Lipase Activity in Oats—Distribution, pH Dependence, and Heat Inactivation

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Cereal Chem. 69(4):379-381

ABSTRACT

The lipase activity in oat kernels at different pHs, at different stages of development, and in different parts of the oat kernel was studied. The measurement of lipase activity was performed by means of fluorescence spectrophotometry. Lipase activity was higher in the aleurone layer and embryonic tissues than it was in the starchy endosperm. This also was shown in flour samples from fractionated milling. In accordance with the lipase distribution found in the oat kernel, the lipase activity was highest in flour fractions close to the superficial bran layer and was lowest in the internal endosperm fractions. The development of lipase activity during germination of the seeds caused an increase in the activity at neutral and alkaline pH, whereas the activity at acid pH remained relatively constant. This indicated that the lipase activity at alkaline pH was more related to the metabolic processes initiated by the growth of the embryo. Three different Swedish cultivars were analyzed. Lipase activity varied only slightly and was not correlated to fat content. Lipase inactivation was studied in model laboratory studies. These showed the importance of good heat transport into the sample, not only high temperature, in inactivating the enzyme.

The fat content of oats (*Avena sativa* L.) is relatively high (3–9%) (Youngs 1978, Sahasrabudhe 1979), but the lipid composition is similar to that of other cereals. The lipase activity is high even at early stages of development and increases during germination (Matlashewski et al 1982, Urquhart et al 1984). In the production of oat products for human consumption, it is generally considered necessary to inactivate lipase activity by means of various hydrothermal treatments before processing (Vorwerck 1988). Otherwise, a degradation of the fat due to hydrolysis and oxidation will lead to rancidification, which is the main limiting factor for storing and handling oat products. The time course of rancidity depends on the process design and the type of final product. Therefore, it is important to study the mechanisms of fat oxidation and its biochemical background, e.g., its relation to endogenous lipase activity. In this study, the distribution and stability of lipase activity in oats were analyzed.

Lipase activity is not evenly distributed in the oat kernel. It is associated with the parts located close to the surface of the caryopsis, i.e., in the aleurone layer (Urquhart et al 1983). To relate the enzyme distribution in single tissues in the oat kernel to the enzyme content in large-scale flour fractions, we analyzed fractions dissected from single kernels as well as from flours produced by fractionated milling.

The postharvest treatment and storage and the processing of oat kernels might damage the tissues. This would lead to increased contact between the enzyme and the fat substrate and would, therefore, increase hydrolysis. The content of free fatty acids in oat raw material arriving at the mill could vary quite considerably (Youngs et al 1977), depending on cultivar, climate, handling, etc. The drying process on the farm was imitated in the laboratory to measure its effect on lipase activity.

The structural changes in the fat deposits and the lipid membranes attributable to mechanical treatment, as well as the chemical modifications during heat treatment, all contribute to expose or protect the fat components in oats. This is the basis of the design of some steps in the hydrothermal treatment of oats aimed at the inactivation of lipase. The development of rancidity occurs during the storage of the final oat product. At that stage, the lipase is mostly inactivated because of the process heat treatment. This does not mean that the concentration of free fatty acids cannot increase, but it means that the effects of lipase will have implications later on in the production process. Laboratory heat inactivation studies were performed to follow the changes in lipase activity.

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MATERIALS AND METHODS

Plant Material

Oat samples were obtained from specially cold stored lots prepared by the farmers organization (Västsvenska Lantmän, Lidköping, Sweden). For the study of lipase distribution, different fractions of the oat grains were prepared by Bengt Bodin at the Swedish University of Agricultural Sciences, Uppsala.

Chemicals

Chemicals were of analytical grade, and a commercial preparation of wheat germ lipase (Sigma Chemical, St. Louis, MO) was used as a reference in the lipase measurements.

Assay for Lipase Activity

Oat grains were disintegrated in a commercial coffee mill, and the samples were extracted after 10 min with $0.2M$ Tris-HCl buffer, pH 8.5. Lipase activity was measured by the fluorescence method used by Heltved (1984), with 4-methylumbelliferyl heptanoate (Serva AG, Heidelberg, Germany) as a substrate. The samples were diluted in a $0.2M$ Tris-HCl buffer, pH 8.5. The substrate solution was $0.01M$ dissolved in 95% ethanol. In the cuvette, 3 ml of the buffer containing the sample was mixed with 25 μl of substrate solution. The reaction was followed at 20°C, the excitation wavelength was 330 nm, and the emission wavelength was 450 nm. The measurements were performed on a Shimadzu RF-5000 spectrofluorophotometer. One unit of lipase activity was defined as the amount of enzyme releasing one micromole of free fatty acid per hour. This was calculated with pure 4-methylumbelliferone as standard and compared with titration data.

