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Effect of Heat Treatment on Lipid Stability in Processed Oats

Pekka Lehtinen†*, Katja Kiiliäinen†, Ilkka Lehtomäki‡ and Simo Laakso†

†Helsinki University of Technology, Laboratory of Biochemistry and Microbiology, PO Box 6100, FIN-02015 TKK, Finland; ‡Avena Group, Avena Oat Ingredients, Kansakoulukatu 1 B, FIN-00100 Helsinki, Finland

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ABSTRACT

The shelf life of processed oat products and the usability of oats in modern food formulations are in many cases still limited by the lipid-associated deterioration. To elucidate the role of lipase inactivation in the development of rancidity in oats, heat treatments varying in severity were applied. Effects of these treatments on lipase activity and lipid oxidation were studied either directly after processing by mixing the fractions in water or after a long-term storage of dry fractions. A trend was found, that the lower the residual lipase activity in whole kernels or kernel fractions, the higher was the oxidation of lipids and evolution of volatile oxidation products during prolonged storage of the dry fractions. If bran was heat-treated to zero lipase activity, the amount of headspace hexanal detected after 12-month storage was 5 to 7 times larger than detected in non-heat treated bran. This formation of hexanal was linked to the oxidation of polar lipids. If the heat treatment was totally omitted, the oxidation of unsaturated fatty acids in polar lipids did not occur even during prolonged storage. The oxidation of polar lipids suggests heat-induced disintegration of membrane structures and inactivation of heat labile antioxidants. This study identifies heat treatments as critical control points in obtaining oat products with enhanced self-stability.

Keywords: oats, heat treatment, lipase, lipid oxidation.

INTRODUCTION

Cereal lipids consists of (1) storage lipids, composed of triacylglycerols and hydrolysis products thereof, which serve as a reservoir for energy and building blocks for germinating seeds and (2) amphipathic phospholipids in membranous structures, which help to maintain microstructure in grain. In the intact, non-germinated grain these lipids are stable, and time-dependent changes in lipids are small. However, during and after milling of grain, lipids undergo many reactions to an extent that is perceived as a sensory flaw[1,2].

Unlike the reactions during seed germination, the reactions after the breaking of non-germinated grain are uncontrolled, and various enzymatic and non-enzymatic reactions are known to occur simultaneously[3,4]. The spectrum of deteriorative reactions in processed cereal products is wide, and means to achieve adequate shelf life varies according to the specific applications.

For certain purposes shelf stability is achieved by inactivating key enzymes responsible for initiation of the deteriorative reactions. Rice bran and oats both have high lipase activity, and stabilization of products made from them relies traditionally on inactivation of lipase[5,6]. Without this inactivation, the products can be stored for only short periods of time. Otherwise rapid lipid hydrolysis and formation of a bitter taste renders the material unusable. Most cereals also contain lipoxygenase that oxidizes 1,4-pentadiene moieties in unsaturated fatty acids. The need to inactivate the lipoxygenase in order to enhance storage stability is, however, ambiguous

ABBREVIATIONS USED: DUS = degree of unsaturation.

* *Corresponding Author: E-mail: Pekka.Lehtinen@hut.fi*

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and is of concern mainly in malt and beer production[7]. Also, the lack of the most abundant lipoxygenase isoenzyme is reported to reduce the formation of stale flavours in rice[8]. Often the lipoxygenase reaction is, however, considered to have a positive role in the development of dough structure[9,10].

Oxidation of unsaturated fatty acids is the most intensively studied non-enzymatic deteriorative reaction of cereal lipids. The means to control it include introduction of exogenous antioxidants[11,12] and the avoidance of oxidative processing stages. An interesting approach would be to induce the formation of inclusion complexes or other lipid forms with improved stability[13,14]. When the product is to be stored for extended periods, the storage conditions can also play a role. In this situation similar actions can be employed that are used to preserve vegetable oils, such as modification of the atmosphere or the moisture content in the package. Furthermore, the exposure of lipids to reactive oxygen species can be reduced by packing the products in materials with low transmission of UV-radiation.

