ESPOO 2003 VTT PUBLICATIONS 494

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Influence of processing on the flavour formation of oat and rye

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VTT Biotechnology

ACADEMIC DISSERTATION

To be presented with the permission of the Faculty of Science of the University of Helsinki, for public criticism in Auditorium XIII at the University's Main Building, Unioninkatu 34, on June 13th, 2003, at 12 noon.

ISBN 951–38–6042–6 (soft back ed.)
ISSN 1235–0621 (soft back ed.)

ISBN 951–38–6043–4 (URL: http://www.vtt.fi/inf/pdf/)
ISSN 1455–0849 (URL: http://www.vtt.fi/inf/pdf/)

JULKAISIJA – UTGIVARE – PUBLISHER

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Technical editing Leena Ukskoski

Otamedia Oy, Espoo 2003

Our philosophy has been that sound methodology for the sensory analysis of foods rests on a thorough knowledge of sensory physiology and an understanding of the psychology of perception. Essential in addition is careful statistical design and analyses of the data. Finally, new understanding of sensory judgment is to be sought through correlation with physical and chemical data.

- Amerine, Pangborn & Roessler 1965

Heiniö, Raija-Liisa. Influence of processing on the flavour formation of oat and rye. Espoo 2003. VTT Publications 494. 72 p. + app. 48 p.

Keywords oat, rye, processing, germination, sourdough fermentation, sensory profiling, flavour, volatile compounds, multivariate analysis

Abstract

The use of whole grain foods, such as oat and rye, beneficial to one's health, could be substantially extended if their flavour properties and flavour stability were better known, and they could be modified in a controlled way. The aim of this study was to specify how the flavour of oat and rye is formed, and which processing factors influence it. The understanding underlying the perceived flavour formation of grain was considerably improved through this thesis.

This study showed that the relatively mild flavour of native grains was considerably adjusted by applying different processing techniques, such as milling fractionation, sourdough fermentation and baking, germination and subsequent heat treatment as well as extrusion cooking. The flavour components were unevenly distributed in the rye grain with the innermost endosperm being the mildest, and the outer bran layers being the most bitter and intense in flavour. The shorts fraction in the middle of the rye grain that had high bioactivity proved to be most interesting in further applications of new products by having a cereal-like flavour without any obvious bitterness. The importance of the grain fractions on the flavour was still obvious when the fractionated rye flour was used in baking bread.

Sourdough baking and germination were used to increase the amounts of phenolic compounds, most of which are beneficial to one's health. In addition, the phenolic compounds considerably influenced the perceived flavour. The sourdough fermentation of rye resulted in sour and intense flavour notes. Heat treatments, such as baking or extrusion cooking of sourdough-fermented rye, further modified the flavour without losing the sourness. The identity and overall acceptance of sour rye bread, when evaluated by consumers, were affected by the acidity and the salt content, even though the effect actually depended on the

wheat to rye flour ratio used in the bread. The determination of the various simultaneous interactions between the process parameters was a novel approach.

A new, short germination procedure was applied to oat and rye. The germinated oat dried at high temperatures was perceived to have flavour characteristics of roasted, sweet and nutty, and in this study these sensory characteristics were clearly related to particular volatile compounds, such as dimethyl sulphide and isobutanol. An important discovery was that the roasted flavour in oat was gained without any apparent Maillard reaction. Germination and drying extended the shelf-life of crushed oat in comparison with native oat, and the development of the undesired sensory attributes, such as bitterness and rancidity, were closely related to the accumulation of free fatty acids and volatile compounds originating from lipid oxidation. Studies on the flavour formation of germinated rye are exceptional.

The statistical data treatment used to relate the perceived flavour of processed oat and rye to the flavour-active volatile substances proved to be successful in linking the results of these techniques, and useful information for developing new cereal products was obtained.

In conclusion, the flavour attributes of grain were highly varied. To tailor these attributes according to consumer expectations, appropriate processing techniques need to be chosen to attain the desired flavour characteristics, e.g. roasted or sour. New tools for developing novel palatable whole grain foods with high bioactivity, such as breakfast and snack products, were introduced in this thesis.

Academic dissertation

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Preface

The first time the idea of researching the flavour formation of cereal products was expressed to me by Professor Kaisa Poutanen in the spring of 1998. The development of the decision to write a doctoral thesis on this topic, however, was born considerably later, two years ago, to be more precise.

The study was carried out on its entirety at VTT Biotechnology. The majority of the research was part of the national projects 'Biotechnical processing of oats for novel food ingredients' (1998–2000), 'Bioactive compounds of rye – influence on healthiness and flavour' (2001) and 'Production of novel bioactive rye products through processing and choice of variety' (2002). These projects were included in the VTT Research Programmes 'Future Foods' and 'Tailored Technologies for Future Foods', and were also part of the national oat and rye programmes of Ministry of Agriculture and Forestry (Finland), National Technology Agency (Tekes, Finland) and the participating Finnish cereal companies, whom I would like to gratefully acknowledge for partially funding the research.

I am most grateful to Professor Juha Ahvenainen for providing the support and excellent working facilities for completing this work. I wish to sincerely thank Professor Carl G. Gahmberg for his straight and flexible attitude during the writing process of this thesis. I am very much obliged to the reviewers of this thesis, Dr Conor Delahunty and Professor Rainer Huopalahti.

I owe my warmest thanks to Professor Kaisa Poutanen for initiating, supervising and guiding my work among all her other duties. The fruitful, encouraging discussions with her always led to the work progressing further. The realisation of the idea to a complete thesis would not have been possible without the support of several colleagues. I wish to express my appreciation for all the other co-writers involved in Publications I–V. They are my collaborators at VTT Biotechnology Dr Kirsi-Marja Oksman-Caldentey, Dr Kirsi-Helena Liukkonen, Kati Katina, Olavi Myllymäki, Kyösti Latva-Kala, Tiina Rajamäki, Annika Wilhelmson, Nina Urala and Dr Jukka Vainionpää; Professor Hely Tuorila from the University of Helsinki; and Dr Pekka Lehtinen from the Helsinki University of Technology. The skilful assistance of Ulla Österlund, Pirkko Nousiainen, Heidi Eriksson, Ulla Vornamo and Anna-Liisa Ruskeepää is particularly appreciated. I am very grateful for the valuable comments and suggestions that Dr Liisa Lähteenmäki and Dr Kirsi-Marja Oksman-Caldentey gave on this thesis. I owe my warmest thanks to Dr Marjaana Suutarinen for her friendly support during the work. In addition, I would like to extend my deepest appreciaton to my other colleagues at VTT Biotechnology and in the ESN group (European Sensory Network) for their collaboration and encouragement.

Finally, I owe my dearest thanks to my family, relatives and friends. I am deeply grateful to my father Leo and to my late mother Lilli, who was always so eager to study. I also wish to express my special thanks to my husband Seppo for his support and for taking on more than his share of the everyday responsibilities during the writing process. The bright light and the enjoyment created by all my children, Lasse, Anni, Maikki and Olli, will be always remembered and appreciated.

Science – to know so much about so little.

Espoo, April 2003

Raija-Liisa Heiniö

List of original publications

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

I Heiniö, R.-L., Oksman-Caldentey, K.-M., Latva-Kala, K., Lehtinen, P. and Poutanen, K. 2001. Effect of drying treatment conditions on sensory profile of germinated oat. *Cereal Chemistry* 78 (6), 707–714.

II Heiniö, R.-L., Lehtinen, P., Oksman-Caldentey, K.-M. and Poutanen, K. 2002. Differences between sensory profiles and development of rancidity during long-term storage of native and processed oat. *Cereal Chemistry* 79 (3), 367–375.

III Heiniö, R.-L., Liukkonen, K.-H., Katina, K., Myllymäki, O. and Poutanen, K. 2003. Milling fractionation of rye produces different sensory profiles of both flour and bread. *Lebensmittel-Wissenschaft und -Technologie* (in press).

IV Heiniö, R.-L., Katina, K., Wilhelmson, A., Myllymäki, O., Rajamäki, T., Latva-Kala, K., Liukkonen, K.-H. and Poutanen, K. 2003. Relationship between sensory perception and flavour-active volatile compounds of germinated, sourdough fermented and native rye following the extrusion process. *Lebensmittel-Wissenschaft und -Technologie* (in press).

V Heiniö, R.-L., Urala, N., Vainionpää, J., Poutanen, K. and Tuorila, H. 1997. Identity and overall acceptance of two types of sour rye bread. *International Journal of Food Science and Technology* 32, 169–178.

The author of the present thesis was responsible for the planning of the research, the sensory studies, the statistical treatments of the results and the interpretation of the results in all publications, with the following exceptions: interpretation of the results of oat stability was done with Pekka Lehtinen in II, and the sensory analysis was carried out together with Nina Urala and Hely Tuorila and the statistical analysis was performed by Jukka Vainionpää in V.

Contents

Appendices of this publication are not included in the PDF version. Please order the printed version to get the complete publication (http://www.vtt.fi/inf/pdf/)

List of symbols

AEDA	Aroma extract dilution analysis
ANOVA	Analysis of variance
DMS	Dimethyl sulphide
FD factor	Flavour dilution factor
FFA	Free fatty acid
HS/GC/MS	Headspace gas chromatography – mass spectrometry
GC/olfactometry	Gas chromatography – olfactometry (sniffing)
n	Number of assessors/subjects in sensory/consumer studies
OAV	Odour active value
p-value	Statistical significance of difference between the samples
PCA	Principal component analysis
PLS regression	Partial least squares regression
RSM	Response surface method
Tukey's HSD test	Tukey's honestly significant difference test

1. Introduction

I want to reach that state of condensation of sensations which constitutes a picture.

- Henri Matisse

Cereal products are of the utmost importance in the daily diets of consumers. Wheat, maize and rice are the leaders worldwide (Bushuk 2001), and they are commonly used when they have been fractionated. Recently the enthusiasm for the whole grain ideology has increased considerably, which is mainly related to the increased evidence of the positive physiological functions of whole grain (Mälkki & Virtanen 2001, Poutanen 1999, Poutanen & Liukkonen 2000). The health benefits of oat are especially based on its cholesterol-lowering function, and rye is known from its very high fibre content and high amount of bioactive phytochemicals. Both oat and rye are traditionally used as whole grains. By finding tools for modifying their flavour in a controlled way, it would be possible to find totally new and versatile applications for oat and rye. In addition, new target groups of consumers might be reached with these products. Although not used worldwide, rye is an important cereal in the German, Polish, Russian and Scandinavian diets, in particular.

Oat is perceived as palatable, it has a favourable lipid composition and valuable technical properties related to its ability to form highly viscose solutions. By biotechnical processing techniques, it is possible to fine-tune the oat flavour to be even more attractive than that of the native oat. Flavour deterioration of oat during storage may result from both the volatilisation of desired flavour attributes and the development of undesired off-flavours. Due to its high lipid content, a bitter off-flavour and rancidity, in particular, are easily formed in unprocessed oat during storage.

Sourdough fermentation is the most studied processing method for rye, and rye is commonly consumed as soft bread or crispbread (Bushuk 2001). Related to the flavour, this fermentation process in particular is used to strengthen the rye-like flavour. However, all consumers might not be familiar with this foreign rye-like flavour. Rye consumption in Europe or worldwide might increase if the strong, specific rye-like flavour could be modified to be slightly milder, without decreasing the high fibre content and other health effects of rye, such as its high bioactivity.

Different processing techniques for fine-tuning the cereal flavour are available. Of the biotechnical processes, germination has mainly been used for barley in beer production until now, although it is also an effective method for adjusting the flavour of other cereals. The bioactivity of grains may be highly increased by the germination process, and the subsequent heat treatment process is an important factor in the formation of flavour. Another processing technique applicable to cereals is sourdough fermentation, which is also known to increase the amounts of bioactive compounds. After baking the products are perceived as being even more intense in flavour. In addition, extrusion cooking is one of the high temperature processes for modififying the flavour of cereal.

Current research represents a new approach to the flavour analysis of cereal products by having the starting point for the study in sensory perception, that is combined with an instrumental flavour analysis, instead of the traditional approach that is based only on researching flavour-active volatile compounds. However, the sensory perception of cereal products is highly influenced by the volatile compounds of the product. In addition, phenolic compounds and amino acids may contribute to the flavour. It is, thus, important to know the impact of the precursors and enzymes of the native grain on the flavour formation.

In general, sensory profiling is used to describe the perception of a product. However, the basis for sensory quality should lie in designing the desired flavour and other sensory attributes found most attractive by consumers, rather than just measuring the sensory profiles of products. The item should be regarded as a challenge, and the main aim should be to modify the flavour (Fig. 1). By using different processing techniques and knowing their influence, the flavour and texture of these products may be exactly adjusted in the desired direction.

