FLAVOR CHARACTERISTICS OF SALTINE CRACKERS

by

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INTRODUCTION

Commercial saltine cracker production by the sponge and dough process requires approximately twenty-four hours of fermentation to produce a tender, crispy and flavorful cracker. It has long been desired to eliminate or substantially reduce this lengthly fermentation while maintaining the same or better cracker with good physical properties as well as acceptable flavor. It was based on this idea and many years of research that Johnson developed Flavol (1), a bread flavor enhancer which is a mixture of gluten amino acids and organic acids compensating for normal fermentation. With adjustment of the amino and organic acids, a Flavol for cracker production was developed (2). By use of this Flavol, crackers of acceptable flavor were produced with a minimum of fermentation.

It is well known that the flavor of baked products results mainly from the Maillard non-enzymatic browning reaction during oven baking. Among the numerous products, carbonyl compounds are major contributors to flavor of baked products.

The purpose of this investigation was to compare carbonyl compounds of commercial saltine crackers made with twenty-five hours fermentation and experimentally made crackers with Flavol and a minimum fermentation. In order to correlate chemical analysis with flavor properties, sensory evaluation by comparison tests were employed.
REVIEW OF LITERATURE

In saltine cracker fermentation, yeast and bacteria are the major biological agents. After fermentation activity reaches an optimum (ten to fifteen hours), the yeast and bacteria activity are retarded. Bacterial growth is retarded to a greater extent than yeast in the latter stages of fermentation. This suggests that there is a symbiotic relationship between bacteria and yeast. Bacteria and yeast activity in fermentation affects pH, temperature rise, and flavor of the finished product. (3)

Yeast Fermentation

It took more than one hundred years of research by numerous investigators to unravel the sequence of enzymatic reactions involved in the fermentation of carbohydrates by yeast to alcohol and carbon dioxide. The basic chemical equation expressing the changes that occurred during the dissimilation of carbohydrates were put forward by Gay-Lussac in 1815:

\[ C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 \]

It was not until 1897, when Bühner in Germany prepared the first yeast extract, that it was shown that fermentation without intact yeast cells was possible. Shortly thereafter the work of Harden and Young, in England, implicated phosphorylated derivatives of the sugars in alcoholic fermentation. This can be expressed as the Harden-Young equation:

\[ 2C_6H_{12}O_6 + 2Na_2HPO_4 \rightarrow 2C_2H_5OH + 2CO_2 + C_6H_{10}O_4(PO_4Na_2) + H_2O \]
As a consequence of these vitro investigations, a series of enzymatic reactions representing the dissimilation of sugar to alcohol was proposed, in which the participation of phosphorus was considered as indispensable and thus, the glycolytic sequence was recognized. (4)

The reactions involved in fermentation (anaerobic) of glucose to pyruvate are identical with those utilized in respiration (aerobic). In yeast metabolism, pyruvate is mainly decarboxylated to acetaldehyde by pyruvate decarboxylase. The acetaldehyde formed is reduced to ethanol by alcohol dehydrogenase and NADH. The reaction catalyzed by glycer-aldehyde 3-phosphate dehydrogenase, is the only oxidative step in the glycolytic sequence leading to pyruvate. In that reaction, NAD is reduced and must be regenerated for glycolysis to proceed due to a limited pool of NAD. The last step in alcoholic fermentation, reduction of acetaldehyde to ethanol, accomplishes this function. However, if for any reason the alcohol dehydrogenase reaction is not operative or when the concentration of acetaldehyde is not sufficient to support the alcohol dehydrogenase reaction at the beginning of glucose fermentation, regeneration of NAD can occur by an alternative reaction. Reduction of dihydroxyacetone-phosphate to glycerol 3-phosphate is the main alternative. Glycerol 3-phosphate is then hydrolysed to glycerol by a specific phosphatase which is released from the cell. When yeast is in an alkaline medium (optimum pH 8.7), acetaldehyde dehydrogenase diverts acetaldehyde from its usual route to ethanol resulting in production of acetic acid. The total equation can be expressed as follows:

\[ 2 \text{Glucose} + \text{H}_2\text{O} \rightarrow 2 \text{CO}_2 + \text{Acetic Acid} + \text{Ethanol} + 2 \text{Glycerol} \]
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Under certain conditions (absence of nitrogen), pyruvate and glycerol can accumulate as products because of repressed pyruvate decarboxylase (5). The equation can be expressed as follows:

\[ \text{Glucose} \rightarrow \text{Pyruvate} + \text{Glycerol} \]

However, yeasts do not metabolize all the glucose they consume through the Embden-Meyerhof glycolytic pathway, but channels some through the pentose phosphate cycle (6). In this pathway, energy is channeled not to the formation of ATP, but to the reduction of NADP. Hommes (7) observed that excess carbohydrate tends to push yeast toward fermentation rather than to respiration. The "Crabtree effect," in which an excess of glucose represses respiration, tends to offset the "Pasteur effect" which suppresses fermentation under aerobic conditions.

Rose (8) broadly divided transport mechanisms into active and passive forms. Deierkauf et al. (9) concluded that active transport is the mechanism for the glucose entry into yeast, and further, that a carrier phosphate-glucose complex is formed. Another aspect of substrate entry into the cell has to do with penetration of organic acids into the yeast cell. Since yeast carboxylase is intracellular, a keto-acid molecule, to be decarboxylated by yeast carboxylase, must first penetrate the cell wall and plasma membrane (10). Since the plasma membrane limits the rate of permeation, the rate of carbon dioxide liberation measures penetration of the keto-acid into the yeast cell.

Although homolactic and alcoholic are the most common fermentation mechanisms, other pathways are known; most of them are variations of the Embden-Meyerhof scheme. In other types of sugar fermentations, propionic
acid, butyric acid, succinic acid, and acetone are end products. Fatty acids and amino acids undergo fermentations by different mechanisms (11).

**Bacteria Fermentation**

In cracker sponge and dough, acids are produced continuously throughout fermentation causing a gradual lowering of the pH of the sponge and dough. The initial pH of the sponge, when freshly mixed, is approximately 5.60. As fermentation proceeds, pH decreases until a final value of 3.95 may be reached at the end of twenty hours of fermentation. This gradual change in pH is principally due to acids produced by lactic and acetic bacteria. Lactic is the principle acid produced. Acetic acid is produced to a lesser degree and other long and short chain fatty acids are formed in minute amounts (2).

It was reported that during initial work with liquid ferments, titratable acidity and lactic acid content increased with time and this was attributed to proliferation of *Lactobacillus* bacteria. The majority of organisms surviving the first six hours of fermentation belong to the genus *Lactobacillus*, a group that is tolerant to highly acidic conditions and frequently found in dough (12).

*Clostridium thermoaceticum*, isolated by Fontaine et al. (13) ferments glucose, fructose, and galactose to yield 85 percent of the carbon as acetate. By fermentation mechanisms (i.e., $C_6 + 2C_3$) only 60 percent of the carbon could appear in acetate.

Wieringa (14, 15) showed that *C. aceticum* fermented hydrogen and carbon dioxide to acetate as the sole product, thereby demonstrating a net synthesis of acetate from carbon dioxide and suggesting the source of the third acetate in the *C. thermoaceticum* fermentation. Evidence
for the condensation of CO₂ to acetate in C. thermoaceticum is based on (a) conversion of xylose solely to acetate (16), and (b) incorporation of C¹⁴O₂ essentially equally into both carbon atoms of acetate during glucose fermentation (17). In (b) the decrease in specific activity in acetate over that of the CO₂ added indicated that only one of the three moles of acetate came from carbon dioxide. When glucose-1-C¹⁴ was fermented, the acetate was methyl-labeled. In the fermentation of glucose-3, 4-C¹⁴, the isotope did not enter the acetate directly. Thus, there is evidence that the Embden-Meyerhof pathway functions in producing two acetate molecules while the third is formed by carbon dioxide fixation. (18)

The identification and association of lactic acid-producing organisms with fermentation was established by numerous investigators including Bondeau, Pasteur and Schutze, and Lister. (19) All members of genera Streptococcus, Pedioccus, Microbacterium, a large number of lactobacilli, certain bacilli, and Rhizopus species ferment glucose predominantly to lactic acid with formation of trace amounts of volatile acids, ethanol and carbon dioxide.

The mechanism of microbial lactic fermentation has been considered as an Embden-Meyerhof glycolysis wherein pyruvic acid is reduced to lactic acid rather than being decarboxylated and reduced to ethanol as in the yeast fermentation. The earliest evidence of Embden-Meyerhof intermediates in lactic bacteria was that of Stone and Werkman (20) who reported that Lactobacillus plantarum accumulates phosphoglyceric acid when glucose is fermented in the presence of fluoride and acetaldehyde or other hydrogen acceptors.
THIS BOOK CONTAINS NUMEROUS PAGES WITH DIAGRAMS THAT ARE CROOKED COMPARED TO THE REST OF THE INFORMATION ON THE PAGE.

