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REVIEWS

Enzymes and Bread Flavor†

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Enzymes used in breadmaking and their influence on bread flavor are reviewed. Components of bread flavor originate from fermentative and thermal reactions. Enzymes provide precursors for both processes, and their influence should be controlled. Enzymes are now replacing other improving additives in breadmaking, indirectly affecting bread flavor. Amylases produce reducing sugars, which are (1) fermentable substrates for fermentative microflora leading to numerous aromatic compounds, those of lower volatility remaining in bread, and (2) precursors of many components (mainly carbonyls) after reacting with amino acids in nonenzymatic browning reactions. Proteases produce peptides and amino acids, which, like sugars, participate in metabolic and thermal reactions and can occasionally be a source of bitter peptides. Lipoxygenase from soy or faba flour, used in some breadmaking processes, gives unstable products decomposing to carbonyl compounds generating off flavors in bread. In addition, the ingredients, the breadmaking process, and the baking conditions modify enzymatic activity and bread flavor.

Keywords: *Bread flavor; enzymes; fermentation*

INTRODUCTION

Flavor is one of the most appreciated sensory attributes in bread (Caul, 1972), the term comprising the total sensation experienced by the consumer: aroma and taste perceptions, tactile sensations (freshness, masticability) in mouth (Caul, 1972; El-Dash, 1971), and even, according to some authors, external aspects (El-Dash, 1971). Bread flavor, qualitatively described as discrete and subtle (Drapron and Richard-Molard, 1979) and very appealing to a great number of consumers (Coffman, 1965), is composed of a large number of components, many of them with very distinctive olfactive characteristics (Drapron and Richard-Molard, 1979). As with other foods, none of them can singly be considered the key component of bread aroma (Coffman, 1965; Drapron and Richard-Molard, 1979), but they seem to act in a synergistic way, with their relative proportions being determinant (Drapron and Richard-Molard, 1979). On the other hand, the presence of a determined substance in bread does not mean that it participates in flavor; however, the concentrations must exceed the detection threshold (Johnson et al., 1966), which, in turn, can be modified by other substances present. Even today, bread flavor remains as a challenge for scientists. Characteristic features of bread flavor can not be assigned to any of compounds identified, but they appear to result from the combination of

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chemical constituents found in bread crumb (Rothe and Thomas, 1959; Richard-Molard et al., 1978; Martínez-Anaya et al., 1990), and until now, comparisons between chemical and sensory aspects have not reached definitive conclusions (Hironaka, 1986; Richard-Molard et al., 1978).

Industrial enzyme preparations have been used for a long time in breadmaking. Most of them have a role in flavor formation, often as a secondary effect. Rates of utilization of exogenous enzymes by the breadmaking industry have sharply increased in the last years, as they are replacing other more controversial additives such as oxidants or emulsifiers (Van Hartingsveldt, 1995; Mutsaers, 1996). The enzyme industry is producing a large number of new products having new and/or improved technological effects. Trends point toward the use of complex mixes of enzymes, which can be composed of amylases, lipases, oxidases, proteases, and/or xylanases; they act on different flour components in a single or synergistic way (Si, 1996; Mutsaers, 1996). Although their improving effect, mainly on bread volume, texture, and some rheological characteristics, has been described, there are no studies evaluating the influence of these interactions on bread flavor, although expected consequences can be important to the consumer. This review aims to summarize existing information on the main enzymes involved in bread flavor, the processes in which they participate, the nature of bread flavor, and the factors influencing flavor characteristics.

DISCUSSION

Origin of Bread Flavor. According to Jackel (1969), bread flavor results from four categories of factors: ingredients, fermentation, degradations, and thermal reactions. Ingredients used in breadmaking, mainly flour, have peculiar aromatic characteristics, but they need to undergo several changes in order to produce the complete flavor (Jackel, 1969). Flour also furnishes a small amount of volatile compounds and aroma precursors, although their participation in bread aroma is estimated to be small (Drapron and Richard-Molard, 1979).

Fermentation of sugars by yeasts leads to a large number of volatile compounds that are supposed to be responsible for distinctive characteristics associated to bread flavor. This is evidenced by the fact that bread from unfermented doughs has a different aroma from that produced with fermented dough (Jackel, 1969). Lactic acid bacteria, of paramount importance in long fermentation processes (Spicher, 1983) and common also in yeast-leavened products (Robinson et al., 1958; Calvel, 1981), strongly enhance sensory flavor.

Mechanical and enzymic degradations are necessary to eliminate the starchy residual taste of flour, which can be partly obtained by partial starch gelatinization and by adequate relaxation of gluten (Jackel, 1969). Thermal reactions, including caramelization and nonenzymatic browning during baking, promote crust flavor and color (Drapron and Richard-Molard, 1979; Jackel, 1969).

Fermentation and baking are the main sources of the flavor of bread (Baker et al., 1953; Coffman, 1965), and both steps are essential (Baker et al., 1953). Volatile compounds are generated from previous precursors present in ingredients or resulting from enzymatic or mechanic degradations (El-Dash, 1971; Drapron and Richard-Molard, 1979). Most of identified compounds come from two categories of precursors: sugars and amino acids (El-Dash, 1971; Spicher and Nierle, 1988).

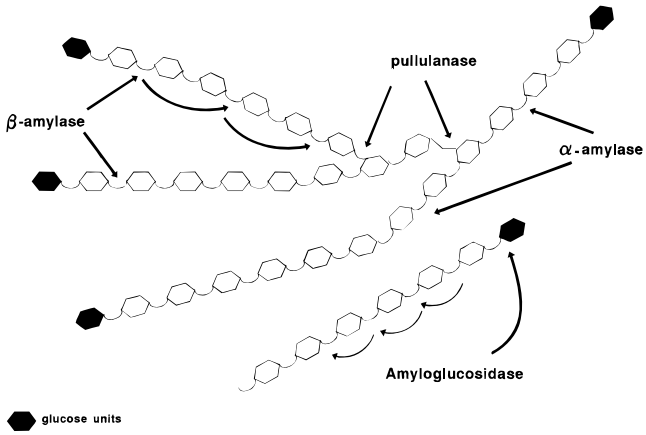

Figure 1. Enzymatic hydrolysis of α-1,4-linkages of starch chains by amylases (α, β) and amyloglucosidase and α-1,3- or α-1,6-linkages by debranching enzymes (pullulanase) (information from Stauffer, 1994a,b).

Part of the crust flavor, when the loaf leaves the oven, permeabilizes to the whole piece, or at least to the zones near the crust (Johnson et al., 1966; Drapron and Richard-Molard, 1979). During cooling and resting, bread undergoes rapid flavor losses due to oxidation and volatilization (Johnson et al., 1966; Coffman, 1965). However, the restoration of bread aroma after reheating leads to the assumption that some aroma components can be trapped (blocked) into the linear structure of starch (Coffman, 1965).

