8.70a	8.33a	0.370	2676a
TRT7 (C)	5.18b	8.10b	7.93b	0.170	2567a
TRT13 (NC)	5.30a	8.40a	8.15a	0.250	2640a
TRT13 (C)	5.18b	7.90b	7.66b	0.240	2466b
TRT15 (NC)	5.35a	8.60a	8.24a	0.360	2734a
TRT15 (C)	5.15b	8.10b	7.86a	0.240	2555b

<sup>1</sup>NC - Not chlorinated  
<sup>2</sup>C - Chlorinated  
Unlike letters within the same treatment are significantly different, p < 0.05.

allowed to cool for 2 hours. Cakes were depanned and heights were measured (Hgt.<sub>(D)</sub>) [Table 17]. After another 1 1/2 hours, the height was measured again (Hgt.<sub>(C)</sub>) [Table 17]. This last height was used in the formulation for volume.

Statistical analysis indicated significant difference in the depanned heights and the cooled heights of the angel food cakes. Only one flour treatment (Treat. 6) showed no significant difference between unchlorinated and chlorinated flours. The heights of the cakes indicated the influence of protein content (or fertilization) on the baked cake. Once the cake was cooled, the cake had settled to a specific height. Chlorination of flour lowered the heights of cakes but had a stabilizing effect on the heights.

The difference figure (Hgt.<sub>(D)</sub> - Hgt.<sub>(C)</sub>) gives an indication of the stabilizing effect of chlorination on the baked cake. It is not so much the collapse of the cake, but the shrinkage that occurs. There was a difference between the unchlorinated and chlorinated flours. The unchlorinated flour showed more shrinkage than the chlorinated flours. There were two exceptions: (a) Treatment 6 showed no difference between unchlorination and chlorination; and (b) Treatment 4 showed the opposite effect.

The volume (Table 17) of the angel food cakes indicated two things: (a) the effect of chlorination shows a significant decrease in the volumes of the baked product; and (b)

fertilization (or protein) caused a decrease in volume of baked cakes, with an increase in protein content.

A graph (Figure 41) was plotted showing volume of baked angel food cakes ( $\text{cm}^3$ ) versus % protein content. The chlorinated and unchlorinated flours were plotted in order to perceive a clearer picture of the events occurring. The unchlorinated flours showed a fluctuation of the volumes, but no differences between chlorination treatments. Notice the linear relationship of the chlorinated flours. This is another indication of the stabilization effect of the chlorination of the flour properties. As the protein increased, the volume of the cakes decreased. A significant correlation coefficient exists here. For the chlorinated flours, the coefficient is a  $-0.89$  while for the unchlorinated it is  $-0.32$ . Treatment 15, which had a change in its protein content after chlorination, fell in line with the volume-protein relationship. Gaines and Donelson (1985) found a significant relationship among flour protein content and angel food cake height. A significant difference in angel food cake height required a change in protein content of 2.7 and 2.3% respectively, for the flours used.

The increase in volume in the unchlorinated flour can be attributed to the stretched cells, causing irregular thick walled cells. The chlorinated flours produced smaller even cells that were closely packed together. This area will be

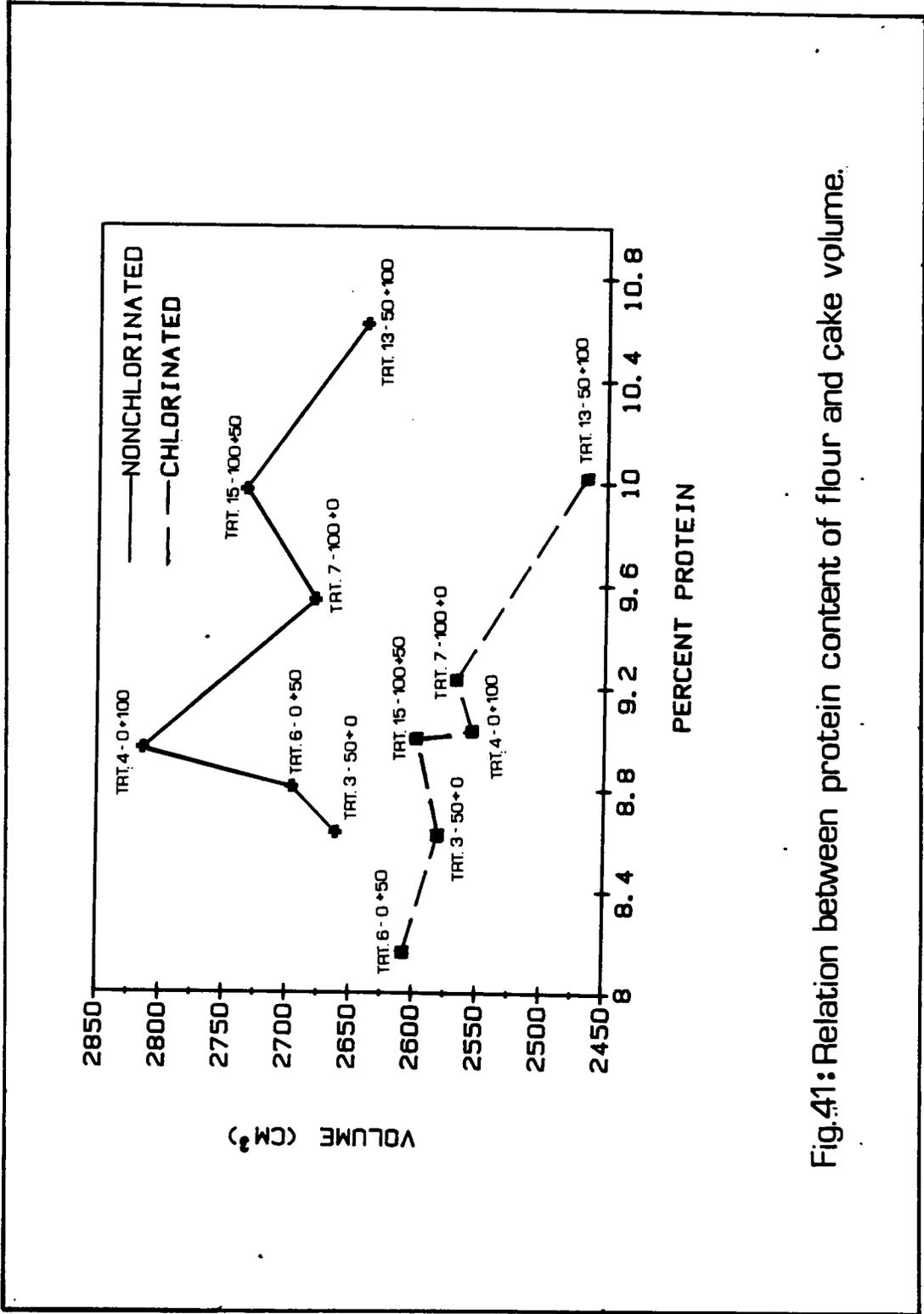


Fig.41: Relation between protein content of flour and cake volume.

explored fully in the next section, because structure will affect the final volume of the angel food cake.

#### *4.06c Summary*

Volume and batter pH were analyzed together to identify their influence on the baked cake structure. Strong correlation existed between the pH of the flour and pH of the batter (0.78). Chlorination had an effect on the pH of the flour which caused a decrease, and this is evident in the decrease in the batter pH. As the batter pH increases, there is a correlation that the volume increases (0.82).

Interrelated with these results are the heights derived from the baked cakes. The batter pH and heights are in correlation: (a) chlorinated ( $Hgt_{(D)} = 0.810$  and  $Hgt_{(C)} = 0.48$ , respectively) and (b) chlorinated ( $Hgt_{(D)} = 0.59$  and  $Hgt_{(C)} = 0.46$ , respectively).

The cell structure is another factor which affects final volume. The evenness and unevenness of the cells is another feature that is correlated well and will be investigated and explained in the next section. The correlation, is negative for even and positive for the uneven cell. Uneven is associated with the unchlorinated flour, and the even is associated with chlorinated flour.

The mixing stability from the farinogram was another set

of data that had a correlation that bears mentioning to tie in the volume of the baked angel food cake. The pH of the batter had a strong correlation to the mixing stability (0.632,  $P < 0.05$ ). Chlorination had an effect in lowering the mixing stability. This is in direct relation with the glutenins and bands (4:00, 5:45) which were reduced. Their correlation to the volume in the unchlorinated state were (0.616 and 0.502) and to the mixing stability are (0.491 and 0.614).

#### *4.06d Differential Scanning Calorimetry (DSC) of Batter*

When a material undergoes a change in physical state such as melting or transition from one crystalline form to another or when a material reacts chemically, heat is either absorbed or liberated. The instrumental technique used to study these transition phenomena is referred to as differential scanning calorimetry (DSC).

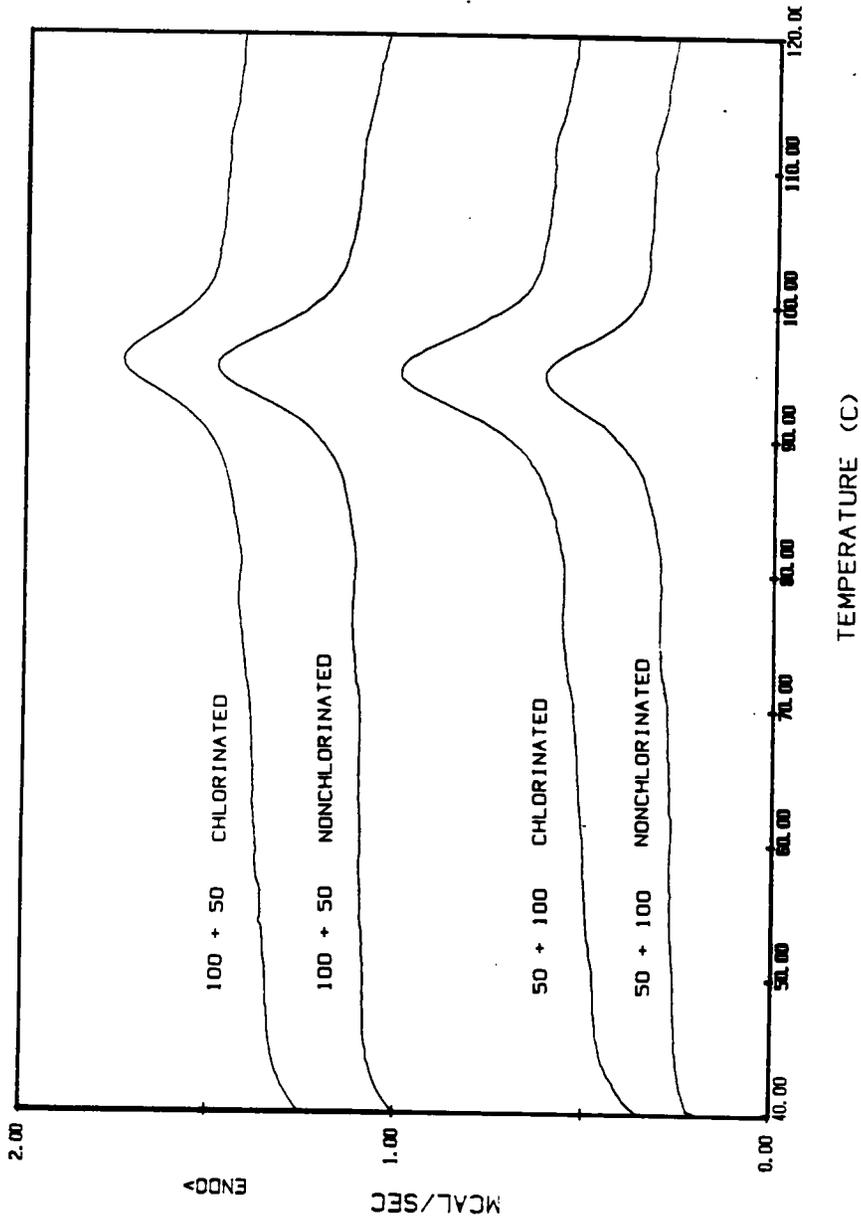
Table 18 lists the maximum temperatures of each flour treatment derived from the thermograms (Figures 42, 43, 44). Statistical analysis concluded the differences in temperatures were non-significant. Only one flour (Treatment 6, C) had a lower maximum temperature than the others, but again this was non-significant.

These results are in agreement with Donovan (1977) and

TABLE 18  
Maximum Temperatures From Flour Thermograms

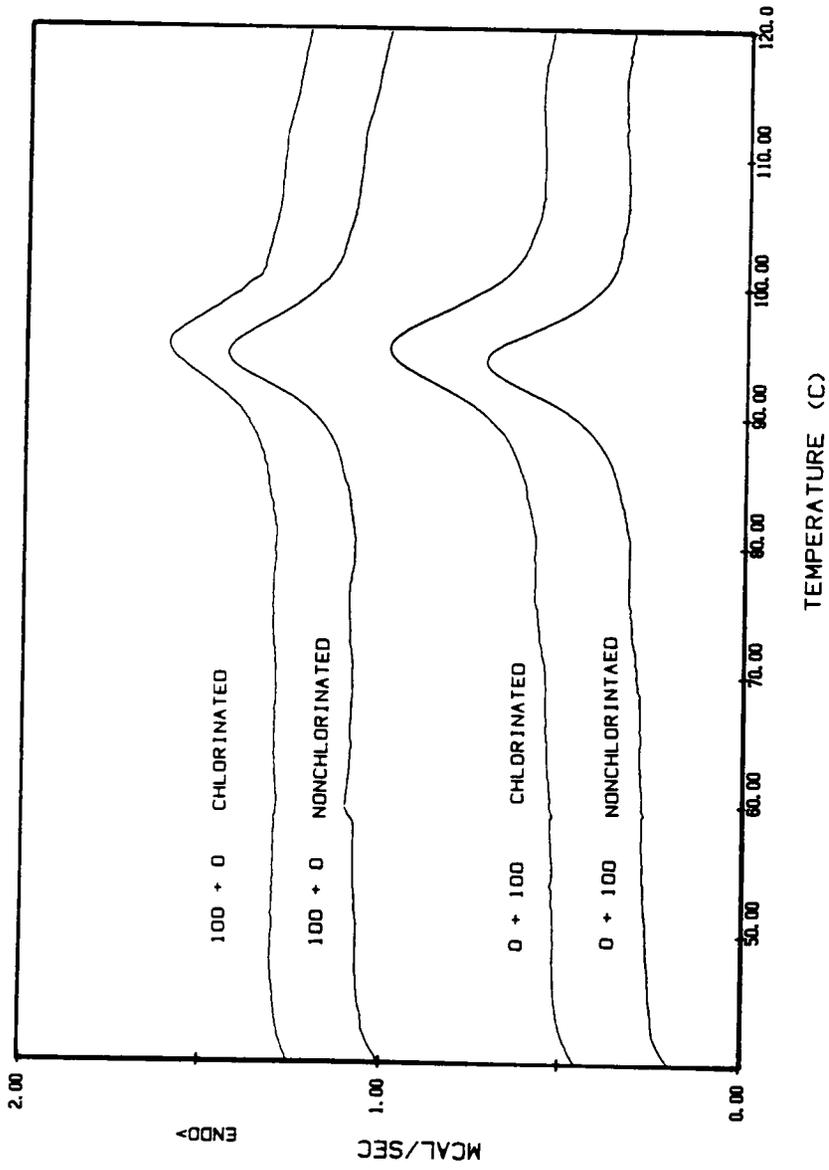
Flour	Max Temp. (°C)
Control	95.9a
TRT3 (NC) <sup>1</sup>	96.5a
TRT3 (C) <sup>2</sup>	96.1a
TRT6 (NC)	96.3a
TRT6 (C)	93.4a
TRT4 (NC)	94.9a
TRT4 (C)	95.7a
TRT7 (NC)	94.9a
TRT7 (C)	95.2a
TRT13 (NC)	96.1a
TRT13 (C)	94.9a
TRT15 (NC)	95.7a
TRT15 (C)	95.9a

<sup>1</sup>NC - Not chlorinated  
<sup>2</sup>C - Chlorinated  
a - No significant difference,  $p > 0.05$ .



