Nonenzymatic Browning Reactions in Breaded Fried Chicken and in Model Food Systems.

Mohd Yusop bin Abu

Louisiana State University and Agricultural & Mechanical College

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NONENZYMATIC BROWNING REACTIONS IN BREADED FRIED CHICKEN AND IN MODEL FOOD SYSTEMS.
THE LOUISIANA STATE UNIVERSITY AND AGRICULTURAL AND MECHANICAL COL., PH.D., 1979

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NONENZYMATIC BROWNING REACTIONS IN BREADED
FRIED CHICKEN AND IN MODEL FOOD SYSTEMS

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
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requirements for the degree of
Doctor of Philosophy

in

The Department of Food Science

by

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ABSTRACT

Breaded fried chicken was prepared according to specific commercial procedures to study the effect of variable amounts of buttermilk solids in the batter formula on the color of the final products. The procedure includes precooking of chicken parts by boiling in water for 15 min, followed by draining and cooling to 40°F, and battering with formula consisting of flour and buttermilk powder up to 20%. The solid-water ratio was 1:1.5. After breading with cracker crumbs the breaded chicken parts were blanched in soybean oil at 395°F for 20 sec. The products were then sealed in polyethylene bags and frozen stored at -4°F for 30 days. The products were deep-fat fried in soybean oil at 360°F for 6 min.

According to sensory evaluation of color it was concluded that the 20% buttermilk treatment produced the same intensity of browning in both the dark (thigh) and white (breast) pieces, which was highly significant (p < 0.01) than the control. Although the 20% level gave undesirable dark color, the final products showed higher yield, higher moisture content, and a higher percentage of crust than the control. In addition, the buttermilk solids imparted a characteristic 'buttery' flavor which is a desirable organoleptic quality for the breaded fried chicken.

However, the undesirable dark color formation by the nonenzymatic browning reactions was successfully inhibited by the use of sulfite in the form of sodium metabisulfite up to 2500 ppm initially in the 20% buttermilk batter mixture. Thus, by incorporating sulfite, the production
of breaded fried chicken with acceptable color together with the above-mentioned quality attributes was made possible.

The results lead to the conclusion that the Maillard reaction, caramelization, sugar acid reaction, and reactions involving lipids and phospholipids had participated in that order to some extent producing browning intermediates (furfurals and other reactive compounds) in the skin-batter complex of the breaded fried chicken pieces until the time for final frying. During the frying process the effect of high temperature on the reactants and intermediates, became most important. Caramelization was believed to be most prevalent during the deep-fat frying process; and it accentuated the effects of the other three types of non-enzymatic browning reactions, particularly the Maillard reaction, contributing to the final dark color of the buttermilk-battered and breaded fried chicken.

In the model food systems the relative rate of reducing sugar reacting with casein was galactose > glucose > lactose. The rate of sugar loss was faster with galactose, followed closely by glucose, and much slower by lactose. The same order of reactivity was indicated by the temperature, moisture content, and water activity treatments. Generally, the Maillard browning rate was increased by the increase in temperature, moisture content, and water activity up to a certain level. Galactose and glucose indicated faster browning rates between $a_w 0.62 - 0.84$, while lactose between $a_w 0.62 - 0.75$. 
INTRODUCTION

Precooked, ready-to-eat, breaded fried chicken as a convenience food is very popular in the United States, and sales have increased tremendously in the past twenty years. It is a growing industry in many developed countries, such as Japan, and also in certain developing countries in Southeast Asia like Malaysia.

There are many methods of preparing this type of chicken product depending on the choice of the processor. Essentially, the process involves precooking of chicken parts (breast, thigh, drumstick, and wing), battering and breading, and deep-fat frying until done. A modification of the process would include a blanching procedure just after the battering and breading step. Blanching the breaded chicken parts in deep-fat at 395°F for 20 sec, for example, would stabilize the coating around the chicken pieces and promotes easy handling of the products, so that they can be frozen stored for more than a month, during which time the products can be transported and distributed to various eating establishments and chain food stores. Before consumption, the products are to be deep-fat fried until done.

Mixtures for battering or breading may be prepared from individual components or they may be purchased ready-mixed. Batter formulation includes starches and/or flour with other optional ingredients like egg solids, corn meal, preservatives, seasonings, and milk solids.

Recent innovation in the fried chicken industry is the formulation of a special batter-breader mixture which, among other things, contains
hydrogenated cottonseed oil and/or hydrogenated soybean oil, and soybean lecithin (for uniform dispersion of oils) such that the breaded chicken pieces can be 'fried' in the oven at 400 - 425°F for 50 - 60 min using only 2 - 4 tablespoons of cooking oil per 2½ pounds of chicken pieces, instead of deep-fat frying. The finish product is thus easily prepared and it is comparable in organoleptic quality as in the deep-fat fried chicken. Maintaining the uniformity of desirable quality attributes in the finish products is a very important factor for a food processor to survive in the free enterprise business.

This investigation was undertaken to improve the quality attribute of color in the breaded fried chicken containing up to 20% buttermilk solids in its batter formulation. The buttermilk powder was included to impart a desirable characteristic flavor in the finish products. The utilization of up to 20% cultured buttermilk solids has been reported by a commercial food processor in Arkansas to result in excessive browning during deep-fat frying of breaded fried chicken.

The color, texture, toughness, fat and moisture, and general appearance of the cooked products, among other things, can be influenced by batter ingredients, solid-water ratio of the batter, coating procedure, and cooking method. These influencing factors were predetermined and controlled in the present investigation.

The breading compositions determine the color shade of the final products; the temperature and time of frying are prime factors on the cooking quality of fried products because they affect the weight loss, fat absorption, color shade and hue, and also the crispness of the finish product. Cracker meal imparted a bright golden brown color and desirable organoleptic qualities to fried food products (Rao and Novak, 1975).
Buttermilk powder contains 40% lactose, a reducing sugar, which plays a major role in nonenzymatic browning reactions. Lactose may break down to glucose and galactose during the cold storage period and especially during a high temperature processing such as deep-fat frying. These simple sugars undergo the Maillard reaction more rapidly than the disaccharide itself.

The chicken meat including the skin and the breading materials contain proteins (amino acids), lipids and phospholipids, and carbohydrates which participate in the nonenzymatic browning reactions. The utilization of these components were studied in effort to elucidate the extent of each type of nonenzymatic browning reactions in contributing to the final dark color of the breaded fried chicken.

Therefore, this investigation was performed to achieve the following objectives:

1. To study the effect of buttermilk solids up to 20% in the batter formula on the color and flavor of the breaded fried chicken.
2. To conduct a sensory evaluation of the color of the finish products for an average consumer's preference.
3. To study the interaction of lactose and its degradation products in the breading materials with protein, carbohydrate, and lipids in chicken skin and meat leading to nonenzymatic browning reactions.
4. To determine the effective level of a chemical inhibitor (sulfite) for the prevention of nonenzymatic browning reactions in the products.
5. To study the nonenzymatic browning rates between reducing sugars and amino acids in aqueous solutions and with casein in model food systems.
Nonenzymatic browning reactions may include the Maillard reaction, caramelization, sugar acid reaction, and oxidation. The Maillard reaction is the reaction of carbonyl compounds with amino compounds resulting in pigment formation such as in milk and milk products, fish, meat, egg, and baked products (Lee, 1975). Caramelization is the reaction of carbonyl compounds or more accurately the nonamino browning of sugars such as in heated sugar and baked products (Greenshields, 1972, and Lee, 1975). The sugar acid reaction is the reaction of sugars with organic acids in food system such as fruit juices (Reynolds, 1975). The oxidation reaction involves the polyphenol or polycarbonyl compound derivatives of unsaturated fatty acids, such as in fish oils and fats (Koch, 1962).

The Maillard reaction occurs more commonly in natural food systems. This reaction was first reported by Maillard in 1912 as the reaction of amino acids and proteins on sugars. Hodge (1953) called this reaction the carbonyl-amine reaction due to the fact that compounds reacting with amine or amino groups usually have a carbonyl or a potential carbonyl group. Thus we can define the Maillard reaction as the aminocarbonyl reaction including the reactions of aldehydes, ketones, and reducing sugars with peptides, proteins, and amino acids.

Lee (1975) outlined the stages in the Maillard reaction leading to formation of brown pigments known as melanoidins, which is represented in the Hodge scheme in Figure 1 (Hodge, 1953).

1) Initial stage (colorless, with no absorption in or near the ultra-violet range).
Figure 1. The Hodge scheme (Hodge, 1953)
A. Sugar-amine condensation
B. Amadori rearrangement

2) Intermediate stage (colorless or yellow, with strong absorption in or near the ultra-violet range)
C. Sugar dehydration
D. Sugar fragmentation
E. Amino acid degradation (Strecker degradation)

3) Final stage (highly colored)
F. Aldol condensation - nitrogen-free aldols, in general, are likely to react with amino compounds, aldimines, and ketimines to form nitrogenous melanoidins.
G. Aldehyde-amine polymerization and formation of heterocyclic nitrogen compounds (melanoidins).

The Maillard Reaction

The major features of the Maillard reaction is represented in Figures 2A and 2B. Figure 2A shows the initial condensation of glucose with glycine to form glycosylamine in a low moisture system. The aldosyl derivative immediately undergoes an Amadori rearrangement to form ketoseamine (MFG) catalysed by the amino acid itself (McWeeny et al., 1974). The MFGs are stable compounds but are more reactive than ketoses and will decompose under cold alkaline conditions. Further reaction of ketoseamine with glucose, followed by a second Amadori rearrangement, results in deketoseamines (DFC).

Figure 2B shows the analogous products of a ketose-amino acid reaction. In this instance, they are monoglucoseglycine and diglycine-
Figure 2A. Initial stages in the Maillard reaction (McWeeny et al., 1974)

Figure 2B. Products of fructose/glycine reaction and DFG decomposition (McWeeny et al., 1974)
Figure 2C. Degradation of ketoseamines by 1,2-enolisation (McWeeny et al., 1974)

Figure 2D. Degradation of ketoseamine by 2,3-enolisation (McWeeny et al., 1974)
glucose.

Then, the amino-sugars are degraded to amino and nonamino-containing compounds (Figure 2Bb). At pH 5.5, the DFG decomposition rate is at a maximum, yielding fructoglycine and other carbonylic compounds such as 3-deoxyhexosuloses and the cis- and trans-forms of unsaturated hexosuloses. These are reactive intermediates which would undergo further reaction to form brown colors and/or off-flavor. (McWeeny et al., 1974).

Sugar dehydration in the sugar-amine browning reaction can take place in two ways, a) In neutral or acid solutions, furfurals are formed; b) In the dry state or in non-aqueous solvents when amines are present, reductones are formed (Lee, 1975).

The mechanism of the decomposition of the ketoseamines has been postulated to follow the 1,2-enolisation (Figure 2C) and 2,3-enolisation (Figure 2D). The 2,3-enolisation is facilitated when the ketoseamines are derived from an aldose and a strongly basic secondary amine in the presence of tertiary amine. Thus, the 2,3-enolisation is limited when the pH of foods is lower and the bases weaker; but a low yield of its degradation products may produce distinctive flavors. The 2,4-diolose can undergo intramolecular condensation with oxygen heterocycles (such as pyrones and furans) or condensation with amino compounds, to form nitrogen containing heterocycles (such as pyrazines). Pyrazines are important flavor components (McWeeny et al., 1974). Decomposition of diketoseamine to the reactive intermediates promotes a faster browning reaction than a ketoseamine alone.

Finally, the brown pigments (or melanoidins, the highly colored,
fluorescent macromolecular pigments) are formed by the condensation of
the carbonylic intermediates either with each other or with amino
moieties.

Intermediates in the system containing aldoses and amines are
shown in Figure 3 (Reynolds, 1965). Color can be generated though
slowly in the absence of amino compounds, but when they are present,
they 1) facilitate the production of highly reactive carbonylic inter-
mediates, and 2) combine with the intermediates to form melanoidins
(McWeeny et al., 1974).

The 3-deoxyhexosulose is regarded as an important intermediate in
the formation of brown pigments, and it can react with amino groups
rather quickly and undergo further condensation reactions to form brown
pigments of higher molecular weight (Reynolds, 1965).

The extent of browning progress depends on factors like moisture
content, pH of the system, temperature, and concentrations of the
reacting species. These are variable factors during processing, storage,
and cooking of foods.

The nonenzymatic browning reactions are considered deleterious as
a result of the production of unwanted colors and off-flavors from
food, or through losses in solubility and nutritive value through
reactions of the alpha amino groups of lysine moieties and the binding
of free amino groups of other essential amino acids thus impairing the
Biological Value of the proteins in food (Rao and Rao, 1972). Desirable
application of the nonenzymatic browning reactions is sought in controlled
production of color and flavors during toasting, roasting, frying, baking,
and caramelization process.
Figure 3. Intermediates in systems containing aldoses and amines (Reynolds, 1965)
Figure 4. Amadori rearrangement products (Eskin et al., 1971)
**Amadori Rearrangement**

The Amadori rearrangement is the isomerization of the N-substituted glucosylamines, involving the transition from an aldose to a ketose sugar derivative (Eskin et al., 1971), which is 1-amino-1-deoxy-2-ketose (Figure 4). Glucose reacts with glycine to produce fructoglycine (Lee, 1975); and Fructose reacts with glycine to produce 2-amino-2-deoxy-\(\alpha\)-D-glucopyranose through the Heyns rearrangement which brings about the same transformation as the Amadori rearrangement.

The main pathway for the browning of foods appeared to go through the Amadori rearrangement (degradation and condensation by way of the 1, 2-enol forms of the aldose and ketose amines (Lee, 1975).

**Intermediates in Brown Pigment Formation**

Furfurals have been suggested as intermediates in the formation of brown pigments. A furfural like (5-hydroxymethyl)-2-furaldehyde may react with glycine to produce brown colors. The addition of furfural to apricot concentrate increased the rate of browning. Pyruvaldehyde and diacetyl (in distillates of sugar and amino acid solutions) and other reactive 3 and 4 carbon sugar fragments have been suggested as possible intermediates. Such compounds would react more rapidly with amines than the osulososes or furfurals, but there is no evidence that they are formed in significant amount in foods (Reynolds, 1963).

It seems likely that the pigment is formed from the reaction between the amino compound and deoxy- and unsaturated osones in reactions containing an excess of aldose to amine and buffered at pH 6. Even in mixtures buffered at about pH 3.5, the osones could be the main reactants.
In systems containing an excess of amino acid to sugar ratios the formation of a ketoseamine can be assumed, but nothing is known about any other intermediates. Intact furane rings were not necessarily present in the pigment (Reynolds, 1963 and 1965).