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Evidence from biochemical studies supports the view that the homolactic fermentation utilizes the glycolytic system, whereas the heterolactic fermentation follows a hexose monophosphate pathway. Since homolactic organisms form other products in major amounts under certain conditions and may contain enzymes of the hexose monophosphate pathways, a more precise definition of "homolactic" has been sought.

The most convincing evidence for Embden-Meyerhof glycolysis has been provided by Gibbs et al. (21) by showing that *L. casei*, *L. pentosus* (plantarum), and *S. faecalis* ferment glucose-1-C\textsuperscript{14} to methyl-labeled lactate with a 50 percent dilution of the specific activity in the methyl group over that of carbon atom 1 of glucose. Similarly, the fermentation of glucose-3, 4-C\textsuperscript{14} yielded carboxyl-labeled lactate without dilution of the specific activity. The labeling pattern is shown below:

![Chemical Structure](image)

Glucose  \rightarrow  2 Pyruvate  \rightarrow  Lactate  \rightarrow  Ethanol + Carbon dioxide

Since these labeling patterns exclusively fit the distribution of carbon atoms expected from the Embden-Meyerhof pathway, there is no doubt that
the glycolytic pathway is the quantitatively significant mechanism functioning in the homolactic fermentation.

The stereoconfiguration of the lactic acid produced varies with the genus or species involved. Lactic acid of D-configuration is produced by members of the genus Leuconostoc, whereas the L-configuration is produced by Streptococci. The lactate-producing Bacillus calidolactis, Bacillus coagulans, and Microbacterium and Rhizopus species also form L(+)-lactate, whereas pediococci and heterolactobacilli produce mixtures of isomers. Among the homofermentative lactobacilli, great variation exists in the type of lactate produced. (22)

In addition to glucose, other hexoses such as fructose, mannose, galactose, disaccharides including lactose, maltose, sucrose, starch and dextrins among the polysaccharides serve as substrates for the homolactic fermentation. It is presumed that these sugars are converted to intermediates of the glycolytic system by inducible enzymes. (23)

More than sixty aromatic compounds are produced by dough fermentation and baking (24) but many are dissipated during either late stage of fermentation or baking. Some react further during baking and produce new flavor stimuli. (24, 25) Cole et al. (26) identified organic acids, carbonyls and alpha-keto acids in preferments. Ketoglutaric, pyruvic and alpha-keto-isovaleric acids were present in measurable amounts with accumulation of pyruvic acid being the largest. Johnson et al. (27) found acetic and lactic acids in preferments. Lactic acid continues to develop slowly, while acetic acid production ceases with extended fermentation time. Hunter et al. (28) found the most common short-chain fatty acids in preferments to be formic, acetic, propionic, butanoic, isobutyric, valeric, isovaleric, caproic, and isocaprylic acids. Esters
were formed from fatty acids and alcohol. Johnson et al. (27) and Smith and Coffman (29) identified ethyl acetate and ethyl lactate in preferments.

Smith and Coffman (29) and Johnson et al. (27) found ethyl, isobutyl, amyl and isoamyl alcohols in preferments and vapors from preferments. Guymon et al. (30) stated that the major higher alcohols produced by yeast appeared to be overflow products from the synthesis of isoleucine, valine and leucine, which have several common enzymatic steps.

Miller et al. (31) studied the volatile constituents formed during fermentation and found it is possible to isolate and identify formaldehyde, acetaldehyde, acetone, iso-butyraldehyde, n-butyraldehyde, methylethlyketone, 2-methylbutyraldehyde, iso-valeraldehyde, and n-valeraldehyde from bread preferment. In addition to these carbonyl compounds, propionaldehyde and n-hexaldehyde were isolated from preferments by Linko et al. (32)

Kohn et al. (33) in a study of normal dough fermentation demonstrated that acetaldehyde, acetone, benzaldehyde, n-butyraldehyde, n-hexaldehyde, pyruvaldehyde, isovaleraldehyde, and 2-hexanone were produced independently of bacterial action.

Enzyme Supplement

Addition of malt or amylase to flour at the mill, or to dough and sponges in the bakery is aimed chiefly at increased alpha-amylase activity (34). According to Reed (35), the American baking industry, with an annual production of 100 to 120 million hundredweights of flour, uses some 56 to 60 million pounds of wheat malt and barley flour for
supplementation at the mill, the addition generally being held to .2
to .4 percent based on flour weight.

Johnson, Dirks and Shellenberger (36) compared gassing power,
maltose value and viscometric values as methods of controlling of
flour supplementation with enzyme preparations from different sources.
According to these workers, the baking test remains the final criterion
of proper supplementation.

Both amylase and protease are reported to improve the crust color
of bread (37). This is attributed to free sugars and amino acids made
available by these enzymes for the browning reaction. Morimoto (38)
found that when protease was added to the cracker sponge and the sponge
was allowed to ferment for eighteen hours, there is an increase in
free amino acids content of the baked cracker. Protease has been
reported to improve physical characteristics of both dough and cracker.
With cracker doughs being more pliable and easy to machine due to pro-
tease action, the finished crackers become more uniform and tender. (39)

Both cereal and fungal amylase supplements are employed in cracker
formulation (40-45). Since sugars are not commonly added to cracker
sponges, diastatic activity on available starch of flour seems to be
the main factor in gas production of the sponge.

Miller and Johnson (46) investigated the differential stability
of alpha-amylases and proteases employing malted wheat flour, malted
barley, and commercial fungal concentrates. Results of their study
suggested that protease action is restricted to the mixing and fermente-
tation periods since a complete inactivation of enzymes was achieved
at a relatively low temperature thus precluding any appreciable action
of protease enzymes during the baking period.
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Browning Reaction

It is recognized today that browning of baked products can be classified into two types, caramelization and Maillard or Melanoidin browning (47, 48, 49). The first type occurs when sugars are heated to about 275°F. The compounds formed in the early stages of caramelize- tion are not well established. In latter stages of caramelizeation, brown, unsaturated, complex polymers are formed which in some respects are similar to products from Maillard-type browning because of the high temperatures of activation required for the former (50). The flavors and odors of the compounds produced in the two types of browning are also different. (47)

Carmelization is a reaction which does not require an amine. Sugars show carmelization when heated to relatively high temperature. It was found that organic acids and their salts accelerate caramelizeation of sugars by promoting enolization of the sugar. Enolization occurs in both acid and alkaline media (23) but far more easily with increasing alkalinity. The enolized sugar is easily dehydrated, and fragmentation products contain unsaturated osones, dehydroreductones and δ-hydroxycarbonyl compounds.

These fragmentation products will undergo browning in aqueous solutions, and, in the presence of amino compounds, browning is greatly accelerated. The most highly reactive compounds are glycolaldehyde, glyceraldehyde, pyruvaldehyde, acetol, dihydroxyacetone, acetoin, and diacetyl. Acetaldehyde is only slightly less reactive, aldol is still a little slower and propionaldehyde is a very slow reactant. Even slower to react are the keto acids, pyruvic and levulinic, while the saccharinic acids, lactic and acetic acids do not react with amino compounds. Formaldehyde is not only inactive but is actually an
inhibitor of furfural formation, carmelization, and carbonylamino types of browning.

There is one outstanding difference between the browning of non-amino (caramelization) and carbonyl-amino systems; acceleration of browning is appreciably greater through glycosylamine enolization (Amadori rearrangement) than through enolization of the unsubstituted sugar (Lobry de Bruyn-Alverda van Ekenstein Transformation). The main reactions occurring in carbonylamino browning also occur in non-amino browning. For example, 1,2-enolization dehydration to furfurals, and fission are known caramelization reactions. As the Amadori rearrangement is known to provide a more labile configuration of the 1-amino sugar chain, so the 1,2-enolization of reducing sugars provides the labile sugar chain (the C-C bond to the carbonyl group) for non-amino browning reactions.

