



Kati Katina

**Sourdough: a tool for the improved
flavour, texture and shelf-life of
wheat bread**

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Sourdough: a tool for the improved flavour, texture and shelf-life of wheat bread

Kati Katina

VTT Biotechnology

ACADEMIC DISSERTATION

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Abstract

The aim of this thesis was to develop means to optimize the biochemical activity of sourdough to achieve improved bread flavour, texture and shelf-life, and to determine how the structure of fresh and aged bread is altered by the use of sourdough. The influence of process conditions of prefermentation on subsequent bread quality is clarified through this thesis.

The importance of an optimised sourdough process in improving the flavour and texture of subsequent bread was demonstrated. The sourdough process had to be optimised in a strain-specific manner to obtain improved flavour and texture. Lactic acid bacteria fermented sourdoughs were more effective in tuning bread quality compared to yeasted preferments if the appropriate conditions were utilised. The ash content of flour and fermentation time were the main factors regulating bread flavour and texture in all of the sourdough types studied. The possibility to improve bread flavour by utilising sourdough with moderate acidity and an enhanced level of amino acids was demonstrated in this study. Bread volume and shelf-life were also improved by sourdough, which was fermented with low ash content flour and with optimised fermentation time.

A new type of sourdough was presented: bran sourdough, which could effectively compensate the negative effect of added wheat bran on bread volume and shelf-life in high-fibre baking. An altered microstructure (improved protein network, enhanced swelling of starch and modified degradation of cell wall components) of bran sourdough breads, especially if made with enzymes, was related to improved volume. A reduced staling rate of bran sourdough breads was further explained due to reduced starch retrogradation and a slower loss of molecular mobility.

In conclusion, wheat bread flavour and texture were effectively modified using optimised sourdough. Bran sourdough was introduced as a potential tool for the future development of technologically and nutritionally superior raw materials for all cereal foods, such as bread, breakfast and snack products.

Academic dissertation

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Preface

This study was carried out in VTT Biotechnology during the years 1998–2005. The research was part of VTT research programme "Future Foods" and was mainly made in the projects "Functional wheat bran in baking (1997–2000)" and "Functional wheat sourdough (1997–2002)". National Technology Agency (Tekes, Finland) and the participating Finnish cereal companies are gratefully acknowledged for partially funding the research.

I sincerely thank Professor Juha Ahvenainen for providing excellent working facilities. I want to warmly thank the "cereal power" professor, Hannu Salovaara and his research team for introducing the world of cereal technology to me, and their support and co-operation during this work. I am very much obligated to the reviewers of this thesis, Professor Simo Laakso and Dr. Clas Lönner for constructive criticism.

I am deeply grateful to Professor Kaisa Poutanen for supervising, guiding and supporting this work among all her other duties. I am most grateful for supervision and guidance of this work to Dr. Karin Autio. My special thanks to Dr. Marjatta Salmenkallio-Marttila, who strongly encouraged me to complete this work and had always time to discuss and help with the topics related to this work. This work would have not been possible without support and co-operation of several colleagues. I wish to warmly thank rest of my co-authors at VTT Biotechnology in Publications I–V: Dr. Raija-Liisa Heiniö, Dr. Pirkko Forssell, Riitta Partanen, Professor Tiina Mattila-Sandholm (currently at Valio), Hanna-Leena Alakomi and Marketta Sauri. The skilful and devoted assistance of Arja Viljamaa, Leena Pulkki, Tiina Järvinen and Leila Kostamo is particularly appreciated. I want to warmly thank my working group, and especially the baking team (Laura, Leena and Arja) for creating encouraging and pleasant environment for this work.

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List of original publications

The present thesis is based on following Publications, which will be referred to in the text by their Roman numerals (I–V). Unpublished data is also presented.

- I Katina, K., Poutanen, K. and Autio, K. (2004). Influence and interactions of processing conditions and starter culture on formation of acids, volatile compounds, and amino acids in wheat sourdoughs. *Cereal Chemistry* 81 (5), 598–610.
- II Katina, K., Heiniö, R.-L., Autio, K. and Poutanen, K. (2005). Optimisation of sourdough process for improved sensory profile and texture of wheat bread. *Lebensmittel Wissenschaft und Technologie*, in press.
- III Salmenkallio-Marttila, M., Katina, K. and Autio, K. (2001). Effects of bran fermentation on quality and microstructure of high-fibre wheat bread. *Cereal Chemistry* 78 (4), 429–435.
- IV Katina, K., Salmenkallio-Marttila, M., Partanen, R., Forssell, P. and Autio, K. (2005). Effects of sourdough and enzymes on staling of high-fibre wheat bread. *Lebensmittel Wissenschaft und Technologie*, in press.
- V Katina, K., Sauri, M., Alakomi, H.-L. and Mattila-Sandholm, T. (2002). Potential of lactic acid bacteria to inhibit rope spoilage in wheat sourdough bread. *Lebensmittel Wissenschaft und Technologie* 35 (1), 38–45.

The author of the present thesis was responsible for planning the research, biochemical and textural analysis, baking experiments, experimental design, statistical treatment of the results and the interpretation of the results in all Publications, with the following exceptions: design and interpretation of sensory evaluation was performed with Raija-Liisa Heiniö in II, interpretation of the microstructure was performed with Marjatta Salmenkallio-Marttila in III and IV, analysis of NMR and DSC results was performed by Riitta Partanen in IV, cultivation of microbes (I, II, V) and turbidometer measurement (V) were performed by Marketta Sauri and Hanna-Leena Alakomi.

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List of symbols

AEDA	Aroma extract dilution analysis
ANOVA	Analysis of variance
CFU's	Colony forming units
d.b	On dough basis
DF	Dietary fibre
f.b	On flour basis
FD	Flavour dilution factor
GMO	Gene modified organism
HS/GC/MS	Headspace gas chromatography – mass spectrometry
LAB	Lactic acid bacteria
P-value	Statistical significance of difference between samples
RSM	Response surface method
TTA	Total titratable acidity
Tukey's HSD test	Tukey's honestly significant difference test

1. Introduction

The art of bread-making is ancient; Egyptians had over 50 different types of cakes, unleavened breads, and bread leavened with beer foam or sourdoughs (Jacob 1997). Traditionally, sourdough breads were made from brown or wholemeal flours due to a lack of advanced milling technologies for the production of endosperm wheat flour. Industrial wheat bread production started at the beginning of last century, after the introduction of baker's yeast as a superior leavening agent for bread production instead of using sourdough or brewing yeast for leavening. Over decades, many different bread-making processes have been developed, which have the common aim of converting wheat flour, made from the endosperm part of kernel, and other ingredients into a light aerated and palatable food.

The most important quality characteristics for wheat pan breads are high volume, soft and elastic crumb structure, good shelf-life and microbiological safety of the product (Cauvain 2003). In wheat products, textural characteristics are mainly based on the formation of gluten network, which has the ability to extend and keep the gas from yeast fermentation and makes a direct contribution to the formation of a cellular crumb structure. Unfortunately, wheat bread is a perishable product and fresh product quality starts to deteriorate immediately after baking. Bread becomes stale largely because of the physical changes that occur in the starch-protein matrix of the bread crumb.

The introduction of new methods for bread baking such as frozen dough baking and partly baked products have created new challenges for obtaining good product quality. There is a massive selection of baking additives such as emulsifiers and enzymes, which can effectively improve particularly texture and shelf-life of the breads on the market. However, most of these substances are either E-numbered additives or produced with GMO-organisms, which does not tally with current consumer trends preferring GMO-free, high-quality products baked without chemical additives. Sourdough baking is an alternative to the use of additives. Furthermore, recent results demonstrate the effectiveness of sourdough fermentation in improving the nutritional value of cereal products (Liukkonen et al. 2003, Kariluoto et al. 2004).

In addition, the flavour of wheat bread has suffered greatly from intensive, shorter bread-making methods and the flavour of bread made with traditional longer processes is considered superior in comparison to short processes. The overall flavour of bread is typically process-induced, and formed from relatively tasteless raw materials during processing. Bread flavour is formed from hundreds of different compounds, which themselves originate from the different stages of the bread-making process such as raw materials, fermentation (both preferments and fermentation during proving) and baking. Due to the complex nature of bread flavour, it still remains a challenge for researchers after 30 years of intensive research. The means to enhance the desired flavour attributes of bread would be highly appreciated by the baking industry.

Wheat is the world's most important food grain. Wheat kernel can be divided into three main parts: i) The endosperm, which forms 83% of the kernel, ii) the bran which forms 14%, and iii) the germ which forms 3%. In general, wheat is mainly consumed in bakery products manufactured from endosperm flour, which has unique technological properties for creating superior, consumer appealing product quality in terms of flavour and texture.

The endosperm part of cereal kernel, however, is nutritionally inferior to the whole grain. Recent epidemiological findings have indicated a protective role of whole grain foods against several western diseases (Jacobs et al. 1998, Liu et al. 2000, Pereira et al. 2002). Dietary fibre has long been considered the major health protective component of grains. There is now increasing evidence also of other protective compounds, such as oligosaccharides and phytochemicals, which together with DF are concentrated in the outer layers of the grains. There is consumer interest in the health aspects of food, including functional food products with specific physiological functions of health relevance. However, good sensory properties remain a prerequisite for any successful food, and consumers expect food to fulfil other criteria such as safety and convenience. Thus, the concept of whole grain baking, or at least enrichment with nutritionally valuable parts such as wheat bran, would be highly beneficial also in wheat baking.

The most common source of DF in wheat baking is cereal bran, especially wheat bran. However, additions of cereal bran, especially in such amounts that health benefits can be expected, cause severe problems in the flavour and texture. In

addition, bran fraction is rich in spoilage organisms such as rope forming bacteria *Bacillus subtilis* which increases the challenges to retain microbiological safety of wheat breads supplemented with bran.

Acceptable loaf volume and shelf-life of high-fibre breads is difficult to accomplish. Bran supplementation usually weakens the structure and baking quality of wheat dough and decreases bread volume and the elasticity of the crumb. The firmness of fresh fibre enriched bread has been reported to be 41% higher compared to bread without bran (Laurikainen et al. 1998). It has been suggested that the deleterious effects of fibre addition on the dough structure are due to the dilution of the gluten network, which in turn impairs gas retention rather than gas production. However, this well known effect of bran inclusion in reducing the volume of bread cannot be explained simply in terms of the dilution of gluten forming proteins (Gaillard and Gallagher 1988). According to Gan et al. (1992), the bran materials in expanded dough appear to disrupt the starch-gluten matrix and also restrict and force gas cells to expand in a particular dimension. This greatly distorts the gas cell structure and may contribute to the resultant crumb morphology, which is an important element of crumb texture. Thus, the supplementation of dietary fibre, such as wheat bran, requires changes in processing techniques for the production of baked goods with good consumer quality.

Sourdough has a natural, additive-free image and lactic acid bacteria have been used in food for thousands of years and are "generally regarded as safe". The introduction of sourdough would be particularly suitable in cereal foods containing dietary fibre rich plant tissues as both nutritional and technological quality could be considerably enhanced in these products by utilising sourdough.

Sourdough is ancient way to improve flavour, texture and microbiological shelf-life of bread, and is widely utilised in whole grain rye baking (Lorenz and Brummer 2003). In wheat baking, the use of sourdough is optional (Röcken and Voysey 1995) and less common, most likely due to the milder flavour of products made from endosperm wheat flour, which limits the amount of acidic sourdough that can be used in wheat products. Contrary to acidic rye breads, the majority of consumers accept only mild acidity in wheat breads. Thus, control of the acidity level of wheat sourdoughs and subsequent breads is a premise for the improvement of quality.

The effects and mechanisms of sourdough fermentation are, however, complex and numerous, and not yet fully understood. In the future, effective use of sourdough, for example in additive replacement purposes, will require a designed fermentation processes in which complex biochemical changes during sourdough fermentation can be controlled to obtain improved product quality. It has been shown for some time, however, that the fermentation process does remarkably more to the properties of bread than simply produce acids (Wood et al. 1975). Furthermore, profound changes in the flour and dough matrix created during the sourdough process can have both desired and undesired consequences in final product quality; improved product quality is obtained only under the optimised conditions of sourdough fermentation (Clarke et al. 2003).

The current study was initiated to improve conditions of using sourdough technology in wheat bread baking and extend the application range of sourdough technology to wheat bran.

1.1 Sourdough: microbiology and the process

1.1.1 Microflora of sourdough

Sourdough is a process in which flour and water (and other ingredients) are fermented with microbes originating from preceding sourdough, commercial starter culture, bakery equipment or from flour.

Spontaneous dough fermentation starts by mixing flour with water without adding a starter culture or portion of a preceding sourdough (mother dough). The microflora of such dough depends on the microflora of the raw materials used and the prevailing hygienic conditions, and is variable in terms of kind, origin and storage conditions of the flour, as well as the technological parameters of the fermentation process applied. For example, whole grain cereals and 100% extraction rye flour may contain 10^4 – 10^6 CFU of unspecified bacteria per gram; in which 10^2 – 10^3 CFU^g belongs to genus *Lactobacillus* (Salovaara 2004). The dominating microbes in spontaneously fermented doughs are homofermentative lactobacilli and *pediococci*, which are found both in wheat and rye sourdoughs at the level of 3×10^8 – 3×10^9 CFU^g. Typical homofermentative lactic acid bacteria (= LAB) in spontaneous sourdoughs are *Lb. casei*, *Lb. delbrueckii*, *Lb.*

farciminis, *Lb. plantarum*, *Pc. pentosaceus*. Typical heterofermentative LAB are *Lb. brevis*, *Lb. buchneri*, and *Lb. fermentum* (Stolz 2003). Various yeast strains have also been isolated from spontaneous fermentations such as *S. cerevisiae* and *Pichia satoi* (Beech and Davenport 1971).

Commercial sourdough processes do not rely on fortuitous flora but on the use of commercial starter cultures or a portion of the preceding sourdough (mother dough). Inoculation of the sourdough with a starter increases the number of lactic acid bacteria to 10^7 – 10^8 CFU^{-g}, which gives little possibility for growth of contaminating organisms, including those which are imported from flour. The range of commercial starter culture includes (1) pure starter cultures in powder form, i.e., freeze-dried single strain or mixed strain cultures, and (2) starter cultures that are active sourdoughs. The use of pure start cultures has not yet gained wide acceptability among bakers, and back-sloping methods (using seed from previous sourdough as a starter) are much more common, probably due to both economical and technological reasons. The sourdough fermentations are performed as single- or multistage processes. During the week, the mother dough is removed from the active sourdough, which is used to start the sourdough fermentation for the next day (Stolz 2003).

Sourdough bacteria cannot be seen as an independent group of bacteria existing only in sourdough. Rather they can be described as a group of specially adapted varieties of LAB that are also common in other habitats. The most relevant bacteria isolated from sourdough belong to the "genus" *Lactobacillus* (Stolz 2003). Isolated lactobacilli strains can be obligate homofermentative strains, facultative heterofermentative lactobacilli or facultative heterofermentative strains. Homofermentative strains ferment hexoses to produce mainly lactic acid. Many heterofermentative strains can also ferment pentosans to produce lactic acid, acetic acid and ethanol. The formation of end products with heterofermentative strains is dependent on the processing conditions of sourdough and type of heterofermentative strain (Röcken et al. 1992).

The most common lactic acid bacteria species found in sourdoughs are *Lb. acidophilus*, *Lb. farciminis*, *Lb. delbrueckii* (obligate homofermentative), *Lb. casei*, *Lb. plantarum*, *Lb. rhamnosus* (facultative heterofermentative), *Lb. brevis*, *Lb. sanfransicensis* and *Lb. fermentum* (obligate heterofermentative) (Salovaara 2004).

Yeast flora of sourdough is more homogenous. Universal sourdough yeast appears to be *Candida milleri* or strains closely related to it. *Saccharomyces cerevisiae* is also often reported (Stolz 2003).