Strecker Degradation

It is the oxidative degradation of \( \alpha \)-amino acids by dicarbonyl compounds (\( \alpha \)-dicarbonyls and other conjugated dicarbonyl compounds) resulting in the formation of aldehydes containing one less carbon atom than the amino acid, carbon dioxide, carbonyl compounds, and amines (Eskin et al., 1971, and Lee, 1975).

\[
\begin{align*}
R - C &= 0 + NH_2 & R - C &= NH_2 + R'CHO + CO_2 \\
R - C &= 0 & R - C &= 0
\end{align*}
\]

| dicarbonyl compound | \( \alpha \)-amino acids | carbonyl compounds | aldehyde carbon dioxide |

The dicarbonyl compounds (osones) can be produced from the decomposition of ketose amines. The amino group in the Strecker degradation products may finally appear as ammonia or it may be combined, depending on the nature of the reactive carbonyl compound.

The formation of aldehydes from \( \alpha \)-amino acids is usually associated with high-temperature reactions leading to changes in food flavor. However, it can also be formed at low temperature.

The Strecker degradation is a secondary effect of the reaction between aldoses and amines, providing further essential reducing compounds for nonenzymatic browning reactions.

Pyrazine compounds with different amounts of substitution had been
reported to be formed in carbonyl-amine reactions (Eskin et al., 1971). These compounds can cause Strecker degradation of the amino acids. A pyrazine, 2,5-dimethylpyrazine, had been isolated from a mixture of glucose with glycine:

![2,5-dimethylpyrazine](image)

**Reactions of Aldoses with Proteins**

The rate of reaction of different aldoses and aldose derivatives with casein varies considerably. The rate of free amino nitrogen loss was highest with xylose and arabinose, much lower with glucose and galacturonic acid, and lower still with lactose, maltose, and glucuronic acid. The rate of development of color was high with xylose, arabinose, and glucuronic acid, much lower with galacturonic acid, and lower again with glucose (Reynolds, 1963); no color developed with lactose and maltose after 20 days at 37°C and 70% relative humidity, when the amino-aldehyde reaction had virtually ceased (Lewis, 1950). These effects were similar to those found with simple amino acids and aldoses (Reynolds, 1963).

The rate of reaction between galactose and casein was similar to the rate for glucose and casein. In the reaction between 2-deoxygalactose and casein, the rate of loss of free amino nitrogen was much lower, but the rate of browning was much higher and there was no initial lag period (Lea and Rhodes, 1952). According to Anet (1957) since 2-deoxygalactose is an aldol, it could be dehydrated to an unsaturated aldehyde that could readily enter into browning reactions.
Jenness and Patton (1959) reported that the initial reaction, at least in the dry state, between glucose and casein involved a 1:1 reaction of the glucose with the ε-amino group of lysine. In addition, other basic amino acids, arginine and histidine, may be involved secondarily.

Reynolds (1963, 1965, and 1969) discussed in some detail the sugar-amine reactions in nonenzymic browning. The isolation, purification, and properties of pigments from protein-carbonyl systems were studied by Clark and Tannenbaum (1970).

**Lactose and Casein in Browning Reactions**

Concentrated and dried forms of milk, when stored for more than 30 days, gradually developed a brown color. Coulter et al., (1951) reported that the browning process was brought about by the reaction of free amino groups of milk proteins with the aldehyde group of lactose. The reaction is accelerated by increase in temperature, pH value, and water content of the protein containing material, or in the relative humidity to which a solid protein is exposed.

The lactose-casein reaction appeared to be similar to those between casein and formaldehyde, not only in that the primary reaction involved aldehyde and free amino groups, but also in that there is a secondary reaction, which, in this instance produced color and decreased the dispersibility of the protein (Patton, 1955).

At first, lysine is the amino acid principally involved, but later, arginine, histidine, methionine, and tyrosine are attacked. With the binding of free amino groups of these essential amino acids, the Biological Value of milk protein is impaired.
Glucoseamine reacted with casein and resulted in a rapid loss of free amino groups; a brown color developed immediately. The free amino groups of casein were not involved (Lea and Rhodes, 1952), but the amino and aldehydeic groups of glucoseamine were simultaneously destroyed. Glucoseamine reacted rapidly with acetylated casein, with the loss of free amino groups and the development of color. A mixture of N-acetylglucoseamine and casein browned very slowly, with a negligible loss of free amino nitrogen (10% after 100 days at 37°C). However, the stored product gave the Morgan and Elson test for N-acetylglucoseamine without the usual preliminary treatment with hot 0.5N sodium carbonate that causes isomerization and condensation to an oxazole ring (Lea and Rhodes, 1952).

Anet (1959) when describing the preparation and structure of D-fructoseglycine, pointed out that the formation and decomposition of diketoseamine provided a mechanism for the conversion of aldose to more reactive compounds, thus explaining the catalytic role of amines in browning reaction; McWeeny and Burton (1963) proposed the same mechanism.

Browning reactions in a mixture of glucose and glycine (8:1) and citrate buffered at pH 6.5, would be expected to follow the course described for the mixture of difructosaglycine and glycine. In the mixture buffered at pH 3.6, the 3-deoxyosulose accumulate about 25 times as fast as hydroxymethylfurfural (Reynolds, 1963). The decomposition products of difructosaglycine browned twice as fast, with glycine, as did hydroxymethylfurfural (Anet, 1959), suggesting that less then 20% of the pigment formed at pH 3.6 was derived from hydroxymethylfurfural.
On the basis of amino nitrogen binding capacity, the relative order of reactivity of sugars toward casein is xylose > arabinose > glucose > maltose > lactose > fructose (Lewis, 1950).

A catalytic decomposition of lactose, through dehydration, fragmentation, and condensation by the amino groups of casein leads to the formation of melanoidins, the brown pigment. Milk serum proteins and the phosphate salts may make minor contributions to the browning of milk system (Janness and Patton, 1959).

The mechanism of lactose degradation and its by-products have been discussed by Adachi (1959, 1961). Ferretti et al., (1970), and Ferretti and Flanagan, (1971) did some work on the lactose-casein model system.

Caramelization

Caramelization is the process of the heat decomposition of sugars as a function of pH and buffers, in the absence of amino compounds. It requires a relatively high order of activation energy.

On the other hand, the initiation of the Maillard (amino-sugar) reaction requires a relatively low order of activation energy, in the range of 21 to 42 kcal (Reynolds, 1963), and exhibits autocatalytic qualities once it has started (Jeness and Patton, 1959).

According to Hodge (1953), the caramelization includes two types of reactions, 1) dehydration reactions which produce furfural derivatives, and 2) oxidation-reduction leading to the fragmentation of the sugar molecule with the formation of reductones and other enediols.

When milk is heated, lactose is destroyed and decomposes to various compounds such as furfural, furfural alcohol, hydroxymethyl furfural, maltol, acetol, methyl glyoxal, and acetaldehyde. But, galac-
toose, not glucose, accumulated (Jenness and Patton, 1959). The frag-
ments probably arose from the glucose portion of lactose molecule.

Alpha lactose caramelizes at 160–180°C and melts at 202°C with
molecular decomposition (Aurand and Woods, 1973). Due to the high
energy requirements, the caramelization usually occur at elevated tem-
peratures. However, the activation energy requirements can be lowered
by the presence of catalysts such as alkalis, carboxylic acids and their
salts, phosphate ions, and metallic ions—especially Fe^{++} and Cu^{++}
(Hawthorn and Leitch, 1962).

The caramel pigments contained carbonyl, carboxyl, and enolic,
together with hydroxyl groups of varying basicity, and phenolic hydroxyl
groups (Lee, 1975). Jenness and Patton (1959) reported that the brown
pigments, melanoidins, formed in milk system, appeared to be highly
complex and probably a mixture of polymers. The pigments appeared to be
unsaturated and the presence of reductones (R-COH=COH-R) and carboxyls
(R-CHO and R-CO-R) gave its capacity to absorb light.

Melanoidins can be decolorized by bromine which is indicative of
the presence of unsaturated and reducing groups in the complex. In
simplified system, the brown pigment contained a significant amount of
nitrogen.

It should be noted also that compounds other than sugars that form
brown pigments when heated in the absence of amine groups include poly-
saccharides, polyhydroxycarboxylic acids, reductones, α-dicarbonyl com-
pounds, and quinones (Hawthorn and Leitch, 1962).
**Sugar Acid Reaction**

Reducing sugars are degraded to furfurals either by mineral acids or organic acids. Pentoses yield 2-furaldehyde which is relatively stable. Hexoses yield 5-hydroxymethyl-2-furaldehyde which can be degraded to levulinic acid (CH$_3$COCH$_2$CH$_2$COOH) (Reynolds, 1965).

Acids and bases both increase the browning of sugar solutions, with alkalis being particularly effective (Hawthorn and Leitch, 1962). Acids generally promote dehydration reactions, with the production of furfural derivatives, while alkalis induce isomerization and fragmentation of the sugar molecules. Alkalis act as catalysts in the caramelization of sugars, and are eventually neutralized by some of the reaction products, rather than reacting directly with the sugars.

The rate of browning of sugar solutions with organic acids are in the order of fructose ≈ sucrose ≈ galactose ≈ glucose (Hawthorn and Leitch, 1962).

The mechanism of the formation of furfurals from sugars has been discussed in detail by Anet (1964).

**Ascorbic Acid Oxidation**

The decomposition of ascorbic acid, under aerobic or anaerobic conditions, leads to the formation of brown pigments, and the production of carbon dioxide. Ascorbic acid plays an important role in the browning of juices, for example, lemon juice and orange juice. Eskin (1971) outlined a possible pathway for the decomposition of ascorbic acid to furfural (Figure 5).
Figure 5. Decomposition of ascorbic acid to furfural (Eskin et al., 1971)
Reactions Involving Lipids and Phospholipids

Reactions Involving Lipids

Oxidative degradation of polyunsaturated fatty acids in lipids readily provides carbonyl groups. These reducing groups can participate in nonenzymatic browning through carbonyl-amino reactions. Compounds like aldehydes, peroxides, ketohydroxy and epoxy compounds during the course of fatty acid oxidation, are capable of reacting with the amino groups of amino acids or proteins. Malonaldehyde, one of the main products of fatty acids autooxidation, is capable of reacting with various food constituents including amino acids and protein through the Maillard reaction leading to darkening and reduced nutritional value of proteins (Labuza, 1971).

Labuza (1971) also indicated that besides the production of objectionable odors and flavors, the free radicals and peroxides produced by lipid oxidation through a free radical mechanism can react with pigments bleaching them. They can also react with protein causing toughenings and reducing digestibility, they can destroy vitamins, and they can lead to the production of possibly toxic material.

Cooked chicken meat, on the average, contained 4.3% fat and 28.5% protein (Mountney, 1966). The intramuscular fat of poultry meat contained 17.6% linoleic and linolenic acids which are unsaturated fatty acids. The skin contained about 40% total lipid, of which 32% was hexane extractable. Total carbonyls comprised 50u mole/10g of the hexane extractable lipid (Thomas et al., 1971).

Kawasaki et al., (1972) studied some effects of radiation on the amino-carbonyl reaction. Radiation effects in poultry meat has been reported by Urbain and Gidding (1971). Davidkova and Khan (1967) studied
the changes in lipid composition of chicken muscle during frozen storage.

According to Pipper et al., (1960), about 60% of the carbonyl compounds remained in the broth of chicken meat which was heated in boiling water. The 2,4-dinitrophenylhydrazones of the volatile carbonyls collected in the steam distillate over four hours, were fractionated and identified to be acetaldehyde, n-hexanal, n-deca-2,4-dienal, and diacetyl and/or acetoin. Sixteen other carbonyls, including n-alkanals, 2-alkenals, and 2-alkanones were tentatively identified. These compounds do not have flavor characteristics like that of cooked chicken. The volatile components of cooked chicken that have been identified include ammonia, hydrogen sulfide, and mono- and di-carbonyls.

Thomas et al., (1971) reported that the fatty acids in the larger amounts in all broiler tissues were palmitic, stearic, oleic, and linoleic, depending on age and ration. Lipids from cooked tissues contained a larger amount of 18-carbon unsaturated fatty acids than the other fatty acids combined.

Braddock (1972) investigated chicken muscle stored at -10°C, and reported that during storage there was a decrease in phosphatidyl ethanolamine and phosphatidylcholine, and increase of lysophosphatidylcholine and free fatty acids. This suggested that since lipid hydrolysis occurred throughout frozen storage, lipid hydrolysis and protein may be interdependent phenomena. Hydrolysis of the phospholipids is catalyst by phospholipases in the muscle thus releasing free fatty acids. The free fatty acids could then cause denaturation of proteins in the flesh. Wladyka and Dawson (Eskin et al., 1971) demonstrated proteolysis in poultry carcass held at -18°C for up to 90 days. It was concluded that
Phospholipids and lipoproteins can react, through their amino groups, with aldehydes and reducing sugars; under oxidizing conditions reactive carbonyl groups formed in the lipid moiety can initiate browning reactions. Deterioration involving phospholipids and lipoproteins have been reviewed by Lea (1957). Lipid oxidation in a food may be accelerated if the water content is reduced to a level that inhibits amine-aldehyde reactions.

Amino compounds promote the browning of oxidized lipids, but it is not certain whether they are incorporated in the brown pigments formed in natural systems.

Marion and Woodroof (1965) reported that breast muscle lipids of chicken broiler, were highest in proportions of phospholipids to neutral lipids, followed by thigh muscle and skin tissue lipids. GLC analysis showed that phospholipids contained higher levels of 18-C saturated and 20-24-C unsaturated fatty acids than triglycerides which contained higher levels of 18-C mono and di-unaturated fatty acids than phospholipids. The fatty acid composition of similar lipid fractions did not vary appreciably with tissue location; only the proportion or level of lipid fraction varied in different tissues.

Pokorny et al., (1973) observed that during heating of mixtures of methyl esters of polyunsaturated fish oil fatty acids and egg albumin under conditions simulating roasting and frying of fish, the peroxides present in the lipid fractions were rapidly decomposed and formed both liposoluble and insoluble brown pigments with free amino groups of albumin. Small amounts of lipids in the mixture have a great effect on
the development of color. The reaction is very rapid at temperatures >
100°C and the brown pigments are not decomposed by prolonged heating.

Pokorny et al., (1973) studied the NEB produced by oxidized poly-
unsaturated lipids on storage with protein in the presence of water. He
noted that in model systems the recovery of extractable lipids was lower
from the mixture containing the more oxidized lipids, whereas the degree
of browning was independent of the peroxide value. Prolonged storage
increased the intensity of browning. Changes appeared to be similar at
4°C and 60°C, but proceeded more rapidly at 60°C.

Reactions involving phospholipids

According to Katz (1966) the ratio between neutral lipid to phos-
pholipids is approximately 79:21 in muscle lipids, and 98:2 in skin
lipids of chicken.