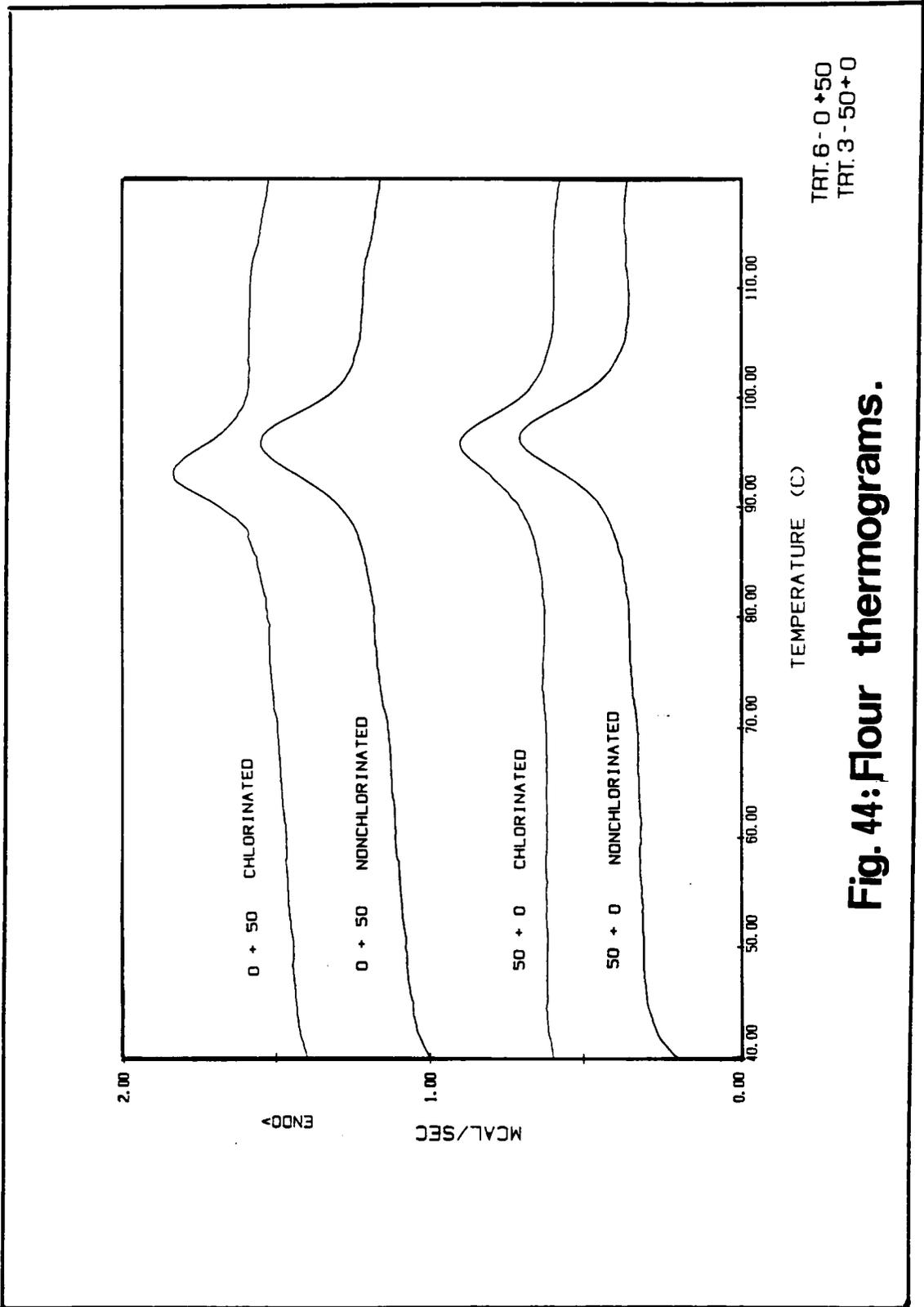
TRT. 15 - 100 + 50  
TRT. 13 - 50 + 100

**Fig.42: Flour thermograms.**



TRT. 7 - 100 + 0  
TRT. 4 - 0 + 100

**Fig. 43; Flour thermograms.**



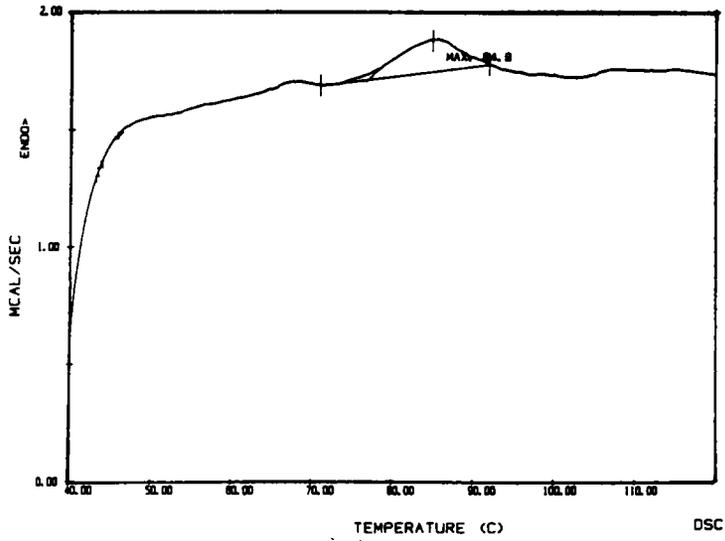
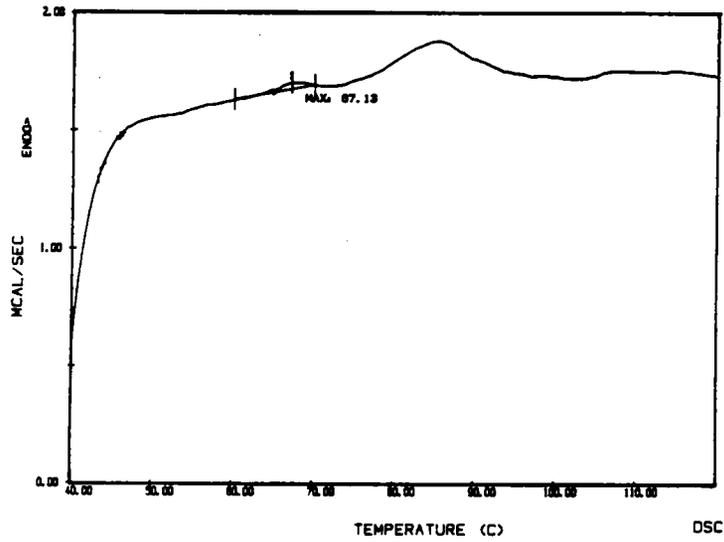
**Fig. 44: Flour thermograms.**

Jacobsberg and Daniels (1974) who researched the role of gelatinization of starch in baked products. Donovan (1977) worked especially with angel food cakes. He found that the gelatinization of the starch occurred roughly simultaneously with the denaturation of the major portion of the protein in the egg white. The batter has attained maximum volume when the starch and protein intermesh simultaneously to form the "structural framework" of the cake.

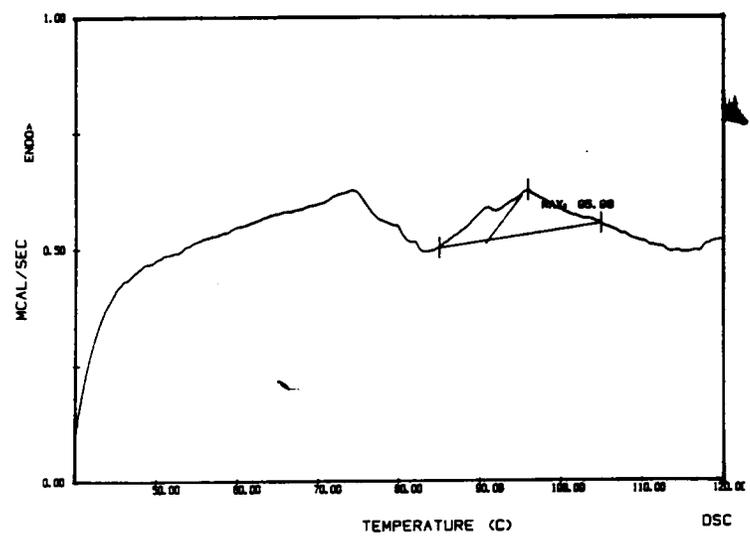
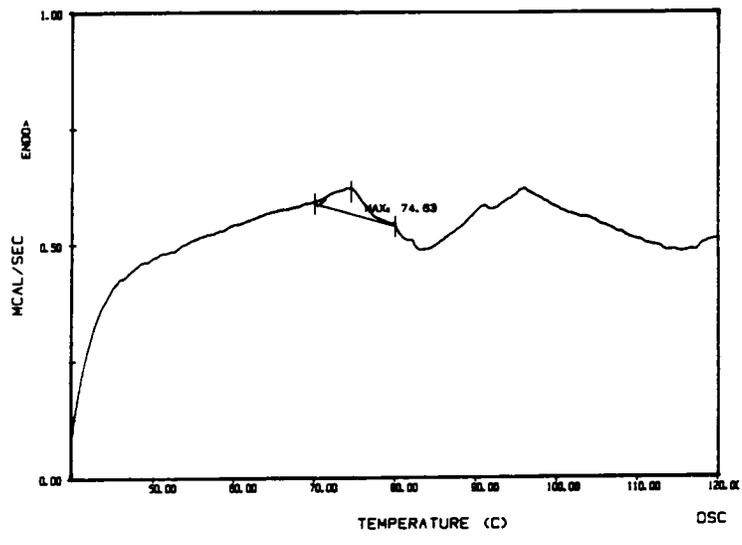
Figures 45 and 46 illustrate the heating effect on egg white alone and egg white and sugar. The two prominent endotherm peaks at 65 degrees and 84 degrees C are due to the denaturation of conalbumin and ovalbumin respectively (Figure 45). When sugar was added, denaturation temperature of the protein is raised to 75 degrees and 94 degrees C, respectively (Figure 46). The gelatinization of the starch occurs simultaneously with the denaturation of the major portion of the protein. The batter has attained maximum volume when the protein and starch intermesh simultaneously to form the structural network.

The starch has two functions during the final gelatinization (Allen, 1977): (a) swelling takes place to form "bricks" with viscoelastic properties characteristic of the final cake composite ("crumb") and (b) excess water is then removed from the crumb.

Chlorination had no significant effect upon the



**Fig. 45: Thermogram of egg white only.**



**Fig. 46: Thermogram of egg white & sugar.**

gelatinization temperature. Some researchers have examined the effect of chlorination on the gelatinization of wheat starch. Jacobsberg and Daniels (1974) found that chlorination did not alter the gelling temperature. Frazier and co-workers (1974) also found that chlorinating flour increased rheological properties of the batter. This treatment prevented collapse of the baked product.

Researchers studied cake batters continuously during the baking process. The formation of a more or less solid matrix appears to occur at 80 degrees C, owing to gelatinization of starch and the formation of a protein gel. Abbett (1987) found that the rheological behavior of the batter which is dependent on the amount of water present can have a direct influence on cake structure. For high values of batter viscosity, convective heat transfer was limited. Due to this limitation poor heating of the center region which would lead to collapsing.

Gough (1978) observed viscosity difference between unchlorinated and chlorinated flours in the batter system. The unchlorinated batter expanded more, then fell whereas, the chlorinated batter rose steadily to a lower height that was maintained. Presumably, the lower viscosity of the unchlorinated sample provided for greater expansion and a weaker structure.

The microscopic studies of the flour under the light and

scanning electron microscopes indicated a veil-like covering over the unchlorinated flour starch granules. The chlorinated flour samples appear to have this veil removed, thus exposing the starch granules. It has been proposed that the degree to which starch gelatinization progresses in the cooking cake batter probably determines the strength of the cooked crumb structure, and therefore, the cake's ability to resist collapse at the end of baking. It is further suggested that the improving action of chlorine is due to the way in which it influences the starch gelatinization behavior.

By reacting with the minor components (chiefly lipids, but possibly some proteins) associated with the surface of the granule, chlorination promotes changes in gelatinization. These changes occur in the loss of granule birefringence. The increase in granule swelling and/or amylose leaching, although slight, is quite noticeable in the cake. The swelling is sufficient to strengthen cake crumb structure and prevent collapse.

#### *4.06e Microscopy of Baked Product*

A true picture of the structure of the films that form the gas cell walls in bread and cake would be a valuable contribution to many baking studies. Microscopic techniques have proved to be valuable research tools for studying

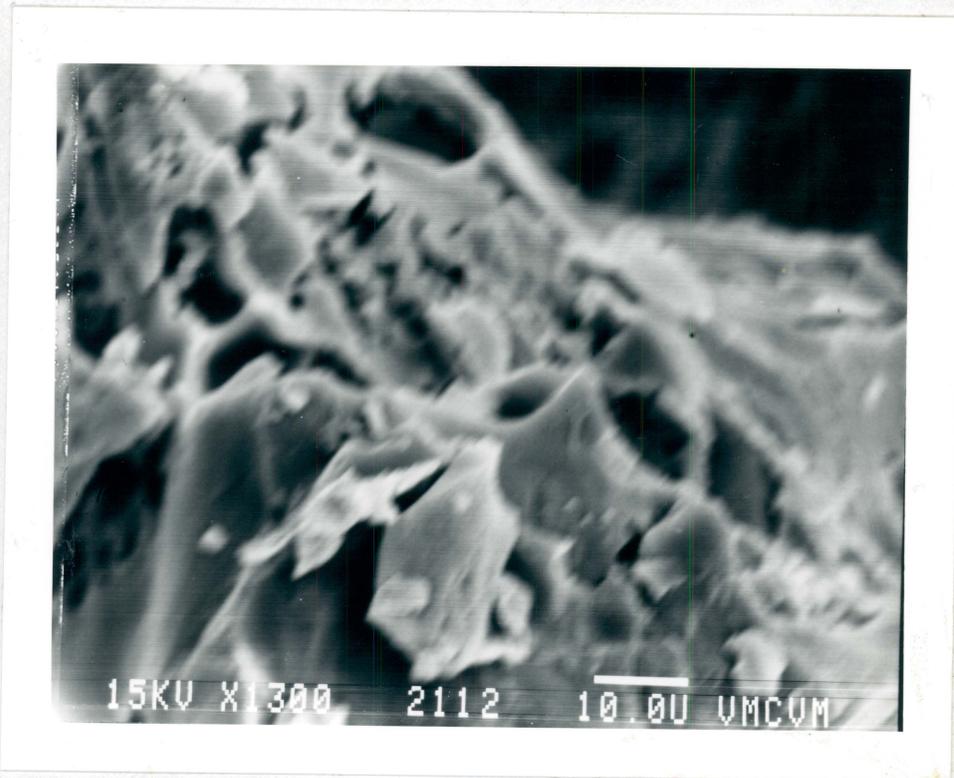
leavened baked products. Microscopy is a particularly useful and powerful instrument for studying the ultrastructure and functional relationships of the interactions that occur in situ.

Scanning electron microscopy (SEM) was used to examine the baked product. All 13 angel food cakes were examined under SEM. The samples were freeze-dried, fractured and placed on a stud with adhesive. Samples were gold-coated (see section 3.10K).

The changes as a result of baking were observed by SEM. Baking acts as a fixative by setting the final structure of denatured protein and gelatinized starch. Free water is absorbed by the starch. When the water is absorbed, the properties of the starch granules control the final physical characteristics of the baked cake (M. Taranto, 1983). Other components (e.g. protein) also compete with the starch for water and affect the final product.

Figures 47, 48, 49 and 50 illustrate a selection of angel food cakes viewed by SEM made from unchlorinated and chlorinated flours.

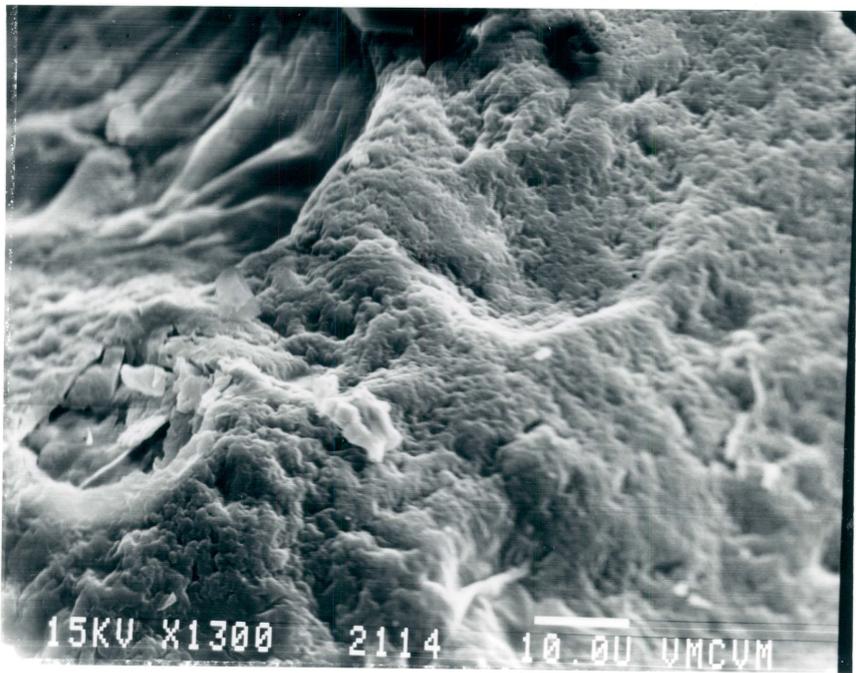
The cakes from unchlorinated samples (Figures 47 and 49) show extragranular material as clumps on the surface and between the starch granules. The cakes from chlorinated samples (Figures 48 and 50) show evidence of the protein matrix more fully developed. The protein-starch material



TRT 7: Unchlorinated (1300X)

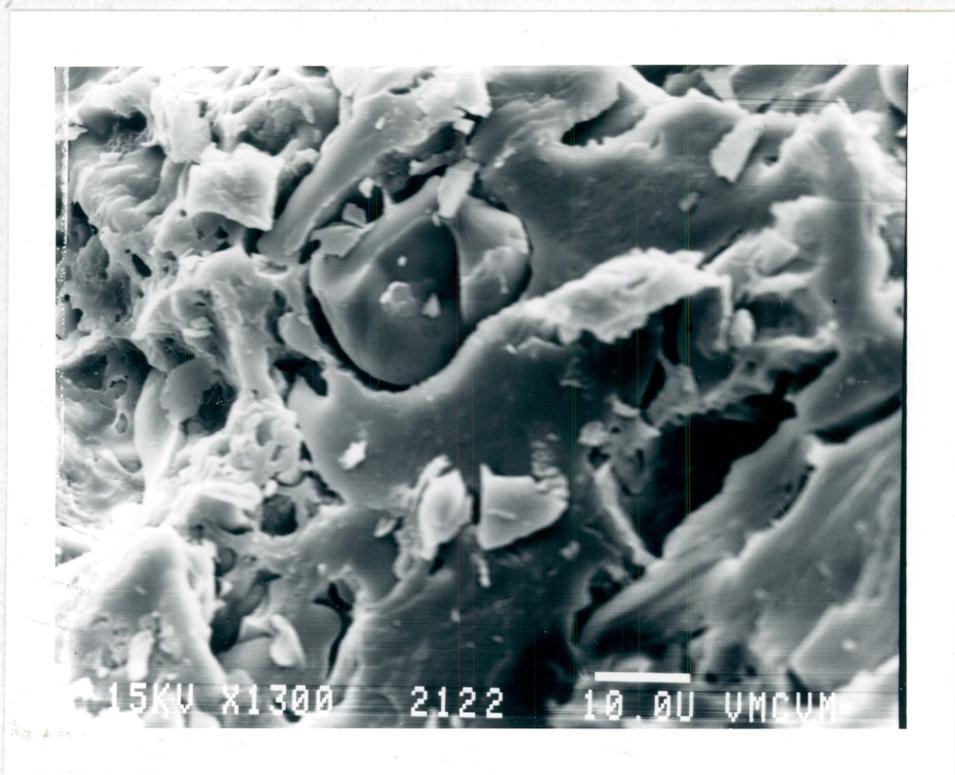
Fig47: SEM observation of angel food cake.