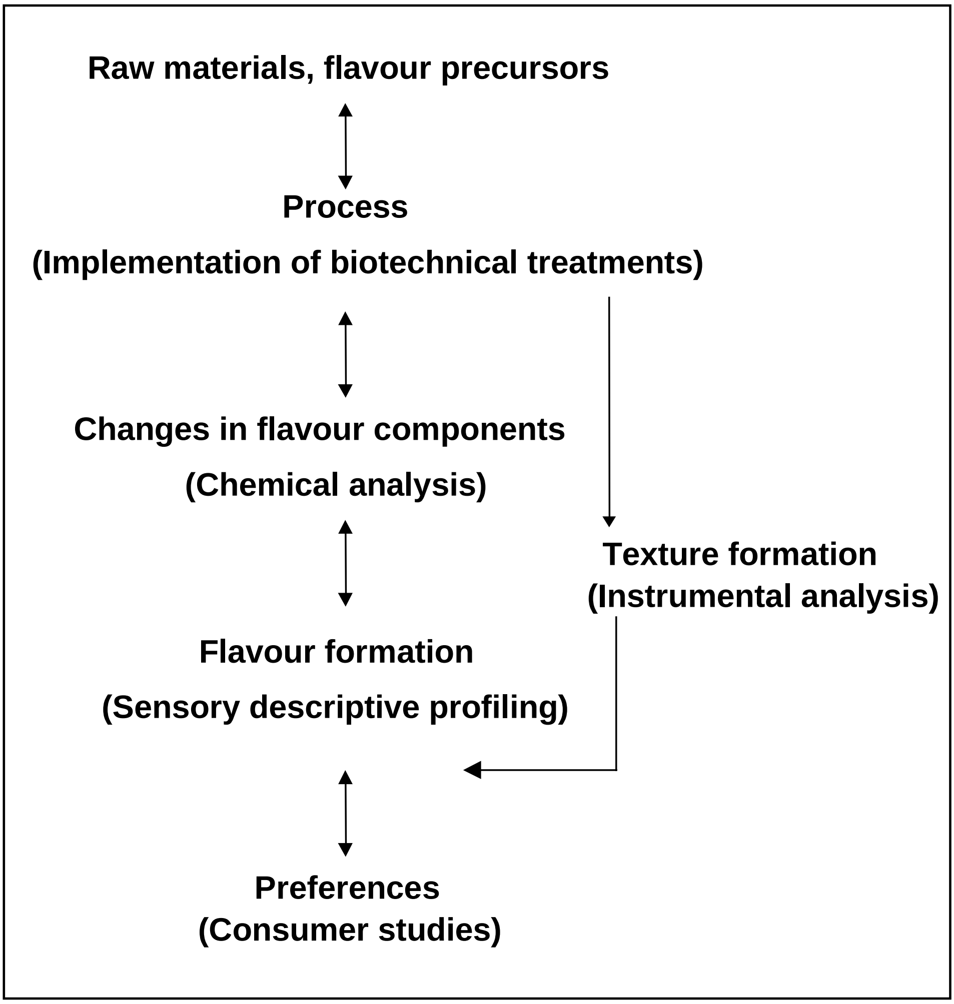

Figure 1. Flavour formation and determination.

1.1 Sensory perception of flavour

To better understand the flavour formation of cereal materials and to be able to optimise the cereal flavour, it is important to recognise certain basic principles of senses, sensory perception and flavour release.

In general, sensory evaluation is a multidisciplinary challenge, requiring an understanding of food science, statistics, chemistry, nutrition, physiology and psychology. Sensory analysis is 'a scientific discipline used to evoke, measure, analyse and interpret reactions that are the characteristics of foods and other materials as they are perceived by the senses of sight, smell, taste, touch and hearing'. This is a slightly modified version of the definition of Stone and Sidel (Stone & Sidel 1993).

The term flavour covers the impressions perceived via the chemical senses from a product in the mouth (Meilgaard *et al.* 1999). While defined in this manner, flavour includes the olfactory perceptions caused by volatile substances perceived in the nasal cavity, the gustatory perceptions caused by soluble substances perceived as basic tastes in the mouth, and chemical feeling factors (astringency, spice heat, cooling, bite) stimulating the nerve ends.

Thus, flavour is simultaneous perception of taste, odour and trigeminal nerve response (Lawless & Heymann 1999). The taste receptors are located on the surface of the tongue, clustered into taste buds, and saliva is necessary as a carrier for the taste perception. The classical four basic tastes include sweet, salty, sour and bitter, but suggestions for others, such as umami and metallic, have been widely accepted. In general, uniform agreement on the subsistence of these basic tastes among the sensory scientists does not, however, exist. The interactions of the four basic tastes are complicated and far from unambiguous (Brannan *et al.* 2001, Breslin 1996, Keast & Breslin 2002). In addition to the basic taste compound, the binary taste interactions depend on the concentration of the taste solution (Fig. 6 in Keast & Breslin 2002). For example, at moderate concentrations salts and acids are known to enhance each other, but at high concentrations they suppress the effect of each other (Breslin 1996).

The diversity of flavours is caused by volatile compounds in the headspace of the product, and these molecules are sensed by the olfactory receptors located very high in the nasal cavity. This diversity is mediated by smell. The olfactory perception is based on the transduction mechanism (Bell 1996). In transduction the odour-binding proteins transport the odorous compounds through the nasal cavity. The odorants are then recognised by the membrane-bound receptors, which finally change the chemical information into nervous activity. Usually when talking about taste, we, in fact, mean flavour, as related to the retronasal perception. Volatile compounds must exceed their specific odour thresholds to be perceived. The odour thresholds describe 'the limits of the sensory capacities', and may be categorised to detection, recognition, difference and terminal thresholds (Meilgaard *et al*. 1999). However, the odour perception of a product is caused by several different volatile compounds, and their relative amounts in the headspace are dominating. In addition, it should be remembered that a wide variation between the odour thresholds exists within and across individuals. Different types of odours and tastes tend to mask or suppress each other, which is often called mixture suppression. On the other hand, tastes may also increase the apparent intensity of odours, or odours may increase the apparent intensity of taste (Noble 1996).

The third factor influencing the flavour perception is the trigeminal nerve ending irritation. This chemical irritation is affected by different irritants causing sensations, such as pungency, burn or tingling. The concentration of the irritant is relevant for the perception; e.g. even ordinary salt, sodium chloride, may be irritative at high enough concentrations.

While considering the sensory interactions between taste sensations and aromas or volatile flavours, certain rules may be generalised (Lawless & Heymann 1999). As adapted from Lawless and Heymann (pp. 67–70), 1. Concerning the different senses, 'sensation intensities are additive'. For example, simultaneous stimulation of odour and taste senses have shown to increase the intensity ratings of odour, taste and overall perceptions, without any apparent interaction between the odour and taste sensations. 2. Taste and odour perceptions are often difficult to distinguish by the individuals, who e.g. may perceive some volatile compounds as a taste. 3. In general, unpleasant tastes suppress and pleasant tastes enhance the volatile flavour. For example, sucrose may be used to suppress bitterness, sourness or astringency. 4. ’Interactions change depending on the taste/flavour combinations'. For example, same sugar concentrations may be perceived sweeter with fruity odour than with nutty or caramel-like odour. 5. 'Interactions vary according to the instructions given to subjects', depending on that, where the attention of the subject is directed. All these interactions should be carefully considered while determining flavour perception.

Flavour release is a very complex process (Buettner & Schieberle 2000, Dattatreya *et al.* 2002, Laing & Jinks 1996, Linforth *et al.* 2002, Plug & Haring 1994, Taylor 1996, Taylor & Linforth 1996). The release of volatile substances is extensively studied whereas the release of semi-volatile or food-bound volatile compounds contributing to the flavour is not as well understood (Dattatreya *et al.* 2002). In general, while eating, the flavour perception is determined by the nature and amount of the flavour components, the availability of these to the senses as a function of time, and the mechanism of perception. The flavour release is mainly influenced by the individual shear-force during chewing, saliva volume and composition, breathing rate and volume, diffusion of molecules, binding, pH of the solution and solubility of the flavour molecules. In addition, the stimulus caused by the flavour compound varies depending on the chemical and physical properties of the volatile substances, the texture, shape and hydrophobicity of the material, interactions between the food components, and the state of the food (Guichard 2002).

Although standardised procedures are used in sensory evaluation, the perception may be influenced by factors difficult or impossible to control, such as earlier experiences, learning and operation of the senses of odour and taste. Individual differences between the subjects in a sensory panel should not be ignored either.

While understanding the mechanisms by which the flavour compounds are formed, flavour formation may be controlled by optimised methods of food processing.

1.2 Flavour of native grains

In general, the chemical composition of native grains varies significantly depending on the environment, the genotype and their interactions. These may substantially contribute to the perceived flavour, at least indirectly; for example, the nitrogen content of the soil greatly influences the composition of amino acids and, through them, the protein composition of the grain. Variations of the composition related to differences in harvesting and post-harvesting conditions or in treatments of the grain prior to its use may also arise. The season is also a dominant factor, especially for protein and fat production.

The grain consists roughly of protein, fat, carbohydrate or starch, fibre and ash, their relative amounts being dependent e.g. on the variety of grain. The protein content of oat is approximately 11%, fat content 5%, and carbohydrate content 59% (Welch 1995), whereas the protein content of rye is approximately 12%, fat content 3%, and carbohydrate content 63% (Shewry & Bechtel 2001), respectively. The high fibre content, being about 14–15%, is dominant for all kind of grains.

Very little literature is available on the perceived flavour of native oat or rye, whereas the flavour of processed cereal products is far better documented. The reason for this might be that native grains are not used as such, but some kind of processing is always required before their consumption. Most of the literature is definitely focused on documenting the volatile compounds identified in processed cereal products, especially in rye bread.

The main features of oat are its high fat content, high quality protein, and the composition of fibre, which contains significant amounts of soluble β-glucan (Welch 1995). All these indirectly influence flavour perception. Oat's high fat content makes it particularly sensitive to oxidation, resulting in a rancid flavour caused by the non-volatile free fatty acids (FFA) (Welch 1995). In particular, storage and processing have an effect on the amount of FFA. Amino acids may act as important flavour precursors. In addition, the flavour perception is influenced by phenolic compounds. The non-volatile phenolic acids have a high antioxidative function.

The mouthfeel is determined by the unbranched polysaccharide, oat gum ((1→3),(1→4)-β-D-glucan), which also significantly influences the flavour release. As a blood cholesterol-lowering agent, it has received special attention among the functional foods (Mälkki & Virtanen 2001). The special feature of β-glucan is that it forms highly viscous water solutions. Related to the viscosity, the flavour release is different for oat gum than for other hydrocolloids, such as guar gum and carboxymethyl cellulose. Oat gum was perceived as being the sweetest, and it tended to retain slightly more of its flavour in intensity and in duration than the water solutions of other thickening agents at equivalent viscosity levels (Mälkki *et al.* 1993). The flavour of oat groats was perceived as being raw oat, weedy-hay and grassy resembling, whereas the flavour of oatmeal has been described as being mild oat, hay-weedy and browned (Heydanek and McGorrin 1986).

The characteristics for rye are its high fibre content and its high amount of phytochemicals, which are known to increase the bioactivity of rye. The phenolic compounds significantly influence the rye-like flavour (Weidner *et al.* 1999).

The flavour of native rye is relatively mild when compared to the flavour of processed rye. The components of the soil influence the amino acid, lipid and sugar composition of the grain, which also indirectly influences the perceived flavour. Based on that, the effect of the season and cultivation area might be more dominant for the flavour formation than for example the rye cultivar.

1.3 Flavour formation in processing of grains

Biotechnical processing, such as sourdough fermentation and baking or germination combined with different thermal processes, may considerably modify the relatively plain flavour of native oat and rye grain. On the other hand, enzymatic reactions, such as oxidation or hydrolysation of lipids, may take place and result in non-desired flavour notes.

1.3.1 Sourdough fermentation and baking

Sourdough fermentation is mainly applied to rye prior to baking the dough into bread, although it is also optional in baking wheat. Rye is traditionally consumed as soft bread or in the form of crispbread, and sourdough fermentation is the most studied method of processing rye (Hansen 1995, Hansen *et al.* 1989, Lund *et al.* 1989).

Sourdough fermentation produces flavour components mainly in the breadcrumb, whereas the baking process mainly influences the flavour of the bread crust. The oxidation of lipids and enzymatic and heating reactions are the key reactions influencing the flavour formation of rye bread (Hansen *et al.* 1989, Schieberle 1996). Volatile compounds evaporate from flour as a consequence of the oxidation reactions. Enzymatic reactions produce flavour-active compounds both in the sourdough fermentation and at the beginning of the baking. The enzymes may originate from the flour, from the yeast or lactic acid bacteria, or from the additives. The flavour compounds produced during the baking process are possibly the most essential compounds for the flavour of rye bread, and they mainly form during the heat treatments, such as the Maillard reaction and caramelisation. The perceived sensory flavour depends on the combined effects of the volatile, phenolic and other flavour-active compounds, and their relative proportions, which are eventually determined by the processing technique applied.