Enzymes Involved in the Formation of Bread Flavor. Three sources of enzymes can be considered in breadmaking: those existing in flour, those associated with metabolic activity of yeasts and lactic acid bacteria, and those intentionally included in formulation. Enzymes are of paramount importance in generating bread flavor. Their more or less intensive action can result in positive or negative effects on desirable bread flavor (Drapron and Richard-Molard, 1979). Enzymic activities can be more intensive in one or another breadmaking stage, but all of them start when flour is hydrated during mixing and proceed in a steady way until temperature during baking degrades protein structure. Three main enzymic systems are accounted for in breadmaking, related to bread flavor: amylases (mainly α and β), proteases, and lipoxygenases, although other secondary activities (invertase, oxidases, lipase) can contribute to a lesser extent. Direct role of enzymes in bread flavor only can be expected from the formation of flavoring peptides; their main role is to produce precursors direct or indirectly (the most likely) related to flavor-forming processes. These precursors, the reactions in which they participate, the products that they originate, their implications in flavor, and the changes due to processing variables will be discussed in subsequent sections.

Amylases. Amylases act by hydrolyzing α-1,4-glycosidic bonds of starch, amylose, amylopectin, and, at a lower rate, maltodextrins and oligosaccharides (Figure 1). β-Amylase has a saccharifying action, producing β-maltose units from nonreducing ends of chains and small amounts of β-maltose. It acts upon gelatinized starch, being unable to split sound starch and slowly acting on starch damaged by milling. α-Amylase is a dextrinizing enzyme, acting at random on gelatinized and damaged starch, even that not attacked by β-amyl-

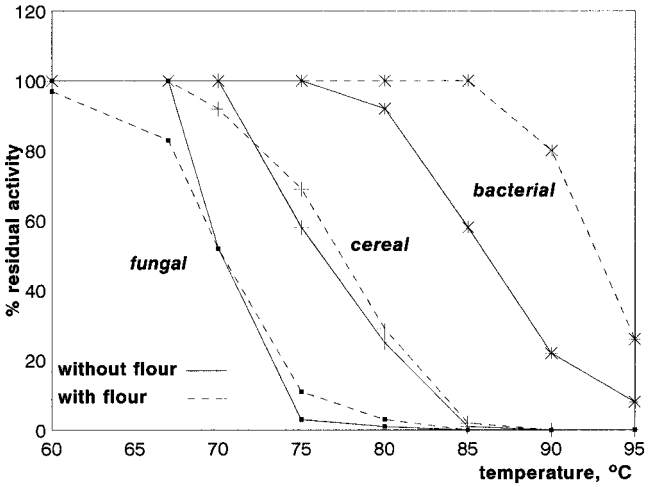

Figure 2. Thermostability of α-amylases.

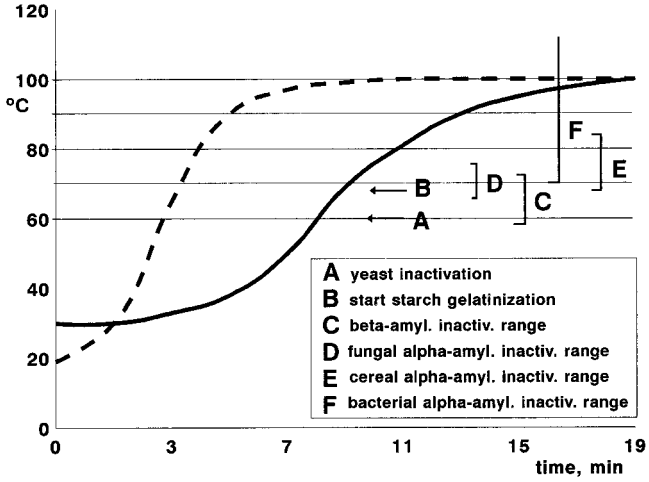

Figure 3. Critical period of amylase activity during baking.

ase (Pomeranz and Finney, 1975; Stauffer, 1994a,b). Unsprouted wheat flour contains adequate levels of β-amylase and low quantities of α-amylase. Production of the amounts of sugars necessary for fermentation relays on an equilibrate action of both enzymes or on the external supply of sugars. Amylase addition is a common practice in breadmaking, affecting dough consistency and fermentative activity and leading to changes in oven transformations, bread color and flavor, and overall quality (Pomeranz and Finney, 1975). Exogenous amylases considerably differ in thermostability, an essential property during baking (Figure 2). Fungal amylases are less stable to heating than those from cereal sources, being that of bacterial origin the most thermostable. The length of the critical step in baking, from β-amylase to α-amylase inactivation (Figure 3), marks the time during which the latter acts upon partly gelatinized starch, wholly susceptible to the attack. Sugar and dextrin production during this period is thus variable and, if excessive, will result in a too dark crust of tart flavor and a sticky and chewy crumb, as is the case of some bacterial amylases (Pomeranz and Finney, 1975); maltogenic amylases of intermediate thermostability are lately being used as antistaling agents (Si, 1995, 1996; Valjakka et al., 1994).

Residual glucose and maltose from amylase action, as well as the effect of enzyme concentration, depend on amylase origin (Beck et al., 1957). Sugars included in formulation also influence amylase activity (Pomeranz and Finney, 1975). On the other hand, type and

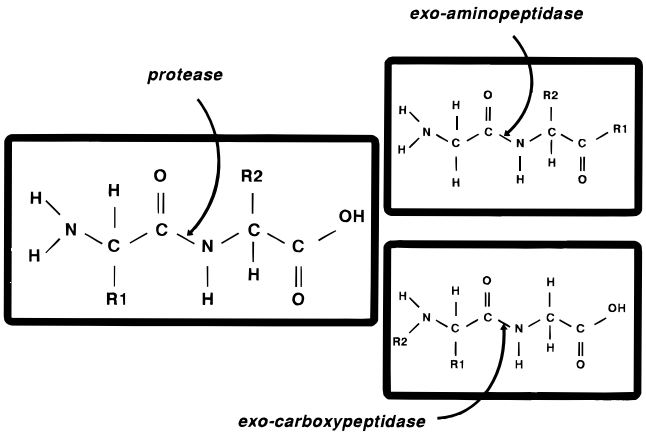

Figure 4. Hydrolytic action of proteases on peptidic bonds of proteins and peptides: (left) endo- and (right) exo-actions.

quantity of sugars produced will influence the rate of formation of specific compounds during fermentation, as well as the residual sugars that will participate in browning reactions during baking (Maga, 1974).

Proteases. Proteases hydrolyze peptidic bonds in proteins, polypeptides, and peptides in an endo- or exo-action (aminopeptidases, carboxypeptidases) (Figure 4), producing oligopeptides, peptides, and amino acids. Sound flour has low proteolytic activity, but flours can be infested by pests in field, resulting in proteolytic activity excessively high and of very difficult control. Proteases are used in breadmaking in order to regulate physical dough properties and bread quality (Keiffer et al., 1990; Rashed et al., 1990). Dough becomes more extensible and develops at a faster rate. Proteases are added to very strong flours or when special characteristics of pliability and extensibility are required (Faridi and Johnson, 1978), and they must be carefully controlled because an excessive amount weakens the dough and lowers gas-retaining ability. Dose should be experimentally adjusted by recording the desired rheological changes (Keiffer et al., 1990; Rashed et al., 1990). The addition of proteases also increases amino acid and peptide levels; the former are intermediate products in the production of aromatic components (Desmazeaud, 1983; Baker et al., 1953), whereas the latter are potential oxidants, taste enhancers, sweeteners, and bitter agents (Baker et al., 1953). The type of protease can influence the kind of carbonyl compounds produced (El-Dash and Johnson, 1967), but the controlled addition improves bread aroma (El-Dash, 1971), providing that they are free of lipases, which would generate off flavors (Baker et al., 1953).