CHIEFTAIN BOND



TRT 7: Chlorinated (1300X)

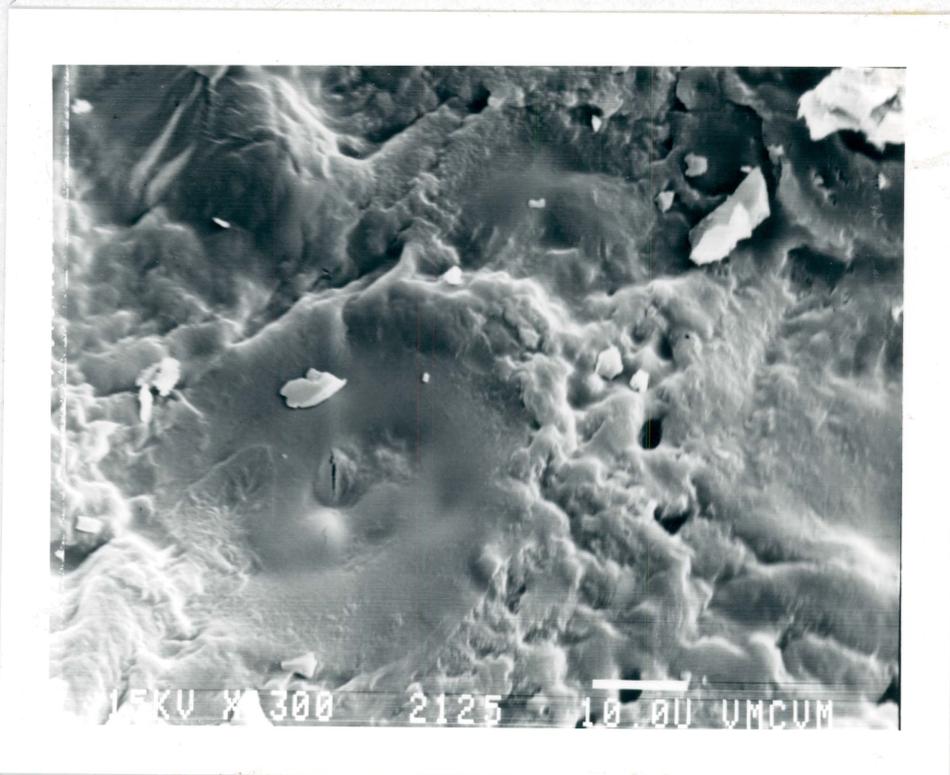
Fig. 48: SEM observation of angel food cake.



TRT 13: Unchlorinated (1300X)

Fig.49: SEM observation of angel food cake.

GHATERAIN DANDU  
50% COTTON FIBER



TRT13: Chlorinated (1300X)

Fig.50: SEM observation of angel food cake.

formed a more continuous network between the granules. This is in agreement with the results of Grider and co-workers (1983).

The continuous matrix formed had a cementing effect in cakes with chlorinated flour. The increased association between the starch granules resulted in a greater build up of the structural units. This cementing of the components into larger building block ultimately influence the final cake structure (Allen, 1977).

Moisture is a key factor of the structural component of the baked product. The experimental formula contained 268% moisture and 285% sugar on a flour basis. Derby and co-workers (1975) found in their microscopic studies that the amount of water absorbed by starch in different concentrations of sugar was based on the assumption that all the water in excess of the control amount was available for reaction with the starch.

Figures 48 and 50 show deformed and folded starch granules indicative of gelatinization. Lineback and Wonsrikasem (1980) found that starch isolated from angel food cake was extremely deformed and folded. The granules lost their fullness and were aggregated similar to those in scanning electron photomicrographs of starch gelatinized in excess water.

Similar results were shown by Hosney and co-workers

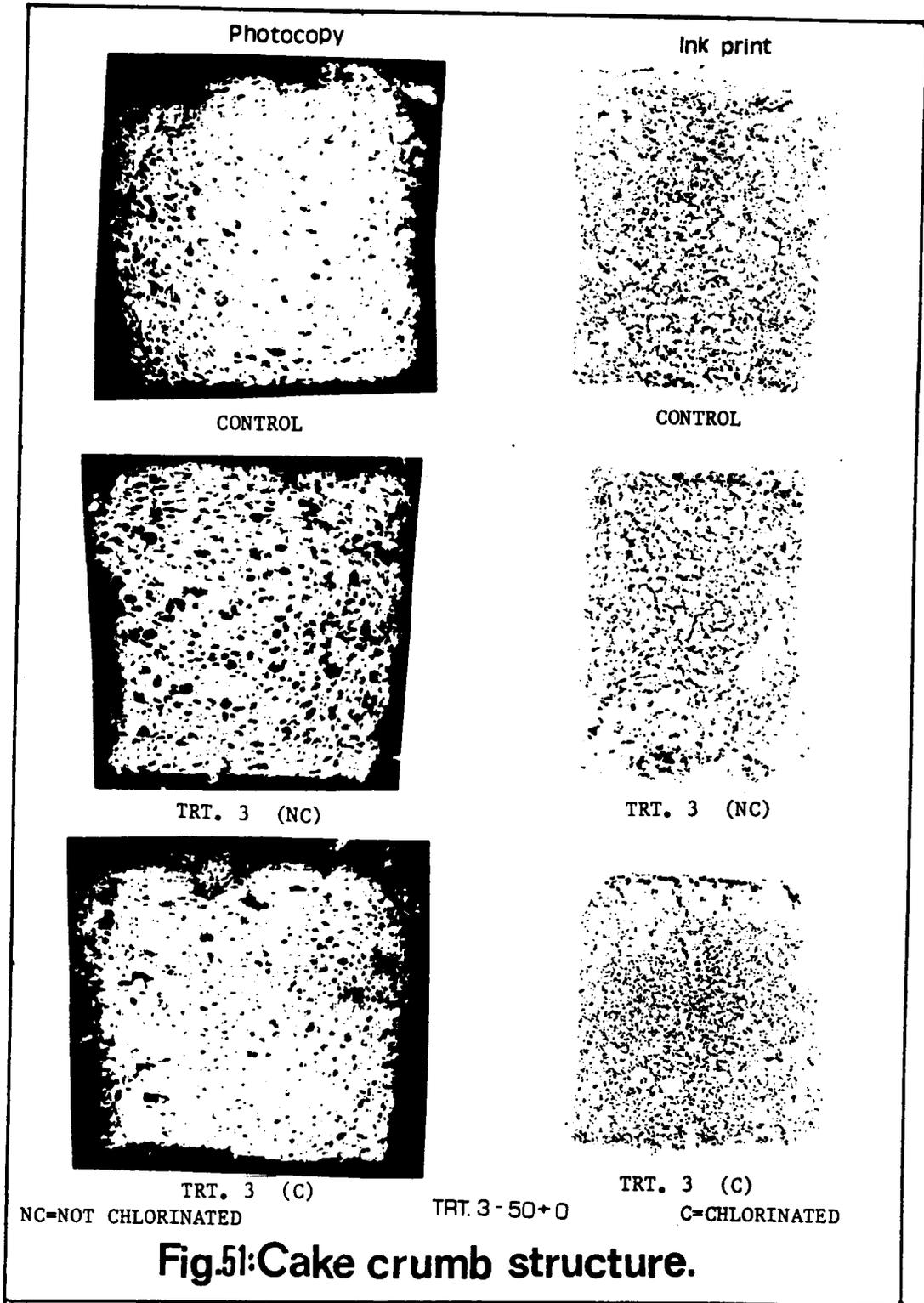
(1977, 1978). They examined the effect of chlorination had the finished baked product. Chlorinated flour observed under SEM and light microscope had the protein veil removed, thus allowing the starch to be more exposed and more vulnerable to reactions during baking.

The SEM study showed the veil-like protein sheet enveloping the starch granules in cakes. The protein sheets completely denatured permitting complete water uptake by the starch when chlorinated flour was used. The brittleness of the granule that appeared in cakes made with unchlorinated flour samples was explained by no protein-starch interaction. This was established by Pomeranz and Myer (1984) when they studied bread structure.

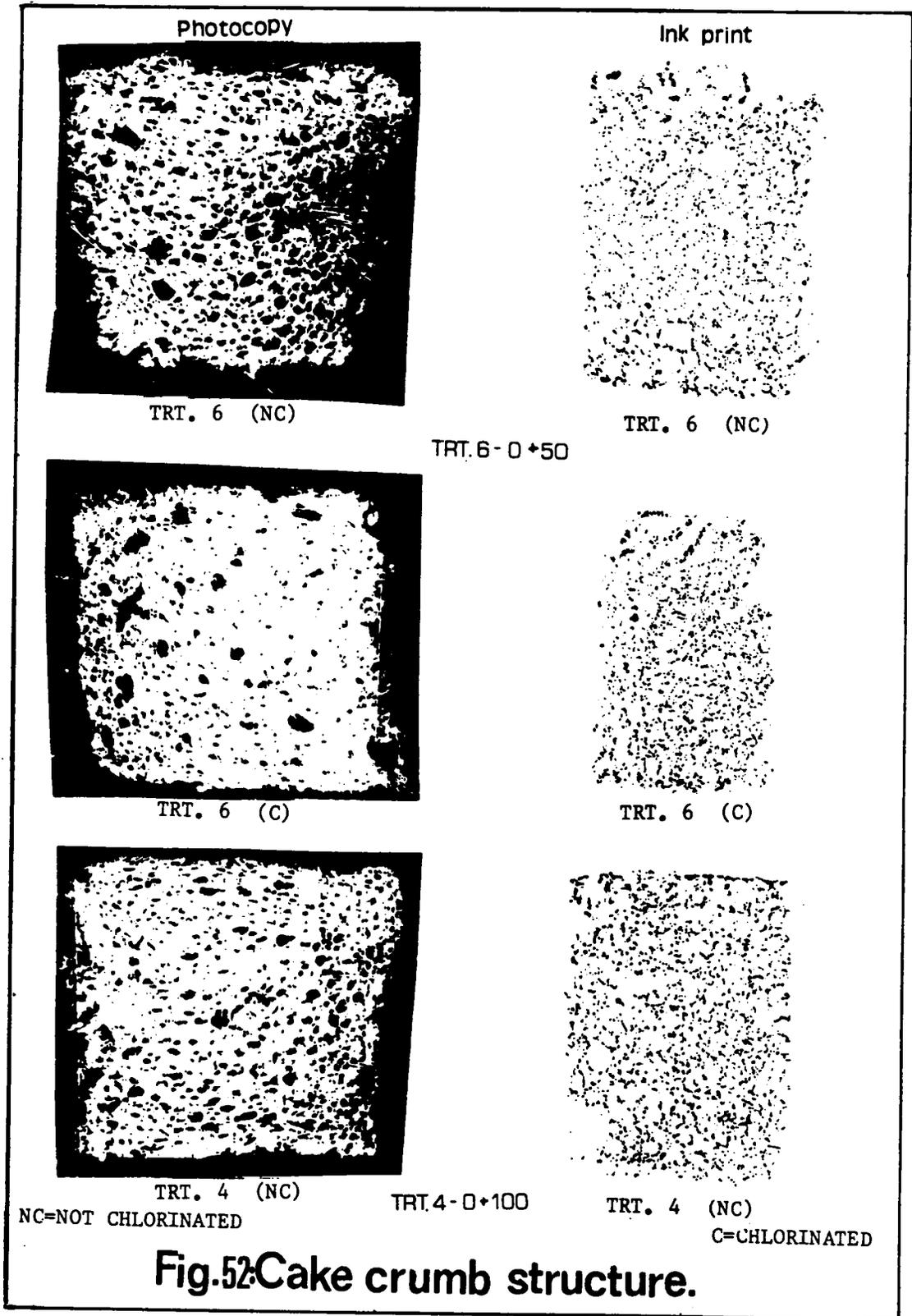
#### *4.06f Cell Structure of the Baked Product*

The cell size and uniformity of the angel food cake is the main focus of this section. Once the batter has expanded and set, the cells are formed which will characterize the product.

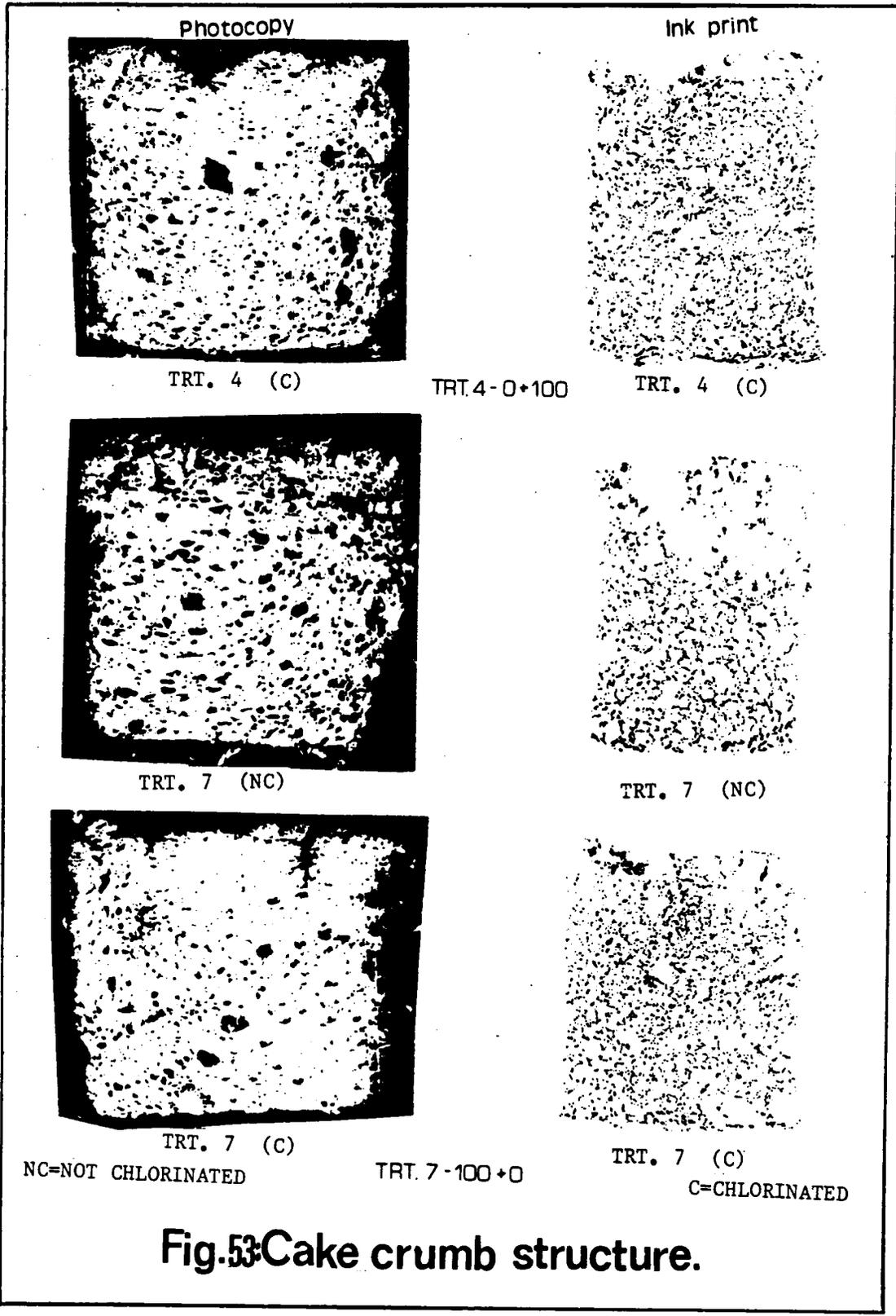
Figures 51, 52, 53, 54 and 55 illustrate the structure of the cakes in two different ways: by photocopy and ink print. These methods serve as a permanent record for the structural make-up of the cake. The cake cells in the unchlorinated flour were large, open, thick cell walled. The



**Fig.51: Cake crumb structure.**

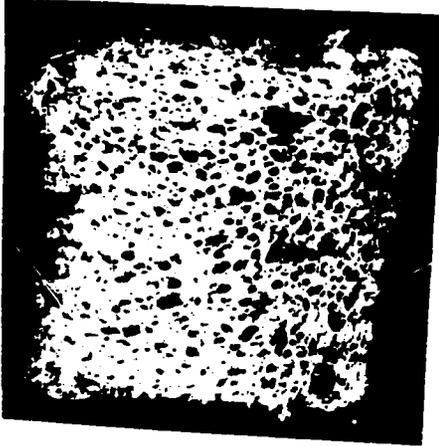


**Fig. 52: Cake crumb structure.**



**Fig.53 Cake crumb structure.**

Photocopy

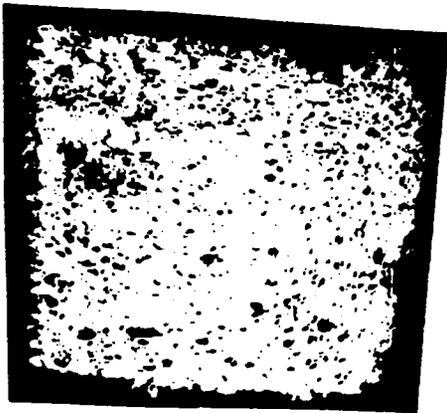


TRT. 13 (NC)

Ink print



TRT. 13 (NC)



TRT. 13 (C)



TRT. 13 (C)

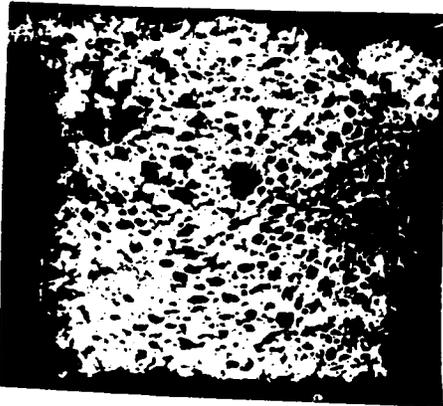
NC=NOT CHLORINATED

TRT. 13 - 50 \* 100

C=CHLORINATED

**Fig 54: Cake crumb structure.**

Photocopy

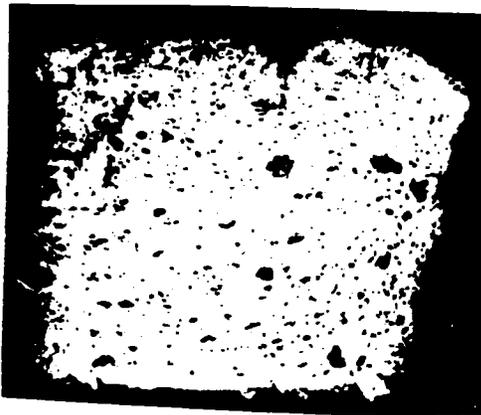


TRT. 15 (NC)

Ink print



TRT. 15 (NC)



TRT. 15 (C)



TRT. 15 (C)

NC=NOT CHLORINATED

C=CHLORINATED

TRT. 15 - 100+50

**Fig.55: Cake crumb structure.**

cells appeared elongated and stretched which contributed to the greater volume of the cake. There were flat, slightly collapsed cells underneath the crust supporting a flat macrostructure. There was no symmetry or slightly rounded contour.