Phospholipids were more important in lipid deterioration based on
the higher losses of fatty acids during cooking and during frozen
storage (Lee and Dawson, 1973). The high proportions of phosphatidyl-
choline and phosphatidylethanolamine is a characteristic of chicken
muscle fat (85.2% together with sphingomyelin).

The loss of phospholipids may be due to both the chemical deterio-
ration and physical rendering of fats from muscle during cooking. The
chemical deterioration of phospholipids may be characterized as autoxi-
dation, hydrolytic decomposition, lipid browning reactions, and lipid-
protein copolymerization reactions (Lee and Dawson, 1976). Hydrolysis
also occurred in muscle phospholipids during cooking in re-used corn oil.

Phosphatidylethanolamine has been related to lipid browning dete-
rioration; its decrease may have been a consequence of involvement in
the dark color in cooked chicken found after storage. It appeared that the mechanisms for destruction of phospholipids in cooked chicken muscle during frozen storage are somewhat different from those of uncooked chicken. Chemical reactions which occur in chicken cooked in fresh corn oil may vary with different tissues and oil treatment. Chemical changes and physical rendering of lipids from chicken muscle and skin occurred during cooking and during frozen storage. The use of reheated cooking oil accentuated these changes, thus the chicken cooked in re-used corn oil was less stable during storage than that cooked in fresh corn oil (Lee and Dawson, 1976).

Factors Affecting Nonenzymatic Browning Reactions

1) pH and Buffers;

The carbonyl-amino reactions can occur in both acidic and alkaline media; although the reaction is favored under the more alkaline conditions. There is an increase in reaction rate with rise in pH (Eskin et al., 1971). Occurrence of strong-base catalysis in the range of pH 6.5 to 8.5, and solvent or weak-base catalysis in the range of pH 3 to 5. The rate of ketoseamine formation increases with increasing pH and subject to acid-base catalysis.

Phosphate, citrate, and/or acetate buffer salts influenced the ionic environment and thus accelerated the sugar-amino reaction rate. Saunders and Jervis (1966) reported that the buffering capacity role of sodium phosphate and sodium citrate buffered the acidic products formed during nonenzymatic browning reaction thereby maintaining an alkaline environment favoring the reaction.
2) Temperature;

There is an increased rate of reaction with an increase in temperature. According to Eskin et al., (1971), there is a linear relationship between the rate of reaction and temperature within the range of 0 - 90°C, conforming to the Arrhenius equation: \( k = a \cdot e^{-\frac{\Delta E}{RT}} \) or \( \ln k = \ln a - \frac{\Delta E}{RT} \), where \( k \) = the equilibrium constant, and is equal to the ratio of the forward and reverse reaction rate constants, \( a \) = the frequency factor, which has the units of specific reaction rate, usually based upon the units of concentration, and is thus dependent on the order of the reaction, \( E \) = the energy of activation in kcal/g mole, \( R \) = gas constant, and \( T \) = absolute temperature in °K. Reynolds (1963) reported that the activation energy for Maillard reactions is in the range of 21 - 42 kcal.

Ionizing radiations also promote the Maillard reactions (Hawthorn and Leitch, 1962).

3) Moisture content and water activity;

Nonenzymatic browning reaction proceeds readily in aqueous solutions. According to Lea and Hannan (1950), the maximum reaction rate between casein and glucose occurred at 13% moisture content, where the reactants were still in comparatively dry state. Thus the optimum moisture content favoring the nonenzymatic browning reaction is at fairly low percent (Eskin et al., 1971). At high moisture content the decreasing rate of browning reaction is due to the dilution effect on the reacting substances and the inhibitory effect to water due principally to the law of mass action (Eichner, 1975). Water is formed during browning, up to 3.5 mole of water per mole of sugar, and it causes a slowing of the condensation stages in the reaction complex (Eichner and Karel, 1972).
The term "Water activity," $a_w$, has been coined to express the degree of availability of water in foods (Scott, 1957). It was defined as the ratio of partial pressure of water in the food to the vapor pressure of water at the given temperature:

$$
aw = \frac{p}{p_o} = \frac{\% ERH}{100},
$$

where $a_w$ = water activity, $p$ = vapor pressure of water in food system, $p_o$ = vapor pressure of pure water, and $\% ERH = equilibrium relative humidity$, that is the relative humidity at which food neither gains nor loses moisture to the atmosphere. A 1-molal solution of a perfect solute in water would reduce the vapor pressure of the solution 1.77% over that of pure water, or the vapor pressure of the solution would be 98.23% of that of pure water, of the $a_w$ would be 0.9823 (Nickerson and Sinskey, 1972; Labuza, 1975).

At higher water activity, the browning reaction rate decrease has been attributed to dilution of the reacting substances. The decreased reaction rate at low water activity, when the amount of mobile water is greatly reduced, has been ascribed to an increasing diffusion resistance which lowers the mobility of the reactants (Labuza, 1975).

4) Sugar and amino acid;

Reducing sugars provide the carbonyl groups for the interaction with the free $\alpha$-amino groups of amino acids. The concentration of each reactant influences the rate of the browning reaction. At higher concentrations, more primary reactants are available for the browning reaction, and thus increased the rate of pigment formation.

The rate of browning varies with different sugars and different amino acids. The reactivity of sugars in model systems has been reported to be $xylose > arabinose > galactose > mannose > glucose > lactose$. 
> maltose. Pentoses react more rapidly than hexoses to produce keto-
seamine and brown pigments (Reynolds, 1963 and Hawthorn and Leitch,
1962).

In the reaction of glucose with amino acids, the maximum browning
effect was the reaction with lysine, followed by tryptophan, and argi-
nine. The minimum browning rate was shown by glutamic acid or proline.
Lactose reacted most readily with tryptophan (Hawthorn and Leitch, 1962).
MATERIALS AND METHODS

Materials for Preparing Breaded Fried Chicken

Flour - all purpose enriched wheat flour, bleached and presifted - purchased from a local food store.
Cracker Crumbs - purchased from a local food store.
Buttermilk Powder - obtained from the Modern Main Food Products., Inc., New York.
Chicken - chicken for the purpose of this investigation were purchased from a local food store.

Preparation and Processing of Breaded Fried Chicken

Whole chicken were purchased from a local food store and brought to the Food Science Department where the birds were cut into four parts, namely; breasts, thighs, drumsticks, and wings. Excessive fat was trimmed from the parts, particularly from the thigh pieces. The cut pieces were cleaned and packed in polyethylene bags and stored in the cold room at -20°C (-4°F) until ready for use.

Precooking. Prior to precooking, each bag of chicken parts was thawed in a 38°C water bath. The cooking methods consisted of precooking in boiling water for 15 min. Then the cooked pieces were drained for 10 min and cooled to 4.4°C (40°F) before being dredged in batter preparation.

Precooking and cooling of chicken parts prior to battering promoted better adhesion of batter and breading material. The amount of coating material or crust is limited to less than 30% of the finish products (United States Department of Agriculture, Poultry Division,
Postry Inspector's Handbook, 1964). It also has been reported that breading increased fat absorption when cooked by methods where fat was used (Baker et al., 1972).

Precoking of chicken parts can be achieved by steaming, simmering, boiling in water, or microwave cooking. According to Baker et al., (1972), that simmering at 82°C produced better products than by steaming or boiling methods.

**Batter Formulation**

In order to study the effect of buttermilk solids in producing undesirable dark color in breaded fried chicken, variable amount of the buttermilk powder was added in the batter formula (up to 20% on dry weight basis). A simplified batter formula was used to minimize other factors which could influence the color of the final products.

**Table I. Batter Formulation**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Percent Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A (Control)</td>
</tr>
<tr>
<td>Flour</td>
<td>100</td>
</tr>
<tr>
<td>Buttermilk powder</td>
<td>0</td>
</tr>
</tbody>
</table>

1 Solids to moisture ratio of 1:1.5

The above is a partial list of formulation ingredients used in industry. It must be emphasized that it is almost impossible to determine the exact proportions of the ingredients used in the commercial
formulations because each processor uses a different combination and amount of ingredients. Other ingredients would include whole milk, egg white, salt, papper, antioxidants, monosodium glutamate, and other flavorings.

The batter ingredients, solid-water ratio of the batter, coating procedures, and/or cooking methods were found to influence the colour, texture, toughness, fat and moisture, and general appearance of the cooked chicken parts (Hanson and Fletcher, 1963).

The batter ingredients were mixed with water in a mixing bowl, maintaining the solids to moisture ratio of 1:1.5. The mixture was stirred very thoroughly to produce a batter with an even consistency.

**Battering and Breading.** The chicken parts which have been pre-cooked and cooled to 40% were dredged in the respective batter preparation according to their batches. After one minute in the batter the pieces were drained for one minute and then rolled over a breading material consisting of cracker crumbs in a tray. The parts were shaken lightly by hand to remove excess breading material. After weighing, the breaded chicken parts were placed in a container lined with aluminum foil and taken to the kitchen at the LSU union. Ample spacing was provided between each part in every batch so that they did not stick to each other. Great care was observed during handling and transportation of the breaded chicken parts.

The breading process promoted easy handling of the products because it reduced the tendency of the parts to stick together.

**Blanching and Frozen Storage.** The breaded parts were blanched in deep-fat (soybean oil) at 395°F (201.6°C) for 20 seconds. This step helped to stabilize the crust around the products. They were cooled
to room temperature and placed in polyethylene bags according to parts and treatments. Each bag was sealed and properly labelled before being transferred into the cold room maintained at -20°C for a period of 30 days or more.

Deep-Fat Frying. After the 30-day period of frozen storage, the products were brought back to the kitchen and deep-fat fried in soybean oil at 360°F (182.2°C) for 6 minutes. The breaded fried chicken were drained for 15 minutes and cooled to room temperature before being subjected to color score by 10 judges.

Other methods of processing by other workers have included pre-soaking in up to 3% phosphate (a food grade mixture of sodium tripoly-phosphate, tetrasodium pyrophosphate, and sodium acid pyrophosphate) for 16 hours. Marination in 1 - 3% of phosphate and salt was reported to improve flavor and yield, reduced shear values, and increased total moisture (Baker, et al., 1972).

Further information relative to producing fried poultry products can be obtained from the U.S. Department of Agriculture, Poultry Division, Poultry Inspector's Handbook (1964).

Selection of Wrapping Material

Wrapping material and packaging served to protect the product against physical damage due to handling, and to preserve the product in good condition during storage when exposed to air circulation, light, and off-odors.

Polyethylene bags meet these requirements. Therefore, they were used to wrap the samples which were stored frozen in a forced air circulation cold room. Beside being more economical than paper, the transparent bag facilitated identification of its content.
Storage at Frozen Temperature

Precooked chicken parts which have been battered, breaded, and blanched were sealed in polyethylene bags and stored in a forced air circulation type cold room at -20°C for 30 days or more. The frozen storage period duplicated the conditions similar to those of the commercially-produced products. Therefore, the keeping stability of the breaded chicken parts, particularly the coating material, could be comparatively observed.

It is necessary to maintain a constant low temperature of storage to avoid formation on the surface of the breaded chicken parts and their consequent adhesion. When there is a rise in the temperature to near the melting point the product may stick and ice crystals may form on the surface of the foodstuff.

Deep-Fat Frying of Breaded Fried Chicken

Deep-fat frying is the process whereby the properly prepared product is cooked by immersion in a body of heated edible fat or oil for a certain temperature and time.

In order to most nearly simulate commercially available equipment and conditions, the breaded chicken parts were banched and deep-fat fried in a kettle in the kitchen of the LSU union. The kettle had the following favorable characteristics: a) Thermostatic control of the frying oil, b) Filtering frying oil system, c) Stainless steel construction, d) Heat exchange by means of tubes in the middle of the fryer tank, e) Low degree of aeration.

By means of the thermostatic control it was possible to maintain a narrow range about the chosen temperature of frying, and thus avoid the
use of superheated oil. Since the exact time and temperature are very important to maintain throughout the frying process so that every chicken part receives equal cooking treatment. Variable time/temperature would introduce another factor in affecting the color intensity of the final product.

Blanching was carried out at 201.6°C (395°F) for 20 seconds and the final frying was conducted at 182.2°C (360°F) for 6 minutes.

According to the U.S. Department of Agriculture, fat used for frying marine products (fish, including shell fish) is not satisfactory for purposes of frying poultry although there is no objection to the use of fat for frying poultry which has been used for the frying of potatoes. Therefore, no attempt was made to add fresh oil or phosphate in the frying oil. The commercially available soybean oil was used in the frying process, and it has been used to fry potatoes for one day during the normal operation of the kitchen. The commercially available oil already contains antioxidants (BHA and BHT) for the purpose of retarding breakdown of the fat into free-fatty acids and other noxious materials.

Breaded chicken parts were arranged in a mesh-wire basket and immersed in the frying oil for the selected time and temperature. In order of frying was first the control, followed by batches B, C, D, E, and the three levels of sulfite 1000, 2000, and 2500 ppm.

**Sensory Evaluation of Color**

The sensory evaluation of color was selected to represent the average consumer's preference. Ten students volunteered to serve the color panel and no effort was made to select the panelists for their visual acuity, since differences in shade of color detected by the
average person were sought. The panelists consisted of 4 females and 6 males ranging from 20 to 30 years of age. They were representatives of four disciplines, namely; food science, dairy science, home economics, and horticulture. The panelists were to judge the color of a breast and a thigh piece of the breaded fried chicken for the five treatments: A, B, C, D, and E. A thigh piece was presented for each of the three sulfite treatments. The total color score compiled was 130.

The samples were placed on a white background in a room lighted by fluorescent lamps. Each panelist, in the absence of others, judged the samples for color by evaluating the whole piece from each treatment and scoring according to the following rank:

   Color score 1. Too dark
   2. Slightly dark
   3. Golden brown (Just right)
   4. Slightly light
   5. Too light

The apparent color of a food depends on (1) the wavelengths (frequencies) of the incident light, (2) the wavelengths reflected or transmitted by the food (resulting from its selective absorption of light), (3) the background conditions, and (4) eye and brain functions.

Components of the light striking the food will be reflected, absorbed, or transmitted. If white light strikes an opaque substance (like bread) and all wavelengths are reflected, a food sample appears dark gray or black. In case of breaded fried chicken the incident light will be reflected and/or absorbed. The reflected light coming from the food is the visual stimulus. The wavelengths for visible light range from 400 to 750 mu (frequencies $10^{14}$ to $10^{15}$ cycles/sec).
The physical structure of the food, as well as the chemical nature of its components, affect reflectance.

Scientifically, color is described by three attributes: (1) Spectral color (hue) - redness, greenness, blueness, or their intermediates; (2) Saturation (purity or chroma) - the strength of hue, or freedom from mixture with white; (3) Brightness (lightness or value) - associated with the luminous energy transmitted or reflected by the substance.

