Helsinki University of Technology
Department of Chemical Technology
Espoo 2003
Applied Biochemistry and Microbiology Report I/2003

REACTIVITY OF LIPIDS DURING CEREAL PROCESSING

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Dissertation for the degree of Doctor of Science of Technology to be presented with due permission of the Department of Chemical Technology for public examination and debate in Auditorium KE 2 (Komppa Auditorium) at Helsinki University of Technology (Espoo, Finland) on the 12th of June, 2003, at 12 noon.

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ISBN 951-22-6476-5
ISSN 1236-2697
Otamedia Oy
Espoo 2003

Lehtinen, Pekka. Reactivity of lipids during cereal processing. Espoo 2003, Helsinki University of Technology
Keywords: *Avena Sativa*, oat, oxidative rancidity, hydrolytic rancidity, lipase, lipoxygenase, shelf-life

A B S T R A C T

The study elucidates factors affecting the reactivity of lipids in multiphase food systems, such as processed cereal food products. By using oat and oat products as model materials, both enzymatic and non-enzymatic lipid reactions were studied in aqueous suspensions and in dry flour. The information obtained can be used to improve existing cereal processing schemes and to develop new processing technologies for obtaining high quality food products with enhanced shelf stability.

In aqueous cereal processes the most important factors affecting the rate of enzymatic lipid reactions were identified to be the availability of lipid substrate to lipolytic enzymes and the total lipolytic enzyme activities present in the system. The ratio between the lipid substrate and flour material was critical in determining the reaction kinetics. When the ratio of lipid to flour was low, the reaction kinetics was set by the availability of substrate whereas, in the opposite case, the reaction kinetics was controlled by lipolytic activities. Different cereals processes represent examples of these two cases. In different cereal materials, the change from substrate limited to an enzyme limited kinetics occurred at different lipid to flour ratios. A simple oat fractionation scheme was identified where the substrate limited kinetics persisted also at high lipid concentrations. Such a fraction is well suited as a processing aid for aqueous processes in which the reactivity of lipids needs to be controlled.

In water activities that are encountered during storage of dry flour, two reactions of lipids were identified in processed oat: enzymatic hydrolysis of acylglycerols, and non-enzymatic oxidation of unsaturated fatty acid moieties acylated to polar lipids. The general practice to prevent enzymatic reactions by hydrothermal inactivation was noticed to actually provoke non-enzymatic lipid oxidation. This, in turn, leads to the development of oxidative rancidity upon prolonged storage of oat products. Thus, in order to prevent the unwanted reactions of lipids upon storage at low water activities, the careful control of hydrothermal treatment is crucial and should meet two requirements: elimination of the endogenous lipolytic activity, and prevention of the subsequent lipid oxidation.

P R E F A C E

The present work was carried out in the Laboratory of Biochemistry and Microbiology at Helsinki University of Technology.

I am grateful to Professor Simo Laakso for his encouragement and help during the work. Considered, that the work presented in this thesis is based on the research carried out during several years, the work has been influenced by numerous people to whom I express sincere gratitude. Especially, I would like to mention M.Sc., MBA Ilkka Lehtomäki, Docent Pekka Reinikainen and Ph.D. Markku Mikola for providing the view from the cereal processing industry. Also I would like to express my warmest thanks to my colleague Dr Anu Kaukovirta-Norja for her valuable knowledge and enthusiasm. I am very grateful to Professor Katrina Nordström for suggesting various improvements into the thesis and original papers.

I thank M.Sc. Katja Kiiliäinen, M.Sc. Satu Laakso and M.Sc. Sanna Jaakola for sharing the devotion to cereal lipids and Ms Ulla Åhman for the contribution in practical experiments. Also I would like to thank co-authors in publication IV, especially Dr Raija-Liisa Heiniö.

The financial support of the Eemil Aaltonen and Antti Wihuri foundations, Applied Bio Science - graduate school, and Research Foundation of Helsinki University of Technology is gratefully acknowledged.

I am most grateful to my wife Marja-Liisa for her never-ending love and support and to relatives and friends for their encouragement.

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II **Lehtinen, P.** and Laakso S. Effect of extraction conditions on the recovery and potency of antioxidants in oat fibre, Journal of Agricultural and Food Science, **1998**, 46, 4842-4845.

III **Lehtinen, P.** and Laakso, S. Inhibition of linoleic acid oxidation by fatty acid binding oat protein fraction, Journal of Agricultural and Food Science, **2000**, 48(11), 5654-5657.

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

V **Lehtinen, P.**, Kiiliäinen, K., Lehtomäki I. and Laakso, S. Effect of heat treatment on lipid stability in processed oat, accepted in Journal of Cereal Science, **2003,** 37(2), 215-221.

The author has been responsible for planning the studies, except for publication IV, which the author planned together with Dr Heiniö and for publication V, which the author planned together with M.Sc. (Techn.) Kiiliäinen. Excluding the sensory- and multivariate-analysis in publication IV, the author was responsible for interpretation of data in all publications. In publication IV, the introduction and results sections were written together by the author and Dr Heiniö.

C O N T E N T S

1 INTRODUCTION

Lipids are involved in all biological structures, and together with proteins and carbohydrates, lipids constitute the majority of dry matter in biological systems. In the seeds of plants, as in all living cells, lipids serve many crucial biochemical functions. Lipids provide an extremely simple construction of compartments which maintain their contents unmixed within the aqueous interior of cells. For many higher organisms lipids also serve as a highly concentrated storage form of energy, and approximately 37 kJ/g is obtained upon oxidation of storage lipids compared to that of 15 kJ/g obtained from oxidation of carbohydrates. In addition to these two major functions, lipids also serve as messengers helping cells to recognize and communicate with each other.

Unlike proteins or carbohydrates, the meaning of the word lipid is somewhat obscure and is conventionally based on the biosynthetic or functional relationship between substances, rather than the actual chemical structure. Accordingly, lipids are by nature hydrophobic, e.g. they are poorly soluble in water, but are readily soluble in apolar solvents. From the chemical point of view, the presence of a hydrophobic carbon chain containing a carboxylic group is a characteristic feature of lipids (figure 1).

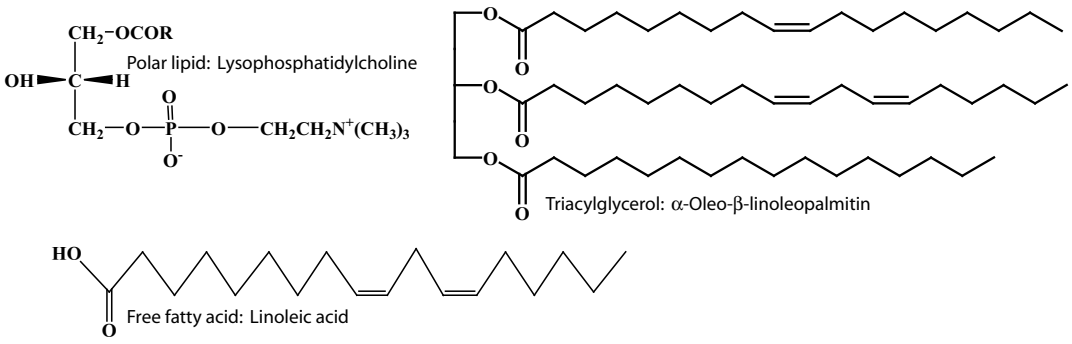

Figure 1. Common lipid structures found in cereals. In the structure of lysophosphatidylcholine R represents fatty acid moiety.

Even though many cereals, including oats, are palatable as harvested, they are usually processed to consumer products with increased nutritional, technological or commercial value. However, such processing will inevitably induce changes in the natural organization of seed lipids. Many of these changes render lipids more susceptible towards deteriorative reactions. On the other hand, processing techniques based on novel information on lipid reactions can also be used to enhance the stability of cereal lipids. This can be achieved not only by inactivating the deteriorative enzymes, but also by introducing processing steps by which the favorable molecular organization and phase distribution of lipids are enhanced.

2 REACTIONS OF CEREAL LIPIDS

Reactions of cereal lipids during processing can be divided into reactions which are catalyzed by the enzymes present in cereals, and reactions which occur without the involvement of enzymes. Most of the enzymatic reactions documented in the scientific literature are related to hydrolytic or oxidative pathways or to non-oxidative isomerization of carbon-carbon double bonds structures. Non-enzymatic reactions are limited to the reactions related to oxidative pathways and isomerizations, as the non-enzymatic lipid hydrolysis is exceedingly slow at ambient pH and temperature values typically encountered during cereal processing. However, for certain lipid compounds intermolecular acyl migration within glycerol structures has been reported to occur without enzyme activity also at ambient conditions (Plueckthun and Dennis, 1982).

2.1 ENZYMATIC REACTIONS

2.1.1 HYDROLYTIC PATHWAY

Most of the fatty acids found in plant seeds are esterified to a specific alcohol molecule, glycerol. The trans-esterification reaction in which the acyl group is transferred from the glycerol to water is generally referred to as lipid hydrolysis (figure 2). This, as well as the reverse reaction, the synthesis of acylglycerols from glycerol and free fatty acids, is catalyzed by lipase enzyme (EC 3.1.1.3).

The water activity has an utmost important role in determining lipase activity. Water affects both the enzyme activation and the thermodynamical equilibrium of the reaction. Different lipases have different water activity values at which activation is established and the literature on microbial lipases suggests that these values are well below a water activity of 0.3 (Wehtje and Adlercreutz, 1997). The water amount required is much smaller than for most other enzymes, corresponding roughly to a mono or multiple adsorption layer of water surrounding the enzyme (Caro *et al.*, 2002). As water is also a reactant in the lipase reaction, it also affects

on the equilibrium of the reaction. The reaction equilibrium has been reported to change from the synthesis to the hydrolysis of esters in the water activity range of *ca.* 0.2 to 0.3 (Svensson *et al.*, 1994). However, the equilibrium is also a function of other substrates involved, namely free fatty acids, glycerol and different acylglycerols. Thus the reaction equilibrium in a cereal matrix can not be deduced solely based on the water content. It is, however, evident that in most situations encountered upon cereal processing, where water activities lie well above 0.4, the lipid hydrolysis is a thermodynamical downhill. Consequently, the hydrolysis of esterified lipids in enzyme active cereal products can easily proceed to an extent that is perceived as sensory flaw. In water activities above 0.8 the amount of free water becomes remarkable, and the lipase catalyzed hydrolysis can either increase or decrease as a function of water activity, depending on the substrate concentration, distribution of substrates between aqueous and oil phases and probably also on the source of the lipase (Adlercreutz *et al.*, 2002; Ma *et al.*, 2002).