Bryce and Greenwood (51) found that 300°C caused pyrolysis of volatiles from sucrose, maltose, glucose, and starch. These findings support the contention that sucrose, glucose, fructose, and maltose form similar caramel. Diemar and Hala (52) showed that maltose, when heated at 190°C for about one hour, was split to glucosyl radicals which built up to disaccharides, trisaccharides, tetrasaccharides, and dextrins. Under these conditions, the flavor compound maltol (3-hydroxy-2-methyl-4Hpyran-4-one) was produced from maltose, but not from glucose. Mineral salts catalyzed the maltol formation. Müller and Täufel (53) and Schneider (54) also showed disaccharides and dimeric sugar anhydride formation in caramelization. Hirschmüller (55) reported the following sucrose caramelization products formed at 180°C: carbon dioxide, carbon monoxide, methanol, acetaldehyde, benzaldehyde, acetone, acrolein, formic
acid, acetic acid, furfural, and unidentified phenol derivatives. Lukesch (56) melted sucrose at 150°C. (the temperature of baking bread crusts) powdered the cooled melt, and extracted it with organic solvents. The solvent-free extracts when chromatographed on paper showed glucose, fructose, glyceraldehyde, dihydroxyacetone, pyruvic acid, pyruvaldehyde, and 5-(hydroxymethyl)-2-furaldehyde.

Bollmann and Schmidt-Berg (57) examined the first decomposition products of dry-heated sucrose and found glucose (at 130°C., fifteen minutes) reversion products (above 150°C.), fructose and its anhydrides (160°C.) and 5-(hydroxymethyl)-2-furaldehyde (above 170°C.). Among the di- and trisaccharides formed were 6-ketose and other fructosylsucroses.

Flavor production by nonenzymatic browning in foods develops mainly from reactions of reducing sugars with amines, amino acids, peptides, and proteins.

Considerable evidence is available indicating that Maillard-type non-enzymatic browning occurs significantly during baking, toasting, and roasting (58). Rooney et al. (59) found that concentrations of the aliphatic aldehydes formed from each amino acid were enhanced only in the crust, which illustrates the importance of the Maillard reaction in crust browning and the formation of carbonyl compounds. They also found that they type of amino acids influenced the kind of carbonyl compounds formed in model systems, whereas the type of sugar influenced the amount of carbonyl compounds formed (60), participation of amino acids and sugars in producing flavors and brown colors has been demonstrated for many foodstuffs. (61) Generally, browning occurs both in acidic and alkaline media. However, the rate of the reaction decreases at low pH values, low temperatures and high or low moisture contents.
Katchalsky and Sharon (62) showed that the extent of the reaction decreased with an increase in hydrogen-ion concentration. However, proteins, peptides and aromatic amino acids are more reactive toward glucose than are the aliphatic amino acids at the lower pH values found in nature. (63)

The effect of the position of the amino group on the browning intensity as measured by absorbance at 500 μm, was reported by Lento et al. (64); as the amino group was moved from the proximity of the carbonyl group, browning occurred at a faster rate. Also, Kubotz (65) found that the carboxyl group had an inhibiting effect on the reactivity of the amino group. Esterification of the carboxyl group resulted in an increase in the reaction velocity. The reaction rates of different amino acids vary greatly. Salem et al. (60) placed the amino acids in the following order of decreasing reactivity toward D-glucose: valine, glycine, glutamic acid, leucine and tyrosine. Rothe and Thomas (66) reported that the most reactive amino acids during the non-enzymatic browning reaction are, in the order of decreasing reactivity, isoleucine, leucine, valine and methionine. Ellis (67) indicated that the order of reactivity of the various amino acids with sugars, even under the same conditions, depends on the methods used in determining reactivity.

According to Hodge (68), three pathways of the Maillard reaction are clearly defined. From the main stem of sugar-amine condensation, enolization of the glycosylamines, and the Amadori transformation to 1-amino-1-deoxy-2-ketoses (69), two branches diverge. In one branch, 3-deoxyhexosones are formed from the 1,2-eneaminol by elimination of the hydroxyl group on C-3 (70). In the other branch, the 1-amino-1-deoxy-2-ketose enolizes 2,3 irreversibly and eliminates the amine from
C-1 to form a methyl 3-dicarbonyl intermediate (71). Dehydration occurs in branches yielding 3, 3-unsaturated-, 3-dicarbonyl compounds (and their amino analogs) which presumably condense and polymerize to melanoidins (72). 2-Furaldehydes are a distinctive end product of the first branch, whereas C-methyl aldehydes, ketonleohydes, ketols, and reductones are products of the second. Both branches of the reaction scheme provide active reagents for the degradation of 3-amino acids to aldehydes and ketones of one less carbon atom (the Strecker degradation), which is the third pathway of the Maillard reaction that is well-defined and important for the origin of flavor in foods.

The so-called oxidative degradation of 3-amino acids to aldehydes of one less carbon atom by compounds such as alloxan, ninhydrin, 2-furaldehyde was more precisely defined by Schünberg et al. (73). They demonstrated that the amino group must contain a - C : O-C : O or a - C : O - (CH:CH) -C : O - grouping. The reaction is illustrated by the following equation:

$$R\cdot CO\cdot CO\cdot R' + R''\cdot CH(\text{NH}_2)\cdot COOH \rightarrow R''\cdot CHO + CO_2 + R\cdot CH\cdot (\text{NH}_2)\cdot CO\cdot R$$

If the hydrogen on the 3-carbon of the amino acid is substituted, a ketone is produced. For example, acetone is formed from 3-aminoiso-butryric acid, $\text{CH}_3\cdot C(CH_3)\text{NH}_2\cdot COOH$. If the dicarbonyl compound is pyruvic acid, it would be converted to 3-alanine during the reaction. Such transformations incorporate nitrogen into the sugar-derived dicarbonyl compounds and eventually into the melanoidins.

The most reasonable mechanism for the Strecker degradation, according to Schünberg and Moubacher (74), provides for Shiff base
formation in the first step to form, for example, from pyruvaldehyde and β-alanine:

\[
\begin{align*}
\text{CH}_3\text{C}=\text{C}-\text{N}\text{=N}\text{C}=\text{O} + \text{H}_2\text{O} & \rightleftharpoons \text{CH}_3\text{C}=\text{C}-\text{N}\text{=N}\text{C}=\text{O} + \text{H}_2\text{O} \\
\text{CH}_3 & \quad \text{CH}_3
\end{align*}
\]

The enolic tautomeric form is an ε-imino acid, known to decarboxylate readily. Decarboxylation would yield:

\[
\begin{align*}
\text{OH}_2 + \text{H} & \\
\text{CH}_3\text{C}=\text{C}-\text{N}\text{=N}\text{C}=\text{H}_2 + \text{CO}_2
\end{align*}
\]

The eneaminol could self-condense to brown polymer or hydrolyze to amino-acetone, \( \text{CH}_3\cdot\text{C}=\text{O}\cdot\text{CH}_2\cdot\text{NH}_2 \), and acetaldehyde, \( \text{CH}_3\cdot\text{CHO} \). Thus, a small amount of aldehyde of one less carbon atom than the ε-amino acid would be liberated to contribute to flavor, along with other carbonyl compounds that would be generated from the sugar. Aldehydes corresponding to the Strecker degradation products of amino acids have been isolated from bread. (75)

Cracker Flavol—Its Function and Application

It has long been believed that lengthy fermentation is essential to produce a baked product with good physical properties and desirable flavors. While sacrificing material losses, manufacturing time, electricity, equipment, space and labor costs. Commercial cracker doughs are prepared by the sponge and dough process which usually requires a total fermentation period of approximately twenty-four hours to attain a crispy, tender and rich flavor of soda cracker.
Johnson and Bailey (76) in a fundamental study of cracker dough fermentation has questioned the credibility of the prolonged sponge fermentation. They found that the only significant changes accomplished by the extended fermentation are the degradation of gluten proteins and the saturation of dough with carbon dioxide. They suggested to use increased amounts of yeast and suitable proteolytic enzyme to compensate for lengthy fermentation.

Since then, many efforts have been made trying to eliminate or substantially reduce such a long term fermentation while attaining rich flavor and good physical properties of final product.

Addition of amino acids to improve bread flavor has been reported by many investigators. (77, 78, 79, 80)

It was after years of research, Johnson (1) found that a combination of gluten amino acids and a mixture of organic acids (flavor compounds) can function as a fermentation compensator, that is, it compensates for or provides changes which normally require fermentation to achieve. Flavol is a patented bread flavor enhancer that, with proper modifications, can be used for manufacturing other yeast-raised products; such as rolls, crackers, pizza dough and sweet dough which are normally subjected to pre-fermentation before baking. (1)

Studies of Flavol as a flavor precursor or fermentation compensator in a non-fermented baking process was reported to yield bread possessing essentially the same or better physical properties and flavor characteristics as conventionally fermented bread. (81)

It was based on this concept that cracker flavol was developed.
Chemical Analysis of Flavor

Analysis of carbonyl compounds in saltine crackers included both volatile and non-volatile. Trace amounts of volatiles, in some instances, can have profound effects on odor sensations. According to Teranishi et al. (82), chemical analysis of flavor usually includes sample preparation, isolation and concentration of total volatiles, separation of mixtures and identification of components.