The high amount of unsaturated fatty acids in oats makes them nutritionally beneficial, but at the same time renders oats particularly susceptible towards formation of rancidity. Despite actions taken to improve the storage stability of oat products, such as heat treatment, the shelf life of oat products is typically shorter than that of equivalent products from other cereals with lower lipid content. The overall mechanism of lipid deterioration in oat products is not completely understood nor are the lipid species susceptible to these reactions identified. The proper understanding of the factors affecting the formation of rancidity in oat products would enable the production of oat products with improved stability.

The present study was performed to explain the role of heat-induced enzyme inactivation on the formation of free fatty acids and volatile oxidation products upon the storage of oats and oat fractions. The rate of enzymatic reactions of lipids in cereal systems is determined by the availability of lipids to the endogenous hydrolytic and oxidative enzymes and on the activity of the enzymes themselves. By following the changes occurring in endogenous lipids during processing and storage, the overall susceptibility of oat material towards enzymatic deterioration can be estimated. This was the experimental approach throughout the present study.

MATERIALS AND METHODS

Oat material

Dehulled, non heat-treated oat groats, cv *Freja*, were obtained from Avena Oat Ingredients, Finland. The heat treatments of varying severity were achieved by introducing a flow of steam into groats according to Table I. Thereafter the groats were air dried to a moisture content of 11%. The non heat-treated and heat-treated groats were milled and the wholemeal flours separated according to size and density in a classifier to obtain bran- and endosperm-enriched flour fractions. In the bran fraction 91% of the flour was retained on a 132 micron sieve, whereas 88% of the endosperm fraction passed a 100 micron sieve. The bran fraction contained 16% β-glucan and 25% starch, whereas the corresponding values for the endosperm fractions were 4 and 66%, respectively. The bran fraction obtained from non heat-treated kernels was used either as such or after enzyme inactivation by extrusion (Table I). These fractions were water treated (see below) or stored in closed, brown paper bags at 20 °C for 12 months. At specified intervals changes in lipid compositions and in formation of headspace hexanal were measured.

Lipids

Lipids were extracted from flour samples twice in 19 volumes of dichloromethane-methanol (2:1, v/v) as described by Heiniö *et al.*[1]. The action of endogenous lipolytic enzymes, the lipid composition of whole meal flour samples was followed by analysis after a water treatment. For this, samples were mixed in five volumes of water at 25 °C for 15 h, after which the lipids were extracted as described above. The lipids in the extracts were separated into the major classes by thin layer chromatography as described by Liukkonen *et al.*[15]. Analysis of fatty acid composition of separated lipid classes was done by converting fatty acids to methyl esters and analysing

Table I The heat treatment scheme

	Moisture during heat-treatment (%)	Average temperature (°C)	Duration (min)
Mild heat-treatment of whole groats	17	90	20
Intense heat-treatment of whole groats	20	100	40
Heat-treatment of bran by extrusion	25	130	2

the latter by gas chromatography essentially as described by Suutari *et al.*[16]. The degree of unsaturation in different lipid classes was calculated by adding together the values obtained by multiplying the proportion of individual fatty acid of the total fatty acids present in the lipid class with the number of double bonds in the acyl chain. In order to obtain the degree of unsaturation of storage lipids, the data for triacylglycerols and free fatty acids were pooled together. The reported values are the means of two measurements (standard deviation < 5%).

Volatile compounds

The amount of hexanal released into the headspace of oat samples was determined by static headspace measurement and analysis of the headspace composition by GC/MS technique[1]. To compensate for the variation in the performance of the headspace injector and the MS-detector during the 12-month storage period, all detector responses of duplicated samples were standardized to the response of an external standard (40 ppm *iso*-butanol in 0·4 mL of water). Results are reported as arbitrary detector responses and values are the means of two measurements (standard deviation < 7%).

RESULTS

Heat treatment of whole kernels prior to processing

To test the extent to which lipid deterioration could be prevented by enzyme inactivation, oat kernels were heat-treated to different degrees, then milled and soaked in excess water for 15 h and thereafter analysed for changes in lipid composition. As expected, the extent of lipid hydrolysis was less the more severe the heat-treatment of the whole kernels. Nonetheless, a minor residual hydrolysis (11%) was observed even after the most severe heat treatment (Table II).

During the water treatment, lipid oxidation was also observed, but only in the flour from non-heat-treated kernels. Polar lipids were oxidised to a lesser extent than storage lipids, and this oxidation was only partially reduced by the heat treatments (Table II). These observations support the need for the general practice of inactivating kernels prior to water processing.

