Flavour in sourdough breads: a review

Salim-ur-Rehman*, Alistair Paterson and John R. Piggott

Centre for Food Quality, Department of Bioscience, University of Strathclyde, 204 George Street, Glasgow G1 1XW, Scotland, UK (Permanent address: Institute of Food Science and Technology, University of Agriculture, Faisalabad, Punjab 38000, Pakistan. Fax: +92 41 9201105; +44 141 553 4124; e-mail: drsalim_rehman@yahoo.com)

Flavour compounds are key elements for consumer acceptance and product identification in bread. One category of speciality breads, the sourdoughs have a fermentation process affected by a complex microflora of yeasts and lactic acid bacteria which confer specific flavour characteristics. Although yeasts have the primary leavening role, lactic acid bacteria (LAB), with trophic and non-trophic relationships, produce important flavour components. Sourdoughs are becoming important as consumers move away from pan breads towards speciality products. However, successful new product development requires an understanding of variations in carbohydrates’ metabolism, roles of endogenous enzymes and interactions of microorganisms for generation of non-volatile and volatile flavour compounds. The potential of sourdough baking remains to be developed through specifications and optimisations of process conditions and introduction of exogenous enzymes and other ingredients. With effective new product development sourdough characteristics could be matched to relate with consumer tastes.

Microorganisms of sourdough sponges

The microbial ecology of the sourdough fermentation is complex and varied with identification of more than 50 species of LAB, mostly of the genus Lactobacillus, and more than 20 species of yeasts, are dominated by the genera Saccharomyces and Candida. Sourdough microflora have primarily stable associations with lactobacilli and yeasts, having important metabolic interactions contributing towards production of flavour compounds (Vuyst & Neyesens, 2005). Sourdough flavour components identified as produced by yeast (Table 1) and LAB (Table 2) fall into many classes of compounds.

Several yeasts are found in sourdoughs but Saccharomyces cerevisiae is considered the dominant organism for leavening of bread (Corsetti et al., 2001) (Table 1). It has been suggested that numbers of S. cerevisiae are overestimated due to the lack of robust systems for quantifying individual yeast in this habitat (Vogel, 1997). Important yeasts in sourdough starters include Saccharomyces exiguus (physiologically similar to Candida milleri), Candida krusei, Pichia norvengensis and Hansenula anomala. Other yeasts isolated from sourdough sponges include

* Corresponding author.

1995; Onno & Rouseel, 1994; Ottogalli, Galli, & Foschino, 1996). The key groups of fermenting organisms, in addition to the yeasts, are strains of the lactic acid bacteria (LAB). In traditional sourdough systems initially part of the flour is mixed with all the yeast and sufficient water to make “sponge” that is allowed to ferment for some hours, typically overnight, exposed to the atmosphere. The sponge is then mixed with rest of the flour, water, salt and fat to a suitable consistency, and then given a short period for fermentation before final proving and baking. The outcome is considered to be bread of a richer flavour due to bacterial souring of the sponge through airborne contamination or incorporation of 2—5% of sponge from the previous batch as a microbial inoculum (Bruemmer & Lorenz, 1991).

In good bakery practice, a sponge should contain metabolically active lactic acid bacteria at 10³—10⁵ cfu g⁻¹ and yeasts at 10⁶—10⁷ cfu g⁻¹, primarily responsible for acidification and leavening action of dough, respectively. However, the LAB may either originate from natural flour contaminant, a fermented dairy product or from a commercial starter culture containing characterised strains of LAB produced in batch fermentation (Vuyst & Neyesens, 2005).
Saccharomyces delbrueckii, Torulopsis holmii and Torulopsis unisporus (Gül, Özcelik, Sadıc, & Certel, 2005).

It is generally considered that in sourdoughs, the ratio of LAB to yeast should be 100:1 for optimal activities (Gobbetti, Corsetti, Rossi, La Rosa, & De Vincenzi, 1994). Although, Saccharomyces exiguus is a dominant species in starters, strains do not ferment maltose and grow by fermenting glucose released by heterofermentative lactic acid bacteria. In return, the yeast supplies an electron source, fructose, to the LAB that assist both growth and acetic acid production: a co-operation with important technological and sensory consequences. Production of acetic acid improves quality in leavened dough and other bakery products (Vernocchi et al., 2004).