Assay for Peroxidase Activity

Peroxidase activity was measured by spectrophotometry according to Shindler and Bardsley (1975). The oxidation of the substrate 2,2′-azino-di-(3-ethyl benzthiazolin sulfonate) (ABTS) was followed as the increase in absorbance at 412 nm. One hundred microliters of sample was mixed with 2.5 ml of 1 mM ABTS in $0.05M$ sodium acetate buffer (pH 5.0), and the reaction was started by addition of 100 μl of 2.5 mM H_2O_2. One unit of peroxidase activity was defined as the amount of enzyme necessary to oxidize one micromole of substrate per minute.

Heat Treatment

The laboratory heating experiments to study the inactivation of lipase were performed in a conventional and a microwave oven. The air-drying experiments to a defined water content were performed at the Swedish University of Agricultural Sciences.

RESULTS AND DISCUSSION

Lipase activity was assayed by fluorescence spectrophotometry, using an ester between the fluorescent probe 4-methylumbel-

liferone and heptanoic acid (Heltved 1984). This is a model substrate differing in structure from the neutral and polar lipids, which are the native substrates for the lipolytic enzymes. There are several reasons for using a fluorescent substrate, however. The method is very sensitive and rapid, it tolerates turbid samples, and it avoids extraction with organic solvents. We have compared this method with the use of radioactive-labeled substrates and the copper-soap method and have chosen the fluorescent substrate method to obtain an estimate of lipolytic activity in samples from different parts of the oat kernel and taken from various processes. In a comparison with the copper-soap method, Miller et al (1989) found the fluorescence assay less specific; however, it showed fairly good correlation to the former method in screening a large number of cultivars. Four fluorescent substrates varying in chain length in the carboxyl acid moiety were compared in our laboratory. The heptanoic acid ester was chosen as being the most suitable with regard to sensitivity and solubility.

Distribution of Lipase in the Oat Kernel

The distribution of lipase activity in the different parts of the oat grain shows that the lipase activity is highest in the embryonic part (Fig. 1). The lipase activity in the aleurone layer also was relatively high, and in considering the total amount, the aleurone layer makes the largest contribution to total lipase activity. Lipase activity is high in the parts of the kernel where the tissues are metabolizing fat, i.e., in the aleurone layer and the embryo. The lipase activity in the starchy endosperm is comparatively low.

Lipase Distribution in Flour Samples from Fractionated Milling

In Table I, the lipase and peroxidase activities for flour fractions are presented. The flour samples were prepared by fractionated milling with or without prior heat treatment (steaming). The bran fraction showed the highest lipase activity. This is in good agreement with the results from the more carefully prepared fractions of the oat kernel, shown in Figure 1. However, in this preparation of flour fractions, it is probable that some parts of the embryo also will be included with the outer fractions.

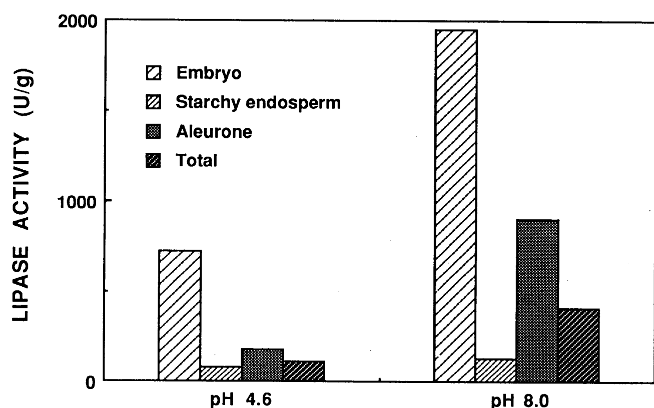

Fig. 1. Distribution of lipase activity in different parts of the oat kernel at two pH levels.

TABLE I
Lipase and Peroxidase Activities in Samples from Fractionated Milling

Sample	Yield (kg)	Lipase (U/g)	Peroxidase (U/g)
Without steam preparation			
Bran	95	1,120	0.73
Flour 1[a]	88	480	1.12
Flour 2[a]	200	600	1.13
With steam preparation			
Bran	135	0	0.02
Flour 1	147	20	0.04
Flour 2	233	20	0.04

[a] Flour 1 and 2 represent outer and inner fractions obtained by fractionated milling.

pH Dependence and Changes During Growth

Although the lipase activity at acid pH remained relatively constant during three days of growth, the lipase activity at neutral and alkaline pH increased considerably (Fig. 2).

There are many different types of lipolytic activities in plants with different specificities and pH optima. The neutral and alkaline lipase activity has been reported to be effective in hydrolyzing not only triglycerides but also di- and monoglycerides (Lee and Hammond 1990) and more effective in hydrolyzing oleic acid than stearic acid (Piazza et al 1989). Its increased activity during growth might indicate that it is involved in fat metabolism in the growing embryonic tissues. The acid lipase activity should then not be so intimately correlated to the increased metabolic activity during growth. For the practical situation of oat processing, acid lipase activity existing before growth would be important, especially because it is also (to a considerable degree) situated in the superficial aleurone layer, giving raise to more exposed hydrolysis products for further steps in the rancidification.