Not much literature is concerned with the sensory perception of the flavour of rye bread, the cereal flavour having mostly been explained by the volatile compounds of the product. One of the most complete sensory studies concerns sensory perception and hedonic responses to rye breads and bread-spread combinations with varying NaCl and acid contents (Hellemann 1991). The acid content dominated the overall flavour of rye bread. If the acid content of the rye bread was increased, the NaCl content of the bread could be decreased. Perceived sourness of rye bread is shown to be strongly related to the concentrations of lactic and acetic acids (Hellemann *et al.* 1988).

Salt-free bread has a bland taste. Thus, salt is used in foods for improving the taste, texture and stability, and for strengthening its flavour (Hellemann 1992). The perception of salt results from cations, and is highly food-dependent.

Preference for saltiness is learnt. However, habits may be slowly changed, even though salt-free food may not correspond to the expectations of the consumer, at least in the beginning. According to nutrition guidelines, it is still recommended that salt intake would be reduced. At high NaCl concentrations, the saltiness and sourness of rye bread tended to compete with each other, and sourness could be masked by saltiness (Barylko-Pikielna *et al.* 1990). Thus, it was suggested that the NaCl concentration could be significantly lowered (as much as 0.75% of the dry weight) without significant changes in sour rye bread flavour.

Twenty of the most salient sensory attributes were generated for rye bread (Hellemann *et al.* 1987). Related to appearance, they were leavening, cracking and floury crust, and darkness of crumb and crust. Important odour attributes were overall aroma and malty odour, and essential taste attributes were bitterness, saltiness, sourness, rye-like and flour-like taste and overall intensity of taste. The texture descriptors were hardness of crumb, graininess, moistness, chewiness and toughness. Pleasantness and freshness were important for the overall impression. The pleasantness rating of rye bread was most clearly related to the characteristics of sour taste, leavening, moistness, rye-like odour and taste, overall intensity of taste, darkness of the crust and saltiness (Hellemann *et al.* 1987). However, although these attributes might be considered sensible, the number of subjects in the consumer panel was low (n = 30), which makes the pleasantness results somewhat debatable.

The fermentation process is used for strengthening the rye-like flavour, and thus rye bread is perceived as being strong and bitter in flavour (Hellemann *et al.* 1987, 1988, Rothe and Ruttloff 1983). Many consumers are not familiar with the somewhat foreign, strong flavour of rye. For consumers, the most dominating sensory attribute of rye bread is its rye-like flavour, but sourness and saltiness are substantially associated with the rye bread (Hellemann 1992, Hellemann *et al.* 1988, Rothe and Ruttloff 1983).

1.3.2 Germination, thermal processes and Maillard reaction

The grain flavour may be considerably modified by several thermal processing techniques, such as germination and subsequent drying, extrusion cooking, autoclave, puffing and roasting techniques, or microwave heating. All of these have a high processing temperature in common, although any possible pre-treatment prior to heating may, of course, also significantly influence the flavour.

The Maillard reaction is one of the major reactions influencing the flavour and colour of a product (Fayle & Gerrard 2002). It consists of several complex reactions, and not all of them are even known yet. Heat and dry atmosphere accelerate it. At high temperatures, free amino acids (or small peptides) together with free sugars are especially important flavour precursors, since they form flavouring volatile compounds, such as pyrazines, pyrroles and furfurals, as a result of the Maillard reaction. Most of these compounds are perceived to contribute to a desired roasted (or toasted), caramelised, somewhat sweet flavour.

To date, germination has mainly been used for barley in beer production, but it can also be an effective method for adjusting the flavour of other cereals, and the subsequent heat treatment completing the process is a particularly important factor for flavour formation. Generally, moisture, temperature and germination time are the major variables in germination, which is completed by a drying process. Germination is used both to evoke the bioactivity of the grain and to form new flavour components for it. During germination, the content of β-glucan of oat decreases, followed by the decrease of the viscosity. Simultaneously the amount of reducing sugars increases, resulting in perceived sweetness, and the amount of FFA rises, increasing the risk for rancidity (Peterson 1998). Oat oil, reducing sugars and FFA are important precursors for the flavour compounds. In germination, alkaline pH of the medium promotes the formation of compounds yielding a caramel-like odour (Przybylski and Kaminski 1983). Germinated grains, such as rye, wheat and barley malt, are a good source of free amino acids and sugars, which act as flavour precursors for the odour-active compounds (Przybylski and Kaminski 1983).

Germination has shown to substantially change the flavour profile of native barley from fruity and hay-like, to the burnt, bread-like, malty and chocolate-like flavour of germinated, dried barley (Beal & Mottram 1993). However, germination is also an effective method for adjusting the flavour of other cereals, and the subsequent heat treatment process, in particular, is an important factor for flavour formation (Przybylski & Kaminski 1983). In germination, alkaline pH promotes the formation of compounds yielding a caramel-like odour. Germinated grains are a good source of free amino acids and sugars, which act as flavour precursors for the flavour-active compounds.

The desired, oat-like flavour results from a heat treatment, which simultaneously inactivates the activity of lipolytic enzymes (Molteberg *et al.* 1996b). However, the thermal treatment of oat with hulls resulted in rancid and bitter flavour notes, whereas oat without hulls was perceived as being fresh and oat-like in flavour (Molteberg *et al.* 1996b). In addition to temperature, moisture content might be critical for the flavour formation. Cooked oatmeal was described with the flavour attributes of toasted, sweet, cereal and chemical (Lapveteläinen and Rannikko 2000). The toasted and cereal-like flavour has also been reported to have been found in heat-treated oat flakes (Sides *et al.* 2001). The flavour of cooked oats has been reported to become oat-like, nutty, browned and burnt in flavour. The nutty flavour of toasted oat originates mainly from carbonyl compounds and amines (Heydanek and McGorrin 1986). Maillard reaction products, e.g. heterocyclic pyrazines, pyrroles, furans and sulphur-containing compounds, are abundant in high-temperature, low-moisture extruded oats, and the products are described as toasted in flavour (Parker *et al.* 2000).

Extrusion is one of the most widely adapted high-temperature processes of cereal products. So far it has mainly been applied to wheat, but applications for oats have also been reported (Bredie *et al.* 2002; Liu *et al.* 2000; Parker *et al.* 2000; Pfannhauser 1993; Reifsteck and Jeon 2000; Sjövall *et al.* 1997). As a heat treatment, it is very effective in modifiying the cereal flavour, resulting in a roasted flavour and crispy texture.

1.3.3 Oxidation and hydrolysis of lipids

The fat fraction of grain consists of triglycerides, phospholipids, glycolipids, FFA and sterols, the dominant fatty acids of grain being linoleic, oleic and palmitic acids. The fat content of oat is 4–6%, which is significantly higher than that of other grain varieties. The special characteristics of oat make it exceptionally sensitive to stability during storage.

The sensory perceptions of rancid and bitter off-flavours, have been shown to result from the oxidative or hydrolytic degradation of lipids of a fatty grain, such as oat, although deterioration of proteins and reactions of phenolic acids should not be excluded. During storage, two distinct reactions for oat lipids take place. First, the hydrolytic deterioration where triacylglycerols or phospholipids are converted to FFA, and the second, the oxidative deterioration where polyunsaturated fatty acids are converted to hydroperoxides and further on to secondary oxidation products. Oat is exceptionally high in lipase, catalysing the hydrolytic deterioration, whereas the lipoxygenase activity, catalysing the oxidative deterioration is low (Ceumern and Hartfield 1984).

Oxidative rancidity develops as a result of enzymatic or non-enzymatic processes. Accordingly, the hydrolytic rancidity is a result of lipase activity in the outer layers of the grain, where fat degrades to FFA (Welch 1995). Lipase, lipoxygenase and peroxidase may be activated by the physical disruption of the grain. They can be inactivated by heat treatment, although if the grains are inappropriately stored or treated, e.g., in high moisture, the level of FFA may still rise (Ekstrand *et al.* 1993, Molteberg *et al.* 1996a). In addition to high moisture content, a light, elevated temperature (38°C) and a high oxygen concentration have shown to increase the development of rancidity in oat during storage (Larsen 2002, Molteberg *et al.* 1996b). Only through proper storage conditions can the rancidity be prevented or at least decreased considerably.

However, the stability of oat flavour depends on the lipid composition and resistance to oxidation. Phenolic compounds, such as avenanthramides and caffeic acid, might act as antioxidants by delaying the lipid oxidation, and thus prevent the development of rancidity (Molteberg *et al.* 1996b).

1.4 Chemistry behind the flavour formation

From the components of a grain, volatile compounds, phenolic compounds, free amino acids, small peptides and sugars may significantly influence the perceived flavour (Fig. 2). In addition, lipids indirectly influence the flavour, first as hydroxy fatty acids resulting from the oxidation of lipids, and second as FFA resulting from the lipase-catalysed hydrolytic oxidation of lipids.

There are two ways how the composition of a grain may influence the perceived flavour of a processed grain. First, the grain itself contains certain flavour-active volatile compounds, such as aldehydes, alcohols and ketones (Hansen 1995). Second, the grain contains important flavour precursors, such as amino acids, fatty acids and phenolic compounds, which produce various flavours during processing (Hansen 1995).

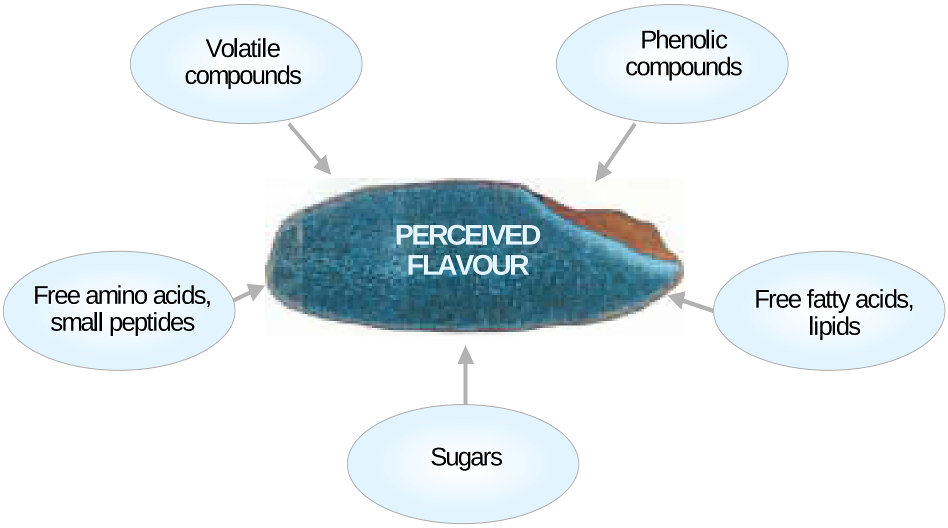

Figure 2. Chemical components of grain influencing the perceived flavour.

1.4.1 Volatile compounds

The origin of the 'cereal' flavour is unclear and complex (Sides *et al.* 2001). Similar volatile compounds are found both in native and processed grains, and the main difference seems to be in their relative proportions quantified in the end products. However, several attempts have been made in determining volatile compounds of native and processed grains, and some compounds seem to be dominant (Table 1). Volatile compounds identified in oat, rye and other grains have been already reported by Maga 25 years ago (1978). The volatile substances identified from oat and rye are mainly aldehydes, ketones and alcohols; as a result of heat treatment, derivatives of pyrazines and pyridines are often found, too.

For native grains, pentan-1-ol has been isolated from raw oats (Sides *et al.* 2001). In general, rye flour has shown to contain only a few flavour-active compounds, and in small amounts (Hansen 1995). These compounds have been identified as being aliphatic aldehydes, such as pentanal and hexanal, formed as oxidisation products from fatty acids, and the corresponding alcohols, pentanol and hexanol. Some of the carbonyl compounds possess a green odour note, with a pungent flavour. Another oxidation product, benzaldehyde, and some microbe-induced compounds, such as ethanol and ethyl acetate, are found in rye flour (Hansen 1995). In addition to pentanal, another volatile compound that has been associated with the rye-like flavour is 2-methylpropanal (Hougen *et al.* 1971). By using aroma extraction dilution analysis (AEDA), 1-octen-3-one, methional and 2-nonenal have been found to be most odour-active in fresh rye flour (Kirchhoff & Schieberle 2002). However, the weakness of an extraction method is that volatile compounds elute solvent-specifically, and some of the odour-active compounds may disappear.

Related to the sourdough fermentation of rye, new flavour compounds are mainly formed as a result of enzymatic reactions by the microflora (Hansen 1995). The volatile compounds are numerous, and although they are often identical, their amounts and relative proportions may vary significantly depending on the process parameters of the sourdough fermentation, thereby resulting in different flavours; for example, increasing the fermentation time may result in an increase in the amount of pyrazines originating from amino acids in rye bread (Schieberle & Grosch 1985). Important flavour compounds in sourdough are alcohols, esters and carbonyls (Hansen *et al.* 1989, Lund *et al.* 1989). Yeast fermentation has been shown to produce ethyl acetate, 2-methylpropanol and 2- or 3-methylbutanol in homofermentative cultures (Hansen *et al.* 1989). Other salient volatile compounds formed in sourdough

Table 1. Volatile compounds identified in cereal oat and rye products.

