# Role of Nonvolatile Compounds in Bread Flavor

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Bread flavor is a mingled, unitary experience of the consumer which includes sensation of smell, taste, and often texture, mechanical eating quality, color, warmth, cold, and mild pain. No single substance is responsible. Many flavor compounds react together synergistically, additively, or subtractively, and relative proportions of bread constituents, volatile and nonvolatile, influence bread taste. The components of the bread formula, except sugar and salt, contribute relatively little to the taste of white bread compared to the effect of microbial action during fermentation and the thermal effects of baking. Interaction between reducing sugars and free amino groups during baking produces a large number of nonvolatile and volatile compounds which contribute to bread flavor.

Interest in bread flavor probably is as old as the history of baking. That interest has grown in recent times is due to three major factors: development of a science of baking, progress in chemistry and research techniques, and bread's rapid perishability and loss of flavor.

Bread firms and becomes progressively stale during storage, resulting in a much less appealing product. This has forced bakers to remove stale bread from store shelves after the second or third day in spite of economic loss. Flavor stabilization is thus important to the baking industry. Modern bread production methods are believed to produce less flavorful bread. Many other economic and technological factors contribute to a need for research on bread flavor.

Recently, a new and urgent aspect has been added to flavor research as a result of the world's population explosion. The United Nations has stated that the most important single world food need is for an increase in proteins containing the essential amino acids. Whether these proteins are derived from fish, meat, algae, or by chemical means, to contribute nutritional value they must be eaten. Not only must new foods be created but they must have acceptable flavor. UNICEF has taken the lead in introducing new foods to hungry people, but the problem of acceptance of those new products must be solved through education and improved flavor. Fifty to 80% of total food intake in most developing and underdeveloped countries consists of bread; so bread is of prime importance in introducing new sources of proteins. Improving bread flavor has significant value in overcoming world hunger and malnutrition.

The true role of all compounds related to bread flavor is still unknown. Many volatile and nonvolatile compounds in bread serve as flavor stimuli. This paper discusses the contribution of nonvolatile compounds to bread flavor.

Johnson and El-Dash (1967) defined bread flavor as a complex phenomenon involving psychological and physiological senses. Flavor of bread includes the total sensations experienced by the consumer, such as aroma, taste, warmth, freshness, appearance, and mechanical eating qualities. Aroma is described as an olfactory experience resulting from a stimulation of olfactory receptors in the nasal passages by volatile material and unaffected by action of other senses of the skin and mouth. Taste receptors, by contrast, are located in the mouth, activated by a large variety of chemical compounds in solution, and nonvolatile compounds, in particular.

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Flavor of bread is a function of both processing and ingredients.

## ROLE OF PROCESSING IN BREAD FLAVOR

The complex chemical and enzymatic reactions occurring during fermentation and baking give rise to a number of volatile and nonvolatile organic compounds which with certain physical and chemical changes contribute to the flavor of the finished product. The importance of both fermentation and baking to formation of bread flavor stimuli was clearly shown by Baker and Mize (1939) and Baker *et al.* (1953), who found that neither an improperly fermented dough baked with crust formation, nor properly fermented dough baked without crust formation, has acceptable flavor. Many compounds produced during fermentation and baking are flavor stimuli in their own right. When added to chemically leavened bread in any combination, however, these compounds are unable to produce the balanced unique flavor of white bread created by yeast fermentation (Schuldt, 1967). Thomas and Rothe (1960) found crust-forming conditions essential to full flavor development. They concluded that substances arising directly from fermentation play but a small part in the complex of flavor compounds. Cornford (1962), using organoleptic tests, found no difference in bread flavor whether bread was produced mechanically without fermentation or by a normal fermentation process. Collyer (1966) considers that the products of fermentation contribute little or no flavor to bread.

The term "bread" usually implies a product produced by yeast fermentation. Wiseblatt (1957) baked bread from a yeasted dough not given time to ferment but leavened by chemical means. Such bread had acceptable grain and texture, but was deficient in taste and aroma.

Metabolic activities of yeast during fermentation produce some aromatic substances which may differ in nature, depending upon the substrate. Many of these products are relatively unstable and may be transformed into other flavor compounds and/or volatilized in later stages of fermentation and baking. The main end products of normal dough fermentation are ethanol, carbon dioxide, and various nonvolatile and volatile organic acid and carbonyl compounds. The role of these volatile compounds in bread flavor is considered elsewhere (Johnson *et al.*, 1966; Rooney *et al.*, 1967; Salem *et al.*, 1967; Smith and Coffman, 1960; Wiseblatt, 1961).