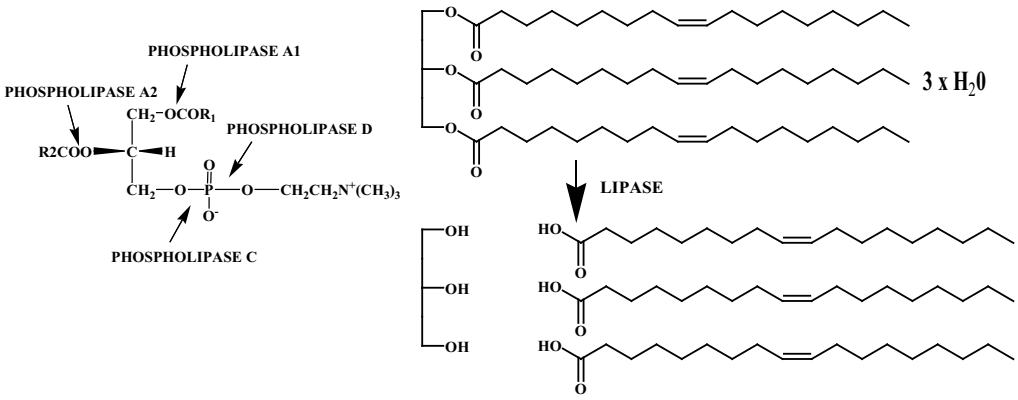

Figure 2. Hydrolysis of polar membrane lipids and neutral storage lipids.

Different enzymes are responsible for the hydrolysis of neutral triacylglycerols and polar phospho- and glycophospholipids. Cereal seeds contain apparently only 1 or up to 3 different isoenzymes of lipases acting on storage lipids (Baxter, 1984; O'Connor *et al.*, 1989; Peterson, 1999; Edlin *et al.*, 2002). On the other hand, the hydrolysis of phospholipids in plant membranes is a far more controlled process involving the induction of plant defense, and multiple isoenzymes of each phospholipase classified in figure 2 (Wang, 2001). During various processes, cereal

lipids may also be exposed to microbial lipase and the role of microbial enzymes in cereal lipid reactions is a controversial subject (O'Connor *et al.*, 1992).

The synthesis of cereal hydrolytic enzymes is induced by hormone signals from embryo tissue, leading eventually into protein synthesis or proenzyme activation in the aleuronic and apparently partly also in the endosperm tissues (Tavern *et al.*, 1969; Laidman and Tavern, 1971; Gallie and Young, 1994). Mature oat grains have a remarkable lipase activity even if the germination is not started (O'Connor *et al.*, 1992). During fractionating of oat, the lipase activity has been found to be present both in the aleuronic rich bran fraction as well as in the endosperm fractions obtained from the inner parts of grains (Ekstrand *et al.*, 1992; Hutchinson *et al.*, 1951). The presence of high lipase activity in the endosperm fraction of non-germinated oat is puzzling. Assuming that the presence of the activity is related to incipient germination, the presence of induction, synthesis and transport mechanisms would be expected also for other hydrolytic activities. In non-germinated oat these activities are, however, not detected consistently with lipase activity. Also the fact that neither lipid hydrolysis nor an increase in lipase activity is observed during early germination, suggests that the lipase activity present in mature oat grain is not related to the germination process (Peterson, 1999; Outinen, 1999). More likely, the activity represents either a residual activity originating from the lipid synthesis upon the seed development or is related to some other biological function such as defense systems (Urquhart *et al.*, 1983).

Many microbial lipases discriminate between the different acyl groups and have different affinities for fatty acids acylated to different OH-groups of glycerol. For wheat and oat lipases the provided data is somewhat conflicting. When endogenous lipolysis in oat products is followed, no such specificity has been reported (O'Connor *et al.*, 1992; Heiniö *et al.*, 2002). In these cases the slight difference in the proportion of fatty acids moieties in triacylglycerol and free fatty acid pools is likely a sign of further oxidative reactions of unsaturated free fatty acids rather than of lipase specificity towards unsaturated fatty acids (Warwick *et al.*, 1979).

Furthermore, the lipid hydrolysis in oat proceeds apparently without any accumulation of di- or monoacylglycerols. Rather, once the triacylglycerols are accessible to lipase, all three acyl groups are subsequently rapidly converted to free fatty acids (Liukkonen *et al.*, 1993). However, when the hydrolysis of supplied 1,2,3-trihexanoylglycerol by oat lipase was studied, a strong positional specificity of hydrolysis was observed (Yasuhide *et al.*, 1997).

Even though the hydrolysis of neutral storage lipids in oat is faster than in other cereals, the hydrolysis of polar lipids during oat processing and storage is minimal and detailed information on the hydrolysis of oat polar lipids is sparse (Liukkonen *et al.*, 1992). However, in barley and especially in barley malt, the hydrolysis of polar lipids occurs swiftly once the seed is milled and the water content is increased. In such a case phospolispases show notable specificity for different acyl groups in such a manner that unsaturated fatty acids are most easily hydrolyzed (Kaukovirta *et al.*, 1998).

2.1.2 OXIDATIVE PATHWAY

Fatty acids in cereal lipids contain 0-3 double bonds. The presence of these double bonds adds extra reactivity to the lipid compounds, and enoic groups can undergo different isomerization or oxidization reactions (figure 3). Lipoxygenase (EC 1.13.11.12) is an abundant enzyme in plants and catalyzes the non-reversible oxidation of *cis,cis*-1,4–pentadiene moieties in acyl groups to a respective acyl hydroperoxide. The lipoxygenase reaction rate in different cereals varies greatly and barley and wheat have high lipoxygenase activity, whereas in rye and oat the reaction is slow (Fretzdorff *et al.*, 1986; Lehtinen *et al.*, 2000). Cereals contain multiple isoenzymes with lipoxygenase activity and cDNA sequences of two lipoxygenases from germinating barley have been determined (Hugues *et al.*, 1994, Van Mechelen *et al.*, 1999; Shiiba *et al.*, 1991). These isoenzymes have apparently different substrate specifity, different distribution in various tissues and produce different hydroperoxide isomers in different proportions, but otherwise the

biological role of these isoenzymes is unknown (Feussner and Wasternack, 1998; Schmitt and Van Mechelen, 1997).

Enzymatic oxidation of double bonds in acyl chains consists of series of reactions. In many plant tissues the hydroperoxides formed by the lipoxygenase reaction are further cleaved by hydroperoxide lyase (EC 4.1.2.-), an enzyme which has been extensively studied in cucumber, tomato and beans (Noordermeer *et al.*, 2001; Matsui *et al.*, 2000; Suurmeijer *et al.*, 2000). The presence of hydroperoxide lyase in cereal grains has not been published in the scientific literature, but the presence of typical reaction products of this enzyme suggests that it is abundant also in cereals (Sides *et al.*, 2001; Sjövall *et al.*, 2000; Parker *et al.*, 2000). In oat, a lipoperoxidase (EC 1.11.1.-) activity is responsible for the conversion of hydroperoxides to relevant hydroxyacids (Biermann and Grosch, 1979). These hydroxyacids are suggested to be partially responsible for the bitter taste associated with the enzymatically active oat (Biermann *et al.*, 1980).

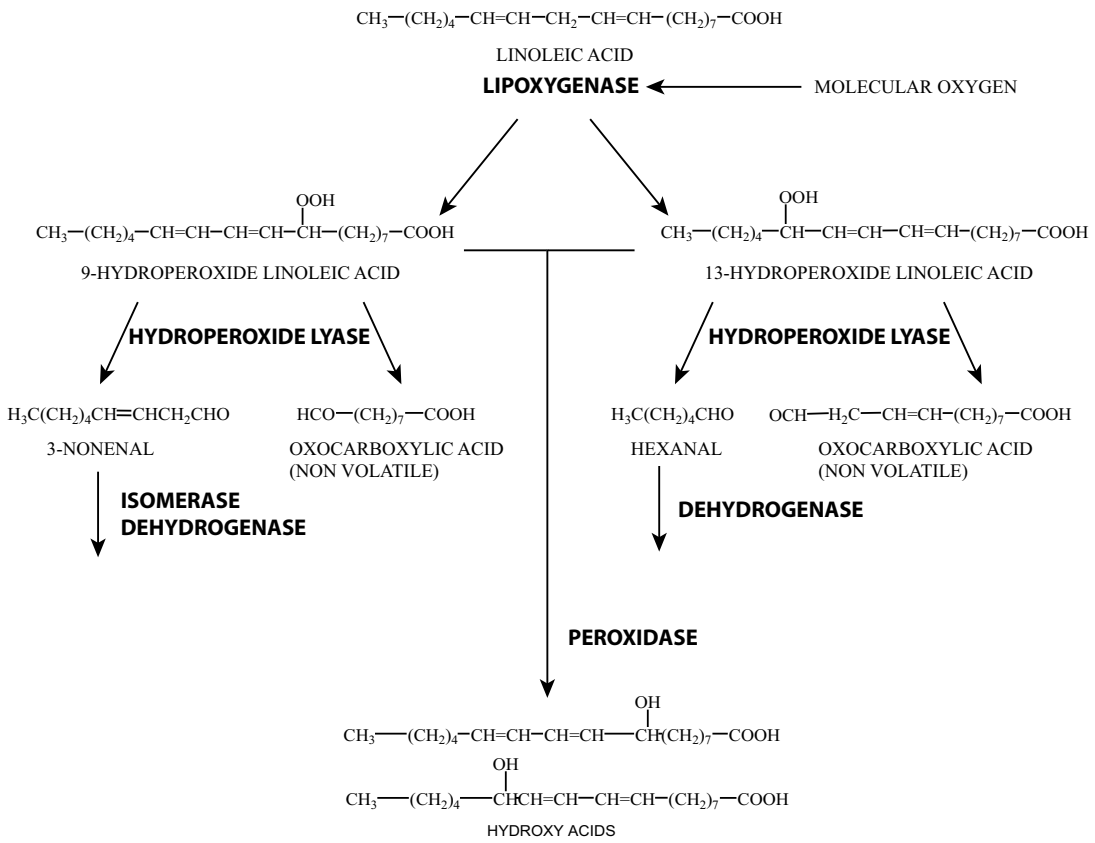

Figure 3. Simplified scheme of the reactions in the enzymatic oxidation of linoleic acid in cereals (modified after Noordermeer *et al.*, 2001 and Biermann *et al.*, 1980).