Oats are also widely processed and stored without addition of water. In such dry processing, the water activity varies typically between 0·4–0·6, and the enzymatic reactions are likely to be substantially slower than in excess water. To clarify the role of heat treatments on the extent of lipid deterioration in oat flours stored with such ambient water activities, heat-treated and non heat-treated kernels were milled and separated into bran- and endosperm-enriched fractions by the dry process and lipid compositions were measured immediately after processing and compared to those measured after prolonged dry-storage.

Processing of non-heated kernels yielded fractions in which the proportion of free fatty acids was higher than in the fractions from heat-treated kernels. However, independent of heat treatment the level of free fatty acids was always lower in the bran

Table II Effect of heat treatment on the degree of hydrolysis and degree of unsaturation of oat polar and storage lipids. Measurements were done from the dry whole meal flour (native) and from the same flour pretreated with water to enable the action of endogenous lipolytic enzymes

	No heat-treatment	Mild heat-treatment	Intense heat-treatment
Degree of hydrolysis[a]			
Native	4%	3%	2%
Water-treated	64%	23%	11%
Degree of unsaturation[b]			
Polar lipids			
Native	1·36	1·36	1·37
Water-treated	1·27	1·30	1·34
Storage lipids			
Native	1·32	1·32	1·31
Water-treated	1·11	1·35	1·32

[a] Proportion of free fatty acids from total fatty acids.
[b] Average number of double bonds per fatty acid.

Table III Effect of storage time on the degree of hydrolysis and degree of unsaturation of oat polar and storage lipids in bran and endosperm enriched oat fractions

		Endosperm enriched flour		Bran enriched flour	
	Storage time	No heat-treatment	Mild heat-treatment	No heat-treatment	Mild heat-treatment
Degree of hydrolysis[a]	Beginning of storage	13%	6%	11%	2%
	1 month	24%	11%	20%	3%
	12 months	–[c]	–[c]	77%	6%
Degree of unsaturation[b]					
Polar lipids	Beginning of storage	1·37	1·35	1·39	1·41
	1 month	1·36	1·33	1·38	1·38
	12 months	–[c]	–[c]	1·34	1·11
Storage lipids	Beginning of storage	1·32	1·31	1·31	1·32
	1 month	1·30	1·32	1·31	1·30
	12 months	–[c]	–[c]	1·23	1·19

[a] Proportion of free fatty acids from total fatty acids.
[b] Average number of double bonds per fatty acid.
[c] Not determined.

fractions, so that even the mild heat treatment applied to whole kernels was sufficient to prevent the formation of free fatty acids in bran fraction almost totally (Table III). The subsequent storage of the fractions at 20 °C, revealed that lipolysis had proceeded also in fractions from heat-treated kernels, although again at a slower rate in the bran than in the corresponding endosperm fraction. Consequently, even the mild heat treatment of whole kernels can be considered sufficient to control the hydrolysis in the bran fraction, but not in the endosperm fraction.

The storage of the two bran fractions was continued for up to 12 months. During this period, lipid hydrolysis had occurred especially in the bran obtained from non heat-treated kernels (Table III). As reported earlier[1], this hydrolysis occurred exclusively in the storage lipids, whereas the polar lipids remained un-hydrolysed for the entire storage period (data not shown).

Even though slight lipid hydrolysis had occurred during the fractionation, especially in the non-heated fractions, the fractionation did not cause a decrease in DUS, reflecting lipid oxidation. The sensory problems associated with lipid oxidation may, however, originate from lipid oxidation that is too low to be detected by conventional fatty acids analysis. Therefore, the bran fractions originating from non heat-treated and mildly heat-treated kernels were tested further for their ability to release hexanal, a volatile compound indicative of lipid oxidation[17]. Unexpectedly, already immediately after processing, the formation of hexanal was more pronounced in bran originating from the mildly heat-treated kernels, than in the bran obtained from non heat-treated kernels (Table IV).