The flavor constituents of dough resulting from fermentation may be affected by several factors, including duration of fermentation, temperature, sugar concentration, salt concentration, strain of yeast, and bacteria. Bread produced by the sponge dough method has better flavor than that produced by the straight dough method. Differences in fermentation probably are chiefly responsible for flavor differences between the two types of bread. Long fermentation time produces greater quantities of acetic and lactic acid and is generally regarded as producing more flavorful bread. Lüers (1949) reported that overfermented dough in which sugars were depleted produced bread of poor flavor compared with dough which allowed adequate Maillard reaction during baking. Sugar added to the overfermented dough rectified this failing, but only if the action of yeast had not used the free amino compounds in the dough. Analysis showed that aromatic substances were present in smaller amounts when overfermentation had depleted the sugars. The amount of fermentation products remaining after baking depended on the temperature reached by the interior of the loaf during baking. Morimoto (1966) studied the changes in free amino acids in sponges and doughs during fermentation and baking and reported that free amino acids decreased markedly during a 6-hour period of straight dough fermentation. Lüers (1949) stated that with higher fermentation temperatures and longer fermentation periods, there is more amino acid degradation leading to the formation of higher alcohols and esters, and concluded that the fermentation process, if carried too far, could lead to loss of flavor.

Yeast varieties differ markedly in their ability to produce acids, alcohols, and esters. Therefore, yeast genera, species, and variety are important in determining some of the final bread flavor constituents. The role of microorganisms other than yeast in bread flavor has been examined by many investigators. Robinson *et al.* (1958) stated that several bacteria enhance bread flavor. Carlin (1968) used bacteria-free yeast and found that such bread had much less flavor than normal yeast bread, suggesting that supplementation with the right microorganisms might improve flavor. In particular, *leuconostic* and strains of lactic acid bacteria were favorable to flavor. A buttery and sometimes pleasant flavor emerges through use of a particular microbial fermentation. However, comment by baking technologists reported by Schuldt (1967) was: "It tastes interesting but it doesn't taste like bread." Several investigators are looking further for a yeast strain which can enhance bread flavor.

Crust-forming conditions are essential for full flavor production in spite of the fact that yeast metabolism can produce most of the suspected bread flavor compounds. Thomas and Rothe (1960) concluded that substances arising directly from fermentation actually play a part in the complex of flavor compounds by forming preconditions necessary for their subsequent formation under influence of oven heat. Baker *et al.* (1953) demonstrated that bread cooked without the formation of a brown crust possesses little desirable bread flavor and stated that crust formation is necessary for a desirable brown crust taste and development of bread aroma. Bread flavor is no doubt formed in the oven and under the influence of heat. Upon its completion, the mingled unitary experience of bread flavor results.

When dough is placed in an oven at proper temperature, the exterior layer turns pale yellow, amber, orange, brown, and finally dark brown to form the brown crust layer. Actual crust temperature is reported not to exceed 150° C., while loaf interior temperature does not exceed 95° C. During crust formation, water vapor and other gas pressures increase inside the loaf and force it to expand. Resulting effects are smooth side breaks, large volume, soft texture, and brown crust. During crust formation several organic compounds are formed, some lost and carried away with oven gases, and

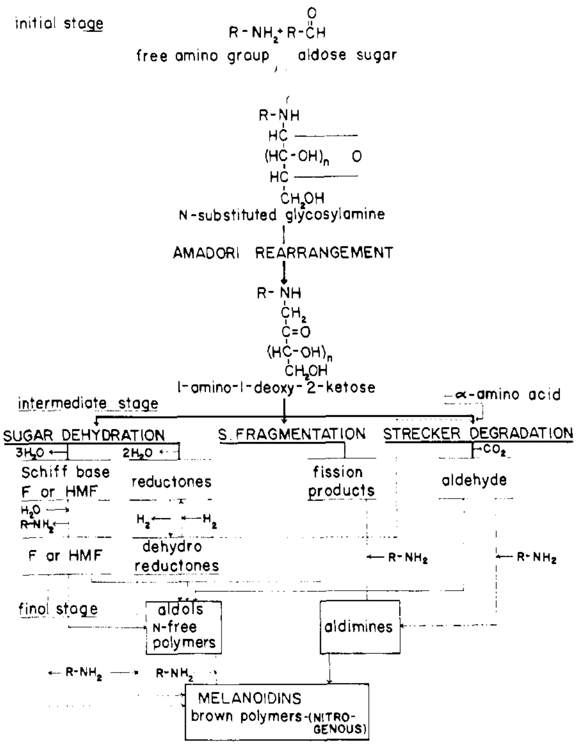


**Figure 1. Formation of brown pigments in bread crust**

others trapped within crust structure and cells. Melanoidins remain to bring about crust color and unique bread crust taste.