As a consequence of these reactions, wide spectrums of products are formed. In many cereals linoleic acid is the most abundant substrate for the lipoxygenase reaction. The lipoxygenase reaction produces mainly two different isomers of hydroperoxide linoleic acid, namely 9- and 13- hydroperoxide linoleic acids. The cleavage of 9-hydropreoxide linoleic acid further, yields mainly 8-10 carbon monoenoic compounds, whereas the cleavage of 13 hydroperoxide linoleic acid yields 5-7 carbon compounds. Upon cleavage both isomers yield also the oxo-carboxylic acid compound with 8 to 12 carbons (Olias *et al.,* 1990; Galliard and Matthew, 1977).

2.2 NON-ENZYMATIC REACTIONS

2.2.1 OXIDATION AND ISOMERIZATION OF ENOIC STRUCTURES

Whereas the enzymatic oxidation of enoic structures uses molecular, triplet state oxygen as a primary substrate, the non-enzymatic oxidation occurs only after pre-

formation of reactive acyl or oxygen species. Thus non-enzymatic oxidation is initiated by factors such as radicals, metal-ions and photons with an energy level capable of triggering endogenous photosensitive molecules. However, once the reaction is initiated, the reaction itself can provide the radicals that will cause the reaction to continue. The presence of endogenous antioxidants in cereals has a marked effect on the onset of non-enzymatic oxidation due to their capability to quench these reactive molecule species into non-reactive form.

Many products of the non-enzymatic oxidation are the same as in the enzymatic oxidation (figure 4). However, the fatty acid hydroperoxides may accumulate if neither hydroperoxide lyase nor lipoperoxidase are present. In this case, the rate of hydroperoxide decomposition is set by the molecular environment of fatty acid hydroperoxides, *i.e.* the presence of antioxidants and metal-ions or other radical forming compounds, phase distribution and presence of amino acids and sugars (Nishiike *et al.*, 1999; McClements and Decker, 2000; Mäkinen *et al.*, 2000). Certain antioxidants slow the decomposition of hydroperoxides, which can reduce the overall rate of lipid oxidation, as the decomposition of hydroperoxides will provide less radicals for the initial H^* abstraction from the fatty acid. However, the role of antioxidants is not straightforward, as in some cases antioxidants can actually increase the decomposition of hydroperoxides, possibly by reducing metal ions into more active form (Mäkinen *et al.*, 2001). Lipid may also form polymeric compounds upon oxidation. These are formed mainly in the highly unsaturated oils such as fish or linseed oils, or in oils that have been excessively heated such as frying oils (Shukala *et al.*, 1991; Neff *et al.*, 1988).

The lipid oxidation products may also undergo different isomerization reactions. The occurrence of trans-2-nonenal (T2N) is associated with the cardboard flavor in beer (Jamieson *et al.*, 1970; Noeel *et al.*, 1999). The formation of the T2N has been suggested to involve an isomerase reaction in which the 3-nonenal is isomerized to T2N (Galliard *et al.*, 1976). However, neither 2- or 3-nonenal products have been reported in rancid oat or wheat germ or, if present, are detected at very low

abundance (Sjövall *et al.*, 2000; Heiniö *et al.*, 2001). Instead, 2-pentyl furan is detected in stored cereals and may represent the major product from the cleavage of 9-hydroperoxide linoleic acid. The conversion of straight chain structure of nonenal into cyclic furan structure can be initiated by the singlet oxygen as shown by Min and Boff (2002), and it seems plausible, that the oxidative stress in cereal matrix is responsible for the instability of 3-nonenal. Interestingly, studies in which purified 9-hydroperoxide linoleic acid has been used as a substrate, the 3-nonenal formed via the hydroperoxide lyase reaction, appears to be stable at least for analytical purposes (Gargouri *et al.*, 1998).

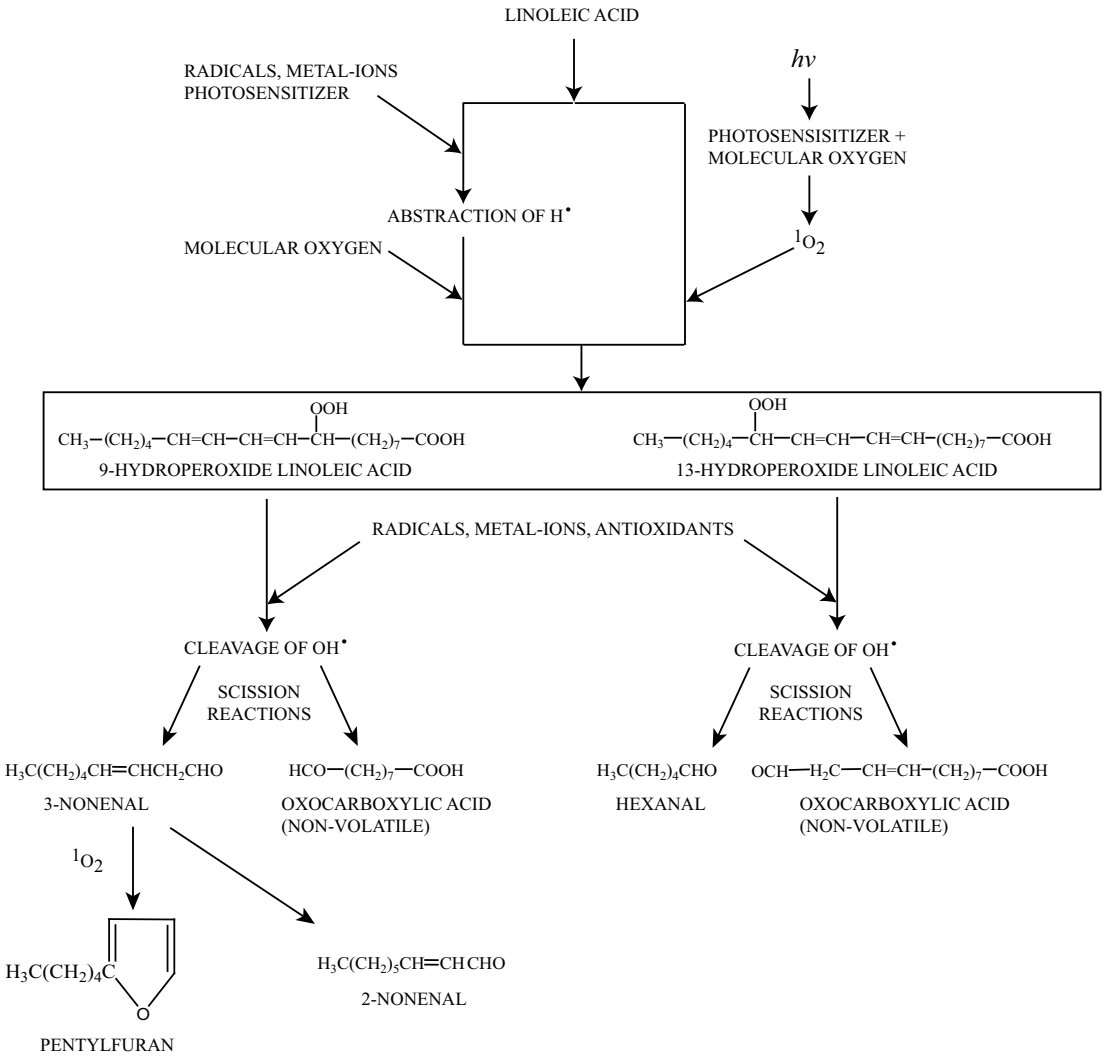

Figure 4. Simplified scheme of the reactions in the non-enzymatic oxidation of linoleic acid in cereals (modified after Min and Boff, 2002). In addition to 9-and 13-hydroperoxides also 10- and 12-hydroperoxides are formed upon photo-oxidation of linoleic acid.

The enoic structures found in unsaturated fatty acids do not undergo spontaneous isomerization at the ambient processing conditions met during cereal processing. However, in the presence of catalyst such as transition metal ions or at temperatures above 200°C, both cis/trans and positional isomerization can occur in unsaturated fatty acids (Kemeny *et al.*, 2001; Wolff, 1993). During thermal processing of cereals temperatures are usually well below 200°C and for example upon extrusion, the formation of trans fatty acids is relatively low, 1 - 1.5 % of total unsaturated fatty acids (Maga, 1978).

2.3 RELEVANCE OF LIPID REACTIONS TO FOOD QUALITY

The most significant result of lipid reactions is their effect on sensory and rheological properties of cereal products (Jacobsen, 1999; Cumbee, 1997). A loss of nutritive value (Andersen *et al.*, 1986) and even cytotoxicity (Esterbauer, 1993) has also been associated with extensive oxidation of unsaturated fatty acids (figure 5). Formation of rancid flavor due to lipid oxidation is a relatively well known phenomenon, whereas relevance of lipid hydrolysis, disruption of cellular structures and lipid interaction with other flour components is less evident.

Analytically the extent of lipid oxidation in food materials is characterised by using parameters such as the amount of remaining intact unsaturated fatty acids, presence of fatty acids hydroperoxides and presence of secondary oxidation products. These parameters are valuable tools for studying the lipid oxidation and for developing products with increased storage stability. However, the relevance of these parameters in explaining the sensory properties is limited, as the sensory impact of different food products differs widely (Jacobsen, 1999). Hexanal is the most abundant and easily detectable secondary oxidation product and thus often used as a marker of lipid oxidation (Frankel *et al.*, 1989; Fritsch and Gale, 1977). Still, it is clear, that neither hexanal nor any other volatile lipid oxidation product is solely responsible for the perceived rancidity. Rather, flavors such as paint- or cardboard-like odor associated with the rancidity are apparently caused by a combination of different volatile carbonyl compounds (Zhou *et al.*, 1999; Heydanek and McGorrin, 1981). These compounds are reported also in rancid oat products in which their concentration in headspace of cereal sample increases approximately 2 to 100 fold during storage (Heiniö *et al.*, 2002; Sjövall *et al.*, 2000).