Associations of yeasts and lactic acid bacteria are often encountered or used in the production of beverages and fermented foods (Gobbetti 1998). The most well known example of stable co-existence of yeast and lactic acid bacteria is the presence of *Candida milleri* and *Lb. sanfransiscensis* in San Francisco sourdough (Kline and Sugihara 1971), in Dutch wheat sourdoughs (Nout and Creemers-Molenaar 1987) and in German commercial sourdough (Böcker et al. 1990). Interaction of these two micro-organisms is based on the fact that *Lb. sanfransiscensis* mainly ferment maltose and leave glucose for the use of yeast, which in turn is incapable of assimilating maltose. Furthermore, the low pH of sourdough has little effect on the growth of *C. milleri* (Gänzle et al. 1998).

1.1.2 Different types of sourdoughs/preferments

Two main types of wheat preferments exist in industrial use (Lorenz and Brummer 2003):

- 1) Yeasted preferment (or yeasted sourdough), in which yeast is used to ferment wheat flour for 4–16 hours at room temperature (22–26 °C). Acidity development is modest and originates from the natural flora of flour, yeast or bakery equipment.
- 2) Actual sourdough in which lactic acid bacteria (+ yeast) are used to ferment flour for up to 24 hours at 25–35 °C.

Yeasted preferments are always produced in a one stage process, but the utilisation of actual sourdough can be achieved in one stage, multiple stages or in a continuous processes. The current trend, however, is towards one stage sourdoughs (Lorenz and Brummer 2003).

1.1.3 Use of sourdough/yeasted preferments in wheat products

Yeasted preferments are used more commonly than acidic wheat sourdoughs. A typical example of yeasted preferment is the sponge and dough method, which is widely used in the United States. In this procedure, 2/3 of the total amount of flour, part of the water and the yeast is mixed loosely and fermented for up to 5 hours. Then, the sponge is combined with the rest of the formula ingredients and mixed into developed dough. Another example of a yeasted preferment is the "sur poolish" method in France. In this method, the prefermentation method consists of a semiliquid mixture of flour and water (50/50), with approximately one third of the total flour quantity used at this stage. Baker's yeast at levels of about 0.5 to 1.0% (based on flour) is added to the mixture and fermented for five hours at 22 °C (Molard et al. 1979). Similar types of yeasted preferments are commonly used in Finland and in Germany (Valjakka et al. 2003, Lorenz and Brummer 2003). It is noteworthy, however, that yeasted preferment benefits lactic acid fermentation of fortuitous flora originating from flour if the fermentation conditions (mainly extended fermentation periods, higher temperature and use of flour with higher ash content) allow the build-up of natural bacteria population.

Traditional acidic sourdoughs are used in Mediterranean countries and in the area of San Francisco in the United States. Examples of wheat products, in which particular product quality is based on the use of sourdough, are Italian Christmas cake, Panettone (Sugihara 1977), San Francisco sourdough bread (Kline et al. 1970) and soda crackers (Sugihara 1977). Wheat sourdough is used currently in Italy (Corsetti et al. 2000), Germany (Seibel and Brummer 1991) Spain (Barber and Baquena 1989) and France (Infantes and Tournier 1991). In Finland, acidic wheat sourdoughs are utilised in only a few bakeries. At the start of the 21st century, bread-making with sourdough constitutes a very small fraction of all bread manufactured; it is estimated e.g., in France, that only 3% of all bread produced is manufactured by using sourdough (Poitrenaud 2003). Successful utilisation of wheat sourdough demands skilled personnel and careful control over the process to obtain high quality products and is thus a much more demanding way to improve product quality in comparison to baking additives. However, the effectiveness of wheat sourdough in improving flavour, texture and nutritional quality is unique, and the utilisation of sourdough has gained popularity in recent times (Kulp 2003).

Amount of preferment/sourdough to be used in subsequent bread dough varies usually between 5–40% (of dough weight); the lowest values being typical for acidic sourdough and highest for yeasted preferment. A special type of sourdough bread is known in Italy and in Spain, in which microbes are added directly to the bread dough and whole dough is fermented up to 9 hours before baking.

1.2 Influence of sourdough fermentation on wheat flour and dough; biochemical changes

The sourdough process depends on numerous factors including, among other things, the composition of microflora, fermentation and enzymatic activities and flour characteristics as presented in Figure 1. These factors do not act separately but in an interactive way, adding to the complexity of the system. Thus, many factors simultaneously affect the processes involved in sourdough fermentation such as the formation of acidity, the production of volatile compounds and the degradation of carbon and nitrogen compounds (Martínez-Anaya 1996b). The level and intensity of these modifications during sourdough fermentation determines subsequent bread quality.

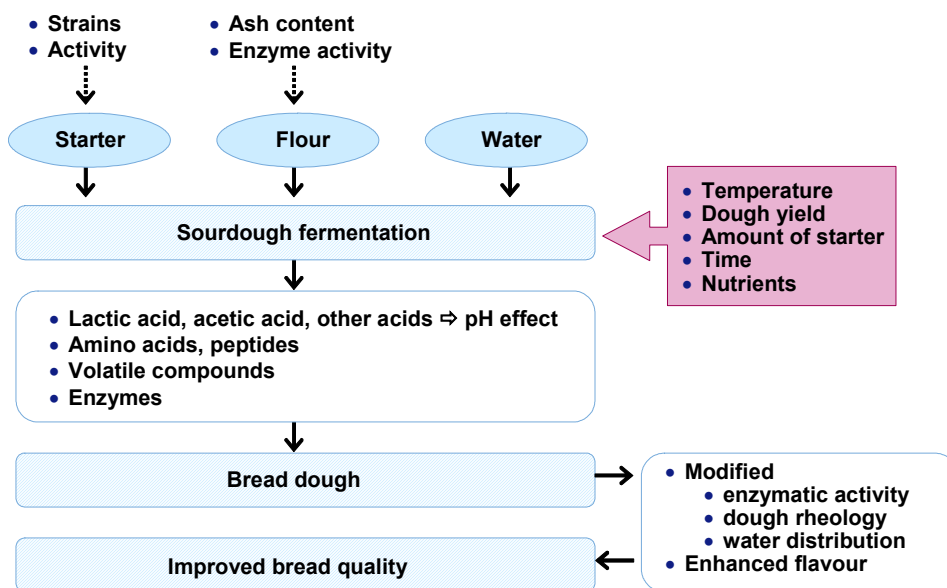


Figure 1. Sourdough fermentation and influencing factors.

1.2.1 Acidification

Sourdough fermentation is based on lactic acid and alcoholic fermentation depending on the composition of microflora and fermentation conditions. Typical pH and TTA values of acidic wheat sourdough are 3.6–3.8 and 8–13, respectively (Brummer and Lorenz 1991). The typical content of lactic acid is 600–800 mg/100 g sourdough and for acetic acid 80–160 mg/100 g sourdough (Barber et al. 1992, Hansen and Hansen 1994b). For yeasted preferment, typical pH and TTA-values are 4.7–5.8 and 3–9, respectively.

The main factor regulating acidification is the amount of fermentable carbohydrates. White flours have very low quantities of free sugars, about 1.55–1.84% (sucrose, maltose, glucose, fructose and oligosaccharides) but the endogenous α -amylase activity, started during mixing, increases initial maltose levels by ten- to fifteenfold. The alfa-amylase activity of wheat flour depends on the extraction rate and quality of flour; wholemeal flour and especially the bran fraction having the highest enzyme activity (Martínez-Anaya 2003).

Sugars used by lactic acid bacteria as an energy source vary by species and even by strain. The most common lactic acid bacteria identified in sourdoughs are capable of fermenting pentoses, hexoses, sucrose and maltose, although some species such as *Lb. sanfransiscensis*, are specific to maltose. Furthermore, *Lb. sanfransiscensis* hydrolyzes maltose and accumulates glucose in the medium in a molar ratio of about 1:1 (Martínez-Anaya 2003). Some lactic acid bacteria common in sourdough systems are fructose negative and grow faster in maltose than in glucose, *Lb. plantarum* prefers maltose and glucose over fructose for rapid growth and weakly ferments sucrose. Heterofermentative lactobacilli such *Lb. sanfransiscensis*, *Lb. brevis* and *Lb. fermenti* are stimulated by oxygen, which shifts the metabolic pathway from ethanol to an acetate route and thus enhances acetic acid production. Proton acceptors, such as fructose, have a similar type of effect as they push the metabolism towards the acetate kinase pathway, producing traces of mannitol and an increase in acetic acid. The efficiency of fructose as a proton acceptor depends on concentration, temperature, and dough consistency (Martínez-Anaya 2003).

In alcoholic fermentation, various yeast strains produce carbon dioxide and ethanol in anaerobic conditions according to the Emden-Meyerhof-Pathway

from the same sugars as lactic acid bacteria. Glucose, fructose and sucrose are fermented similar rates by *S. cerevisiae*, because yeast contains active invertase rapidly hydrolyzing sucrose into glucose and fructose already at the dough mixing stage. Yeast strains (e.g *Torulopsis holmii*) lacking invertase do not ferment sucrose. Maltose is fermented by yeast only at later stages of fermentation, after the major portion of glucose and fructose has been utilised (Kulp 2003). Typical sourdough yeasts, *C. milleri* and *S. exiguus*, do not ferment maltose.

As typical sourdough often consists of both yeast and lactic acid bacteria, the interaction of yeasts and lactobacilli is important for the metabolic activity of sourdough. When *Lb. sanfransiscensis*, *Lb. brevislinderi* or *Lb. plantarum* are associated with maltose negative yeast such as *S. exiguus*, the lactobacilli completely takes up maltose and bacterial cell yield increases and acid production is not inhibited. In association with *S. cerevisiae*, a decrease in bacterial metabolism occurs due the faster consumption of maltose and particularly glucose by the yeast, which reduces the availability of glucose when both micro-organisms grow together (Martínez-Anaya 2003). The presence of yeast has been reported to diminish acid production (Brummer 1988).

The production of acids depends also on other things such as fermentation temperature, time and dough yield. Optimum temperatures for the growth of lactobacilli are 30–40 °C depending on strain (Stanier et al. 1987) and for yeasts 25–27 °C. In general, a higher temperature, a higher water content of sourdough and the utilisation of wholemeal flour enhances the production of acids in wheat sourdoughs (Brummer and Lorenz 1991, Lorenz and Brummer 2003).

1.2.2 Proteolysis

The proteolytic enzymes present in the sourdough system degrade various cereal proteins (Spicher and Nierle 1988, Thiele et al. 2002). This proteolysis produces free amino acids, which may act as flavour precursors (Spicher and Nierle 1984, Schieberle 1990a, Gobbetti et al. 1995). Gluten proteins determine, to a great extent, the rheological properties of wheat doughs and texture of wheat breads. The proteolytic degradation of gluten proteins also alters the formation of the gluten network (Kawamura and Yonezawa 1982), which can result in weak and

sticky dough. Even minor changes in the gluten structure can cause considerable changes in dough properties (Pizzinatto and Hoskeny 1980).

Contradictory theories have been proposed about whether the proteolytic activity in sourdoughs originates from the LAB or from the cereal materials present in the sourdough. Spicher and Nierle (1988) concluded that only one third of the proteolytic activity in a rye sourdough originated from cereal enzymes. Other studies have also shown that proteinases from LAB can liberate soluble protein hydrolysates from gluten proteins (Gobbetti et al. 1996, Wehrle et al. 1999). However, recent results indicate that the proteolytic activity of lactobacilli is negligible compared to that of the wheat flour in a wheat sourdough system (Loponen et al. 2004, Thiele et al. 2002, 2003, 2004). The endogenous cereal proteases of flours have been shown to degrade cereal prolamins under acidic conditions (Kawamura and Yonezawa 1982, Brijs et al. 1999). In addition, the role of cereal proteolytic enzymes on the rheological properties of dough has been well established (Pizzinatto and Hoskeny 1980, Kawamura and Yonezawa 1982, Lin et al. 1993).

Substantial hydrolysis of gliadinin and glutenin proteins occurs during sourdough fermentation due to pH-mediated activation of cereal enzymes; especially aspartic proteinase appears to be active in the conditions of wheat sourdough (Thiele et al. 2003, Loponen et al. 2004). Furthermore, sourdough fermentation results in a solubilisation and depolymerisation of the gluten macropolymer (Thiele et al. 2004). Cereal proteinases have been shown to be active at pH 3.7, but show no activity at pH 5.5. Thus, proteolysis during sourdough fermentation is highly dependent on formation of acids. Lactic acid bacteria contribute to overall proteolysis during sourdough fermentation by creating optimum conditions for activity of cereal proteinases. Furthermore, lactic acid bacteria with high proteolytic activity contribute to the hydrolysis of wheat proteins in a strain-specific manner as e.g. substrate specificity varies between different LAB strains (Di Gagno et al. 2003), and liberation of certain amino acids such as ornithine may require specific LAB strain to be utilised (Thiele et al. 2002).

Increased proteolysis during sourdough fermentation leads to the liberation of amino acids in wheat and rye doughs (Spicher and Nierle 1988, Collar et al. 1991, Gobbetti et al. 1994). Generally, sourdough fermentation with lactic acid

bacteria results in an increase of amino acid concentrations during fermentation, whereas dough fermentation with yeast reduces the concentration of free amino acids (Thiele et al. 2002). The level of individual amino acids in wheat doughs depend on the pH level of the dough, fermentation time and the consumption of amino acids by the fermentative microflora (Thiele et al. 2002). An accumulation of amino acids can occur only if proteolysis exceeds the demand of amino acids for the growth of microbes. Glutamic acid, isoleucine and valine are essential for the growth of *Lb. brevis* and *Lb. plantarum*. Each individual amino acid, except for lysine, cysteine and histidine, is suitable for the growth of yeasts, which thus expresses a much stronger demand on amino acids and low molecular weights peptides during fermentation. At the beginning of fermentation, proteolytic activity is low due to non appropriate pH conditions; at this stage, yeast follows a log phase of growth that induces a strong demand for nitrogen. Lactic acid bacteria also have a long lag phase and develop metabolic activities at a slow rate during the first 4 h. Thus, the accumulation of amino acids is not observed until the later stages of fermentation (Martínez-Anaya 2003).

In wheat sourdoughs, *Lb. brevis linderi*, *Lb. sanfranciscensis*, *Lb. brevis* and *Lb. plantarum* have been reported to increase the levels of aliphatic, dicarboxylic, and hydroxyl amino acids (Collar et al. 1991, Gobbetti et al. 1994). The yeasts *S. cerevisiae* and *S. exiguus* decrease the total level of amino acid in a similar way, the latter being more effective in amino acid removal from the dough (Spicher and Nierle 1984). The combination of yeast and lactic acid bacteria shows intermediate values for total amino acid levels. The estimated content of free peptides of sourdough with *Lb. brevis* or *Lb. plantarum* is lower than the estimated amount of amino acids. Reactivity of peptides is higher during fermentation in comparison to amino acids, and both of the above-mentioned strains reduce the content of peptides during fermentation, especially if *S. cerevisiae* is associated with these LAB (Mascaros et al. 1994). Furthermore, LAB fermentation has been reported to affect the size distribution of peptides; the presence of lactobacilli decreases the content of larger peptides and increases that of smaller molecules such as dipeptides and amino acids (Thiele et al. 2003).

The proteolytic activity of wheat sourdough depends on the microbial starter and the processing conditions. For wheat sours, the extraction rate of flour and the fermentation temperature have been reported to be the main factors with positive influence on the level of free amino acids and on the accumulation of

hydrophobic and basic amino acids. Dough yield has also been reported to influence the level of amino acids; soft doughs contain a lower amount of amino acids (Martínez-Anaya 2003). The positive influence of wholemeal flour on amino acid liberation is probably based on location and a higher activity of cereal aspartic proteinase, as well as other cereal proteases in the outer layers of the cereal kernel (Loponen et al. 2004).