Salient volatile compounds	**OAT PRODUCT** Native	Groats	Heat proc. groats	Toasted	Extruded flour	Flakes	Stored	Stored extruded
Pentan-1-ol	x[1]						x[2]	
3-Methylbutanal		x[2]						
2,4-decadienal		x[2]						
Pentanal		x[2]					x[2]	
Hexanal		x[2]				x[1]	x[4, 2]	x[5]
2-Methylpropanal		x[2]						
Nonanal						x[1]		x[5]
Decanal						x[1]		
Benzaldehyde		x[2]						
2-Butanone		x[2]						
3-Pentanone		x[2]						
3,5-Octadien-2-one							x[2]	
Limonene		x[2]						
Methylbenzenes						x[1]		
Pyrazines				x[2]	x[3]			
2-Methyl pyrazine			x[2]					
2,5-Dimethylpyrazine			x[2]					
Pyridines				x[2]				
Oxazoles				x[2]				
Pyrroles					x[3]			
Furans					x[3]			
2-Pentylfuran						x[1]	x[4]	x[5]
Sulphurous compounds					x[3]			
Dimethylsulphide		x[2]						
DMDS		x[2]						
Alkyl thiazoles				x[2]				

References: [1] Sides *et al.* 2001, [2] Heydanek & McGorrin 1986, [3] Parker *et al.* 2000, [4] Molteberg *et al.* 1996a, [5] Sjövall *et al.* 1997.

Salient volatile compounds	**RYE PRODUCT** Native flour	Sourdough	Breadcrumbs	Breadcrust
2-Methylpropanol		x[2]	x[2]	
2/3-Methylbutanol		x[2]	x[2, 3]	
Benzylalcohol			x[6]	
2-Phenylethanol			x[6]	
Methional	x[1]	x[1]	x[3, 5]	x[2, 4, 5]
(E)-2-nonenal	x[1]		x[3, 4, 5]	x[2, 4, 5]
Hexanal	x[1]		x[3]	
3-Methylbutanal		x[1]	x[3, 5, 6]	x[2, 4, 5]
Vanillin		x[1]	x[3]	
(E,E)-2,4-decadienal		x[1]	x[3, 4, 5]	x[2, 4]
Phenylacetaldehyde			x[3, 4, 5]	
2,3-Butanedione		x[1]	x[3]	
2-Propanone			x[6]	
3-Hydroxy-4,5-dimethyl-2*(5H)*-furanone			x[3, 5]	
4-Hydroxy-2,5-dimethyl-3*(2H)*-furanone				x[4, 5]
2-Acetyl-1-pyrroline				x[5]
2-Ethyl-3,5-dimethylpyrazine				x[4]
Ethylacetate		x[2]		
3-Methylbutanoic acid		x[1]		
Acetic acid		x[1, 2]	x[2, 3, 5]	x[2, 4]
2- and 3-methyl butanoic acid			x[3, 5]	

References: [1] Kirchhoff & Schieberle 2002, [2] Hansen 1995, [3] Kirchhoff & Schieberle 2001, [4] Schieberle & Grosch 1994, [5] Grosch & Schieberle 1997, [6] Hansen *et al.* 1989.

fermentation are pentanol, hexanol, 2-methylbutyl acetate, ethyl lactate, ethyl n-hexanoate, diacetyl and hexanal (Lund *et al.* 1989). In addition, acids, especially lactic and acetic acids, are important flavour-active products of sourdough fermentation, and may be influenced by temperature and the starter culture.

After sourdough fermentation, totally new flavour-active volatile compounds are formed in baking rye bread, mainly related to the Maillard reaction: different alcohols, acids, aldehydes, hydrocarbon-substituted furans, ketones, lactones, pyrazines, hydrocarbon substituted pyrroles and sulphur compounds. The perceived flavour and the flavour-active volatile compounds of the crumb are significantly different from those of the crust on rye bread (Grosch & Schieberle 1997, Kirchhoff & Schieberle 2001; Schieberle & Grosch 1985, 1994), and by modifying the process parameters in sourdough fermentation and baking, they may be effectively influenced. To be more specific, most intense and bread-like flavour notes of sourdough rye bread crumbs have been associated with volatile compounds of 2-propanone, 3-methyl-1-butanal, benzylalcohol and 2-phenylethanol (Hansen *et al.* 1989). 2-Phenylacetaldehyde has been suggested to be a key odorant for rye bread crumb, and methional for rye bread crust (Schieberle 1996).

In germination, certain volatile compounds appear although they have not been identified since the germination process requires a heat treatment to be completed. The circumstances in the germination, however, influence the formation of volatile substances. At lower processing temperatures and higher moisture levels, different types of volatile compounds have been identified when compared to those formed at higher temperatures and lower moisture levels. After kiln drying, oat flavour components, such as terpenes, alkylbenzenes, aldehydes, alcohols and heterocyclic compounds, develop (Dimberg *et al.* 1996).

In extruded oat, two main groups of flavour-active volatile compounds have been identified. Firstly, the compounds formed in the Maillard reaction, e.g. pyrazines, pyrroles, furans and sulphur-containing compounds, which have toasted cereal sensory descriptions, and secondly, the compounds originating from lipid oxidation, e.g. hexenal and hexanol (Parker *et al.* 2000; Pfannhauser 1993; Sjövall *et al.* 1997). High processing temperatures during extrusion enhance the Maillard reaction, and these extrusion products have been described as toasted and cereal (Parker *et al.* 2000). The major volatiles found in moistened, extruded oat flour stored at 32°C were hexanal, decane, 2-pentylfuran and nonanal (Sjövall *et al.* 1997). The extrusion temperature and the alkalinity of the system have been shown to significantly influence the flavour generation from wheat flour by extrusion cooking (Bredie *et al.* 2002). Almost half of the volatile compounds formed contained sulphur, and nitrogen-sulphur-containing heterocyclic compounds formed at high temperatures and alkaline conditions were described as being sulphury and rubbery in odour when analysed by GC/olfactometry (Bredie *et al.* 2002).

Flavour deterioration of cereals during storage is influenced both by the reduction of the desired flavour attributes through volatilisation, and by the development of undesired off-flavours (Zhou *et al.* 1999). Interactions of volatile compounds with other food constituents are important for the flavour characteristics, and for their intensity and stability. The long-chain hydroxy fatty acids, as free or as monoglycerides, cause a bitter perception. In addition, the N-heterocyclic compounds that form during processing may cause off-flavours in oat (Bierman & Grosch 1979, Welch 1995). Aldehydes, ketones and alcohols cause the rancid flavour of oat. Hexanal is the main one, although pentanal, 1-pentanol and 3,5-octadien-2-one are also culprits (Heydanek & McGorrin 1986). Hexanal, which is a very abundant volatile compound in oat, is one of the dominating oxidative breakdown products of linoleic acids resulting from extreme heat treatments. Hexanal accumulates only partly in food products because it may evaporate or be quenched into non-volatile compounds (Zhou & Decker 1999). It is, however, always present in oat that has an acceptable flavour character for human consumption. Thus, rancidity in oat-containing foods is due as much to the change of the concentration of hexanal than its absolute presence (Sides *et al*. 2001). In addition, 2-pentylfuran in high concentrations is related to rancidity (Sides *et al*. 2001).

1.4.2 Phenolic compounds

The function of phenolic compounds is somewhat unclear; many of them have proven to have antioxidative activity, and some of them may be salient in defending against diseases (Decker *et al.* 2002, Welch 1995). In spite of all, more evidence on the efficiency of bioactivity of phenolics is still required. Some sensory attributes may be related to foods beneficial to one's health.

However, consumers cannot perceive the healthiness connected to the bioactivity of cereal products.

Nonvolatile phenolic compounds may also contribute considerably to the flavour of cereal products (Dimberg *et al.* 1996, Peterson 2001). In general, for the flavour of cereals the most abundant phenolic acid is ferulic acid, but other phenolics, such as sinapic acid, *p*-coumaric acid and caffeic acid, are shown to be essential as well (Andreasen *et al*. 2000, Weidner *et al.* 1999). Although free phenolic acids are minor compounds in cereals, they may influence the flavour perception of oat even in small amounts (10–90 mg/kg) (Dimberg *et al.* 1996). Phenolic compounds are mainly concentrated in the outer layers of the grain, in the bran fraction (Decker *et al.* 2002, Shewry & Bechtel 2001). However, significant variation between the rye varieties and seasons of harvest has been observed (Andreasen *et al.* 2000).

In oats, ferulic acid is the major phenolic acid; caffeic and syringic acids are also found (Welch 1995). Caffeic and ferulic acids as well as avenanthramides, which are *N*-cinnamoyl-substituted alkaloids, are antioxidative. Avenanthramides have proven to be relatively stable compounds thermally, and germination increased their amount (Pihlava & Oksman-Caldentey 2001). In addition to phenolic acids, phenolic aldehydes, antioxidative vanillin and avenanthramides of oats may contribute to its flavour. The release of phenolic compounds depends highly on the moisture content, temperature and time during processing (Dimberg *et al.* 1996, Molteberg *et al.* 1996b). In addition, the hulls of the grain may play an important role for the phenolic compounds. Resulting from a heat treatment, the levels of vanillinic acid, vanillin, and especially *p*-coumaric acid, *p*-hydroxybenzaldehyde and coniferyl alcohol increased significantly in oat processed with hulls but not in hulless oat. The level of ferulic acid increased on both occasions, whereas the amounts of caffeic acid and avenanthramides tended to decrease. In general, storing unprocessed oat produces an increase in the amounts of phenolic acids and aldehydes. Some of the phenolic compounds (*p*-coumaric acid, vanillin, *p*-hydroxybenzaldehyde and coniferyl alcohol) may also contribute to the rancid, bitter, intense flavour of oat (Molteberg *et al.* 1996b).

In rye grain, the 5-alkylresorcinols (5-alkyl-1,3-dihydroxybenzenes) are important phenolic compounds (Shewry & Bechtel 2001). Rye grain contains up to 0.5% of 5-alkylresorcinols, which is more than twice the amount found in wheat.

Related to their strong specific flavour, free phenolic acids of rye may also dominate the bread flavour by intensifying the total rye-like flavour. Heat or microorganisms may decompose the phenolic acids to chemical compounds with a strong flavour; for example, ferulic acid is a source of 2-methyl-4-vinylphenol, which is described as having a burnt or tar-like flavour (Hansen 1995).

1.4.3 Amino acids

Proteins of grains are usually characterised either by the amino acid composition or by the solubility-based protein fractionation pattern. The soluble fractions of proteins are albumins (water-soluble), globulins (soluble in dilute aqueous salt solutions), prolamins (soluble in aqueous alcohol) and glutelins (soluble in dilute acid or alkali). There are substantial differences in the amino acid composition of the fractions. In native oat, globulins are the main proteins and comprise approximately 50% of proteins (Welch 1995), whereas prolamins, called secalins, are not necessarily the most abundant, although they are most important in native rye in relation to their location in the grain (Shewry & Bechtel 2001). The storage proteins of rye, the prolamins, contain a high proportion of glutamate, glutamine and proline (Shewry & Bechtel 2001).

Free amino acids may influence the perceived flavour as such or as flavour precursors. When baking bread, the proteases produce free amino acids as flavour precursors to the sourdough, to react together with free sugars in the Maillard reaction during baking. The heat treatment following germination and other thermal processes also use amino acids for flavour formation.

The amount of amino acids is higher in rye than in wheat, etc. (Hansen 1995). Amino acids form flavour-active volatile compounds, such as pyrazines, pyrroles and furfurals, as a result of the Maillard reaction at high temperatures. The free amino acids may also be a source of nitrogen for yeast, resulting in higher alcohols, such as 3-methylbutanol.

In wheat sourdough, D-alanine, D-glutamic acid and traces of other D-isomers of amino acids were observed, but the contents of free total D- and L-amino acids decreased by more than 44% after baking the sourdoughs (Gobbetti *et al.* 1994). From the amino acids, ornithine, phenylalanine, leucine and methionine are considered to be the most important flavour precursors to generate flavour compounds of bread (Schieberle 1996).

The conditions during germination considerably influenced the reactivity of the amino acids (Kaminski *et al.* 1981). The amounts of lysine, arginine, glutamic acid and proline decreased significantly within 15 minutes of heating at 120°C. High losses in all amino acids were noticed at extreme pH values, below 5 and above 7.

1.5 Relation between perceived flavour and volatile compounds

The perceived odour is never a sum of different volatile compounds identified in a product, because complex synergistic and other reactions are involved. Most volatile compounds have specific odour descriptions and odour thresholds (Van Gemert 1999). The odour thresholds of volatile compounds are important in the attempt to explain the perceived flavour, though they should always be considered as indicative, qualified and regarded with some precaution. Some may go even further in doubting the existence of sensory thresholds at all (Meilgaard *et al.* 1999). Very often the threshold values may easily differ 100- or 1000-fold depending on the method of analysis (Meilgaard *et al.* 1999, Van Gemert 1999, Zhou *et al.* 1999). For example the odour threshold of dimethyl sulphide (DMS) varies between 0.0003 and 20.6 mg/m^3 in air and between 0.00008 and 0.03 mg/m^3 in water (Van Gemert 1999). Often it is not reported if the threshold is the detection or recognition value.