The cakes made with chlorinated flours had a smaller, more rounded, compact uniform cellular structure. The cells had more closeness than those of cakes made with unchlorinated flours. Cakes with chlorinated flours had more uniformity in the rise and were lower in volume (Table 17). The volumes of the chlorinated cakes were more linear with regard to protein content than the unchlorinated (Figure 41). The structure could be a contributory factor along with the protein content.

Cakes of unchlorinated flours had a 3:1 ratio of uneven to even cell size. Cakes made with chlorinated flours had a reversal in the ratio of 1:2 uneven to even cells. There was a significant difference in ratio of uneven to even cells when chlorinated flour was used (Table 19).

The chlorinated cake samples observed under SEM showed complete gelatinization of the starch and protein matrix (Section 4.06e). This is one explanation for the total closeness of the crumb that is observed.

There are strong correlations of the effect of lipid on the evenness and unevenness of the cake crumb.

TABLE 19

## Cell Uniformity of Angel Food Cakes

Flour	Uneven	Even	Ratio Uneven:Even
Control	17.25	29.50	1:2
TRT3 (NC) <sup>1</sup>	27.50a	10.75a	3:1
TRT3 (C) <sup>2</sup>	17.50b	34.50b	1:2
TRT6 (NC)	20.50a	7.50a	3:1
TRT6 (C)	20.75a	32.20b	1:2
TRT4 (NC)	28.25a	10.00a	3:1
TRT4 (C)	16.00b	24.75b	1:2
TRT7 (NC)	23.25a	9.75a	2:1
TRT7 (C)	17.25b	26.00b	1:2
TRT13 (NC)	27.00a	9.25a	3:1
TRT13 (C)	19.75b	29.75b	1:2
TRT15 (NC)	23.25a	8.00a	3:1
TRT15 (C)	20.25a	32.50b	1:2

<sup>1</sup>NC - Not chlorinated  
<sup>2</sup>C - Chlorinated  
 Unlike letters within the same treatment are significantly different, p < 0.05

Chlorination affected the free fatty acid content of the flour (Section 4.04a (ii)). Fatty acids located on the flour's surface and are susceptible to the effects of chlorination. Chlorination resulted in a decrease of unsaturated fatty acids but the saturated fatty acids remained unchanged. There was a significant correlation between the evenness of the cell and the presence or absence of certain fatty acids.

Free fatty acid content had a direct correlation with the cell structure. The fatty acids involved were 18:1, 18:2 and 18:3 (Table 20). The values were based on correlations derived from unchlorinated flour (uneven cells) and chlorinated flour (even cells). Chlorination caused a decrease with the FAME content of the unsaturated lipids. The results have shown that there was a positive correlation with the unevenness of the cells in unchlorinated flour. The evenness correlation indicated that as the fatty acid content decreased with chlorination there was an increase in cell evenness.

One explanation is the gluten-lipid interaction taking place within the cake-batter system. A batter is a colloidal dispersion of several dispersed phases such as air cells, starch granules and other solid particles. The entire system is identified as a continuous matrix.

The continuous matrix of dough consists of a concentrated

TABLE 20

Correlation of Unsaturated Fatty Acids to Crumb Structure

Fatty Acid	<u>Crumb Structure</u>	
	Evenness	Unevenness
C 18:1	-0.822	0.523
C 18:2	-0.843	0.575
C 18:3	-0.807	0.632

aqueous lamellar phase of wheat storage protein. The gas cells of a dough have a lipid monolayer on the surface of the matrix. Equilibrium is dependent upon the degree of dispersion of the lipid in the continuous gluten phase (Chung and Pomeranz, 1981).

When polar and non-polar lipids are mixed in the batter they are dispersed into a lamellar phase (Larson, 1987). The lamellar phase is ideal for stabilization of gas cells and contributes to the gas holding capacity of the batter. When heat is applied this gas is released, the batter rises and the cells are formed.

Polar lipids unlike non-polar lipids interact with water giving liquid-crystalline phases. Their use as functional additives in foods is usually related to their aqueous interaction. Lipid-water phases are able to stabilize foam structures in foods. When the dough is formed there is a dispersion of starch granules and air bubbles in a continuous gluten phase.

The lamellar liquid crystal phase forms small aggregates so called liposomes in an excess of water type environment. These small particles are ideal to stabilize expanding gas/water interface.

Gelatinization takes place if amylose molecules can leach out from the starch granules to the water phase. If lipid monomers form an insoluble surface film on the granule,

gelatinization should be inhibited. The surface coating by lipid offers a possibility to increase the gelatinization temperature and reduce water uptake. Kissell (1979) suspected that the enhancing effect of lipids had on cake quality was due to the relocation of the lipids to the surface of flour particles. They are then readily available to act as functional hydrophilic emulsifiers during batter preparation.

The three fatty acids (C18-1, C18-2, C18-3) were associated in the crumb makeup of the baked product. Chlorination indicated that these fatty acids were in a critical position on the starch granule not only by their reduction but in the outcome of the cell structure.

#### **4.06g      *Tenderness of the Baked Product***

Tenderness is defined as having a soft or yielding texture. It is something that is easily broken, cut or damaged: delicate or fragile. Slices of angel food cake were examined for tenderness with the Baker's Compressimeter (Appendix L). The instrument measures the amount of pressure applied to the cake piece. The value (gm) derived is the tenderness of the cake (Table 21).

Tenderness was affected by fertilization and chlorination. The cakes prepared with unchlorinated flour showed a decrease in tenderness as the fertilization of the

TABLE 21  
Tenderness of Angel Food Cakes

Flour	Tenderness (Gm)
Control	16.79
TRT3 (NC) <sup>1</sup>	7.43a
TRT3 (C) <sup>2</sup>	14.707b
TRT6 (NC)	8.59a
TRT6 (C)	15.42b
TRT4 (NC)	10.29a
TRT4 (C)	16.45b
TRT7 (NC)	10.60a
TRT7 (C)	16.73b
TRT13 (NC)	13.38a
TRT13 (C)	15.55b
TRT15 (NC)	11.81a
TRT15 (C)	15.85b

<sup>1</sup>NC - Not chlorinated  
<sup>2</sup>C - Chlorinated  
 Unlike letters within the same treatment  
 are significantly different,  $p < 0.05$

grain increased. Cakes of chlorinated flour were significantly less tender than those of unchlorinated flour. Chlorination stabilized the product whereby, the tenderness values within flour treatments were about constant. This same type of stabilization effect was seen with the volumes and cell evenness ratio of the baked product.

The decreased tenderness of cakes made with chlorinated flours may be related to gelatinization and cell structure in the unchlorinated flour. Starch granules of unchlorinated flour do not gelatinize and swell sufficiently to make a mutual contact before the batter sets. There are the large open cells and the shrinkage of the cake due to the fragility or lack of support that the cells have in the baked product.

There were significant correlations to show the effect of chlorination on tenderness. The pH of the flour and batter (-0.87 and -0.65) were negatively correlated with tenderness. Chlorination decreased the pH and when this occurred, the tenderness decreased.

The height after cooling (Hgt (c)) and volume (-0.59 and -0.58, respectively) were negatively correlated with tenderness. As the volume and Hgt (c) decreased there was a decrease in tenderness.

The evenness and unevenness of the cells (0.83 and -0.73, respectively) had a significant correlation to tenderness. As the even cells increased, due to chlorination, there was

a decrease in tenderness. To further involve the evenness of the cells, the tenderness correlation was significant to the presence of three fatty acids: C18:1, C18:2, C18:3 (-0.78, -0.74 and -0.70, respectively). The decrease in the free fatty acids had an influence on the evenness of the cell structure.

#### *4.06h Cohesive Index*

The cohesiveness of the cake crumb was estimated from repeated penetrations under a constant load by a similar procedure of Kamel and Rasper (1986). Cohesive indices were calculated by using the penetrometer (Appendix K). The results (Table 22) indicated there were no significant difference in the Cohesive index between the unchlorinated and chlorinated flours. The trend was, though not significant, that cakes from chlorinated flour had a lower cohesive index. The crumb in a cake with chlorinated flour was less tender, and thus making it less cohesive.

#### *4.06i Color of Internal Crumb*

Human vision responds to a tristimulus in the sensory perception of color. The eyes possess three types of light-sensing devices each corresponding to a different band of

TABLE 22

## Cohesive Index of Angel Food Cakes

Flour	Cohesive Index
Control	0.6417a
TRT3 (NC) <sup>1</sup>	0.8416a
TRT3 (C) <sup>2</sup>	0.6133a
TRT6 (NC)	0.6748a
TRT6 (C)	0.6951a
TRT4 (NC)	0.7482a
TRT4 (C)	0.6747a
TRT7 (NC)	0.6490a
TRT7 (C)	0.6372a
TRT13 (NC)	0.6647a
TRT13 (C)	0.5641a
TRT15 (NC)	0.7552a
TRT15 (C)	0.5984a

<sup>1</sup>NC - Not chlorinated  
<sup>2</sup>C - Chlorinated  
a - No significant difference,  $p > 0.05$

wavelengths. The XYZ values of the International Commission on Illumination (CIE) are the numerical representatives of red, green and blue and have been adopted as international standards.

The Hunter Labscan Spectrophotometer was used to measure the internal crumb color of the angel food cakes (Table 23). The values used for evaluation were : L, b, and  $\Delta E$ . These values represented: the lightness, yellowness and difference from the standard, respectively. With one exception, there was a significant difference in lightness between the unchlorinated and chlorinated flours and between cakes of chlorinated and unchlorinated flours. The L values for cakes of Treatments 4 and 7 (0 + 100, 100 + 0, respectively), did not change.

The color of the crumb depends on two factors according to Francis and Clydesdale (1975). The factors are: color of the flour and the method of preparation. The cakes made with unchlorinated flour had a coarse uneven structure that was darker in color as measured by L and  $\Delta E$  values. Even cells were associated with cakes of chlorinated flour and brighter color. The evenness of cells was significantly correlated to the L values of 0.78 and to the  $\Delta E$  values of 0.75. One explanation may be that small cells produce more multiple reflections and, thus, appear lighter.

The degree of yellowness (b) of the crumb is dependent

TABLE 23  
Color of Internal Crumb

Flour	L <sup>3</sup>	b <sup>4</sup>	ΔE <sup>5</sup>
Control	88.56	+13.49	89.53
TRT3 (NC) <sup>1</sup>	86.29a	+14.76a	87.49a
TRT3 (C) <sup>2</sup>	88.06b	+13.44b	89.04b
TRT6 (NC)	85.68a	+15.19a	86.98a
TRT6 (C)	87.55b	+13.98b	88.60b
TRT4 (NC)	86.25a	+14.62a	87.67a
TRT4 (C)	85.71a	+14.29a	86.85a
TRT7 (NC)	86.91a	+14.62a	88.10a
TRT7 (C)	85.71a	+14.62a	86.85a
TRT13 (NC)	85.47a	+15.23a	86.72a
TRT13 (C)	87.69b	+13.99b	88.49b
TRT15 (NC)	85.05a	+14.86a	86.29a
TRT15 (C)	87.57b	+13.99a	88.60b

<sup>1</sup>NC - Not chlorinated  
<sup>2</sup>C - Chlorinated  
<sup>3</sup>L - 100 = white; 0 = black  
<sup>4</sup>b - + = yellow; - = blue  
<sup>5</sup>ΔE =  $\sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$   
 Unlike letters within the same treatment are significantly different, p < 0.05

upon the amount of carotene and xanthophyll in the original wheat and the natural and artificial bleaching of the flour. Croes (1961) emphasized that when expressing the visual impression of whiteness, the lightness alone is inadequate, since the degree of yellowness should also be taken into account.

There was generally a significant difference between the b values of unchlorinated and chlorinated flours (Table 23) and the cakes made with these flours. There was no significant change in the b value of the flour and cake of Treatments 4, 7, and 15.

Hunter and Harold (1987) emphasized that when using the L, a, b system, the b dimension measuring yellowness is the most critical of the three. This statement is a very important one, considering the results of the study.

Hunter and Harold (1987) also felt that the two dimensions, L and b, are sometimes adequate for the identification of a white color.

Other variables which affect the color perception of the baked product are the level and color of the illumination and size and flatness of the specimen. The light source can easily pass through the porous structure of angel food cake and the results would be in error. Therefore, the thickness of the slice was important.

#### 4.07 *Sensory Evaluation*

##### 4.07a *Sensory Characteristics*

The cakes were rated by QDA for: color, cell size, cell uniformity, chewiness, cohesiveness, and moistness. The two senses used for rating sensory attributes were vision and mouthfeel. The discussion of this section will be divided according to these senses.

##### 4.07b *Sense of Vision*

###### 4.07b (i) *Color*

Vision can be regarded as the process of seeing whereas appearance is the recognition and assessment of the properties. This would include the surface structure opacity and color associated with the object seen.

The cakes of flour treatments 13 and 3 were significantly whiter when made with chlorinated flour (Table 24). The effect the color had on the visionary aspect of the judges was surprising. There is no explanation why these two treatments were whiter while all treatments showed a change in the chlorinated state.

Francis and Clydesdale (1975) reported on the color of

TABLE 24

Sensory Attributes for Baked Cakes I  
(Vision)

Flour	Crumb Color <sup>3</sup>	Cell Size <sup>4</sup>	Cell Unif. <sup>5</sup>
Control	9.57	9.55	8.24
TRT6 (NC) <sup>1</sup>	6.38a	4.81a	6.54a
TRT6 (C) <sup>2</sup>	8.65a	8.81b	8.07a
TRT3 (NC)	6.15a	6.45a	7.12a
TRT3 (C)	9.42b	10.71b	9.51b
TRT4 (NC)	7.03a	4.86a	5.91a
TRT4 (C)	8.77a	9.38b	8.78b
TRT7 (NC)	6.64a	4.13a	5.62a
TRT7 (C)	8.76a	8.66b	8.25b
TRT13 (NC)	5.49a	4.91a	6.15a
TRT13 (C)	9.54b	11.12b	9.70b
TRT15 (NC)	6.96a	4.44a	5.84a
TRT15 (C)	9.35a	9.99b	8.80b

<sup>1</sup>NC - Not chlorinated

<sup>2</sup>C - Chlorinated

<sup>3</sup>0 - Creamy; 14 - White

<sup>4</sup>0 - Large; 14 - Small

<sup>5</sup>0 - Uneven; 14 - Even

Unlike letters within the same treatment are significantly different,  $p < 0.05$ .

bread crumb. A properly fermented bread had a fine crumb with soft texture, bright color and a good sheen. An improperly fermented bread had a coarser structure and appeared darker in color.

The correlation coefficient for the b value of the angel food cakes made with unchlorinated flour had a -0.67 correlation coefficient with the sensory color analysis. As the b value increased due to yellowness the value for the sensory decreased (creamy). The b value decreased when chlorinated, thus affecting the whiteness of the product.

#### *4.07b (ii) Cell Size and Uniformity*

Cell size (Table 24) was rated significantly different among the cakes made with unchlorinated and chlorinated flours. Cakes made with unchlorinated flour had a large cell size, while cakes made with chlorinated flour had a small cell structure.

The sensory panel scores were in agreement with evaluation of the photocopies of the baked cakes (Figures 51-55). Cakes made with unchlorinated flours had large, opened, deformed cells. Cakes made with chlorinated flour had cells with a smaller, rounder, and closer structure.

The cell uniformity of the angel food cakes was also examined by panel members. There was a significant difference

between the cakes made with unchlorinated and chlorinated flour (Table 24). According to the orthogonal contrast applied to the statistical analysis, chlorination had a strong effect on decreasing the size and increasing evenness of the cell structure.

The sensory analysis concerning evenness of the cells complements the data derived from the uneven:even ratios from the photocopies. Chlorination decreased the amount of uneven cells and significantly increased even cells in the baked product.

There were significant correlation coefficients derived from the sensory data. The unevenness and evenness of the cells were significantly related to cell size and cell uniformity of the sensory data. The chlorination effect of the flour batter is the strong characteristic for this analysis. Chlorination lowered the pH of the batter. For chlorinated flour, the correlation coefficient is  $-0.74$  for the sensory cell size. As the pH decreased, the value for the cell size increased which indicated small size. There was also a similar coefficient,  $-0.78$ , for cell uniformity. Cakes made with chlorinated flour had a lower pH and greater uniformity.

The evenness and unevenness of the cells demonstrated a significant correlation with the chlorination effect. The correlation coefficient for cell size (sensory) was  $-0.72$  and

0.95 for uneven and even cells respectively. When considering cell uniformity for the chlorinated flour the correlating coefficients were -0.74 and 0.92 for uneven and even cells, respectively.

The  $\Delta E$  value derived from the chlorinated flours had a significant correlation with cell size and cell uniformity based upon chlorinated flours (0.72 and 0.68, respectively). Chlorination caused an increase in the whiteness of the cake crumb. The size and uniformity of the cake cell was important when considering the internal crumb color.