Table IV Effect of heat treatment of bran-enriched oat fraction on the development of volatile rancidity measured as headspace hexanal (arbitrary detector responses)

	No heat-treatment	Mild heat-treatment
Non-processed groats	0·3	0·3
Bran-enriched flour		
Beginning of storage	0·3	0·8
Stored for 1 month	0·4	2·0
Stored for 3 months	0·7	4·8
Stored for 6 months	2·0	9·4
Stored for 12 months	6·1	30·0

During the storage of the oat fractions, the oxidation of storage and polar lipids were differently affected by the degree of heat treatment. The remarkable decrease in the DUS of polar lipids was noticed in the bran obtained from heat-treated kernels but not in the bran from non heat-treated kernels. In turn, the traces of oxidation detected in the storage lipids appeared irrespective of the heat treatment (Table III). Also the formation of hexanal during storage was increased by heat treatment, and the concentration of hexanal after 12 months storage was around 5-fold higher in the heat-treated compared to non heat-treated bran (Table IV). It was concluded that heat treatment of kernels reduced the rate of formation of free fatty acids in processed fractions but rendered especially the acylated fatty acids in the polar lipid fraction more susceptible to oxidation.

Table V Effect of fractionation of whole meal oat into bran and endosperm enriched fractions on the degree of hydrolysis and degree of unsaturation of oat polar and storage lipids

		Whole meal flour	Endosperm enriched flour	Bran enriched flour	
	Storage time	No heat-treatment	No heat-treated	No heat-treated	Heat-treated after bran preparation[d]
Degree of hydrolysis[a]	Beginning of storage	6%	61%	18%	12%
	12 months	–[c]	–[c]	53%	15%
Degree of unsaturation[b]					
Polar lipids	Beginning of storage	1·27	1·26	1·27	1·30
	12 months	–[c]	–[c]	1·21	1·07
Storage lipids	Beginning of storage	1·28	1·19	1·26	1·28
	12 months	–[c]	–[c]	1·24	1·23

[a] Proportion of free fatty acids from total fatty acids.
[b] Average number of double bonds per fatty acid.
[c] Not determined.
[d] Heat-treated by extrusion.

These observations support the view that hydrolysis and oxidation of lipids occur independently during dry storage of oat fractions. But rather heat treatment used to prevent lipid hydrolysis actually rendered the polar lipids susceptible to oxidative deterioration. Based on the data shown in Table III, DUS in the polar lipids of bran made from non heat-treated oat kernels had decreased from 1·39 to 1·34. This means that only 1 in every 28 double bonds in the polar lipids was oxidised during the 12-month storage whereas in storage lipids of the same sample one in every 16 double bonds was oxidised. In the same fraction from the heat-treated kernels, lipid oxidation was more severe and the corresponding figures 1 in every 5 and 10 double bonds for the polar lipids and storage lipids, respectively. Thus it is evident that heat treatment of kernels induces lipid oxidation especially on the polar lipid fraction.

Heat treatment of processed fractions

The heat treatment experiments described involved whole oat kernels, which were then processed into two fractions. Since in the bran fraction only minor lipid hydrolysis occurred during dry processing, inactivation of lipase activity could be made in the finished product instead of the intact groats. Accordingly, operations that include a heat treatment, such as extrusion, would be applicable to oat fractions prepared from non-heated or mildly heat-treated kernels. To test this, wholemeal flour from non-heated kernels was processed into bran and the residual flour fractions as described above. The bran was extruded to inactive lipase.

In agreement with the results above, lipid hydrolysis occurring during and shortly after the fractionation process was more intensive in the endosperm-enriched flour fraction than in the bran fraction; around 60% of the fatty acids present in the former fraction were free fatty acids, whereas in the bran the similar figure was from 12 to 18%. Also slight oxidation of storage lipids was evident in the endosperm fraction (Table V).

Extrusion almost completely prevented lipid hydrolysis in the bran during 12-months dry-storage whereas in the corresponding non-extruded product the increase in the proportion of free fatty acids during the storage was from 18–53% (Table V). Again, the extent of lipid oxidation did not follow that of lipid hydrolysis during the same storage period. Based on the DUS-values, the storage lipids in both the extruded and non-extruded bran attained the same level of lipid oxidation. However, extrusion led to an increase in the oxidation of polar lipids during storage. Concurrently with reduction in DUS of polar lipids, an increase in the headspace hexanal was observed (Table VI).