High polymer products (melanoidins) and carbonyl compounds produced in nonenzymatic browning reaction are thought to have high significance in bread flavor. Many investigators now believe that the nonenzymatic browning reaction involves condensation of reducing sugars and free amino groups and is the most single important reaction responsible for bread crust formation and flavor. Caramelization of sugar appears to play a nonsignificant role in bread flavor compared to nonenzymatic browning, because of the low temperature required for the latter reaction to take place. The nonenzymatic browning reaction takes place through three stages (Hodge, 1953). The initial stage involves sugar amino acid condensation, followed by an Amadori rearrangement. The intermediate stage involves sugar dehydration, fragmentation, and amino acid moiety degradation. There is little or no color development at this stage. The final stage involves polymerization and formation of heterocyclic nitrogen compounds and is characterized by dark brown colors of varying intensity.

The interrelationship of these reactions is shown in Figure 1.

## ROLE OF COMPONENTS IN BREAD FLAVOR

LIPIDS. Mecham (1964) stated that relatively little is known of changes that may occur in flour lipids beyond the dough mixing stage. Improved bread flavor was reported by Kleinschmidt *et al.* (1963) upon addition of the products of lipoxidase action on certain fat components to dough. Intensity of flavor was described as wheaty or nutty and was proportional to degree of peroxidation. However, it was not known whether this effect was direct or indirect through other chemical reactions during the baking process. Fats or oils added to bread formula can serve as flavor carriers (Otterbacher, 1959), make crust more tender and crumb softer, and contribute richness to taste. Desirable carbonyl compounds can also arise from oxidation of fats during baking (Collyer, 1964).

SALT. Salt taste is a function of both cation and anion. An absolute correlation between taste and physical and chemical properties of salt has not been established, but, in general, low molecular weight salts are salty and high molecular weight salts cause a taste shift to bitter. NaCl is the most widely used salt in the baking industry. Salt is added to the bread formula, to impart and enhance bread taste. It also exerts a controlling effect on enzymes and on microbial functions during fermentation, and strengthens and tightens the dough gluten proteins.

SUGARS. Sugars in bread dough come from three sources: originally present in the flour; produced from oligosaccharides or polysaccharides by enzyme action during fermentation; and dough ingredients intentionally added. Sugars are added chiefly to obtain the desired sweetness in baked products, to maintain the fermentation process at a desirable level, and to bring about the most distinct flavor of baked products through the reaction with free amino groups in the nonenzymatic browning reaction.

Johnson (1952) believes that sweetness in bread may not be important as far as intrinsic flavor is concerned. While survey of public opinion may indicate preference for bread of higher residual sugar, this may not be necessarily associated with sweetness but rather with the total intensity of flavor substances in the bread resulting from accumulation of fermentation and baking product flavor compounds. The residual sugar content of bread depends on the quantities and kinds of sugars added in the formula, kind and amount of alpha-amylase present in the dough and fermentation, and the baking process. Rice (1938) and Bohn (1954) observed larger quantities of maltose than glucose and fructose in bread made by straight dough, while bread made with sponge dough contained less maltose.

Barham and Johnson (1951) studied the effect of sugar levels up to 10% on bread crumb firmness and reported that a minimum of crumb firmness after 3 days' storage was observed with 2 to 4% sugar for glucose, fructose, and sucrose; as the sugar level was increased above 4%, the crumb became increasingly firm. None of the sugars decreased the rate of firming upon storage. Several other investigators (Ewald and Perlin, 1959; Johnes, 1955) have shown that the sugars have only a slight effect upon retention of bread crumb softness during storage. On the other hand, the shelf life of sugar cookies could be extended as much as 70% by adding 5% dextrose to the formula (Griffith and Johnson, 1957). Longer shelf life was attributed to formation of reductones, the products of nonenzymatic browning reaction which may function as fat antioxidants.

Production of bread flavor substances by caramelization and Maillard browning reactions has been cited. Although less attention has been given to the caramelization reaction in bread flavor, many investigators now believe the Maillard browning reaction is more important for flavor formation.