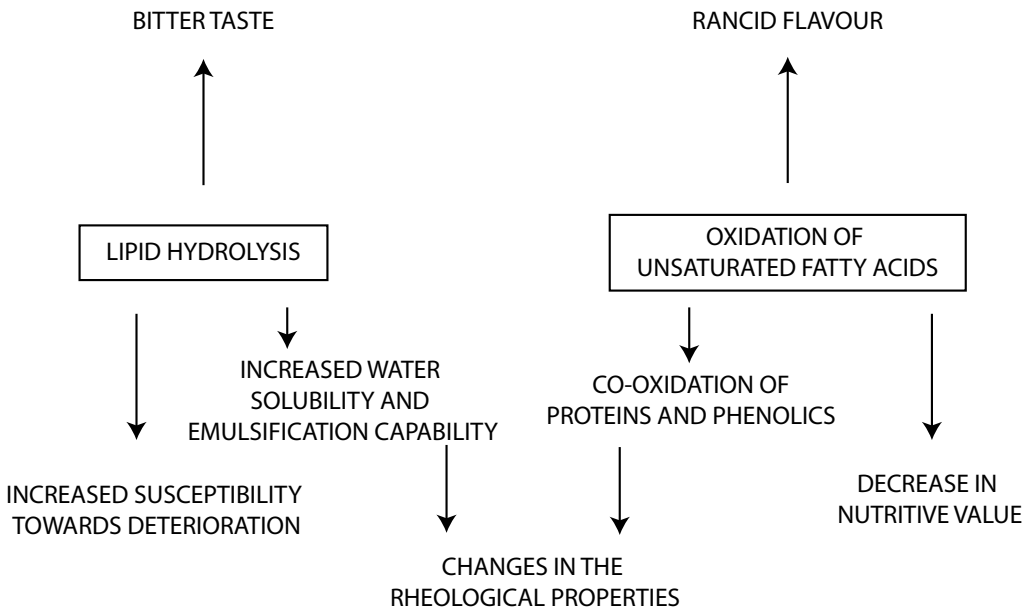

Figure 5. Lipid reactions causing sensory and rheological changes in cereals.

The consequences of the hydrolysis of triacylglycerols to free fatty acids and partially esterified glycerols are reflected both on changes in technical properties and on changes in perceived sensory properties. The prior dogma has been that the hydrolysis of acylglycerols renders the unesterified fatty acids moieties susceptible towards oxidation and thus increases the risk for rancidity.

The free fatty acids in cereals formed via hydrolysis have such long carbon chains that they are virtually non-volatile. The bitter taste of free unsaturated fatty acids, mainly linoleic and linolenic acids, has been observed in the aqueous emulsions at concentrations above 0.7 and 0.1 mg/g respectively (Stephan and Steinhart, 2000). In dry oat material, in which extensive lipid hydrolysis has occurred, the concentrations of these acids is *ca.* 10 and 0.5 mg/g respectively (Sippola, 2002). However, the bitter taste in such oat is suggested to be due to the presence of long chain hydroxyacids rather than to free fatty acids (Biermann *et al.*, 1980). Biermann (1980) also postulated that the bitter taste associated with non-heat treated oat products results from the formation of a hydroxygroup in the carbon chain of monoglyceridelinoleate. However, the hydrolysis of neutral storage lipids is well characterised in the scientific literature, and it appears that the hydrolysis proceeds rapidly to glycerol and free fatty acids. The accumulation of partially

hydrolyzed triglycerols, such as monoglyceridelinoleate has not been observed (Liukkonen *et al.*, 1992). One possibility is that the hydroxy fatty acid is formed while the fatty acid is still acylated to glycerol and that the formed hydroxyacylglycerol is discriminated by oat lipase and thus accumulates into the monoglyceride pool.

3 PROCESSING INDUCED INTERACTIONS OF LIPIDS WITH OTHER CEREAL COMPONENTS

Many seed components are enveloped by membrane structures consisting of mono- or bilayer of amphiphatic phospholipids. Neutral storage lipids, on the other hand, are concentrated as 0.2-2.5 μm oil bodies consisting of a continuous lipid phase of triacylglycerols covered by a protein rich membrane of polar lipids (Huang, 1994; Huang, 1992). In addition to membranes and oil bodies, lipids in the intact seed are also found as inclusion complexes with amylose (Morrison *et al.*, 1993). Upon processing, these organized microstructures are destroyed and lipids are attached to new molecular organizations based on the interactions between lipids and other flour components. Water content is an important factor in affecting the lipid mobility taking place in grain or in flour (figure 6). Inside the whole mature grain the water activities range between 0.4 and 0.8 even if the seeds are kept soaked in excess water. Milling and other dry processing is generally carried out at these ambient water activities, corresponding to a moisture content of 6-15 %. On the other hand, during dough mixing, wet-fractionation processes and wort preparation, the water activities can approach the value of 1.0 and in these processes the flour components are distributed between aqueous and particulate phases.

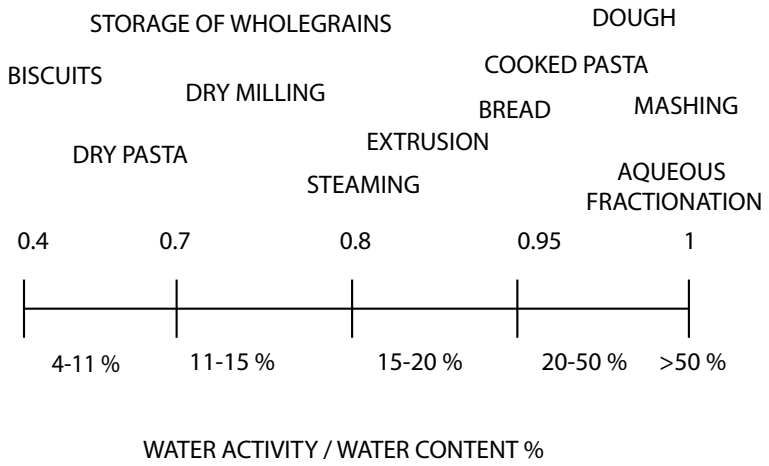

Figure 6. Water activities and moisture content of typical cereal products.

3.1 INTERACTIONS INDUCED BY DRY PROCESSING

Processing induced interactions between lipids and other flour components are enforced by mechanical and thermal energy. Cereal lipids themselves are liquid over the ambient temperature range, and thus no phase changes are expected to occur in the continuous lipid phase during processing. The viscosity of lipids does, however, change dramatically in the temperature range encountered during cereal processing (Abramovic and Klofutar, 1998). The low viscosity in the elevated temperatures increases the mobility of lipids and thus increases the chances of disintegration of native molecular organization. Concurrently, the disruption of grain structure upon milling brings the lipolytic enzymes and lipids into close contact. Especially non-inactivated oat is known to form a bitter taste due to enzymatic deterioration induced by milling. In the typical milling processes, subcellular components such as starch granules and oil-bodies are likely to remain intact. However, heating, introduction of extensive mechanical energy, enzymatic treatments and various aqueous treatments can cause the individual components to lose their integrity and as such lead to changes in molecular mobility (Lehtinen, 1994).

3.2 INTERACTIONS IN EXCESS WATER

3.2.1 SELF-ORGANIZATION OF LIPIDS IN WATER

The solubility of neutral lipids, such as triacylglycerols, in water is sparse. Nevertheless, if flour is mixed in water, a certain amount of lipids is moved into the aqueous phase, and for example after mashing the aqueous wort contains few percents of lipids originally present in malt (Anness and Reed, 1985). Only a small proportion of such lipids in aqueous phase are found as the monomolecular, soluble, lipids. Instead, the majority of lipids are present in differently organized molecular structures such as fat globules, micelles or premicellar aggregates or attached to water-soluble molecules such as proteins and polysaccharides. Technically, the aqueous phase always contains some amount of small particles, and it seems likely that an aliquot of the lipids present in the aqueous phase are actually attached to the surface of cell wall fragments, starch granules and protein bodies. In an aqueous suspension these fragments, granules and oil bodies can provide lipids with a less hydrophilic environment than water does.

From a chemical point of view, only lipids that are found as monomolecular in water should be referred as soluble. The critical micelle concentration (CMC) is the highest possible concentration in which the lipids are present in monomolecular form. If the concentration of lipid increases above the CMC, the surplus of lipid is found either in the micellar form or as a separate lipid phase. In systems such as water-flour suspensions, containing lipids with a different degree of solubility in water, polar lipids tend to form mixed micelles containing more than one lipid species. In such instances the CMC cannot be explicitly assigned. However, the CMC value for palmitoyl-lysolesitin, a partially hydrolyzed polar lipid component abundant in cereal starches, is *ca.* 3 mg/L (Stafford *et al.*, 1989). During aqueous processing of cereals the content of this lipid species can be considerably higher and is therefore predominantly present in micellar form.

The water solubility for very hydrophobic compounds such as neutral lipids is very low. Such lipids do not spontaneously form micelles, but are present as fat globules. In the intact cereal matrix, most of the storage lipids are present as oil bodies similar to these globules. By ferocious mixing, the globules can be introduced into the aqueous phase. However, even within the aqueous phase the lipids remain as small droplets of continuous oil phase, and the system approaches an oil in water emulsion.

3.2.2 LIPIDS ASSOCIATED WITH WATER SOLUBLE MOLECULES

Water-soluble molecules such as proteins and polysaccharides contain hydrophobic cavities, which are able to accommodate hydrophobic lipids. As a result, these molecules can act as host molecules for lipids in aqueous phase and provide an apparent increase in the solubility of lipids.

The binding of lipids into protein structures results mainly from weak interactions, and is thus often reversible (Alzagtat and Alli, 2002). Due to their poor solubility in water, the lipids in the aqueous biological systems are transported by specific lipid transfer proteins, of which serum albumin is most comprehensively characterised (Peters, 1985). In plants various lipid transfer proteins have also been identified. The biological role of these proteins has been assigned to involve the regulation of fatty acid pools and membrane biogenesis (Yamada, 1992). On the other hand, the discovery of a secretor signal peptide associated with these proteins suggests that they also possess a role in defense mechanisms (Kader *et al.*, 1996; Blein *et al.*, 2002). It is yet to be determined, to which extent the lipid binding observed during cereal processing is non-specific binding by hydrophobic protein regions and to which extent specific binding by lipid binding proteins with an established biological role (Zawistowska *et al.*, 1986).

Water soluble carbohydrates carry extensive amounts of polar hydroxy-groups and the capability of simple sugars to enhance the solubility of lipids is sparse. However, the polysaccharides with helix-like structure may possess a hydrophobic

interior, which can accommodate lipids. A most comprehensively studied polysaccharide – lipid interaction is the amylose-lipid complex (Morrison *et al.*, 1993). Unlike the protein-lipid complexes, the amylose-lipid complex is not reversible at room temperature and heating in aqueous alcohol is needed to break the complex (Morrison, 1989). The small oligosaccharides obtained from starch hydrolysis can be modified into closed ring structures, i.e. cyclodextrins, which can act as a host molecule for hydrophopic compounds. Unlike the non-dissociable amylose lipid-complex, the exchange of the hydrophopic guest compounds in cyclodextrins occurs easily even at room temperature (Bru *et al.*, 1997).