The first two attributes together describe chromaticity. Adding brightness, they describe the color (Marion, 1972).

The composition of the breading material will largely affect the chromaticity, and the combination of time and temperature of cooking will affect the brightness attribute primarily. The composition of the coating material is the major variable of the breaded fried chicken being investigated.

The organoleptic quality (color, texture, and flavor) of a food is the primary factor for the food to be accepted by the consumers. Then, it is appropriate to conduct the sensory evaluation of color of the breaded fried chicken to represent an average consumer's preference, under controlled conditions.

**Inhibition of Nonenzymatic Browning Reactions by Sulfite**

Complex chemical reactions are involved in the nonenzymatic browning system. The effectiveness of sulfite in controlling these reactions so that the development of the melanoidins is either inhibited or retarded, is variable depending on the nature of the browning system, the time of introducing the sulfite into the system, and the quantity of the sulfite being introduced. Sulfite as a nonenzymatic browning inhibitor has been
reported by Gehman and Osman (1954), Burton and coworkers (1963), Anet and Ingles (1964), Hurst (1972), and McWeeny et al., (1974).

The sulfite have also been used for other purposes: (a) as a preservative, (b) as a reducing agent for color retention and for splitting disulfite bonds in flour for biscuit manufacture and in spinning of vegetable proteins, (c) in controlling enzymatic browning reactions (McWeeny et al., 1974).

An investigation was also conducted to study the effectiveness of sulfite in controlling the nonenzymatic browning reactions in the breaded fried chicken which contained up to 20% buttermilk solids in its batter formulation.

Theoretically, sodium metabisulfite ($\text{Na}_2\text{S}_2\text{O}_5$, MW=190.11) contains 67.4% sulfite ($\text{SO}_2$). The amounts of sodium metabisulfite needed per 100 g of batter mixture to contain three levels of sulfite were calculated and included in the 20% buttermilk batter. The sulfite levels were arbitrarily selected as 1000, 2000, and 2500 ppm.

A thigh and a drumstick were battered in each level of sulfite-batter mixture. All other processing procedures were the same as previously described. The final products were color-scored by the same panel members.

Nonenzymatic Browning Reactions in Aqueous Solutions

Five percent aqueous solutions of glucose, galactose, and lactose with lysine in distilled water were prepared. Potassium sorbate was added as an antimicrobial agent at the rate of 0.3%. The solutions were buffered with phosphate and the pH was adjusted to 6.0 using 0.1 N HCl or 0.1 N NaOH when applicable. Each solution was poured into glass tubes,
tightly capped, and stored at 27°C. The progress of nonenzymatic browning reactions were determined by measuring the intensity of the brown pigments being formed. The light transmittance (or absorbance) of the solutions were measured at three-day intervals using the Bausch and Lomb spectronic 20 at 420 μm. Distilled water was used as a blank for 100% light transmittance.

A second set of aqueous solutions (1% concentration) was also prepared containing lactose-lysine and lactose-glutamic acid. The procedure was the same as the above. The lactose-lysine solution was adjusted to pH 5.5 and the lactose-glutamic acid solution was adjusted to pH 3.5. Sample tubes of each solution were stored at 4.4°C and 27°C. The light transmittance of the solutions at 420 μm was measured at weekly intervals.

The third set of 1% aqueous solutions contained glucose-lysine and galactose-lysine. Following the same procedure as the above, the pH of the solutions was adjusted to 4.0. Sample tubes were stored at 4.4°C and 27°C and light transmittance was recorded at weekly intervals.

Chemicals for the Study of the Rate of Nonenzymatic Browning Reactions in Aqueous Solutions and in Model Food Systems.

Glucose, galactose, and lactose were the reducing sugars for the Maillard browning reactions; the amino acids as well as casein were the sources of free amino groups for the Maillard reactions; potassium sorbate, a preservative, was used for antimicrobial agent; glycerol was used for liquid humectant to control the water activity and plasticity of model food systems; since microcrystalline cellulose is inert to Maillard reaction it served as a solid support for the model systems; TRI-SIL-Z, a prepared reagent, was used to derivatize the sugars for the gas chromatographic analysis.

The formula for the intermediate moisture model food systems was modified from the intermediate moisture food formula by Desrosier (1970) and the model food formula by Warmbier et al., (1976).

Nonenzymatic Browning Reactions in Model Food Systems

For the purpose of this study, model food systems were formulated to contain three different sugars and casein as the source of amino acids. Each formula contained one type of reducing sugar and casein as the basic ingredients. The total ingredients are presented in Table II.

The ingredients were weighed and added in the same order as listed in Table II. Direct mixing of water into the mixture was followed by thorough stirring. After measuring the pH of the systems, each formula was poured into 5 sample bottles, loosely capped, and placed in a dessicator over a saturated solution of magnesium nitrate (\( \text{Mg(NO}_3\text{)}_2 \cdot 6\text{H}_2\text{O} \)), water activity \( a_w 0.52 \) for 24 hr. After the water activity equilibration, the bottles were sealed tightly. Representative sample bottles were kept at 27 and 36°C. Samples were taken at regular intervals to measure
### Table II. Model Food Systems

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Composition (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A (Glucose)</td>
</tr>
<tr>
<td>1. Potassium sorbate</td>
<td>0.3</td>
</tr>
<tr>
<td>2. Reducing sugar</td>
<td>10</td>
</tr>
<tr>
<td>3. Glycerol</td>
<td>20</td>
</tr>
<tr>
<td>4. Casein</td>
<td>30</td>
</tr>
<tr>
<td>5. Microcrystalline cellulose (Avicel)</td>
<td>10</td>
</tr>
<tr>
<td>6. Water</td>
<td>60</td>
</tr>
</tbody>
</table>

### Table III. Model Food Systems for Water Activity Treatments

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Composition (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D (Glucose)</td>
</tr>
<tr>
<td>1. Potassium sorbate</td>
<td>0.3</td>
</tr>
<tr>
<td>2. Reducing sugar</td>
<td>10</td>
</tr>
<tr>
<td>3. Glycerol</td>
<td>20</td>
</tr>
<tr>
<td>4. Casein</td>
<td>20</td>
</tr>
<tr>
<td>5. Microcrystalline cellulose (Avicel)</td>
<td>10</td>
</tr>
<tr>
<td>6. Water</td>
<td>40</td>
</tr>
</tbody>
</table>
brown pigment accumulation and the amount of sugar left in each formula.

The brown pigments were extracted and the light transmittance was measured using the Spectronic 20 at 420 μm, with distilled water as the blank. Sugar was determined by gas chromatography, using the TRI-SIL-Z as the derivatizing agent.

Samples were analysed on the same day or kept frozen at -20°C until further use.

For the study of the effect of water activity on the rate of nonenzymatic browning reactions in model food system a second set of model food formulation was also prepared as shown in Table III. The ingredients were weighed and mixed in the order as listed. Direct mixing of water was adopted. After mixing thoroughly, each formula was poured into glass bottles. Two sample bottles from each formula were kept in a desiccator over one type of saturated salt solution for water activity equilibration by humidification process (see Table IV). Different water activity levels were achieved by the equilibration (humidification) process in vacuum dessicators containing saturated salt solutions (Rockland, 1960) which provided constant equilibrium relative humidities or water activities at 27°C. Appropriate samples were taken at regular intervals for sugar content determination.

Table IV. Saturated Salts Solutions for Water Activity Equilibration

<table>
<thead>
<tr>
<th>Saturated Solutions</th>
<th>Water activity (aw)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Magnesium nitrate (Mg(NO₃)₂·6H₂O)</td>
<td>0.52</td>
</tr>
<tr>
<td>2. Sodium nitrate (NaNO₂)</td>
<td>0.62</td>
</tr>
<tr>
<td>3. Sodium Chloride (NaCl)</td>
<td>0.75</td>
</tr>
<tr>
<td>4. Potassium chromate (K₂CrO₄)</td>
<td>0.84</td>
</tr>
</tbody>
</table>
Pigment Extraction and Light Transmittance Determination

Pigment formation in Maillard browning reaction involves the formation of increasingly unsaturated multi-carbonyl compounds. In dry systems and in dilute solutions containing protein, the pigment causes cross-linking between protein chains (Clark and Tannenbaum, 1970). Therefore, in the extraction procedure the insoluble protein-bound pigment was treated with a protein splitting enzyme namely trypsin. During this digestion period the protein chains are broken down and thus releasing the pigment into the solution.

A 2-g sample was dispersed and/or dissolved in 50 ml of distilled water. It was followed by the addition of 2 ml of a 10% trypsin solution. After mixing by stirring, the solution was incubated at 45°C for 2 hr. The pH of the solution was in the range of the trypsin activity. After the digestion period, 2 ml of 50% trichloroacetic acid was introduced into the mixture to precipitate out the protein. Before filtering through a filter paper (No. 589), 0.1g of celite analytical filter aid was added into the mixture. The filtrate was measured for its light transmittance at 420 nm, with distilled water as the blank at a 100% reading, using the Bausch & Lomb Spectronic 20.

Proximate Analyses

Moisture. The percent moisture content of each sample was calculated after drying 2 - 5g duplicate samples in a vacuum oven at 70°C and 25 mm Hg for 24 hr.

Fat. The percent crude fat in the moisture-free sample was determined by extracting the sample with ethyl ether in a Goldfisch fat extraction apparatus using the A.O.A.C. procedure (1970).
Protein. Percent total protein was determined by the macro-Kjeldhal method (A.O.A.C., 1970). The percent nitrogen was multiplied by 6.25 to obtain total protein.

Ash. Percent ash content of the moisture-free sample was determined by the A.O.A.C. procedure (1970).

Carbohydrate. Percent total carbohydrate was calculated by deducting the percentages of moisture, fat, protein, and ash from 100. On the dry basis, only the dry percentages of the last three components were considered (Triebold and Aurand, 1963).

All pH measurements were taken by using a Corning pH meter, model 7.

Total Plate Count of Microorganisms

Serial dilutions were employed in order to determined the number of bacteria per gram of sample.

Under aseptic conditions, dilutions of samples from $10^1$ to $10^5$ were pipetted and dispensed into 1 ml amounts in sterile Petri dishes to which Standard Methods Agar (Hausler, 1972) was added. Duplicate plates of each dilution were made and incubated at 37°C for 48 hr. The total bacterial growth (Standard Plate Count) was recorded after the incubation period.

Free Fatty Acids

Free fatty acids were determined as acid value. The acid value is defined as the number of mg of KOH necessary to neutralize 1 gram of fat or oil. The free fatty acids content is expressed as percentage by weight of oleic acid (MW=282). Acid value = 1.99 X % Free Fatty Acids (expressed as oleic).
The amount of free fatty acids present in the cooking oil indicates the degree of denaturation, and toxicity of the sample (Pomeranz and Meloan, 1971).

Color of Cooking Oil

The oil samples were filtered through Whatman No. 2 filter paper in order to remove particles which would interfere with the color measurement. The light absorbance (or transmittance) was measured at 460 mu using the Bausch & Lomb Spectronic 20 with a CCl₄ blank at 100% reading.

Qualitative Tests

Carbonyl compounds: Positive tests are indicated by the formation of a precipitate after mixing the sample with 2,4-dinitrophenylhydrazine in 2N HCl.

Furfurals: The formation of red color after mixing the sample with xylidine or glacial acetic acid indicates a positive test.

Amadori rearrangement products: Positive tests are indicated by a reduction of potassium ferricyanide or 2,6-dichlorophenol indophenol by the sample.

Determination of Sugar Content by Gas Chromatography

Reagent: TRI-SIL-Z - available from Pierce.

This determination involves a reagent solvent system where Trimethylsilylimidazole (TSIM) is the active silylating reagent and dry pyridine is the solvent (v/v, 50:50). This one step derivatizing system is a
powerful formulation for quickly derivatizing hydroxy, polyhydroxy, and
carboxyl compounds either dry or in aqueous solution. Therefore, it is
a reagent of choice for carbohydrates - wet or dry. It will not deri-
vatize amino groups.

The TRI-SIL-Z is a prepared reagent which will silylate sugars
very rapidly and smoothly and with less anomerization, even in the
presence of moderate amount of water.

The TSIM formula is \((\text{CH}_3)_3\text{SiNCH=NCH=CH}\).

Derivatizing time. Many compounds silylate as soon as dissolved,
and most hydroxyl compounds derivatize completely within 5 min. For
determining the derivatizing time, the silylating reagent and sample
are observed in the chromatograph at the following intervals: 5, 15, 30
min and 1, 4, and 8 hr. When there is no increase in product peak in 3
successive chromatograms, the lowest time is taken at which the peak
is reached as the derivatizing time for the compound.

Column. A stainless steel column appears to be quite satisfactory.
The transition from liquid to gas phase, in the presence of metal, is
the most critical breakdown point. Once the gas phase has been attained,
the derivatives appear quite stable in the presence of stainless steel.

Stationary phase. Silicones are the most useful stationary phase
for this purpose, because they are quite inert, stable, and have excel-
 lent separating characteristics. Their ability to perform at higher
temperatures greatly extends the range of the chromatograph. Their
ability to react at various ranges of polarity among the silicones
broadens their applicability and contributes to their specificity. The
most widely useful stationary phase is SB-30 ultraphore, a high purity,
nonpolar phase useful to 350°C.
Substrate for column packing. The one reliable substrate that will withstand the rugged conditions of high temperatures and prolonged programming is chromosorb W(HP). It is a diatomise-based and thoroughly treated material (Pomeranz and Meloan, 1971).

Column conditioning. The column used for TSIM derivatives should be thoroughly conditioned before use. The reagent is repeatedly injected until a stable base line is attained. New columns were conditioned at 275°C for 36 hr with the carrier gas nitrogen flowing at a full rate.

Handling precautions. Contact of reagents with the skin and inhalation should be avoided, (particularly the eyes). Some individuals could be sensitive to the reagent. Working areas should be well ventilated.

Procedure for Simple Sugars

A 10 - 15 mg sample was dissolved in 1 ml TRI-SIL-Z in a screwcap septum vial. The vial was shaken for 15 sec at 2 min intervals. it may be heated to 60 - 70°C for 5 sec if necessary. Sugars are usually completely silylated when dissolved. Using the proper syringe, 0.1 - 0.5 ul of the reacted mixture was injected directly into the gas chromatograph. Working parameters were stabilized and presented in Table V.