Correlation coefficients between the derived sensory data (color, cell size and cell uniformity) and chlorination treatments were significant. The color of the crumb indicated a strong correlation with cell size and cell uniformity (0.94 and 0.88, respectively). The correlation coefficients was 0.97 for cakes made from chlorinated flour; for cakes from unchlorinated flours the coefficient correlation was 0.91.

The panel members were able to assess the size and evenness of the cake crumb with greater accuracy than the color of the cake crumb. The way the eye interprets color was highly variable especially between individuals. Shadows on the cell structure of the sample may have affected the visual rating of color. The size and uniformity of the crumb played an important function for color interpretation.

#### 4.07c *Role of Texture in Sensory Testing*

The size and shape of the sample presented to the panelist was standardized. Cake pieces at room temperature were served to the panelists 24 hours after baking.

##### 4.07c (i) *Chewiness*

This and the next two sections deals with the mouthfeel of the cake samples. Chewiness was defined by the sensory judges as the amount of force used to masticate the cake piece when tasted.

Chlorination of the flour significantly increased the chewiness of the angel food cake (Table 25). Cakes from fertilization treatment (TRT 6, TRT 7, and TRT 15) were significantly different. There was a trend of increased chewiness with chlorinated flour.

The crumb structure was rated firmer due to the chlorination effect. The firming effect was attributed to a less tender cake structure which was confirmed by compressimeter (Section 4.06g).

The pH of the batter had a weak correlation coefficient -0.39, but there is an indication that chlorination of flour has an effect on the chewiness of the cake. Even cell structure was associated with cakes made with chlorinated

TABLE 25

Sensory Attributes for Baked Cakes II  
(Textural)

Flour	Chew. <sup>3</sup>	Cohesive <sup>4</sup>	Moist <sup>5</sup>
Control	8.52	7.44	5.24
TRT6 (NC) <sup>1</sup>	8.23a	6.88a	9.58a
TRT6 (C) <sup>2</sup>	8.23b	7.53a	7.29b
TRT3 (NC)	5.70a	7.34a	9.60a
TRT3 (C)	6.42a	6.64a	7.30b
TRT4 (NC)	6.27a	7.01a	8.60a
TRT4 (C)	7.43b	7.15a	6.60b
TRT7 (NC)	5.36a	7.17a	9.33a
TRT7 (C)	7.02a	6.89a	7.23b
TRT13 (NC)	6.13a	7.22a	9.17a
TRT13 (C)	7.13a	6.97a	6.95b
TRT15 (NC)	5.86a	6.96a	8.85a
TRT15 (C)	7.29b	7.47a	7.33b

<sup>1</sup> NC - Not chlorinated

<sup>2</sup> C - Chlorinated

<sup>3</sup> 0 - Less; 14 - More

<sup>4</sup> 0 - Less; 14 - More

<sup>5</sup> 0 - Less; 14 - More

Unlike letters within the same treatment are significantly different,  $p < 0.05$ .

flours. The correlation coefficient for even and uneven cells were 0.79 and -0.61, respectively. These structural characteristics were characteristic of chewiness.

The tenderness scores derived with the Baker's Compressimeter were strongly correlated with chewiness (0.79). A high score with the compressimeter was indicative of being less tender. Therefore, the effect of chlorination had a strong relation to decreasing tenderness and increasing the chewiness of the baked product.

#### 4.07c (ii) *Cohesiveness*

The panel members rated the cake pieces for cohesiveness. The criterion was defined by chewing and the difficulty in swallowing. The way the cake balled up in the mouth or stuck to the side of the mouth was also considered. Statistical analyses of the ratings indicated no significant difference (Table 25). Cohesiveness was the only attribute that was not significantly different among treatments.

This category is a good example for the term being a descriptor, but not a discriminator. According to Powers (1984), if the F value is non-significant then the descriptor can only be characterized as being a descriptor. If the F-value is significant, then the term is a discriminator. The term may also fail to be a discriminator because the attribute

exists in all the samples at approximately the same intensity.

The difference of cohesive index that was used for the baking analysis was also found to be non-significant. The action of chlorine on the flour used did not have any effect with the sensory panel or objective measures.

There was a strong correlation coefficient (0.87) between the sensory attribute of chewiness with cohesiveness for cakes made with chlorinated flour. The panel members correlated chewiness with the cohesiveness of the chlorinated cakes.

#### 4.07c (iii)

#### *Moistness*

The last attribute dealing with texture was moistness. There was significant difference among treatments (Table 25). Cakes made with unchlorinated flour were more moist than cakes with chlorinated flour. The cake made with the commercially chlorinated cake flour was less moist than the experimental chlorinated flour. The increased moistness of cakes made with chlorinated flours was supported by SEM examination. The starch of cakes made with chlorinated flours was fully gelatinized and the starch and protein have formed a more complete matrix.

The full gelatinization that took place also inferred

that there was less free water in the cake make-up, thus making it less moist. When flours were viewed under the light-microscope and SEM, there was a veil-like covering over the starch granules. When the flour was exposed to the chlorination process, the veil was removed, the starch is exposed and, available to compete with the protein for the free water.

The structure of the crumb was established due to these reactions taking place. The cakes with chlorinated flour had a less tender crumb than cakes made with unchlorinated flour. The sensory analysis of moistness correlated strongly ( $-0.87$ ) with the instrumental data derived on tenderness.

#### *4.07d Circular Graphs of Sensory Analysis*

From the sensory data generated, circular graphs (Figures 56, 57, 58, 59) were plotted. Each flour treatment is represented by a figure with the unchlorinated (broken line) and chlorinated flour (solid line). Each spoke of the web represents a sensory attribute.

The graph was constructed to permit visualization of the difference between flour treatments. The distance from the center of the spoke to the end point is in proportion to the mean. The profiles presented the data in a summarized form and serve as a visual aid for interpreting the data.

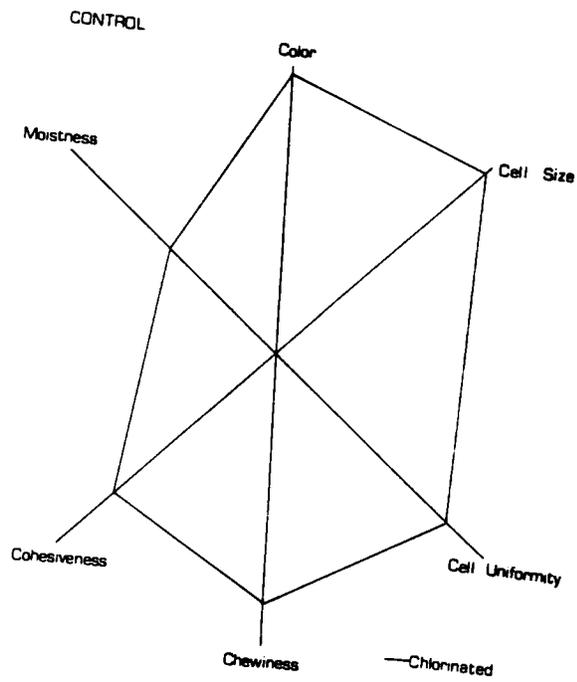


Fig. 56: Circular graphs for sensory analysis

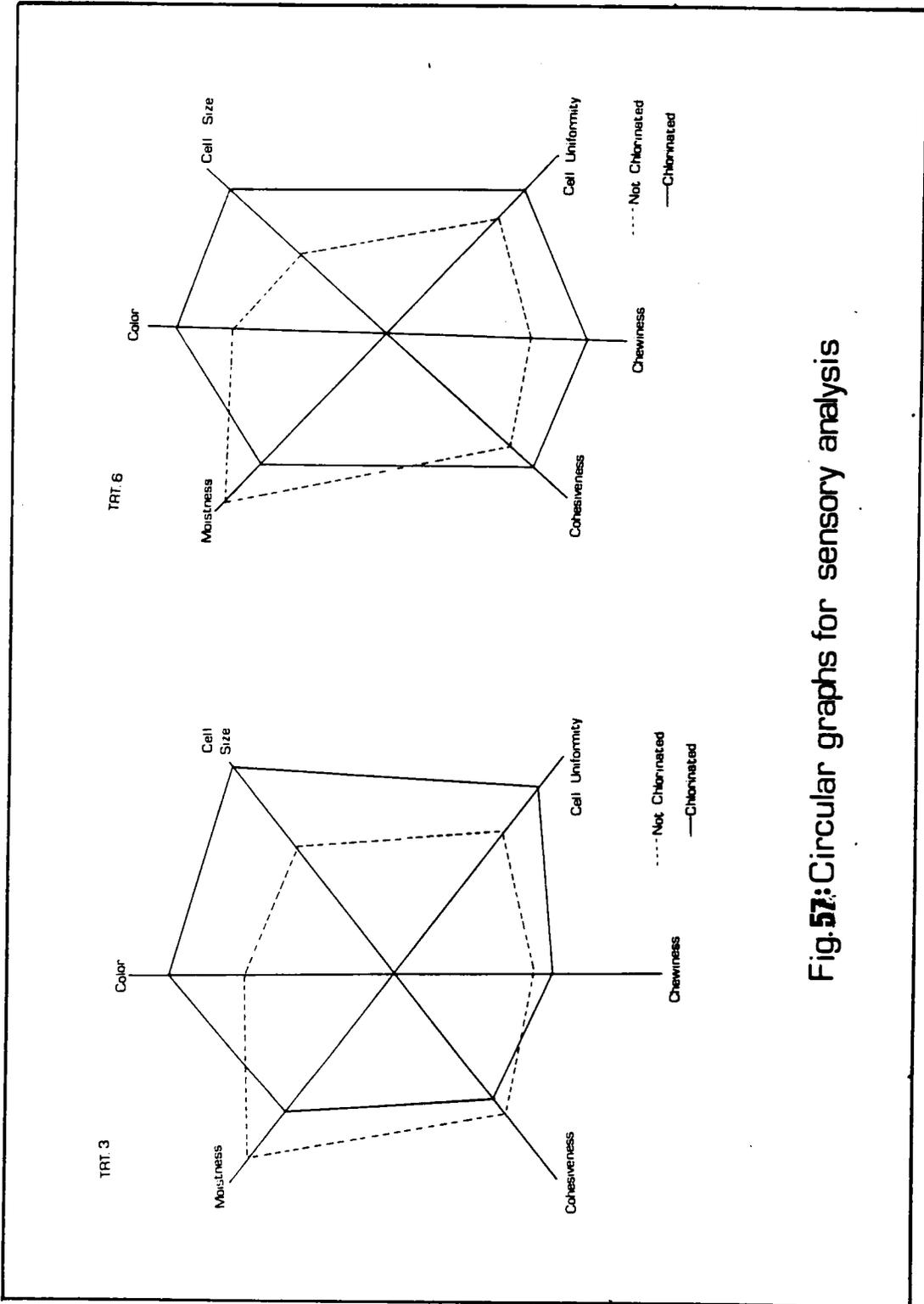


Fig. 57: Circular graphs for sensory analysis

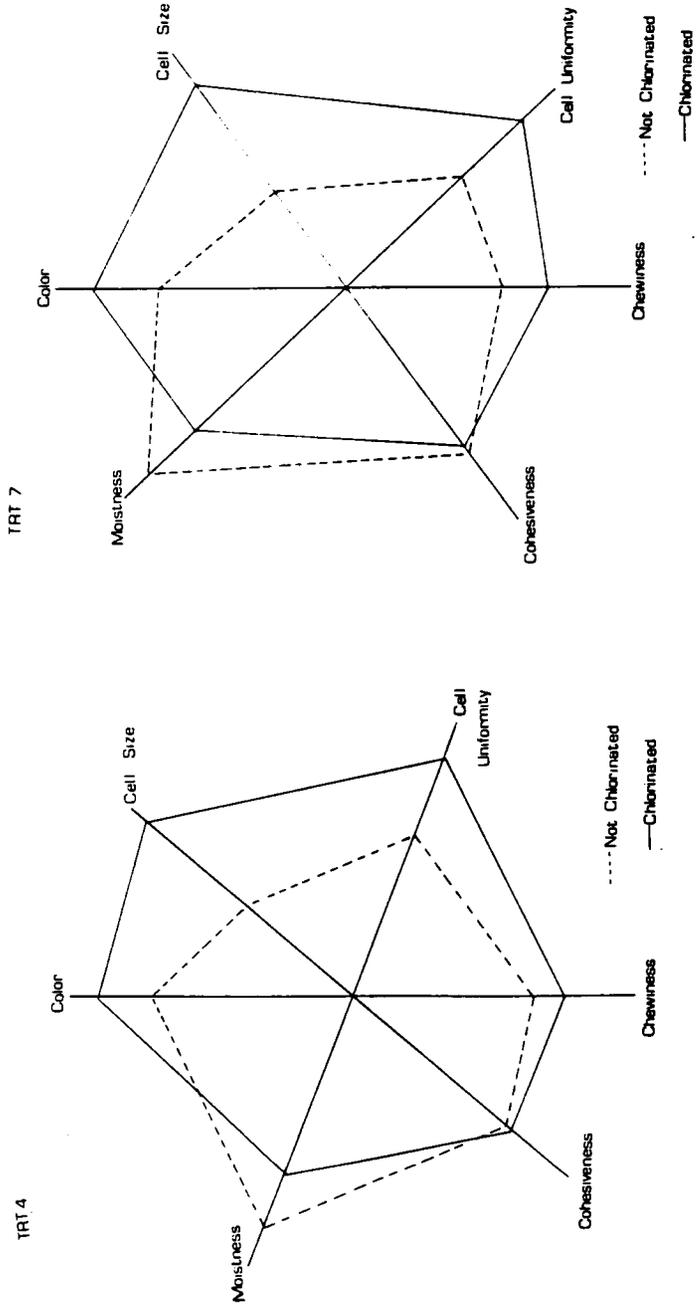


Fig. 58: Circular graphs for sensory analysis

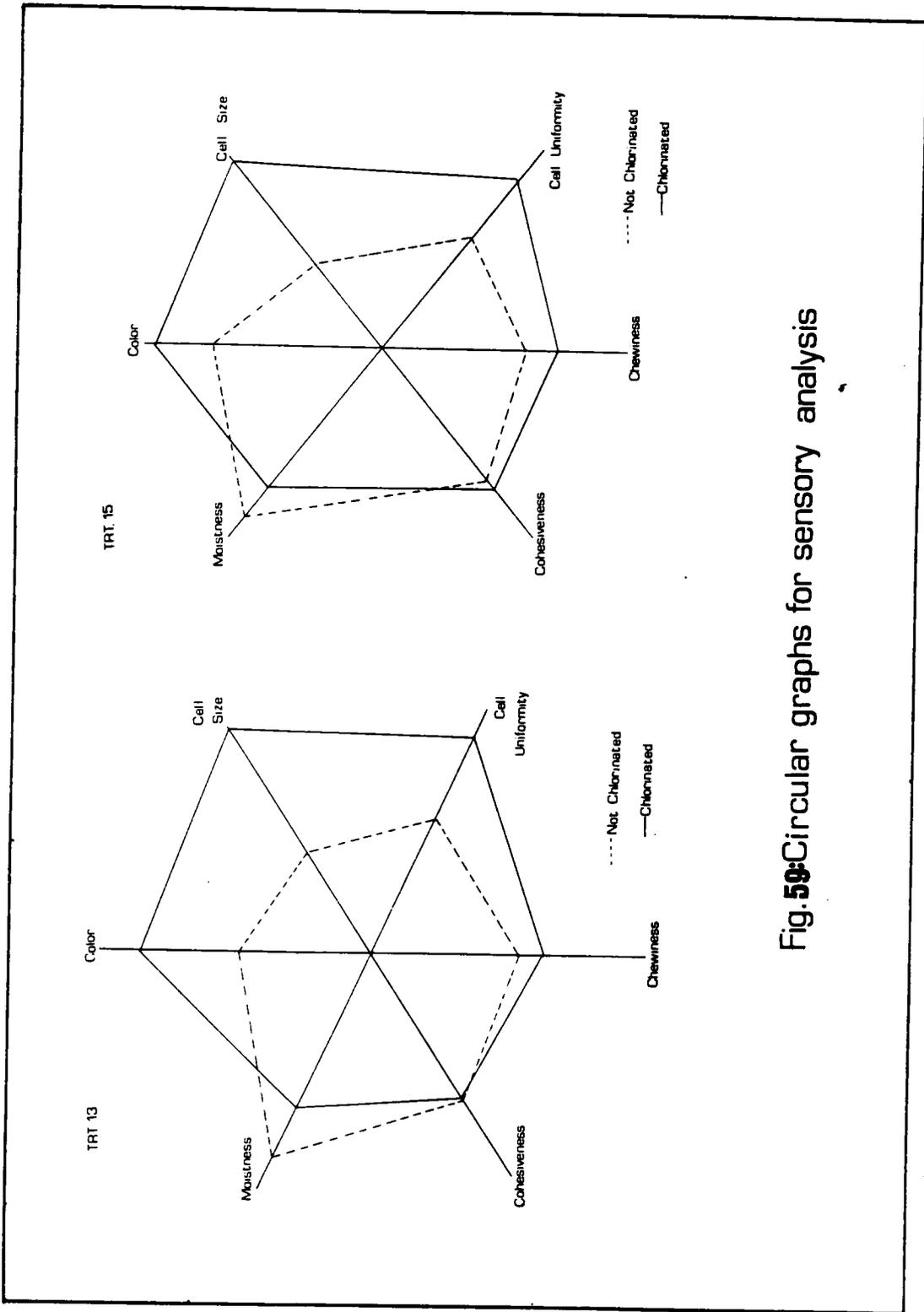


Fig. 59: Circular graphs for sensory analysis

Sensory analysis demonstrated that chlorination had a significant effect on the following attributes: color, cell size, cell uniformity, chewiness and moistness. The strongest effect involved cell size, cell uniformity and moistness. Color and chewiness showed difference between chlorinated and unchlorinated flours, but were not significant for all treatments.

Cohesiveness was the only attribute non-significant among the unchlorinated and chlorinated flours. Both cake types were rated at midpoint on the rating scale.