1.2.3 Production of volatile compounds

Volatile compounds are produced both in lactic acid fermentation and in alcoholic fermentation, but the levels of these compounds are much higher in yeast fermentation (Hansen and Hansen 1994b, Meignen et al. 2001). Lactic acid bacteria produce volatile compounds in a strain-specific manner; each strain has its own profile of volatile compounds. Homofermentative lactobacilli are characterised by the high production of diacetyl, acetaldehyde, hexanal and heterofermentative strains are characterised by the production of ethyl acetate, alcohols and aldehydes. Isoalcohols (2-methyl-1-propanol, 2,3-methyl-1-butanol), with their respective aldehydes and ethylacetate, are characteristic volatile compounds of yeast fermentation (Damiani et al 1996). Association of lactic acid bacteria and yeast results in a synergistic increase of alcohols in comparison to fermentation with yeast alone. The main bacterial volatile compounds, ethylacetate and carbonyls are significantly decreased (Martínez-Anaya 2003). For example, the association of *Lb. brevis ssp linderi*, or *Lb. plantarum*, and *S. cerevisiae* increases the formation of yeast fermentation products such as 1-propanol, 2-methyl-propanol and 3-methylpropanol and the number of aroma compounds detected (Gobbetti et al. 1995). All volatiles formed do not affect the final flavour of the bread. Generally, it is believed that compounds having a high flavour dilution factor (FD) will have a significant impact on the final odour (Schieberle 1996). 3-methylbutanol and 2-phenylethanol have been reported to be the most important flavour active compounds formed in yeast fermentation (Gassenmeier and Schieberle 1995). Acetic acid, butanoic acid, phenylacetic acid, 2- and 3-methylbutanoic acid, and pentanoic acid have been reported to be important flavour active compounds formed during sourdough fermentation (Czerny and Schieberle 2002).

It is possible to control the formation of volatile compounds, besides choosing the appropriate starter culture, by adjusting fermentation conditions such as time, temperature, flour extraction rate and dough consistency. In sourdough fermented with mixed cultures, raising the temperature from 25 °C to 35 °C, increased yeast fermentation. At 25 °C, ethyl acetate, acetic acid and lactic acid were formed; whereas at 30 °C, ethanol, 2-methyl-1-propanol and 3-methyl-1-butanol were typical products. However, increasing the temperature further to 35 °C did not modify the flavour profile (Gobbetti et al. 1995). In firm dough, volatile compounds are formed by lactobacilli, whereas in soft doughs, ethanol and ethylacetate are predominant with high levels of isoalcohols in dough containing heterofermentative strains. These compounds are assumed to be formed due to yeast fermentation, which can proceed further without the formation of acetic acid. A high ash content of flour has been reported to increase the amount of volatile compounds in mixed fermentations (Hansen and Hansen 1994a, Czerny and Schieberle 2002). This has been proposed to be due to the presence of different levels of certain flavour components already occurring in the flour and to the metabolic changes that take place during lactic acid fermentation. Information about the effect of fermentation time on the formation of volatile compounds is limited, even though there is a general assumption that longer processing (fermentation) times enhance the flavour of the final product. Gobbetti et al. (1995) reported an increase in the levels of volatile compounds in acidic sourdoughs when the fermentation time was extended from 3 to 9 hours. However, after 24 hours of fermentation, the amount of volatile compounds had reduced, probably due to the evaporation of volatile compounds from the dough. Schieberle (1996) reported that during 16 hours of fermentation in the yeasted preferment, the amount of 3-methylbutanol and 2-phenylethanol increased during first 8 hours and then ceased during last 8 hours. The lack of amino acid precursors was proposed to explain the phenomenon.

1.2.4 Modification of dietary fibre components

The influence of sourdough on fibre content (both soluble and insoluble) and quality is important from both nutritional and technological points of view. From a nutritional point of view, for example, the molecular weight of β -glucan (the main soluble fibre in oats and barley) has been postulated to be relevant for the

cholesterol lowering effect of oats. From a technological point of view, high molecular weight soluble arabinoxylans have been linked to improved volume and softness in wheat baking (Courtin and Delcour 2002).

The fermentation of barley and oat fibre concentrates (rich in β -glucan) with LAB has been reported to decrease the content of insoluble fibre (IF) in barley and oats. Based on the measured viscosities, the content of soluble fibre in barley fibre concentrate did not change during fermentation, whereas the content of soluble fibre in oat fibre concentrate decreased. However, the molecular weight of β -glucan was apparently not affected. (Lambo et al. 2005). Opposing results were obtained by Degutyte-Fomins et al. (2002), who reported an increased solubility of beta-glucan in fermented oat bran suspension and a decreased viscosity of water-soluble fraction in bran ferment. The controversial results might be due to the different acidity levels obtained (pH 4.0 for oat bran concentrate and 5.2 for oat bran) and the differences in the chemical composition and enzyme activity of the preferments. Boskov-Hansen et al. (2002) reported reduced dietary fibre content and increased solubility of arabinoxylans during imitated rye sourdough fermentation. The mechanism for reducing the content of DF is no clear, but it is postulated to be due to the ability of microorganisms to produce extracellular enzymes that are either cell-free or cell-associated (Schwarz 2001).

The sourdough fermentation of rye increased the amount of soluble pentosans and decreased the molecular size of pentosans (Härkönen, unpublished results) most likely due to a lower pH (Härkönen et al. 1995). In wheat sourdough baking, the influence of commercial pentosanases on the molecular weight of arabinoxylans was shown to be dependent on starter type (Devesa and Martínez-Anaya 2001). The influence of processing conditions or starter culture on insoluble or soluble pentosans during wheat sourdough fermentation is not known. However, the addition of pentosans extracted from wheat bran has been reported to improve bread volume (Zhen et al. 2003).

1.2.5 Production of exopolysaccharides

Recently, it has been reported that certain lactic acid bacteria are able to produce exopolysaccharides, which might have a positive affect on bread volume and shelf-life (Korakli et al. 2001, Tiekling et al. 2003). Dough should contain, for

example, xanthan and dextran between 0.1–2% d.w in order to induce positive changes in bread texture. As sourdough or preferments are generally used at the level of 5–40% (depending on the sourdough type), the content of exopolysaccharides should be rather high. Furthermore, the acidity level of sourdough can only be moderate to avoid negative effects on texture.

Microbial exopolysaccharides (= EPS) are utilised in the food industry, particularly the dairy industry. Microbes produce slime to protect themselves from drying out and other stress factors (Salkinoja-Salonen and Lounatmaa 2002). It is generally agreed that EPS influence product texture, mainly due to their ability to influence viscosity of the product. For example, the typical texture of Finnish milk product "viili" (pudding type fermented milk product) is due to exopolysaccharides produced by certain lactic acid bacteria (*Lactococcus lactis* ssp. *cremoris* and *Leuconostoc mesenteroides*). The best known microbe-originated EPS's are dextran, xanthan and levan and are produced by bacteria. The production of these compounds in sufficient amounts during sourdough fermentation would create the possibility to replace hydrocolloids in baking. Hydrocolloids have been reported to improve bread quality (Rosell et al. 2001). As Tiekking et al. (2003) characterised the ability of several sourdough originated LAB to produce EPS, some of the reported benefits of sourdough on bread quality may be based on the formation of these compounds. More research, however, is needed to clarify the role of EPS producing strains in sourdough baking.

1.2.6 Influence of sourdough on dough rheology

The utilisation of sourdough has fundamental effects on dough rheology at two levels: in sourdough itself, and in subsequent bread dough containing sourdough (at the level of 5–40%). At the sourdough level, fermentation causes decreased elasticity and viscosity (Kawamura and Yonezawa 1982, Wehrle and Arendt 1998, Clarke et al. 2004). At the final dough level, the addition of sourdough has been reported to cause less elastic and softer doughs; effect of being more pronounced with longer fermentation times (Clarke et al. 2004). A decreased resistance to extension and increased extensibility has been reported for doughs containing sourdough (Di Gagno et al. 2003).

Enzymatic activity, particularly protease activity, and the formation of acids are the main causes for observed rheological changes both in sourdough and in final bread doughs. Chemical acidification has been shown to increase the solubilisation of proteins due to a positive net charge in acidic conditions (Maher Galal et al. 1978). Under optimal mixing conditions, chemically acidified doughs showed more elastic behaviour (Wehrle et al. 1997) and increased softness and extensibility of gluten (Schober et al. 2003). However, in the sourdough process, acidity development is progressive over time (not instant as in chemically acidified doughs) allowing cereal (and microbe) originated enzymes to affect dough texture. According to recent results (Clarke et al. 2004, Thiele et al. 2004), the main factor determining the rheology of doughs containing preferment is the activity of cereal proteases with acidic optima. As the actual weakening of the gluten structure has been shown to take place in doughs containing sourdough (Thiele et al. 2004), it is very likely that solubility of pentosans and the formation of exopolysaccharides have a major role in determining dough and subsequent bread texture in products containing preferment. Also, there is a strong indication that the level of rheological changes taking place in these doughs (and subsequent influences on bread quality) can be controlled by adjusting fermentation time (Clarke et al. 2004) and the ash content of flour during the prefermentation process (Collar et al. 1994b).

1.3 Sourdough as a tool for tailoring bread quality

1.3.1 Flavour

Flavour is simultaneous perception of taste, odour and trigeminal nerve response (Lawless and Heymann 1999). The four classical basic tastes are sweet, salty, sour and bitter. The diversity of flavours is caused by volatile compounds in the headspace of the product and mediated by smell. Different types of odours and tastes tend to mask or suppress each other, which is often called mixture suppression. Tastes may also increase the apparent intensity of odours, or odours may increase the apparent intensity of taste (Noble 1996).

Flavour is one of the most appreciated sensory attributes in bread (Caul 1972). Bread flavour is composed of hundreds of volatile and non-volatile compounds, which originate from different parts of the baking process such as fermentation

and the baking step, and from ingredients. Many alcohols, aldehydes, ketones, acids, esters, ether derivatives, furan derivatives, hydrocarbons, ketones, lactones, pyrazines, pyrrol derivatives and sulphur compounds serve as flavour stimuli (Maga 1974, Folkes and Grahamshaw 1981, Schieberle 1996). Bread flavour can be divided into crumb and crust flavour; their role in the overall flavour impression of bread is still controversial even though crust flavour seems to dominate overall flavour due to its intensity in the aroma of fresh bread. Some potential compounds that influence bread flavour are presented in Table 1. The pyrazine and pyrrol derivatives have been found to contribute strongly to the flavour of bread crust (Schieberle and Grosch 1987, Schieberle and Grosch 1989). When bread is eaten, the overall sensory impression is also influenced by the colour and textural attributes of the bread.

None of the identified compounds can singly be considered as the key component of bread aroma (Drapron and Molard 1979), but they seem to act in a synergistic way, with their relative proportions being determinant. On the other hand, the presence of a determined substance does not mean that it participates in creating the flavour; the concentration must exceed the detection threshold which, in turn, can be modified by other substances present (Meilgaard et al. 1999).

Bread flavour is formed during processing which occurs when relatively non-aromatic flour undergoes several changes during the baking process. White wheat flour also furnishes a small amount of volatile compounds and aroma precursors, although their contribution to bread flavour is estimated to be small (Drapron and Molard 1979). With wholemeal flour, the amount of volatile compounds as well as amino acids is considerably higher (Czerny and Schieberle 2002). Fermentation and baking are the main sources of flavour of bread and both steps are essential (Hansen and Schieberle 2005). Volatile compounds are generated from previous precursors present in ingredients or resulting from enzymatic or mechanical degradations (El Dash 1971, Drapron and Molard 1979). The most important precursors of the identified compounds are sugars and amino acids (El Dash 1971, Spicher and Nierle 1988, Martínez-Anaya 1996b, Thiele et al. 2002). The formation of bread flavour during the baking process is illustrated in Figure 2.

Fermentation of sugars by yeasts during the bread-making process leads to a large number of volatile compounds that are supposed to be responsible for the distinctive characteristics associated with bread flavour: bread from unfermented doughs has a different aroma from that made from fermented dough (Jackel 1969) and has a much smaller amount of volatile compounds (Frasse et al. 1993). Fermentation is essential in the creation of normal bread flavour: if baker's yeast is replaced with baking powder, the crumb structure and the baking times are normal, but the bread has a completely unacceptable flavour in comparison to yeast-raised bread (Hansen et al. 1989, Schieberle 1989). With bread leavened with baking powder, the crust of the bread particularly shows an odour note reminiscent of day-old bread and the cracker-like, roast odour note is lacking. When the flavour compounds of chemically and yeast leavened breads are compared, one striking difference is that 2-acetyl-1-pyrroline is a minor component in chemically leavened bread, but a major component in yeast leavened bread. 2-acetyl-1-pyrroline was identified as one of most important character impact compounds of bread crust flavour and the presence of this compound creates roasty, cracker-like flavour in the crust. (Schieberle and Grosch 1985). Yeast also converts free amino acids by Erlich's mechanism to flavour compounds such as alcohols. These alcohols have one carbon less than the corresponding amino acids. For example, valine, leucine and phenylalanine are converted respectively to isobutanol, 3-methylbutanol and 2-phenylethanol (Molard 1994, Gassenmeier and Schieberle 1995).

During baking, thermal reactions such as caramelisation and the Maillard reaction, promote crust flavour and colour (Drapron and Molard 1979, Schieberle 1989). The important role of baker's yeast as a source of the precursors in the formation of 2-acetyl-1-pyrroline and 2-acetyltetrahydropyridine (important character impact flavour compounds of crust) has been well elucidated (Schieberle 1990a, Schieberle 1990b). These compounds are formed both inside and outside the yeast cell when sugars and amino acid degradation products react with each other during the Maillard reaction in baking. According to Schieberle (1990a), 2-acetyl-1-pyrroline is created in the reaction between pyruvaldehyde (generated by yeast sugar metabolism) and 1-pyrroline (a product of the Strecker degradation of either proline or ornithine).

Table 1. Some of the important flavour compounds of wheat bread.

Compound	Location		Flavour origin
	Crumb	Crust	
Acetic acid	x ^{2,4}	x ⁵	Fermentation
Methional	x ⁴	x ^{4,5}	Baking, Stecker's degradation
(E)-2-nonenal	x ^{1,4}	x ⁴	Degradation of lipids
(E,E)-2,4 decadienaal	x ^{1,4}		Degradation of lipids
Diacetyl	x ^{1,2,3,4}	x ^{4,5}	Fermentation
Phenylacetaldehyde	x ⁴	x ⁴	Baking, Stecker's degradation
Acetaldehyde	x		Fermentation
2-methylpropanol	x ²		Stecker's degradation
3-methylbutanol	x ^{1,2,3}	x ⁵	Baking, Stecker's degradation
3-methylbutanal		x ^{1,4,5}	Baking, Stecker's degradation
2-methylbutanal		x ⁵	Baking, Stecker's degradation
Benzylethanol	x ²		
2-phenylethanol	x ^{2,3}		
Ethanol	x ²		Fermentation
1-octen-3-one	x ^{1,4}	x ⁴	Degradation of lipids
2-acetylpyridine		x ⁵	Baking, Maillard's reaction
4-(Z)heptenal		x ^{4,5}	Degradation of lipids
2-acetyl-1,4,5,6-tetrahydropyridine		x ⁴	Baking, Maillard's reaction
4-hydroxy-2,5-dimethyl-3(2H)-furanone		x ^{1,4}	Baking, Maillard's reaction
2-acetylpyrroline		x ^{1,4}	Baking, Maillard's reaction

References: ¹Schieberle 1996, ²Hansen and Hansen 1996, ³Frasse et al. 1993, ⁴Schieberle and Grosch 1991, ⁵Folkes and Grahamshaw 1981)

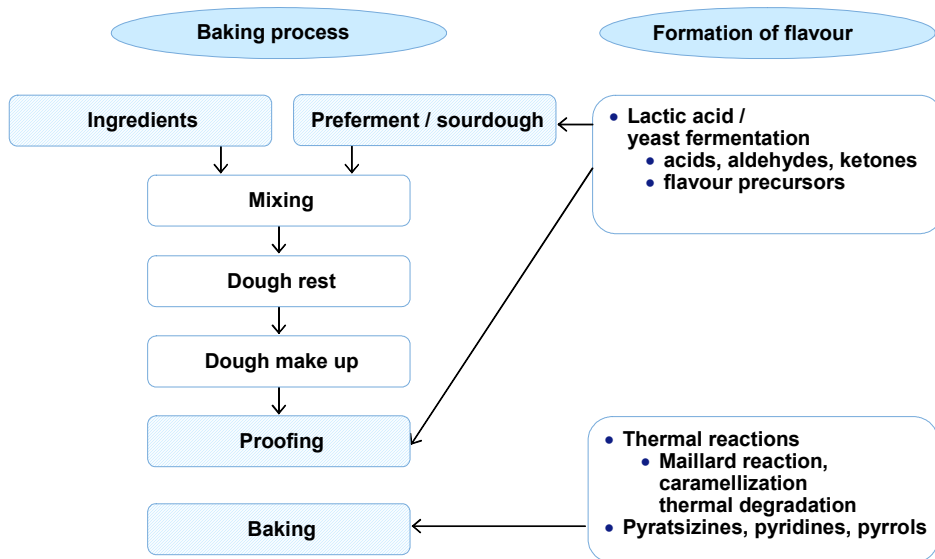


Figure 2. Formation of bread flavour during the baking process.