Sample Preparation

Obtaining the sample represents only a small portion of the total effort expended in the investigation of a natural flavor, but it is an all important step.

1. Analysis of Total Volatiles by Distillation and Extraction. A flavor concentrate will be obtained, hopefully free of artifacts, that contains all the volatile compounds that are present in the food.

2. Analysis of Head Space Volatiles. Horstein et al. (83) employed a trapping coil cooled in liquid nitrogen to concentrate head space volatiles. Modification of this system have been used to collect volatile compounds from a variety of substracts (84, 85).

Isolation and Concentration of Total Volatiles

Distillation, extraction and adsorption are usually applied to isolate all volatile materials from a food. Distillation usually can be carried out either at atmospheric pressure, reduced pressure or under vacuum by using simple distillation, steam distillation or fractional distillation method. Concentration of total volatiles can be effected by either extraction, freeze concentration or charcoal adsorption. If the solution is not too dilute, extractions with organic solvents can be
made directly. If the solution is extremely dilute, one may use freeze concentration prior to extraction. Charcoal adsorption may also be used to concentrate very dilute solutions. Charcoal is not deactivated by water and has a great capacity for adsorbing organic compounds.

Separation of Mixtures by Gas Chromatography

Gas Chromatography is one of the very powerful methods used in aroma analyses for separating small amounts of complex mixtures and is versatile enough to handle head space volatiles or flavor concentrates.

Resolution in GC depends on the differences, under identical conditions of chromatography, among the partition coefficients of the compounds being separated and on the efficiency of the column. The efficiency of a chromatographic column is a measure of peak broadening as the solutes pass through the column. The greater the efficiency of the column, the better the resolution of compounds whose partition coefficient may differ only slightly. Temperature programming is almost always used, because of the wide range in the boiling points of flavor volatiles. Generally, a preliminary GC separation on a nonpolar column should be made and fractions collected between arbitrarily selected temperatures. These fractions should be evaluated organoleptically to establish their contribution to the flavor under study (82).

Identification of Flavor Components

Some information can be gained from relative retention data. The Kovats (86) retention index is widely used and expresses the retention behavior of an unknown using the normal paraffins as standards. Retention indices are most useful for identifying monofunctional compounds of low molecule weight. When larger molecules of multiple functionality
are isolated, additional identification means are generally necessary.

Infrared Spectroscopy (IR)

Infrared Spectroscopy (IR) is the most widely used spectrometric method and is a powerful tool for determination of functional groups such as hydroxyl, amino, carbonyl, double bonds, etc. Its accuracy and sensitivity are adequate for most flavor research problems. Usually about 100 grams of a compound are required. For some compounds, and with instruments equipped with scale expansion, beam condensers, etc., samples in the 1 to 10 ug. range can give useful information. (82)

Nuclear Magnetic Resonance (NMR)

Nuclear Magnetic Resonance (NMR) is a potential tool for elucidating structure but it has not been used widely in flavor research for two primary reasons:

1. Sample size requirements have been too large for most flavor research problems, and

2. most attention has been given to relatively simple compounds (82). However, since instrumentation and techniques have been improved, NMR can be used in flavor research.

Mass Spectroscopy (MS)

Mass spectroscopy is the most sensitive of the analytical methods used in combination with GC. Combined GC-MS analysis provides the flavor chemist with a means for rapid analysis and identification of large numbers of components present in the mixtures that he isolates. The application of the GC-MS combination is a major advance in the analysis of the volatiles contributing to the aroma of foods.
A GC-MS system is capable of recording spectra on full range flavor concentrates, head gas samples, and essences, separated on high efficiency GC column; prefracton by preparative GC, TLC, or column chromatography is usually desirable to simplify these mixtures prior to GC-MS analysis.

According to Teranishi et al. (82), by combining the GC-MS system with computer analysis of data and searching of reference files, it is possible to identify rapidly large numbers of simple compounds which occur in these complex mixtures. The investigators can pursue more difficult structure problems after elimination of these compounds.
MATERIALS AND METHODS

Preparation of Saltine Crackers

Saltine crackers were baked at the KSU laboratory. Commercial saltine crackers were purchased locally.

The cracker and cracker flavor formula were given in Table I (81) and Table II respectively. Ten batches of crackers were made by the straight dough procedure, after two hour dough fermentation at 84–86°F. ± 88–90 percent relative humidity. The whole piece of dough was used to make three successive bakes. In each bake a small piece of dough was rounded and slightly flattened by hand, then passed four to five times through "Anets-Model-MDR-45" roller to reduce its thickness to about 1.5 (0.061 inch). The reduced sheet of dough folded in six layers and passed again four to five times through the roller to reduce its thickness to about 0.79mm (0.031 inch). The sheet of dough was then cut, salt sprinkled and baked at 510°F. for 3 to 3-3/4 minutes. Crackers (500 g) were randomly sampled for analysis of their carbonyl contents.

Analysis of Carbonyl Compounds

Isolation of Carbonyl Compounds

Carbonyl compounds were isolated from crackers by extraction with chloroform and hexane. The carbonyl compounds were reacted with 2,4-dinitrophenylhydrazine (2,4-DNPH) in phosphoric acid on a Celite
TABLE I

SALTINE CRACKER FORMULA

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Percent (Flour Basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flour&lt;sup&gt;a&lt;/sup&gt;</td>
<td>100</td>
</tr>
<tr>
<td>Yeast (compressed)</td>
<td>1.0</td>
</tr>
<tr>
<td>Shortening</td>
<td>12.0</td>
</tr>
<tr>
<td>Sodium stearoyl-2-lactylate (SSL)</td>
<td>0.3</td>
</tr>
<tr>
<td>Lactose</td>
<td>2.0</td>
</tr>
<tr>
<td>Soda</td>
<td>0.5</td>
</tr>
<tr>
<td>Salt</td>
<td>1.0</td>
</tr>
<tr>
<td>Dry diastatic malt</td>
<td>1.0</td>
</tr>
<tr>
<td>Cysteine</td>
<td>0.01</td>
</tr>
<tr>
<td>Soluble starch</td>
<td>0.25</td>
</tr>
<tr>
<td>Fungal amylase</td>
<td>11.0 SKB units/100g.</td>
</tr>
<tr>
<td>Fungal protease</td>
<td>62.0 H.U./100 g.</td>
</tr>
<tr>
<td>Cracker Flavel&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.5</td>
</tr>
<tr>
<td>Water&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Variable</td>
</tr>
</tbody>
</table>

<sup>a</sup> A 95 percent soft wheat flour, 5 percent light rye flour.

<sup>b</sup> As recommended by Hamed Faridi Araghi (2).

<sup>c</sup> Varied with ingredients and processing conditions increasing at a rate of 1 percent with each 5 percent of added rye flour.
<table>
<thead>
<tr>
<th>Compounds</th>
<th>Weight (g/1000 g Cracker Flavol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Promate-200</td>
<td>534.51</td>
</tr>
<tr>
<td>Calcium lactate</td>
<td>224.63</td>
</tr>
<tr>
<td>Sodium acetate</td>
<td>25.52</td>
</tr>
<tr>
<td>Sodium propionate</td>
<td>4.72</td>
</tr>
<tr>
<td>Sodium isobutyrate</td>
<td>7.25</td>
</tr>
<tr>
<td>Sodium butyrate</td>
<td>1.98</td>
</tr>
<tr>
<td>Sodium isovalerate</td>
<td>5.28</td>
</tr>
<tr>
<td>Sodium valerate</td>
<td>2.87</td>
</tr>
<tr>
<td>Sodium caproate</td>
<td>1.28</td>
</tr>
<tr>
<td>Carrier starch</td>
<td>191.97</td>
</tr>
</tbody>
</table>
column (87). After evaporation of chloroform-hexane under vacuum, the residue was dissolved in benzene-methanol and the non-carbonyl compounds were removed from the solution by using a strongly acidic cation exchange resin, AG-50W-X4 (88). The carbonyl derivatives were adsorbed on activated magnesia while eliminating the bulk of fat or oil, followed by desorption of the derivatives (89). The derivatives were fractionated on weak alumina (89). Total carbonyls, dicarbonyls, ketoglycerides and monocarbonyl fractions were determined by a modified Henick method (90) using a Beckman DB-G grate spectrophotometer.

Large quantities of solvents; benzene, hexane and chloroform were purified rapidly and continuously on a column of Celite 545 impregnated with concentrated sulfuric acid (91). Concentrated sulfuric acid (60 ml) was slurried with 100 g of Celite 545 (Fisher Scientific Co., Fairlawn, New Jersey). The chromatographic tube was approximately 3.4 x 60.5 cm. Anhydrous granular sodium sulfate (reagent grade) was added to a depth of approximately 10 cm. and then 160 g. of the sulfuric acid–Celite adsorbent was added with tamping. This section of the column was approximately 30 cm. long. The column was topped off with 7 to 8 cm. of crystalline sodium sulfate.