Lactic acid bacteria used in breadmaking processes can have a proteolytic activity of their own. In general, it suffices to produce the necessary amino acids for growth (Gilliland, 1985). Proteases excreted by fermentative microorganisms are considered less significant than those of fungal sources added (Faridi and Johnson, 1978). Proteolytic activity is a quality inherent to strain, not to species, often being of exopeptidase character, liberating low molecular weight compounds (Tourneur, 1972). Many strains possess membrane-bound activity to facilitate transport into the cell and endocellular activity of wide specificity, both of peptidase type (Tourneur, 1972; Lawrence et al., 1976). Extracellular proteinase activity is less frequent (Lawrence et al., 1976), and it usually has certain degree of specificity (Gilliland, 1985). It is generally accepted that participation of lactic acid bacteria to the overall

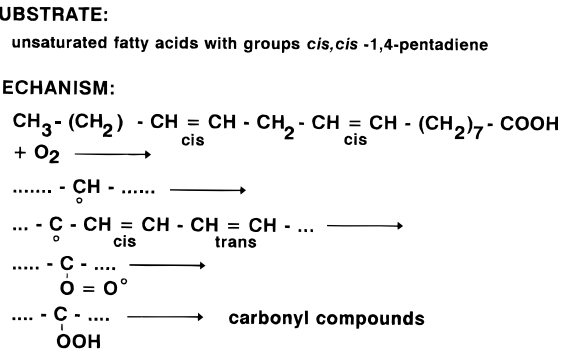

Figure 5. Summary of oxidative action of lipoxygenase. Active oxygen on unsaturated fatty acids leads to the production of free radicals that induce the formation of peroxides and hydroperoxides.

proteolysis of the process is limited, mainly when using whole wheat or rye flour, that contains very high protease activity (Kratochvil and Holas, 1984; Rouzaud, 1994; Collar et al., 1991), but they can bring noticeably qualitative effects (Rouzaud, 1994) due to their specificity.

Lipoxygenases. Wheat flour contains very little lipoxygenase activity that is located in germ and bran, but it is abundant in soy and faba beans and peas. Some traditional processes use small amounts of soy or faba flours to bleach flour, improve dough machinability, and increase dough volume (Drapron and Richard-Molard, 1979). Lipoxygenase oxidizes unsaturated fatty acids containing *cis,cis*-1,4-pentadiene groups (linoleic, linolenic, and araquidonic acids) in the presence of molecular oxygen (Figure 5). The oxidative reaction leads to the production of free radicals, which induce peroxide and hydroperoxide formation that destroys carotenoid pigments, oxidizes sulfhydryl groups of proteins to disulfide groups, and decomposes to carbonyl compounds (Stauffer, 1994a,b). Multiple action of lipoxygenase results in (1) bleaching of flour and dough giving whiter crumb bread, (2) strengthening of gluten structure increasing its mixing tolerance and producing loaves of better volume and texture, (3) production of carbonyl compounds that influence bread flavor, and (4) destruction of liposoluble vitamins (provitamin A) and essential fatty acids.

Other Enzymes. Yeast invertase hydrolyzes sucrose and oligofructans from flour. Splitting ability of the enzyme toward these substrates decreases with increasing molecular weight (Nilsson et al., 1987). Its action produces fructose and glucose that are used as substrates for fermentation and in browning reactions.

Microbial lipases from endogenous microflora of flour or proceeding from commercial enzyme preparations induce changes in lipid composition and production of short chain fatty acids (Lawrence et al., 1976; Galal et al., 1978). Lipases with specific activities are recently being used as potential improvers in breadmaking; overdosage should be avoided to prevent generation of off flavors (Si, 1995).

New enzyme preparations consisting of oxidases (glucose oxidase, sulfhydryl oxidase) intended to strength the gluten network can lead to further oxidations with undesirable effects on flavor. Other hydrolyzing enzymes (xylanases, hemicellulases, pentosanases) in expanding use nowadays have no effects on components related to bread flavor; however, they are usually used in conjunction with other enzymes (amylases, oxidases, and/or lipases), and synergistic effects on technological properties have been observed (Si, 1995, 1996). Their possible implication in bread flavor has not been considered and will require complementary research.

Fermentation Process. *Carbohydrate Fermentation*. Bread dough fermentation is a complex phenomena, involving several fermentative processes. Alcoholic and lactic fermentation are the only desirable ones, and the ones likely to happen under correct processing conditions. Predominance of one or the other, or their simultaneity, depends on the breadmaking process and the microbial starter used. Alcoholic fermentation predominates in white bread made from commercial yeast. Lactic fermentation is common in sourdough processes, made from wheat and rye flours. Peculiarities from each type of processes and their particular advantages on bread characteristics have been summarized elsewhere (Lorenz, 1981; Oura et al., 1982; Spicher, 1983; Martínez-Anaya, 1994).

Yeasts. Flour sugars, these intentionally included in formulation and that coming from amylolytic degradation of starch, are potential substrates for yeast fermentation (Pomeranz and Finney, 1975). Flour contains very low amounts of sugars, about 1.55−1.84% (0.19−0.26% sucrose, 0.07−0.10% maltose, 0.01−0.09% glucose, 0.02−0.08% fructose, and 1.26−1.31% oligosaccharides (fructosans and maltooligosaccharides)), but amylase activity starting during mixing increases maltose levels 10−15-fold its initial value (Pomeranz and Finney, 1975). Yeast species common in breadmaking have saturated kinetics for hexoses and maltose, and all possess α-glucosidase and β-fructosidase (invertase) (Antuña and Martínez-Anaya, 1993). Hexose transport occurs by an active metabolism-bound mechanism (Pomper, 1969), whereas the maltose transport system is inducible (Harris and Thompson, 1960a,b; Hautera and Lövgren, 1975; Lövgren and Hautera, 1977; Siro and Lövgren, 1979); the synthesis of the maltose permeases is the limiting step in maltose fermentation (Pomper, 1969). Glucose and fructose act to repress the induction of maltose permeases, glucose being more effective (Lövgren and Hautera, 1977; Siro and Lövgren, 1979).

Glucose and fructose are fermented by yeast at the same rate, but glucose is consumed faster than fructose when both sugars are present at similar levels (Koch et al., 1954). Sucrose is hydrolyzed about 200 times faster than the resulting hexoses are fermented, and it is not detected after mixing. Maltose accumulates at first and only starts to be used when the adaptative transport system is induced, this occurring when levels of monosaccharides are low (Koch et al., 1954; Lee and Geddes, 1959; Oura et al., 1982). When yeasts becomes adapted to maltose fermentation, this sugar is used as quickly as the hexoses, in spite of their concentration.