The exact pH optima were not determined, because the activity was measured in the tissue, and the lipase enzymes were not purified and further characterized. The result of this pH screening of the lipase activity showed maximum lipase activity at neutral pH, in agreement with earlier studies (Martin and Peers 1953, Matlashewski et al 1982).

Lipase Activity in Different Cultivars

The lipase activity was measured in different cultivars ranging in fat content from 5 to 7%. There was no correlation between fat content and lipase activity and only slight variation between cultivars, as can be seen in Table II. However, there are reports in literature about greater differences in lipase activity between cultivars (Miller et al 1989, Lee and Hammond 1990).

Effects of Drying and Heat Treatment

As can be seen in Table I, the enzyme activities after steam treatment are barely detectable and indicate practically total inactivation.

There might be changes occurring in the lipase activity even during the postharvest handling on the farm. This is attributable to different climate conditions that require additional drying with

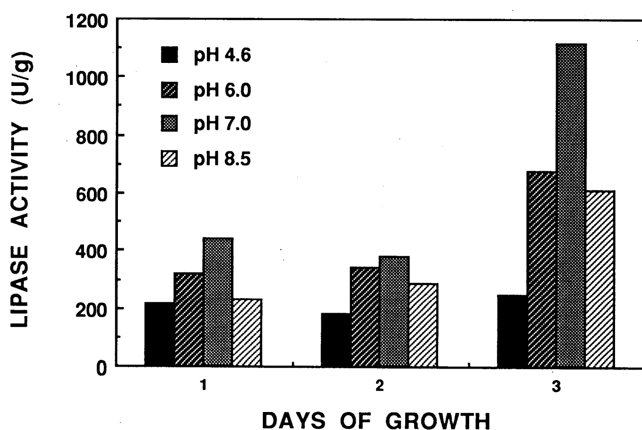

Fig. 2. Lipase activity at different pH levels in samples grown for one, two, or three days.

TABLE II
Lipase and Peroxidase Activities in Samples from Different Oat Cultivars

Cultivar	Lipase (U/g)	Peroxidase (U/g)	Fat[a] (%)
Vital	220[b]	0.72	5.04
Sang	268	0.77	6.02
Magne	247	0.94	7.10

[a] Percentage of dry matter.
[b] Data represent mean of two replicates.

TABLE III
Lipase and Peroxidase Activity[a] in Oat Samples Air-Dried at Different Temperatures

Temperature	Lipase (U/g)				Peroxidase (U/g)
	pH				
	4.6	6.0	7.0	8.5	
Ambient	214	374	740	304	15.1
40° C	186	398	500	252	16.8
60° C	106	186	200	154	15.5
80° C	56	62	60	44	15.3

[a] Mean value of duplicates.

heated air. Besides the mechanical treatment, the temperature reached during the drying and the duration of the drying process might vary considerably. To study the effects of the drying process on lipase activity, samples of oats were carefully harvested and kept in a moist environment where their water content was slowly raised to 30%, after which they were air-dried in an oven at different temperatures until a water content of 16% was reached. The results (Table III) indicate that lipase activity decreased by all drying treatments, but only at higher temperatures (60–80° C) did the lipase activity change considerably. As a comparison, the peroxidase activity was measured, showing its greater thermal stability.

A number of experiments have been performed in our laboratory that show the importance of air humidity in the inactivation of the lipase. Direct contact with water causes a much more effective heat transfer and thereby a more complete inactivation of the enzyme. When oat kernels were treated for 10 min with steam with a final sample temperature of 72° C, more than 70% of the activity remained; when kernels were immersed in water at 80° C, giving a final sample temperature of 80.5° C, there was only 2% residual lipase activity. This indicates that dry heat treatment in a process would have quite different effects on lipase activity and, therefore, on lipid stability than would wet heat treatment. The use of conventional or microwave heating showed no differences in this respect.

There is now increasing evidence for the importance of the release of free fatty acids for the properties of flour or other cereal products. For wheat, Galliard and co-workers have shown the effect on baking quality of lipid hydrolysis (Tait and Galliard 1988). This implies that in mixed products, the combined situation resulting from the properties of different components must be considered.

ACKNOWLEDGMENTS

We are grateful to the Västsvenska Lantman (Lidköping) for advice and practical handling of the oat samples and to Bengt Bodin, the Swedish University of Agricultural Sciences, Uppsala, for many interesting and enlightening discussions and for kindly preparing well-defined oat kernel fractions. The project was financed by the Swedish Council for Forestry and Agricultural Research.

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[Received July 17, 1991. Accepted January 22, 1992.]