Haney (1952) used methylated derivatives of glucose at the 5% level in the cookie formula. Browning and flavor production were inhibited, compared to cookies made with 5% glucose, which were brown and had a distinct flavor. Pomeranz *et al.* (1962) followed the rate of browning of 20 sugars and derivatives with glycine or lysine in a diluted buffer solution heated at 114° C. in an autoclave. Pentoses were most reactive, followed by hexoses and reducing disaccharides. Sugars without a reducing group failed to show browning. Arabinose, ribose, and xylose imparted a dark brown color to the bread crust (Rubenthaler *et al.*, 1963). Salem *et al.* (1967) reported that the crust of bread made with xylose–amino acids was darker than that of bread made with glucose–amino acids because of the high reactivity of xylose in browning. Lactose and melibiose produced a darker crust than any of the other disaccharides (Rubenthaler *et al.*, 1963). Linko *et al.* (1962) observed that increase of the initial sucrose concentration in bread formula increased production of hydroxymethylfurfural in the crust. Raffinose in sugar cookies and in dilute sugar solution produced little browning, but imparted a dark color to bread crust. This seems to result from change of the trisaccharide by yeast to fructose and melibiose, both of which darken the color of bread crust. Menger (1962) studied the reaction between the soluble carbohydrates of durum wheat and the nonenzymatic browning of wheat pastes, and suggested that browning colorations in pastes prepared from durum wheats were the results of condensation products involving the soluble carbohydrates.

STARCH. The bread staling phenomenon is a good example of direct effect on senses other than taste and smell—i.e., texture and mechanical eating quality—of bread flavor and consequently consumer acceptance or preference.

The staling process represents a series of slow changes in crust and crumb layers. Staling of the crust as distinguished from the crumb of bread is of great importance in the unsliced loaf. Fresh bread has a dry and crisp crust. Slowly with movement of moisture from the interior of the loaf and with absorption of water vapor from the surrounding atmosphere, crust becomes tough and leathery. Staling of bread crumb is attributed to physiochemical changes within the starch and dextrins. At the moisture level of white bread, the amorphous structure of starch is transformed into a more crystalline form with attendant firming of structure resulting in a significant change in bread texture and mechanical eating quality.

Herz (1965) reported that starch is mainly responsible for changes observed in bread staling; changes in the gluten might exert a noticeable effect beginning a few days after baking. Gilles *et al.* (1961) and Prentice *et al.* (1954) concluded that firming and other physical changes in bread on staling are caused by retrogradation of branched molecules of the starch.

Only fragmentary work has been done on changes in taste and aroma of baked bread after the loaf leaves the oven. A variety of views have been expressed to explain loss of aroma and flavor upon staling. Some researchers believe aromatic substances are lost by volatilization or possibly by chemical reaction such as oxidation. Freshness of bread, however, may be partially renewed by the simple process of heating. Volatilization or oxidation may take place in case of volatile aromatic substances and could be considered partially responsible for decrease of aromatic compounds upon aging. Schoch (1965) maintained that linear molecules of amylose form helical complexes with flavor elements, rendering them insoluble and hence imperceptible to taste buds. Upon heating, the complex is dissociated and flavor elements are released and become available to the taste buds. Schoch's suggestion may be valid in the case of aromatic substances consisting mainly of relatively low molecular weight molecules affecting the sense of smell. However, that taste-affecting elements of high polymers and sugars form a complex with amylose is probably unlikely.

Changes in texture and mechanical eating quality of bread may be attributed to physical changes in amylopectin molecules within the swollen granules of starch. In fresh bread, amylopectin molecules (branched molecules) are extended as much as swelling of starch granules will permit, giving starch granules, and consequently the crumb, a soft and extensible texture. In staling, amylopectin molecules gradually associate with each other and fold up within the swollen granules, resulting in a slow hardening of the crumb (Schoch, 1965). Such physical change is reversible by heating.

PROTEINS AND AMINO ACIDS. The rate of Maillard reaction is dependent on several factors, including the concentration of free amino groups and reducing sugars. However, results obtained by adding amino acids to the bread formula are rather uncertain and none too clear except for proline. Rooney *et al.* (1967) concluded that because no component seems to predominate in determining bread aroma, a mixture of amino acids would be most likely to give an acceptable bread flavor. The ratio of amino acids required for acceptable bread flavor is critical and is not known. Pence (1967) recently reported that chemists are yet uncertain about the relative importance of amino groups in doughs in production of bread flavor stimuli.

The type of amino acid affects the kind of carbonyl compounds formed during reaction with sugar, whereas sugar type influences the amount of carbonyl compounds in model systems (Rooney *et al.*, 1967). The same conclusion was reached by Salem *et al.* (1967) using bread. They stated that the type of amino acid significantly affected bread aroma and addition of amino acids to bread formula increased the content of total carbonyl compounds in bread crust but only slightly in bread crumb. Kiely *et al.* (1960), in an early report, said that sugar influenced the rate of the Maillard reaction, but odor was controlled by the amino acid. Moreover, addition of amino acids increases the intensity of crust color (Salem *et al.*, 1967; Zenter, 1961). Increasing free amino groups by means of proteolysis in the dough markedly increased crust color and intensified bread aroma but did not influence bread taste (El-Dash and Johnson, 1967).