Metabolism of hexoses by yeasts usually follows the glycolytic or Embden−Meyerof−Partnas (EMP) pathway (Prescott and Dunn, 1981). Under aerobic conditions pyruvate degrades through the Krebs or tricarboxylic acid (TCA) cycle (Prescott and Dunn, 1981), but under limited oxygenation, as in the case of bread dough, fermentation occurs and carbon dioxide and ethanol molecules are produced from glucose, with lower energetic efficiency (Pomper, 1969).

Lactic Acid Bacteria. Sugars used by lactic acid bacteria as energy sources vary within species and even strain. The most frequent lactic acid bacteria identified in sourdoughs are able to ferment pentoses (Savola et al., 1982), hexoses, and the disaccharides sucrose and

tyrosine	tirosol
triptophane	triptofol
phenylalanine →	β -phenylethanol
valine	2-methyl-1-propanol
leucine	1-pentanol
alanine	ethanol

Figure 6. Amino acid precursors in alcohol production (from information given by Maga, 1974).

maltose (Savola et al., 1982; Martínez-Anaya et al., 1989; Antuña and Martínez-Anaya, 1993), although some species, such as *Lactobacillus sanfrancisco*, usual in the San Francisco sourdough French bread process, are specific for maltose (Saunders et al., 1972). Lactic acid bacteria common in doughs have saturated kinetics for hexoses and present linear incorporation of sucrose and maltose, and all of them have α-glucosidase and β-fructosidase (Antuña and Martínez-Anaya, 1993). Maltose transport uses the phosphotransferase phosphoenolpyruvate dependent system (PTS−PEP) (Antuña and Martínez-Anaya, 1993); however, some species use a maltose phosphorylase to facilitate maltose uptake (Wood and Rainbow, 1961; Lohmeier-Vogel et al., 1983).

Homofermentative strains use the EMP pathway, but heterofermentative strains, lacking aldolase and triose phosphate isomerase, use the hexose monophosphate path (Prescott and Dunn, 1981; Spicher and Stephan, 1987). Lactic acid bacteria produce lactate from pyruvate by a lactate dehydrogenase (Oura et al., 1982). Lactate is the main product of homolactic fermentation, while heterolactic fermentation gives, in addition, small amounts of formic and acetic acids, ethanol, and carbon dioxide (Stamer, 1979). Oxygen or proton acceptors (such as fructose) increase acetic acid production at the expense of ethanol in heterolactic strains (Lawrence et al., 1976; Martínez-Anaya et al., 1994). Limited amounts of glucose (Montville et al., 1987) or growth of cells in maltose (Lohmeier-Vogel et al., 1983) causes a shift in metabolic pathways from homolactic to heterolactic fermentation, leading to the subsequent changes in the composition of volatile byproducts.

Metabolism of Nitrogen Compounds. Amino acids stimulate growth and activity of yeasts and lactic acid bacteria (Gilliland, 1985). Membrane-bound peptidases hydrolyze extracellular peptides to facilitate transport across the cell wall, allowing their assimilation, after transformation in amino acids by endocellular peptidases, usually of wide specificity (Lawrence et al., 1976). Mechanisms for amino acid utilization are based on the loss of one or two carbon atoms with aldehyde formation, which can afterwards be oxidized to acids or reduced to alcohols (Spicher, 1983; El-Dash, 1971; Johnson et al., 1966; Watanabe et al., 1990; Drapron and Richard-Molard, 1979). Some high molecular weight alcohols produced from amino acids by this mechanism are shown in Figure 6 (Drapron and Richard-Molard, 1979).

Metabolism of Other Substrates. Lactic acid bacteria can anaerobically metabolize other substrates, such as citrate, fumarate, gluconate, malate, 2-oxoglutarate, and pyruvate, producing flavor-related compounds (acetoin, diacetyl, and 2,3-butyleneglycol) (Radler and Brohl, 1984; Cogan, 1980). Wheat and rye flours contain small amounts of these substrates (Spicher, 1983). Neither yeasts nor lactic acid bacteria produce diacetyl from acetoin; in fact, some microorganisms that form acetoin are unable to produce diacetyl (Collins, 1972; De Cárdenas et al., 1980). Both groups of microorganisms produce diacetyl by the same mechanism, which differs from that used to form acetoin (Collins, 1972; De Cárdenas et al., 1980).

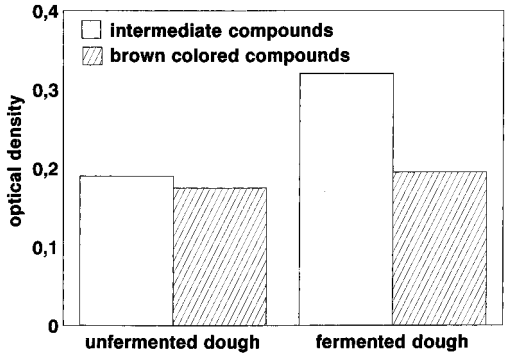

Figure 7. Influence of fermentation on the intensity of nonenzymatic browning reactions during bread baking (results from El-Dash, 1971).

Bread Baking. The rate of enzymatic reactions rises during the first minutes of baking, mainly that coming from α-amylases, whose intensity depends on enzyme thermostability. This results in the enhanced production of reducing sugars and dextrins by action on partly gelatinized starch; final concentrations vary with the length of the critical period (Figure 3). The most thermolabile enzymes (amylases from cereal or fungal sources, proteases, lipoxygenases, and others) are inactivated by heat, and its action is limited during this step.

After protein denaturation, browning of crust starts. Two main thermal reactions occur, in which participate amino acids and/or sugars remaining in the dough surface: nonenzymatic Maillard and caramelization reactions. Heating causes sugars and, in a lesser extent, polysaccharides, particularly starch, to be transformed to colored degradation products, as well as to numerous volatile products, carbonyl type, and furfurals. The caramelization process is less understood than Maillard reactions, and it is difficult to distinguish the contribution of each reaction to crust aroma; however, it is widely accepted that nonenzymatic browning reactions are predominant (Drapron and Richard-Molard, 1979).

Reducing sugars and free amino acids form an addition compound that transforms in a Schiff's base; this isomerizes in N-substituted glycosamine, which undergoes an Amadori rearrangement. A fission reaction produces carbonyl compounds, and a dehydration process conducts to the formation of furfurals and dicarbonyl components; after oxidation to dehydroreductones, the Strecker degradation produces aldehydes having the structure of the initial amino acid (Baker et al., 1953; Johnson et al., 1966; El-Dash, 1971; Drapron and Richard-Molard, 1979; Kaminski et al., 1981). Thus alanine results in acetaldehyde production, glycine in formaldehyde, isoleucine in 2-methylbutanal, leucine in isovaleraldehyde, methionine in methional, phenylalanine in phenylacetaldehyde, threonine in 2-hydroxypropanal, and serine in glyoxal (Johnson et al., 1966). This degradation essentially constitutes the step generating crust aroma, more so than carbonyl proceeding from fermentation or from hexanal liberated by lipoxygenase action (Drapron and Richard-Molard, 1979). Compounds formed from furfurals or 1-amino-1-deoxy-2-ketose fission have a lesser quantitative importance in aroma (Drapron and Richard-Molard, 1979).