In summation, the following conclusions were derived from the profiles. Cakes made with chlorinated flour had a whiter color, small, even cells, and were less tender and moist. Cakes made with unchlorinated flour had creamy color, large, uneven cells, and were more tender and more moist.

## CHAPTER V

### CONCLUSIONS

The following are the conclusions of this study:

- (1) Increased levels of fertilization caused an increase in protein content. Timing had no effect on protein content.
- (2) Chlorination lowered the protein content of some treatments. There was a significant decrease for treatments 0 + 50 and 100 + 50.
- (3) Chlorination altered glutenin composition in the lower molecular weight regions. Gliadin was not affected by chlorination.
- (4) Chlorination caused a weakening of the gluten structure demonstrated by rheological studies: low mixing time stability and a high mixing time index.
- (5) Lipid content constituted a minor part of the flour but contributed to the quality of the baked product. Unsaturated fatty acid levels (16:1, 18:1, 18:2, 18:3) were significantly decreased due to chlorination.
- (6) Three unsaturated fatty acids (18:1, 18:2, 18:3)

were associated with cake cell size. Phospholipids which were qualitatively assessed also appeared to be implicated.

- (7) Chlorination of the flour decreased the volume of the baked product which caused less shrinkage and more stability.
- (8) Chlorination increased starch gelatinization as viewed by SEM. The light microscope was a complementary tool in viewing the biochemical makeup of the flour granule.
- (9) Cakes made with chlorinated flour had a more even cell structure; less tender; whiter in color.
- (10) Protein, lipid and starch contributed to baking quality. For cake baking, low protein levels (8-9%) and decreased fatty acid content (18:1, 18:2, 18:3) had a positive effect on the baked product.

The purpose of the study was to evaluate the baking quality of soft red winter wheat (Coker 916) grown under different fertilization treatments and evaluate the effect of chlorination of flour. Ammonium nitrate was used for fertilization of the wheat. The increments used as fertilizers were 0 + 50 (TRT 6); 50 + 0 (TRT 3); 0 + 100 (TRT 4); 100 + 0 (TRT 7); 50 + 100 (TRT 13) and 100 + 50 (TRT 15). Fertilization had a significant effect on the protein content

of the flour. Protein content of the flour increased with increasing levels of fertilization. The rates were more effective than the timing.

Fertilization was applied at growth stages 25 and 30 of the wheat growth cycle. Protein contents of the flour ranged from 8 - 11%. Chlorination caused a reduction in the protein of the flour. There were two instances where the higher fertilization levels at the later growth stage increased protein content (TRT 6 and TRT 13). There was one instance where early fertilization increased protein content (TRT 7) (Table 8).

Rheological studies of the dough indicated that the mixing ability was altered by chlorination. Mixing time index and mixing stability of the flours were significantly affected. Chlorination caused a decrease in the mixing time stability and increased the mixing time index.

Gel studies focused on the gliadin and glutenin content of the various flours. Gliadin gel scans revealed no significant differences between the bands in the various flours. Chlorination had no effect on the gliadins. This may be due to the gliadin arrangement. Gliadins are arranged in a tertiary form whereby the helix that is formed is tightly bound. There is intramolecular bonding involving the disulfide bonds. This intramolecular bond would also affect the functional properties. Gliadin was analyzed to have 21

bands. This was in agreement with other researchers which contribute this to a varietal characteristic (Lookhart, 1981).

Glutenin was analyzed by SDS-PAGE electrophoresis. This system was analyzed with standard markers that were used in quantifying the various glutenin subunits. Gliadin electrophoresis (PAGE) did not lend itself to have such a system. Two of the markers (Phosphorylase b and Glyceraldehyde 3-P dehydrogenase) did not enter the system. There were some theories offered which considered the pH and chemical make-up of the buffer system. Enzymes at times could be hindered by such factors.

Glutenin are large sized molecules and a gradient gel was used to separate the molecules into their various subunits. The results were consistent with the type of wheat studied. Bands in the upper regions were weakly stained which indicated the lack of high molecular weight subunits. The intensity of the staining was greater in the low molecular weight region. These results were typical due to the protein content of the flour (8-11%).

Chlorination had an effect on certain regions in the glutenin which was not found in the gliadin. The effect was found in three bands that eluted at 2:82, 4:00 and 5:45. Bands at 4:00 and 5:45 had the most significant changes occurring (Table 9). The area of significant change occurred in the lower molecular weight region of 40,000 - 50,000

daltons.

The cause for this change could be explained by the type of bonds that are connecting the subunits together. Gliadin and glutenin are both held together by disulfide bonds due to the presence of the amino acid cysteine. Gliadins have a very tight structure that is held by intramolecular bonding. Glutenins are held together by inter- and intra- molecular bonding. Chlorination indicated where these bonds were lying among the molecular subunits of the glutenin structure, and the strength that held these bonds together. The high molecular weight regions appeared to be held by intramolecular bonds and chlorine had no effect in this region.

Glutenins also were prepared without Beta-mercaptoethanol. This reducing agent was used to break up the disulfide bonds, and thus helped in the elution of the subunits during electrophoresis. The purpose was to determine the strength of the bonds themselves with or without chlorination. Gel scans were conclusive that there was no elution in the high molecular regions. The high molecular weight units were held by strong bonds. There was an implication of strong intramolecular bonds in this region. The strength of these bonds were weaker in the lower molecular region, but intramolecular bonds were also present because intensity of the peaks are not as great as those scans which included Beta-mercaptoethanol in the analysis.

The presence of intramolecular bonds appear to be associated with more stability than intermolecular bonds. Gliadins contain intramolecular disulfide bonds which confer stability on folded random coil structures. Glutenins are capable of forming both intra- and inter- polypeptide disulfide bonds. The presence of glutamine residues and the solubility of a portion of the glutenin in SDS indicated that both hydrogen bonding and hydrophobic interactions are also involved in the association of glutenins.

The relationship of the protein content of the flour with the volume of the baked product was shown in Figure 41. Cakes made with flours of increased protein content had a decrease in volume. The relationship was more linear (-0.89) with chlorinated flour than with unchlorinated flour (-0.32). Chlorination had a stabilizing effect on the volume. Even though volumes were lower due to chlorination the product was more stable than one made with unchlorinated flour.

The three glutenin bands (2:83; 4:00; 5:45) were correlated with the cooling height of the baked product (0.57, 0.58 and 0.46, respectively). Upon bleaching, bands at 4:00 and 5:45 indicated negative correlations with the cooled heights (-0.84 and -0.68, respectively), and affected the quality of the baked product. Correlations between the presence of certain HMW subunits of glutenin and indices of breadmaking quality have been observed in several studies of

European wheats. The different HMW subunits have been ranked in order of their influence on breadmaking quality (Schofield, 1986).

Protein content was the main contributor to the baking quality, but there were other factors which offered a supporting role to the baked product. Lipids constituted a small fraction (1 - 2%), but were associated with the basic framework of the baked product. Free lipids (non polar) were analyzed on a quantitative level, while the bound lipids (polar) were qualitatively analyzed. The unsaturated fatty acids (16:1, 18:1, 18:2 and 18:3) were reduced due to chlorination. All flours contained a large amount of linoleic acid (18:2). The affect of chlorination not only caused a reduction, but had a stabilizing effect on the fatty acid content. The values of 18:1 after chlorination were 89.6 - 93.5 mg, TRT 15 was the only exception with a value of 80 mg / 100 gm flour. The 18:2 value ranged from 467.9 - 548.9 mg / 100 gm flour. The 18:3 value was between 21.3 - 22.7 mg/100 gm flour.

There was no apparent change with the phospholipids as determined by TLC. This was concluded because of the position of the triglyceride on the starch granule. The triglycerides are more on the surface of the starch granule, while the bound lipids (polar lipids) were inside. The chlorine was used up at a fast rate and was not able to attack any further. The

polar lipids investigated were phosphatidyl - choline, - ethanolamine, and - glycerol (PC, PE, PG). PC was found in all the flours, while PE, and PG were found with less frequency.

Chlorination caused a decrease in the surface lipid content. Chlorination produced a more even crumb size which may be the result of a decrease in lipid content. Cells became more even and uniform when the fatty acid content decreased with the application of chlorine. The fatty acids involved were 18:1, 18:2, and 18:3. A more rounded crumb structure was the result, and due to the evenness and compact structure, there was also a lower volume. This was opposed to cakes with an uneven, opened structure that were made with unchlorinated flour.

The ratio of uneven to even cells in cakes made with chlorinated flour was 1:2. Linoleic acid which had the greatest range in value would appear to be the controlling factor for evenness of the cake cells. TRT 6 ranked in the middle while TRT 15 had the lowest fatty acid values and the finest cellular structure according to the cake photocopies (Figure 51-55). Chung (1989) reported a decrease in cake volume and excessively fine cells resulted from removing free lipids. Although free lipids play an important functional role in cake quality, their improving mechanism in soft wheat products must differ from that in breadmaking. Lipid binding

in dough or gluten formation is critically important in breadmaking, whereas lipid-protein interaction in gluten formation should be avoided in cake making (Chung, 1989).

There was no significant difference between the gelatinization temperatures (93-96 degrees C) of batters made with unchlorinated or chlorinated flours (Table 18). By SEM observation the cakes made with chlorinated flour had a complete matrix between the starch and protein of the flour. The starch was more completely gelatinized.

The use of the light microscope and SEM were also useful in observing the starch granule itself. The starch granules of unchlorinated flours appeared to have a veil - like covering. This veil - like covering was identified as protein which was stained with Ponceau 2-R and fuchin dye. Chlorinated flours showed an absence of this veil - like covering. The starch's surface was more exposed and complete gelatinization would be the result.

Another theory concerning more complete gelatinization would be free fatty acid involvement. The free fatty acids are present on the surface of the starch granule. Chlorination cause a reduction of the free fatty acids. With this reduction, starch was more completely gelatinized and even cells were the result. There was a complete netting of the starch, protein and free fatty acids. Fitchett and Frazier (1986) reported that the oxidation reactions that the

lipids are involved with are responsible for the improved baking quality of chlorinated cake flours. The researchers also proposed that the effect of chlorine may be in altering the structure coat on starch granules, thereby allowing greater water absorption.

The course of starch gelatinization depends strongly on the quantity of water availability. The gelatinization process usually begins at a lower temperature, and progresses more completely as water is present. As emphasized earlier, the chlorine treatment had no influence on the temperature at which gelatinization occurred but research (Fitchett and Frazier, 1986) showed chlorination enhanced exudation of amylose enhanced. This caused the crumb to become more firm and prevented collapsing upon cooling.

Cakes made with unchlorinated flour experienced collapsing in the center when removed from the oven. It was not a severe collapsing, but a shrinkage of the baked product. There was less shrinkage with cakes made with chlorinated flour. Cakes made with unchlorinated flour were found to be less tender than cakes made with non - chlorinated flour. Less tender cake was associated with even cell structure ( $r = 0.83$ ) and was also rated less moist by the sensory panel ( $r = 0.79$ ).

Protein, lipids and starch all play an integral role in the quality of the baked product. Increased levels of protein

were found to have a detrimental effect on the volume of the product. Cakes with protein contents of 7.8-9% were highest in volume. Lipids had an effect on the crumb structure which also affected volume and textural quality of the baked product. Decreased values of 18:1, 18:2, and 18:3 were associated with a fine crumb structure. The role of the polar lipids was investigated but to a certain extent. Starch's gelatinization properties were also an important asset. The proteins and lipids were also involved with gelatinization of the starch. Chlorination played a key role in its effect on the protein, lipid and starch. It had a stabilizing effect on the values derived, but also stabilized the baked product.

Soft wheat flour has been used in cakes, pastry and biscuits. The information gathered in this study is useful to the agronomist in understanding the relationship between grain nitrogen management and specific end uses of flour. Lower fertilization levels (0+50, 50+0) were found to be more complementary to cake-making.

Flour chlorination improved the cake's structural quality. Chlorination caused cakes to be less tender. This factor should be considered by the food industry especially those involved in mass production. Cakes made with unchlorinated flour were more tender, and hence, more fragile and cannot be packaged as easily.

The rheological properties of the dough were affected by

nitrogen fertilization and also by chlorination. Chlorination caused a break-down in gluten structure. This effect enhance the cake baking in most instances, but would not be recommended for bread-making.

*CHAPTER VI*  
*SUGGESTIONS FOR FURTHER RESEARCH*

The study investigated the major components of the flour: protein, lipids and starch. Nitrogen fertilization played an integral role in its effect on the protein content and baking quality of Coker 916 flour. The results suggested that split application of fertilizer is preferable for optimizing baking quality. The effects of timing of fertilization on wheat and the flour should be explored further.

A different system or different markers may be needed to separate the gliadins of flour by their molecular weights. Amino acid content is another area to be evaluated. The effect of nitrogen fertilization on the N:S ratio of the grain and the gliadin and glutenin make-up of the flour should be investigated.

Flour lipids were involved with the structural make-up of the flour. Polar lipids, especially glycolipids and phospholipids should be investigated further as to the functional role in the baked product.

The use of chemical improvers, especially bleaching agents should be tested and compared to chlorine gas. Effects of these chemical agents on protein, lipid, and starch, should be investigated as well as the baking quality.

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***APPENDICES***

***APPENDIX A***  
***SAMPLING PROCEDURE***

*SAMPLING PROCEDURE*

Lees (1968) gives the following method for sampling powdery or granular materials which will be used in this research project. The flour will be poured out onto a large paper sheet and mixed with a spatula. A cross will be drawn over the pile of material. Remove two diagonally opposite segments and return them to the package. Remix with the spatula and again draw a cross over the heap of powder. Remove the two opposite segments and return them to the original package. This procedure will be conducted until a sample size of approximately two pounds is reached for each flour sample. Each flour sample will be placed in plastic bag, sealed, labelled and placed in a box subsequent to testing.

**APPENDIX B**  
**CRUDE PROTEIN**  
**KJELDAHL METHOD, BORIC ACID MODIFICATION**  
**AACC METHOD 56-12**

*CRUDE PROTEIN-KJELDAHL METHOD-BORIC ACID MODIFICATION:  
AACC METHOD 56-12*

*Apparatus*

1. Kjeldahl flashes, Pyrex, 800 ml. capacity, used for both digestion and distillation.
2. Digestion heaters, 600 W (more or less, depending upon voltage). Heater unit should boil 250 ml. water starting at 77 degrees F in 5 minutes with hot burners.
3. Digestion unit; consists of electric heaters, large lead tube, fume stack (plastic), and suction fan capable of exhausting toxic fumes to outside air.
4. Distillation unit; to consist of Iowa State-type connecting bulbs (traps) 36 x 100 mm., Pyrex glass condenser tubes, pure gum-rubber stoppers and tubing, electric heating units (600 W), condenser tubes capable of being kept cool with adequate amounts of cool water

during distillation and with thermo-water control on stills. Upper ends of bulbs or traps connect with high-quality rubber tubing to condenser tubes and lower ends with rubber stoppers to 800 ml. distillation flask. Lower ends of condenser tubes have rubber-connected glass or polyethylene tubes that lead to:

5. Receiving bottles or flasks, 300 ml. capacity
6. Proper burets for dispensing;
  - a. concentrated  $H_2SO_4$
  - b. concentrated NaOH
  - c. boric acid indicator solution
  - d. class A buret for dispensing 0.1 n  $H_2SO_4$ .

### *Reagents*

1.  $H_2SO_4$ , concentrated, containing approximately 96%  $H_2SO_4$  (specific gravity, 1.84, nitrogen free).
2. Catalyst. Polyethylene packets containing 15 g. potassium sulfate, 0.7 g. mercuric oxide, and approximately 0.10 pumice stone.
3. Antibumping agent. Either zinc metal, 20-mesh, or pumice stone (if pumice is not already combined in catalyst mixture).

4. Concentrated NaOH solution (specific gravity 1.48 at 80 degrees F) for liquid NaOH already prepared. Since mercury is used as a catalyst, add 80 g. sodium thiosulfate per L to NaOH solution to precipitate mercury.
5. Methyl red-methylene blue indicators. Mix 2 parts 0.2% alcohol methylene red solution with 1 part 0.2% alcohol methylene blue solution. Other indicators may be used satisfactorily.
6. Standard  $H_2SO_4$ , approximately 0.1 N.
7. Boric acid-methyl red-methylene blue receiver solution. Add 360 g. boric acid crystals and 48 ml. methyl red-methyl blue indicator to 18 L water.

#### *Procedure*

1. Weigh quickly and accurately 1 g. finely ground sample. Place in digestion flask. (Sample may be placed in nitrogen free paper to prevent clinging to sides of flask). Add polyethylene packet of catalyst, or equivalent, and 25 ml. concentrated  $H_2SO_4$  to flask (reagent 1). Digest until solution is clear and then 30 minutes longer; remove and cool but do not allow to crystallize.

2. Place 300 ml. bottle or flask containing 50 ml. boric acid-methyl red-methylene blue indicator solution (reagent 7) under condenser with tip of condenser tube immersed under surface of solution. Add to original flask that is cooling 200-300 ml. tap water) and anti-bumping agent, if not previously added. Gently add 50 ml. concentrated NaOH (reagent 4), connect to condenser with tight-fitting rubber stopper, and swirl. Boil until ammonia has distilled (at least 150 ml. of distillate), and then set receiving bottle down so that condenser tube is completely drained. 3. Titrate distillate to neutrality with standard 0.1 N H<sub>2</sub>SO<sub>4</sub>, using buret graduated in 0.1 ml. Read ml. of acid used, directly from buret.
4. Run blank determination periodically, using all ingredients except sample. Correct buret reading for nitrogen in reagents as shown by blank.

*Calculation*

$$\% \text{ Protein} = \frac{(\text{ml Std. H}_2\text{SO}_4 \times \text{N of H}_2\text{SO}_4) \times 1.4007 \times 5.7}{\text{sample weight (g)}}$$

where factor for wheat, flour, and bread = 5.7

**APPENDIX C**  
**ASH - BASIC METHOD**  
**AACC METHOD 08-01**

*ASH - BASIC METHOD: AACC METHOD 08-01**Apparatus*

1. Electric muffle furnace provided with pyrometer for indicating temperature.
2. Ashing dishes, preferably of platinum or silica. A shallow relatively broad disk is desirable.