4 EFFECT OF PROCESSING ON LIPID REACTIVITY

The processing of cereal grain into consumer products induces disintegration of native molecular organization of cereal components, and the formation of new interactions. These in turn effect on the molecular mobility of lipid compounds, lipolytic enzymes and on the susceptibility of lipid components to pro- and antioxidants. Molecular mobility of lipids, water, enzymes and other catalysts increases as a function of temperature and the degree of dis-integration of biological structure. A contrary process occurs when mechanical energy is brought to the protein network. In this case the formation of inter- and intramolecular cross-linking decreases the extractability of lipids (Chung and Chen, 1975; Laignelet and Dumas, 1984).

The lipase catalyzed hydrolysis of acylglycerols starts swiftly once the cereal grain, with endogenous lipase activity, is milled. The cause for this onset of hydrolysis is probably due to many factors. In order to catalyze the lipid hydrolysis, lipases need the presence of an oil water surface. Thus the overall reaction rate can be expected to be a function of the effective surface area and the diffusion rate of reactants and lipase into the surface. The diffusion of lipids within the oil bodies is fast and can not be expected to limit hydrolysis rate. On the other hand, intact oil bodies can be relatively resistant towards lipase action, as is demonstrated by the stability of non-homogenized milk (Tarrasuk and Frankel, 1955). The onset of lipid hydrolysis upon milling, as well as during milk homogenization, may partly be explained by the dis-integration of oil bodies or by the partial denaturation of surface of oil bodies. Milling may also increase the mobility of lipase and enable the diffusion of the lipase into the effective oil-water interface. Furthermore, the localization of water itself can be altered by milling in such a manner that the oil-water surface is established.

Whereas lipase acts on the oil-water interface, lipoxygenase prefers the monomolecular form of free fatty acids in aqueous phase (Brash, 1999). Thus, the

rate of oxidation of unsaturated fatty acids by lipoxygenase reaction is expected to increase as a result of lipid hydrolysis. This has been observed in aqueous processing of barley (Kaukovirta *et al.*, 1993). Similarly, any processing that reduces the amount of monomolecular linoleic acid can be expected to reduce the enzymatic oxidation rate. This occurs, for example, during mixing of flour water suspension or dough. During such mixing, linoleic acid does not change chemically, but the amount of monomolecular form is reduced due to lipid binding, and the rate of lipoxygenase reaction is consequently reduced (Graveland, 1970; Lehtinen *et al.*, 2000). Linoleic acid has a pKa value above 7, and is thus found as non-dissociated in most food products. Then the low water solubility of non-dissociated linoleic acid may hinder it's availability towards lipoxygenase (Kanicky and Shah, 2002; Glickman and Klinman, 1995; Brash 1999). The flour matrix consists of many hydrophobic phases with greater affinity for linoleic acid than that of the aqueous phase. As will be pointed out in present thesis, these hydrophobic phases may retain linoleic acid, whether formed by hydrolysis of acylglycerols or as supplemented exogenous acid, unavailable towards lipoxygenase.

5 AIMS OF THE PRESENT THESIS

The study focuses on the reactions of lipids in processed multiphase food materials and identifies factors that are critical for such reactions. In traditional food processing practice the control of lipid reactions relies largely on empirical experiences and dogmatic principles, rather than on profound understanding of the underlying mechanisms. However, in today's global food markets, the industry faces strict challenges in the development of new processes and applications, for which the prior experience is unsatisfactory or totally unavailable. Therefore, a series of studies were conducted to expand knowledge of the phenomena that influence the reactivity of lipids under processing conditions and thereby affect the overall quality of the products. The experiments were mainly carried out using oat and its fractions as models since this material has an inherent, well known property of developing lipid-associated quality problems, is rich in lipid modifying enzymes but, on the other hand, is gaining increasing interest as part of a healthy diet.

The specific targets of the study were 1) to elucidate the relative significance of enzyme-catalyzed and non-enzymatic lipid reactions in cereal processing, 2) to reveal the significance of limited substrate availability for the rates of lipid reactions, and 3) to use the above information to create new strategies to control of processes against adverse lipid reactions.

6 MATERIALS AND METHODS

The general overview of the experimental approach of the study is given below. Detailed experimental design and analytical methods are described in the original publications I-V.

6.1 ENZYMATIC OXIDATION IN EXCESS WATER (PUBLICATIONS I-III)

The studies focused on the susceptibility of supplied unsaturated fatty acid towards enzymatic oxidation in aqueous suspensions of different cereal materials. The system consisted of 1) flour sample to be studied suspended in aqueous buffer, 2) supplied micellar unesterified linoleic acid, and 3) supplied purified soybean lipoxygenase. In such a system lipoxygenase oxidizes linoleic acid by using molecular oxygen dissolved in the buffer solution. The rate of this reaction was followed by monitoring the oxygen level of the solution. The possible inhibition of linoleic acid oxidation caused by the flour sample could then be evaluated by comparing the deoxygenating rates of such a system. If the studied flour inhibited the oxidation reaction, *i.e.* if it possessed any antioxidative effect, the observed deoxygenation rate was consequently slower as compared to the identical system in the absence of flour. To enable the quantification of the antioxidative effect, the concept of an inhibitory unit was applied. Accordingly, the amount of flour sample capable of reducing the deoxygenation rate by 50 % in the system studied was defined to contain one inhibitory unit. This inhibitory unit was employed in a manner similar to enzyme activities during enzyme purification procedures.

Oat fractions with high capability to inhibit the oxidation, *i.e.* with high inhibitory unit content per g dry matter, were purified by aqueous processing techniques and these fractions were chemically characterised. In addition, the mechanism of inhibition was characterised by studying the kinetics of inhibition. This was achieved by varying the concentrations of components, the order in which the components were mixed, and the contact time between the components. The

oxidation reaction is initiated only after both linoleic acid and lipoxygenase are present. Thus, the contact time between the flour sample and lipoxygenase as well as the contact time between the flour sample and supplied linoleic acid could be varied prior to starting the oxidation.

6.2 PROCESSING AND STORAGE RELATED CHANGES IN LIPIDS (PUBLICATIONS IV-V)

These studies draw together the different processing techniques of oat with respect to the lipid reactions occurring during the processing and subsequent storage. This data was, in turn, interrelated to the development of volatile compounds and to sensory properties of these cereal materials[1]. The processing schemes included crunching, germination and subsequent drying, steaming, fractionation into bran and endosperm enriched fractions and extrusion. Some of the processes were such that they partly or totally inactivated the enzymatic activities, whereas in others all enzyme activities were retained in the product.

The changes in lipid composition occurring during processing and subsequent storage of these oat products were followed by analyzing the formation of volatile oxidation products of unsaturated fatty acids, formation of free fatty acids due to lipid hydrolysis and changes in fatty acid composition of each lipid class: free fatty acids, triacylglycerols, diacylglycerols and polar lipids. The observed changes were then compared with the processing history of the material.

[1] author has no credit for the sensory analysis presented in original publication IV, and this part of the study is not included as a part of this academic thesis.

7 RESULTS AND DISCUSSION

7.1 ENZYMATIC OXIDATION OF UNSATURATED FATTY ACIDS IN AQUEOUS SUSPENSION OF CEREALS (PUBLICATIONS I-III)

In complex mixtures of biological material, the rate of enzymatic reactions depends on the total enzyme activity present in the system and on the amount of substrates available for the enzyme. In the pure aqueous systems with water soluble substrates, the kinetics is rarely limited by diffusion. Then the amount of substrates available for the enzyme is equal to the total concentration of substrates. However, for aqueous systems containing free fatty acids, the water soluble pool represents only a relatively small proportion of total concentration of the fatty acids. Furthermore, in the flour-water suspension, the distribution of free fatty acids between soluble and non-soluble phases is further complicated by the presence of different soluble and particulate matter. In the study, the enzymatic oxidation reaction of linoleic acid was followed in water flour suspension. In these suspensions, the relative significances of the activity of oxidizing enzyme and the availability of substrate on the oxidation rate were determined. This was achieved by following the rate of deoxygenation in aqueous suspensions of cereals supplemented with varying amounts of exogenous unesterified linoleic acid to mimic lipase action and exogenous oxidative enzyme, lipoxygenase.

It was shown that when linoleic acid is mixed with flour water suspension it is distributed in different pools, with different capability to serve as a substrate for lipoxygenase reaction. From the literature it is evident, that of the different forms of linoleic acid only the monomolecular, water soluble pool is oxidized by lipoxygenase (Prima-Hartley *et al.,* 2000; Schilstra *et al.*, 1994; Galpin and Allen, 1977; Lagocki *et al.*, 1976). The monomolecular pool is in rapid equilibrium with the micellar form and together they form the total apparent reactive pool. In water flour suspension the amount of linoleic acid in this reactive, rate determining, pool is much less than the total amount of applied linoleic acid (figure 7).