Prior to weighing and derivatizing, each sample was dried in a vacuum oven at 60°C and 25 mm Hg pressure for 16 hr. The sample was then pulverized to loosely fine particles.
Table V. Parameter of Gas Chromatography for Sugar Analysis

**Carrier gas:** Nitrogen at 40 psi, flow rate = 3 cm³/min.

**Detector gas:** Hydrogen at 30 psi, and Compressed Air at 25 psi.

**Gas Chromatography:** PERKIN ELMER 990

- Temp. programming: 170 - 240°C
- Prog. rate: 2°C/min
- Injection port temp.: 270°C
- Initial time: 2 min
- Detector temp.: 300°C
- Cool rate: Med.
- Column temp.: 170 - 240°C
- Final time: Hold
- Amp. Attenuation: X 8
- Amp. Range: X 10

**Automatic Digital Integrator:** INFOTRONIC Model CRS-208

- Recorder: Linear
- Track rate: 300 uV/min
- Min. peak width: 3 sec
- Recorder attn.: 20
- Max. peak width: 100 sec
- Peak sensor gain: 4

**Column:** Stainless Steel; 6'X1/8' S.S. 3% SE-30 M on Varaport 30

- 100 - 120 mesh.

**Recorder:** Varian Linear Type

- Chart speed: 0.5 in/min
- Applied voltage: 1 mv
RESULTS AND DISCUSSION

Breaded Fried Chicken

The deep-fat frying time and temperature and other processing conditions for the production of the breaded fried chicken were those of the processing parameter being used by a commercial food processor in Arkansas. The conditions were duplicated in our laboratory resulting in a good golden brown color for the control product which did not contain buttermilk solid in its batter formulation. However, the final color for the breaded fried chicken containing up to 20% buttermilk solids in its batter formulation proved to be too dark for consumer's acceptance. Nevertheless, it was noted that the buttermilk component contributed to a desirable characteristic flavor in the breaded fried chicken.

The color panelists judged the color of the final products according to 1 to 5 hedonic scale ranking; number 1 being too dark in color and number 5 representing the other extreme, too light in color. The center scale, number 3, reflected the golden brown color which was the most preferred color for the breaded fried chicken or any other fried products for that matter. The results are represented in Table VI.

The judges' scores indicated no significant difference in color of the breaded fried chicken between the dark meat (thigh) and the white meat (breast) for the control treatment (p > 0.05). The mean color scores for the two parts were 2.8 and 2.9, respectively. The color scores also revealed that there was no significant difference in color between
<table>
<thead>
<tr>
<th>Batter Formula</th>
<th>Chicken Part</th>
<th>Judge Number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 2 3 4 5 6 7 8 9 10</td>
</tr>
<tr>
<td>A (Control)</td>
<td>Dark Meat</td>
<td>3 3 3 3 3 2 3 3 2 3</td>
</tr>
<tr>
<td></td>
<td>White Meat</td>
<td>3 3 3 3 3 3 3 3 2 3</td>
</tr>
<tr>
<td>B (5% BM)</td>
<td>Dark Meat</td>
<td>3 2 2 3 2 2 3 2 2 2</td>
</tr>
<tr>
<td></td>
<td>White Meat</td>
<td>3 3 2 2 2 3 2 3 2 2</td>
</tr>
<tr>
<td>C (10% BM)</td>
<td>Dark Meat</td>
<td>2 2 2 2 1 1 1 2 2 2</td>
</tr>
<tr>
<td></td>
<td>White Meat</td>
<td>2 2 1 2 2 1 2 2 2 3</td>
</tr>
<tr>
<td>D (15% BM)</td>
<td>Dark Meat</td>
<td>1 1 1 1 1 1 1 2 2 1</td>
</tr>
<tr>
<td></td>
<td>White Meat</td>
<td>1 2 1 1 1 2 2 2 2 1</td>
</tr>
<tr>
<td>E (20% BM)</td>
<td>Dark Meat</td>
<td>1 1 1 1 1 1 1 1 1 2</td>
</tr>
<tr>
<td></td>
<td>White Meat</td>
<td>1 1 1 1 1 2 2 2 2 2</td>
</tr>
</tbody>
</table>

BM - Buttermilk solids
Color Scores:
1 - Too dark
2 - Slightly dark
3 - Golden brown (Just right)
4 - Slightly light
5 - Too light
Table VII. Mean Color Scores of Breaded Fried Chicken

<table>
<thead>
<tr>
<th>Batter Formula</th>
<th>Judge Number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>A (Control)</td>
<td>3.0</td>
</tr>
<tr>
<td>B (5% BM)</td>
<td>3.0</td>
</tr>
<tr>
<td>C (10% BM)</td>
<td>2.0</td>
</tr>
<tr>
<td>D (15% BM)</td>
<td>1.0</td>
</tr>
<tr>
<td>E (20% BM)</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Table VIII. Grand Mean Color Scores of Breaded Fried Chicken

<table>
<thead>
<tr>
<th>Batter Formula</th>
<th>Grand Mean Color Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (Control)</td>
<td>2.85</td>
</tr>
<tr>
<td>B (5% BM)</td>
<td>2.35</td>
</tr>
<tr>
<td>C (10% BM)</td>
<td>1.70</td>
</tr>
<tr>
<td>D (15% BM)</td>
<td>1.35</td>
</tr>
<tr>
<td>E (20% BM)</td>
<td>1.15</td>
</tr>
</tbody>
</table>

BM = Buttermilk solids

Color Scores:
1 - Too dark
2 - Slightly dark
3 - Golden brown (Just right)
4 - Slightly light
5 - Too light
Table IX. Analysis of Variance of the Mean Color Scores

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>S.S...</th>
<th>M.S.</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batter formulation</td>
<td>4</td>
<td>20.09</td>
<td>5.02</td>
<td>34.96**</td>
</tr>
<tr>
<td>Replication (Judges)</td>
<td>9</td>
<td>5.03</td>
<td>0.56</td>
<td>4**</td>
</tr>
<tr>
<td>Error</td>
<td>36</td>
<td>5.17</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
<td>30.28</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** Highly significant at 1% probability level

$F_{.01}$ with 4 & 36 d.f. = 3.89 (Batter formula)

$F_{.01}$ with 9 & 36 d.f. = 2.95 (Replication)
the two types of chicken parts at the 5 and 20% levels of buttermilk solids in their batter formulations. The mean scores for the former are 2.3 and 2.2, respectively, and the later are 1.1 and 1.2, respectively (P > 0.05).

However, the panel scores indicated a significant difference in color between the dark and white pieces at the 10 and 15% of buttermilk solids in their batter formulations (P < 0.05). The mean color scores at the 10% level are 1.7 and 1.9 for the dark and white pieces, respectively. While at the 15% level they gave mean color scores of 1.2 for the dark pieces and 1.5 for the white pieces. Both buttermilk solid levels showed that the dark meat were darker in color than the white meat. However, at the 20% level the final products appeared too dark in color shades and that the judges could not detect the difference in color, if any, between the two types of chicken meat. The mean color scores are presented in Table VII.

The judges mean color scores for the different levels of buttermilk solids content in the batter formulation ranged from 2.85 for the control to 1.15 for the 20% level.

The mean score for each judge in this category was calculated and presented in the increasing order from 1.7 to 2.5:

<table>
<thead>
<tr>
<th>Mean Score</th>
<th>1.7</th>
<th>1.8</th>
<th>1.9</th>
<th>2</th>
<th>2.1</th>
<th>2.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Judge number</td>
<td>3</td>
<td>5</td>
<td>6, 7, 9</td>
<td>4</td>
<td>1, 2</td>
<td>10, 8</td>
</tr>
</tbody>
</table>

Four judges had mean scores from 1.9 to 2.1. Five of the judges had scores below this range and one judge had a score above it.

The calculated F value for judges was 4 which was highly significant (P < 0.01). The critical values at d.f. 9/36 were $F_{0.05} = 2.15$. 
and $F_{.01} = 2.95$.

From the analysis of variance, Table IX, it was concluded that there was a highly significant difference in color of the breaded fried chicken between the levels of buttermilk solids content in their batter formulation ($P < 0.01$). The $F$ value was 34.96. The critical values at d.f. 4/36 were $F_{.05} = 2.63$ and $F_{.01} = 3.89$. This indicated that the breaded fried chicken containing 20% buttermilk solids in its batter formulation showed significantly darker color shade than the control which did not have the buttermilk treatment. By the same token, it is also concluded that the addition of buttermilk solids up to 20% in the batter mixture produced a significantly darker colored product than those without it.

Variable which could play some roles or influence the final color of the breaded fried chicken either directly or indirectly were studied. In order to concentrate the study to unknown variables (composition of chicken meat and skin-batter complex and the utilization of each component by the nonenzymatic browning reactions), most of the known variables were predetermined and controlled, namely; the processing procedure and conditions including the time and temperature for cooking and storing, batter ingredients, and solid to water ratio in the batter preparation.

The buttermilk powder composition (Table X) and wheat flour composition (Table XI) were reproduced from Webb (1965) and Hlynka (1964), respectively. Percent composition indicated that the buttermilk powder contained 40% lactose, the milk sugar. Therefore, in the formula E which contained 20% buttermilk solids, there was 8% lactose contributed by the buttermilk powder. The wheat flour contained 1.2% total sugars
Table X. Percent Composition of Buttermilk Powder

<table>
<thead>
<tr>
<th>Component</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>1.9</td>
</tr>
<tr>
<td>Protein</td>
<td>38.7</td>
</tr>
<tr>
<td>Fat</td>
<td>5.9</td>
</tr>
<tr>
<td>Lactose</td>
<td>40.0</td>
</tr>
<tr>
<td>Ash</td>
<td>7.7</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>5.9</td>
</tr>
</tbody>
</table>

Table XI. Percent Composition of Wheat Flour

<table>
<thead>
<tr>
<th>Component</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>12.0</td>
</tr>
<tr>
<td>Protein</td>
<td>19.5</td>
</tr>
<tr>
<td>Fat</td>
<td>1.0</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>76.1</td>
</tr>
<tr>
<td>Ash</td>
<td>0.43</td>
</tr>
</tbody>
</table>

Table XII. Percent Composition of Batter Formula

<table>
<thead>
<tr>
<th>Component</th>
<th>Formula A (Control)</th>
<th>Formula E (20% Buttermilk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>1.2</td>
<td>0.96</td>
</tr>
<tr>
<td>Lactose</td>
<td>0</td>
<td>8.0</td>
</tr>
<tr>
<td>Protein</td>
<td>10.5</td>
<td>16.14</td>
</tr>
<tr>
<td>Fat</td>
<td>1.0</td>
<td>1.98</td>
</tr>
</tbody>
</table>
Figure 6. Percent buttermilk solids vs initial sugar-protein ratio
Figure 7. Percent buttermilk solids vs mean color score
(measured as glucose) and the rest of the carbohydrate portion was starch, a polysaccharide. The flour contributed 0.96% sugar in the formula E. Thus the total amount of sugars in the 20% buttermilk batter formulation was calculated to be 8.96%, mostly lactose. Formula A (Control), therefore, contained 1.2% sugar in the form of glucose.

The buttermilk powder contained 38.7% protein and the wheat flour contained 10.5% protein. Therefore, formula E would contain a total of 16.15% protein and it was only 10.5% protein in the control formula. The weight ratio of sugar to protein would be

\[
\frac{1.2}{10.5} = 0.1143 \text{ for formula A, and } \frac{8.96}{16.14} = 0.5551 \text{ for formula E.}
\]

The control product gave a mean color score of 2.85 and the product of treatment E gave a mean color score of 1.15. The perfect golden brown color would give a mean score of 5. Graphic representation of the relationship between the levels of buttermilk solids in the batter formula and the initial sugar-protein ratio and the mean color scores, is shown in Figures 6 and 7, respectively. The calculated composition of the dry batter formula is presented in Table XII.

The poultry meat was analysed after precooking and after the final frying. The bones and the skin-batter portion were removed prior to sampling for analysis. The results are presented in Tables XIII and XIV. The dark meat (thigh) contained a higher percentage of fat, 7.31%, than the white meat (breast), 1.3%, in the precooked meat. After the final frying the percentages changed to 10.50 and 3.37%, respectively. The dark meat still maintained the higher percentage of fat than the white meat. The increase of fat content in the meat of the final product is attributed to two factors: 1) moisture loss, and 2) fat absorption during
Table XIII. Percent Composition of Cooked Chicken Meat (Without Bones and Skin-Batter Complex)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>White Meat (Breast)</th>
<th>Dark Meat (Thigh)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>68.15</td>
<td>67.04</td>
</tr>
<tr>
<td>Fat</td>
<td>1.30</td>
<td>7.31</td>
</tr>
<tr>
<td>Protein</td>
<td>30.42</td>
<td>25.41</td>
</tr>
</tbody>
</table>

Treatment E - After Final Frying

<table>
<thead>
<tr>
<th>Parameter</th>
<th>White Meat (Breast)</th>
<th>Dark Meat (Thigh)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>57.25</td>
<td>58.88</td>
</tr>
<tr>
<td>Fat</td>
<td>3.37</td>
<td>10.50</td>
</tr>
<tr>
<td>Protein</td>
<td>38.20</td>
<td>30.50</td>
</tr>
</tbody>
</table>

Table XIV. Loss and Gain of the Cooked Chicken Meat (Without Bones and Skin-Batter Complex)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>White Meat (Breast)</th>
<th>Dark Meat (Thigh)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture Loss</td>
<td>10.90</td>
<td>8.16</td>
</tr>
<tr>
<td>Fat Absorption</td>
<td>2.07</td>
<td>3.19</td>
</tr>
<tr>
<td>Protein Gain</td>
<td>7.78</td>
<td>5.09</td>
</tr>
</tbody>
</table>
the deep-fat frying process. The total fat absorption was 2.07% for the white meat and 3.19% for the dark meat, however, the total moisture loss was 10.90% for the white meat and 8.16% for the dark meat. The breast, evidently, lost more moisture than the thigh; although the breast contained slightly more percentage of moisture in the precooked meat. Such discrepancies can be explained by the nature of the chicken parts; The thigh meat was relatively smaller in size than the breast meat, and it was well covered by the batter and breading material that gave protection against moisture lost. The breast meat had more exposed meat area, particularly on its edge, apparently received less protection from the coating material. Therefore, the breast would be more liable to moisture loss or absorption.

In contradiction with the above argument, the thigh meat absorbed more fat (3.19%) than the breast meat (2.07%), a difference of 1.12%. This phenomenon could be attributed to the original fat content of the thigh meat (7.31%). The breast contained only 1.3% fat. Once absorbed, the fat was well contained and not easily lost.

The differences in protein content were due mostly to the moisture loss during frying. The final products were found to be dryer than those before frying. Some protein was bound to be lost due to denaturation and leaching during cooking and also due to the role of amino acids, particularly free amino acids, in the nonenzymatic browning reactions with sugars resulting in a darker colored product. Comparatively, however, the breast contained a higher percentage of protein (30.42%) than the thigh (25.41%), in the precooked meat.