*Procedure*

1. Weigh 3-5 g. (+ 0.01 g.) of well mixed sample into ashing disk that has been ignited, cooled in desiccator, and weighed soon after attaining room temperature.
2. Place in muffle furnace at 550 degrees for soft wheat flours, or 575-590 degrees for hard wheat flours. Incinerate until light gray ash is obtained or to constant weight. Ash must not be allowed to fuse. Cool in desiccator and weigh soon after room temperature is attained.
3. If desired, ash may be transferred to small counterpoised watch-glass and weighed directly. To transfer ash, invert dish; usually ash will be transferred completely

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to watch-glass by this procedure. If ash sticks, it usually can be removed with point of spatula.

*Calculation*

$$\% \text{ Ash} = \frac{\text{weight of residue}}{\text{weight of sample}} \times 100$$

**APPENDIX D**  
**HYDROGEN - ION ACTIVITY (Ph) - ELECTROMETRIC METHOD**  
**AACC METHOD 02-52**

*HYDROGEN-ION ACTIVITY (pH) - ELECTROMETRIC METHOD*  
*AACC METHOD 02-52*

***Apparatus***

Use electrodes and potentiometric equipment that have been checked against buffer solution of known hydrogen-ion activity.

***Procedure***

Place 10 g. flour (or some multiple thereof) in dry E-flask and add for each 10 g. flour, 100 ml. cool, recently boiled water at temperature of 25 degrees C.

**APPENDIX E**  
**USE OF THE HUNTER COLORIMETER**  
**MODEL D-25**

*USE OF THE HUNTER COLORIMETER, MODEL D-25*

1. Turn meter on. The apparatus must warm up for at least 1 hour (the heater light will go off when the colorimeter is ready).
2. Take out the white tile. This is the standardizing tile. Put in on the platform and raise the platform up to the light source.
3. In the left hand column (this is the standardizing column) set the numbers that are on the tiles in the spaces provided.
4. Turn the knob on the bottom to L; adjust the L knob in the standardizing column.
5. Next turn the knob to a; turn the knob on the standardizing column until the needle in the "a" is in the middle.
6. Turn the knob to b; proceed as in Step 5.
7. Place sample on a piece of wax paper; bring it up to the light source; turn knob to "L"; turn knob on right side marked "L" until needle is in the center. Turn knob to "a" setting the turn "a" that is on the right side to where needle is in the middle. Turn knob "b" setting and

turn knob "b" on the right until needle is in the middle.

L= lightness (100: white; 0: black)

-a= greenness

+a= redness

-b= blueness

+b= yellowness

**APPENDIX F**  
**SDS-PAGE ELECTROPHORESIS OF GLUTENIN SUBUNITS**

*SDS-PAGE ELECTROPHORESIS OF GLUTENIN SUBUNITS**Procedure*

1. Assemble glass plates using 1.5 mm spacers in the casting stand.
2. Prepare separating gel solution and mix briefly.
3. Mark a line on the glass plates that is 4.5 cm from the top.
4. Divide into two equal portions, then add 0.1 ml of fresh ammonium persulfate, solutions are delivered by way of pump.
5. Overlay with about 0.3 ml of water immediately to achieve a good interface. (Water lay 1 gel before pouring another).
6. Polymerization should take place for 24 hours.
7. Remove water layer by inverting the gels.
8. Prepare stacking gel solution and mix briefly.
9. Add 0.25 ml of fresh 10% ammonium persulfate and swirl gently.
10. Use a small amount of the stacking gel solution to rinse the surface of the separating gel and discard.

11. Pour the stacking gel and insert combs, making sure no bubbles are caught under the wells.
12. Polymerization should be complete within 1 hour.
13. Carefully remove combs and rinse with tank buffer.
14. Put the upper buffer chamber in place and lock in clamps.
15. Fill chamber with tank buffer.
16. Load wells using a Hamilton syringe or a micropipet. (Amount will vary according to number of wells and protein concentration of sample.)
17. Place upper buffer chamber in lower buffer chamber which contains about 3.5 L of tank buffer and has been cooled to 12 degrees C.
18. Place on lid with red to red and black to black on power supply.
19. Run on constant current 20 mA/gel until dye front reaches the bottom, about 5.5 hours.
20. After run disassemble the glass plates and put gels into Stain and allow to stain overnight.
21. Pour off stain and save for later use.
22. Using Destaining solution I, allow to shake for 3 hours.
23. Pour out and replace with Destaining solution II. Can be stored indefinitely in this solution.



4X Running Gel Buffer (1.5 tris (base) - Cl pH 6.8)

Tris                                    12.1 g.  
10% SDS                                8.0 ml.  
Water                                    to 200 ml.

(Adjust to 8.8 using conc. HCl)

4X Stacking Gel Buffer (0.5 M Tris (base) - pH 6.8)

Tris                                    12.1 g.  
10% SDS                                8.0 ml.  
Water                                    to 200 ml.

(Adjust to 6.8 using conc. HCl)

2X Treatment Buffer (0.125 tris - Cl pH 6.8, 4% SDS, 20% glycerol, 10% 2-mercaptoethanol). Store Frozen.

Tris                                    2.5 ml. of stacking Gel Buffer  
SDS                                    4.0 ml. of 10% SDS  
Glycerol                                2.0 ml.  
2-mercaptoethanol                    1.0 ml.  
Water                                    to 10 ml.

Tank Buffer (0.025 M tris pH 8.3, 0.192 M glycine, 0.1% SDS)

Tris                                    12 g.  
Glycine                                57.6 g.  
SDS                                    40 ml. of 10% SDS  
Water                                    to 4 liters

Stain Stock (1% Coomassie blue R-250)

Coomassie blue R-250      2.0 g.  
Methanol                      to 200 ml.

Mix for about 20 minutes. Filter before using.

Stain (0.125% Coomassie blue R-250, 50% methanol, 10% acetic acid)

Coomassie blue R-250      62.5 ml. of Stain Stock  
Methanol                      250 ml.  
Acetic Acid                      50 ml.  
Water                              to 500 ml.

Destaining Solution I (50% methanol, 10% acetic acid)

Methanol                      250 ml.  
Acetic Acid                      50 ml.  
Water                              to 500 ml.

Destaining Solution II (70% acetic acid, 5% methanol)

Acetic acid                      35 ml.  
Methanol                        25 ml.  
Water                              to 500 ml.

Separating Gel Solution: Gradient Gel 18%/8%.

<u>8%</u>		<u>18%</u>
3.75 ml.	Buffer 4X Running	3.75 ml.
4.00 ml.	30% Monomer	9.00 ml.
7.20 ml.	Water	2.20 ml.
10 u1.	TEMED	10 u1.
40 u1.	Ammonium Persulfate (10% Soln.)	40 u1.

Stacking Gel Solution

4X Stacking Gel Buffer	4.125 ml.
Distilled water	10.295 ml.
30% Monomer solution	2.105 ml.
TEMED	16.5 u1.
Ammonium persulfate (10% Soln.)	123 u1.

**APPENDIX G**  
**PAGE ELECTROPHORESIS OF GLIADINS**

## PAGE ELECTROPHORESIS OF GLIADINS

Gel procedure for 6% or 7.5% Polyacrylamide Gel Electrophoresis.

1. Make gel solution for two gels:

	<u>Wheat</u>	<u>Oats</u>
	6%	7.5%
Aluminum Lactate (purified)	0.50 g.	
Ascorbic Acid	0.048 g.	
Bis-acrylamide	0.50	0.75 g.
FeSO <sub>4</sub> 7 H <sub>2</sub> O	500 ul. of 10 mg./10 ml.	

Dissolve this in a beaker for 20 minutes, then pH to 3.1 with Lactic acid. Bring solution to 200 ml in a volumetric flask. (100 ml makes one 3.0 mm gel).

2. Using a vacuum filter the gel solution through a Whatman #1 qualitative filter. Divide into two portions by pouring in 250 ml. erlenmeyer flasks. Use immediately or store in refrigerator overnight. (Bring to room temperature before using.)
3. For tank buffer dissolve 5.0 g of aluminum lactate in 400 ml beaker or 5.625 g for 4500 ml beaker. When that is

dissolved, pH to 3.1 with lactic acid.

4. Glass plates should be soaked overnight in dilute aqua regia, then washed and rinsed. Allow them to air dry.
5. Set up gel apparatus according to Biorad directions.
6. Add 100 ul of fresh  $H_2O_2$  (3%) to the gel solution. Stir well (30 sec.), then pour gel solution into plated, put in slot former making sure no bubbles are in the slots. Allow to polymerize for 15 minutes.
7. Remove slot former (comb) and pipette out excess fluid. Fill slots with tank buffer.
8. Start water bath, temperature should be set at 20 C, tip cooling system a few times to allow air bubbles to escape.
9. Next, place the sample in the slots. Two extraction methods that yield a gliadin concentration that works well are:
  - a. Weigh into test tube 0.25 g ground wheat or flour, add 250 ul of 70% ethanol, vortex briefly. Place in sonicator for 30 minutes or let stand one hour centrifuge at 4500xg for 10 minutes. Remove supernatant and place in sample bottle. To this add a drop of methyl green dye\* and 5 drops glycerin. Mix Discard pellet. \*The methyl dye is just an undetermined amount of methyl green dissolved in

water.

- b. A more qualitative method is to weigh into a test tube 0.25 g of ground wheat or flour, add 750  $\mu$ l of 70% ethanol. Vortex briefly, sonicate and centrifuge as above. To 250  $\mu$ l of the supernatant add 5  $\mu$ l of methyl green dye and 100  $\mu$ l of glycerin. Mix.
  - c. For single seed extraction grind a seed using a mortar and pestle, place in a small centrifuge tube (500  $\mu$ l capacity). Add 100  $\mu$ l of 70% ethanol and proceed as above. Then add 5  $\mu$ l methyl green dye and 1 drop glycerin. Mix. The amount of sample used per slot is 5  $\mu$ l-20 slot, 10  $\mu$ l-10 slot, or 15  $\mu$ l-10 slot for single seed. This amount may need to be increased or decreased depending on protein concentration.
10. Use a constant voltage of 500 volts and five the amperage dial an extra 1/8 turn after it reaches the maximum amperage to ensure enough current to maintain the voltage throughout the run.
  11. Allow gels to run 2 1/4 hours.
  12. An hour before the gel is to be removed make the gel staining solution:  
For one gel:

75 ml. of 50% Trichloroacetic Acid (1 kg. of TCA to 1 L. H<sub>2</sub>O)

9 ml. of 1% Coumassie Brilliant Blue in EtOH

Bring to volume of 300 ml. After thoroughly dissolved filter the solution through a Whatman #1 qualitative filter.

13. After run turn off power and disconnect water hoses. Pop the gels apart using a spacer and by squirting water underneath the gel, it should slide off the plate into the staining tray. Add 300 ml of the gel stain. Place tray on a rocker at a gentle rocking speed.
14. Allow gel to stain overnight or until bands become distinct. To destain use the stain recipe minus the BBr. If bands are not dark enough add more concentrated BBr in EtOH.
15. When gel has destained sufficiently place in refrigerator 1-2 days to sharpen the bands. The gel can be stored this way for 1 week or more.

**APPENDIX H**  
**SOXHLET PROCEDURE AND FATTY ACID ANALYSIS**

## SOXHLET PROCEDURE AND FATTY ACID ANALYSIS

### I. Soxhlet Procedure

Principle: The procedure yields the approximate percent of crude fat that is in the sample. The solvent that is used is very important because of the extracting process. Before the Soxhlet procedure is run the moisture of the flour should be determined in order that the crude fat can be determined on a wet weight basis.

### Method

#### Determining % Crude Fat on Wet Weight Basis.

1. Weigh out 50 g. of sample into proper drying pan.
2. Place in a 250 degree F oven for 1 hour.
3. Remove from oven and place in desiccator.
4. Weigh sample and determine the % of moisture.
5. Weigh two pieces of Whatman #1 qualitative filter paper (which has been folding into thirds) plus 2 paper clips.
6. Weigh approximately 3 g. flour.
7. Fold into a very tight package: the inner filter paper, then the outer filter paper; forming a small, tight bundle. Fasten paper clips at each end.

8. Place bundles in the thimble, and place thimble into the reflux.
9. Add petroleum ether; reflux for 24 hours.
10. Samples are then allowed to have petroleum ether evaporated before they are put into drying oven.
11. Samples are placed in drying oven for 24 hours. (Temperature range is between 60-70 degrees C.).
12. Samples are placed in a desiccator for 24 hours until equilibrium is reached and then reweighed again.

Calculation: (Sample Calculation)

1. Weight of sample before extraction
2. Weight of sample after extraction
3. Weight of Crude Fat (Difference between #1 and #2)
4.  $100 - \% \text{ Moisture} = \% \text{ Solids}$
5. Wet weight =  $\frac{\text{Dry weight (Wt. of sample before extraction)}}{\% \text{ solids}}$
6.  $\% \text{ of Crude Fat on Wet Weight Basis} = \frac{\text{Weight of Crude Fat}}{\text{Wet Weight}} \times 100$

*II. Extraction and Formation of Fatty Acid Methyl Ester*

A. Extraction of Non Polar Lipids

1. Weigh out 5 g. flour (dried at 80 degrees C for 24 hours).

2. Add to sample bottle (vial used with a teflon lined cap). Add internal standard; heptodecanoic acid is added: aliquot added that contained 2 mg. of internal standard.
3. Add 18 ml. conc. HCl; stopper the bottle (seal with teflon lined cap). Place sample bottles in a water bath and heat 45 minutes until hydrolysis has taken place.
4. Remove bottles from hot water; cool; open and divide the contents between 2 teflon test tubes.
5. To each tube, add 10 ml. chloroform; shake the tube, open stopper to release pressure; close and shake (50 times).
6. Centrifuge tubes at 7000 rpm for 10 minutes. Allow to rest and remove 5 ml. (bottom part) from each tube.
7. Place in a 20 ml. sample bottle; evaporate to dryness under nitrogen.

B. Methylation

1. To the dried sample add: 6 ml. benzene and 6 ml. boron trifluoride. Stopper with teflon lined caps and boil gently in a water bath for 10 minutes.
2. Add 3 ml. water to stop the reaction.
3. Remove benzene layer and pass it through magnesium sulfate to dry the sample.
4. Evaporate half of the sample under nitrogen.

5. Inject sample into gas chromatograph at a rate of 0.75 ul.

**APPENDIX I**  
**ANGEL FOOD CAKE FORMULA ADAPTED FROM**  
**AACC METHOD 10-15**

*ANGEL FOOD CAKE PROCEDURE ADAPTED FROM AACC METHOD 10-15**Formula and Ingredient Specifications*

<u>Ingredient</u>	<u>Amount(g)</u>	<u>% (Flour basis)</u>
Flour	110.0	100.0
Sugar	314.0	285.0
Dried Egg Albumen	40.0	36.4
Acid Salt (Cream of Tartar)	1.5	1.4
NaCl	3.0	2.7
Water (add 14% mb correction)	295.0	268.0

Method

1. Bring oven to baking temperature (190 degrees C or 375 degrees F). \* Because of altitude cakes will be baked at 400 degrees F.
2. Reconstitution of dried egg albumen. About 18 hours before the bake, add requisite quantity of egg albumen to water (see table) in 10 quart bowl. Mix with whip at low for 5 minutes, scraping at intervals. Transfer solution to beaker(s), cover with plastic wrap, and store overnight at about 2 degrees C. Stock egg solution

should be refrigerated during bake to minimize variation in whipping performance.

*Reconstitution of Dry Egg Albumen*

<u>Batch Size</u>	<u>Egg (g)</u>	<u>Water (ml)</u>
1	42	310
2	84	620
3	126	930
4	169	1240
5	210	1550
6	252	1860
7	294	2170
8	336	2480
9	378	2790
10	420	3100

3. Prior to use, tare mixing bowl and add 335 g. cold stirred egg solution. Add sifted mixture of acid, salt, NaCl, and one-half of sugar (157 g.). Mix for 1.0 minute at low to dissolve solids. Scrape.
4. Set mixer at high speed and whip to end point specific gravity of 0.14-0.13. \*This was changed to .15-.17,

according to Pyler (1973) in order to achieve greater height.

5. Specific gravity determination. Calibrate 1/4 cup measure by taring on sensitive balance, fill capacity with distilled water, and weigh. Normal weight should be 59 g. = 59 ml. capacity. Tare dry cup and fill carefully with foam while holding at 45 degree slant. Carefully overfill cup using steel spatula to eliminate air voids. Scrape off level and remove traces of foam from exterior of cup. Weigh contents to 0.01 g. and calculate specific gravity.

$$\text{Specific Gravity} = \frac{\text{weight foam}}{\text{volume cup}} \quad \text{e.g. } \frac{8.05}{59} = 0.137$$

6. When specific gravity range is reached, place sifted mixture of flour and remaining sugar (157 g.) in weigh scoop. At low speed add mixtures over 20 second period. Remove bowl and whip from mixer and place on low bench. Fold in residual dry flour-sugar mixture by dipping whip into batter while rotating bowl and whip 1/4 turn. Shake off whip and repeat for 10 folds (\* A rubber spatula was used for folding and 25 cut and fold motions were used for incorporating the sugar/flour mixture).

7. Tare tube pan on large capacity balance. Transfer batter with rubber spatula. Rotate pan as needed to achieve uniformity. Eliminate air voids and level top surface by rotating pan with spatula held stationary. Adjust batter weight to 650 g. Large bubbles may be surfaced by dropping 5 times from height of 5 cm. to padded surface.
8. Bake cake at 400 degrees F for 30 minutes. Remove from oven and invert on center tube for cooling; after 40 minutes ring with steel spatula and tap out cake over 10 inch rigid square for support and measure (\*1 1/2 hours elapsed before cake was removed in order to prevent collapsing).