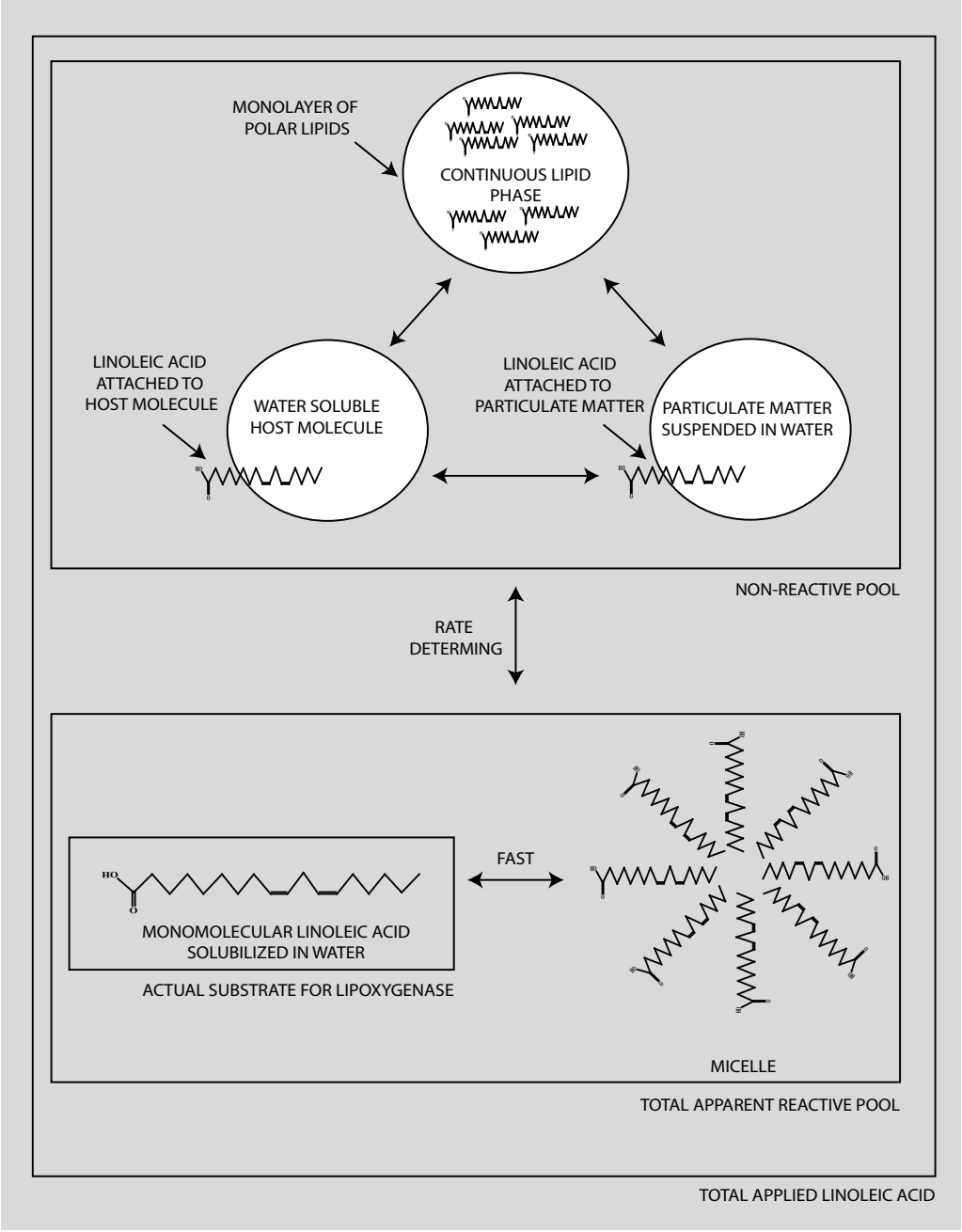

Figure 7. Distribution of applied linoleic acid in aqueous suspension of cereal flour into reactive and non-reactive pools. The micellar phase contains also various surfactant molecules, such as lysophospholipids. For simplicity these have been omitted from the figure.

The distribution of applied linoleic acid between the reactive and non-reactive pools was set by the ratio of linoleic acid to flour (figure 8). When the amount of linoleic acid was less than approximately 0.5-1 % (w/w) of flour, the reactive substrate concentration was negligible and regardless of the enzyme amount, the oxidation reaction was slow. In somewhat higher substrate concentrations, the amount of reactive substrate was still lower than what was observed under similar

conditions, but in the absence of the flour material. If the substrate concentration was further increased so that the amount of linoleic acid was approximately 5-10 % of flour or higher, the amount of reactive linoleic acid was practically the same as in the absence of flour. In flour water suspension, the latter situation is seldom achieved without the addition of exogenous lipids. Thus, in most cases when flour is mixed in water, the substrate availability is expected to restrict the enzymatic oxidation rate of linoleic acid.

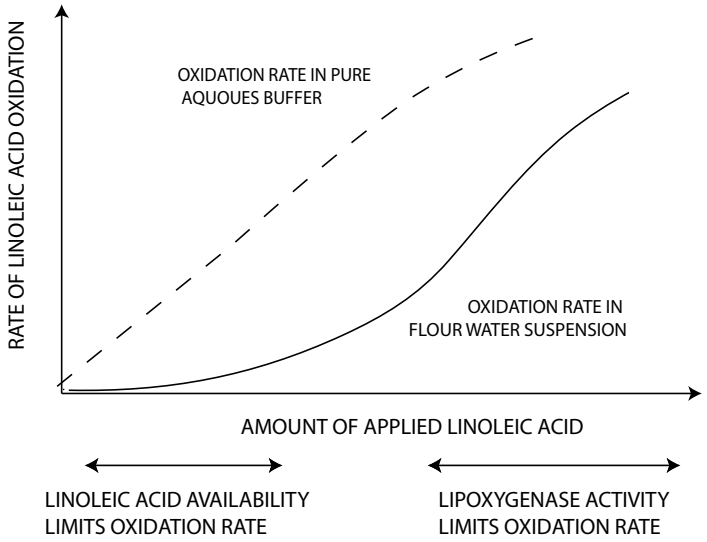

Figure 8 . Effect of linoleic acid concentration on the oxidation rate of linoleic acid in aqueous buffer and in the flour water suspension.

The kinetics of linoleic acid oxidation was similarly affected by all studied cereals and cereals fractions. However, the change from substrate limited kinetics into enzyme activity limited kinetics was established at different amounts of each cereal sample. Accordingly, when linoleic acid was mixed in oat flour suspension, a lower amount of linoleic acid was found in the reactive pool than when the equal amount of linoleic acid was mixed in rye or wheat flour suspension.

The efficiency of the flours to retard the rate of enzymatic oxidation was dependent on the contact time between the flour material and the supplied linoleic acid. During this contact time, the total amount of free linoleic acid was assayed to remain chemically unchanged, but the amount acting as a substrate for lipoxygenase was reduced with increasing contact time. By similar experimental

setup, the effect of contact time between lipoxygenase and flour did not have any effect on the oxidation kinetics. It was therefore concluded, that the flour material interacted with linoleic acid and not with the lipoxygenase. This interaction between the linoleic acid and flour made linoleic acid unavailable as a substrate for the lipoxygenase thus causing the substrate limited kinetics represented in figures 7 and 8.

The capacity of the flour to retain linoleic acid in a non-reactive pool was limited and if the amount of linoleic acid exceeded this capacity, the excess linoleic acid was found in the reactive pool. Fatty acids other than linoleic acid were also noticed to affect this capacity; if these fatty acids were added to flour water suspension prior to the addition of linoleic acid, the capacity of flour to retain linoleic acid was reduced in a competitive manner. An attempt was made to elucidate if the studied flour material showed any specificity towards the fatty acids retained. A slight preference of oleic over palmitoleic acid was noticed, but whether this is true selectivity of the flour material or due to the difference caused by different solubility properties of these fatty acids cannot be explicitly concluded. In either case, the flour material was capable of retaining all studied fatty acids: palmitoleic, oleic and linoleic acids. As the natural lipid sources in cereal systems are mixtures of different fatty acids and other lipid structures, the total lipid, or total free fatty acid, content rather than the amount of unesterified linoleic acid should be considered when present results are used in evaluating the reactivity of lipids in cereal suspensions.

The interaction described above was reversible, and total cessation of linoleic acid oxidation was not noticed with any flour concentration. When the oxidation of linoleic acid was followed in flour suspension, the reactive pool was rapidly oxidized. Once this pool was oxidized, the remaining linoleic acid reacted with a slower rate (B in figure 9). This rate was deduced to represent *in situ* oxidation of linoleic acid dissociating from the non-reactive pool back to a monomolecular / micellar pool. Thus, the oxidation rate observed in such a situation is equal to the

dissociation rate. This hypothesis was supported by the observation that the increase in the enzyme dose had little effect on the oxidation rate once the reactive pool was consumed. At low linoleic acid flour ratios, the initial reactive pool was nearly non-existent and the dissociation rate also set the initial oxidation rate (A in figure 9). On the other hand, if the original amount of linoleic acid in the reactive pool exceeded (mol/mol) the amount of soluble oxygen, the reaction ceased due to the shortage of oxygen and no dissociation limited reaction rate was established (C in figure 9).

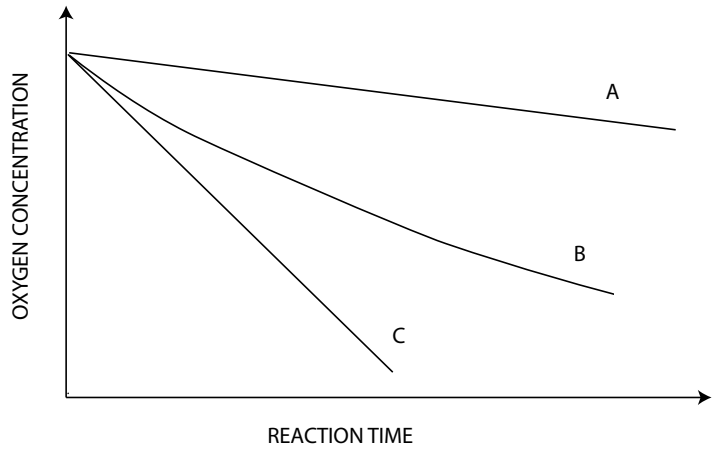

Figure 9. Reaction course of enzymatic linoleic acid oxidation in flour water suspension. (A) Ratio of linoleic acid to flour is low and reaction rate is set by the linoleic acid dissociation from non-reactive pool into reactive pool (B) Small amount of reactive pool is initially present in suspension and once this pool is oxidized the reaction rate approaches the situation in A (C) Amount of linoleic acid in reactive pool is higher (mol/mol) than the amount of soluble oxygen, oxidation proceeds rapidly and the substrate availbility does not restrict the reaction.

7.1.1 TECHNICAL AND CHEMICAL CHARACTERIZATION OF OXIDATION INHIBITOR

All the studied cereals had the same mode of operation. However, the cereals differed in the capacity to retain linoleic acid in the non-reactive pool and of the major cereals whole meal oat was most efficient. When oat was fractionated by wet processing to yield fiber[2], protein, and starch enriched fractions this capability was unevenly distributed among these fractions. Fiber and especially soluble fiber

[2] In publication I, the term fiber refers to a bran enriched fraction obtained from the whole meal oat by aqueous processing

maintained a higher proportion of the capacity than did the starch and protein fractions.

In order to enable quantification of the capacity of flour to retain linoleic acid, each studied flour sample was assigned an $IC_{50\%}$-value. This value corresponds to the amount of flour needed to reduce the deoxygenation rate by 50 % in the defined system. The $IC_{50\%}$-value was, in turn, used to define a concept of inhibitory unit (IU), which was then used to quantify the inhibitory activity in a manner similar to enzyme activity during enzyme purification protocols. By this approach, the oat fiber was assigned to contain 48 IU/g dry matter. This fiber was then used as a starting material for the extraction process to yield fractions with higher capacity to harbor fatty acids *i.e.* higher IU content per g dry matter. By alkaline extraction and pH precipitation, a fraction was obtained which possessed 830 IU per g dry matter. Surprisingly, the recoveries of total inhibitory activity during such purification process vastly exceeded 100 %. This behavior suggests that not only the chemical composition of flour material, but also the molecular and particulate arrangement of flour affects its capacity to retain fatty acids. In other words, the extraction procedure does not only concentrate the active component, but also converts the material into more active form. The high protein content of the most effective fractions and the strong dependence of inhibitory activity on the pH-changes suggest that proteinaceous material is responsible for the observed phenomenon.