In general, the utilisation of wheat sourdough is assumed to improve flavour, but controversial results have been published (Salovaara and Valjakka 1987, Collar et al. 1994a, Hansen and Hansen 1996, Meignen et al. 2001, Thiele et al. 2002). Yeasted preferment is not assumed to enhance bread flavour significantly (Lorenz and Brummer 2003) but other views do exist on the topic (Molard et al. 1979). Research has focused, during recent decades, on the formation of flavour active volatile compounds or precursors during prefermentation processes, and the impact on the sensory profile of subsequent bread has been studied less, particularly by utilising appropriate sensory methods such as quantitative descriptive analysis.

The influence of sourdough on bread flavour is based on three main factors: i) formation of acidity, ii) formation of flavour precursors such as amino acids, iii) formation of volatile compounds. As the amount of sourdough/preferment varies 5–40% of final bread dough, the impact on bread flavour depends on acidity level, level of free amino acids and important flavour compounds in sourdough.

The formation of acidity has a profound effect on bread flavour. A minor amount of acetic acid has been claimed to enhance wheat bread flavour (200 ppm, Molard et al. 1979). Both lactic acid and acetic acid cause a pungent, unpleasant flavour in higher concentrations (Molard and Chagnier 1980, Salovaara and Valjakka 1987,

Lilja et al. 1993). The maximum amount of acids in wheat bread without decreased pleasantness ratings has been reported to be 0.35% (flour weight), which corresponds pH 4.9 and TTA-value 6.0, respectively. Furthermore, increased acidity enhanced overall taste intensity (Lilja et al. 1993).

A high amount of free amino acids in sourdough has been linked to an enhanced intensity of the overall flavour of bread, and enhanced intensity of roasted flavour (Martínez-Anaya 1996a, Thiele et al. 2002). The formation of ornithine during sourdough fermentation has particularly been shown to be a major factor in the enhanced roasted flavour of subsequent bread (Thiele et al. 2002). Proline has also been established to be major precursor for 2-acetyl-1-pyrroline responsible for the roasted flavour of crust. The formation of peptides, with unpleasant sensory characteristics, during sourdough fermentation has also been proposed (Martínez-Anaya 1996b). The levels of leucine and phenylalanine in yeasted preferments have been shown to be key factors enhancing the formation of 3-methylbutanol and 2-phenylethanol responsible for crumb flavour notes (Gassenmeier and Schieberle 1995).

The formation of certain volatile compounds in preferments/sourdoughs is generally considered to be important for the sensory profile of bread. Chemically acidified doughs with corresponding levels of amino acids as in sourdoughs improve bread flavour only slightly (Thiele et al. 2002), which indicates the significant role of sourdough originated volatile compounds in flavour improvement. Intensive formation of volatile compounds, and particularly the formation of flavour active compounds, has been established in yeasted preferments (Frasse et al. 1993, Gassenmeier and Schieberle 1995, Hansen and Hansen 1996). Thus, the compounds responsible for bread flavour are synthesised already during the prefermentation process and the total amount of these compounds in subsequent bread dough is increased by the use of preferment. However, the influence of these yeasted preferments on the intensity of bread flavour has been reported to be minor (Brummer and Unbehend 1997, Lorenz and Brummer 2003), and long yeast fermentation has actually been reported to decrease, for example, the roasted flavour of bread in comparison to a shorter process (Zehentbauer and Grosch 1998). The level of key crumb odourants, 2-phenylethanol and 3-methylbutanol, originating from preferments varies 14–48% depending on the amount of yeast and water in preferments (Gassenmeier and Schieberle 1995). As yeasted preferments usually have

modest levels of acids and amino acids, their impact on bread flavour is most likely purely due to volatile compounds present in the preferment. Yeast fermentation during proofing (at bread-making stage) has clearly a very strong effect on final bread flavour, but volatiles originating from yeasted preferment might have a less important role in final sensory profile of the bread.

The importance of fermentation conditions during the sourdough process for the sensory profile of subsequent bread is obvious. The ash content of flour utilised in prefermentation is a major factor determining the intensity of subsequent bread flavour attributes (Rouzaud and Martínez-Anaya 1997); a higher ash content of flour results in enhanced intensity of taste and aroma. However, sourdough breads had better sensory quality if the low ash content of flour was used in the sourdough process (Rouzaud and Martínez-Anaya 1997, Collar et al. 1994b). Thus, a higher ash content of flour strongly increases the metabolic activity of sourdough (formation of acids, amino acids and volatile compounds) but the resulting stronger flavour of bread is not necessarily accepted and liked by consumers. The influence of fermentation time during prefermentation on the sensory profile of subsequent bread is not well reported, but longer fermentation times are generally assumed to create stronger flavours in comparison to no-time or short time processes in wheat baking (Molard 1994).

1.3.2 Volume and shelf-life

1.3.2.1 Specific volume

Loaf-specific volume is a primary quality characteristic of bread (Maleki et al. 1980). The texture of wheat bread depends heavily on the formation of the gluten network, which traps gas from yeast fermentation, and makes a direct contribution to the formation of the cellular crumb structure of the subsequent bread (Cauvain 2003). Gluten proteins of wheat create unique viscoelastic properties of dough, which allow dough to expand due to the formation of carbon dioxide during fermentation and, at the same time, retain most of this gas inside the dough texture. Also, other biopolymers of flour, starch and pentosans, have to be swollen and solubilised in appropriate amounts to obtain the optimal bread texture.

The utilisation of sourdough has been reported both to decrease (Salovaara and Valjakka 1987; Armero and Collar 1996; Rouzaud and Martínez-Anaya 1997) and to increase (Hansen and Hansen, 1996, Corsetti et al. 1988, 2000, Crowley et al. 2002, Clarke et al. 2002) bread volume. A key to improved volume has been proposed to be a type and level of acidification (Clarke et al. 2002). The utilisation of sourdough bread improved bread volume more efficiently in comparison to its chemically acidified counterpart (Clarke et al. 2002). Also, acidic sourdough has been shown to be more effective in improving bread volume in comparison to yeasted preferment (Corsetti et al. 2000). However, if the acidity of sourdough is further increased, bread volume diminishes (Barber et al. 1992). Even though many sourdough microbes produce carbon dioxide, it is generally assumed that the utilisation of sourdough improves gas retention and not the gas production in bread dough (Hammes and Gänzle 1998, Clarke et al. 2002).

The influence of sourdough on bread volume has been proposed to be mainly due to enzymatic reactions taking place during fermentation. During sourdough fermentation, pH drops gradually allowing amylolytic, proteolytic, lipoxygenases, peroxidases, catalases and polyphenol oxidases to modify dough components over a time period of 8–20 hours. Thiele et al. (2004) demonstrated that gluten macropolymers are solubilised and degraded during sourdough fermentation, which resulted in a softer and less elastic texture of bread dough containing sourdough (Clarke et al. 2002). Clarke et al. (2004) demonstrated also that reduced firmness and elasticity occurs over time in preferments and in subsequent bread doughs irrespective of the presence of acid or lactic acid bacteria. The resulting weaker gluten structure indicates that improved volume in sourdough breads must have other factors than the integrity of gluten network to create better gas holding properties. Weaker gluten might allow the higher expansion of dough, but this usually decreases gas retention. Improved volume in sourdough breads may be partly explained by the solubilisation of arabinoxylans and by the production of exopolysaccharides (Korakli et al. 2001).

Thus, it is possible to improve a specific volume by utilising wheat sourdough if the amount of acidity, as well as the level of other metabolites and the degradation of gluten, is at appropriate levels. Accordingly, fermentation conditions have a significant influence on bread volume: the utilisation of wholemeal flour in sourdough fermentation has been reported to decrease bread volume (Salovaara

and Valjakka 1987, Rouzaud and Martínez-Anaya 1997) and fermentation time has to be optimised in a strain-specific manner (Clarke et al. 2003).

1.3.2.2 Shelf-life

Bread is a perishable commodity, whose shelf-life is normally limited by physiochemical deterioration called staling, leading to a hard and crumbly texture and a loss of fresh-bake flavour. The staling phenomenon has been intensively studied for decades, but a scientific and technological understanding of the mechanism of staling, however, is far from clear (Chinachoti 2003).

Bread texture becomes harder largely due to physical changes that occur in the starch-protein matrix of the bread crumb. Retrogradation is the process by which starch amylopectin reverts to a more ordered state after gelatinisation. The solubility of starch decreases, the structure of starch granules becomes rigid and shrunken and part of the gelatinised starch amylopectin crystallises. The crumb characteristics of the bread change: the crumb becomes hard, coarse and crumbly. Even though starch retrogradation has been shown to be the primary cause of bread firming, other factors such as the state of proteins and the water content of dough affect the staling rate (Martin and Hosenev 1991, Martin et al. 1991, Davidou et al. 1996, Zobel and Kulp 1996). Other flour components, like pentosans and lipids, and added ingredients like fat, emulsifiers, sugars and enzymes affect crumb softness of fresh bread and its shelf-life. Water is also assumed to have a significant role in the staling process. Water is more abundant in the swollen amorphous regions of starch, facilitating local polymer chain mobility (plasticisation) and the subsequent crystallisation and retrogradation (Chinachoti and Vodovotz 2001). Water distribution within regions in gluten, amorphous and crystalline starch are assumed to play an important role in starch and gluten rigidity.

Utilisation of sourdough in wheat baking has been reported both to decrease and increase, or to have no impact, on shelf-life of wheat bread; influence being dependent on fermentation conditions and the process utilised (Armero and Collar 1998, Corsetti et al. 1998, 2000, Crowley et al. 2002, Kulp 2003). The influence of sourdough on bread staling is not completely elucidated and controversial results are partly due to the different use and interpretation of terminology. For example, improved shelf-life is related in some cases to

delayed starch retrogradation even though the actual firmness of sourdough breads after storage is higher in comparison to the control bread (Barber et al. 1992, Corsetti et al. 1998). After all, from a consumer's point of view, increased softness is considered to be reduced staling.

The effect of sourdough is partly based on improved volume, as a positive correlation has been established between softness and volume (Maleki et al. 1980). Pure acidity does not explain improved softness as chemically acidified breads (with comparable acidity levels obtained in sourdoughs) stale faster compared to sourdough breads (Clarke et al. 2003) or at a similar rate to the control bread (Corsetti et al. 2000). However, the acidity level of sourdough and subsequent bread dough seems to be an important factor, as strong acidity provides a harder crumb structure and milder acidity increases softness (Barber et al. 1992, Crowley et al. 2002). However, the rate of starch retrogradation has been reported to be lower for sourdough breads, even with strong acidity (Corsetti et al. 1998, Barber et al. 1992). This might be partly explained by the formation of low molecular weight dextrans in acidic conditions, which have been postulated to interfere with the starch retrogradation process (Rouzaud and Martínez-Anaya 1997). On the other hand, sourdough has been reported to reduce starch hydrolysis by inhibiting endogenous flour α -amylases. This limits the liberation of low molecular mass dextrans, which interfere with starch crystallisation and delay bread staling (Siljeström et al. 1988). Thus, depending on sourdough type and the acidity level obtained, sourdough may either enhance or decrease starch retrogradation.

The significant role of strain-specific properties of LAB has been proposed to explain observed differences in the staling of sourdough breads (Corsetti et al. 1998, 2000), because different sourdough breads, with comparable acidity levels, had varying staling rates (in terms of firmness and starch retrogradation). LAB strains possessing proteolytic and amyolytic properties were most effective in delaying staling (Corsetti et al. 1998). Furthermore, recently Korakli et al. (2003) demonstrated the ability of certain sourdough originated LAB of producing exopolysaccharides, many of which are potential anti-staling substances. Also, the solubilisation of arabinoxylans during sourdough fermentation might reduce bread staling as pentosans have been postulated to prevent starch-gluten interactions responsible for staling (Gray and Bemiller 2003). The combined use of exogenous enzymes (α -amylase and xylanase)

and sourdough in the same baking process have been reported to enhance the rate of acidification, improve bread volume and retard bread staling in white wheat baking (Martínez-Anaya et al. 1998, Corsetti et al. 2000, Di Cagno et al. 2003). In high-fibre baking, the influence of the above-mentioned combination treatment has not been reported.

1.3.2.3 Prevention of rope spoilage

Rope spoilage is the most important spoilage of bread after mouldiness. It is usually caused by *Bacillus* sp., especially *Bacillus subtilis* and *Bacillus licheniformis* (Kirschener and Von Holy 1989). Both *B. subtilis* and *B. licheniformis* cause a potential risk to foodborne illness when present at levels of 10^5 CFU^g in bread crumb (Kramer and Gilbert 1989). This level has been reported to occur after only two days of storage at room temperature (Rosenquist and Hansen 1998). Furthermore, some *B. licheniformis* strains were confirmed to be toxigenic (Salkinoja-Salonen et al. 1999). However, not all *B. subtilis* strains cause ropiness, Röcken and Voysey (1993) reported that 48% of 25 *B. subtilis* strains isolated from bakery sources were able to cause rope within seven days of storage at 37 °C. Rope forming strains have been reported to differ from other strains by higher degrees of heat resistance spores, faster growth rates in bread crumb, and enhanced protease and amylase production during growth (Röcken and Voysey 1993).

In general, rope spoilage is noticed as an unpleasant sweet odour similar to that of rotting melons, following by the discolouration of bread crumb, usually in patches that vary from yellow to brown. Finally, the crumb loses its structure; it becomes sticky and soft. The intensity of symptoms is, however, strain-specific and this sometimes makes it difficult to detect rope spoilage in time (von Holy et al. 1988).

Rope spoilage of wholemeal bread has been reported to happen more frequently than the spoilage of white wheat bread, possibly due to higher spore counts of bran fractions (Eyles et al. 1989). Thus, the control of growth of rope forming bacteria is an important issue when the wholemeal flour of bran fractions are utilised in bakery products. As the current trend is to avoid chemical preservatives also in wholemeal baking, there has been a significant increase in the rope spoilage of wheat bread (Voysey and Hammond 1993).

Control of the growth of *Bacillus* species in bread is a difficult task because they have a common distinctive feature of forming endospores. These are inactive or dormant stages of the organism, which have a unique degree of resistance to environmental stresses such as UV, ionising irradiation, disinfectants, hydrogen peroxide, osmotic stress and heat, as well as hydrostatic pressure (Rosenquist and Hansen 1995). Thus, any sanitary or other means to lower *Bacillus* counts originating from raw materials or bakery equipment are, in most cases inefficient, for lowering the level of *Bacillus* counts in sufficient amounts. Furthermore, the baking stage in the oven usually activates these endospores present in dough to grow instead of killing them (Rosenquist 1996).

One effective means to limit the germination and growth of *Bacillus* is to increase acidity, which creates an unfavourable environment for the survival of endospores. When pH was lowered from 6.86 to 4.62 with acetate, the thermal destruction of spores of *B. subtilis* was increased tenfold (Gradel et al. 1976). Acidity can be increased by adding acidulants or by sourdough fermentation. The most effective acids are propionic acid and acetic acid. Lactic acid has been reported to be less effective (Rosenquist and Hansen 1998) in agar tests. Lactic acid bacteria can also produce antimicrobial compounds (bacteriocins) such as nisin, which have the potential to inhibit germination and the growth of *Bacillus* species (de Vuyst and Vandamme 1993). However, lactic acid bacteria with a capability to produce bacteriocins have not been very effective in sourdough breads, and the inhibitory effect of sourdough has been reported to be mainly due to the production of acids (Rosenquist and Hansen 1998).