The method of analysing volatile compounds from cereals has been shown to significantly influence the compounds recovered, and the results from different methods should be considered carefully (Zhou *et al.* 1999). In particular, when solvent extraction was used, the volatile compounds identified showed multiplicity. In addition, the volatile compounds identified did not necessarily explain the perceived flavour of the grain.

All volatile compounds are not odorous, but their odour-activity depends on the odour thresholds. For example, many long-chain hydrocarbons do not produce any odour perception. For determining the contribution of odour-active volatile compounds to the perceived odour, a few techniques are frequently used. One of them is the calculation of the ratio of the concentration of the volatile compounds to their odour thresholds, thus setting the odour active value (OAV) (Grosch & Schieberle 1997). However, as concluded earlier, the odour threshold values should be considered with reserve.

Another concept involves the screening of potent odour-active volatile substances by aroma extract dilution analysis (AEDA), where the extract obtained from the food sample is analysed by gas chromatography-olfactometry (GC/olfactometry) (Grosch & Schieberle 1997, Schieberle 1996). The result for each odour-active volatile compound is then expressed as a flavour dilution (FD) factor, reflecting the ratio between the concentration of the compound in the initial extract and its concentration in the most diluted extract in which the odour was perceived by GC/olfactometry. However, the use of solvent extraction may cause bias in the results, if the odour-active compound is not extractable in the solvent used.

Related to the simultaneous analysis by sensory and instrumental techniques, GC/olfactometry (GC/sniffing) may also be used without AEDA for producing aroma profiles. Only flavour-active volatile compounds are relevant for perceived flavour, and in spite of appearing at even very high concentrations, the substances having very high odour thresholds should be rejected. In conclusion, only those results obtained from the equivalently implemented trials should be compared with each other directly.

Thus, the sample preparation technique is a factor that greatly affects the appearance of volatile compounds in the grain samples (Ibañez & Cifuentes 2001). Extraction with organic solvents, column fractionation etc. have traditionally been used in sample preparation, which may, however, introduce potential qualitative or quantitative errors in the results. For example, the selection and use of extraction solvents are critical. The risks related to the use of organic solvents in large quantities cannot either be ignored. Headspace sampling methods are solvent-free techniques, and may thus be closer to the occasion in human perception. Headspace solid-phase microextraction (HS-SPME) integrates sampling, extraction, concentration and sample introduction into a single step, and might thus be one of the alternatives in analysing volatile compounds from foods (Ibañez & Cifuentes 2001, Sides *et al.* 2001). Many new methods for measuring volatile release in a way that mimics human interaction with food have been developed. These include model mouths and in-mouth techniques.

Efficient use of advanced statistical techniques is necessary, while combining instrumental and sensory profiling data. The multivariate techniques, such as PLS regression, and correlation methods are useful, while relating the flavour data derived by different analysis methods to better understand how the cereal flavour is formed, and the factors that might influence it. However, for achieving a complete causality of the results would necessitate the use of experimental design or other kind of manipulation of the test conditions.

Descriptive analysis is very suitable for relating compositional data to perception. However, sensory assessment and instrumental analysis produce different types of results; while sensory profiling is focused to form a complete picture from the product through its attributes, instrumental techniques tend to describe the fragments of the product characteristics. Descriptive attributes are often concepts, and they do not represent any single compounds, but rather complex mixtures of compounds. In addition, descriptive analysis does not provide absolute results, but it is a comparative technique. These different aspects specific to the two techniques should not be ignored in the data processing.

Efforts have been made to relate certain perceived odours to certain volatile substances by statistical methods (Molteberg *et al.* 1996b, Zhou *et al.* 2000). PLS regression has been successfully used to explain sensory attributes from chemical data (Molteberg *et al.* 1996b, Tamime *et al.* 1997), or even from volatile compounds (Karlsen *et al.* 1999, Martin *et al.* 2002, Noble & Ebeler 2002). While relating the results of the 'macroworld' of sensory evaluation with volatile compounds determined by the 'microworld' of headspace techniques, a high expertise in sensory, headspace and statistical methods is required in the incidence of these disciplines. Seamless and flexible co-operation between all these parties is necessary.

While using statistical multivariate methods, it should be remembered that the relations observed between the samples and their properties are always relative to each other, although they are always consistent within one experiment or treatment.

1.6 Aims of the study

The success in increasing the use of the whole grains, oat and rye, which are beneficial to one's health, is to a large extent dependent on their flavour or flavour stability. The aim of this thesis was to specify how the flavour of oat and rye is formed, and which processing factors influence it. As means to solve this objective, the volatile substances or other chemical components were related to the flavour of processed oat and rye. The different processing techniques for modifying the cereal flavour were milling fractionation, germination and heat treatment, sourdough fermentation and baking, as well as extrusion.

The specific objectives were:

- To determine the salient flavour attributes of native oat
- To study the flavour in different parts of the rye grain by using the milling fractionation process
- To study the effect of sourdough fermentation and baking on rye flavour
- To study the effect of germination and subsequent heat treatment on oat and rye flavour
- To learn about factors influencing the increasing stability of oat flavour during storage
- To determine the taste factors influencing identity and consumer acceptance of rye bread.

2. Materials and methods

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

2.1 Oat and rye samples

Oat was used as whole grain (cultivars Veli and Lisbeth) in native, germinated-undried and germinated-dried forms using six different heat treatments (I). Its stability was determined from the coarse-ground (cultivar Veli) native and germinated-dried forms (II).

Rye was used as milling fractions (cultivar Amilo) in the form of flour and baked into breads (III), as extruded pellets after pre-treatment (native, germinated-dried and sourdough fermented) (cultivars Amilo and Akusti) (IV, Figure 2), and as soft bread and crispbreads modified with four different process parameters (V).

To gain the most reliable results possible from the studies, the grain samples were studied plain, without any additional treatments prior to the analysis. Thus, no water or solvent extraction was used. This was valid for both sensory and instrumental measurements.

2.2 Sensory and consumer studies

The sensory attributes of oat and rye products were commonly evaluated by using descriptive profiling by trained panels (I–IV), except for the identity and acceptance of products, which were evaluated by a consumer panel (V).

2.2.1 Sensory profiling

The sensory experiments were carried out by quantitative descriptive profiling (Lawless & Heymann 1999, Meilgaard *et al*. 1999). The vocabulary of the sensory descriptors was developed separately for each study reflecting the salient attributes developed in each processing treatment of oat and rye. The assessors were accordingly trained each time. Verbal descriptions or real models fixed definitions for the sensory attributes. The attribute intensities were rated on 10-unit continuous, unstructured line scales, verbally anchored at each end. The vocabulary, training of the assessors and other implementation of the sensory profiling is described exactly in Publications I–IV.

The assessors for the descriptive panel were selected based on their sensory skills. The panel consisted of well-trained assessors, the panel size varying from six to 17, being 10 in most cases. The assessors commonly evaluated the products in duplicate, with some exceptions due to the amount of the sample (II). The samples were presented to the assessors coded and in random order. The results were recorded and collected using computerised data systems (Panel 5, Finland in I–II and Compusense, Canada in II–IV). All sensory work was performed at the sensory laboratory of VTT Biotechnology, which fulfils the requirements of the ISO standards (ISO 1985, 1988).

2.2.2 Consumer acceptance

When measuring the consumer acceptance of soft breads and crispbreads made of rye, four continuous recipe variables: the wheat to rye flour ratio, the bread acidity, the ash content of rye flour and the NaCl content of the bread, as well as the bread type were the parameters in the half replicate of a 2^5-factorial design. The experimental design followed the completely randomised design. The samples were presented to the assessors coded and in random order.

The participants in the consumer panel (n = 79) rated first three attribute intensities of the breads (sourness, saltiness and rye-like flavour). After that they rated, how well the sensory attributes (colour, overall appearance, odour, taste and texture) and the samples as a whole fitted to the impression the subjects have of rye bread. Finally the subjects rated the pleasantness and the purchase intention. All the factors were determined by applying 9-point, end-anchored category scales.

2.3 Instrumental analysis of flavour components

In addition to sensory profiling and consumer acceptance, volatile compounds (I, II and IV), phenolic compounds (I and II), lipids (I and II), and chemical process parameters, such as the NaCl content, bread acidity and lactic to acetic acid ratio (V), were analysed from the oat and rye products. The impact of volatile compounds on perceived flavour was described in the present study, whereas the role of several non-volatile compounds known to considerably influence the cereal flavour, such as different phenolic compounds and amino acids, has been only described here briefly.

2.3.1 Volatile compounds

The flavour-active volatile compounds were analysed using HS/GC/MS (I, II and IV) and GC/olfactometry (IV).

The volatile compounds were determined either by dynamic HS/GC/MS (I and IV) or by static HS/GC/MS (II). For dynamic headspace analysis, saturated sodium chloride solution was used for transferring the volatile compounds from the samples to the headspace (the salting out effect). The volatile compounds were purged from the headspace vials into the dynamic headspace sampler, which was interfaced to GC/MS. The compounds were identified on the basis of their mass spectra and retention time, and their amounts were quantified using selective ions for each compound from total ion chromatograms against standard solution mixture series, prepared into 30% sodium chloride solution. The samples and the standards were analysed in duplicates.

Correspondingly, in static headspace analysis, the volatile compounds were identified and quantified from the headspace of the equilibrated sample vials. The compounds were identified on the basis of their mass spectra and retention indices. To compensate for the variation in the performances of the headspace injector and the MS-detector during the storage period, all detector responses for triplicate samples were proportioned to the response of an external standard (isobutanol in water).

GC/olfactometry deals with the sensory evaluation of odours present in the GC effluents (Dattatreya *et al.* 2002, Van Ruth 2001). The simultaneous sensory sniffing of the compounds by a well-trained four-member sensory panel was combined with the GC, and the volatile compounds were separately identified by GC/MS. The samples were prepared for the sniffing analyses by steam distillation. At the end of the capillary column, the effluent was split so that one fifth of the gas carrier was directed to the GC FID-detector, and four fifths were directed to the four separate sniffing ports equipped with glass funnels. The sensory panel used verbal descriptors for characterising the odorous compounds. The odour descriptors used by the four assessors were combined with the MS-identified volatile compounds to create an aromagram for each sample separately.

2.3.2 Phenolic compounds

The concentration of total phenolic compounds was determined as gallic acid equivalents using a slightly modified Folin-Ciocalteu method (I).

2.3.3 Lipids

The lipids were extracted from the samples, and evaporated to dryness under N_2. The lipid class separation was done by thin-layer chromatography (Liukkonen *et al.* 1992). The fatty acid composition analysis of extracts and separation into the lipid classes were performed by converting the fatty acids to methyl esters and analysing the latter by gas chromatography (Suutari *et al.* 1990).

2.3.4 Chemical analysis of process variables of rye bread

The NaCl content of bread was analysed according to V. The bread acidity was determined by titrimetric analysis (Standard Methoden für Getreide, Mehl und Brot 1978). The lactic acid and acetic acid were extracted from the bread samples, and the amounts were analysed by high-pressure liquid chromatograph (V).

2.4 Data analysis

The data were analysed using the standard statistical procedures as described in the individual publications (I–V). The statistical methods are summarised in Table 2.

Table 2. Statistical methods used to analyse the results in Publications I–V.

Publ. No.	Applied for data of	Statistical methods
I	Sensory profiling Sensory profiling & volatile compounds Sensory, volatile & phenolic compounds, FFA	ANOVA, Tukey's HSD test, PCA PLS regression Correlation analysis
II	Sensory profiling Sensory profiling & volatile compounds Sensory, volatile & phenolic compounds, lipids Volatile compounds & lipids	ANOVA, Tukey's HSD test, PCA, *t*-test PLS regression Correlation analysis *t*-test
III	Sensory profiling	ANOVA, Tukey's HSD test, PCA
IV	Sensory profiling Sensory profiling & volatile compounds	ANOVA, Tukey's HSD test, PCA PLS regression, correlation analysis
V	Consumer data & process variables	RMS

2.4.1 Statistical analysis of sensory profiling data

An analysis of variance (ANOVA), Tukey's honestly significant difference (HSD) test ($P<0.05$), a paired *t*-test and a principal component analysis (PCA) were used for statistically analysing the sensory profiling data (Table 2).

ANOVA was used to test the statistical differences in the sensory attributes between the samples, the significance of each descriptive attribute in discriminating between the storage periods, and the statistical differences between the sensory sessions ($P<0.05$). When the difference in ANOVA was statistically significant, pairwise comparisons of the attributes between the samples were conducted by Tukey's test. The significance of the difference between the native and processed oat groats for each separate storage time was executed by a paired *t*-test ($P<0.05$). ANOVA, Tukey's HSD test and a paired *t*-test were performed using the SPSS software package (SPSS Ver. 8.0.2 or 10.0, SPSS Inc.).