The decrease of pH produced during fermentation seems to positively influence browning reactions, mainly the formation of intermediate components (Figure 7),

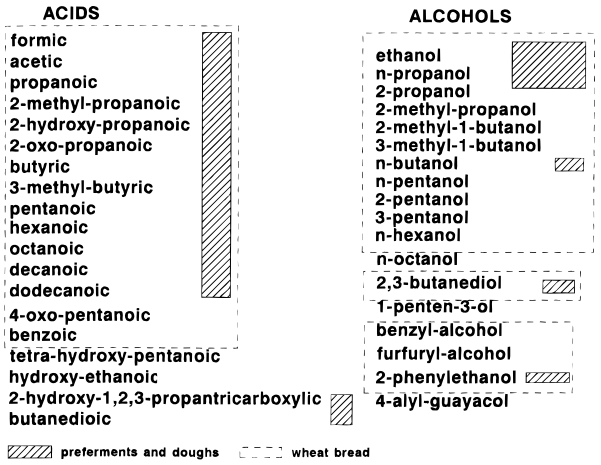

Figure 8. Summary of compounds identified in preferments, doughs, and breads: acids and alcohols (information from Maga, 1974; Schieberle and Grosch, 1984, 1985, 1986). (Compounds not marked have been found in rye products.)

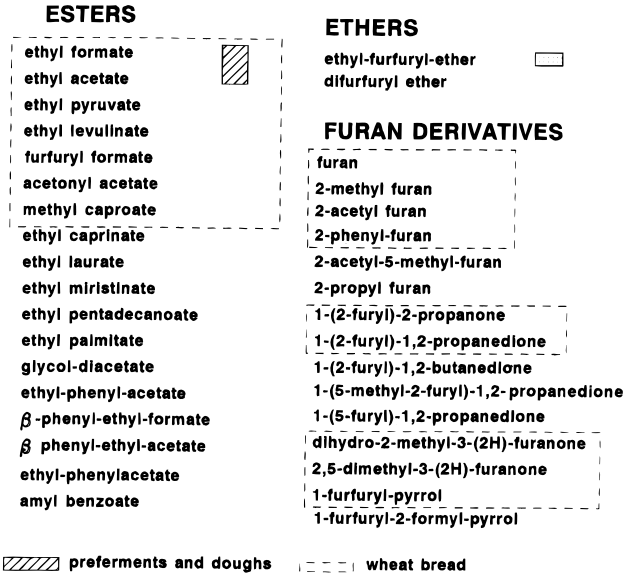

Figure 9. Summary of compounds identified in preferments, doughs, and breads: esters, ethers, and furan derivatives (information from Maga, 1974; Schieberle and Grosch, 1984, 1985, 1986). (Compounds not marked have been found in rye products.)

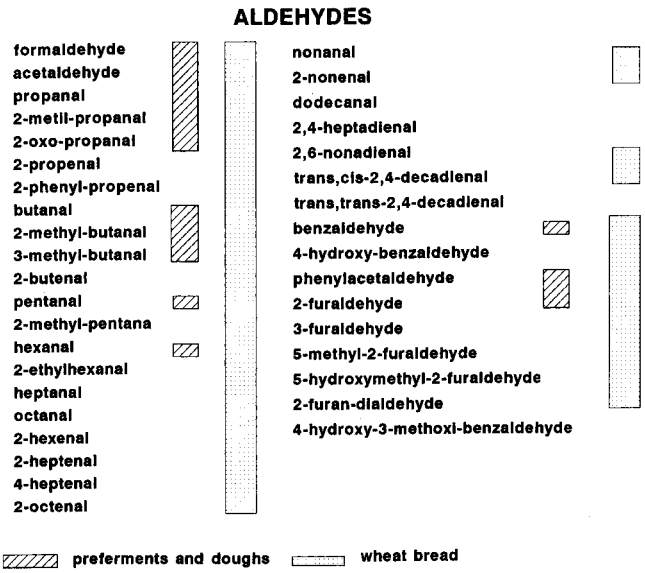

Figure 10. Summary of compounds identified in preferments, doughs, and breads: aldehydes (information from Maga, 1974; Schieberle and Grosch, 1984, 1985, 1986). (Compounds not marked have been found in rye products.)

probably because Amadori rearrangement requires a H^+ from the medium to start the process (Johnson et al., 1966; El-Dash, 1971).

The total amount of amino acids and reducing sugars and their relative proportions on the surface of dough during baking are the limiting factors in crust aroma quality. Both depend on their formation from enzymatic activities and/or their involvement in metabolic processes. Intensity of reaction depends on temperature and moisture in the oven (Drapron and Richard-Molard, 1979; Kaminski et al., 1981). The type of aroma is influenced by the amino acid structure (El-Dash, 1971), so glucose plus leucine, arginine or histidine gives a fresh bread aroma, while dihydroxyacetone plus proline give a cracker type aroma (Coffman, 1965). Sugar type affects reaction rate more than aroma (El-Dash, 1971).

Bread Flavor Components. *Compounds Identified as Constituents of Bread Flavor*. Many compounds have been detected in doughs and breads (crust and/or crumb), and they have been previously summarized (Coffman, 1965; Maga, 1974; Drapron and Richard-Molard, 1979; Schieberle and Grosch, 1984, 1985, 1987; Hansen and Hansen, 1994a,b; Hansen et al., 1989a,b; Lund et al., 1989). Wheat and rye flour doughs and breads contain many common components, but rye flour leads to additional components not identified in wheat products (Maga, 1974; Schieberle and Grosch, 1984, 1985, 1987). Figures 8−12 summarize components identified in preferments, doughs, and breads, grouped according to their chemical nature, in acids, alcohols, aldehydes, esters, ethers, furan derivatives, ketones, pyrrole derivatives, pyrazines, and sulfur compounds (Maga, 1974; Schieberle and Grosch, 1984, 1985, 1987).

From components identified as alcoholic fermentation byproducts, ethanol constitutes about 95% (Jackel, 1969), and the remaining 5% is mainly composed of isobutyl, propyl, and phenylethyl alcohols, acetaldehyde, and acetone, the remaining compounds existing at trace levels (Drapron and Richard-Molard, 1979). From the neutral fraction, 2,3-butanediols make up about 98%.

Lactic fermentation mainly leads to the production of lactic acid. Heterofementative lactic acid bacteria produce 50−65% lactic acid plus acetic acid and other byproducts, while more than 85% is composed of lactic acid in homofermentative species, with production of small amounts of formic and acetic acids, ethanol, and carbon dioxide (Spicher and Stephan, 1987). Additionally, lactic acid bacteria, by proteolytic activity, can produce bitter peptides from nonbitter polypeptides, as has been noticed in dairy products (Lawrence et al., 1976). This property depends on strain and process conditions controlling proteolytic activity, mainly pH (Sullivan et al., 1973; Thomas and Mills, 1981). In some strains, production of bitter peptides can take place at faster rates than that of nonbitter peptides (Lawerence et al., 1976). Although some species have peptidases able to split these peptides, their activity decreases with lowering the pH, as happens during lactic fermentation (Sullivan et al., 1973; Thomas and Mills, 1981).