The acidity level of sourdough and subsequent bread dough seems to be important for the inhibition of rope spoilage. The addition of 10% of sourdough with pH-values of subsequent bread from 4.96–5.24 and TTA-valued from 3.0–4.7 did not prevent rope spoilage. The addition of 15% of sourdough with pH-values of subsequent bread from 4.55–4.77 and TTA-valued from 4.6–5.1 prevented rope spoilage (Rosenquist 1996). It is noteworthy that the effective acidity level of enhanced microbial shelf-life does not meet the acidity criteria for a good flavour of wheat sourdough bread, which have been set to TTA values from 2.8 to 4.34 (Collar et al. 1994a).

1.4 Aims of the study

Sourdough fermentation has been studied intensively during recent decades, but is still not a well-understood process due to its complicated nature, and designed sourdough processes are not easily achieved. An understanding of acidity formation and its relation to other biochemical changes during sourdough fermentation, in particular, would create the possibility to control the activity of sourdough and subsequent bread quality attributes such as flavour and texture.

The aims of this thesis were

- To develop means to optimise sourdough fermentation at the biochemical level during the prefermentation process itself and at the product level of subsequent bread to obtain improved flavour, texture and shelf-life.
- To establish the most important biochemical changes during sourdough fermentation for improved bread flavour.
- To establish potential of bran fermentation to compensate negative effects of bran supplementation in high-fibre baking.
- To relate the texture and shelf-life of high-fibre sourdough wheat bread to structural changes in the starch-protein matrix and the retrogradation of starch.

2. Materials and methods

Raw materials and experimental procedures are described in detail in the original Publications I–V, and only a brief summary is presented below.

2.1 Raw materials and process conditions

2.1.1 Flours and wheat bran

Two different white wheat flours were used in Publications I–V. Furthermore, wholemeal wheat flour, wheat flour with medium ash content and wheat bran were used in the preparation of sourdoughs in Publications I–V. The properties of different flours are presented in Table 2. The amount of total and soluble fibre, total pentosans, soluble pentosans and beta-glucan for WF 2 and wheat bran are presented in Publication III. Methods for the determination of chemical composition are presented in Publication III. Farinograph measurements were performed in duplicate according to the AACC standard method (1998).

Table 2. Properties of wheat flours used in Publications I–V.

Quality test	WF1*	WF2	WMF	MaF
Protein content (N*5.7), %	11.7	12.03	11.86	14.6
Ash content, %	0.61	0.7	1.82	1.22
Wet Gluten	27.2	28.0	nd**	
Falling number	286	275	nd	271
Farinograph				
Absorption, %	61.5	63	nd	66.2
Dough development time, min	1.5	2	nd	5.75
Stability, min	4	8	nd	8.0
Publication	I, II	III, IV, V	I, II	I, II

* WF= wheat flour, WMF= wholemeal wheat flour, MaF=wheat flour with medium ash content, ** nd= not determined

2.1.2 Recipe and other raw materials

Reference breads

Three different types of reference bread were used in this study:

- a) Reference bread for sourdough breads (Reference 1). This bread contained 75% white wheat flour. A detailed recipe is presented in Publication II.
- b) Reference breads (2) for wheat breads supplemented with 20% wheat bran (f.b). These included white wheat bread (reference 2a) and wheat bread containing 20% untreated bran (reference 2b). Detailed recipes are presented in Publications III and IV.
- c) Reference breads (2) for breads containing *Bacillus* bacteria. These included reference white wheat bread without added *Bacillus* bacteria (Reference 3a) and reference bread with added *Bacillus* spores (3b). Detailed recipes are presented in Publication V.

Sourdough breads

Three different types of sourdough bread were used in this study:

- a) Sourdough breads in Publication II. These breads contained 20% sourdough on dough basis. Microbial strains, as well as the ash content of flour, fermentation time and temperature during the sourdough process were varied according to the experimental design detailed in Publications I and II.
- b) Bran sourdough breads with and without enzymes. These breads contained 20% bran sourdough on flour basis as detailed in Publications III and IV. Furthermore, a combination of bran sourdough and enzymes was included in Publication IV.
- c) Sourdough breads with varying degrees of acidity (Publication V). These breads contained 10–20% sourdough fermented with different antimicrobial strains to obtain high or low levels of acidity with a particular strain. Detailed recipes are presented in Publications V.

2.1.3 Process conditions

Sourdough processes

3 different sourdough types and processes were used in this study:

- a) Wheat sourdoughs with varying degrees of flour ash content, length of fermentation time and level of fermentation temperature. The above mentioned parameters were varied according to experimental design and sourdoughs were fermented with *Lb. plantarum*, *Lb. brevis*, *S. cerevisiae* or combinations of yeast and lactic acid bacteria. Sourdough had a constant dough yield and amount of added microbial strain. Details of these sourdoughs are presented in Publications I and II.
- b) Bran sourdoughs (2) were prepared by fermenting milled bran with yeast or with yeast and *Lb. brevis*. Bran sourdough was fermented for either 4 hours at 28 °C (yeasted bran preferment) or 16 hours at 25°C (bran sourdough with yeast and lactic acid bacteria). Details of these sourdoughs are presented in Publications III and IV.
- c) Wheat sourdoughs with varying degrees of acidity. The acidity of sourdoughs was controlled by adjusting the ash content of flour, fermentation time and temperature according to the experimental design. Details of these sourdoughs are presented in Publication V.

Ready sourdoughs were stored for a maximum of 1 hour at 4 °C and used in subsequent baking without delay.

Dough processing

Flour, water, sourdough (If included in the recipe) and other ingredients were mixed with a Diosna spiral mixer or Kenwood table mixer (Publication V) to obtain optimal dough consistency. The optimal mixing time and water absorption were determined by farinograph and with test bakings for every dough type. After 20–30 minutes floor time at a constant temperature and humidity, the dough was divided into pieces of 400 g, which were moulded into loaves with a conical rounder and bread moulder. In Publication V, moulding was performed by hand.

Loaves were proofed in tin pans at a constant temperature and humidity for 45–60 minutes according to bread type and baked at 200–220 °C for 20 minutes. Details of the baking process for each bread type are presented in Publications II–V.

2.1.4 Microbes and utilised microbial methods

The lactic acid bacteria and the yeasts utilised in sourdough fermentations are summarised in Table 3. In rope spoilage studies, target organisms were *Bacillus subtilis* (VTT E-96699) and *Bacillus licheniformis* (VTT E-978813).

Table 3. Microbial strains utilised in the study.

Microbial strain	Origin	Publications
<i>Lb. brevis</i> L 62	Dried commercial starter, C.H. Hansen, Denmark	III, IV, V
<i>Lb. brevis</i> VTT E-P5612	VTT's culture collection, isolated from rye sourdoughs	I–II
<i>Lb. plantarum</i> VTT E-78076	VTT's culture collection, isolated from rye sourdoughs	I–II, V
<i>S. cerevisiae</i> VTT B-81047	VTT's culture collection, isolated from rye sourdoughs	I–II
Baker's yeast	Commercial dried yeast Fermipan, Gist-Brocades, Netherlands	III, IV
<i>Pc. pentosaceus</i> VTT E-90390	VTT's culture collection, isolated from rye sourdoughs	V

Commercially dried lactic acid bacteria and yeast were added to sourdoughs as such (Publications III and IV). Commercial LAB culture contained approximately 2×10^{10} CFU/ g of culture (according to the manufacture), and utilised amount (0.162 g /450 g of bran sourdough) provided addition level of 7.2×10^6 CFU/g of sourdough for LAB in III and IV. In I, II and V in-house grown cultures were added at the level of 10^7 CFU/g of sourdough and yeast at

the level of 10^6 CFU/g of sourdough, respectively. Amount of each LAB was at the level of 10^7 CFU/g and for yeast at the level of 10^6 CFU/g when sourdough was fermented with yeast+LAB. Preparation of the culture filtrates for sourdoughs (strains from VTT culture collections) was performed as described in I, II and V. The growth ability of selected LAB in sourdoughs was ensured with AP-PCR and cultivation methods.

Screening of antimicrobial activity of *Lb. brevis* L 62, *Lb. plantarum* VTT E-78076, *Pediococcus pentosaceus* VTT E-90390 against several *Bacillus* species was performed by turbidometer tests, described in detail in Publication V.

2.2 Biochemical analysis of sourdoughs

Several chemical parameters were measured in sourdoughs: pH, TTA (I–V), content of lactic acid and acetic acid (I), concentration of amino acids and the concentration of selected volatile compounds (I). The impact of these compounds on subsequent sourdough bread flavour and texture, as well as microbiological shelf-life, were described in this study.

2.2.1 Acidity measurements

The pH and TTA were measured with titrimetric analysis (Standard Methoden für Getreide, Mehl und Brot 1978) with a TitroLine Alpha 471217 (I–V). Lactic acid and acetic acid were extracted from sourdoughs and the amounts determined enzymatically using Boehringer Mannheim kits (I) or high-pressure liquid chromatography (V). All samples were analysed in duplicate.

2.2.2 Amino acids

Free amino acids were extracted with water from sourdoughs and proteins were precipitated with sulphosalicylic acid. 19 Amino acids were quantified by high-pressure liquid chromatography and post column derivatisation was performed with phthaldialdehyde according to Dong and Gant (1985). The samples were analysed in duplicate (I).

2.2.3 Volatile compounds

The potential flavour active volatile compounds were analysed in sourdoughs using dynamic HS/GC/MS (I). For dynamic headspace analysis, a saturated sodium chloride solution was used to stop the fermentation before freezing and to transfer the volatile compounds from the samples to the headspace (the salting out effect). Sourdough samples were frozen immediately after adding the salt solution and analysed later. Before analysis, samples were thawed and allowed to stabilise for 2 hours at room temperature (25 °C).

After being thawed, stabilised samples' volatile compounds were purged from the headspace vials into a headspace sampler, which was interfaced with GC/MS. Compounds were identified on the basis of their mass spectra, and their amounts were quantified using selective ions for each compound from total ion chromatogrammes against a standard solution mixture series, which were prepared into 30% sodium chloride solution. The samples and standards were analysed in duplicate.

2.3 Analysis of bread texture

Both macroscopic and microscopic methods were utilised to study the structure of fresh and aged breads. The specific volume and hardness (II, III, IV) and microstructure (bran breads) were studied from fresh breads. The staling of bread was followed by measuring crumb firmness (II, III, IV). Staling of bran breads were also measured in IV by following changes in the crystallisation of amylopectin (DSC), increase in the signal from the solid phase (NMR) and light microscopy.

2.3.1 Instrumental analysis of bread texture

After 2 hours of cooling, loaf volume (determined by rape seed displacement) and weight were measured (II, III, IV). The specific volume was calculated by dividing the volume by weight. A minimum of three parallel loaves were measured and the average loaf volume was calculated for each sample.

The crumb firmness of fresh and stored breads was measured to assess the potential shelf-life of the breads (II, III, IV). Bread crumb firmness during storage was determined as the maximum compression force (40% compression, AACC 1998, modified method 74–09) using the Texture Profile Analysis (TPA) (Texture Analyser, Stable Micro Systems, Godalming, England). Eight bread slices (originating from 3 loaves) were measured and the results were expressed as mean values. The height of each bread slice was 2.5 cm and the edges of the slices were cut off before measurement.

2.3.2 DSC and NMR

For the measurement of starch retrogradation, fresh and stored breads (supplemented with 20% bran) were cut into four pieces, and a sample (10 mg) was taken from each one and tightly packed into an aluminium pan. The pan was closed with a lid and weighed. Calorimetric measurements were performed with Mettler DSC 820 within the temperature range of 10–90°C. The heating rate was 10°C/min. The endothermic peak was integrated and enthalpy (J/g dry starch) was calculated (IV).

For NMR measurements (IV), fresh and stored bran breads were cut into four pieces, and a sample was removed from each and placed into a glass tube. The sample was packed tightly with a glass rod and the tube sealed. The measurements were performed with Maran Ultra 23 MHz NMR spectrometer using VT gradient probe. The FID signal of the NMR measurement was resolved into three components: one fast decaying (T₂ typically of the order of tens of microseconds), and two components with a slower decay (T₂ up to a few milliseconds). The intensity of the fast decaying component was taken as the solid signal and the intensities of the two others were combined to represent the liquid signal. Staling was seen as an increase in the S/L ratio indicating the increased rigidity of macromolecules in bread.

2.3.3 Microscopy

The microstructure of breads supplemented with bran (III, IV) was studied by light microscopy. Both fresh (III, IV) and aged breads (IV) were studied in order to examine the starch-protein matrix under the microscope in different bran

bread during storage. Pieces of bread crumb were removed from the middle of the loaf, embedded in agarose, fixed in glutaraldehyde in phosphate buffer, dehydrated in a series of ethanol solutions (50%, 70% and 95%), embedded in Historesin and sectioned with a microtome. For fluorescence microscopic examination, the bread sections (4 μm) were stained with specific fluorochromes. Protein was stained with aqueous Fuchsin Acid and β -glucan was stained with aqueous Calcofluor White. Calcofluor stains intact cell walls blue. Fuchsin Acid stains proteins red. Starch remains unstained and appears black.

For bright field microscopy, the sections were stained with Light Green and with diluted Lugol's iodine solution. Light Green stains protein green. Iodine stains the amylose component of starch blue and amylopectin brown, respectively. The samples were examined with an Olympus BX-50 microscope (Tokyo, Japan). Micrographs were obtained using a SensiCam CCD camera and the image analysis program.

2.4 Analysis of sensory attributes of sourdough bread

The sensory experiments of wheat sourdough breads (II) were carried out using quantitative descriptive profiling (Lawless and Heymann 1999). The vocabulary of the sensory descriptors was developed separately reflecting attributes typical of wheat bread. The selected attributes of the sensory profile described the texture and flavour (simultaneous perception of odour, taste and trigeminal nerve response) characteristics of the different sourdough breads as extensively as possible. The attribute intensities were rated on continuous unstructured, graphical intensity scales by the panel. The vocabulary, training of the assessors and implementation of the sensory profiling is described exactly in Publication II.

The descriptive panel consisted of ten trained assessors with proven skills. All sensory work was carried out at the sensory laboratory of VTT Biotechnology, which fulfils the requirements of ISO standards (ISO 1985, 1988). All assessors of the internal sensory panel have passed the basic taste test, the odour test and the colour vision test. They have been trained in sensory methods during numerous sessions over several years, and their evaluation ability is routinely checked using individual control cards for each assessor. The panel was particularly familiar with the sensory descriptors and the attribute intensities by

using verbal definitions describing the ends of the intensity scales of the attributes. The same panel has also frequently been used in our previous studies on cereals.

The samples were presented to the assessors coded and in random order. The control bread without sourdough was thus introduced in evaluations as a hidden, randomised sample among other samples. Scores were recorded and collected using a computerised data system (Compusense Five, Ver 4.2, CSA, Computerized Sensory Analysis System, Compusense Inc, Canada).

2.5 Experimental design and data analysis

The data were analysed using standard statistical procedures as described in the individual Publication (I–V). The statistical methods are summarised in Table 4.

Table 4. Statistical methods used to analyse the results in Publications I–V.

Publication number	Applied for data of	Statistical methods
I	Amino acids pH, TTA Acetic acid and lactic acid Volatile compounds	RSM correlation analysis
II	Sensory profiling Specific volume Hardness of bread	RSM ANOVA, Tukey's HSD correlation analysis
III	Specific volume Hardness of bread	ANOVA, Tukey's HSD
IV	Specific volume Hardness of bread Starch retrogradation (DSC) Ratio of liquid/solid protons (NMR)	ANOVA, Tukey's HSD
V	pH, TTA, CFU's Biochemical analysis of sourdough and sensory profiling	ANOVA Bivariate correlations

2.5.1 Statistical analysis of instrumental measurements and sensory profiling data

The mean calculations for the raw data obtained were calculated. In II–IV, the significance of each instrumental measurement/descriptive attribute in discriminating between the samples was analysed using an analysis of variance (ANOVA), and Tukey's Honestly Significant Difference (HSD) test (significance of differences at $p < 0.05$). A two-way ANOVA was applied as the general linear model (GLM) procedure for the bread samples using SPSS software (SPSS Ver. 10.0, SPSS Inc.). ANOVA was used to test the statistical differences in instrumental measurements/sensory attributes between the samples, and the statistical difference between the sessions in sensory evaluations. When the difference among the samples in ANOVA was statistically significant, pairwise comparisons of these samples were analysed using Tukey's test. In V, ANOVA was used to estimate the probability that pH, TTA-values or CFU's of sourdoughs/sourdough breads made with different strains were statistically different at a confidence level of 95%.