Little information is available on the kind and amount of bread aroma stimuli produced by the Maillard reaction with different amino groups. Chichester *et al.* (1952) found 24 different compounds produced by the reaction of glycine with glucose. Linko *et al.* (1962) and Salem *et al.* (1967) reported an increase of acetone and formaldehyde in the crust upon addition of glycine, while lysine increased the concentration of all carbonyl compounds in bread crust approximately three- to fourfold over the control. Lysine-fortified bread has a darker crust and slightly different flavor (Collyer, 1964). Lysine at levels recommended for bread enrichment did not affect the loaf volume of standard white bread, but crust browning increased with increasing lysine concentration (Zenter, 1961). On an equimolar basis, lysine was essentially equal to glycine on its effect on crust browning. Linko *et al.* (1962) added xylose and leucine to bread dough. The bread crust contained several times the quantity of 3-methylbutanal present in the control bread crust, but 3-methylbutanal of the crumb was not affected. These results were confirmed by Salem *et al.* (1967). An odor resembling that of crackers was obtained by reaction of triose sugars with isoleucine (Wiseblatt and Zoumut, 1963). This product, after oxidation, appeared to be identical with a substance isolated from stale bread. Another possible influence on bread flavor is glutamic acid. Among the various amino acids, only L-glutamate has been known to have a characteristic activity as a flavor potentiator (Kuninaka, 1967). (Flavor potentiators are compounds having no sensory effect in small quantities but intensifying effects of other agents.) The amount of free glutamic acid in bread is extremely small, and therefore it is likely that glutamic acid as a flavor potentiator in bread is without great significance.

Proline has little effect on the crust color (Rooney *et al.*, 1967; Salem *et al.*, 1967). Wiseblatt and Zoumut (1963) reported that a bread flavor constituent was produced by reaction of proline and dihydroxyacetone. Reaction of proline with reducing sugars has been also shown to produce compounds having a distinct bread-like or cracker-like aroma (Wiseblatt and Zoumut, 1963). When proline was added to bread formula, bread aroma appeared to be increased (Wick *et al.*, 1964).

Sulfur-containing amino acids of flour and yeast proteins were reported to produce methanethiol during baking (Collyer, 1964). While phenylalanine, tyrosine, and serine were lower in concentration in bread than in unbaked constituents, losses of amino acids, particularly phenylalanine, tyrosine, and lysine, were greater in crumb. Addition of alanine or valine to the formula increased the acetone content as well as Strecker degradation aldehydes in crust. Histidine increased the content of acetone and acetaldehyde three to four times in crust over the control (Salem *et al.*, 1967). Kiely *et al.* (1960) stated that a distinct bread-like aroma was produced by the reaction of leucine, histidine, or arginine with glucose. These workers suggested that addition of leucine and arginine or histidine improved the flavor of chemically leavened bread.

Very little information is available on the effect of proteins on bread flavors. Bertram (1953) was one of the first to show that certain Dutch low-protein flours producing gray-like bread crust were notably improved by addition of gluten protein or egg albumin. Wick *et al.* (1964) added a selected group of amino acids, proteins, and sugars to dough and evaluated their effect on bread flavor by organoleptic tests. However, the control bread flavor was superior. Stenberg and Geddes (1960) studied browning of wheat gluten, starch, and sucrose mixtures stored in sealed tin cans at 70° C. for long periods of time. They found 17 acidic and 13 neutral carbonyl compounds in the brown extract. It has been suggested that the water-insoluble proteins (the gliadin and glutenin) are more important than free amino acids as browning reactants in breadmaking (Horvat *et al.*, 1962). The present investigation was undertaken to study the relative importance of proteins in formation of bread aroma.

## MATERIALS AND METHODS

A straight bread dough consisting of flour, water, 2% salt, 3% sugar, and 2% compressed yeast was fermented for 3 hours at 30° C. At the end of the fermentation, 100 grams of dough was dispersed in 300 cc. of 70% ethanol with an Omni-mixer for 3 minutes at 6000 r.p.m. The suspended dough was designated as fraction I. A portion of the suspension was cooled to 3° to 5° C. and centrifuged for 50 minutes at 4500 r.p.m. to remove the insoluble protein and starch. The clear ethanol extract was concentrated under vacuum and contained ethanol-soluble amino acids, peptides, or proteins (fraction II). Kjeldahl analysis of fraction I showed it to contain 6.25% ethanol-soluble proteins and 7.95% insoluble proteins (dry basis), while fraction II contained 14.47% ethanol-soluble protein (dry basis). No attempt was made to fractionate the flour dough proteins according to classical definitions of albumins, globulins, gliadin, or glutenin. It was recognized that the 70% ethanol extract would contain most of the free amino acids and the gliadin proteins, and that fraction I contained the entire range of wheat proteins and free amino acids.