Typical sourdough prokaryotes are members of the LAB genus Lactobacillus with obligately homofermentative, and facultatively or obligately heterofermentative strains with strains of Lb. sanfranciscensis (Catzeddu, Mura, Parente, Sanna, & Farris, 2006) (synonym Lb. brevis subsp. lindneri), Lb. plantarum and Lb. brevis most frequently isolated (Trüper & de Clari, 1997). Certain strains, initially classified as Lb. brevis, are recently allotted to the new species Lb. Pontis (Vogel et al., 1994). Other LAB strains including Carnobacterium divergens (Lb. Diversens, Lb. amylobillus, Lb. sake, Lb. acetotolerans, L. plantarum, Pediococcus pentosaceus and P. acidilactici, and Tetragenococcus halophilus (Pediococcus halophilus) have been isolated from sourdoughs (Gül et al., 2005). In wheat sourdough, mixed cultures were estimated as: Lb. Sanfranciscensis — 20%; Lb. Alimentarius — 14%; Lb. Brevis — 12%; Leuconostoc citreum — 7%; Lb. Plantarum — 6%; Lactococcus lactis subsp. lactis — 4%; Lb. fermentum, Lb. acidophilus and Weissella confusa — each 2%; Lb. delbrueckii subsp. delbrueckii (Corsetti et al., 2001). Although, Lb. sanfrancisco is considered a key sourdough lactic acid bacterium (Gobbetti & Corsetti, 1997) in rice sourdough, Lb. fermentum, Lb. gallinarum, Lb. kimchii, Lb. plantarum, Lb. pontis, Lb. paracasei, and Lb. paralimentarius have been reported to dominate (Merotha, Hammesa, & Hertela, 2004).

Mixed cultures of LAB and yeasts vary in composition in sourdough sponges. The use of mixed cultures has a number of important advantages, such as improved flavour and texture and retained freshness for longer compared to baker’s yeast bread (Meignen et al., 2001). In such mixed cultures, yeasts act mainly as leavening agents, while LAB contribute mainly to the flavouring compounds of bread. Kefir is a natural mixed culture which can produce bread of good quality. Many microorganisms have been isolated from kefir microflora, sharing symbiotic relationships including yeasts (Kluyveromyces, Candida, Torulopsis and Saccharomyces sp.), lactobacilli (Lb. brevis, Lb. acidophilus, Lb. casei, L. helveticus, and Lb. delbrueckii), streptococci (Streptococcus salivarius) lactococci (Lc. Lactis ssp. Table 1. Volatile and non-volatile compounds present in wheat flour sourdough fermented with various yeast strains (Damiani et al., 2001)
thermophilus, Leuconostoc mesenteroides and L. cremoris) and occasionally acetic acid bacteria (Plessas, Pherson, Bekatou, Nigam, & Koutinas, 2005; Simova et al., 2002). The phenotypic characterisation of the isolates identifies the presence of 80% as facultative heterofermentative LAB (Lb. plantarum, Lb. paracasei, Lb. casei) and 12% as heterofermentative LAB (Lb. brevis and L. mesenteroides) in sourdough of Altamura bread (Ricciardi, Parente, Piraino, Paraggio, & Romano, 2005).

The conditions and substrates of the sourdough sponge are fundamental with respect to microbiological stability of dough. Mixed commercial starters containing Lb. brevis and S. cerevisiae (baker’s yeast), fermented at 30 °C for 20 h, showed reduced yeast growth and ethanol production in dough but more glycerol (80%) and acetic acid (55%) whereas lactic acid production was unchanged (Meignen et al., 2001). In contrast to the production of durum wheat bran flour sourdough breads, more than 95% strains were of the yeasts Candida humilis and C. krusei and S. cerevisiae were dominant in rice sourdough (Merotha et al., 2004). While in Triticum aestivum wheat flour sourdough, 58 strains as S. cerevisiae, five as Candida colliculosa, four as Candida lambica, three each as C. krusei and Candida valida and two as Candida glabrata were identified (Succi et al., 2003).

Factors affecting sourdough volatiles and non-volatile compounds

Sourdough sponges vary in proportions of LAB in different speciality breads. The primary metabolic products of LAB are l-lactic and acetic acids with lesser amounts of citric and malic acids. Ratio of lactic acid to acetic acid is important for final product flavour (Linko, Javanainen, & Linko, 1997). LAB multiply and produce lactic and acetic acids more slowly in the mixed culture with yeasts than in monocultures (Meignen et al., 1995).