Source of Compounds Identified as Part of Bread Flavor. Figure 13 shows the course of formation of some aromatic components during glycolysis and/or amino acid metabolism (Drapron and Richard-Molard, 1979; Prescott and Dunn, 1981). Intermediate compounds

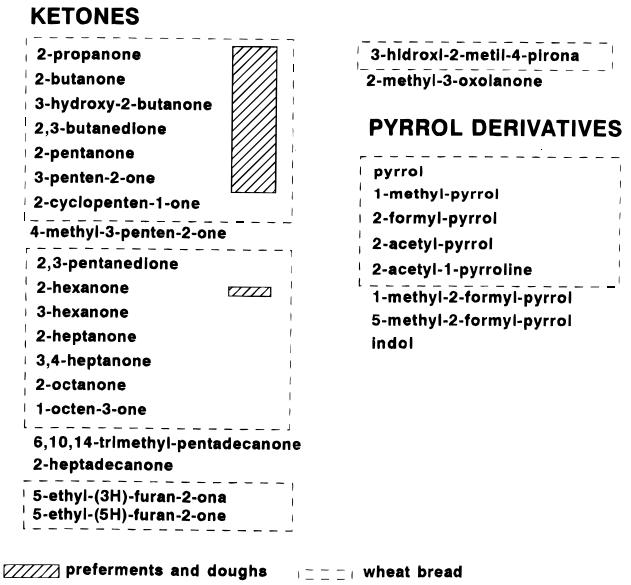

Figure 11. Summary of compounds identified in preferments, doughs, and breads: ketones and pyrrole derivatives (information from Maga, 1974; Schieberle and Grosch, 1984, 1985, 1986). (Compounds not marked have been found in rye products.)

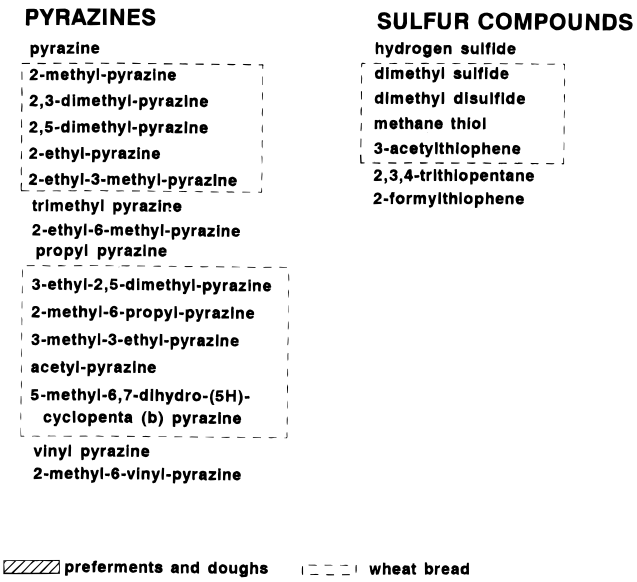

Figure 12. Summary of compounds identified in preferments, doughs, and breads: pyrazines and sulfur compounds (information from Maga, 1974; Schieberle and Grosch, 1984, 1985, 1986). (Compounds not marked have been found in rye products.)

interact in a complex way to produce a large number of products, many of which have been included in Figures 8−12.

Acetic and lactic acids, as mentioned before, mainly proceed from lactic fermentation (Spicher and Stephan, 1987). Saturated short chain organic acids (C2−C5) can come from several sources: fermentation of sugars by yeasts and lactic acid bacteria, decarboxylation of α-keto acids, amino acid transamination and oxidation of resulting aldehydes, and lipolytic activities (Johnson et al., 1966; Galal et al., 1978; Lawrence et al., 1976; Faridi and Johnson, 1978). The most probable way seems to be from amino acids (Lawrence et al., 1976), and the least likely is lipolysis, mainly in sourdough processes, where the low pH generated by fermentation is far from the optimum pH for lipase activity (7−8) (Galal et al., 1978). As new lipases are being marketed for specific hydrolysis of fats, their optima pH should be considered.

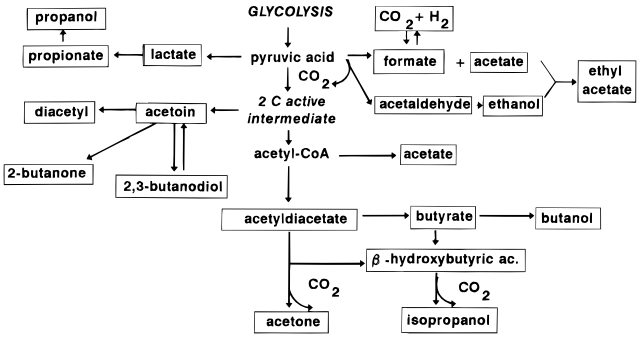

Figure 13. Summary of volatile compounds produced from glycolysis (information from Drapron and Richard-Molard, 1979).

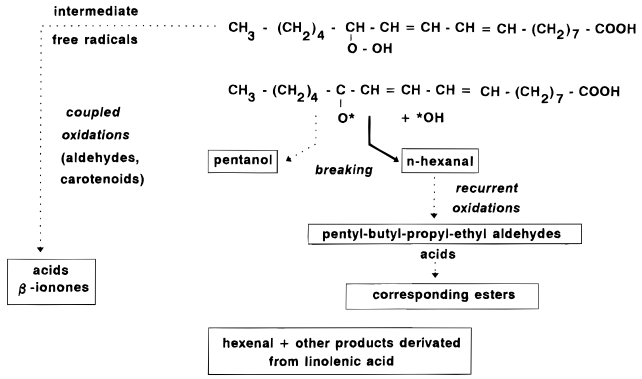

Figure 14. Volatile compounds resulting from lipoxygenase action (information from Drapron and Richard-Molard, 1979).

Short chain alcohols proceed from sugar fermentation and those of higher molecular weight from amino acid metabolism (Maga, 1974). Short chain organic acids, more so than long chain acids, are the source of esters by reaction with alcohols (Maga, 1974), of which ethyl esters are the most abundant (Baker et al., 1953; Maga, 1974; Lawrence et al., 1976). Ethyl acetate and others with low volatility disappear during baking.

Carbonyls (aldehydes and ketones) can result from many different reactions in breadmaking (Maga, 1974). Some proceed from fermentation, such as acetoin or diacetyl (Lawrence et al., 1976), but the majority are produced during nonenzymatic browning reactions (Johnson et al., 1966). Also some carbonyl compounds generated during fermentation can volatilize during baking and appear again during browning reactions (Johnson et al., 1966). Bread crust usually contains a larger number of carbonyl compounds than bread crumb (El-Dash, 1971). Lipoxygenase is a source of carbonyls by decomposition of hydroperoxides. Thus, linoleic acid produces hexanal, besides pentanol and other products from the partial oxidation of hexanal (Figure 14). Linolenic acid produces hexenal, and carotenoid pigments of flour lead to the production of β-ionones (Drapron and Richard-Molard, 1979). Like aldehydes, ketones are mainly formed during Maillard reactions (Maga, 1974). All amino acids promote acetone formation in the crust (Maga, 1974), which is also formed during fermentation, as well as 2-butanone (Maga, 1974).