Starch beds were prepared by making a starch paste consisting of 50% water with purified wheat starch. The paste was placed in 8-inch aluminum dishes and comparable nitrogen levels of fractions I and II were poured over the starch beds. The starch beds were baked in an oven at 221° C. for 28 minutes, as was the control loaf of bread. The aroma, color, and carbonyl compounds were estimated for each fraction compared to the control bread, to establish whether any free amino acids or gliadin proteins were responsible for development of flavors in baked bread.

Crust color was determined by measurement with a Photovolt reflectometer, with a white standard of 100 and a black standard of zero. An organoleptic panel of five members was instructed to rate the aroma of the baked products containing fractions I and II with the loaf of bread as a standard. Carbonyl compounds were extracted from the baked starch containing fractions I and II and bread crust with carbonyl-free chloroform. 2,4-Dinitrophenylhydrazine (2,4-DNPH) derivatives were formed by refluxing chloroform extracts with 1% 2,4-DNPH solution. Gas-liquid chromatography of the isolated carbonyl compounds was performed with an Aerograph A90-p instrument equipped with a Model A-500B hydrogen flame ionization detector and a 5-mv. Speedomax,

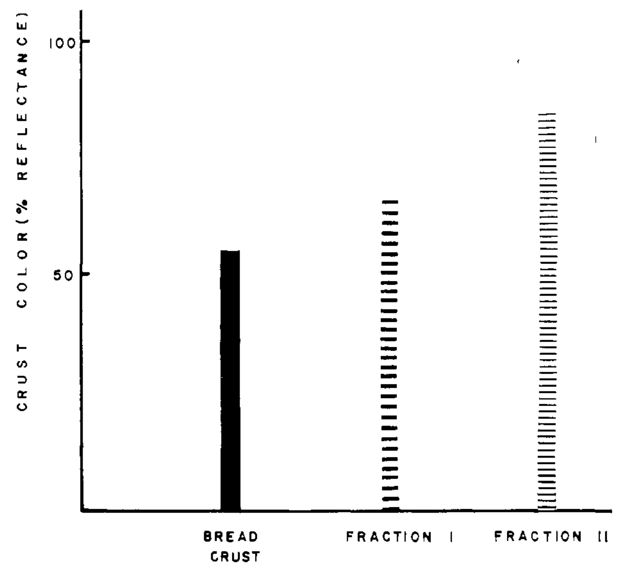


**Figure 2. Effect of different protein fractions on color development**

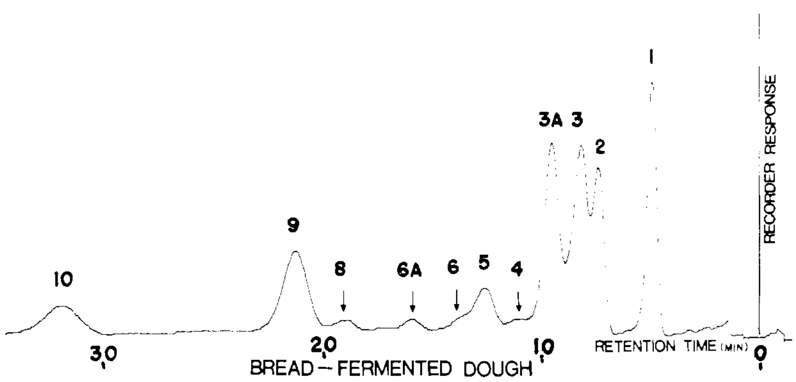


**Figure 3. Carbonyl compounds formed in bread crust**

Column temperature 100°C., injection port temperature 200°C., helium flow rate 37.5 ml. per minute, helium pressure 8 p.s.i.g., hydrogen pressure 17 p.s.i.g.

1. Ethanal
2. Propanal
3. 2-Methylpropanal and 2-propanone
3A. Unknown
4. Butanal
5. 2-Butanone
6. 2-Methylbutanal and 3-methylbutanal
6A. Unknown
7. 2,3-Butanedione
8. Pentanal
9. 2-Methylpentanal
10. Hexanal