**APPENDIX J**  
**MEASURING STANDING HEIGHT OF BAKED ANGEL FOOD CAKE**  
**AACC METHOD 10-15**

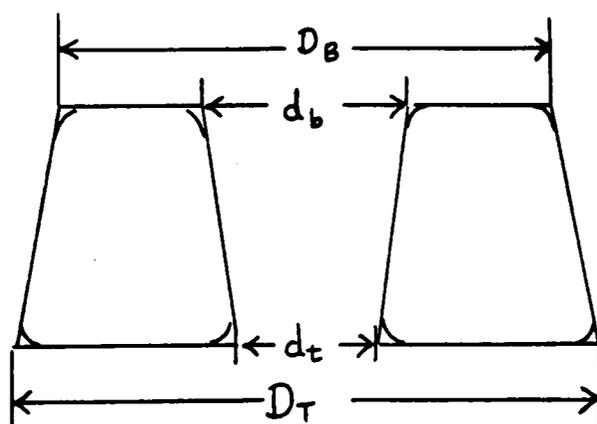
*MEASURING STANDING HEIGHT OF BAKED ANGEL FOOD CAKE,  
AACC METHOD 10-15*

Measure and record cake height after depanning and again after 3-4 hours, after cooling (hc) as an index of shrinkage. Measure height at center of hole, using 30 cm. scale as straight edge and 15 cm. scale as depth gauge. Hold crossed scales together firmly and remove for reading. Repeat measurement 4 times, rotating straight edge for 45 degrees for each reading.

Volume of Angel Food Cake

1. Measure and record cake height after depanning, and again after 2. hr., cooling (hc) as an index of shrinkage.
2. Measure height at center of hole, using 30 cm. scale as straight edge and 15 cm. scale as depth gauge. Hold crossed scales together firmly and remove for reading. Repeat measurement 4 times rotating edge 45 degrees for each reading.
3. Cut cake on diameter to cause minimum deformation. Score internal characteristics as required.

4. After scoring, measure maximum and minimum outside (D) and inside (d) diameters. (See below):



Consider cake as geometric volume, ignoring local shrinkage and imperfections. From mean diameters,

$$\bar{D} = \frac{(D_B) + (D_T)}{2} \quad \text{and} \quad \bar{d} = \frac{(d_b) + (d_t)}{2},$$

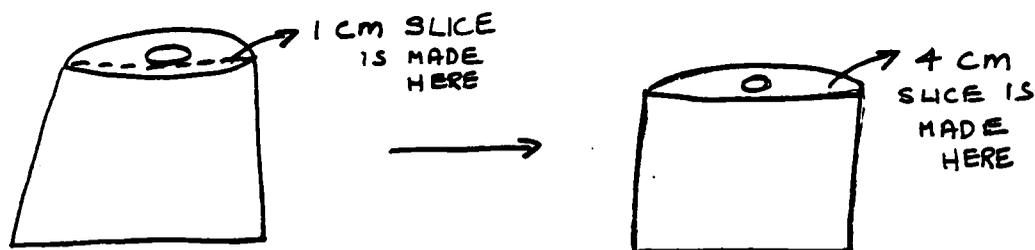
and cold-cake height ( $h_c$ ) compute ring volume

$$\text{Vol (v)} = \pi \frac{h_c (\bar{D}^2 - \bar{d}^2)}{4}$$

**APPENDIX K**  
**METHOD IN USING THE PENETROMETER TIME,**  
**LAB-LINE INSTRUMENT, INC., MELROSE PARK, ILL.**

*METHOD IN USING THE PENETROMETER TIME,  
LAB-LINE INSTRUMENT, INC., MELROSE PARK, ILL.*

1. Uniform slices must be used in order to get acceptable results. The size reached was 4 cm. x 4 cm.
2. In order to get uniform pieces, the cake will be cut in the following manner:



Care must be taken in slicing the cake in order to avoid breaking down texture or structure of the cake.

3. 4 x 4 cm. pieces are cut and each piece will be placed under the plunger.
4. The knob is depressed for 2 seconds and then released. When a "click" is heard, the top handle is depressed and a reading is then taken from the dial (in mm.).
5. At least 3 readings should be taken and then a mean value calculated.

*APPENDIX L*  
*METHOD IN USING BAKER COMPRESSIMETER,*  
*F. WATKINS, CORP.*

*METHOD IN USING BAKER COMPRESSIMETER, F. WATKINS, CORP.*

Principle: The instrument is used for measuring the relative compressibility of bakery products and similar items. Its level system permits the observation, on a direct reading scale, of the strain (distortion) of a test sample resulting from the application of stress (force applied).

Standard Test Procedure

1. Select a sample of suitable size and thickness to be representative and prepare the sample in such a manner that it provides a flat horizontal surface for uniform contact with the plunger.
2. Place the sample on the test platform beneath Plunger A and center it so that the plunger will contact a representative portion of the sample. With the plunger resting freely on the test piece and the string hanging loosely, raise or lower the lever assembly to compensate for the thickness of the sample by turning nut B until the pointer on scale D reads zero. Scale C indicates the thickness of the sample.

3. Start the motor and when the test piece has been compressed, a pre-selected amount as indicated on scale D, turn off the motor and read the force applied in grams on scale J.
4. Several tests may be required to determine the amount of compression that gives the most reliable results with the particular materials being tested. Record the reading on all scales. Unwind the drum E to relieve the tension. Raise the levers and remove the test sample. \* Baked and allied products show great variations in compressibility and firmness. It is suggested that the operator make the necessary adjustments and develop suitable operating techniques to suit his own conditions.

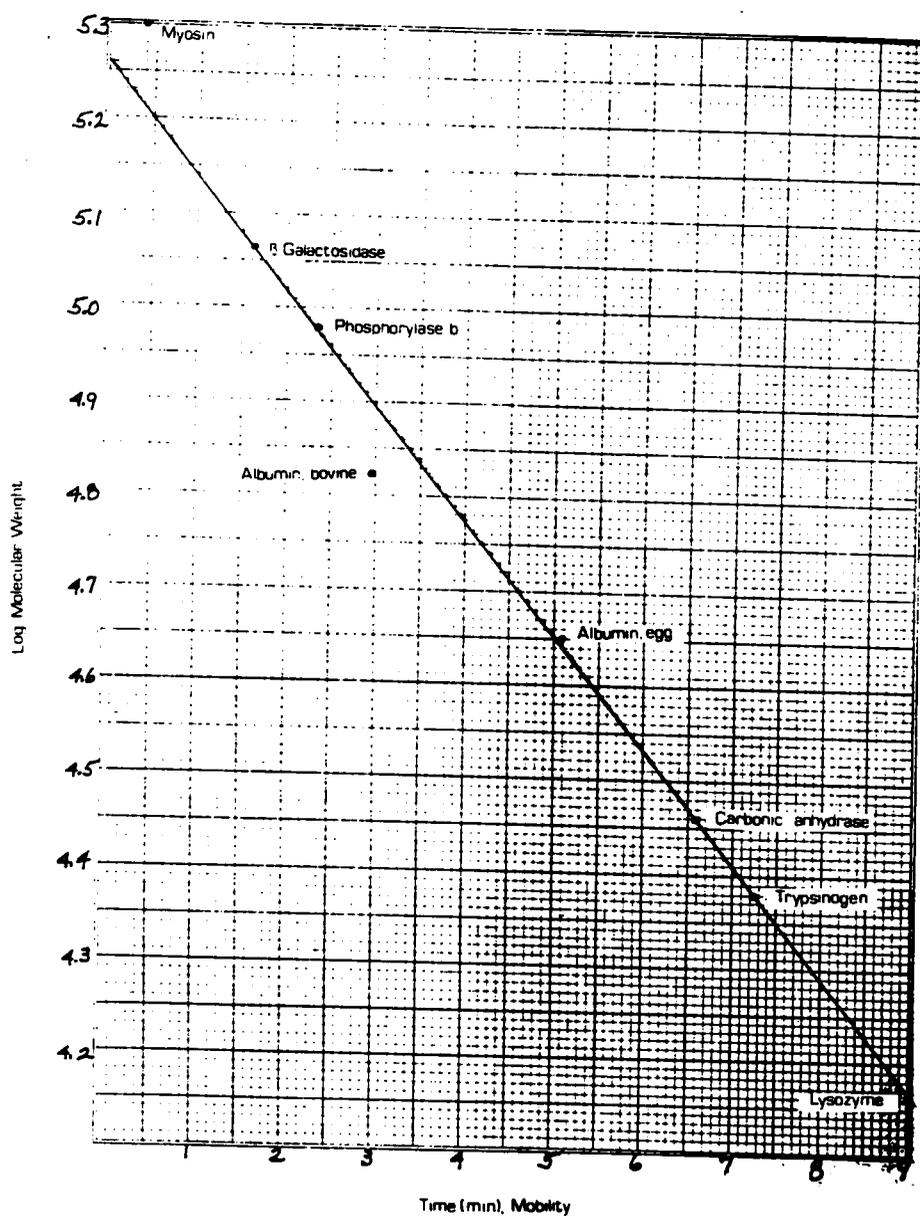
<u>Plunger Position</u>	<u>Mechanical Advantage</u>
1	12.10
2	6.06
3	4.04

If soft products are to be investigated, the mechanical advantage of the level system may be reduced to decrease the force applied to the sample by the spring movement. To accomplish this, move plunger A to position 3 on lever H. It will be necessary to rebalance lever H by adjustment of the counterweight M if the position of the plunger is shifted. To determine the actual force applied to the test sample by

the plunger, multiply the reading in grams on scale J by the mechanical advantage of the respective plunger positions.

**APPENDIX M**

**SDS - PAGE MOLECULAR WEIGHT CALIBRATION CURVES**



**Fig. 18: Molecular weight calibration curve.**

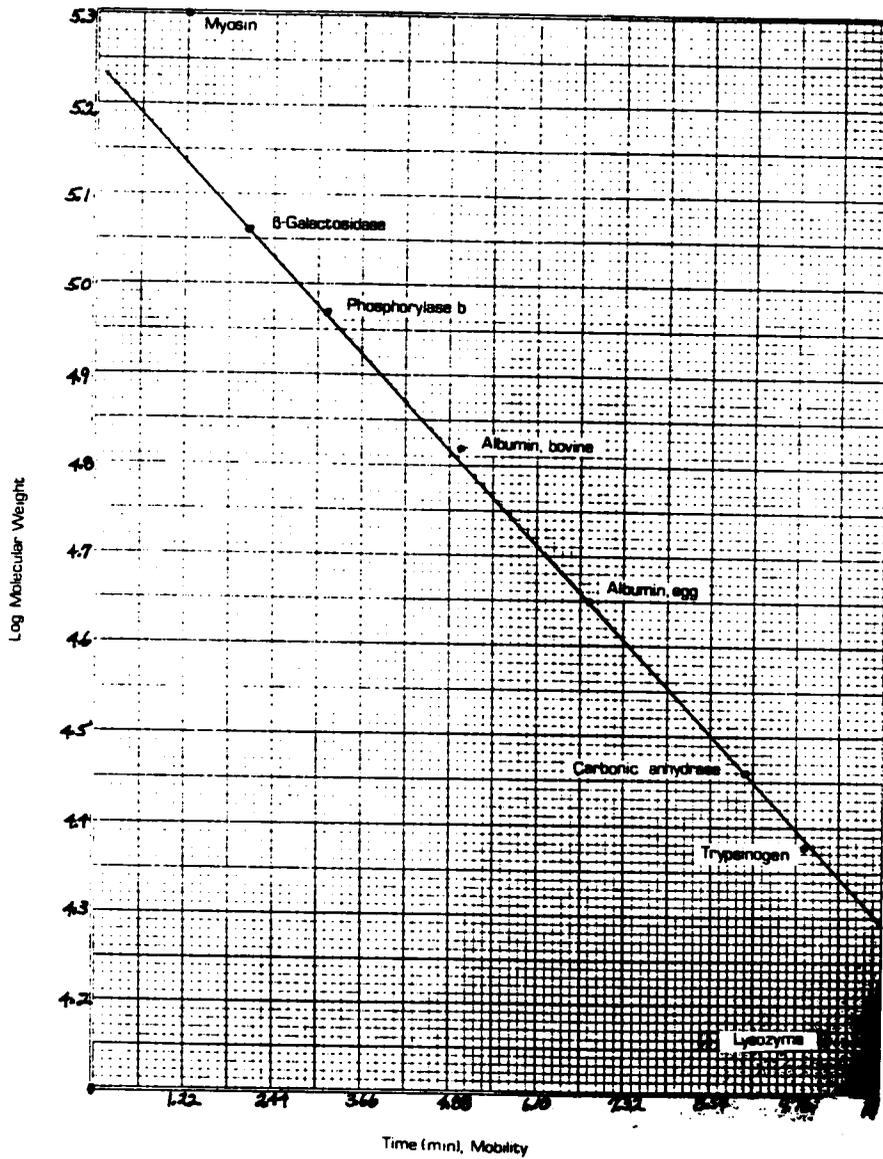


Fig.19: Molecular weight calibration curve without mercaptoethanol.

*APPENDIX N*  
*THIN LAYER CHROMATOGRAPHY OF POLAR LIPIDS*

*THIN LAYER CHROMATOGRAPHY OF POLAR LIPIDS**A. Extraction*

1. Weigh out 3 g. of flour into 20 ml. sample bottles.
2. Add 16 ml. water saturated n-butanol (made up 65:35 (v/v). This should be mixing when aliquots are taken.
3. Add to flour; seal thoroughly and vortex.
4. Place bottles in a boiling water bath; shake every now and then for 1 hour.
5. Remove supernatant; repeat; adding 16 ml. water saturated n-butanol; repeat steps 2-4.
6. After 1 hour, remove supernatant.
7. Repeat steps 2-5.
8. Centrifuge the supernatant tubes and the tubes with the flour.
9. Centrifuge @ 7000 rsm for 10 minutes.
10. Decant off the supernatant and place in round bottom flasks.
11. Evaporate with a rotary evaporator under nitrogen until solvent is evaporated.

12. Add 0.25 ml. chloroform; swirl to remove lipids from the sides of the flask.
13. Place into small vials; seal and freeze to preserve.

*B. Developing of Polar Lipids*

1. Use silica plates G.
2. Heat plates at 110 degrees C for 1 hour in order to activate.
3. Plates were spotted with micropipettes at 15 ul.
4. Place in developing tank with the following solvent mixture:
  - 70 ml. chloroform
  - 20 ml. methanol
  - 2 ml. 30% (v/v) ammonia solution.
5. Seal tank with lid to prevent evaporation of developing solution.
6. Allow developer to travel up the plate to within 2 cm. from the top.
7. Remove plate; allow to dry for 5 minutes. Spray plate with zinzadze reagent. The appearance of spots indicates the presence of polar lipids.

**APPENDIX O**  
**FARINOGRAPH PROCEDURE**

*FARINOGRAPH PROCEDURE*Constant Flour Weight: Small bowl

1. Turn on the thermostat and circulating pump at least 1 hour prior to using instrument.
2. Determine the moisture content of flour as directed in any oven method for flour. (Keep flour samples in moisture-proof containers. Accurate moisture values are very important).
3. Place in bowl 50 + 0.1 g. flour.
4. Fill small buret with water at room temperature, making sure that tip is full and automatic zero adjustment of buret is functioning properly.
5. Turn on machine and run 1 minute until zero minute line is reached. At this instant begin adding water to right front corner of bowl from buret to volume nearly that of expected absorption of flour. When dough begins to form, scrape down sides of bowl with plastic scraper, starting on right side, front and working counterclockwise. Cover with plate. If it appears that mixing curve will level off at value larger than 500 BU, cautiously add more water.

6. For the final titration, add all water within 25 sec after opening buret stopcock. Permit machine to run until adequate curve is available for evaluation.
7. Report absorption values to nearest 0.1%. Calculate absorption on 14% mb, determined with small bowl, using equation:

$$\text{Absorption \%} = 2 (X + Y - 50);$$

where X = ml water to produce curve with maximum consistency centered on 500-BU line and Y=g flour used, equivalent to 50 g., 14% mb.

**APPENDIX P**  
**STAINING OF WHEAT FLOUR**

### STAINING OF WHEAT FLOUR

#### A. General Preparation

1. A 6% solution of flour (3 gm flour + 50 ml. water). Vortex at high speed. With a plastic pipette take up solution. Do not stop mixing.
2. Place a drop of the mixture on the slide. Smear the drop across, but do not bring it to the end of the slide.
3. Allow the smear to air dry.
4. Use slide for the following stains.

#### B. Protein Staining

1. Stain with 0.1% aqueous Ponceau 2R containing 3-4 drops of 1 N  $H_2SO_4$  per 50 ml. stain for 10 minutes.
2. Rinse in two changes of distilled water.
3. Dehydrate in alcohol.
4. Clear in xylene.
5. Place 2-3 drops of a synthetic mountant; apply cover slip with no air bubbles.

*C. Starch Staining*

1. Stain with 0.5% aqueous chlorazol black E.
2. Place prepared slide in stain for 10 minutes.
3. Rinse in two changes of distilled water.
4. Dehydrate in alcohol.
5. Clear in xylene.
6. Place 2-3 drops of a synthetic mountant; apply cover slip with no air bubbles.

*D. Starch Staining II*

1. Stain with Basic fuchin: 0.5 toluidine blue and P<sup>1</sup>P<sup>1</sup>, P<sup>1</sup>,<sup>1</sup> triaminophenylmethylen for a 1% solution.
2. Follow procedures for Starch Stain I.

**APPENDIX Q**  
**USE OF HUNTER LAB LABSCAN SPECTROCOLORIMETER**

*USE OF HUNTER LAB LABSCAN SPECTROCOLORIMETER*

1. Turn on machine at least 30 minutes before using in order for it to warm up.
2. Take white tile off. Make sure aperture is wide open.
3. Place black tile on aperture. Press STD. "Working" will come on; then "ZERO".
4. Take white tile; place it on the aperture; press STD; after it is standardized open box to the left and press XYZ.
5. Take green tile and place it on the aperture. Press read; then press LAB.
6. Take cake piece: should be 6 X 6 X 4 cm. Take readings.
7. Instrument gives the following readings:

L, a, b,  $\Delta E$ , C

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