The differences in the moisture, fat, and protein contents seemed to give no distinctive effect in the final color of the breaded fried
chicken. At the 20% buttermilk solids level, it was shown that there was no significant difference between the dark and white pieces \((P > 0.05)\). On the average, they both scored 1.15 on the hedonic color scale.

From the review of literature it was noted that poultry meat contained water, protein, fat, vitamins, minerals, and small amounts of carbohydrate. The intramuscular fat contained 17.6% linoleic and linolenic acid. The muscle contained thiamine, riboflavin, ascorbic acid, and a high proportion of nicotinic acid (Mountney, 1966).

According to Thomas et al., (1971), the poultry skin contained 20g of total lipid per 50g skin. The hexane extractable was 32% and total carbonyls amounted to 50 u mole/10g of the hexane extractables. These carbonyls could contribute to the amino-carbonyl reactions resulting in the formation of brown pigments in the breaded fried chicken. Although the major proportion of the carbonyls would be derived from the sugars present in the batter formulation. Formula E was calculated to have 8.0% lactose and 0.96% glucose. This treatment produced a darker colored breaded fried chicken than the control which contained only 1.2% glucose.

For the purpose of studying the changes that took place in the outer layer of the breaded fried chicken, compositional analysis was performed on the skin-batter complex. The thigh was chosen to be the representative part because it provided a good supply of the complex from around it. Furthermore, the thigh meat indicated a relative ratio of moisture, protein, and fat content \((67:25:7\), respectively, after precooking, and \(59:31:11\), respectively, after the final frying). In addition, it was found that the removal of the skin-batter complex was much easier from the thigh part than, for instance, from the wing part.
Analysis was carried out on the skin-batter complex after the blanching process and also after the final frying. The results are presented in Tables XV and XVI. The pH and the Total Plate Count of the complex were also determined after the 30-day period in the frozen storage, prior to the deep-fat frying operation. The results were presented in Table XVIII. The loss and gain of the complex were compared between the control and the 20% buttermilk solids treatment, and the result is presented in Table XVII.

The skin-batter complex was removed by cutting with a knife and tearing it apart from the meat by hand. Special care was observed to collect everything that was removed. Using a pair of scissors, the complex was sliced into small pieces. After the moisture determination, the dried sample was used for the fat and protein determination. After determining the ash content, the percentage of carbohydrate was calculated. Percentages on dry and wet basis were calculated and tabulated.

The final moisture content of the skin-batter complex was 23.63% for the treatment E, and 21.89% for the control. The 1.74% difference in favor of the treatment E was attributed to the characteristic water holding capacity of the milk protein (casein) and the milk sugar (lactose), present in the buttermilk component. The moisture loss during the frozen storage period and during the final deep-fat frying process was calculated to be 6.92% for the treatment E and 6.24% for the control. In this respect, treatment E indicated a greater moisture loss than the control, by 0.68%. Analysis of the meat portion indicated 8.16% moisture loss (see Table XIV). The total moisture loss was attributed to 1) the loss during frozen storage period, 2) moisture evaporation during high temperature frying, and 3) moisture being utilized by the
### Table XV. Percent Composition of the Skin-Batter Complex of the Breaded Fried Chicken

<table>
<thead>
<tr>
<th>Parameter</th>
<th>A Thigh Piece of Treatment A (Control)</th>
<th>After Blanching</th>
<th>After Final Frying</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Dry</td>
<td>Wet</td>
</tr>
<tr>
<td>Moisture</td>
<td></td>
<td>28.13</td>
<td>21.89</td>
</tr>
<tr>
<td>Fat</td>
<td>41.58</td>
<td>29.88</td>
<td>42.95</td>
</tr>
<tr>
<td>Protein</td>
<td>8.50</td>
<td>6.11</td>
<td>7.25</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>49.60</td>
<td>35.65</td>
<td>49.48</td>
</tr>
<tr>
<td>Ash</td>
<td>0.42</td>
<td>0.23</td>
<td>0.32</td>
</tr>
</tbody>
</table>

### Table XVI. Percent Composition of the Skin-Batter Complex of the Breaded Fried Chicken

<table>
<thead>
<tr>
<th>Parameter</th>
<th>A Thigh Piece of Treatment E (20% Buttermilk)</th>
<th>After Blanching</th>
<th>After Final Frying</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Dry</td>
<td>Wet</td>
</tr>
<tr>
<td>Moisture</td>
<td></td>
<td>30.55</td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td>40.13</td>
<td>27.87</td>
<td>42.77</td>
</tr>
<tr>
<td>Protein</td>
<td>9.77</td>
<td>6.79</td>
<td>8.15</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>48.20</td>
<td>33.47</td>
<td>47.18</td>
</tr>
<tr>
<td>Ash</td>
<td>1.90</td>
<td>1.32</td>
<td>1.90</td>
</tr>
</tbody>
</table>
### Table XVII. Loss and Gain of the Skin-Batter Complex of the Breaded Fried Chicken

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment A (Control)</th>
<th>Treatment E (20% Buttermilk)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dry</td>
<td>Wet</td>
</tr>
<tr>
<td>Moisture Loss</td>
<td>6.24</td>
<td></td>
</tr>
<tr>
<td>Fat Absorption</td>
<td>1.37</td>
<td>3.67</td>
</tr>
<tr>
<td>Protein Loss</td>
<td>1.25</td>
<td>0.45</td>
</tr>
<tr>
<td>Carbohydrate Loss</td>
<td>0.12</td>
<td>3.00(^a)</td>
</tr>
<tr>
<td>Total pH Drop</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td>pH Drop During Storage</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>pH Drop During Final Frying</td>
<td>0.45</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) – Carbohydrate Gain
nonenzymatic browning reactions. The moisture content of the breaded fried chicken were 59.5% for treatment E and 55.8% for the control. In essence, there was a greater loss of moisture in the skin-batter complex of the 20% buttermilk treatment than the control; but, the final moisture content of the complex and the whole breaded fried chicken was higher for the same treatment than the control. It was concluded that the addition of 20% buttermilk solids in the batter formula resulted in better retention of moisture in the final product, thus a more juicy breaded fried chicken than without it.

On a dry basis, the final composition of the skin-batter complex for treatment E was fat 42.77%, protein 8.15%, carbohydrate 47.18%, and ash 1.9%. The complex from the control piece gave fat 42.95%, protein 7.25%, carbohydrate 49.48%, and ash 0.32%. From Table XVII it is clear that the skin-batter complex of treatment E had greater fat absorption, greater protein loss, and greater carbohydrate loss than that of the control. Percentage-wise, 2.64, 1.63, and 2.20%, respectively, for treatment E, and 1.37, 1.25, and 0.12%, respectively, for the control. Such differences indicated greater activities of the nonenzymatic browning reactions by utilizing greater amount of substrates leading to the formation of greater amount of brown pigments in the buttermilk breaded fried chicken than the control. This is supported by the different mean color scores received by the treatment E (1.15) and by the control (2.85). The perfect golden brown product would receive a mean score of 3.0.

A greater proportion of the carbohydrate loss would be from lactose being utilized in the Maillard browning reactions with amino acids from the protein content of the protein content of the buttermilk formula.

On a wet basis, the carbohydrate content seemed to increase by 3%
in the skin-batter complex of the control, and 2.56% in the buttermilk treatment. The percentage increase was due to the significant reduction of the moisture content in the final product and the carbohydrate content, a major component, was greatly affected. Actually, some carbohydrate was lost as shown by the figures on a dry basis.

The pH of the skin-batter complex was also determined. The result indicated a drop of the pH value from 6.10 to 6.08 during the frozen storage period of the treatment E product, and the control seemed to maintain the same pH 6.15 throughout the 30-day frozen storage period (Table XVIII). The lower pH reading for the treatment E was attributed to the lactic acid content of the buttermilk component. The batter formula E was calculated to have 1.18% lactic acid. A noticeable drop of the pH reading was recorded for the control after the final deep-fat frying process. The final pH reading was 5.7, while the final pH of the buttermilk treated product was lowered to 5.69. Obviously, the deep-fat frying process resulted in the lowering of the pH reading by 0.45 for the control, and 0.39 for treatment E (Table XVII).

Table XVIII gave the Total Plate Count of the skin-batter complex after the frozen storage period and prior to the final frying operation. The buttermilk treatment showed 2800 TPC and the control showed 2500 TPC, indicating that the products were handled with relatively good care throughout all stages of the processing procedure.

Table IX indicated the yield of the breaded fried chicken. The yield was calculated from the average weight of the four parts, namely; breast, wing, thigh, and drumstick. The addition of 20% buttermilk solids in the batter formula resulted in a product with higher final yield, higher total moisture, and a higher percentage of crust than the control
### Table XVIII. The pH and Total Plate Count of the Skin-Batter Complex of the Breaded Fried Chicken

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment A (Control)</th>
<th>Treatment E (20% Buttermilk)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>After Blanching</td>
<td>Before Final Frying</td>
</tr>
<tr>
<td>pH</td>
<td>6.15</td>
<td>6.15</td>
</tr>
<tr>
<td>TPC</td>
<td>2500</td>
<td>2800</td>
</tr>
</tbody>
</table>
Table XIX. Percent Yield of the Breaded Fried Chicken

<table>
<thead>
<tr>
<th>Parameter</th>
<th>A (Control)</th>
<th>B (20% Buttermilk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breaded yield(^1)</td>
<td>110.4</td>
<td>114.2</td>
</tr>
<tr>
<td>Final yield(^1)</td>
<td>95.4</td>
<td>98.1</td>
</tr>
<tr>
<td>Total moisture</td>
<td>55.8</td>
<td>59.5</td>
</tr>
<tr>
<td>Percent crust(^2)</td>
<td>11.5</td>
<td>15.2</td>
</tr>
</tbody>
</table>

1 Yield is expressed as a percentage of the weight before precooking

2 Percent crust was determined by its removal after the final frying:
   A known quantity of breaded fried chicken was washed under a tap water with gentle scraping by hand for 15 min and then the parts were blotted dry and reweighed. Percent weight loss was considered as the percent crust.

Table XX. Free Fatty Acids and Color of the Cooking Oil (Soybean Oil)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before Frying</th>
<th>After Frying at 360°F for 6 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Free Fatty Acids (as oleic)</td>
<td>0.035%</td>
<td>0.072%</td>
</tr>
<tr>
<td>2. Light Absorbance at 460 mu</td>
<td>0.08%</td>
<td>0.95%</td>
</tr>
</tbody>
</table>
The breaded chicken yield was also greater for the buttermilk treated product. However, the greater mass of the batter and breader around the 20% buttermilk treated sample allowed more proportion of cooking loss which is the difference between the breaded yield and the final yield. The cooking loss for the control was 15%, and the treatment E indicated 16.1% of cooking loss which is 1.1% higher than the control. Nevertheless, treatment E still had 3.7% more percentage of crust, than the control.

The 20% buttermilk solids batter formula contained 8.96% reducing sugar (lactose being 8.0% and 0.96% glucose), 16.14% protein from wheat flour and casein, and 1.98% fat. The control batter formula contained 1.2% glucose, 10.5% protein from the flour, and 1.0% fat. The ratio of sugar to protein was 1:8.75 for the control and 1:1.8 for the 20% buttermilk treatment. Obviously, the later formula had a relatively good supply of the primary reactants for the Maillard reaction, and they were about the same concentration. While the control formula contained too low concentration of reducing sugar (1.2%) and also it was devoid of casein, the activity of nonenzymatic browning is thus limited to some extent. The greater browning activities in the buttermilk batter were substantiated by the dark colored breaded fried chicken.

Chemical changes in the cooking oil. The soybean oil which was used in the frying operation was sampled before and after the process. Chemical test on its acid value was determined. The acid value was converted to free fatty acids content expressed as oleic acid. Light absorbance was also determined. The result is presented in the Table XX.

Chemical changes during heating depends on at least four factors:
1) the length of time a specific triglyceride is exposed to heat, 2) the temperature, 3) the presence of prooxidants, 4) the mixed fatty acid composition and the position of the fatty acids in the triglyceride. In terms of commercial operations, the length of time a specific triglyceride molecule is exposed to heat is dependent on the amount of fat absorbed on the cooked food item. This turnover rate is minimum for foods which contain substantial amounts of fat such as steak and chicken and maximum for foods such as potato chips which absorb 40% of the frying fat or oil (Kummerow, 1962).

However, it was found that the frying process resulted in the increase of the free fatty acids (as oleic) by 0.037% and the light absorbancy by 0.87%. The increase of free fatty acids was also reflected by the lowered pH of the breaded fried chicken. The amount of the free fatty acids also reflected the toxicity of the used oil. The value in excess of 2% would declare the frying oil as unwholesome. Filtering the sediment, and adding fresh oil will remedy the situation.

**Inhibition of NEB Reactions by Sulfite**

For the purpose of this study, three sulfite levels were arbitrarily chosen and included in the formula that contained 20% buttermilk solids. From the previous result it was certain that the addition of buttermilk solids up to 20% in the batter formula produced darker colored products. The thigh piece was chosen as the test piece for this experiment due to the relative proportion of moisture, fat, and protein content in the thigh meat.

Sodium metabisulfite was weighed to the nearest milligram to provide the three levels of sulfite (SO₂) initially in the batter formula.
Table XXI. Color Scores of Breaded Fried Chicken Treated with Sulfite

<table>
<thead>
<tr>
<th>Batter Formula</th>
<th>Judge Number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>E (Control)</td>
<td>1</td>
</tr>
<tr>
<td>F (1000 ppm)</td>
<td>2</td>
</tr>
<tr>
<td>G (2000 ppm)</td>
<td>2</td>
</tr>
<tr>
<td>H (2500 ppm)</td>
<td>3</td>
</tr>
</tbody>
</table>

Table XXII. Mean Color Scores of Breaded Fried Chicken Treated with Sulfite

<table>
<thead>
<tr>
<th>Batter Formula</th>
<th>Mean Color Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>E (Control)</td>
<td>1.10</td>
</tr>
<tr>
<td>F (1000 ppm)</td>
<td>1.70</td>
</tr>
<tr>
<td>G (2000 ppm)</td>
<td>2.40</td>
</tr>
<tr>
<td>H (2500 ppm)</td>
<td>2.80</td>
</tr>
</tbody>
</table>

Color Scores:
1 - Too dark
2 - Slightly dark
3 - Golden brown (Just right)
4 - Slightly light
5 - Too light
Table XXIII. Analysis of Variance of the Mean Color Scores

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>S.S.</th>
<th>M.S.</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfite level</td>
<td>3</td>
<td>17</td>
<td>5.67</td>
<td>37.8**</td>
</tr>
<tr>
<td>Replication (Judges)</td>
<td>9</td>
<td>3</td>
<td>0.33</td>
<td>2.25*</td>
</tr>
<tr>
<td>Error</td>
<td>27</td>
<td>4</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>39</td>
<td>24</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** Highly significant at 1% probability level
* Significant at 5% probability level

\[ F_{0.01} \text{ with } 3 \text{ & } 27 \text{ d.f. } = 4.6 \text{ (Sulfite level)} \]
\[ F_{0.05} \text{ with } 9 \text{ & } 27 \text{ d.f. } = 2.25 \text{ (Replication)} \]
\[ F_{0.01} \text{ with } 9 \text{ & } 27 \text{ d.f. } = 3.15 \text{ (Replication)} \]
Figure 8. Initial sulfite (ppm) vs mean color score
The levels of sulfite were 1000, 2000, and 2500 ppm. The sulfite was mixed with the dry batter mixture then the same amount of water was added to each formula, F, G, and H. All other stages of handling and processing were the same as previously explained.