Heterofermentative LAB species can be discriminated from homofermentative on the basis of volatile compounds (Table 2). Certain aldehydes are derived both from enzymatic oxidation or autoxidation of the lipid fraction of the wheat (Frankel, 1982; Hann & Morrison, 1975) and bacterial metabolism. Homofermentative LAB, with the exception of Lb. delbrueckii subsp. delbrueckii strains are

<table>
<thead>
<tr>
<th>Compounds</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
<th>I</th>
<th>J</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactic acid</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ethanol</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1-Propanol</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2-Methyl-1-propanol</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3-Methyl-1-butanol</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2-Methyl-1-butanol</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1-Pentanol</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2-Methyl-1-pentanol</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1-Hexanol</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3-Hexen-1-ol</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1-Heptanol</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1-Octanol</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3-Methyl-1-butanal</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2-Methyl-1-butanal</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hexanal</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3-Methyl-hexanal</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Heptanal</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Trans-2-heptenal</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Octanal</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Nonanal</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Benzaldehyde</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Diacetyl</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hexane</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Heptane</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Octane</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+, Present and –, not present. A — Lactobacillus brevis lindneri; B — Lactobacillus brevis; C — Lactobacillus fructivorans; D — Lactobacillus fermentum; E — Lactobacillus cellobiosus; F — Lactobacillus plantarum; G — Lactobacillus farciminis; H — Lactobacillus alimentarius; I — Lactobacillus acidophilus; and J — Lactobacillus delbrueckii.

The composition of sourdough flavour compounds is, however, not only influenced by microbial composition, but also interactions between bread making processes and ingredients (Collar, 1996). These are important for process stability and production of variety of regional specialities that meet consumer and commercial demands (Gobbetti, 1998). In sourdoughs produced from Triticum aestivum (Succi et al., 2003) and Triticum durum wheat flours, a number of lactic acid bacteria and yeasts range from ca. log 7.5 to log 9.3 colony forming units (cfu)/g and from log 5.5 to log 8.4 cfu/g, respectively (Corsetti et al., 2001). In slack doughs, there are changes in yeast and lactic acid bacterial growth with more abundant flavour compounds. Stimulating effects on yeasts of NaCl can indirectly be attributed to a reduction in competition with lactic acid bacteria for available sugars (Ney sens, Messens, & Vuyst, 2003), and with content of other soluble carbohydrates, in the cereal flours at typical fermentation temperatures.

Ingredients in sourdough bread influence generation of volatile compounds, volatile compounds of germinated, sourdough fermented, and native rye substantially different, variable even after the second treatment (Heiniö et al., 2003). Type of cereal flours, endogenous and exogenous cereal components and processing steps such as the heat treatment in baking has significant effects on the generation of flavouring compounds in breads (Hansen, 1995). One key driver of flavour is the carbon source available to the microorganisms, notably low-molecular weight sugars that is, at least in part, flavour precursors (Martinez-Anaya, 1996).

Sucrose addition to wheat dough stimulates both yeast and LAB growth and increases bacterial production of lactic and acetic acids (Corsetti, Gobbetti, & Rossi, 1994). However, titratable acidity (TTA) of the dough increases in proportion to the addition of sucrose over the range of 0–6%, ascribed to acetic acid accumulation which affects the generation of flavouring compounds (Simonsøn, Salovaar, & Korhola, 2003). San Francisco sourdough breads are produced with specific cultures of Lactobacillus (Linko et al., 1997; Seitz, Chung, & Rengarajan, 1998). A lack of competition between Lb. sanfranciscensis and S. exigus for maltose consumption was noted which is fundamental for the stability of both the microorganisms for the production of good quality San Francisco French bread (Sugihara, Kline, & McCready, 1970). However, LAB growth and lactic and acetic acid productions may be reduced by more rapid consumption of maltose and, notably, of glucose by S. cerevisiae in an association with Lb. sanfranciscensis in synthetic media (Collar, 1996). Even so, imbalances between yeast consumption and starch hydrolysis by endogenous flour enzymes lead to rapid depletion of soluble carbohydrates during wheat sourdough fermentation which, in turn, decreases LAB acidification due to microbial competition (Rouzaud & Martinez-Anaya, 1993). This situation is less pronounced in rye dough fermentation due to the greater flour enzyme activity, which increases the availability of soluble carbohydrates (Röcken & Voysey, 1993). In wheat sourdough fermentations, maltose remains between 2 and 5 g/kg (Martinez-Anaya, Pitarch, & Benedito de Barber, 1993) as yeast preferentially depletes available glucose and fructose (Barber, Baguena, Benedito de Barber, & Martinez-Anaya, 1991).