2.5.2 Statistical analysis of sensory profile and instrumental measurements of bread and the process variables of sourdough

In I–II, the response surface method (RSM) was applied to study the influence of selected process parameters (ash content of flour, fermentation time and temperature) on the biochemical activity of sourdoughs (acidity, amino acids and volatile compounds) as well as the sensory and texture properties of subsequent sourdough breads.

RSM is a regression analysis method, which predicts the value of response variables (e.g. the level of amino acids) based on the controlled values of the experimental factors (process parameters of sourdough). All of the factors in RSM were quantitative, based on the results obtained from the experimental design. The average values of each response were analysed with the multiple regression method (MLR or PLS), which described the effects of variables in second order polynomial models. Regression analysis was calculated and the response surfaces were plotted with the Modde 4.0 and 6.0 (Umetrics AB, Umeå, Sweden).

The fit of model to the experimental data was given by the coefficient of determination, R^2 , which explains the extent to which the variance in a modelled variable can be explained by the model. Each model was also validated by calculating the predictive power of model, Q^2 , which is a measure of how well the model will predict the responses for a new experimental condition. The replicates at the centre point made it possible to estimate the pure error of the analyses, which was used to predict whether the models gave significant lack of fit. The reproducibility of models was evaluated by comparing the variation of the response under the same conditions (pure error), at the centre points to the total variation of the response with the following equation: $1 - (\text{Mean square (Pure error)} / \text{Mean square (total SS corrected)})$. Only models with high reproducibility and with no significant lack of fit were included in this study.

2.5.3 Statistical analysis in seeking relations between the sensory attributes of bread and biochemical activity of wheat sourdough

The biochemical changes in sourdoughs were related to the subsequent bread flavour by determining the Pearson's coefficients of correlation between the scores of sensory attributes, amount of amino acids, amount of volatile compounds and level of acidity ($P < 0.05$).

3. Results and discussion

3.1 The influence and interactions of process parameters and starter culture on the metabolic activity of wheat sourdough (I)

Controlled sourdough processes require an understanding on the effects of the process parameters of fermentation on the most relevant biochemical changes during sourdough fermentation. To obtain this goal, the influence and interactions of the ash content of flour, fermentation time and temperature on the formation of acidity, amino acids and volatile compounds were studied by using experimental design and mathematical modelling. The influence of different process parameters on biochemical activity of sourdough is summarised in Tables 5a and 5b. There are no reported studies in which the formation of lactic acid and acetic acid, amino acids and volatile compounds in response to process variations would have been simultaneously studied in the same sourdough.

The linear influence of the ash content of flour, fermentation time and temperature on formation acidity has been well documented in the literature (Spicher and Nierle 1984, Martínez-Anaya 1994, Rio et al. 1996) and confirmed also in this study. The most important parameter for increased acidity was the fermentation time. Fermentation time and temperature had significant interaction on the formation of acidity with LAB fermented sourdoughs indicating that high levels of acidity in sourdoughs required both elevated temperature levels and longer fermentation times, and preferably the use of flour with a high ash content (wholemeal wheat flour). In earlier studies, however, development of acidity has not been linked to formation of amino acids and volatile compounds, which is premise for the improved flavour in wheat sourdough baking. If a lower acidity level would be preferred in sourdoughs, shorter fermentation time and reduced temperature would provide moderate acidity levels in all of the studied sourdough types. It is noteworthy, that temperature had major impact on the acidity formation but had only small effect on formation of amino acids and volatile compounds in LAB fermented sourdoughs. Furthermore, interaction of time and temperature had very strong influence on acidity formation, but only small influence on formation of amino acids or volatile compounds.

The level of amino acids was demonstrated to be highly dependent of the ash content of flour in all of the studied sourdough types. The most intensive proteolysis occurred in sourdoughs with wholemeal flour and with LAB started sourdoughs. An increased fermentation time effectively enhanced the levels of amino acids when wholemeal flour was used, as significant interactions were observed between these parameters in LAB started sourdoughs. The significant correlation between TTA and levels of amino acids ($r = 0.50\text{--}0.86$) obtained in all sourdoughs agrees with the recent hypothesis that the pH dependent activation of cereal proteases (the highest level present in wholemeal flour) at low pH is the main reason for proteolysis during sourdough fermentation (Thiele et al. 2004, Loponen et al. 2004). It is noteworthy that an intensive accumulation of amino acids did not take place if the low ash content of flour (0.6%, white wheat flour) was used in sourdoughs, even though long fermentation times and high temperatures would be used. Thus, the milling rate of flour had great importance in the regulation of amino acids levels in sourdoughs if process conditions allowed the pH dependent activation of cereal proteases.

The influence of process parameters on the formation of volatile compounds was clearly different for yeast and lactic acid bacteria. With pure LAB fermented sourdoughs, maximum amount of volatile compounds was obtained by using 18 hours fermentation and wholemeal flour, as the linear influence of these parameters was observed in the formation of volatile compounds. However, the content of volatile compounds was much smaller in LAB fermented sourdoughs (10%) in comparison to preferments/sourdoughs fermented with yeast (pure yeast fermentation or combined yeast and lactic acid fermentation). In yeasted sourdoughs, frequent interactions and the quadratic effects of the process parameters were observed, indicating that there was an optimum time window and level of temperature during fermentation for the formation of a particular compound. For instance, the optimum formation of 3-methylbutanol (3-MB), which is one of the important identified flavour active compounds in crumb flavour (Gassenmeir and Schieberle 1995), was obtained after 14 hours of fermentation in yeasted preferment, and after longer fermentation time, the formation of 3-MB decreased. Thus, the optimum formation of volatile compounds during sourdough fermentation required an adjustment of the process conditions in a strain-dependent manner. So far, studies on the influence of fermentation time on the formation of volatile compounds during the prefermentation process are rare.

The high acidity of sourdough limits its use due to a negative effect both on bread flavour and texture (Salovaara and Valjakka 1987, Barber et al. 1992). According to the results of this thesis, the optimum sourdough for flavour improvement would contain moderate acidity with high levels of amino acids and certain volatile compounds. Only a few authors (Mori et al. 2001) have proposed a means to achieve such a designed fermentation process. According to the results of this thesis, this goal could be obtained by two main approaches: 1) to use LAB started sourdoughs made with wholemeal flour (ash content of flour >1.6%) and with fermentation time of 14 hours and fermentation temperature of 22–24 °C. This type of sourdough would contain increased levels of amino acids with moderated acidity. 2) To use *S. cerevisiae* fermented sourdough made with white wheat flour (ash content of 0.6%) with fermentation time of 18–20 hours and temperature of 32 °C. This type of sourdough would contain a high amount of volatile compounds with low levels of acidity.

Even though the exact optimum fermentation conditions are valid for particular strains of this study, strong influence and interactions of process parameters and their strain-dependent behaviour on the metabolic activity of sourdoughs is likely to be similar for other sourdough strains as well. Thus, any industrial sourdough or yeast fermentation process could be designed to produce maximum amount of flavour compounds or precursors without strong acidity by using optimised length of fermentation time, level of temperature and ash content of flour for particular strains present in the system.

Table 5a. Influence of sourdough process parameters on metabolic activity of sourdough and sensory attributes of subsequent bread fermented with pure LAB strain.

Strain/measured response	Process parameters of sourdough fermentation		
	Ash content of flour	Time	Temperature
<i>Lb. plantarum</i>			
Sourdough			
Total AA*	+++***	+	+
Total VC**	+++	+	+
pH		+++	++
TTA	++	+++	++
Bread			
Pungent flavour	+++	++	+
Fresh flavour	++	+++	
Intensity of flavour	++	+	
Roasted flavour of crust	++	+++	++
Aftertaste	+++	++	+
<i>Lb. brevis</i>			
Sourdough			
Total AA	+++	+	+
Total VC	+++	++	+
pH		+++	+++
TTA	++	++	++
Bread			
Pungent flavour	++	++	
Fresh flavour	++	++	
Intensity of flavour	+++	++	
Roasted flavour of crust	++	++	
Aftertaste	++	++	

*Total AA= total amount of amino acids, **Total VC= total amount of volatile compounds*** +++ = parameter has a big influence on formation of metabolite in sourdough or sensory attribute of bread. + = parameter has a small influence on formation of metabolite of sourdough or sensory attribute of bread.

Table 5b. Influence of sourdough process parameters on metabolic activity of sourdough and sensory attributes of subsequent bread fermented with yeast or with yeast+LAB.

Strain/measured response	Process parameters of sourdough fermentation		
	Ash content of flour	Time	Temperature
<i>S. cerevisiae</i>			
Sourdough			
Total AA*	+++***		
Total VC**	+	+++	++
pH		+++	+++
TTA	+++	++	++
Bread			
Pungent flavour	+++	++	
Fresh flavour	+++	++	+
Intensity of flavour	+++	++	++
Aftertaste	++	++	
<i>S. cerevisiae</i>+LAB			
Sourdough			
Total AA	+++	+	+
Total VC	+++	++	+
pH		+++	++
TTA	++	+++	++
Bread			
Pungent flavour	++	++	
Fresh flavour	++	++	
Intensity of flavour	+++	++	
Roasted flavour of crust	+	+	
Aftertaste	+++	++	

*Total AA= total amount of amino acids, **Total VC= total amount of volatile compounds*** +++= parameter has a big influence on formation of metabolite in sourdough or sensory attribute of bread. += parameter has a small influence on formation of metabolite of sourdough or sensory attribute of bread.

3.2 Effect of sourdough process on bread flavour (II)

The effect of process parameters of sourdough fermentation on subsequent bread flavour and texture was studied by baking same sourdoughs as in 3.1.1 into wheat sourdough breads. Thus, the influence and interactions of ash content of flour, fermentation time and temperature on bread flavour and texture were also studied using experimental design and mathematical modelling. The influence of different process parameters in sensory attributes of bread is summarised in Tables 5a and 5b. This approach also enabled to evaluate the impact of biochemical changes of sourdough in subsequent bread quality.

In all of the studied sourdough breads, the most important parameter influencing flavour attributes was the ash content of flour, as the utilisation of low ash content of flour in sourdoughs resulted in only very minor modifications in bread flavour in comparison to the control bread without sourdough; as seen in Figures 1–3 in Publication II. However, modification of bread flavour by utilising sourdough was also strongly dependent on the fermentation time. Short fermentation times did not significantly modify most of the bread flavour attributes, even though a high ash content of flour and higher temperature were used. The ash content of flour has also earlier been reported to be a major factor in determining the intensity of the sensory attributes of sourdough bread (Collar et al. 1994a), sensory scores being highest if low or medium levels of ash content of flour were used (Rouzaud and Martínez-Anaya 1997). However, if white wheat flour was utilised, sourdough did not contribute significantly to e.g. the intensity of the aroma and taste of bread (Martínez-Anaya et al. 1993), which is in accordance with the results of this thesis.

The potential of wholemeal flour in the modification of flavour is based mainly on the following facts: i) intensive proteolysis producing amino acids in wholemeal sourdoughs due the activation of cereal proteases at a low pH if longer fermentation times are utilised, ii) higher amount of volatile compounds present in wholemeal flour (Cerny and Schieberle 2001), iii) possible liberation of phenolic compounds of wholemeal flour during fermentation (Liukkonen et al. 2003), iv) more intensive acidification taking place with wholemeal flour.

One of the major results of this work was the high correlations between different sensory attributes, both desired and undesired, in all sourdough types (Table V

in Publication II). This indicates that fermentation conditions creating e.g the maximum level of roasted crust flavour and maximum intensity of crumb flavour and aftertaste (for example, in Figure 1 in Publication II) will inevitably also result in a very intense pungent flavour and strongly reduced fresh crumb flavour. Thus, biochemical changes during fermentation, particularly if wholemeal flour is utilised, are responsible for both desired and undesired modifications in subsequent bread flavour. Using sourdough as a flavour improver requires therefore carefully optimised fermentation conditions providing moderate acidity and enhanced level of amino acids and probably enhanced level of certain volatile compounds for balanced bread sensory profile.

Furthermore, strain-specific influences on bread flavour were evident in this study. In general, LAB-containing sourdoughs more effectively modified bread flavour, both desired and undesired sensory attributes. Sourdoughs containing yeast modified bread flavour less effectively and roasted crust flavour could not be enhanced at all with yeasted sourdoughs. Diminished roasted flavour due to the utilisation of yeasted preferment has also been reported by Zehentbauer and Grosch (1998). Yeasted preferment has been reported to either improve flavour (Thiele et al. 2002) or have no effect on bread flavour (Lorenz and Brummer 2003), the opposite results being most likely due to the different processing conditions of prefermentation and the variation of measured sensory attributes in different studies.

The strong influence of processing conditions of sourdoughs in subsequent bread flavour was common for all of the studied sourdoughs. Without optimised conditions for a particular strain, the utilisation of sourdough did not improve bread flavour or could easily even create inferior bread flavour in comparison to the same bread without sourdough. Thus, wheat sourdough fermentation should be always optimised and run with careful process regime to avoid negative effects on subsequent bread flavour.

According to the results of this thesis, improved flavour is obtained by using LAB fermented sourdough containing moderate level of acidity with enhanced levels of amino acids. A model for such optimised sourdough process is presented in Figure 3. Balanced flavour could be obtained by choosing e.g *Lb. brevis* for a starter and the utilisation of wholemeal flour (high ash content of flour) for 20 hours at 24 °C. High temperature in combination with long

fermentation time is a key factor for intensive acidification in LAB fermented sourdoughs but less important for intensive proteolysis or formation of volatile compounds, or for sensory attributes of subsequent bread. Thus, utilisation of lower temperature allows controlling acidity development in sourdoughs and at the same time high ash content of flour and long fermentation time promotes proteolysis. Above mentioned model sourdough will enhance intensity of overall flavour of bread, intensity of aftertaste and roasted flavour of bread crust without intensive pungent flavour or reduced fresh flavour. Such sourdough would be, however, in an intensive phase of metabolism and would need an instant method (such as cooling down or addition of salt) to slow down the fermentation when optimum level of metabolites is reached.

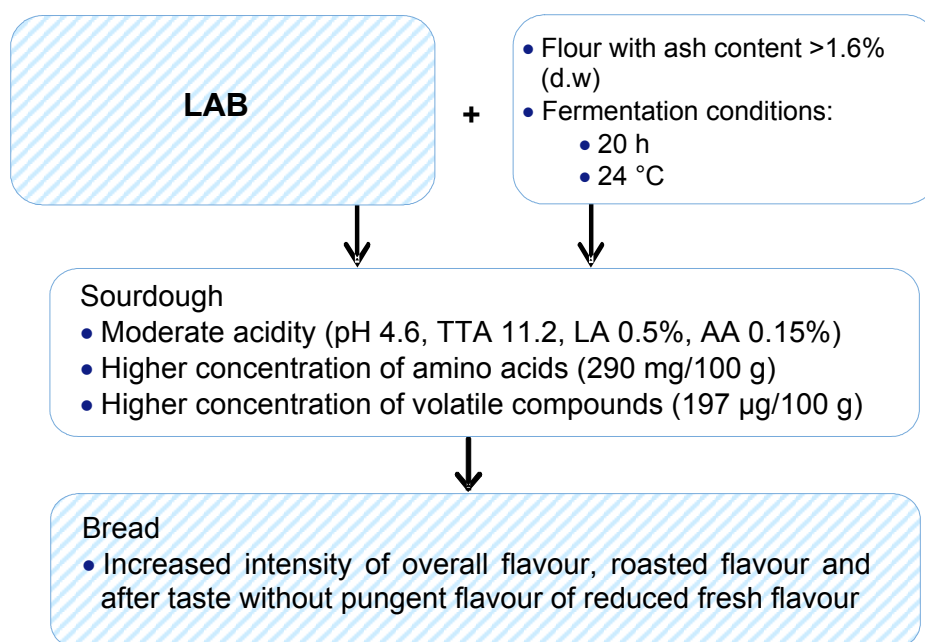


Figure 3. A model sourdough (*Lb. brevis*) for improved bread flavour. LA = lactic acid, AA= acetic acid.