A multivariate analysis method, PCA, was used to describe the variation among the sensory data by compressing the data to its most dominant factors (Martens & Naes 1998). The score plot shows the location of the samples, and the loading plot the location of the sensory attributes on the usually two-dimensional map. The map is used to show the similarities, differences and groupings of the samples and their attributes. The sensory profiling data used for PCA were means calculated over all assessors. PCA was performed for the sensory results using the Unscrambler software package (Unscrambler Ver. 7.5 or 7.8, CAMO ASA).

2.4.2 Statistical analysis in seeking the relations between sensory and instrumental data

The impact of chemical flavour-active components, mainly volatile compounds, was related to the perceived flavour by statistical methods. A multivariate analysis method, a partial least squares (PLS) regression, and a correlation analysis and a paired *t*-test were used to statistically relate the sensory profiling data and instrumental data (Table 2).

While PCA is a format of representation for sensory, instrumental data, etc., PLS regression was used for relating the sensory profiling data (dependent y variables) with the most relevant, selected volatile compounds (independent x variables) (Martens & Naes 1998). The selection of the volatile compounds was based on their odour thresholds. The sensory profiling data used for the multivariate analysis were means calculated over all assessors. The relative peak areas (I and II) or absolute amounts (IV) of the chosen volatiles were used for the PLS regression. The model was validated by cross-validation. PLS regression was performed using the Unscrambler software package (Unscrambler Ver. 7.5, CAMO ASA).

The Pearson's coefficients of correlation between the scores of sensory attributes, and the relative amounts of selected volatile compounds, lipid composition and total amount of phenolic compounds were determined ($P<0.05$).

However, it should be recognised that the correlation coefficients as well as the results from PCA or PLS regression always vary according to the data matrix used, and are thus only valid for that data used in the statistical analysis.

The amounts of lipids and volatile compounds of native and corresponding processed oat were compared by paired *t*-test ($P<0.05$).

2.4.3 Statistical analysis of consumer data and process variables

The response surface method (RSM) was applied for statistically relating the consumer data and the data of the process variables of bread (Table 2).

The RSM is a regression analysis method, and it projects the value of a response variable (dependent variable = consumer data) based on the controlled values of the experimental factors (independent variables = process variables) (Meilgaard *et al.* 1999). All the factors in the RSM experiment were quantitative, based on the results obtained from the experimental design. The average responses of the consumer data were submitted to a stepwise regression analysis, which resulted in a predictive equation that related the consumer response values to the values of the process variables. The predicted relationship was displayed in contour plots. The RSM analysis was performed using the Statgraphics Plus software package (Statgraphics Plus, Ver. 7.1, Manugistics Inc.).

3. Results and discussion

3.1 Salient flavour attributes of native oat (I)

In general, all varieties of cereals have a characteristic flavour. In our study, native oat was perceived as being cereal-like in flavour, the texture being described as tough and hard (Table 3 in thesis). In addition, slight differences in the flavour were found to be dependent on the cultivar (I); for example, the flavour of the oat cultivar Veli was perceived to be more cereal-like and sweet than that of the cultivar Lisbeth.

Table 3. Summary of the main sensory descriptions obtained as a result of different processing in the studies (Publications I–V).

Publ.	Grain and its treatment	Main sensory descriptions
I	Native oat	Cereal-like flavour; tough, hard texture
	Germinated-undried oat	Moist, musty, earthy flavour; soft, moist texture
	Germinated-dried oat	Roasted, nutty, sweet, intense flavour, intense aftertaste; crispy, hard, brittle texture
II	Native, stored oat	< 6 mo: Tough texture; 12 mo: Bitter, rancid; musty flavour
	Germinated-dried, stored oat	< 6 mo: Roasted, sweet, nutty flavour; 12 mo: Musty flavour
III	Rye flour from endosperm	Mild flavour
	Rye flour from shorts	Cereal-like flavour
	Rye flour from bran	Bitter, intense flavour, intense aftertaste
	Rye bread from endospermic fraction	Mild flavour
	Rye bread from shorts fraction	Cereal-like flavour
	Rye bread from bran fraction	Bitter, intense flavour, intense aftertaste
IV	Rye flour extrudate	Mild flavour; tough texture
	Germinated rye extrudate	Fresh, cereal-like flavour; hard texture
	Sourdough fermented rye extrudate	Sour, intense flavour, intense aftertaste; porous texture
V	Soft and crisp rye bread	Rye-like, sour flavour

The native grains were evaluated as such, without any pre-treatment in the present experiment, in contrast to the other reported sensory investigations. In general, only the water extract of the product has been subjected to sensory evaluation (Heydanek and McGorrin 1981, Heydanek and McGorrin 1986, Molteberg *et al.* 1996a, 1996b), or at least the sample has been pre-moistened (Parker *et al.* 2000). Any difficulties that may have arisen from the sensory evaluation of native grains as such were not observed. The objective in the evaluation of the whole grains as such was to gain as reliable knowledge as possible on natural perception of the sensory attributes of the product.

One reason for the sparse information on the flavour of native grains might be related to the very simple fact that tasting plain native grains is rather challenging and not as attractive when compared to the evaluation of processed grains.

3.2 Distribution of flavour components in rye grain (III)

In this study, the flavour components were unevenly distributed in the rye grain (Table 3 in thesis). The endosperm was perceived as being the mildest, with the outer bran layers being the most bitter and intense in flavour (Fig. 1 in III). The middle shorts fraction was described as cereal-like in flavour without any obvious bitterness. Thus, the optimal flavour of the shorts fraction makes it very interesting for further applications related to its high bioactivity.

The main products in milling fractionation are presented in Fig. 3. The bran fraction is the outermost layer of the grain, the shorts fraction lies in the middle, and the endosperm is the innermost part of the grain.

The effect of grain fractions on the perceived flavour was consistent even when fractionated rye flour was used in baking mixed bread, where 20% of the wheat flour was replaced by the rye fraction (Table 3b in III). The endospermic fraction induced a very mild flavour in the mixed wheat bread, resembling pure wheat bread, but the bitter, intense flavour and aftertaste were perceived as being characteristics of the bread containing the bran fraction. In addition, the shorts was a very important fraction, in producing a cereal-like but not bitter flavour in bread, consistent with flour. The colour intensity of the bread samples increased from the endospermic layer to the bran layer. Thus, it was obvious that one fifth of the wheat flour replaced by the milling fraction was enough to significantly show the influence of the rye fraction, when the sensory profiling was conducted by a trained sensory panel. However, the amount of rye flour in the mixed bread was not the only explanation for the perceived rye-like flavour, when wholemeal rye was used in sourdough baking, and when the assessments were performed by a consumer panel (V). However, sourdough fermentation was not used while baking the breads from the rye fraction flours (III).

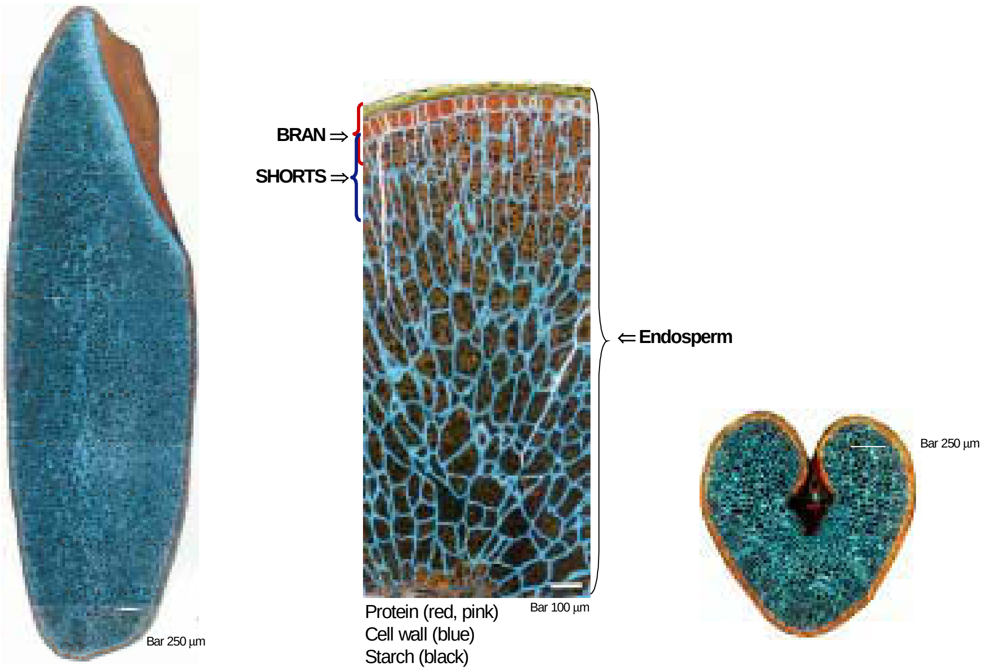

Figure 3. The rye grain (left), the main milling fractions of rye grain (middle), and the cross-section of rye grain (right).

Whole grain fibre is, in general, considered a primary factor contributing to the health effects. In addition to dietary fibre, rye grain is a source of many bioactive compounds that have potential health benefits. It has been shown that the bioactive compounds of sterols, folates, tocopherols and -trienols, alkyl-resorcinols, lignans, phenolic acids and total phenolics are concentrated in the bran layers of the grain, and are present only at low levels in the endosperm. The levels of easily extractable phenolic compounds tend to increase in germination and sourdough baking (Liukkonen *et al.* 2003).

In general, phenolic compounds are known to influence the flavour of oat (I, Molteberg *et al.* 1996b). In addition, it is possible that alkylresorcinols may contribute to the flavour perception. In the present study the relative amounts of total phenolic compounds were distributed among the different layers of the rye grain in close accordance with the perceived bitterness and flavour intensity of the milling fractions of rye (III, Liukkonen *et al.* 2003). In the bran fraction, a 6-fold amount of phenolic compounds was detected when compared to the endospermic flour, and a 3-fold amount when compared to the shorts fraction. Alkylresorcinols, alkenylresorcinols, lignans and phenolic acids form a substantial amount of the total phenolic compounds.

3.3 Effect of sourdough fermentation and baking on rye flavour (IV & V)

In the current study, sourdough fermentation resulted in the extrudates having a sour and intense flavour and aftertaste (Table 3 in IV, Table 3 in thesis), with the texture of the sourdough-fermented extrudates being porous. The effect of rye cultivar on the sensory profiles was unsubstantial; the sourdough-fermented extrudates of the two rye cultivars, Amilo and Akusti, produced similar profiles (Table 3 in IV). Even after extrusion, the flavour of the sourdough-fermented rye still varied to a great extent from those of germinated and native rye (Fig. 1 in IV). Thus, the sourdough fermentation process was completed by a heat treatment, either by extrusion cooking or by baking as reported below.

Rye-like, sour flavour was also perceived to be important in soft bread and crispbread by consumers (Table 3 in thesis). The salt content had slightly more of an effect on the perceived sourness of rye bread at high (1.8%) than low (0.7%) ash content (Fig. 3 in V). The different ash contents of rye flour were achieved by mixing rye endosperm and rye bran flour according to the experimental design. The smaller the ash content of rye flour was, the more it contained rye endosperm. The perceived rye-like flavour was significantly influenced by the ash content of rye flour ($r = 0.64$) and by the bread acidity ($r =$

0.77) ($p < 0.05$). These results are well in accordance with the earlier observations (Hellemann *et al.* 1987, 1988).

In addition to sourness, saltiness is strongly associated with rye bread, although the sodium chloride content was not critical for the flavour of sour rye bread in our study. Both the salt content and the bread acidity influenced the perceived saltiness of sourdough-fermented rye bread, whereas the effect of the ash content on it was insignificant. A similar intensity of perceived saltiness was reached in several combinations of acidity and salt content. However, sourness and saltiness have been shown to compensate for each other (V, Hellemann 1992, Hellemann *et al.* 1988, Rothe & Ruttloff 1983).

The flavour of plain rye flour, even after extrusion, is rather mild (IV). Although rye has more intensive flavour than oats, the strong rye-like flavour was developed in processing. In general, sourdough fermentation is known to enhance the rye-like flavour (Hansen 1995, Hellemann *et al.* 1987, 1988, Rothe & Ruttloff 1983). In baking the sourdough-fermented dough into bread, an even broader spectrum of new flavour components appears, mainly as a result of the Maillard reaction. Thus, the flavour components of the breadcrumbs and breadcrust are different (Schieberle 1996, Schieberle & Grosch 1994). In the present study (V), the flavour of the breadcrumbs and crust was not studied separately.

3.4 Effect of germination and heat treatment on oat and rye flavour (I, II & IV)

Germination of rye seemed to be rather unique, since no reported studies on the flavour formation of germinated rye were available. A new, short germination procedure was applied to both oat and rye (Wilhelmson *et al.* 2001), using only two days instead of the usual eight days.