Co-fermentations are other metabolic strategies to enable sourdough LAB to use non-fermentable substrates, increasing adaptability. Co-fermentation of fructose and maltose or glucose has been observed specifically in a fructose-negative strain of Lb. Sanfranciscensis (Gobbetti, Corsetti, & Rossi, 1995; Stolz, Böcker, Hammes, & Vogel, 1995), as has co-metabolism of citrate and maltose or glucose (Gobbetti & Corsetti, 1996). Moreover, changes in the availability of citrate and fructose can influence the ratios of acetic acid and lactic acid and micro-structural rheological features of the doughs (Gianotti et al., 1997), through change in acetic acid production (Calderon, Loiseau, & Guyot, 2003). Certain strains of S. cerevisiae are sensitive to the acetic acid produced by LAB, especially at the normal sourdough pH of 4.0–4.5 that favours the undissociated lipophilic and membrane-diffusible form of the organic acid. Thus, it is necessary to add baker’s yeast to compensate for poor survival of wild-type yeasts in consecutive sourdough fermentations (Suhkó & Makenin, 1984).

Lb. sanfranciscensis hydrolyses maltose and accumulates glucose in the medium in a molar ratio of about 1 maltose to 1 glucose (Gobbetti, Corsetti et al., 1994; Gobbetti, Simonetti et al., 1994; Stolz, Böcker, Hammes, & Vogel, 1993). A study of maltose uptake and glucose excretion in Lb. sanfranciscensis (Neubauer, Glaasker, Hammes, Poolman, & Konings, 1994) showed that once the maltose is depleted, consumption of the excreted glucose is initiated. Glucose excreted during fermentation may be used by maltose-negative yeasts such as S. exigus or induced glucose repression of maltose catabolism in competitors from using maltose, thereby giving an ecological advantage to Lb. sanfranciscensis (Nout & Creemers-Molenaar, 1987). However, among the Lactobacillaceae, only certain species — Lb. sanfranciscensis, Lb. pontis, Lb. reuteri, and Lb. fermentum (Vogel et al., 1994) — phosphorylate maltose and the enzyme maltose phosphorylase may be considered the key for LAB growth during sourdough fermentation.

Conversely, with sucrose and Lb. plantarum and S. cerevisiae or S. exigus in co-culture (Gobbetti, Corsetti, & Rossi, 1994a) cell yield and lactic acid production increase (Robert et al., 2006). Invertase hydrolysis of sucrose by yeasts into glucose and fructose, may enhance LAB metabolism (Aksu & Kutsal, 1986) because yeasts can hydrolyse sucrose to about 200 times faster than the released monosaccharides are fermented (Martinez-Anaya, 1996), with rapid sucrose depletion during sourdough fermentation (Seppi, 1984). S. exigus preferentially utilise glucose or sucrose and shows a high tolerance for the acetic acid
from heterolactic LAB metabolism (Suihko & Makinen, 1984). Flavour component release may also be influenced by the environmental conditions such as temperature. Yeast and LAB growth increase in a direct relationship with temperature between 15 °C and 27 °C. Optimal growth temperature of the two Candida milleri strains was between 26 °C and 28 °C in monoculture. Nevertheless, decrease in the growth of yeast may be accompanied by a similar reduction in the growth of LAB (Neyens et al., 2003; Simonson et al., 2003).

Processing conditions such as proofing time, temperature and slackness of sourdough may also affect the aroma volatiles. Low temperature (25°C) and sourdough firmness are considered appropriate for LAB souring activities but limited yeast metabolism. Raising the temperature to 30 °C and semi-fluid sourdough fermentation can generate more complete volatile profiles. While at 3 h fermentation, the sourdough may mainly be characterised by iso-alcohols but an increase of leavening time of up to 9 h can produce volatiles about three times higher than that at 5 h as a result of LAB contribution (Lund, Hansen, & Lewis, 1989).