Furan derivatives result from thermal degradation of sugars in bread crust. Some are intermediate compounds in Maillard reactions undergoing condensation with amino acids. Their characteristics are more in-

Table 1. Factors Influencing Bread Flavor

flour type (white, whole, extraction rate) (Stephan, 1982; Maga, 1974)
enzymic activity (amylolytic, proteolytic) (Stephan, 1982; Spicher and Nierle, 1984a–c, 1988)
fermentation temperature (Stephan, 1982)
fermentation time (Baker et al., 1953; Hironaka, 1986)
number of fermentation stages (Stephan, 1982)
percent of sourdough added (Stephan, 1982)
percent of yeast added (Baker et al., 1953; Stephan, 1982)
mixing conditions (Baker et al., 1953; Drapron and Richard-Molard, 1979; Hironaka, 1986)
dough consistency (Spicher, 1983; Lund et al., 1989)
breadmaking process (Drapron and Richard-Molard, 1979; Richard-Molard et al., 1978, 1979)
oxygenation of dough (Drapron and Richard-Molard, 1979)
percent of sugar added (Baker et al., 1953; Maga, 1974)
oxidants (Baker et al., 1953)
preservative agents (Stephan, 1982)
baking conditions (Baker et al., 1953; Stephan, 1982)
postbaking treatments (Baker et al., 1953)

fluenced by amino acid than by sugar type (Maga, 1974). Pyrrole derivatives, pyrazines, and sulfur compounds proceed from Maillard reactions too; some pyrazines can be provided by flour or milk solids included in formulation (Maga, 1974).

Participation of Identified Compounds in Bread Flavor. The presence of a determined compound in bread does not necessarily mean its participation in bread flavor (Johnson et al., 1966); on the other hand, aromatic substances essential in one food can not play any role in another (Gilliland, 1985). The way in which the volatile fraction from fermentation influences bread flavor is not well known; it has been suggested that some components such as organic acids or ethanol can affect the perception of other substances (Spicher, 1983) or retain them in bread (Johnson et al., 1966).

Acetic and lactic acids are of importance in wheat and rye bread flavor (Hansen and Hansen, 1994a,b; Spicher and Stephan, 1987), being the lactic/acetic ratio determinant in typical rye bread taste (Spicher and Stephan, 1987). Besides, acetic acid acts as flavor enhancer (Richard-Molard et al., 1978, 1979; Richard-Molard and Cahagnier, 1980), sensitizing the consumer toward other aromatic components, the effect related to its actual concentrations (Helleman et al., 1988). Small variations in acetic acid concentration are more important in flavor than the same changes in lactic acid; however, differences in sensory acidity can not be exclusively attributed to acetic acid because other volatile constituents and/or interactions between both acids can be responsible for changes in acidity (Hellemann et al., 1988). Short chain organic acids (C2–C5), although constituting about 1% of titratable acidity, can play a decisive role in bread flavor because they are formed in bigger amounts in long fermentation processes that are considered as having more appealing and intense flavor (Faridi and Johnson, 1978). Short chain isoacids, on the contrary, have a negative effect in bread flavor (Richard-Molard et al., 1978, 1979).

From the alcoholic fraction, the greater part of ethanol is evaporated during baking, but higher aromatic alcohols (e.g., amylic) having a characteristic aroma and taste can influence bread flavor (Maga, 1974; Johnson et al., 1966). Hironaka (1986), correlating sensory and chemical data, found negative correlations between the flavor preferred by the consumer and the amounts of ethyl and isobutyl alcohols remaining in the bread. It is believed that esters present in bread must contribute to flavor because of their strong and appealing aroma (Maga, 1974). Carbonyl compounds also possess strong sensory properties, seeming to affect more so aroma than taste (El-Dash and Johnson, 1967; Maga, 1974). Isobutyraldehyde, propylaldehyde, and 2-butanone have positive influence in sensory flavor (Hironaka, 1986). Short chain carbonyls (C2–C4) can be the cause of improvement and defects in the flavor of dairy products, according to their concentration (Lawrence et al., 1976).

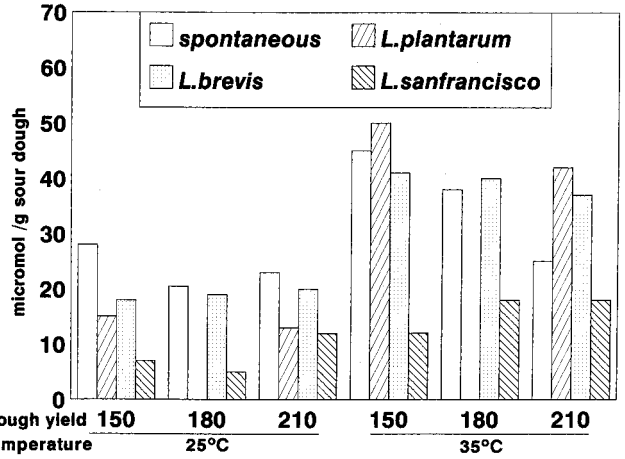

Figure 15. Influence of processing conditions on total amino acid content of sourdoughs (results from Spicher and Nierle, 1988).

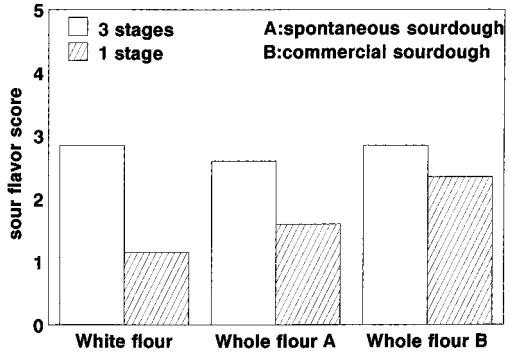

Figure 16. Effect of sourdough and breadmaking process on sensory sour flavor (results from Grönman and Möttönen, 1982).

Diacetyl imparts positive characteristics to bread flavor (Baker et al., 1953), being essential in dairy products; however, acetoin and 2,3-butyleneglycols are not aromatics (Cogan, 1980). Aldehydes proceeding from lipoxygenase activity, mainly hexanal, due to its concentration, generate off flavors in bread (Drapron and Richard-Molard, 1979).

Pyrazines resulting from thermal reactions also have typical aroma features able to be imparted to bread (Maga, 1974). Finally, the least volatile fractions, such as melanoidins, dihydroxyacetone, ethyl succinate, and succinic and lactic acids, contribute more to the taste than to the aroma of bread (Baker et al., 1953).

Influence of Processing Conditions on Bread Flavor Components. Numerous factors influence the composition of bread flavor; the most relevant are included in Table 1. Some examples will illustrate the extent of expectable changes.