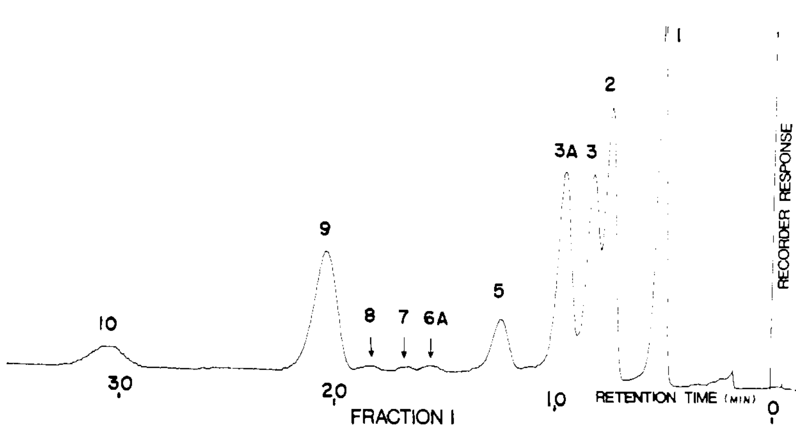


**Figure 4. Carbonyl compounds formed in fraction I**

For explanation see Figure 3

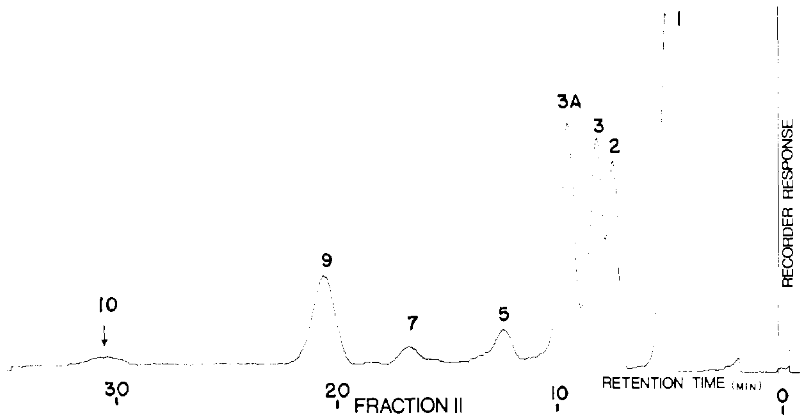


**Figure 5. Carbonyl compounds formed in fraction II**

For explanation see Figure 3

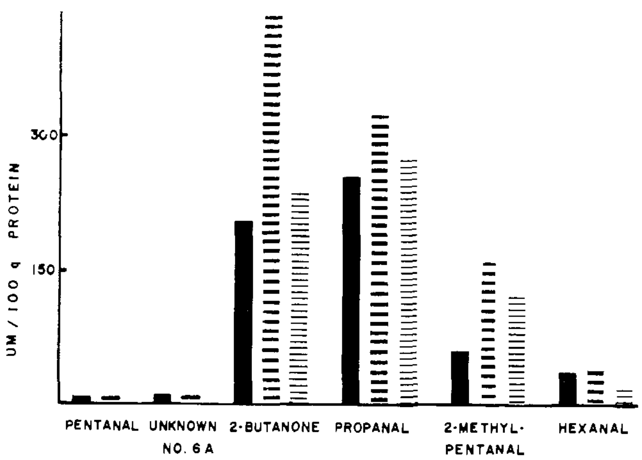


**Figure 6. Effect of different protein fractions on production of carbonyl compounds**

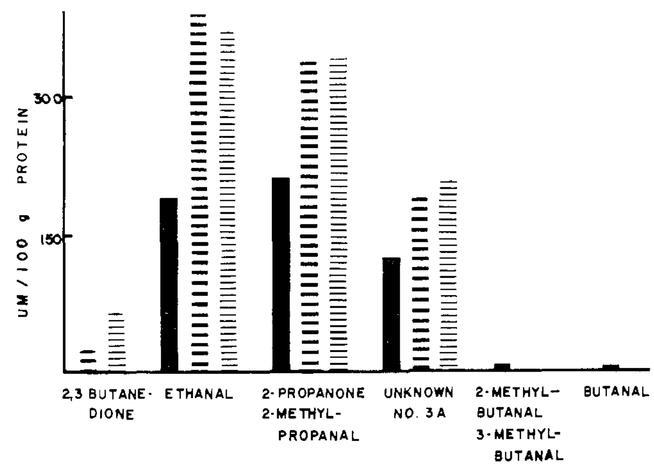


**Figure 7. Effect of different protein fractions on production of carbonyl compounds**

Model s-600 recorder. A stainless steel column (10-foot × $^{1}/_{8}$-inch i.d.) packed with 60- to 80-mesh Chromosorb-P coated with 20% Carbowax 20-M was used. The modified flash-exchange technique of Stephens and Teszler (1960) was used to release the aldehydes and ketones upon the reaction of 2,4-DNPH derivatives with alpha-ketoglutaric acid at 250° C. for 30 seconds in a capillary tube connected to the injection port of the instrument.