Types of flavouring compounds in sourdough breads

There are two categories of flavour compounds, produced during sourdough fermentation. Non-volatile compounds including organic acids produced by homo- (Gobbetti, Corsetti, & De Vincenzi, 1995a) and heterofermentative bacteria (Gobbetti, Corsetti, & De Vincenzi, 1995b) which acidify, decrease pH and contribute aroma to the bread dough (Barber, Benedetto de Barber, Martinez-Anaya, Martinez, & Alberola, 1985; Galal, Johnson, & Varriano-Marston, 1978).

The second category — volatile compounds of sourdough bread — includes alcohols, aldehydes, ketones, esters and sulphur. All these compounds are produced by biochemical and biochemical actions during fermentation and contribute to flavour (Spicher, 1983).

Non-volatile compounds

Interactions between lactic acid bacteria and exogenous enzymes influence microbial kinetics of acidification, acetic acid production and bread textural characters. It has been shown to be desirable to augment endogenous enzyme activities in flour and has been obtained from LAB in association with microbial glucose oxidase, lipase, endo-xylanase, -amyloses and proteases. The growth of Leuconostoc citreum 23B, Lb. lactis subsp. lactis 11M and Lb. hilgardii 51B has been influenced by enzyme addition, which increases lactic acid production (Cagno et al., 2003). However, in a continuous sourdough fermentation, an association between Lb. sanfranciscensis and S. cerevisiae has shown optimal production of acetic acid (Vollmar & Meuser, 1992), whereas Torulopsis holmii improved dough acidification in association with Lb. sanfranciscensis and S. cerevisiae and further enhanced acetic acid production if associated with Lb. sanfranciscensis and Lb. plantarum (Spicher, Rabe, Sommer, & Stephan, 1981; Spicher, Rabe, Sommer, & Stephan, 1982).

Temperature optima for co-culture of C. humilis and Lb. sanfranciscensis are 28 and 32 °C, respectively, but reduced production of acetate by Lb. sanfranciscensis has been observed at 35 °C, although, both lactate and ethanol formations are not affected at this temperature (Brandt, Hammes, & Ganzle, 2004). Lactic acid production varied from 3.11 to 5.14 g/kg and traces of acetic acid may be produced by some of the Lb. plantarum and Lb. farcininis strains during fermentation (Table 1).

Volatile compounds

Microbial metabolisms verify production of different volatile compounds for hetero- and homo-lactic LAB (Table 1) fermentations. Abundant products of yeast fermentation are 2-methyl-1-propanol, 2,3-methyl-1-butanol and other iso-alcohols. Heterofermentative LAB products are dominated by ethyl acetate and certain alcohols and aldehydes (Spicher et al., 1982) whereas major homofermentative LAB products are diacetyl and carboxyls (Spicher et al., 1981) (Table 2).

Lactobacillus brevis subsp. Lindneri and Lactobacillus plantarum have been considered to have the most appropriate profiles of flavour components. (Gobbetti, Corsetti, & De Vincenzi, 1995a). However, with the exception of Lb. plantarum DC400—Saccharomyces exiguus M14 association, both hetero- and homofermentative LAB enhance yeast formation volatile compounds (Damiani et al., 1996; Gobbetti, Corsetti, & De Vincenzi, 1995b).

The LAB strains of sourdough vary in metabolism and aroma compounds. Monoculture fermentation of dough for 15 h at 30 °C, followed by mixing and a further 10 h fermentation has shown increase in the production of typical sourdough volatile compounds (Merotba et al., 2004). The production of volatile aroma compounds during fermentation with combinations of single strains of S. cerevisiae and C. guillermondii, Lb. plantarum has been investigated using both wheat doughs and simple substrates including maltose and glucose (Stolz et al., 1993). Using only yeasts in wheat bread, seven volatiles were found abundant: acetaldehyde, acetone, ethyl acetate, ethanol, hexanal, isobutyl alcohol, and propanol. In this fermentation, S. cerevisiae produced more volatiles than C. guillermondii, but quantity of volatile flavour compounds can be improved by the addition of glucose and sucrose less by maltose. It is still not clear whether yeasts produce more flavouring components than LAB in simple substrate which appeared to produce a greater range of volatiles from wheat flour (Hansen, 1995; Torner, Martinez-Anaya, Antuña, & Benedetto de Barber, 1992; Vollmar & Meuser, 1992).