The final product was subjected to color scoring by ten color panelists as previously explained. The result was duplicated in Table XXI. The mean color score was calculated and presented in Table XXII. The mean color scores ranged from 1.1 for the control which did not contain sulfite, to 2.8 for the formula H at the sulfite level of 2500 ppm. The relationship of sulfite treatment with the mean color score is presented in Figure 8.

It was concluded from an analysis of variance (Table XXIII) that there was highly significant difference between the color of the breaded fried chicken of the different levels of sulfite treatment \((P < 0.01)\). The \(F\) value was 37.8. The critical values at d.f. 3/27 were \(F_{0.05} = 2.96\) and \(F_{0.01} = 4.6\). This indicated that the initial 2500 ppm sulfite treatment resulted in a highly significant lighter colored product than those without the sulfite treatment. The mean color score for the 2500 ppm sulfite treatment was 2.8, and the mean color score for the product without the buttermilk and sulfite treatments was 2.85. The perfect golden brown product would receive a mean score of 3.0.

It was concluded, however, that the addition of 2500 ppm of sulfite initially in the 20% buttermilk solids batter formula was sufficient to produce a good golden brown breaded fried chicken which will be more acceptable to the consumers than those without the sulfite treatment.

The mean score for each judge in this category was calculated and presented in the increasing order from 1.5 to 2.25:
Two judges had mean scores of 1.5, four judges had mean scores of 2, and another four judges had mean scores of 2.25.

The calculated F value for judges was 2.25 which was significant (P = 0.05). The critical values at d.f. 9/27 were \( F_{0.05} = 2.25 \) and \( F_{0.01} = 3.15 \).

**The Mechanism of Sulfite Inhibition**

Sodium metabisulfite will form two molecules of sodium bisulfite in the presence of water. The sulfite and/or bisulfite ion reacted and combined with simple sugar molecules making them unavailable for the nonenzymatic sugar-amino browning reactions. The sulfite reactions can be shown as in the following:

\[
\text{Na}_2\text{S}_2\text{O}_5 + \text{H}_2\text{O} \rightleftharpoons 2 \text{NaHSO}_3
\]

\[
\text{R - CHO} + \text{NaHSO}_3 \rightleftharpoons \text{R - C - OH} \quad \text{sodium bisulfite} \quad \text{\( \alpha \)-hydroxy sulfonate}
\]

According to McWeeny et al., (1974), the sulfite can participate with carbonylic intermediates in a number of quite distinct chemical reaction and several of these are equilibrium reactions:

- Reducing sugar \( \rightleftharpoons \) Sugar hydroxysulfonate
- Simple carbonyl \( \rightleftharpoons \) Carbonyl hydroxysulfonate
Di-carbonyl $\xrightarrow{\text{Sulfonylated carbonyl}}$ Dicarbonyl di-hydroxysulfonate

$\alpha\beta$-Unsaturated carbonyl $\xrightarrow{\text{Sulfonated carbonyl}}$

Pigment ("Melanoidins") $\xrightarrow{\text{?}}$

It is also noted that once the pigment has been formed it can be at least partially bleached by the addition of sulfite, thereby, effecting a reduction in color intensity.

Some of the sulfite was undoubtedly lost during blanching at 395°F for 20 sec and during deep-fat frying at 360°F for 6 min. Therefore, the actual effective amount of sulfite in inhibiting nonenzymatic browning reactions in the breaded fried chicken was definitely lower than the 2500 ppm. Due to the nature of the processing procedure, it was necessary to include a relatively high level of sulfite initially to effectively inhibit the browning reactions.

In beef, and also poultry meat, the acidity of the meat could be lowered to pH 5.3 without the taste being objectionable to most person; while rendering a good color of the product. The 2500 ppm is a tolerable level of $\text{SO}_2$ in a cooked beef (Borgstrom, 1968).

The sulfite level was reported to be up to 3000 ppm in dried fruits, but less in dehydrated vegetables which would loose 90% of the sulfite during cooking.

Sulfite above 0.01M was reported to be organoleptically detected in cooked potato. At 1 ppm the sulfite would depress phenolase activity, and at 10 ppm, the phenolase was inactivated (Hurst, 1972 and Borgstrom, 1968).
Rate of Nonenzymatic Browning Reactions in Aqueous Solutions

The development of brown pigments in the aqueous solutions glucose-lysine (5%), galactose-lysine (5%), lactose-lysine (5%), lactose-lysine (1%), lactose-glutamic acid (1%), glucose-lysine (1%), and galactose-lysine (1%) were studied by measuring the intensity of the colored pigments being formed at different pH, different temperatures, and at certain intervals.

The greater the intensity of the brown pigments, the greater is the light absorbance and consequently the lower is the light transmittance. The light transmittance was measured at 420μm using a spectrophotometer, and a water blank at 100% reading.

The rate of brown pigments produced was represented by the graph of the percent loss of light transmittance versus time. Figure 9 represented the rate of nonenzymatic browning reactions of glucose, galactose, and lactose with lysine at 5% concentration, pH 6.0, and at 27°C. Obviously, from the graphs, the relative rates of nonenzymatic browning reactions between the sugars and lysine were galactose > glucose > lactose. The percent light transmittance of the galactose system was reduced to 51% in 30 days, 59.5% for the glucose, and 90% for the lactose solutions during the same period. If the rates at the straight part of the curves were considered by calculating the slope of each line, the relative rates were 1.8, 1.5, and 0.36 for galactose, glucose, and lactose, respectively. It explained that the percent loss of light transmittance from the galactose-lysine solution per day was 1.8, and so forth.

The general tendency of the curves to flatten after the 20-day period was quite obvious. It indicated that the rate of the nonenzymatic reactions began to slow down after the 20-day period at 27°C due to the
Figure 9. Percent light transmittance vs time (days) for 5% aqueous solutions at 27°C and pH 6.0.
Figure 10. Percent light transmittance vs time (weeks) as a function of temperature in 1% aqueous solutions at pH 3.5 and 5.5.
decreasing amounts of reactants, namely; the amino and carbonyl groups, and also the increased amount of pigments being formed.

Figure 10 represented the rates of nonenzymatic reactions for the lactose-lysine solution (1% concentration and pH 5.5) and the lactose-glumatic acid solution (1% concentration and pH 3.5) at 4.4 and 27°C. The relative rates of browning were faster at 27°C than at 4.4°C. The slopes for the straight part of the lines were 0.014 and 0.214 for the lactose-lysine at 4.4 and 27°C, respectively. The lactose-glutamic acid slopes were 0.021 at 4.4°C and 0.257 at 27°C. The rates were calculated from the percent loss of light transmittance per day.

The tendency of the curves to flatten after the 20-day period was noted, particularly at the 27°C storage.

The lactose-glutamic acid solution showed a greater rate of browning than the lactose-lysine solution, and of course at two different pH's (comparative slopes were 0.021/0.014 and 0.257/0.214 at 4.4 and 27°C, respectively). Generally, however, the lower the pH the faster is the browning rate between the amino and carbonyl groups, catalysed by the acid. The aminocarbonyl reaction is much more favorable in the alkaline conditions accompanied by strong-base catalysis.

Free glutamic acid is present in a greater percentage than the other amino acids in the amino acid content of the fresh chicken meat (Mountney, 1966), 3.76 u mole/g in the thigh and 1.44 u mole/g in the breast. Free lysine content is 1.41 and 0.78 u mole/g, respectively. According to Hlynka (1964), the wheat flour protein contained 20.53% glutamic acid and 2.49% lysine. Webb (1965) reported that casein contained 22.4% glutamic acid and 8.2% lysine. Quantitatively, the lactose-glutamic acid represented a major reaction in the buttermilk battered
and breaded fried chicken.

Relative percentage of some amino acids in casein were glutamic acid (22.4%), proline (10.6%), leucine (9.2%), lysine (8.2%), valine (7.2%), aspartic acid (7.1%), serine and tyrosine (6.3%) each, isoleucine (6.1%), phenylalanine (5.0%), and the rest were below 5%. In wheat flour protein the relative percentages were glutamic acid (20.5%), proline (8.9%), arginine (7.64%), leucine (4.66%), serine (4.53%), glycine (3.76%), valine (3.39%), histidine (3.18%), and the rest were below 3% (lysine 2.49%).

Figure 11 represented the browning rate of galactose-lysine and glucose-lysine solutions, at 1% concentration, pH 4.0, and at 4.4 and 27°C. The browning rates seemed to be much faster in the first week for both solutions at 27°C, then the rates slowed down noticeably. The slopes for the first week were 2.571 and 2.643 for galactose and glucose, respectively. They were quite close to one another. Thereafter, the relative slopes were 0.429 and 0.371, respectively. At 4.4°C the relative slopes were 0.143 and 0.114 for galactose and glucose, respectively.

At 5% concentration, pH 6.0, and 27°C the galactose-lysine solution reduced the light transmittance to 51% in 30 days; at 1% concentration, pH 4.0, the light transmittance was reduced to 70% after the same period. The glucose-lysine solution reduced the reading to 60% and 68% in both instances, respectively. Lactose-lysine solution reduced the percent light transmittance to 90% at the 5% concentration, pH 6.0, and 27°C after the 30-day period, and only to 97.5% reading for the 1% concentration solution at pH 5.5.
Figure 11. Percent light transmittance vs time (weeks) as a function of temperature in 1% aqueous solutions at pH 4.0
Rate of Nonenzymatic Browning Reactions in Model Food Systems

In order to investigate further the rates of nonenzymatic browning reactions involving carbonyl and amino groups, in a food, six formulations of intermediate moisture food systems were prepared. Each formula contained one type of sugar, either glucose, galactose, or lactose. The sugar was the source of carbonyl groups were provided by the amino acids from casein. Other ingredients included potassium sorbate, glycerol, microcrystalline cellulose, and water. The function of each ingredient in the model food system was explained previously. Tables II and III showed the amount of each ingredient in the model systems. The two levels of initial moisture content were 46.2 and 40%. The initial ratios of sugar to protein by weight were 1:3 and 1:2. The former ratio was associated with the 46.2% moisture content, and the later with the 40% level.

Samples were taken at regular intervals, and the brown pigments were extracted and measured for their light absorbance (or transmittance) at 42mu. The results were presented in Figure 12. The rate of brown pigments being formed was reflected by the decreasing amount of light transmittance (or increasing light absorption) by the extracts. From the graphs, it was noted that the relative rates of nonenzymatic browning reactions in the model food systems were in the order of galactose > glucose > lactose. The rates at 36°C were noticeably faster than those at 27°C. However, the same order of reactivity was replicated by the two temperatures.

The relative rates of browning in the intermediate moisture model food systems were the same as in the aqueous solutions as previously discussed. It was indicated by the graphs that there was a period of
Initial sugar:casein (1:3)
Initial moisture content 46.2%

Figure 12. Brown pigment production as a function of time (weeks) in model food systems as a function of temperature
induction for the browning reactions during the first week at 27°C as well as at 36°C storage. This phenomenon was in agreement with the results reported by Warmbier et al., (1976).

The slope of each graph at the straight portion of the lines was calculated, and they were 0.915, 0.938, and 0.960 for the lactose, glucose, and galactose systems at 27°C. At 36°C, the slopes were 0.982 (lactose), 1.161 (glucose), and 1.339 (galactose). This explained that the rate of browning reactions as reflected by the percent loss of light transmittance per day was 1.339 for the galactose system at 36°C, and 0.960 at 27°C. These rates were held true at least during the first five to six weeks of the storage period.

Visually, during the fourth week color changes were noticeable in the systems containing galactose and glucose. Brown pigment accumulation was very distinct in the galactose system. This was observed at both levels of storage temperatures. Qualitative tests for carbonyls, furfurals, and Amadori rearrangement products gave positive results.

By the second month at 36°C, the glucose system showed dark color formation. The galactose system was already too dark in color, an indicative of faster browning activities than the glucose system. This was proved by the light transmittance test of their extracts (see Figure 11).

In the fifth week, samples which were stored at 27°C showed little color differentiation by the naked eyes. The lactose system had only changed to yellowish cream in color. However, the lactose system at 36°C showed a darker color than the one at 27°C.

For the different water activity treatment, at 27°C, the relative order of browning was observed to be galactose > glucose > lactose
in each level of the water activity storage.

Analysis of Sugar Content by Gas Chromatography

Standard peaks for glucose, galactose, and lactose. For the purpose of this investigation standard peaks were first established for each sugar. The retention time and the integrated value for each peak were obtained by derivatizing 10 mg of known pure sugar samples in 1 ml of the TRI-SIL-Z reagent. Galactose and glucose were dissolved in five min, but lactose powder took fifteen min to dissolve completely and thus derivatized by the silylation process. After dissolving, 0.5 ul of the mixture was injected directly into the gas chromatograph. The results were duplicated in Figures 13, 14, and 15.

The relative retention time for the sugars was found to be 5 min for α-glucose, 10 min for β-glucose, 9 min for galactose, and 38.5 min for lactose. It was observed that it was necessary to double the amount of lactose mixture to 1 ul for the injection into the gas chromatograph in order to get a good representation of its peak on the chart paper. However, the integrated value for the peak, which was registered by the automatic integrator, also changed with the same proportion.

It was also observed that galactose and glucose were detected when the column temperature was well within the range of temperature programming (170 - 240°C). However, due to the length of the retention time, lactose was detected when the column temperature was at 240°C, just after the temperature programming ended. It was also learned that the carrier gas flow meter had to be adjusted as the column temperature reached over 200°C during the lactose determination, in order to keep a constant carrier gas flow throughout the analysis. At higher temperatures the
Figure 13. Standard peak for galactose
Figure 14. Standard peak for glucose
Figure 15. Standard peak for lactose

(Retention time = 38.5 min)
Figure 16. Calibration curves for reference sugars.
gas flow seemed to be reduced.

Sample preparation for sugar determination. Samples from the model food systems at different temperature storage, different moisture content, and different water activity treatment were taken at regular intervals and analysed for their sugar content.