Hexane and benzene were applied to the column directly without auxiliary treatment while chloroform prior to clean up requires a preliminary wash with water (six times), followed by drying over anhydrous sodium sulfate.

Crackers (500 g.) were fragmented with mortar and pestle and placed in a Waring Blender (Model CB-4, Waring Products Corporation, New York City) which contains 200 ml. chloroform, add 800 ml. of chloroform, blended for twenty seconds with low speed and soak for
thirty minutes with occasional shaking. The slurry was filtered through glass wool with sodium sulfate on top to absorb moisture. Hexane (1000 ml) was added to the filtered cracker residue and blended for ten seconds at low speed and soaked for fifteen minutes with occasional shaking before filtering through glass wool topped with sodium sulfate. The filtered fractions were combined and were passed through the Celite reaction column.

**Reaction of Carbonyls with 2,4-Dinitrophenylhydrazine (2,4-DNPH)**

The significance of carbonyl compounds to the flavor of baked products has long been established. Since minute quantities of carbonyls are generally sufficient to give characteristic odors that can be sensed by human olfactory nerves, many techniques have been developed to analyze those micro quantities of aldehydes and ketones. "Solvent extraction" methods were applied to this study for analyzing carbonyl compounds present in saltine crackers.

Contamination of micro amounts of carbonyls in solvent systems were considered very critical in this study. Therefore, all solvents were purified before use. Solvent purification could be achieved by a reaction column (Celite impregnated with 2,3-DNPH, phosphoric acid and water or by a column of Celite 545, impregnated with concentrated sulfuric acid). In this study, Celite-sulfuric acid column was employed, since its clean-up was quantitative involving little or no auxiliary treatments and was applicable to non-oxygenated and water immiscible solvents commonly used for extraction of the carbonyl compound.

Among solvents used in this study, hexane and benzene could be applied to the column directly while chloroform required a preliminary wash with water, followed by drying over anhydrous sodium sulfate to
remove alcohol which otherwise could be dehydrogenated by 2,4-DNPH to produce acetaldehydes and interfered with this analysis (87).

2,4-DNPH is the most common derivative employed in the presence of an acid catalyst to quantitatively react with the carbonyls present in solvent extracts since it is highly specific, reacts rapidly and yields hydrazones which are relatively acid stable. In the instance where the reagent is insoluble or only slightly soluble in the solvent, other methods must be used.

The reaction column was prepared in the following manner: 0.5 g of DNPH (J. T. Baker Chemical Co., Phillipsburg, New Jersey) was dissolved in 6 ml of 85 percent H$_3$PO$_4$ by grinding in a 4-inch mortar. Distilled water (4 ml) was added to the clear yellow solution and the precipitated DNPH was redissolved by grinding. Ten grams of celite (Johns-Manville, Lompoc, California) was then ground with the solution until a homogeneous damp preparation was obtained. The bright yellow impregnated celite was then transferred to a chromatographic tube (approximately 3.4 cm. i.d. x 65 cm. length) in about ten equal portions. Each portion was tamped tightly before addition of the next. The column was washed with 50 ml. benzene followed by hexane until a colorless effluent was obtained.

The filtrate of chloroform-hexane extract of crackers was added to the column and flow rate adjusted to 46 ml/hr. When the last of the solution entered the column, the sides of the tube were washed down with hexane and the washings allowed to enter the column. The column was then flushed with hexane until the effluent emerged colorless or had the same absorbance (at or near 340 mu) as the effluent from a blank column which was run simultaneously.
Quantitative aspects of reaction column was studied by Schwartz and Parks (87). They studied 40 model compounds (aliphatic monocarbonyls) and found that all of those which contained no other functional group quantitative recovery was achieved and no artifacts were produced.

The effluent from the reaction column contained all of the lipids; the 2,4-DNPH’s of monocarbonyls, semialdehydes and ketoglycerides and other classes of carbonyl compounds whose derivatives were soluble in the fat-chloroform-hexane solution, a small amount of DNPH, and traces of the decomposition products of 2,4-DNPH. Carbonyls whose derivatives were insoluble in the fat-chloroform-hexane solution remained on the column (87). Limited experiments with aromatic carbonyls dissolved in hexane indicated rapid reaction on contact with the reaction column as manifested by the formation of a red band at the top of the column. This reaction occurs only when the aromatic carbonyl is put on a column in which the resulting hydrazone is insoluble or slightly soluble. (87)

The chloroform-hexane-fat solution was evaporated by using a Roto-evaporator-R. (Brinkman Instruments, Cantague Road, Westbury, New York) at 30° C. under aspirator pressure until dried. The residue was dissolved in the minimum of purified methanol and carbonyl-free benzene (1:1).

Selective Removal of 2,4-Dinitrophenylhydrazine on Resin

Schwartz, Johnson and Parks established that DNPH is quantitatively absorbed by the AG-50W-X4 resin. One gram of resin (moisture free basis) will readily absorb 100 mg. of DNPH. If impure DNPH is applied to the resin, colored impurities will pass through. The formation of colored impurities is accelerated in the presence of acid (87). Hydrazones
containing a basic function may be expected to be absorbed by the resin along with DNPH. DNPH is not eluted with 5N H₂SO₄ or 5N HCl or with 1 percent trichloroacetic acid in carbonyl-free benzene (87). This suggested that DNPH is irreversibly held by the resin once exchange has occurred.

The ion-exchange resin used was AG-50W-X4, 200-400 mesh (Bio-Rad Laboratories, Richmond, California). All hydrazones passed through this resin quantitatively while DNPH was taken up by the resin. All solvents used were analytical grade. Deionized water was used to wash the resin. Carbonyl-free methanol was prepared by the following method: to 500 ml. of methanol about 5 grams of 2,4-DNPH and a few drops of concentrated hydrochloric acid were added. After refluxing two hours, the methanol was distilled through a short Vigueux column. If kept tightly stoppered, the methanol remained suitable for use for several months (92). The procedures for preparing ion-exchange column (2.3 cm. i.d. x 39.4 cm) were as follows: AG-50W-X4 cation exchanger was slurried in water and transferred to a chromatographic column. One gram of resin (moisture-free basis) would readily hold 100 mg. of DNPH. The resin in the tube was treated with the following sequence of reagents:

1. Two column volumes of 1.0 N NaOH;
2. Water until effluent was neutral;
3. Two column volumes 1.0 N of HCl;
4. Water until effluent was neutral;
5. Four column volumes of methanol;
6. Two column volumes of purified methanol: carbonyl-free benzene (1:1) (88).
Methanol and benzene must be carbonyl free, otherwise, 2,4-DNPH's will be formed; this is presumably due to the good contact made between the free carbonyls in the impure solvents and the zone of DNPH which builds up on the resin as the methanol-benzene passes through AG-50X being a strong acid, catalyzes the reaction. Since a large amount of fat is present in crackers, the efficiency of ion exchange was impaired; also, two different fats were involved in this study; shortening (in KSU crackers) and lard (in Commercial crackers). The hydrazone residue containing shortening requires five to seven times more solvent to dissolve completely and the exchange velocity is decreased accordingly; so three ion-exchange columns (2.3 cm i.d. x 39.4 cm) were applied continuously at an average speed about 1.5 cc/minute until all 2,4-DNPH had been retained on the column. This could be best visualized after flushing the packing material out of the column with air. The color of the upper portion was much darker than the lower portion. The sample solution was passed through the column at a rate of approximately 1.0 ml/min. The resin was washed with the purified solvent mixture at a rate of approximately 2.0 ml/min. until the effluent was colorless. The effluent was evaporated to dryness at 30°C. by Rotoevaporator under aspirator pressure, and dissolved in a minimum of hexane.

Separation of Dicarbonyl Derivatives on Magnesia

Activated magnesia proved to an excellent adsorbent for 2,4-DNPH's (89). Its affinity for 2,4-DNPH's is presumably due to the ionic species of the m-dinitro groups created by the alkaline environment. The stability of 2,4-DNPH's to an alkaline environment has been amply demonstrated (93) and the recoveries of aliphatic monocarbonyls are above 90 percent. The capacity of magnesia for 2,4-DNPH's depends
on (1) fat concentration; (2) types of carbonyl compounds present
(ketones, aldehydes); and (3) strength of the adsorbent. The nitro-
methane-chloroform effluent from magnesia-Celite 545 contained all
of the monocarbonyls, semi aldehydes, ketoglycerides and probably
other classes of hydrazones. All dicarbonyl bis (2,4-DNPH's), vicinal
and non-vicinal which may have been soluble in the methanol: Benzene
solution and subsequently in hexane solution was kept on the magnesia.
These were manifested by blue, gray, or violet bands near or at the
top of the column (89).