Addition of enzymes to sourdough sponges can also enhance bread volatile compounds (Martinez-Anaya, 1996), lipid oxidations through addition of enzymes in the form of active soya flour increase concentrations of hexanal, 1-hexanol, 1-penten-3-ol, 1-pentanol and 2-heptanone, and
2-heptenal and 1-octen-3-ol have only been reported in breads containing soya flour (Luning, Roozen, Moëst, & Poshumus, 1991). Addition of lipase, endo-xylanase and α-amyloses enhanced acetic acid production by Lb. hilgardii 51B. Textural analyses suggest that such sourdoughs with single enzyme have greater stability and crumb softening than breads produced with only exogenous enzymes (Cagnol et al., 2003). Moreover, addition of fructose and citrate to sourdough enhanced the production of volatiles by LAB including ethanol, which was eliminated in baking and 2-methyl-1-propanol with no effect on lactic and acetic acids’ production (Gobbetti, Corsetti, & De Vincenzi, 1995a; Gobbetti & Corsetti, 1996; Gobbetti, Smacchi, Fox, Stepaniak, & Corsetti, 1996).

In a French yeast sourdough, more than 40 flavour components were identified: 20 alcohols, 7 esters, 6 lactones, 6 acids, 3 alkanes and a single sulphur compound (Frasse, Lambert, Richard-Moland, & Chiron, 1993). Components, except for aldehydes and alkanes, increased in abundance due to greater activity of S. cerevisiae notably those related to formation of fusel alcohols. However, in this fermentation 2,3 butanediol, 3-methyl-1-butanol, 2-methyl-1-butanol, methional, 2-phenylethanol and 2 unidentified compounds with a pungent and mushroom odour were also apparent.

A number of other factors influence the production of volatile compound during production of sourdough bread including free amino acids formed during fermentation through proteolysis which influence volatile compound profile (Collar & Martinez, 1993; Collar, Mascaros, Prieto, & Benedito de Barber, 1991; Gobbetti, Corsetti, & Rossi, 1994b; Gobbetti et al., 1996; Spicher & Nierle, 1984). Thiazolines, thiophenes, thiophenones, thiazoles, polythialcycloalkanes, pyrroles and pyrazines have been shown to relate to acidity in wheat extrusion cooking (Hansen & Hansen, 1994a) and sourdough fermentation (Hansen et al., 1989a).

**Effect of Maillard and Caramelization reactions on the flavour of bread**

The production of volatiles is also influenced by proofing and baking processes (Hansen, Lund, & Lewis, 1989b; Kamiński, Przybylski, & Gruchala, 1981). The Maillard and Caramelization reactions are overwhelmingly responsible for flavour formation in cereal products. Upon heating, the reaction involves between simple the precursors such as amino acids and simple aldose or ketose of sugars (Rothe & Rutloff, 1983). As shown previously, the highest amount of free amino acids was produced during sour dough fermentation with the association between LAB and S. exigus M14 (Gobbetti, Corsetti et al., 1994; Gobbetti, Simonetti et al., 1994; Kratochvil & Holas, 1983; Rothe, 1974). These amino acids are precursors of iso-alcohols (Gobbetti, Corsetti, & De Vincenzi, 1995b; Hansen & Schiebeberle, 2005) that contribute directly to bread flavour during sourdough fermentation and baking of bread (Bredie, Mottram, & Guy, 2002; Damiani et al., 1996). Maillard reaction products from high temperature processing include pyrazines, pyroles, furans and sulphur-containing compounds and lipid degradation products such as alkyls, 2-alkenals and 2,4-alkadienals (Parker, Hassell, Mottram, & Guy, 2000). Moreover, at higher temperatures pyrroles, thiophenes, thiophenones, thiapyrans and thiazolines increase and furans and aldehydes decrease (Bredie et al., 2002). The pyrazines are nitrogen-containing ring compounds that have extremely powerful odour and flavour properties (Ji and Berhard, 1992). Hence, the production of these compounds has a striking effect on the flavour of bread.

**Sensory characteristics of bread**

The sourdough fermentation is central to acceptability in flavour, as chemically acidified bread and breads prepared with pure commercial starter cultures are not well scored in sensory preference assessments (Lund et al., 1989; Rothe & Rutloff, 1983). The synergistic metabolic activities of the microorganisms produce an acidification or souring influencing the final character in the bread, notably the texture, increased shelf-life by reducing the mould growth during storage (Corsetti et al., 2000; Oura, Soumalainen, & Wiskari, 1982; Rücken & Voysey, 1995) and generate typical flavour components yielding typical sourdough sensory attributes (Gobbetti, 1998; Katina et al., 2005).