It was found to be necessary to dry the samples first before the derivatization process so that a good and reproducible peak can be obtained on the chart paper. Therefore, each sample was dried in a vacuum oven at 60°C (25mm Hg), for 16 hr. This condition was kept constant for all samples in order to minimize variation in sample weights. Such variation was minimized and/or eliminated by weighing exactly the same amount (0.1000g) of dried sample, each time. Each sample was injected at least three times in order to get the average integrated value for its peak and hence the amount of sugar present in the sample.

The difference in sugar recovery from the model food samples was also eliminated by taking the sample at zero time as the reference for the integrated value of the peak. The registered integrated value for each peak at the zero time would represent a 10g sugar content. Thereafter, the integrated values were compared with the reference value, using the slope factor from Figure 16.

The slope factor was calculated as the g of sugar per each unit of the integrated value for each sugar. The slope factors were 9999.8^{-1} (galactose), 9997.8^{-1} (glucose), 4973^{-1} (lactose). The amount of sugar present in each sample was obtained by multiplying the average integrated value for the peak with the appropriate slope factor.

Figure 17 represented the rate of sugar (galactose, glucose, and lactose) lost from the model food systems which were stored at 27 and
Figure 17. Reducing sugars loss rates as a function of temperature
$36^\circ C$, with the initial moisture content of $46.2\%$ and the initial equilibrated water activity of $0.52$. The initial weight ratio of sugar to protein was $1:3$. It was noted that the graphs seemed to flatten after the first month scale, particularly so for the $36^\circ C$ storage temperature. The decrease in the rate of sugar loss reflected the decrease in the rate of nonenzymatic browning reactions after the 30-day period. The reactants available for the Maillard reactions were also decreased in their concentrations, resulting in reduced activities. The relative rates of sugar loss were in the order of galactose $> \text{glucose} > \text{lactose}$. The loss rate at $36^\circ C$ was faster than that at $27^\circ C$. This observation proved the results which were obtained by measuring the pigments being formed in the model food systems as well as in the aqueous solutions.

The slope for each graph was calculated as the g of sugar loss per day, during the first 30 days for galactose and glucose. The period for lactose was extended to 60 days for the $36^\circ C$ storage and 90 days for the $27^\circ C$ storage. The relative slope values at $27^\circ C$ were $0.154$ (galactose), $0.137$ (glucose), $0.056$ (lactose), and the values at $36^\circ C$ were $0.241$ (galactose), $0.235$ (glucose), and $0.088$ (lactose).

The results indicated that the rate of nonenzymatic browning reactions were increased by an average factor of $1.62$ for $9^\circ C$ increase of storage temperature. It was also noted that the concentration of primary reactants, namely the reducing sugar and free amino groups, influenced the rate of nonenzymatic browning reactions. The rate was decreased as the concentration of the primary reactants reduced to less than one-third of the original amount. According to Warmbier et al., (1976) the rate of nonenzymatic browning reactions in intermediate moisture food was 33 times faster at $45^\circ C$ than at $25^\circ C$. The system contained 60 - 80%
Figure 18. Reducing sugars loss rates as a function of moisture content

- **Lactose**
- **Glucose**
- **Galactose**

Legend:

- (a) 40% Initial moisture content (Initial sugar:casein (1:2))
- (b) 46.2% Initial moisture content (Initial sugar:casein (1:3))

Storage: 27°C
moisture.

Figure 18 represented the rate of nonenzymatic browning reactions at two levels of moisture content, namely 40 and 46.2%, at 27°C and water activity \( a_w \) 0.52. The graphs indicated that galactose had faster rate of browning than the other two sugars at both moisture levels. However, the rate of galactose loss at 40% moisture seemed to be a little higher than that at 46.2% moisture, with the respective slope value of 0.159 and 0.154, a difference of 0.05. Theoretically, the rate should be higher at the higher moisture content. Following the Brunauer, Emmett, and Teller (B.E.T.) theory of monomolecular layer of adsorbed water, an increased in moisture content would increase the rate of chemical degradation and thus the increase in browning reactions rate. In food systems even the moisture content is decreased, the browning rate could still be increased due to the lipid oxidation.

The slopes for glucose and lactose indicated an increased nonenzymatic browning reaction at the 46.2% moisture than at 40% moisture content. The calculated slope values were 0.116 and 0.053 for glucose and lactose, respectively, at 40% moisture content. The value increased to 0.137 and 0.056, respectively, at 46.2% moisture content. The discrepancy in galactose rate was probably due to individual error during sampling. It should be noted also that the initial weight ratio of sugar to protein was 1:2 for the 40% moisture content, and 1:3 for the 46.2% moisture content.

It is also interesting to note that the glycerol being a liquid it has water-like properties and thus it increases reactant mobility and/or solubility. However, at a higher water activity, the glycerol was reported to decrease the rate of nonenzymatic browning reaction in
the model food system (Warmbier et al., 1976).

At high moisture content and thus high water activity, the rate of nonenzymatic browning reaction could also decrease due to dilution of the reacting substances. The dilution effect could have been partially encountered in the case of galactose systems as described earlier. Considering the B.E.T. monomolecular layer, the dilution effect, and the reactant mobility, it should also be noted that the diffusion of solutes can only take place at the water content above the monomolecular layer value on the moisture sorption isotherm which is a plot of moisture content versus relative humidity. The B.E.T. monolayer value for precooked and dehydrated chicken meat was reported to be 5.48 (Koch, 1962). However, browning can develop even below this moisture level to some extent.

During the process of browning up to 3.5 mole of water is being formed per mole of sugar. The water causes a slowing of the condensation stages in the browning reactions, due to the law of mass action (Eichner and Karel, 1972).

**Effect of water activity on the nonenzymatic browning reactions:**

The effect of water activity on the rate of browning in the intermediate moisture model food system at 27°C was studied. The water activity levels being used were 0.52, 0.62, 0.75 and 0.84. The results are presented in Figures 19, 20, 21, and 22, respectively. The initial moisture content was 40% and the initial weight ratio of sugar to protein was 1:2.

The relative rate of nonenzymatic browning reactions of the three sugars with casein was in the order of galactose > glucose > lactose. The same order was observed in all levels of water activity.
Initial sugar:casein (1:2)
27°C Storage

Figure 19. Reducing sugars loss rates at $a_w$ 0.52
Initial sugar:casein (1:2)
27°C Storage

Figure 20. Reducing sugars loss rates at $a_w$ 0.62
Initial sugar:casein (1:2)
27°C Storage

Figure 21. Reducing sugars loss rates at $a_w$ 0.75
Figure 22. Reducing sugars loss rates at $a_w$ 0.84

Initial sugar:casein (1:2)
27°C Storage

Lactose
Glucose
Galactose
treatment. The graphs for galactose and glucose showed the general tendency to flatten after the six weeks scale, meaning that the rate of browning began to decrease due to the decreasing concentration of the primary reactants namely reducing sugar and amino groups.

The slopes of the graphs were calculated as the g of sugar lost per day during the first 30 days for galactose and glucose. The period was extended to 90 days for lactose. At the water activity of 0.52, the slopes were 0.159 (galactose), 0.116 (glucose), and 0.053 (lactose). At the water activity of 6.2, the slopes were 0.165 (galactose), 0.131 (glucose), 0.061 (lactose). At the water activity of 0.75, they were 0.157 (galactose), 0.119 (glucose), and 0.054 (lactose). Finally, at the water activity of 0.84, the slopes were 0.175 (galactose), 0.136 (glucose), and 0.25 (lactose).

The galactose loss rate was the fastest at the water activity of 0.84 (0.175) followed by treatments 0.62 (0.165), 0.52 (0.159), and 0.75 (0.157).

The glucose loss rate was the fastest at the water activity of 0.84 (0.136), followed by treatments 0.62 (0.131), 0.75 (0.119), and 0.52 (0.116).

The lactose loss rate was the fastest at the water activity of 0.62 (0.061), followed by treatments 0.75 (0.054), 0.52 (0.053), and 0.84 (0.025).

The average loss rate of galactose and glucose combined gave the following values in decreasing rate order: 0.84 (0.156), 0.62 (0.148), and 0.75 with 0.52 (0.138). The difference in slope value between treatment 0.84 and 0.62 was less than 5.5%.

Generally, the rate of nonenzymatic reactions increased with the
Figure 23. Reducing sugars remaining at four $a_w$ levels after 3-month storage at 27°C
increase value of water activity. The rate was at maximum at the water activity of 0.84 for galactose and glucose; but, for lactose the maximum rate of browning was at the water activity of 0.62. Warmbier et al. (1976) reported a maximum rate of nonenzymatic browning reactions within the water activity range of 0.6 to 0.8, and the rate variation was less than 10% within the water activity range of 0.52 to 0.60.

Figure 23 represented the amount of each sugar left in the model food systems after three months storage at 27°C as affected by the different levels of water activity treatment. Generally, about 88% to 90% of the galactose and glucose had been used up in the nonenzymatic browning reactions. The utilization of lactose, at the most, was about 55% at the water activity of 0.62.

Based on the gas chromatographic analysis and the sensitivity of the recorder being used, it was observed that there has been no detectable degradation of lactose to glucose and galactose per se in the intermediate moisture model food system, at least up to 90 days at 36°C. There was only one peak being recorded on the chart paper for every lactose-based model food formula. However, the lactose degradation might have had taken place to some degree, and the more reactive products had already participated in the nonenzymatic browning reactions resulting in the formation of brown pigments, the melanoidins.

The possibility of some lactose degradation in the breaded fried chicken should not be totally ignored. The 20% buttermilk solids batter formula contained 8% lactose, and the final products received a mean color score of 1.15 whereby a color score of 1 would indicate a color of the products which was too dark. According to Aurand and Woods (1973), \( \alpha \)-lactose caramelizes at 160-180°C and it melts with molecular
decomposition at 202°C. During processing, the buttermilk-battered and breaded chicken parts were blanched in deep-fat at 201.6°C (395°F) for 20 sec. Taking into consideration that the temperature variation of the cooking oil was ± 1°C, then the actual blanching temperature had reached 202°C. Therefore, the lactose in the coating material was subjected to molecular fragmentation and possibly the actual degradation of the lactose molecules, particularly the alpha form, to galactose and glucose and eventually to some other reactive components such as furfural alcohol, furfural, hydroxymethyl furfural, and aldehyde derived from the glucose portion (Jenness and Patton, 1959, and Adachi, 1959).

The breaded chicken parts, however, did not show dark color formation after the blanching process as well as after the frozen storage period. It was believed that up to this processing stage the production and accumulation of browning intermediates, particularly in the skin-batter complex, had taken place to some extent. Brown pigment was not yet produced in quantitative amount. During the final deep-fat frying process at 182.2°C (360°F) for 6 min, the formation of dark colored pigments became most evident.

The results suggested that the temperature of frying activated the four types of nonenzymatic browning reactions, particularly the caramelization which became more prevalent during the frying process. The lactic acid presence in the batter formula promoted the caramelization process by reducing its activation energy requirement. The sugar acid reaction and oxidation reaction (of lipids and phospholipids) activities were evidenced by the lower pH value of the skin-batter complex after the final frying process. It was believed that the caramelization process accentuated the effects of the Maillard reaction, sugar acid
reaction, and lipid and phospholipids oxidation in contributing to the formation of brown pigments in the buttermilk-battered and breaded fried chicken.

However, the formation of dark colored pigments in the finish products through nonenzymatic browning reactions was successfully inhibited by the use of sulfite up to 2500 ppm in the batter mixture.
SUMMARY AND CONCLUSIONS

Breaded fried chicken was prepared according to specific commercial procedures to study the effect of variable amounts of buttermilk solids in the batter formula on the color of the final products. The procedure includes cutting the chicken into four parts, namely; breast, thigh, drumstick, and wing. These parts were precooked by boiling in water for 15 min. After draining and cooling to 40°F (4.4°C), the chicken parts were battered with the appropriate batter mixture containing 5, 10, 15, and 20% buttermilk powder. The control batter contained only flour. The ratio of solid to water was 1:1.5. After breading with cracker crumbs, the breaded parts were blanched in deep-fat (soybean oil) at 395°F (201.6°C) for 20 sec to stabilize the coating material for easy handling. The products were then sealed in polyethylene bags and frozen stored at -4°F (-20°C) for 30 days. Following the storage period the products were deep-fat fried in soybean oil at 360°F (182°C) for 6 min.

The results lead to the following conclusions:

1. According to sensory evaluation of color it was concluded that there was no significant difference ($p>0.05$) in color between the dark (thigh) and white (breast) pieces within the same treatment at 0, 5, and 20% buttermilk levels. But, at 10 and 15% levels the two types of meats differ in color significantly ($p<0.05$), the dark meat being darker in color than the white meat.

2. The addition of buttermilk powder up to 20% in the batter mixture resulted in a dark colored breaded fried chicken which was highly significant ($p<0.01$) than the control. However, the 20% buttermilk treatment gave higher final yield, higher moisture content, and a higher percentage of crust than the control. In addition, it was noted that the buttermilk
solids imparted a characteristic 'buttery' flavor which is a desirable organoleptic quality for the breaded fried chicken.

3. The formation of dark colored products in the 20% buttermilk treatment, through nonenzymatic browning reactions, was successfully inhibited by the use of sulfite in the form of sodium metabisulfite up to 2500 ppm initially in the batter mixture.

4. Considering the physical and chemical changes in the skin-batter complex, the concentrations of primary reactants, and the processing parameters, it was concluded that the Maillard reaction, caramelization, sugar acid reaction, and reactions involving lipids and phospholipids had participated in that order to some extent leading to the formation of browning intermediates (furfurals and other reactive compounds) in the skin-batter complex until the time for final frying. During the deep-fat frying the effect of high temperature on the primary reactants and browning intermediates, became most important. It was concluded that caramelization became most prevalent during the final frying process; and it accentuated the effects of the other three types of nonenzymatic browning reactions, particularly the Maillard reaction, leading to the formation of dark colored breaded fried chicken.

5. In the intermediate moisture model food systems the rate of reducing sugar reacting with casein through the Maillard reaction was galactose > glucose > lactose. The rate of reducing sugar loss was faster with galactose, followed closely by glucose, and much slower by lactose. The same order was repeated by the temperature, moisture content, and water activity treatments. Generally, the browning rate of each reducing sugar with casein was increased by the increase in temperature, moisture content, and water activity. However, galactose and glucose indicated faster rates
between \( a_w \) 0.62 and 0.84; but lactose showed faster browning rates between \( a_w \) 0.62 and 0.75. The relative rate of reducing sugars reacting with lysine in aqueous solutions was in the order of galactose \( \rightarrow \) glucose \( \rightarrow \) lactose. Lactose seemed to react with glutamic acid as well as with lysine in acidic solutions.
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Title of Thesis: Nonenzymatic Browning Reactions in Breaded Fried Chicken and in Model Food Systems

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