3.3 The relation between bread flavour and the metabolic activity of wheat sourdough

The influence of the relevant biochemical changes of sourdough fermentation on subsequent bread flavour was estimated by calculating bivariate correlation coefficients between the properties of sourdough and the subsequent bread flavour attributes.

With *Lb. plantarum* fermented sourdough bread, the intensity of pungent flavour, intensity of aftertaste and degree of roasted flavour positively correlated with acidity (TTA and lactic acid), levels of volatile compounds and amino acids of sourdoughs (Table 6). The degree of fresh flavour was negatively correlated with acidity and level of volatile compounds, indicating that the utilisation of sourdough with high metabolic activity results in bread with reduced fresh flavour. A very high correlation of pungent flavour, aftertaste and the degree of roasted flavour of bread was observed with TTA and the total amount of volatile compounds of sourdough ($r = 0.83\text{--}0.93$). This indicates the controversial role of metabolic activities of sourdough in tuning bread flavour, as both undesired and desired flavour attributes were enhanced by sourdough fermentation. The accumulation of amino acids was most highly correlated with the intensity of aftertaste, degree of roasted flavour and with the intensity of pungent flavour. The most important amino acids in tuning bread flavour were glutamic acid, glycine, valine, tyrosine, histidine, lysine, leucine, methionine, phenylalanine and proline ($r = 0.78\text{--}0.85$). Valine, histidine, lysine, leucine, phenylalanine and proline have been linked to improved bread flavour (Collar et al. 1991, Schieberle and Grosch 1991, Fadel and Hegazy 1993, Gassenmeier and Schieberle 1995). Phenylalanine and leucine are precursors for two important flavour compounds of bread crumb, 2-phenylethanol and 3-methylbutanol, respectively (Gassenmeier and Schieberle 1995) Methionine is a precursor for 3-methylthiopropional and proline is a precursor for 1-acetylpyrroline, which is responsible for roasted crust flavour (Schieberle 1989).

However, the influence of amino acids seems to be dependent on the concentration, as higher amounts of e.g valine, glycine and ornithine, have also been linked to undesired flavour attributes (Suyama and Adachi 1980, Collar et al. 1991, Thiele et al. 2002). Thus, the same amino acids can be responsible for the development of a pungent flavour, intense aftertaste, reduced fresh flavour

and a high degree of roasted flavour; the influence being dependent on the concentration. For example, ornithine can increase both the degree of roasted flavour and bitter flavour and the nature of influence is dependent on the concentration of amino acid as demonstrated by Thiele et al. (2002).

With *Lb. brevis* fermented sourdough bread, the intensity of a pungent flavour and the intensity of aftertaste highly correlated to the level of acetic acid and the total amount of volatile compounds ($r = 0.70\text{--}0.81$, Table 7). Acetic acid has been postulated to act as a flavour enhancer in minor amounts (Molard et al. 1979) and also reported to cause an unpleasant flavour in higher concentrations (Molard and Cahagnier 1980). The intensity of aftertaste also highly correlated to the total amount of amino acids ($r = 0.87$). The intensity of crumb flavour and degree of roasted flavour correlated to the total amount of amino acids ($r = 0.74\text{--}0.76$). However, the intensity of pungent flavour also positively correlated to the total amount of amino acids ($r = 0.76$). The most important amino acids in bread flavour regulation were glycine, serine, valine, methionine, leucine, lysine, histidine, phenylalanine, and proline and γ -butyric acid.

With yeast (*S. cerevisiae*) fermented sourdough bread, the correlation coefficients between sourdough and the subsequent bread flavour were generally lower in comparison to LAB fermented sourdoughs breads. Even though an intensive formation of volatile compounds occurred in this sourdough (Publication I), the significant correlation between sensory attributes occurred with the level of amino acids, (especially with ornithine and proline) and with TTA (Table 8). Thus, the intensive formation of volatile compounds in yeasted preferments does not seem to be major factor enhancing subsequent bread flavour. This observation is in agreement with the results of Brummer and Unbehend (1997), who stated that flavour improvement to be unlikely when yeasted preferments are utilised. However, Gassenmeier and Schieberle (1995) have identified the intensive formation of the flavour active compounds 3-methylbutanol and 2-phenylethanol in yeasted preferments, but they did not identify the role of these compounds, in particular, sensory attributes. In yeasted preferments, flour originated acidification and proteolysis during sourdough fermentation seem to be key factors in the subsequent modification of bread flavour.

With yeast and lactic acid bacteria fermented sourdough bread, the intensity of pungent flavour and intensity of crumb flavour and aftertaste correlated

significantly to acetic acid ($r = 0.77$, Table 9). The pungent flavour correlated to the total amount of volatile compounds and especially with 3-methylbutanol and with ethylacetate ($r = 0.72\text{--}0.75$). the intensity of crumb flavour and intensity of aftertaste correlated most to the total amount of amino acids; most determinant amino acids being serine, glycine, valine, methionine, γ -butyric acid, histidine, lysine and proline ($r = 0.75\text{--}0.85$).

The results of this work emphasise the significant role of acidification and proteolysis during sourdough fermentation in the modification of subsequent bread flavour. However, the intensive formation of acidity and amino acids modifies both desired and undesired bread flavour attributes at the same time, which creates a challenge in achieving balanced wheat bread flavour by utilising sourdough. The formation of volatile compounds has a particular impact on pungent flavour and aftertaste, and on degree of roasted flavour. Volatile compounds originating from sourdough have the least effect on the intensity of crumb flavour in all studied sourdough types, which may indicate the significant role of sourdough in producing flavour precursors such as amino acids for actual yeast fermentation during dough proofing. Dough fermentation during the baking process has a major role in determining crumb flavour (Baker et al. 1953). However, the utilised analytical method, GC-MS, limits the selection of volatile compounds to be screened and the utilisation of extract methods such AEDA (= aroma extract dilution analysis, Schieberle 1996) might have revealed more influential volatile compounds in flavour modifications, and allowed differentiation between different volatile compounds and flavour attributes.

Table 6. Correlation coefficients (r)^a between properties of sourdough and scores of sensory attributes in *Lb. plantarum* fermented sourdough bread.

Sourdough**	IP***	DFr	IFl	IA	DR
pH	-0.64*	0.57*	0.29	-0.44	-0.58
TTA	0.85*	-0.81*	0.41	0.83*	0.79*
lactic acid	0.74*	-0.74*	0.26	0.71*	0.71*
ethanol	0.79*	-0.84*	0.31	0.54	0.74*
hexanal	0.69*	-0.51	0.26	0.54*	0.51*
diacetyl	0.74*	-0.56*	0.30	0.75*	0.59*
3-methylbutanol	0.71*	-0.65*	0.52*	0.73*	0.64*
ethylacetate	0.29	-0.52	0.22	0.37	0.22
Total VC	0.93*	-0.77*	0.54*	0.84*	0.78*
aspartic acid	0.30	-0.32	0.64*	0.42	0.26
threonine	0.48	-0.44	0.54*	0.57*	0.52*
serine	-0.11	-0.15	0.27	0.12*	0.48
glutamic acid	0.77*	-0.75*	0.60*	0.81*	0.71*
glycine	0.69*	-0.66*	0.56*	0.77*	0.72*
alanine	0.44	-0.44	0.55*	0.57*	0.48
valine	0.73*	-0.74*	0.59*	0.82*	0.76*
tyrosine	0.69*	-0.71*	0.61*	0.80*	0.74*
methionine	0.66*	-0.69*	0.51*	0.77*	0.73*
γ -aminobutyric acid	0.67*	-0.58*	0.56*	0.72*	0.62*
histidine	0.81*	-0.76*	0.61*	0.87*	0.79*
lysine	0.79*	-0.75*	0.62*	0.76*	0.78*
leucine	0.79*	-0.81*	0.52*	0.85*	0.82*
phenylalanine	0.72*	-0.78*	0.51*	0.81*	0.78*
ornithine	0.59*	-0.55	0.66*	0.67*	0.51*
proline	0.77*	-0.78*	0.57*	0.85*	0.77*
Total AA	0.71*	-0.69*	0.62*	0.81*	0.72*

*For all correlations $P < 0.05$. For values in bold $r \geq 0.70$ (positive or negative). **Total VC= total amount of volatile compounds, Total AA=total amount of amino acids. ***IP = intensity of pungent flavour, DFr = degree of fresh flavour, IFl = intensity of overall flavour, IA= intensity of aftertaste, DR= degree of roasted flavour.

Table 7. Correlation coefficients (*r*) * between properties of sourdough and scores of sensory attributes in *Lb. brevis* fermented sourdough bread.

Sourdough**	IP***	DFr	IFl	IA	DR
pH	-0.55*	0.38	-0.32	-0.44	-0.45
TTA	0.74*	-0.49*	0.67*	0.83*	0.65*
Lactic acid	0.61*	-0.30	0.50*	0.71*	0.52*
Acetic acid	0.70*	-0.45	0.61*	0.81*	0.55*
ethanol	0.45	-0.21	0.31	0.47	0.44
hexanal	0.41	-0.10	0.31	0.009	0.36
diacetyl	0.53*	-0.15	0.49*	0.75*	0.47
3-methylbutanol	0.66*	-0.60*	0.64*	0.73*	0.69
ethylacetate	0.57*	-0.19	0.47	0.74*	0.51
Total VC	0.76*	-0.52*	0.66*	0.80*	0.68*
aspartic acid	0.56*	-0.55*	0.70*	0.77*	0.72*
threonine	0.63*	-0.57*	0.75*	0.77*	0.66*
serine	0.72*	-0.52*	0.74*	0.88*	0.72*
glutamic acid	0.69*	-0.40	0.60*	0.84*	0.63
glycine	0.78*	-0.68*	0.78*	0.83*	0.73*
alanine	0.59*	-0.58*	0.73*	0.76*	0.67*
valine	0.71*	-0.51*	0.73*	0.88*	0.73*
tyrosine	0.65*	-0.54*	0.57	0.53*	0.60
methionine	0.71*	-0.51*	0.78*	0.89*	0.73*
γ-aminobutyric acid	0.72*	-0.71*	0.78*	0.75*	0.71*
histidine	0.69*	-0.49*	0.69*	0.85*	0.74*
lysine	0.58	-0.54	0.72*	0.75*	0.69*
leucine	0.51	-0.51*	0.72*	0.86*	0.73*
phenylalanine	0.69*	-0.42	0.63*	0.85*	0.68*
ornithine	0.66*	-0.45	0.64*	0.84*	0.67*
proline	0.77*	-0.45	0.63*	0.77*	0.63*
TotalAA	0.76*	-0.64*	0.75*	0.86*	0.75*

*, **, *** Abbreviations as in Table 6.

Table 8. Correlation coefficients (r)^{*} between properties of sourdough and scores of sensory attributes in *S. cerevisiae* fermented sourdough bread.

Sourdough**	IP***	DFr	IFl	IA
pH	-0.39	0.04	-0.35	-0.49
TTA	0.84*	-0.51*	0.76*	0.77*
isobutanol	0.32	-0.14	0.20	0.28
2-methylbutanal	0.48*	-0.44	0.29	-0.29
3-methylbutanal	0.09	-0.19	0.11	0.07
ethanol	0.53*	-0.49*	0.37	0.40
hexanal	0.54	-0.18	0.51*	0.51*
diacetyl	0.435	-0.16	0.11	0.08
3-methylbutanol	0.51*	-0.08	0.39	0.36
ethylacetate	0.57*	-0.24	0.62*	0.79*
Total VC	0.47*	-0.14	0.38	0.39
aspartic acid	0.10	-0.41	0.35	0.25
threonine	0.10	-0.47	0.24	0.13
serine	0.44	-0.61*	0.54*	0.43
glutamic acid	0.77*	-0.59*	0.82*	0.92*
glycine	0.78*	-0.51*	0.65*	0.62*
alanine	0.67*	-0.55*	0.59*	0.56
valine	0.73*	-0.49*	0.55*	0.52*
tyrosine	0.50*	-0.42*	0.37	0.28
methionine	0.44	-0.55*	0.41	0.25
γ -aminobutyric acid	0.76*	-0.54*	0.63*	0.58*
histidine	0.77*	-0.71*	0.68*	0.61*
lysine	0.36	-0.53*	0.34	0.19
leucine	0.55*	-0.52*	0.46	0.39*
phenylalanine	0.61*	-0.48*	0.52*	0.49*
ornithine	0.72*	-0.64*	0.82*	0.84*
proline	0.80*	-0.52*	0.82*	0.82*
TotalAA	0.64*	-0.62*	0.62*	0.55*

*, **, *** Abbreviations as in Table 6.

Table 9. Correlation coefficients (r)^{*} between properties of sourdough and scores of sensory attributes in yeast+LAB fermented sourdough bread.

Sourdough**	IP***	DFr	IFl	IA
pH	-0.26	0.24	-0.35	-0.24
TTA	0.75*	-0.67*	0.76*	0.74*
Lactic acid	0.63*	-0.55	0.76*	0.58*
Acetic acid	0.77*	-0.57	0.77*	0.77*
isobutanol	0.61*	-0.62*	0.49	0.57
2-methylbutanal	-0.002	-0.07	-0.07	-0.03
3-methylbutanal	0.3	-0.42	0.15	0.26
ethanol	0.68*	-0.49*	0.60*	0.71*
hexanal	-0.20	-0.05	-0.18	-0.20
diacetyl	0.40	-0.53*	0.40	0.41
3-methylbutanol	0.72*	-0.70*	0.63*	0.66*
ethylacetate	0.75*	-0.51*	0.71*	0.72*
Total VC	0.68*	-0.66*	0.58*	0.63*
aspartic acid	0.72*	-0.57*	0.73*	0.77*
threonine	0.70*	-0.62*	0.72*	0.77*
serine	0.74*	-0.57*	0.80*	0.75*
glycine	0.82*	-0.72*	0.83*	0.85*
alanine	0.68*	-0.58*	0.69*	0.74*
valine	0.73*	-0.57*	0.78*	0.73*
tyrosine	0.65*	-0.55*	0.61*	0.69*
methionine	0.72*	-0.57	0.78*	0.73*
γ -aminobutyric acid	0.82*	-0.75*	0.79*	0.87*
histidine	0.76*	-0.65*	0.85*	0.78*
lysine	0.70*	-0.61	0.69*	0.74*
leucine	0.51	-0.35	0.53	0.78*
phenylalanine	0.52*	-0.36	0.63*	0.52*
ornithine	0.54*	-0.37	0.65*	0.56*
proline	0.65*	-0.48	0.75*	0.69*
TotalAA	0.80*	-0.60*	0.85*	0.82*

*, **, *** Abbreviations as in Table 6.

3.4 Effect of sourdough on bread volume and staling (II, III, IV)

Influence of both wheat sourdough (II) and bran sourdough (III, IV) on bread volume and staling was established in this study.

Specific volume

Specific volume could be improved 9–12% by utilising sourdough both in plain wheat breads (II) and in wheat breads supplemented with 20% of bran sourdough (f.b) (III, IV). The application of sourdough has been reported to either increase (Corsetti et al. 2000, Crowley et al. 2002, Clarke et al. 2003b) or decrease bread volume (Salovaara and Valjakka 1987, Barber et al. 1992); the type of influence being dependent on the acidification level obtained and the microbial strains.