The flavour of sourdough wheat bread is richer and more aromatic than in wheat bread, a factor that can be attributed to the long fermentation time of sourdough (Bruemmer & Lorenz, 1991). The most desirable sensory characteristics are obtained at pH 4.0–5.5 and at 140 °C (Przybylski & Kamiński, 1983). The loaves made with the addition of 5–10% sourdoughs fermented with Lb. plantarum and 5–15% sourdoughs fermented with Lb. sanfranciscus are preferred in odour and taste (Hansen & Hansen, 1996). Sensory evaluation of sourdough of wheat bread crumb showed that bread made from sourdough fermented with the heterofermentative Lb. sanfranciscisens had a pleasant, mild, sour odour and taste, whereas sourdough bread fermented with the homofermentative Lb. plantarum had an unpleasant metallic sour taste. But, when sourdough was supplemented with sourdough yeast S. cerevisiae, the wheat bread received a more aromatic flavour (Katina, Heiniö, Autio, & Poutanen, in press). Chemical analyses of flavour compounds can be combined with sensory analysis of bread. Compounds that have been positively correlated with flavour of wheat crumb are acetaldehyde, 2-methylpropanoic acid, 2,3/methyl-1-butanol, 3-methyl-butanoic acid, isopentanal, 2-nonenal, benzyllethanol, 2-phenylethanol, 2,3-butandione and 3-hydroxy-2-butanoic, dimethyl sulphide and 2-furfural (Hansen & Hansen, 1996; Rothe, 1974). Sensory evaluation of sourdough rye bread crumb suggested that a most intense and bread-like flavour was related to propanone, 3-methylbutanal, benzyl alcohol and 2-phenylethanol (Hansen et al., 1989a).
Effect of type and flour composition on flavour compounds

The interactions between starter cultures and flour components play an important role in the production of flavour compounds in the sourdough breads. A particular favoured cereal for sourdough is rye flour that contains flavour precursors, amino acids, fatty acids and phenolic compounds that are converted into flavour-active compounds in different production stages of baking (Schieberle & Grosch, 1983; Schieberle & Grosch, 1985; Schieberle & Grosch, 1987; Hansen, 1995). In rye, germination imparts cereal and fresh flavour in extruded products. Key flavour components are dimethyl sulphide and 2-methylbutanal which could be related to the sensory attribute intensities. Extruded rye sourdough has been reported to have an intense, sour flavour and porous texture with high contents of furfural, ethyl acetate, 3-methylbutanol and 2-methylbutanol and 2-phenyl-ethanol (Hansen et al., 1994). Other contributors to the overall crumb flavour of rye bread include 3-methylbutanal, 2-nonenal, 2,4-decadienal, hexanal, phenylacetaldehyde, methional, vanillin, 2,3-butan-dione, 3-hydroxy-4,5-dimethyl-2(5H)-furanone and 2- and 3-methylbutanoic acid (Kirchhoff & Schieberle, 2001).

Sourdough breads may vary in flavouring compounds as a result of flour composition and blending of the cereal flours (Schieberle & Grosch, 1985; Schieberle & Grosch, 1992; Schieberle & Grosch, 1994). Wheat flour composition influences the concentration of ethyl acetate and ethanol production in heterofermentative LAB sourdoughs which are more abundant with high-grade flours (Hansen & Hansen, 1994a). Ash contents from 0.55 to 1.00% had positive influences on total volatiles. Wheat bread from sourdough sponges including Lb. plantarum or Lb. sanfranciscensis had a higher content of 2,3-methyl-1-butanol; and through association of LAB and yeasts, bread was considered of enhanced flavour quality with higher contents of 2,3-methyl-1-butanol, 2-methylpropanoic acid, 3-methylbutanoic acid and 2-phenyl-ethanol (Hansen et al., 1998b; Hansen & Hansen, 1996). It is considered that this is the result of the combination of greater bacterial acidification and proteolysis (Gobbetti, Corsetti et al., 1994; Gobbetti, Simonetti et al., 1994; Levesque, 1991) which may be attributed to Lb. sanfranciscensis in the sourdough. Hansen & Hansen (1994b) have suggested an association of Lb. sanfranciscensis, Lb. plantarum and S. cerevisiae as optimal for aroma balance in wheat sourdough breads.