Proteolysis developed during fermentation increases amino acid production. This activity depends on flour

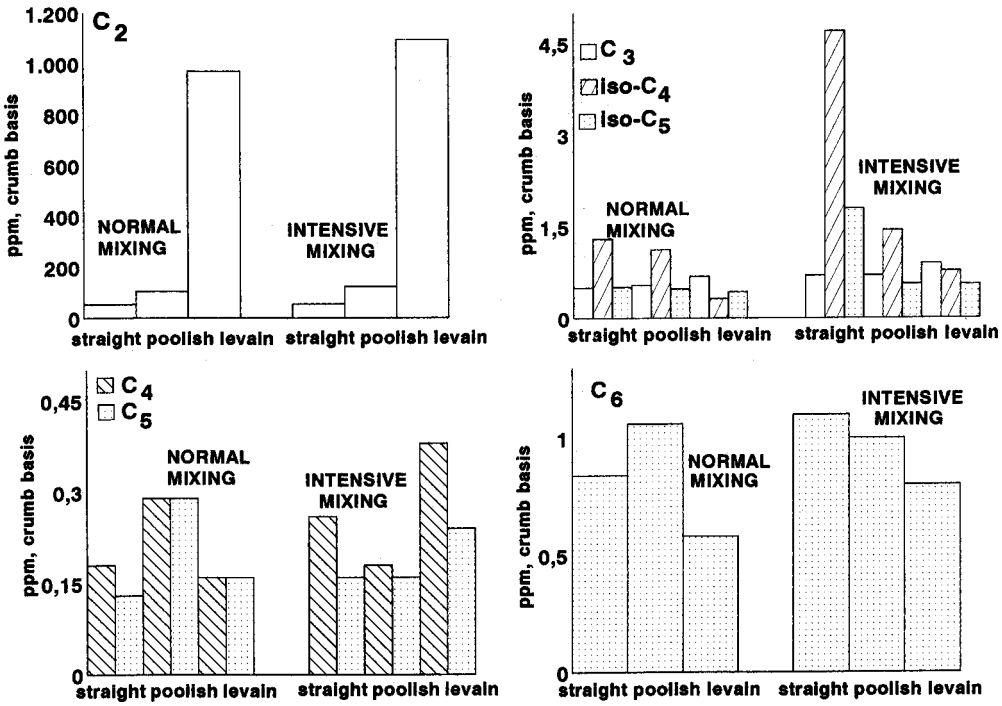

Figure 17. Influence of breadmaking process on short chain organic acid content of bread crumb (results from Richard-Molard et al., 1978, 1979).

and endogenous and exogenous microflora, which, in turn, vary with strain and species of microorganism. Proteolysis is affected by processing conditions, mainly temperature, and to a lesser extent by dough consistency (Figure 15) (Spicher and Nierle, 1984a–c, 1988; Collar and Martínez, 1993).

The type of breadmaking process modifies organic acid production. Acetic and lactic acid levels are a function of temperature, dough consistency, and the number and length of breadmaking stages (Spicher and Stephan, 1987; Grönman and Möttönen, 1983); Figure 16 shows some effects in sensory sour flavor. Comparison of a straight, poolish, and levain breadmaking process, as well as the use of intensive or normal mixing, showed that, although judges differentiate bread flavor among products, the volatile fraction of bread presented very little differences, except in acetic acid and, to a lesser extent, isoacids. The relative amounts of these acids underwent important changes, and their negative effect on bread flavor can be noticeable even at their low concentrations (Figure 17) (Richard-Molard et al., 1978, 1979; Richard-Molard and Cahagnier, 1980).

The effect of microorganism strain and dough consistency on aromatic compounds of sourdoughs is depicted in Figure 18. Dough consistency leads to bigger changes than the type of lactobacilli. In soft doughs, ethanol and ethyl acetate are predominant, with high levels of isoalcohols in doughs containing homofermentative strains; these are attributable to yeasts that spontaneously developed more in the presence of homofermentative species, due to the lower concentrations of acetic acid generated. Tough doughs differ in ester composition; ethyl acetate in heterofermentative sourdoughs and carbonyls in the homofermentative ones are more abundant than in softer doughs (Lund et al., 1989).

Hansen and co-workers (Hansen et al., 1989a,b; Hansen and Hansen, 1994a,b) in a systematic study established the differences in the aromatic fraction of sourdoughs caused by the use of lactic acid bacteria or acidification, as well as whether or not yeast is incorporated. Quantitatively sourdough breads containing lactic acid bacteria have greater amounts of 2-propanone, 3-methylbutanal, benzyl alcohol, and 2-phenylethanol, whereas chemically acidified breads have more hexanal and ethyl lactate; if acidification was made with acetic or lactic acid, bread had more acetoin and diacetyl. High-extraction rate flours increase the amount of ethyl acetate and ethanol when inoculated with heterofermentative lactobacilli, but white flour breads have higher levels of other alcohols. *n*-Hexanal and *n*-pentenal appeared in all breads, but carbonyl compounds were formed in sourdoughs with homofermentative strains (due to yeast action).

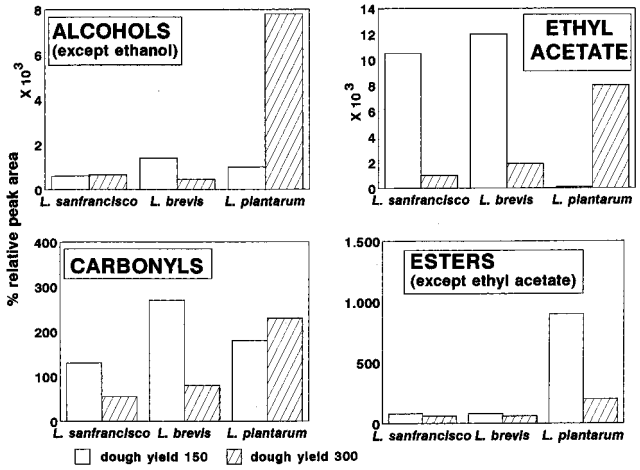

Figure 18. Effect of processing conditions and lactobacilli strain on volatile components in rye bread (Lund et al., 1989, with permission).

Finally, mixing conditions influence oxygen incorporation, thus affecting, if a source of lipoxygenase has been included in formulation, levels of carbonyls and short chain fatty acids. Intensive mixing incorporates greater amounts of air, increasing contacts between lipoxygenase and substrate, resulting in greater amounts of hydroperoxides and oxidation of carotenoid pigments.

Hexanal produced by intensive mixing can be 10-fold that obtained with normal speed mixing; other oxidation byproducts can also be augmented. Hexanal modifies the balance of flavor components, leading to an uncharacteristic flavor of lower consumer acceptance (Drapron and Richard-Molard, 1979).

CONCLUSIONS

Summarized information reveals the complex interactions among flour components, processing conditions, fermenting microflora, and spontaneous and/or exogenous enzymic systems, and their repercussions on bread flavor. Much has been accomplished in understanding the mechanisms responsible for the formation of bread flavor; but which component(s) should be promoted or how to synthesize flavor still remains unknown today. Moreover, new preparations having single or mixed enzymatic activities are intensively being used to improve process and quality, but their direct effects on bread flavor, in which they actively participate, have not been evaluated. Research covering this aspect should be encouraged. It would be very useful to the industry to know the magnitude of the positive or negative expectable changes in bread flavor, depending on the enzymatic sources included, and the breadmaking process used in order to obtain the desirable technological improvement together with a more appealing and characteristic bread flavor.

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