DISCUSSION

The high lipase activity in the mature oat kernel[18] indicates that lipid hydrolysis is the critical reaction that triggers lipid deterioration and explains, at least in part, the decrease in the sensory properties of processed oats during the storage.

A question arose, as to why the bran enriched fraction was not more susceptible to lipid hydrolysis when it is known that lipase is located in the outer

Table VI Effect of heat treatment of bran enriched oat fraction on the development of volatile rancidity measured as headspace hexanal (arbitrary detector responses)

	Bran enriched flour	
	No heat-treatment	Heat-treated after bran separation[a]
Beginning of storage	0·2	3·7
Stored for 1 month	0·3	3·3
Stored for 3 months	1·3	3·9
Stored for 6 months	2·0	9·6
Stored for 12 months	8·5	59·6

[a] Heat-treated by extrusion.

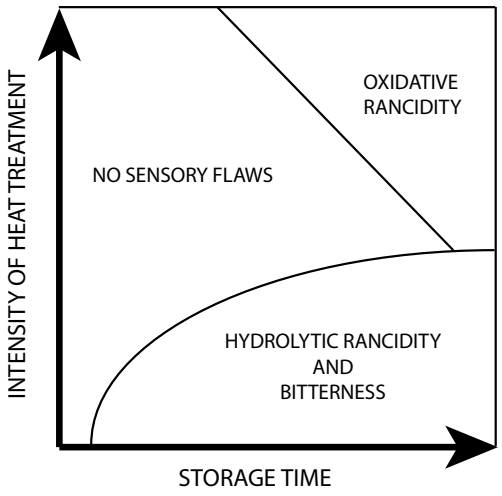

Figure 1 Schematic presentation of the effect of heat treatment on the oxidative and hydrolytic stability of oat lipids.

layers of the kernel and in the embryonic tissue[19–21]. In earlier studies differences in lipase activities was followed using exogenous substrates, whereas in the present study the extent of lipase reaction towards oat endogenous lipids was measured. The present results show that the lipase is present both in bran and endosperm fractions in amounts sufficient to cause extensive hydrolysis of lipids in those fractions. The rate-determining step of lipid hydrolysis may therefore be determined by factors other than amount or activity of lipase present in the fraction. Among these factors, the availability of acyl lipid substrate for lipase plays a critical role. This in turn is determined by the content and chemical structure of acyl lipids and their physical organization and capability to migrate within the tissue matrix to form suitable interfaces for lipase action. Many of these factors are largely determined by water content and the degree of disintegration of kernel structures. Similar substrate limited kinetics has also been suggested for enzymatic lipid oxidation in aqueous slurries of different cereals[22].

The widely applied approach to lipase inactivation has been heat-treatment also used in the present study. It appears however, that in oat products intended for long-term storage, heat-inactivation of lipase had an adverse effect on storage stability. This reduction in stability was traced to oxidation of the polar lipid fraction, a phenomenon that was non-existent in the corresponding non-heated products. Therefore, lipid hydrolysis occurring in non-heated oat products cannot be considered as the primary cause for the appearance of symptoms of lipid oxidation. On the contrary, once the lipid hydrolysis is prevented, the long-term storage stability is determined by the accumulation of lipid oxidation products.

At present, there is no solid explanation as to why, specifically, polar lipids in oats are susceptible to oxidation after heat-treatment. Heat-treatment itself did not cause instantaneous changes in the assayable lipid composition, but this does not exclude the possibility that disintegration of membranous structures or inactivation of heat labile antioxidants had occurred. Byngelsson *et al.*[23] however noticed no significant loss of oat antioxidants upon steaming of oat groats. Upon extrusion, on the other hand, a 63–94% reduction of wheat tocopherols has been reported[24,25].

In spite of the present observations, heat-treatment of oats or their products will probably remain as a common practice for most applications, otherwise the rapid formation of free fatty acids or other enzymatic reactions leading to bitter flavours would occur[26,27]. However, the severity of the heat treatment should be recognised as a key parameter in obtaining the oat products with extended shelf life. The heat treatment should achieve selective lipase inactivation without rendering the polar lipids oxidisable upon prolonged storage (Fig. 1). Another challenge for future research would be an understanding by why heat-treatment of oats does not prevent the formation of volatile secondary products from oxidized lipids and why these volatile products originate specifically from polar lipids.

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