The amount of fermentable carbohydrate in the flour varies with the type of cereal, but in particular with the activities of amylases, xylanases and peptidases enzymes of flour. In wheat flours, totals of maltose, sucrose, glucose and fructose vary from 1.55 to 1.85% depending on extent of starch hydrolysis, and activities of microbial enzymes and microbial contaminants (Martínez-Anaya, 1996).

The flour type has a momentous effect on the production of ethyl acetate and ethanol in sourdough heterofermentative cultures, with the highest amounts detected in sourdough made from wholemeal and low-grade flours (Hansen & Hansen, 1994a). In contrast to white wheat bread, the starch granules are very much swollen in the bran sourdough bread with enzymatic mixture which improves the texture, volume, flavour and shelf-life of bread (Katina, Salmenkallio-Marttila, Partanen, Forsell, & Autio, 2006).

With the high extraction rate (80–100%), the content of nutrients such as B vitamins and minerals increases compared to low extraction rate flour (65–75%), as does the buffering capacity of flour chiefly due to the phytic acid (Batifolier, Verny, Chanliaud, Rèmésy, & Demigné, 2005). These factors stimulate the growth and biochemical activity of the LAB followed by a higher production of acids and flavour compounds (Martínez-Anaya, Benedito de Barber, & Esteve, 1994; Salovaara & Valjakka, 1987). Soya flour, containing a range of enzyme activities, also could influence the bread volatiles, isolated by dynamic headspace techniques and quantified by gas chromatography and gas chromatography/mass spectrometry (Luning et al., 1991).

Wheat and rye flours are mostly used for sourdough making but maize and rice flours can also be used (Buttery & Ling, 1999; Buttery, Orts, Takeoka, & Nam, 1999; Clarke & Arendt, 2005; Rocha & Malcata, 1999; Sanni, Onilude, & Fatungase, 1998). Major volatiles of rice flour include 1-hydroxy-2-propanone, furfuryl alcohol, 2,5-dimethylpyrazine, 2-methylpyrazine, pyrazine, hexanal, furfural, pentanal, 3-hydroxy-2-butanoate and ethyl-3,6-dimethylpyrazine (Buttery et al., 1999) whereas major volatiles identified in corn flour include 2-methylpyrazine, 1-hydroxy-2-propanone, 4-vinylguaiacol, 2-acetyl-4-methylpyrazine and 3-methyl-1-butanol, γ-butyrolactone, furfuryl alcohol and 2,5-dimethylpyrazine (Buttery & Ling, 1999).

Conclusion

In sourdoughs, flavour-active compounds are produced by LAB and yeasts individually and through their interactions. Heterofermentative LAB mainly produce ethyl acetate and certain alcohols and aldehydes, whereas homfermentative LAB synthesise diacetyl and other carbonyls. In contrast, iso-alcohols are products of yeast fermentation but may contribute little towards final bread flavour. Addition of fructose/glucose/maltose or citrate to the dough increases LAB contributions to volatile formation in baking but Maillard and Caramelization reactions are also responsible for flavour formation in baked cereal products. Interactions between microbial strains and ingredients, fermentation temperature, pH and leavening time have all been evaluated. Further research should be conducted on optimization of fermentation process in wheat bread production by varying biotechnical parameters, levels of emulsifiers/improvers including lactose, milk solids and...
oxidants. Effects of dried mixed cultures of yeasts-LAB also need further attention.

References


Gobetti, M., Corsetti, A., Rossi, J., La Rosa, F., & De Vincenzi, S. (1994). Identification and clustering of lactic acid bacteria and
yeasts from wheat sourdoughs of central Italy. Italian Journal of Food Science, 1, 85–94.
Heinio, R., Katina, K., Wilhelmsen, A., Myllymaki, O., Rajamaki, T., Latva-Kala, K., et al. (2003). Relationship between sensory perception and flavour-active volatile compounds of germinated, sourdough fermented and native rye following the extrusion process. LWT-Food Science and Technology, 36, 533–545.
Levesque, C. (1991) ‘Etude des Potentialite`s de la Souche S47 de Saccharomyces cerevisiae a` produire des alcools superieurs a` partir de compos’es organiques identifie`s et des precureurs de la fatine’ in these d’universite`, Nantes.