In Publication II, improvement of volume was demonstrated to be highly dependent on the use of optimised fermentation conditions. In addition, the influence of fermentation conditions was strain-dependent as seen in Figures 4 and 5 in Publication II. In general, the higher ash content of flour resulted in diminished volume, but the influence of fermentation time and temperature was different for LAB or yeast containing starters. Yeast fermented sourdoughs produced the optimum volume with short fermentation times (6 hours) and *Lb. brevis* fermented sourdough after 12–14 hours fermented at 22–24 °C. It is noteworthy that in all sourdough types, utilisation of sourdough did not improve bread volume if optimised conditions were not used. This result is in accordance with the work of Clarke et al. (2003b) who demonstrated that optimised fermentation conditions are strain-dependent and a premise for improved volume.

Optimised conditions for improved volume provided moderate acidity in all sourdough types (pH 4.9–5.2) and for subsequent breads (pH 5.1–5.5) in I and II, which is in agreement with the work of Clarke (2003). However, as chemically acidified counterpart of sourdough has shown to improve bread volume less effectively, it is most likely that enzymatic modifications of flour during sourdough fermentation and subsequent changes in dough rheology are mainly responsible for the improved volume of sourdough breads (Clarke et al. 2002). In this work, combination of α -amylase, xylanase and lipase with bran

sourdough improved bread volume most efficiently, which agrees with proposed significance of enzymatic modifications in sourdough baking.

As a result of this thesis, bran sourdough (bran fermented with yeast and/or lactic acid bacteria) was introduced to effectively improve bread volume in high-fibre baking, especially when used in combination with α -amylase, xylanase and lipase. Based on Figure 3 in III and Figure 6 in IV, the addition of bran fermented with yeast and *Lb. brevis* for 16 hours results in an improved protein (gluten) network, increased phase separation of amylose and amylopectin, as well as a more swollen starch structure. These changes indicate an altered water distribution between dough components, most likely due to the enzyme activity of bran and flour. The bran fraction of flour particularly contains high levels of endogenous enzymes, which might be activated or deactivated during fermentation. For instance, the endogenous proteases of bran are activated at a lower pH (Loponen et al. 2004), which allows the modification of gluten properties in bread dough containing fermented bread and might partly explain improved volume. In addition, several endogenous xylanases have a pH optimum at 4.5–5.0 (Rasmussen et al. 2001) and the activity of enzymes decreases rapidly at higher pH-levels being nearly inactivated at pH 6. Thus, a lowered pH obtained using sourdough activates the xylanases. The resulting increased solubility and formation of arabinoxylans with higher molecular weight has been related to an altered water distribution from the arabinoxylan phase to the gluten in dough, which results in better gluten extensibility and improved volume (Maat et al. 1992). Thus, the fermentation of bran may bring beneficial extra enzymes for bread dough, create optimum conditions for the activity of several enzymes and modify bran particles as such.

The most effective treatment in volume improvement was the combination of exogenous enzymes (α -amylase, xylanase and lipase) with bran sourdough (Publication IV), suggesting the synergist effects of enzymes and sourdough. The pH-level obtained in bran sourdough bread (5.5) was most likely low enough to enhance the effect of xylanases (probably both bran and added ones) and high enough to maintain alfa-amylase activity for optimum volume. Similar positive effects of the combination of enzymes and sourdough has been reported in white wheat baking by Martínez-Anaya et al. (1998) and Di Gagno et al. (2003), but the idea to improve bread texture in high-fibre baking by combining fermentation of bran with usage of enzymes has not been presented before.

Bread staling

After 3–6 days of storage, sourdough bread was 16–30% softer than the control depending on sourdough type (sourdough made with bran or flour, fermented either with LAB or/and yeast) and fermentation conditions as reported in Publications II, III and IV. If bran sourdough was combined with the use of exogenous enzymes, the resulting bread was 70% softer than the control bread after 6 days of storage (Publication IV). The use of sourdough has been reported both to decrease (Armero and Collar 1996, Rouzaud and Martínez-Anaya 1997) and increase shelf-life (Corsetti et al. 1998, 2000); controversial results being probably due to different fermentation conditions and sourdough types in different Publications.

According to the results of this thesis, the influence of sourdough on bread softness during storage depended on fermentation conditions and starter culture. If fermentation conditions were not optimised in a strain-specific manner, the use of sourdough did not improve bread softness or even increased bread hardness (Publication II). In general, the softest bread texture was obtained using a long fermentation time and low ash content of flour in LAB fermented sourdoughs (Publication II). Solubilisation of arabinoxylans during extended fermentation periods is one possible explanation for improved self-life, as high molecular weight arabinoxylans have been linked to improved softness (Courtin and Delcour 2002). Production of dextrans, which have ability to interfere with starch retrogradation, during long fermentation might also explain improved softness (Rouzaud and Martínez-Anaya 1997). In pure yeast fermentation, the elevated temperature was crucial to improved softness (Publication II), probably due to enhanced carbon dioxide production in higher temperatures.

The effect of sourdough on improved softness was partly due to an higher volume, as significant correlation coefficients were established between volume and softness ($r = -0.61-0.96$). Furthermore, in bran sourdough breads (Publication IV) starch retrogradation and the development of molecular rigidity was not inhibited, which further emphasises improved volume as the main reason for a better shelf-life in sourdough breads. However, the softest bread texture was obtained when bran sourdough and use of amylase, xylanase and lipase were combined, which indicates the modification of starch cell wall polysaccharides. The above-mentioned enzyme combination did improve shelf-life by itself, but

the synergistic effects were obvious when enzymes were used with bran sourdough. For example, enhanced xylanase activity leads to the solubilisation and degradation of cell wall components (such as pentosans) (Figure 5 in IV), which could, in turn, lead to an altered water distribution between dough components and create a softer bread texture. The utilisation of microbial strains with exogenous enzyme activity (Gobbetti 1998) or the combined use of sourdough and enzymes (Corsetti et al. 2000) has been reported to retard staling in white wheat baking. Furthermore, fermentation conditions providing the optimum softness of breads create moderate acidity in subsequent bread (pH 4.9–5.6), which is near the optimum conditions for the activity of xylanases and proteases, but does not allow inhibition of α -amylase.

3.5 Inhibition of rope spoilage by wheat sourdough (V)

In this study, the growth of rope-forming *Bacillus* strains was effectively inhibited by using wheat sourdough if the acidity level of the sourdough and subsequent bread was low enough. Antimicrobial activity of sourdoughs fermented with *Lb. plantarum*, *Lb. brevis* or with *Pediococcus pentosaceus* was evident if the pH of sourdough was < 4 and TTA > 12, and the pH of sourdough bread was 4.8–5.1 and TTA 4.8–6.2 and the concentration of lactic acid was 1.5–1.7 g/100g of sourdough. However, the same LAB were ineffective if the pH was higher and TTA was lower (pH 4.1–4.9 and TTA 3.9–5.4 for sourdoughs and pH 5.4–5.6 and TTA 2.8–3.7 for subsequent breads) indicating the strong role of acidity in rope prevention. This result is in accordance with work of Rosenquist and Hansen (1998), as they concluded that the level of acidity and not the properties of LAB are determinant in rope prevention. However, the addition of pure lactic acid in comparable concentrations to reach a pH-level of 4.9–5.0 did not prevent rope spoilage as indicated in Table 6 in V. Thus, the combined effect of the production of antimicrobial compounds by the selected LAB with a low pH was assumed to explain the observed antimicrobial effect.

It is noteworthy that the observed demand of low pH in sourdough breads for antimicrobial effect is not in line with the optimum acidity level for the sensory quality of the same breads. In II, *Lb. plantarum* (VTT E-76) fermented bread had the optimum predicted bread flavour, if fermentation was carried out e.g. at 32 °C for 6 hrs and made with flour having an ash content of 1.6% These

conditions provided a pH 5.7 and TTA 3.8 for subsequent bread and such acidity levels are unlikely to promote microbiological shelf-life.

Mold growth was not reported in Publication V even though appearance of mold was also recorded during visual checking of bread slices to observe rope spoilage. In general, mould growth was not observed in sourdough breads with pH level < 4.9 and higher pH-levels promoted mold growth. Antimould activity of different LAB strains has been reported (Lavermicocca 2000) and some strains appears to be active even with higher pH-levels (Clarke et al. 2004). However, role of acidity in the performance of different antimould strains requires further studies to evaluate potential of sourdough to promote microbiological shelf-life without deteriorating flavour.

4. Conclusion and future outlook

The influence and interactions of sourdough process conditions on the metabolic activity of sourdough and subsequent bread quality (flavour and texture) were studied in this thesis. The levels of acidity, amino acids and volatile compounds of wheat sourdoughs were related to the subsequent bread quality. A new type of sourdough, bran sourdough, was introduced to compensate for the negative effect of bran addition on bread volume and shelf-life. The staling mechanism of high-fibre bran breads was studied using versatile physical methods. The potential of LAB sourdoughs to prevent rope spoilage was also studied.

The use of sourdough as a flavour improver has long been well established. However, according to the results of this thesis, the improved bread flavour required carefully optimised fermentation time, temperature and ash content of flour in a strain-specific manner to achieve a balanced flavour profile between desired and undesired flavour attributes. This fermentation window for balanced flavour must be defined, because the development of desired and undesired flavour attributes of bread were highly correlated in the sourdough process, probably because formation of sensory attributes were based on the same metabolic events during sourdough fermentation. Without optimised conditions, the use of sourdough did not improve bread flavour or resulted in inferior bread flavour. The most effective improvement in flavour was obtained with pure LAB fermented sourdoughs by using long fermentation time, high ash content of flour and reduced temperature. Desired flavour development was related mainly to the moderate development of acidity and enhanced proteolysis during sourdough fermentation. The role of volatile compounds formed during pre-fermentation was less important in flavour enhancement. Proteolysis during sourdough fermentation correlated strongly to the acidity development, which complicates flavour improvement due to the strong role of acidity in the enhancement of pungent flavour and reduced fresh flavour.

Usage of traditional wheat sourdough is limited to 5–10% due to high acidity levels of sourdough. This study introduced designed fermentation process to obtain sourdough with moderate acidity level and enhanced levels of flavour precursors and flavour compounds, which allows increasing amount of sourdough to be used in subsequent bread dough. Accordingly, the amount of flour to be pre-fermented is increased when higher amount of sourdough is used

in the final product. This result is important in future applications of sourdough aiming for improved nutritional value of cereals (either refined or wholemeal flour, or different milling fractions), as higher amounts of mildly flavoured, nutritionally improved fermented flour or milling fraction can be used in subsequent cereal products.

The future prospects of flavour improvement by utilising sourdough could include the combined use of appropriate exogenous proteases, preferably with a higher pH optimum, with sourdough fermentation to obtain more intensive proteolysis at moderate acidity levels. The definite advantage of using proteases at the sourdough stage instead of the dough preparation stage is the more reliable control over the extent of proteolysis in sourdough. The use of proteases at the dough stage easily results in an inferior texture due to the utmost importance of proteins in the structure forming components of wheat bread.

Improved volume and softness of bread during storage was also obtained under optimised fermentation conditions, which were strain-dependent. The optimum volume of all sourdough breads was obtained at moderate acidity, which also creates nearly optimum conditions for several endogenous enzymes. Thus, improved volume and softness is most likely due to the combination of appropriate acidity and favourable modification of dough components (such as proteins and pentosans) by enzymatic activity of flour/bran. Future research challenges will be to more thoroughly understand e.g the state of arabinoxylans in sourdough fermentation and their relations to bread volume and shelf-life. The possibility of enhancing the technological potential of sourdough with the production of exopolysaccharides is one of the future prospects, as well as the combined use of exogenous enzymes and sourdough for improved texture.

A high acidity sourdough was shown to be an effective way of inhibiting rope spoilage in wheat bread and the same influence at a comparable pH level could not be obtained with pure acids. However, the strong acidity required for rope prevention will inevitably lead to undesired flavour characteristics such as a pungent flavour as well as reduced volume. The enhancement of microbial shelf-life with sourdough may be more suitable for wholemeal baking, in which the acidity level for the accepted flavour is less critical, or requires the development of starters with high antimicrobial properties also at higher pH-levels.

This thesis introduced a new method, bran sourdough, to overcome deleterious effect of bran addition in high-fibre baking. Particularly in combination with α -amylase, xylanase and lipase, the utilisation of bran sourdough effectively improved the volume and shelf-life of wheat bread supplemented with 20% bran. The improved macroscopic texture was related to profound changes in the microstructure of bran sourdough breads. Bran prefermentation improved the protein network and the increased swelling of starch granules of bran breads. During aging, the lower staling rate of bran sourdough breads with α -amylase, xylanase and lipase was related to an altered water distribution between dough components, reduced amylopectin recrystallisation and slower loss of molecular mobility.

A method to improve the quality of high-fibre wheat bread using bran sourdough is novel and offers interesting challenges for future applications. There is growing evidence of significant health benefits of whole grain. The major potential of whole grain originates from outer layers of the kernel, especially in the bran fraction, where the most interesting health-promoting compounds are located. Furthermore, according to recent results, sourdough fermentation can significantly enhance levels of potential health-promoting compounds in whole grain foods, especially if combined with the high enzyme activity of the raw material. Thus, the fermentation of bran can effectively modify texture, flavour, and nutritional value of wheat or other cereals.

In conclusion, this study determined fermentation window for balanced bread flavour in wheat sourdough baking by using statistical design and mathematical modelling. Improved, balanced flavour was related to moderate acidity and enhanced levels of amino acids and volatile compounds in sourdough. A novel method, bran sourdough, was introduced to overcome deleterious effect of bran addition in high-fibre baking. In the future, bran sourdoughs or other fermented milling fractions can be designed to produce nutritionally and technologically superior raw materials for all cereal foods, such as bread, breakfast and snack foods.

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Title Sourdough: a tool for the improved flavour, texture and shelf-life of wheat bread			
Abstract <p>The aim of this thesis was to develop means to optimize the biochemical activity of sourdough to achieve improved bread flavour, texture and shelf-life, and to determine how the structure of fresh and aged bread is altered by the use of sourdough. The influence of process conditions of prefermentation on subsequent bread quality is clarified through this thesis.</p> <p>The importance of an optimised sourdough process in improving the flavour and texture of subsequent bread was demonstrated. The sourdough process had to be optimised in a strain-specific manner to obtain improved flavour and texture. Lactic acid bacteria fermented sourdoughs were more effective in tuning bread quality compared to yeasted preferments if the appropriate conditions were utilised. The ash content of flour and fermentation time were the main factors regulating bread flavour and texture in all of the sourdough types studied. The possibility to improve bread flavour by utilising sourdough with moderate acidity and an enhanced level of amino acids was demonstrated in this study. Bread volume and shelf-life were also improved by sourdough, which was fermented with low ash content flour and with optimised fermentation time.</p> <p>A new type of sourdough was presented: bran sourdough, which could effectively compensate the negative effect of added wheat bran on bread volume and shelf-life in high-fibre baking. An altered microstructure (improved protein network, enhanced swelling of starch and modified degradation of cell wall components) of bran sourdough breads, especially if made with enzymes, was related to improved volume. A reduced staling rate of bran sourdough breads was further explained due to reduced starch retrogradation and a slower loss of molecular mobility.</p> <p>In conclusion, wheat bread flavour and texture were effectively modified using optimised sourdough. Bran sourdough was introduced as a potential tool for the future development of technologically and nutritionally superior raw materials for all cereal foods, such as bread, breakfast and snack products.</p>			
Keywords sourdough, bread, flavour, texture, processing conditions, acidity, amino acids, volatile compounds, bran, high-fibre baking			
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Wheat sourdough was shown to be an efficient tool in improving bread flavour and texture. Understanding of biochemical activity and controlled fermentation conditions are a prerequisite for full exploitation of the potential of sourdough technology. This thesis showed how the sourdough process should be optimised to improve bread quality, and examples of optimised conditions were given. Controlled acidity and enhanced proteolysis were shown to be important for balanced bread flavour.

Wheat bran is an important source of dietary fibre and bioactive compounds. However, addition of wheat bran in baking results in inferior bread quality. A novel method of bran sourdough was developed to pretreat bran prior to the baking process. This pre-treatment resulted in significant improvement of bread texture due to modified starch-protein network. Sourdough thus shows promise also for production of nutritionally superior high-fibre raw materials for different cereal foods.

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