Saponification of glycerides was reported to occur to some degree
on the column of magnesia-Celite 545; the magnesium salts thus formed
are also retained on the column (89). On an alumina column, about
2500 ml. of hexane was used to elute fatty material, at least two
alumina columns were applied for separating ketoglycerides and purifying
the monocarbonyls. The adsorbed aliphatic monocarbonyls were eluted
with a 1:1 benzene-hexane solution from an alumina column. Their
quantitative recovery has been established and that no artifacts are
produced (87).

Seasorb 43 (Adsorptive Magnesia, Fisher Scientific Co., Fairlawn,
New Jersey) was used as received. Celite 545 was dried at 150°C for
twenty-four hours. The column was prepared in the following manner.

Equal weights (45 g) of Seasorb 43 and Celite 545 were slurried
with hexane. The slurry was poured into a chromatographic tube con-
taining a plug of glass wool at the constricted portion of the tube.
The slurry was packed under air pressure (1-3 psi) leaving about 1 cm.
of hexane above the surface of the bed. The sides were washed free
of solid material before the sample was applied.
The hexane solution of carbonyl derivatives was introduced to the column. The adsorbed hydrazones appeared as a dark purple zone. Nitrogen pressure was used to force the fat solution through the column. Hexane (1000 ml.) was used to flush part of the fatty material out of the column. Hexane could be reused after evaporation and condensation. At least, two magnesia columns were used continuously for separating dicarboxyls and for purifying hydrazone solutions.

Nitromethane (Spectro Grade, Eastman Organic Chemicals, Rochester, New York) and chloroform (A.C.S. grade) in one to three ratio was used to elute hydrazones. Elution was continued until a colorless or very pale effluent was obtained. The nitromethane-chloroform eluate was evaporated on a water bath at 30°C by using Rotoevaporator under aspiration until the odor of nitromethane was absent. The residue was then redissolved in a minimum of hexane.

**Separation of Ketoglyceride Derivatives on Alumina**

Alumina (Fisher Scientific Co., Fairlawn, New Jersey) was activated by heating for twenty-four hours at 150°C, then partially deactivated by the addition of 6 percent (W/W) distilled water. The wet alumina was allowed to equilibrate overnight at room temperature (87). The alumina column was packed in the following manner: to about 20 ml. of hexane in a chromatographic tube (2.3 cm. i.d. x 39.4 cm) was added 10 g. of alumina, a little at a time, with shaking and the column packed under air pressure (1-3 psi), leaving about 1 cm. of hexane above the top of the bed. A hexane solution of the hydrazones from magnesia-Celite 545 column was applied to the column. Most of the fatty material was flushed out of the column with 2500 ml. of hexane.
Monocarbonyl derivatives were eluted with a mixture of benzene-
hexane (1:1) under nitrogen pressure. Solvent was removed from the
effluent by rotary evaporation in a water bath at 30°C under aspiration.
These conditions usually held, but in case of the carbonyl fractions
present in a large volume of fat, the ketoglyceride fraction may be
mixed with the monocarbonyl fraction. The problem could be solved by
use of either method suggested below:

1. Collect the ketoglyceride and monocarbonyl fraction and after
removal of the solvent, the residue could be rechromatographed on
another column of alumina.

2. Elution of the column with a weaker benzene-hexane (1:3),
removal of solvent and dissolving the residue in a suitable amount of
hexane for rechromatographing.

Quantitative Analysis of Carbonyl Compounds

When the spectrophotometric measurements were made in 1-cm cur-
vettes with the Beckman DB-G Grate spectrophotometer, the analysis
could be calculated by the following equations:

\[
\text{Unsaturated} = \frac{3.861 \ A_{460} - 3.012 \ A_{430}}{0.854}
\]

\[
\text{Saturated} = 3.861 \ A_{460} - 2.170 \ \text{Unsaturated}
\]

This method was modified for analysis of hydrazones in hexane solution (94).

The procedure was as follows: into a 50-ml. volumetric flask was
added 0.5 - 2.0 ml. of 2,4-dinitrophenylhydrazones (2,4-DNPH's), diluted
to approximately 10 ml. with hexane; into the flask 10ml. of ethanolic
KOH was added, the solution was diluted to the mark with absolute ethanol.
The absorbance was determined against a similarly prepared carbonyl-free
blank. Estimation of total carbonyl content was made after the sample solution was put through the cation exchange column. Estimation of dicarboxyls and ketoglycerides was made after the samples were passed through a magnesia-Celite 545 column and alumina columns, respectively.

The monocarboxyl content was made by the difference between total carbonyl content and dicarboxyl plus ketoglyceride content.

Class Pattern of Aliphatic Monocarboxyl Derivatives

Seasorb 43 (adsorptive magnesia), obtained from Fisher Scientific Co., Fairlawn, New Jersey, and Celite 545 (Johns-Manville Co.) were sieved separately; the material passing through a 200-mesh screen was utilized.

For preparation of the plates, 15 g. of Seasorb 43 and 6 g. of Celite were slurried in 45 ml. of 95 percent ethanol in a 250-ml. glass-stoppered Erlenmeyer flask by shaking the flask vigorously until homogeneous. The slurry was poured immediately into the spreader and spread over five 8 x 8 inch plates. The plates were air dried for twenty minutes and then further dried at 120°C for one hour. The plates were stored at room temperature in an air-tight, moisture-free desiccator until needed.

The thin-layer chromatographic plates were prepared as follows: the top and the bottom 1/4 inch of adsorbent was scrapped off evenly with a straight edge prior to spotting. The spotted plate was placed in a tank lined with solvent presoaked Whatman No. 4 filter paper, which had been previously equilibrated against the solvent system for at least sixteen hours. The solvent (80 percent CHCl₃ in Hexane) was permitted to ascend to the top of the plate (about thirty-five minutes).
After removing the plate from the tank, it was air dried and was then placed in another solvent (12 percent Methanol in Chloroform) saturated tank, the solvent was allowed to ascend to the middle of the plate; the plate was removed from the tank and air dried.

Organoleptic Difference Test

Fourteen students all majoring in some aspect of Foods and Nutrition or Food Science who were taking a course "Fundamentals of Food Flavor Analysis" during summer semester 1975 were asked to write down differences they detected between the two samples of saltine crackers. These included such properties as appearance, mouth feel, aroma, flavor including after taste.

Each person examined samples individually without communication. The identity of the samples was not divulged and the test made on two consecutive days.
RESULTS AND DISCUSSION

Determination of Carbonyl Content

Total carbonyls, dicarbonyls, ketoglycerides and monocarboxylics were measured by a modified Henick method (90). They were measured three times and averaged values were shown in Table III.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Experimental Cracker</th>
<th>Commercial Cracker</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μ mole / 500 g</td>
<td>%</td>
</tr>
<tr>
<td>Total Carbonyl</td>
<td>30.859</td>
<td>100</td>
</tr>
<tr>
<td>Dicarbonyl</td>
<td>28.539</td>
<td>92.482</td>
</tr>
<tr>
<td>Ketoglyceride</td>
<td>1.993</td>
<td>6.458</td>
</tr>
<tr>
<td>Monocarboxyl</td>
<td>0.327</td>
<td>1.060</td>
</tr>
<tr>
<td></td>
<td>6.470</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>4.511</td>
<td>69.722</td>
</tr>
<tr>
<td></td>
<td>1.536</td>
<td>23.740</td>
</tr>
<tr>
<td></td>
<td>0.423</td>
<td>6.538</td>
</tr>
</tbody>
</table>

The analysis of carbonyl compounds by this procedure may not reveal the total carbonyl picture. This is partly attributed to the un-eluted other classes of carbonyls, low molecular weight dicarbonyls, oxoacids and saponification products of ketoglycerides. The major advantage of this method gives an accurate measurement of monocarboxylics (above 90 percent recovery) and at the same time gives an approximate analysis of dicarbonyls and ketoglycerides.
Table III shows that experimental cracker contains almost five times greater total carbonyl than the commercial cracker. The major reasons for this may be:

1. Variation in cracker formula and procedure, especially in the levels of enzyme supplementation. Since both cereal and fungal amylase and protease were used in saltine cracker formulation, their activity affects the availability of free reducing sugars and amino acids which, through the Maillard non-enzymatic browning reaction during oven baking can produce carbonyl compounds, melanoidin pigments and other compounds.

2. Difference in fermentation time, temperature and humidity. The consequence of this difference causes a varied extent of dough development and a difference in flavor precursors (carbonyl compounds) produced after fermentation.