7.1.1.1 Remarks on methodology

There are many approved and accurate methods available for analysis of the chemical composition of foods and any other biological material. However, the methods that are used to track molecular organization are often indirect, and as such are easily susceptible towards misinterpretation and non-valid hypotheses. In the study described above, the molecular organization was followed by measuring the differences in the oxidation rate of supplied oxidizible lipid compound. The advantages of this methodology as well as risks related to misinterpretation of results are discussed in the following.

The use of polarographic measurement of molecular oxygen to follow the lipoxygenase reaction is well documented in the scientific literature (Pourplanche *et al.*, 1991). However, the solubility of free linoleic acid is much lower than the solubility of oxygen. In order to obtain measurable changes in oxygen concentration in aqueous systems, linoleic acid needs to be supplied in concentrations exceeding its solubility. This can be achieved, for example, by using micellar linoleic acid substrate or by complexing the linoleic acid to a water soluble host compound, *e.g.* β-cyclodextrin (Lopez-Nicolas *et al.*, 1997). In both cases the monomolecular, dissolved, pool is constantly fed by a rapid equilibrium between the monomolecular and the micellar or complexed pools. In the present study the linoleic acid was supplemented initially as a mixed micellar solution of Tween 20 and linoleic acid (Axelrod *et al.*, 1981). The re-distribution of linoleic acid between the pools accessible and non-accessible for lipoxygenase was then measured when the substrate was mixed with flour suspension.

The experimental assay system allowed the direct measurement of the oxidation reaction, without interference with the reaction itself. However, it differed from the plain flour water mixtures in two respects. First, the catalyst of oxidation was the purified lipoxygenase from soybean. It is possible that the unpurified natural catalysts, e.g. endogenous cereal lipoxygenase, have different localization, mobility and fatty acid substrate requirements in such a suspension. Second, linoleic acid was introduced into the assay system as mixed micelles of linoleic acid and the non-ionic surfactant, Tween 20. In plain flour water mixture, the endogenous linoleic acid originates mostly from the lipase reaction on the water-oil surface. The formation of micellar phase from this free linoleic acid is dependent on the presene of other surfactants such as polar lipid components derived from membranous structures. Consequently, these differences need to be taken into account, when current results are used to guide process development.

7.2 CHANGES IN OAT LIPIDS UPON PROCESSING AND STORAGE (PUBLICATIONS IV-V)

Both the amount of storage lipids and their distribution within the kernel is different in oat as compared to other major cereals. The amount of lipids in oat is up to 5 times of what is present in wheat. Oat lipids are also found distributed throughout the starchy endosperm, whereas in other cereals a higher proportion of lipids is localized in the embryo-axis (Price and Parson 1975; Hargin *et al.*, 1980; Zhou *et al.*, 1999b; Peterson, 2002). These facts, together with the high lipase and lipoperoxidase activities makes oat particularly susceptible towards deteriorative lipid reactions. The factors affecting the lipid hydrolysis and oxidation of unsaturated fatty acid moieties upon processing and storage of oat products are discussed below.

7.2.1 FACTORS AFFECTING LIPID HYDROLYSIS IN OAT

Despite the relatively high lipase activity in mature oat grains, the extent of lipid hydrolysis during the storage of intact grain is negligible. Even if the germination of seed is initiated by increasing the moisture content, no lipid hydrolysis is observed in whole grains (Outinen, 1999). The mechanical de-hulling of oat seeds can cause substantial breakage of groats but during subsequent storage of dehulled groats only minor lipid hydrolysis is observed (Peltonen-Sainio *et al.*, 2003). However, instantaneously after the milling or crunching of grain, the lipid hydrolysis starts rapidly. As the lipase reaction is set on by milling, it is likely, that different milling schemes would provide flours with different tendency to form free fatty acids. Unfortunately, no data is available in the scientific literature on this subject, even though it could enable an additional means to control lipid hydrolysis.

The rate of hydrolysis is affected by the amount of available water. At moisture contents of dry flour, around 7 to 11 %, the hydrolysis of storage lipids is slow, and the hydrolysis takes place within weeks or months. By increasing the water content of oat flour up to 50 %, the rate of lipid hydrolysis is markedly increased and substantial accumulation of free fatty acids occurs already during an overnight

storage (Liukkonen *et al.*, 1992). In the present study a significant correlation was noticed between the degree of lipid hydrolysis in flours after dry storage and in flours treated with excess water for 15 h. Thus, the extent of lipid hydrolysis occurring during dry storage can be effectively predicted by following the accelerated hydrolysis of lipids at high water activities. The main course of lipid hydrolysis was deduced to be the hydrolysis of triacylglycerols to free fatty acids, and apparently no partially hydrolyzed acylglycerols accumulate during this process.

The lipase reaction during processing and storage of oat has traditionally been prevented by inactivating all enzymatic activities. This is most easily achieved by moist heat. This heating is expected to effect different parts of the kernel with different efficiency. In the present study, heat treated oat was fractionated into fractions that originate from different parts of kernel. In accordance with intuitive assumption, the data provided in publication V indicates that lipase was most easily inactivated in outer layers of kernel. Thus, even though the lipase in native mature oat grain is mainly localized in outer layers of kernel, the residual lipase activity in heat inactivated oat is highest in endosperm rich flour.

7.2.2 CRITICAL FACTORS AFFECTING LIPID OXIDATION

At least two different oxidative reactions of unsaturated fatty acid moieties were noticed to occur during processing of oat 1) the enzymatic oxidation of unsaturated fatty acid moieties of neutral storage lipids when the non-heat treated oat fractions are mixed in excess water and 2) the non-enzymatic oxidation of the same moieties in polar membrane lipids during the storage of dry oat fractions after processing. As virtually all oat currently used for various food formulations is enzyme inactivated, the latter reaction appears to determine the shelf life of oat products.

Compared to the other cereals, the assayed lipoxygenase activity in oats is low (Table 1). However, once the non-inactivated oat is mixed in water, it will spontaneously consume the oxygen dissolved in water (unpublished results by the

author). Simultaneously a small proportion of unsaturated free fatty acids are oxidized. This activity is easily inactivated by heat and was thus not detected in any heat treated samples.

Table 1. Lipoxygenase activities of different cereals (Lehtinen *et al.*, 2000).

	Initial rate of lipoxygenase reaction (umol/min) / 100 mg flour
Barley	1200
Rye	290
Wheat	630
Oat malt	<50
Oat	<50
Barley malt*)	<50

*) The lipoxygenase activity of barley malt depends on the kilning level and for certain malt types the activity can be substantially higher.

Non-enzymatic oxidation of unsaturated fatty acid moieties in polar lipids appears to be the major mechanism by which the lipids in enzyme inactivated oat deteriorate. Factors affecting non-enzymatic oxidation in vegetable oils are relatively well characterized in the scientific literature. Molecular oxygen does not react directly with *cis,cis*-1,4–pentadiene structures in linoleic acid, but linoleic acid or the oxygen is first converted into reactive molecular species, such as an acyl radical or singlet oxygen. Accordingly, the oxidation reaction is determined by the formation and elimination of these reactive molecular species. These in turn are determined by oxidative stress from the environment or by the protective effect on endogenous antioxidants. However, in addition to the reactions involved in formation and suppression of these reactive molecular species, factors such as mobility, solubility and molecular organization must be taken into account when stability of cereal products with multiple and discontinuous phases are evaluated.

The intensity of heat treatment applied to the oat kernels was recognized as a key parameter in determining the rate of non-enzymatic oxidation of unsaturated fatty acid moieties during prolonged storage of oat products derived from such kernels.

In non-heat treated oat products, the oxidation of unsaturated fatty acid moieties during storage of dry flour is small and occurs mainly in the free fatty acid pool. However, if the product was otherwise identical, but had been heat treated prior to the storage, noticeable oxidation of unsaturated fatty acids was detected both as a decrease in unsaturated fatty acid content in polar lipids and as an increased formation of volatile lipid oxidation products. No detailed interpretation for this could be drawn from the results, but possible reasons include 1) the increased formation of reactive molecular species, 2) the decreased quenching of reactive molecular species by endogenous antioxidants and 3) increased susceptibility of lipids towards reactive species as a result of heat induced membrane disintegration.

The increased formation of reactive molecular species can be due to the formation of pro-oxidative molecules such as photosensitizers. When oat products obtained from heat treated oat are exposed to light, the oxidation accelerates markedly as compared to the same sample stored in dark. Based on this observation, it can be assumed that the non-enzymatic lipid oxidation in cereals proceeds partly via photo-oxidation. Therefore process design and packing materials that minimize the exposure of cereal material to light should be chosen.

Possible molecular species in cereals that act as photosensitizers include, riboflavin and wide spectrum of maillard reaction products. Riboflavin, vitamin B2, is found in most whole meal cereals as harvested, whereas the maillard reaction occurs after heating mixtures of protein and carbohydrates. The strong dependence of oxidation rate as a function of heat treatment suggests that melanoidins, a complex mixture of maillard reaction products, could play a critical role in increasing the formation of reactive molecular species.

The heat induced inactivation of endogenous antioxidants would result in the decreased capability of flour to quench the radicals. Tocopherol is the most well characterized antioxidant in oat and the total concentration has been shown to remain unchanged over steaming of whole kernels whereas a 63-94 % reduction is reported during extrusion of de-branned flour (Bryngelsson *et al.*, 2002;

Wennermark, 1993; Zielinski *et al.*, 2001; Guth and Grosch, 1994). The inactivation of tocopherol upon these heat treatments appears to be the competitive oxidation with unsaturated lipids (Verleyen *et al.*, 2002). The reactivity and pro- or antioxidative capability of tocopehrol oxidation products thus formed remains to be elucidated. Furthermore, the increased accumulation of volatile lipid oxidation products in heat treated oat may partly result from the inactivation of an enzyme system converting volatile lipid oxidation products to further products (Lehto *et al.*, 2003). In this a case the sensory attributes associated with enzyme active and heat treated products will be different even though the actual lipid oxidation would proceed at equal rates.

The observed difference between the enzyme active and inactivated oat may be a combined effect of all the factors mentioned above. The clarification of the role of individual factors would enable the production of oat products with increased stability.

7.2.3 RELATIONSHIP BETWEEN LIPID HYDROLYSIS, LIPID OXIDATION AND FORMATION OF RANCIDITY

Prior dogma for the progress of rancidity in oat products has tied together the hydrolysis of acylglycerols and oxidation of unsaturated fatty acids. This has been based on the assumption that increased hydrolysis of lipids increases the susceptibility of unsaturated fatty acid moieties towards the oxidation. However, it appears that during oat processing this dogma is valid only for enzymatic oxidation. Considering that the lipoxygenase catalyzed oxidation of linoleic acid has a small or nonexistent relevance in determining the shelf life of oat products, the linkage of hydrolysis and oxidation needs to be re-evaluated.