This study showed that germination and the subsequent drying of oat produced sensory profiles that varied greatly, depending on the processing parameters such as the drying speed and the temperature profile (Table 2 and Figs. 2 and 3 in I, Table 3 in thesis). Germination alone did not produce the desired sensory attributes for oat; most of the positive attributes, however, were formed when heating the germinated oat. During germination, the native oat became moist, musty and earthy in flavour, and soft, less tough, crisper and more brittle in texture (Fig. 1 in I). The heat treatment of germinated oat formed the desired flavour attributes, such as an intense roasted, nutty, sweet flavour, while the moist, musty and earthy flavour disappeared. The roasted, sweet and intense flavour developed in germinated, dried oat, even though the occurrence of the Maillard reaction was vague. The texture of germinated, dried oat obtained attractive characteristics by significantly changing to a hard, crisp and brittle direction, and the moistness and toughness disappeared when compared to the non-heated oat (Fig. 1 in I). Commonly, high temperatures exceeding 85ºC and quick drying after germination were necessary for the formation of the favourable sensory attributes described above. The roasted, sweet flavour that developed in drying the fresh germinated oat stayed for several months in stored oat (Table 1 and Fig. 1 in II).

In general, these results for the flavour of germinated, heat-treated oats parallel the previously reported observations on high-temperature, low-moisture extruded oats that were described as toasted or roasted in flavour (Pfannhauser 1993, Parker *et al.* 2000). Except for the toasted flavour, a crisp textural perception similar to that in germinated, dried oat has been reported to be important in extruded oat (Liu *et al.* 2000). Textural changes probably originate from the germination phase of malting, when most of the β-glucan degrades, resulting in lower viscosity (Peterson 1998). In addition to the Maillard reaction, caramelisation may also occur in high-temperature extrusion, when heating sugars in the presence of acids and salts, which produces somewhat similar flavour characteristics to those that result from the Maillard reaction (Reifsteck & Jeon 2000). Heat treatment of oat flours has also been reported to reduce the intensity of some negatively perceived attributes, bitterness and astringency, in particular, and increase the intensity of the oat-like flavour (Molteberg *et al.* 1996a).

For rye, the sensory perception (and volatile compounds) of germinated, sourdough fermented and native rye were substantially different, and they stayed so even after the second treatment, i.e., the extrusion process (Fig. 1 in IV). The first treatment process (native, germination or sourdough fermentation) seemed to be the most dominant in the formation of the flavour of the rye extrudates.

The rye cultivar had an unsubstantial effect on the sensory profiles (Table 3 in IV).

In this study, the flavour of germinated, extruded rye was described most frequently as fresh and cereal-like, but had no other significant flavour attributes when compared to the other rye extrudates prepared from native or sourdough fermented rye (Table 3 in IV). This result of rye deviates from the study with germinated, dried oat (I, II). Either the extrusion performed after the germination may suppress the roasted, sweet flavour originating from germination and subsequent heat treatment observed in oat, or the roasted, sweet flavour is masked by the strong, bitter, rye-like flavour in the germination of rye. In addition, the total germination time applied to rye was shorter than that for oat. However, the texture of germinated, extruded rye was evaluated as being hard and crisp, which is consistent with the results on germinated, dried oat.

3.5 Stability of oat flavour (II)

According to our study, the stability of the flavour was significantly increased through germination and subsequent heat treatment, and the chemical changes causing rancidity and bitterness developed more slowly in the processed, crushed oat when compared to the corresponding native oat during a 12-month storage period (Fig. 2 in II, Table 3 in thesis). In native oat the deterioration had already occurred after 1 month of storage and was perceived as bitterness, whereas in germinated, dried oat the changes were perceived considerably later (Table 1 in II). In general, oat that had been stored for a long time and had deteriorated was perceived as being musty and earthy in odour, and bitter and rancid in flavour.

In several applications, the use of oat is limited due to its tendency to become rancid and to form a bitter off-flavour during processing and storage. This is a consequence of the high lipid content of oats.

The development of rancidity is commonly negatively related to heating or antioxidative activity. Germination increases the amount of phenolic compounds and the antioxidation activity of oat (Fig. 5 in I). This might explain the better stability of germinated oat when compared to native oat. One reason for the activation of the lipolytic enzymes, which are responsible for the formation of rancid and bitter off-notes in flavour, could be the mechanical crushing of the oat grains. Thus, storage of whole grains would presumably give unequal results for the flavour stability when compared to crushed oat. The chemical rancidity of oat is well documented, whereas the references on rancid flavour were not available. The impact of chemical components on the deteriorated oat flavour is discussed more precisely in Chapter 3.7.

The formation of rancidity is always irreversible, and once the rancid flavour has settled in, it is not a removable perception, and the product will be spoiled and unfit for human consumption.

3.6 Identity and consumer acceptance of rye bread (V)

The results described in Chapters 3.1–3.5 are based on a sensory profiling of oat and rye processed in several ways, performed by trained laboratory panels. However, to learn which sensory attributes consumers combine with the identity and acceptance of a typical rye bread, a consumer study was conducted by using a consumer panel.

In our study, rye-like flavour and sourness showed high intensities in sourdough-fermented rye bread, as concluded earlier in Chapter 3.3. A clear relationship was observed between the perceived sourness and the acidity of the bread, and between the perceived ryeness and the ash content of rye flour, respectively. In addition, the perceived ryeness and bread acidity were closely related. However, the small difference between sour and rye-like perceptions might be difficult to evaluate for consumers. Somewhat surprising was that the perceived saltiness and NaCl content of the bread were not related. The perceived identity of soft rye bread was dominated by its acidity, and of crispbread by the ash content of the flour (Fig. 4 in V).

The acceptance of soft bread and crispbread was mostly enhanced by the ash content of rye flour, and weakened by interactions of the wheat to rye ratio and ash content, as well as the bread acidity and NaCl content (Fig. 5 in V). Among the perceived sensory attributes, ryelike flavour was the most important.

Acceptance ratings of sour soft rye bread and crispbread were rather equal to each other.

The sensory quality of a typical rye bread results from several factors, and especially from their interactions. In conclusion, the most important factors affecting the identity and overall acceptance of rye bread were acidity and NaCl content, the effect being dependent on the wheat to rye flour ratio in the bread (V).

Relationships between the identity and acceptance of rye bread have not been discussed, although the major sensory attributes describing rye bread have been studied in some extent (Hellemann *et al.* 1987). Acceptance is not bound to a certain image of rye bread; the sample may be acceptable without being a typical rye bread. The identity, or 'typicalness', reflects the cultural concept of what the product should be, whether the consumer likes it or not.

In addition to quality expectations before purchasing the product, the quality experience after the purchase influences consumer food choice (Grunert 2002). Consumers today seek variety, expect nutritional benefits, and are neophilic in their attitude toward foods, which requires responding to the demands of these changing eating habits and food preferences (Stone & Sidel 1995). The extent, to which consumer expectations of a product are matched, depends on its sensory properties (Cardello 1994).

The methodological approach in this research was new while studying the influence of four recipe variables simultaneously instead of only one or two variables, as in previous studies (Rothe & Ruttloff 1983, Hansen & Hansen 1994). Valuable information on the interactions between the process variables was gained in this somewhat more complex design. The RSM technique, which is often used in process optimisation, appeared to be a useful tool in the search for the optimal matching of product quality with consumer expectation.

The importance of sourness on the flavour of sour rye bread has been also documented in other studies (Hansen *et al.*, 1989, Hellemann 1992, Hellemann *et al.* 1987, 1988). Reduced sodium chloride levels have been found to be acceptable in sour rye breads (Tuorila-Ollikainen *et al.* 1986), particularly at high acidity levels (Hellemann 1992). A high salt content of rye bread did not seem to be necessary for consumer acceptance, and consumers might accept a less salty rye bread, in which the saltiness could be compensated for by sourness (V). This same conclusion has also been obtained in earlier studies (Barylko-Pikielna *et al.* 1990, Hellemann 1991).

Consumers tend to evaluate products in their totality rather than seeing them as consisting of several, separate attributes, which often makes it very difficult for consumers to distinguish the sensory properties as attributes of a food product clearly from each other, whereas it is very obvious for a trained sensory panel (Lawless 1995). For the trained panel, however, it is difficult or even impossible to predict consumer preferences, even though it has become possible by using a technique called preference mapping, which combines sensory and consumer perceptions (McEwan 1996, Martínez *et al.* 2002). The difference in the perception of sourness, saltiness and the rye-like flavour of rye bread might have been rather challenging for consumers. In addition, the subjects in consumer studies may tend to try and please the interviewer by giving replies that they think the organiser of the experiment is expecting, which may lead to somewhat false results.

3.7 Relation between perceived flavour and chemical components (I, II & IV)

In addition to sensory evaluation, the flavour of a product may be studied through the flavour-active components (Table 4 in thesis), such as volatile substances, phenolic compounds and lipid constituents, influencing it.

Sample handling is of the utmost importance, both in sensory evaluation and in the chemical analysis of the flavour compounds (Stevenson *et al.* 1996). Contrary to other studies, the sensory and instrumental determinations were executed as purely and simply as possible, directly from the samples. The conditions of the instrumental headspace analysis were mimicking the circumstances used in the sensory assessment as closely as possible, and thus the instrumental headspace determinations were executed directly from the samples. The volatile compounds were analysed after salting out from the samples, but without using solvent extraction, as performed elsewhere (Schieberle & Grosch 1985, 1994, Zhou *et al.* 1999). The remote sample treatment made the comparison of the results obtained from different analysis techniques more successful.

Table 4. Summary of the main flavour descriptors and volatile compounds of the samples obtained as a result of different processing techniques in the studies (Publications I and IV).

Publ.	Grain and its treatment	Flavour description	Volatile compound
I	Germinated-dried oat	Roasted, nutty, sweet, intense flavour & aftertaste	Dimethyl sulphide, hexanal, pentanal, isobutanal
IV	Rye flour extrudate	Mild flavour	2-Ethyl furan, 2-methyl furan, hexanal, pentanal
	Germinated rye extrudate	Fresh, cereal-like flavour	Dimethyl sulphide, 2-methyl-butanal
	Sourdough fermented rye extrudate	Sour, intense flavour, intense aftertaste	Furfural, ethyl acetate, 3-methyl-butanol, 2-methyl butanol

Germinated, dried oat. In this study, the total amount of volatile compounds was higher in native than in germinated, dried oat (I). During germination, and particularly during the drying process, the profile of volatile compounds changed. In general, the most abundant compounds responsible for flavour were dimethyl sulphide, hexanal, pentanal and isobutanal (Table 4 in thesis). The relative amount of DMS increased as a function of temperature in drying (Table 3 in I), whereas hexanal, pentanal and isobutanal in addition to several other small ketones, alcohols and esters disappeared during heating.

The roasted, sweet and nutty flavour of germinated oat dried at high temperatures (≥ 85°C) was clearly related to dimethyl sulphides and isobutanol in the PLS regression analysis of sensory and instrumental profiles of selected volatile compounds in our study (Fig. 6 in I). The moist and earthy flavour of freeze-dried oat was associated with cymene, limonene and isobutanal. Phenolic compounds significantly influenced the oat flavour, whereas lipids had a negligible effect (Table 4 in I).

In other studies, the important volatile compounds identified in native oat have been shown to include 3-methylbutanal, 2, 4-decadienal and benzaldehyde, while the flavour was described as being similar to raw oat, weed-hay and grass (Heydanek and McGorrin 1986). The flavour of cooked oat has been reported to become more oat-like, nutty, browned and burnt in flavour. The nutty flavour of toasted oat originates mainly from carbonyl compounds and amines (Heydanek and McGorrin 1986).

The composition of volatile compounds obtained in different studies is dependent on the heat treatment; for example, heterocyclic Maillard reaction products such as pyrazines, pyrroles and furans, which mainly affect the roasted flavour, have been identified in other studies on the heat processing of oat (Pfannhauser 1993, Parker *et al.* 2000). As a consequence of relatively low temperatures and high moisture levels in our studies, these compounds were not formed through the Maillard reaction, which occurs at high temperatures and low humidity levels.

In the present study, the concentration of phenolic compounds had a tendency to increase slightly with the drying temperature. This is well in accordance with the study reported earlier (Molteberg *et al.* 1996b). Avenanthramides, which are important for oat flavour, have been shown to correlate positively with the fresh flavour of oat (Molteberg *et al.* 1996b).

Stability of germinated, dried oat. Our study proved that the development of the bitter and rancid flavour was closely related to the accumulation of FFA and volatile compounds related to lipid oxidation (Table 9 in II). The lipolytic enzyme activity causing rancidity was lower in the processed oat groats than in the native oat. During the storage period an increase in stability was clearly obtained, since the formation of the perceived rancid and bitter flavour, degradation of lipids, and production of volatile oxidation products were slower in the processed oat than in the native oat. The volatile compounds related to protein degradation or the total amount of phenolic compounds showed positive correlations only with the desired sensory attributes, such as roasted odour and flavour (Table 9 in II).