3. Addition of cracker flavol to the experimental cracker dough and subsequently two hours of dough fermentation may increase the amount of total carbonyl compounds. Since cracker flavol contains amino acids, one important reactant in the Maillard non-enzymatic browning reaction, accordingly, controls the level of total carbonyl compounds. Organic acid salts in cracker flavol acts as an acid-base catalyst in the reaction, accordingly, controls the level of total carbonyl compounds in a positive way.

4. Longer storage or more unfavorable storage conditions of baked products are generally responsible for the loss of fresh flavor of baked products. Experimental crackers were wrapped in polyethylene bags twenty minutes after baking and were analyzed within one week of storage at room temperature while the commercial crackers were
wrapped in wax paper and stored at room temperature for unknown
time.

According to Table III, except the fact that experimental cracker
contains approximately five times higher total carbonyls than commercial
cracker. In each sample, there is a gradual decrease in carbonyl
content in an order of dicarbonyl fraction, ketoglyceride fraction and
monocarbonyl fraction.

In both samples, dicarbonyls are the most predominant, contain
approximately 92 percent and 69 percent of total carbonyls in experi-
mental and in commercial cracker respectively; ketoglycerides are the
next predominant, contain approximately 6 percent and 23 percent of
total carbonyls in experimental and in commercial cracker respectively.

This decreasing order of each carbonyl may be due to their
increasing volatility of each fraction; that is, dicarbonyls are the
least volatile carbonyls, ketoglycerides are moderately volative car-
bonyls while monocarbonyls are the most volatile carbonyls. In other
words, the high temperature of oven baking during carmelization and
Maillard reaction may largely account for the above differences.

As far as the relative concentrations of each carbonyl fraction
in both samples are concerned, experimental crackers contain approxi-
mately six times more of dicarbonyl fraction, approximately 1.3 times
more of ketoglycerides fraction and approximately equal or slightly
less monocarbonyl fraction than commercial crackers. In other words,
content of each carbonyl fraction in experimental cracker is generally
higher than that in commercial cracker especially in dicarbonyl fraction.
However, the percentage of ketoglycerides and monocarbonyls fraction of
total carbonyls in commercial cracker is relatively higher than that in
experimental crackers.
Thus, quantitative differences do exist in total carbonyls and their constituents; they are dicarbonyls, ketoglycerides and monocarbonyls. The significance of these differences to cracker flavor cannot be answered by chemical analysis alone.

Purity and Distribution of Monocarbonyl Compounds

Thin-layer Chromatography was employed to investigate the purity and distribution of monocarbonyl components in a standard mixture as well as in two samples.

A standard mixture was composed of eleven alkanals (formaldehyde, acetaldehyde, propanal, isobutanal, pentanal, hexanal, heptanal, octanal, nonanal, decaanal and undecanal), eight ketones (acetone, butanone, pentanone, hexanone, heptanone, octanone, nonanone and undecanone) and six 2-alkenals (pentenal, heptenal, octenal, nonenal, decenal and undecenal), 2,4-alkadienals which is not commercially available.

A standard mixture was separated distinctively by using two solvent systems; 80 percent chloroform in hexane and 12 percent methanol in chloroform. The former solvent was permitted to ascend to the top of the plate (about thirty-five minutes). This solvent separated aldehydes from ketone components both of which have lower polarity in comparison with the enal components. After removing the plate from the solvent-saturated tank, the plate was air dried and was then placed in 12 percent methanol in chloroform system. The solvent was allowed to ascend to the middle of the plate. The purpose of using this solvent system was to separate the enal component which is more polar than ketones and aldehydes. Since the solvent was allowed to ascend only to the middle of the plate, ketone and aldehyde spots were located above the middle of the plate.
A clear separation among ketones, mixture of eight standard compounds, aldehydes, mixture of eleven standard compounds, and 2-alkenals, mixture of six standard compounds, in standard mixture is shown in Figure 1. Since the four classes show different colors on the finished plate, this aided greatly in differentiating one class from another. Methyl ketones were gray, saturated aldehydes were tan, 2-enals were rust red, and 2,4-dienals were lavendar (this class was lacking in standard mixture). Complete separation between ketones and aldehydes was difficult since they were similar in polarity. The characteristic colors of ketones and aldehydes alleviated this problem.

According to Schwartz et al. (95), Thin-Layer Chromatography has been used successfully for the rapid classification of an unknown 2,4-DNPH's, for a check in purity of cuts obtained from column chromatograms and for the rapid scanning a complex mixture obtained from natural sources. The following lower limits of detection were reported by Schwartz (89): methyl ketones, $3 \times 10^{-4}$ μmoles, alkanals, $2 \times 10^{-4}$ μmoles, 2-alkenals, $1.4 \times 10^{-4}$ μmoles, and 2,4-alkadienals, $1.4 \times 10^{-4}$ μmoles.

Carbonyl compounds extracted from commercial cracker and from experimental cracker were also applied to the plate after their repeated purification through ion-exchanger, magnesium column and aluminum columns. The distribution of carbonyl compounds extracted from commercial cracker was shown at C in Figure 1. Concentration of carbonyl compounds in experimental cracker was so low that could not be detected by Thin-Layer Chromatography. Also, the presence of shortening instead of lard renders the analysis much more difficult. In this investigation, three times more hexane was employed to elute fatty material from column
containing experimental cracker extracts than that employed in eluting fatty material from columns containing commercial cracker extracts. However, results cannot be conclusive, therefore, other methods would seemingly be required.

According to Schwartz et al. (96), more positive identification of methyl ketones, saturated aldehydes, 2-enals and 2,4-dienals may be achieved by running sample through a magnesia-celite 545 column. Separation of four classes of 2,4-DNPH’s is effected by using a series of solvents with increasing polarity. Separation could be monitored by a continuous flow analyzer using a 340-nm filter. The effluent was collected in 5-ml fractions using an automatic fraction collector. The tubes comprising a given peak were pooled for further analysis. Characterization of a class can be facilitated by checking the absorbance maximum in chloroform or hexane. The chemistry involved in color formation between adsorbent and 2,4-DNPH’s is not known yet but class separation suggests a specific interaction between the adsorbant and double bonds of 2,4-DNPH’s by formation of coordination complexes between unsaturated compounds or chromogenic compounds and several metal ions (97,98). Strict exclusion of water is essential both for separation of classes and for occurrence of the typical colors.

Thin-Layer Chromatography (TLC) and especially column chromatography were mainly employed in this study for analysis of aliphatic monocarbonyl compounds in saltine crackers. The approach is satisfactory, but the accuracy and sensitivity are questionable. Other sensitive and effective techniques must be employed.

Analysis of 2,4-DNPH’s by Gas-Liquid Chromatography (GLC) has been reported by Soukup et al. (99) and Kallio et al. (100) and Papa
1 KETONES
2 ALDEHYDES
3 2-ALKENALS

et al. (101). They reported that thermal decomposition and column deterioration occurred during chromatography. They also reported an analysis of 2,4-DNPH's by high-performance liquid chromatography (HPLC). The separating power was comparable to that attainable with GC and the estimated sensitivity was 5 nanogram (102). Mulders et al. (103, 104) in a study of volatile bread flavor components, GC was used to separate bread extract into fractions and compounds were identified by GC-MS. Carbonyl compounds were also extracted from powdered bread by several low boiling solvents, isolated by formation of derivatives and identified by TLC. Twenty-four carbonyl compounds were identified, nine of which had not been reported to occur in the aroma of white bread.

A GC-MS system is capable of recording spectra of full range flavor concentrates, head gas samples, and essences, separated on high efficiency GC columns; prefraction by preparative GC, TLC, or column chromatography is usually desirable to simplify these mixtures prior to GC-MS analysis.

According to Teranishi et al. (82), by combining the GC-MS system with computer analysis of data and searching of reference files, it is possible to identify rapidly large numbers of simple compounds which occur in these complex mixtures. The investigators can pursue more difficult structure problems after elimination of these compounds.

Sensory Evaluation by Difference Test

Twenty-eight response sheets were collected at the end of the comparison test. The responses were categorized according to sensory properties, responses were summarized in Table VI. It was surprising to find out that all fourteen panelists were overwhelmingly giving the same responses in appearance, aroma, mouth feel, flavor and after taste.
Results of this comparison test show a qualitative difference of sensory properties exist between the two samples. It also suggests that further improvement in sensory properties of experimental cracker is necessary and the level of cracker flavol (0.4–0.6 percent), obtained from chemical analysis can hardly compensate for commercial twenty-four hour sponge and dough fermentation. A flavor rating system employed by Johnson and Sanchez (105) to investigate flavor acceptibility of bread is highly recommended. By applying this system, it is possible to determine ingredient levels and relative ratios of Promate 200 (wheat gluten hydrolysate), and organic acid salts mixture contained in cracker flavol, to produce a cracker with good physical properties as well as rich flavor.