The current study shows, that the lipid hydrolysis and oxidation do not occur one after the other in oat products. Instead, in the heat treated oat, the unsaturated fatty acids, whilst still acylated to phosphoglycerol, are most susceptible towards oxidation. On the other hand, if the heat treatment is omitted, the free fatty acids

formed via lipase catalyzed hydrolysis of storage lipids are very stable towards non-enzymatic oxidative reactions.

The total amount of fatty acids acylated to phosphoglycerols in oat products is *ca.*10-15 mg per g flour of which *ca*. 50 % is linoleic acid. During the storage of extensively heat treated bran fraction, the amount of linoleic acid moieties in this lipid class decreased by *ca.*20 %. Even though this decrease is relatively small, the consequences in the volatile lipid oxidation products were dramatic: concurrent with such oxidation, the hexanal detected in the headspace of sample increased by 160-1000 %. Thus, it is evident that lipid changes in order of less than 1 mg per g flour, are capable of reducing the quality of material due to the formation of volatile rancidity.

8 CONCLUSIONS

Even though cereal lipids and other cereal components have been an important part of human diet for thousands of years, the incorporation of cereal products in modern food formulations is challenged by the strong reactivity of lipids. This reactivity is partly due to endogenous enzymes in cereals and partly to the oxidative stress from the processing and storage environment (figures 10a and 10b).

During aqueous processing, such as wet fractionation, mashing or different fermentative processes, the reactivity of lipids is restricted by the availability of lipid substrate towards oxidative enzymes. Such availability is different in different cereal materials, and appears also to be a function of processing history. Furthermore, by pH-treatments it is possible to alter the distribution of lipid substrates between the reactive and non-reactive pools. Alternatively, a fraction with enhanced capability to retard lipid reactions can be supplemented to other non-cereal food products in order to reduce the amount of fatty acids in the reactive pool.

If the material is known to contain substantial enzyme activities that were to lead to unwanted lipid reactions during the processing, the enzymatic activities have customarily been prevented by inactivating enzymatic activities by hydrothermal processing, such as steaming and kilning. However, such treatments induce also unwanted changes such as denaturation of nutrients and, as pointed out in the present study, also lead to the increased susceptibility of unsaturated fatty acid moieties towards non-enzymatic oxidation. In order to enhance the stability by inactivating the enzymes, a process design should allow the accurate control over the severity of heat treatment. This is challenged by the high thermal capacity and low thermal conductivity of cereal materials.

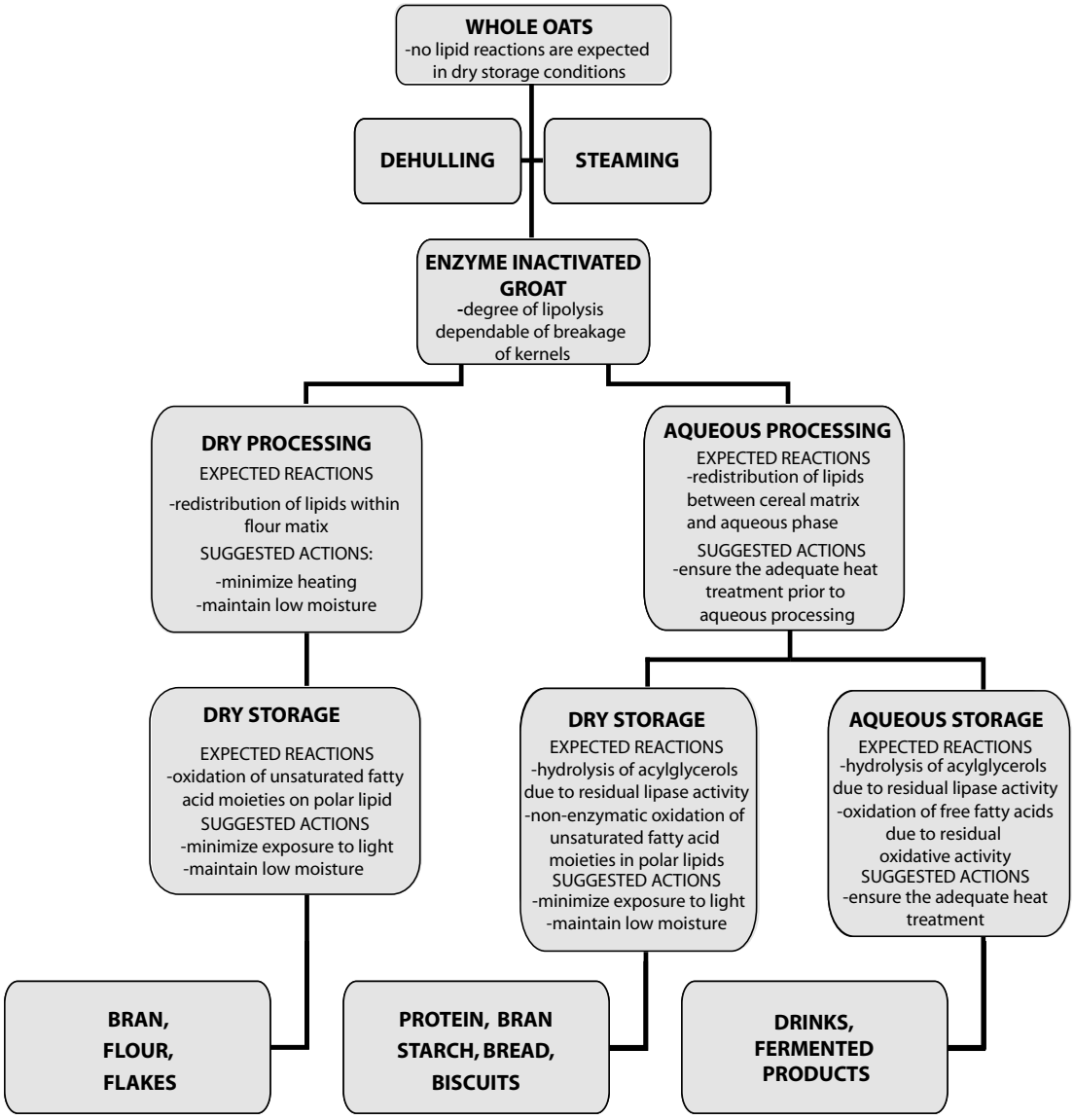

Figure 10a. Schematic presentation of the expected lipid reactions during processing and storage of <u>enzyme inactivated</u> oat products and the suggested actions to minimize unwanted reactions.

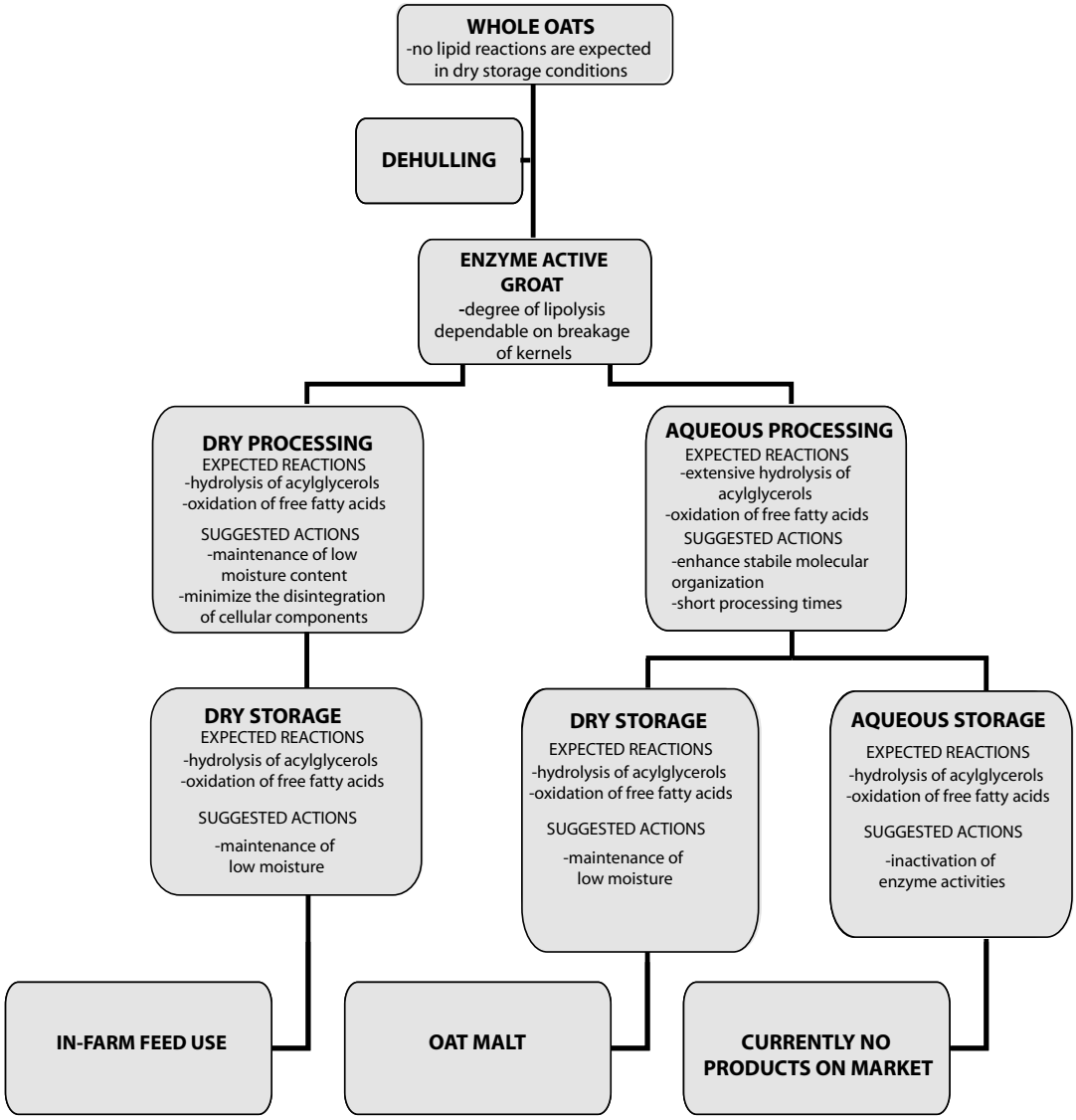

Figure 10b. Schematic presentation of the expected lipid reactions during processing and storage of enzyme active oat products and the suggested actions to minimize unwanted reactions.

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