## RESULTS AND DISCUSSION

Figure 2 shows per cent reflectance of the three baked samples. Reflectometer values are inversely related to color intensity. Bread crust had the darkest color and baked starch with fraction II was most lacking in brown pigments. Baked starch with fraction I was intermediate.

The flavor panel evaluated fractions I and II as inferior to bread in aroma. However, the aroma of fraction I was judged as similar to that of pie crust or biscuits and, in general, similar to baked products but not bread-like aroma. Fraction II was judged as completely lacking bread aroma and unacceptable.

Figures 3, 4, and 5 are chromatograms of the carbonyl compound analysis of the baked bread crust, baked starch with fraction I, and baked starch with fraction II. The chromatograms indicate a predominance of carbonyl compounds in bread crust. Butanal, 2-methyl butanal, and 3-methylbutanal were absent in baked starch with fraction I, while baked starch with fraction II was lacking in butanal, 2-methylbutanal, 3-methylbutanal, unknown 6A, and pentanal. These data indicate that ethanol-insoluble proteins significantly affected the carbonyl compound formation during the baking process.

Carbonyl compounds formed as micromoles per 100 grams of protein as well as the relative molar ratio of carbonyl compounds to ethanol are given in Table I. Figures 6 and 7 show graphically the amount of carbonyl compounds produced in the three fractions. These data suggest that formation of carbonyl compounds as related to its protein precursors may be classified into four groups: Carbonyl compounds formed from ethanol-insoluble proteins include only pentanal and unknown 6A; the carbonyl compound formed mainly from ethanol-soluble proteins was 2,3-butanedione; carbonyl compounds formed mainly from ethanol-insoluble proteins included 2-butanone, 2-methylpentanal, hexanal, and propanal; carbonyl compounds formed from both ethanol-soluble and nitrogen-insoluble proteins included ethanal, 2-propanone, 2-methylpropanal, and unknown 3A.

Substituting ethanol-insoluble proteins for soluble proteins and free amino acids resulted in a decrease of carbonyl compound formation, lack of bread aroma, and lack of crust color development. In conclusion, ethanol-insoluble proteins appeared to have a significant effect on production of bread aroma stimuli and formation of crust color.

Treatment of the flour proteins with 70% ethanol increased formation of carbonyl compounds over the control, as shown

Table I. Effect of Protein Fractions on Production in Carbonyl Compounds

| Compound No. | Carbonyl Compd. | Bread Crust | | Fraction I | | Fraction II | |
|---|---|---|---|---|---|---|---|
| | | μmoles[a] | RMR[b] | μmoles[a] | RMR[b] | μmoles[a] | RMR[b] |
| 1 | Ethanal | 186.9 | 100.0 | 385.6 | 100.0 | 366.1 | 100.0 |
| 2 | Propanal | 149.5 | 79.9 | 315.1 | 81.7 | 269.4 | 73.6 |
| 3 | 2-Methylpropanal | 207.5 | 111.0 | 333.9 | 86.6 | 338.5 | 92.5 |
| 3A | Unknown | 120.2 | 64.3 | 187.4 | 48.6 | 203.8 | 55.7 |
| 4 | Butanal | 3.4 | 1.8 | ... | ... | ... | ... |
| 5 | 2-Butanone | 201.7 | 107.9 | 430.5 | 111.6 | 232.4 | 63.5 |
| 6 | 2-Methylbutanal 3-Methylbutanal | 7.4 | 3.9 | ... | ... | ... | ... |
| 6A | Unknown | 9.2 | 4.9 | 8.2 | 2.1 | ... | ... |
| 7 | 2,3-Butanedione | Trace | ... | 20.6 | 5.3 | 62.9 | 17.2 |
| 8 | Pentanal | 5.5 | 2.9 | 5.4 | 1.4 | ... | ... |
| 9 | 2-Methylpentanal | 68.6 | 36.7 | 158.0 | 40.9 | 120.2 | 32.8 |
| 10 | Hexanal | 33.1 | 17.7 | 37.9 | 9.8 | 15.8 | 4.3 |

[a] Micromoles per 100 grams protein on a dry basis.
[b] Relative molar ratio.

in Figures 6 and 7. This may be explained by the effect of ethanol on the conformation of the albumin and globulin proteins (Singer, 1962). Treatment with 70% ethanol may have caused exposure of additional amino groups which could react with sugars to create more intense crust color, aroma, and carbonyl compound production during the baking process.

Much knowledge must be gained before a thorough understanding about the source of flavor in bread is achieved. Present facts appear to establish that bread flavor is a very complex matter but that variation in bread flavor is related to formulation, fermentation, and baking. Formulation and fermentation influence the final bread flavor which is created during the final baking process. Nonvolatile substances may influence bread flavor directly or indirectly by their effect on the fermentation or baking process.

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