According to Johnson and El-Dash (106), flavor evaluation can be a very powerful marketing tool. Flavor may influence new product development, product and process improvement, processing, storage stability, and marketing.

There are a number of test methods for organoleptic flavor evaluation. However, choice of flavor evaluation tests depends on the object of evaluation.

Many methods, including threshold measurements, difference tests and descriptive tests have been used. It is reasonable to assume that if a substance is present above its threshold, it has significance; but sensory thresholds are not invariant. These values can be influenced by many physiological and psychological factors. Interpretation of threshold data is further complicated by the fact that some compounds modify an odor even if they are present below threshold value. Additive effects, mixture of two or more components each below threshold may produce a detectable odor.
TABLE VI
FLAVOR PROPERTIES OF EXPERIMENTAL CRACKER AND COMMERCIAL CRACKER
EVALUATED BY FOURTEEN SEMI-TRAINED PANELISTS

<table>
<thead>
<tr>
<th>Experimental Cracker</th>
<th>Commercial Cracker</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Appearance</strong></td>
<td></td>
</tr>
<tr>
<td>1. Unevenly browned</td>
<td>1. More evenly browned</td>
</tr>
<tr>
<td>on top and bottom</td>
<td>on top and bottom</td>
</tr>
<tr>
<td>2. Rocky salt topping</td>
<td>2. Fine salt topping</td>
</tr>
<tr>
<td><strong>Aroma</strong></td>
<td></td>
</tr>
<tr>
<td>Salty, doughy (Starchy)</td>
<td>Toasted grain odor</td>
</tr>
<tr>
<td><strong>Mouth feel</strong></td>
<td></td>
</tr>
<tr>
<td>Sticky to mouth</td>
<td>Not sticky</td>
</tr>
<tr>
<td>(Does not moisten readily)</td>
<td>(Moistens quickly)</td>
</tr>
<tr>
<td><strong>Flavor</strong></td>
<td></td>
</tr>
<tr>
<td>1. Pale color</td>
<td>1. Brown color</td>
</tr>
<tr>
<td>2. Salty</td>
<td>2. Slightly salty</td>
</tr>
<tr>
<td>3. Less crispy</td>
<td>3. Crispy</td>
</tr>
<tr>
<td>4. Doughy (Starchy)</td>
<td>4. Toasted grain aroma</td>
</tr>
<tr>
<td><strong>After taste</strong></td>
<td></td>
</tr>
<tr>
<td>Salty and sweet</td>
<td>1. Sweet</td>
</tr>
<tr>
<td></td>
<td>2. Baked</td>
</tr>
</tbody>
</table>
In a study of odor intensities, Guadagni et al. (107) employed the concept of the odor unit. One odor unit is the olfactory threshold concentration. The ratio in a given sample of the actual concentration of a given compound to the threshold concentration of the compound represents the number of odor units contributing by that component. This approach enables one to estimate the contribution of a compound to the overall aroma in terms of odor rather than in terms of concentration.

To describe and recognize an isolates' characteristic aroma quality is also very important, some compounds exhibit different odor qualities at different concentrations. They may have one "character" at threshold levels and quite a different one at suprathreshold concentrations. It is therefore important to learn whether or how odor characteristics change with increasing concentrations of a given pure compound.

All investigation of relationships between molecular structure and olfactory quality are fundamentally dependent on an assurance that the compound of interest has "olfactory purity." That is, that any impurities which may be present have no effect on the odor of the compound. Use of threshold determinations to measure attainment of "constant threshold values" after repeated purification steps can be a powerful tool for achieving olfactory purity.

Although human judgments always provide the ultimate test of sensory quality, particularly in terms of pleasantness, unpleasantness, and overall acceptability, it is very desirable that objective analyses be developed which can take their place. It is much easier to calibrate and learn the accuracy, precision, sensitivity, reproducibility and
reliability of instruments than to establish these same characteristics for people. Thus, correlation of objective techniques with sensory techniques of flavor and odor evaluation is much to be desired.
SUMMARY AND CONCLUSIONS

Investigation of carbonyl compounds present in commercial crackers and in experimental crackers was made.

Ten batches of experimental crackers made by the straight dough procedure, using two hours dough fermentation at 84-86°F and 88-90 percent relative humidity. After fermentation, the dough was sheeted 0.031" thick, the crackers were baked for 3 to 3-3/4 minutes at 510°F. The crackers were randomly sampled and compared to commercial crackers purchased locally.

Carbonyl compounds present in saltine crackers were extracted with carbonyl-free chloroform and hexane. The carbonyl compounds in the extracts were converted to 2,4-dinitrophenylhydrazone (2,4-DNPH's) in a celite reaction column. After eluting them from the column, they were purified through a AG-50W-X4, a strongly acidic cation exchanger. The total carbonyl compound derivatives were separated into dicarbonyls, ketoglycerides and aliphatic monocarbonyl fractions on magnesia-celite 545 and alumina columns, respectively. The recovery of aliphatic monocarbonyl fractions was approximately 90 percent.

The average content of total carbonyls, dicarbonyls, ketoglycerides and monocarbonyl fractions were measured by a modified Henick method using a Beckman DB-G Grate Spectrophotometer. Quantitative differences were found among total carbonyls, dicarbonyls, ketoglycerides and monocarbonyls between two samples.

The purity and distribution of aliphatic monocarbonyl compounds were studies by Thin-Layer Chromatography (TLC) on magnesia-celite 545.
plate. Two solvent systems were employed; 80 percent chloroform in hexane and 12 percent methanol in chloroform. The distribution of ketones, aldehydes, 2-enals and 2,4-dienals can be identified if the carbonyl compound extracts were pure and standard compounds of 2,4-dienal were available.

In order to correlate chemical analysis with flavor properties, sensory evaluation by comparison test was employed. Flavor properties such as appearance, aroma, mouth feel, flavor and after taste were found better in commercial cracker than in experimental cracker. A further improvement of experimental cracker is necessary and a flavor rating system employed by Johnson and Sanchez was recommended.

Great advancement in chemical identification of flavor compounds has been made by using powerful analytical tools, while the problems relating to the physiological basis of odor and taste detection and of the psychophysiology of flavor evaluation are far from solved.
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FLAVOR CHARACTERISTICS OF SALTINE CRACKERS

by

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AN ABSTRACT OF A THESIS

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The primary purpose of this study was to compare the carbonyl compounds present in two different kinds of saltine crackers: one was commercial cracker with twenty-five hours fermentation and another one was experimental cracker produced from the experimental baking laboratory, Department of Grain Science and Industry, Kansas State University, Manhattan, with Flavol mixture additives (a blend of starch carrier, promate 200 and organic acid salts mixture) and a minimum of two hours fermentation.

The carbonyl compounds were extracted with carbonyl-free chloroform and hexane. The extracts of carbonyl compounds were converted to 2,4-dinitrophenylhydrazones (2,4-DNPH's) in a celite reaction column. After eluting them from the column, they were purified through a AG-50W-X4, a strongly acidic cation exchanger. The total carbonyl compound derivatives were separated into dicarbonyls, ketoglycerides and aliphatic monocarbonyl fractions on magnesia-celite 545 and alumina columns, respectively. The recovery of aliphatic monocarbonyl fractions was approximately 90 percent.

The average content of total carbonyls, dicarbonyls, ketoglycerides and monocarbonyl fractions were measured by a modified Henick method using a Beckman DB-G Grade Spectrophotometer. Quantitative differences were found among total carbonyls, dicarbonyls, ketoglycerides and monocarbonyls between two samples. Commercial saltine cracker contains 6.470 μmoles of total carbonyls, 4.511 μmoles of dicarbonyl fraction, 1.536 μmoles of ketoglyceride fraction and 0.423 μmoles of monocarbonyl
fraction per 500 grams of crackers, while experimental cracker contains 30.859 μ moles of total carbonyls, 28.539 μ moles of dicarbonyl fraction, 1.993 μ moles of ketoglyceride fraction and 0.327 μ moles of monocarbonyl fraction per 500 grams of crackers.

The purity and distribution of aliphatic monocarbonyl compounds were studied by thin-layer chromatography on magnesia-celite 545 plate. Two solvent systems were employed: 80 percent chloroform in hexane and 12 percent methanol in chloroform.

In order to correlate chemical analysis with flavor properties, a sensory evaluation by comparison test was employed. Panelists responses show that further improvement of the experimental cracker is necessary and a flavor rating system employed by Johnson and Sanchez was recommended.