In the present study, rancidity, bitterness and musty perceptions were related to certain volatile compounds related to lipid degradation: hexanal, pentanal, n-butylfuran and pentylfuran (Fig. 2 in II). This finding is well in accordance with an earlier study, where the major volatile compounds in stored oat flours were hexanal and 2-pentyl furan (Molteberg *et al.* 1996a). According to Molteberg, both the sensory and chemical stability of oat flour during storage were greatly improved by heat treatment (soaking in water, steaming and drying): the levels of volatiles and the sensory attributes used to describe rancidity, such as grass, hay and paint, remained rather low for up to 5 weeks of storage. The heat treatment used in the present study maintained the original sensory profile of the processed oat groats effectively for a storage period of at least 3 months, whereas significant deterioration of the native oat groats was observed within 1 month (Table 1 in II).

The improved sensory and chemical stability of processed oat groats could be attributed to the reduction of FFA formation at the start of storage and to the reduced formation of volatile lipid oxidation products during the whole storage period. Compared to the heat treatment normally used to inactivate lipase, the process where oat is germinated and dried appears tempting as it improves the oxidative stability, whereas the heat treatment used for lipase inactivation may promote oxidative deterioration (Ekstrand *et al.* 1993). However, a more intensive lipase inactivation would be needed to ensure the storage stability of processed oat. Otherwise the FFA generated by the residual lipase activity will not only produce unpleasant lipid degradation products, but may also inhibit the formation of the more pleasant flavours derived from the Maillard reaction (Parker *et al.* 2000).

Pre-treated rye extrudates. In this study, the flavour-active volatile compounds of native, germinated and sourdough fermented rye extrudates were greatly varied and depended mainly on the pre-treatment used (Table 4 in IV, and Table 4). In the extrudates prepared from rye flour that had a mild flavour, 2-ethylfuran, 2-methylfuran, hexanal and pentanal were the dominating volatile compounds (Fig. 1 in IV). From these compounds, hexanal and pentanal are documented to be salient in rye flour (Hansen 1995). The cereal and fresh flavour of germinated, extruded rye was related to DMS and 2-methylbutanal (Fig. 1 in IV). DMS was also identified as resulting from the germination and drying of oat (Fig. 6 in I). The sour, intense flavour and aftertaste of sourdough fermented, extruded rye was related to furfural, ethyl acetate, 3-methylbutanol and 2-methylbutanol in the experimental conditions that were used (Fig. 1 in IV). These volatile compounds may also result from sourdough fermentation without extrusion cooking.

The results generated from the PLS regression (Fig. 1 in IV) and GC/olfactometry (Table 6 in IV) were essentially parallel. The compounds with very low odour threshold values, e.g., methional, 1-octen-3-ol, benzeneacetaldehyde, hexanal and 2-heptenal, were emphasised in the GC/olfactometry results, which was not as obvious in the PLS regression. The GC/olfactometry results were quantitative only in some extent. Some volatile compounds were succeeded be to related to sensory descriptions. For example, the odour of hexanal was described as being green, fresh or flowery by the panel, methional like a jacket of a baked potato, 1-octen-3-ol mushroom-like and benzeneacetaldehyde flowery, honey-like or bitter almond resembling (Table 6 in IV).

4. Conclusions and future outlook

The flavour formation of oat and rye, processed by germination, sourdough fermentation, extrusion or milling fractionation techniques was studied. Process-induced changes in the sensory profiles of the products were commonly evaluated by using descriptive profiling by trained panels. The impact of flavour-active volatile compounds was related to the perceived flavour by statistical multivariate analysis. In addition, the role of lipids and phenolic compounds influencing the cereal flavour was briefly described.

The flavour of native oat and rye is rather mild, and typical product taste is mainly formed in processing. The flavour components were unevenly distributed in rye grain, the endosperm being mild and the outer bran layers very bitter and intense in flavour. For further applications, the shorts fraction in the middle of the rye grain showed that it was very interesting, by being cereal-like but not obviously bitter in flavour and by simultaneously having high bioactivity. The importance of the grain fractions on the flavour was still obvious when the fractionated rye flour was used in baking bread; sensory profiles for both rye flour fractions and bread baked from these fractions were similar.

Sourdough baking and germination were observed to increase the amounts of phenolic compounds, most of which are known to be beneficial to one's health. In addition, phenolic compounds considerably influenced the perceived flavour.

Sourdough fermentation of rye resulted in a sour and intense flavour and aftertaste. The sensory perception and volatile compounds of native, germinated and sourdough-fermented rye were substantially deviant, and they stayed different even after extrusion. Thus, the biotechnical pre-treatment of the rye grain was more dominant than the second processing step (extrusion cooking). Very probably these bioprocesses were effective in producing flavour precursors, which during the heat treatment then intensified the flavour. By only using extrusion, the flavour of rye was considerably plainer than by using germination and sourdough fermentation as a pre-treatment.

Important factors affecting the identity and overall acceptance of rye bread were evaluated by a consumer panel as being the acidity and the salt content, even though the effect depended on the wheat to rye flour ratio used in the bread. The salt content of sour rye bread was, however, not critical to the flavour, since sourness and saltiness of sourdough-fermented rye bread compensated each other. The determination of the various simultaneous interactions between the process parameters was a new approach, and is a useful tool for the design of bread quality criteria.

A new, short germination procedure was applied to oat and rye. Germination alone did not produce the intense roasted, nutty, sweet flavour and crisp, brittle texture to oat, but these desirable attributes were formed while finishing the germination by a quick heat treatment exceeding 85ºC. The roasted, nutty, sweet flavour characteristics were related to volatile compounds, such as dimethyl sulphide and isobutanol in this study. Roasted flavour developed to germinated, heat-treated oat, although the occurrence of the Maillard reaction, generally necessitating high processing temperatures, was not apparent. Related to its high fat content, oat becomes easily rancid. However, germination and subsequent heat treatment considerably extended the shelf-life and postponed the perceived rancidity and bitterness of crushed oat. The development of these undesired sensory attributes was closely related to the accumulation of FFA and volatile compounds related to lipid oxidation. Studies on the flavour formation of germinated rye are exceptional.

In the current study, the perceived flavour was related to its volatile constituents. This is not a very commonly used approach. The statistical data treatment that was chosen proved to be successful in linking the results of these two techniques. In general, the volatile compounds behind the flavour formation of bread and other cereal products are very well documented, and the flavour is often explained only through volatile substances. At the same time, literature on the perceived flavour of cereal foods is very sparse, and the combination of sensory flavour with the instrumental flavour of processed grains and cereal products can hardly be found. Thus, the relationships between the perceived flavour and the chemical constituents established gave useful information for developing novel cereal products.

In conclusion, the use of whole grains, such as oat and rye, which are beneficial to one's health, could be substantially extended if their flavour properties and flavour stability were better known, and they could be modified in a controlled way. According to this thesis, the rather mild flavour of native grain was significantly evoked by applying different processing techniques, such as milling fractionation, sourdough fermentation and baking, germination and subsequent heat treatment, and extrusion cooking. The understanding underlying the perceived flavour formation of cereal materials was considerably improved. The flavour attributes of oat and rye grain may be tailored by knowing what processing technique to use for attaining the desired flavour, e.g., roasted or sour. Thus, the study showed that completely new possibilities for varying the flavour of cereal could be found for producing novel whole grain products.

Product development should recognise the consumer needs, and, in fact, call for new types of whole grain cereal foods among health-conscious consumers does exist (Liu *et al.* 2003). Dietary fibre and bioactive compounds provide several health benefits. Milling fractionation of rye grain proved to be a promising technique for producing nutritionally valuable whole grain products, which, in addition, have a desired flavour; the inner (endosperm or shorts) layers of the rye grain could be used to considerably add the fibre content of novel, tasty whole grain products. For achieving products that are a rich source of bioactive phytochemicals, nutritionally of good quality, preservable and particularly palatable, germination and sourdough baking provide excellent possibilities for producing novel cereal products by the breakfast and snack industry. Especially by germination of rye completely new applications of cereal foods could be found.

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Author(s)
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Title

Influence of processing on the flavour formation of oat and rye

Abstract

The use of whole grain foods, such as oat and rye, beneficial to one's health, could be substantially extended if their flavour properties and flavour stability were better known, and they could be modified in a controlled way. The aim of this study was to specify how the flavour of oat and rye is formed, and which processing factors influence it. The understanding underlying the perceived flavour formation of grain was considerably improved through this thesis.

This study showed that the relatively mild flavour of native grains was considerably adjusted by applying different processing techniques, such as milling fractionation, sourdough fermentation and baking, germination and subsequent heat treatment as well as extrusion cooking. The flavour components were unevenly distributed in the rye grain with the innermost endosperm being the mildest, and the outer bran layers being the most bitter and intense in flavour. The shorts fraction in the middle of the rye grain that had high bioactivity proved to be most interesting in further applications of new products by having a cereal-like flavour without any obvious bitterness. The importance of the grain fractions on the flavour was still obvious when the fractionated rye flour was used in baking bread.

Sourdough baking and germination were used to increase the amounts of phenolic compounds, most of which are beneficial to one's health. In addition, the phenolic compounds considerably influenced the perceived flavour. The sourdough fermentation of rye resulted in sour and intense flavour notes. Heat treatments, such as baking or extrusion cooking of sourdough-fermented rye, further modified the flavour without losing the sourness. The identity and overall acceptance of sour rye bread, when evaluated by consumers, were affected by the acidity and the salt content, even though the effect actually depended on the wheat to rye flour ratio used in the bread. The determination of the various simultaneous interactions between the process parameters was a novel approach.

A new, short germination procedure was applied to oat and rye. The germinated oat dried at high temperatures was perceived to have flavour characteristics of roasted, sweet and nutty, and in this study these sensory characteristics were clearly related to particular volatile compounds, such as dimethyl sulphide and isobutanol. An important discovery was that the roasted flavour in oat was gained without any apparent Maillard reaction. Germination and drying extended the shelf-life of crushed oat in comparison with native oat, and the development of the undesired sensory attributes, such as bitterness and rancidity, were closely related to the accumulation of free fatty acids and volatile compounds originating from lipid oxidation. Studies on the flavour formation of germinated rye are exceptional.

The statistical data treatment used to relate the perceived flavour of processed oat and rye to the flavour-active volatile substances proved to be successful in linking the results of these techniques, and useful information for developing new cereal products was obtained.

In conclusion, the flavour attributes of grain were highly varied. To tailor these attributes according to consumer expectations, appropriate processing techniques need to be chosen to attain the desired flavour characteristics, e.g. roasted or sour. New tools for developing novel palatable whole grain foods with high bioactivity, such as breakfast and snack productss, were introduced in this thesis.

Keywords
oat, rye, processing, germination, sourdough fermentation, sensory profiling, flavour, volatile compounds, multivariate analysis

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ISBN			Project number
951–38–6042–6 (soft back ed.) 951–38–6043–4 (URL:http://www.vtt.fi/inf/pdf/)			
Date May 2003	Language English	Pages 72 p. + app. 48 p.	Price C

Name of project	Commissioned by
Future Foods, Tailored Technologies for Future Foods	Ministry of Agriculture and Forestry (MMM), National Technology Agency (Tekes), Finnish cereal companies
Series title and ISSN	**Sold by**
VTT Publications 1235–0621 (soft back ed.) 1455–0849 (URL: http://www.vtt.fi/inf/pdf/)	VTT Information Service P.O.Box 2000, FIN–02044 VTT, Finland Tel. +358 9 456 4404, Fax +358 9 456 4374

The use of whole grain foods, such as oat and rye, beneficial to one's health, could be substantially extended if their flavour properties and flavour stability were better known, and they could be modified in a controlled way. The understanding underlying the perceived flavour formation of grain was considerably improved through this thesis.

This study showed that the relatively mild flavour of native grains was considerably adjusted by applying different processing techniques, such as milling fractionation, sourdough fermentation and baking, germination and subsequent heat treatment as well as extrusion cooking. The product development should be consumer-originated, and to tailor the sensory attributes according to consumer expectations, appropriate processing techniques need to be chosen to attain the desired flavour characteristics, e.g. roasted or sour. New tools for developing novel palatable whole grain foods with high bioactivity, such as breakfast and snack products, were introduced in this thesis.

Tätä julkaisua myy	Denna publikation säljs av	This publication is available from
VTT TIETOPALVELU PL 2000 02044 VTT Puh. (09) 456 4404 Faksi (09) 456 4374	VTT INFORMATIONSTJÄNST PB 2000 02044 VTT Tel. (09) 456 4404 Fax (09) 456 4374	VTT INFORMATION SERVICE P.O.Box 2000 FIN–02044 VTT, Finland Phone internat. +358 9 456 4404 Fax +358 9 456 4374

ISBN 951–38–6042–6 (soft back ed.)
ISSN 1235–0621 (soft back ed.)

ISBN 951–38–6043–4 (URL: http://www.vtt.fi/inf/pdf/)
ISSN 1455–0849 (URL: http://www.vtt.fi/inf/pdf/)

VTT PUBLICATIONS 494 Influence of processing on the flavour formation of oat and rye

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