Measurement of Lipase Activity in Single Grains of Oat (*Avena sativa* L.)[1]

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ABSTRACT

Lipase activity (LA) in individual oat grains was measured as the increase in free fatty acid (FFA) content by lipolysis of the endogenous lipid in a crushed sample of seed incubated at 38 C for 30 min. FFA content was determined by a modified copper soap method. The dough stage, about 2 weeks before normal harvest, showed the highest LA. Stored samples of mature grains showed about 45-50% loss of LA in 2 years.

INTRODUCTION

It is generally recognized that oat (*Avina sativa* L.) contains a lipase which is potentially more active than other cereal grains such as wheat, barley and rye (1). Commercially, oat lipase is inactivated by heat treatment (2). In our investigations on the use of oat and oat products, a simple, rapid method for the determination of LA in an individual seed, which could be used for screening varieties of oat for LA, was required. LA in oat products is generally measured by incubating the defatted, powdered sample with an external olive oil substrate and by titrating the released free fatty acids (FFA) with alcoholic sodium hydroxide (3). This procedure is not sensitive enough to be applied to single seeds of oats weighing 28-34 mg.

This paper describes the application of a microquantitative procedure for lipase activity in a single crushed seed of oat using a modified copper soap method. The procedure is also applicable to powdered composite samples of up to 500 mg. LA was measured in oat samples preharvest and after storage for up to 2 years.

MATERIALS AND METHODS

Oat samples (HINOAT) were grown at the Central Experimental Farm, Agriculture Canada, Ottawa. The colorimetric copper soap method described by Shipe et al. (4) for milk was adopted for the determination of FFA in oats. Single grains of oat (28-34 mg) were soaked for 1 min with 40-60 μL of 1 N HCl and then extracted in a polytron (Brinkmann, PJ1oST) with 10 mL of a mixture of chloroform/heptane/methanol (49:49:2, v/v/v) (CHM) for 2 min and allowed to settle. After filtration, the residue was reextracted with 10 mL of CHM for 1 min; the contents were then transferred to a filter and washed with CHM to a total volume of 50 mL. Five mL of the extract was mixed with 2 mL of cupric nitrate-triethanolamine reagent (4), centrifuged at 2,000 x g for 15 min, and 4 mL of the upper layer was mixed with 0.1 mL of sodium diethldithiocarbamate solution (4). The absorbance was measured at 440 nm within 1 hr.

Powdered samples of oat (up to 500 mg) were similarly extracted in a polytron with adjustments in the addition of acid and in dilutions of the sample. FFA content was expressed as mg/100 g sample.

To determine LA using the endogenous triglyceride substrate, an individual grain was soaked in a required volume of buffer in a polytron tube to give a moisture content of 50-100% and was crushed with a glass rod to ensure complete cell fracture. The sample was then incubated for 30 min at 37-38 C in a water bath, acidified with 0.1 mL of 1 N HCl and the FFA extracted in the solvent mixture using a polytron as described earlier. The same procedure of incubation and extraction was applied to a powdered sample of up to 500 mg. LA was expressed as units of FFA in mg/100 g of sample hydrolyzed in 30 min.

RESULTS AND DISCUSSION

The method uses the endogenous lipid as substrate in the determination of lipase activity in single seeds of oat. Oats contain 4.6-11.6% lipid of which 50-56% are triglycerides (5). The optimal pH (7.5) and temperature (37.5 C) were established in studies on purified oat lipase (6). Complete extraction of FFA by CHM was not possible without acidification with HCl; presumably some FFA was bound or entrapped.

Figure 1 shows the development of FFA over a period of 16 hr after inhibition of buffer and maceration in 2 oat samples. Number 1 was a freshly harvested sample whereas number 2 was a 2-year-old sample. Each value of FFA represents an average of 5 single grains. Maximal development of FFA occurred in the first 30 min of incubation at 37.5 C with no further significant change in FFA up to 16 hr. To maintain a moisture content of over 30% during the 16-hr period, it was necessary to add 50 μL of water each 1.5 hr.

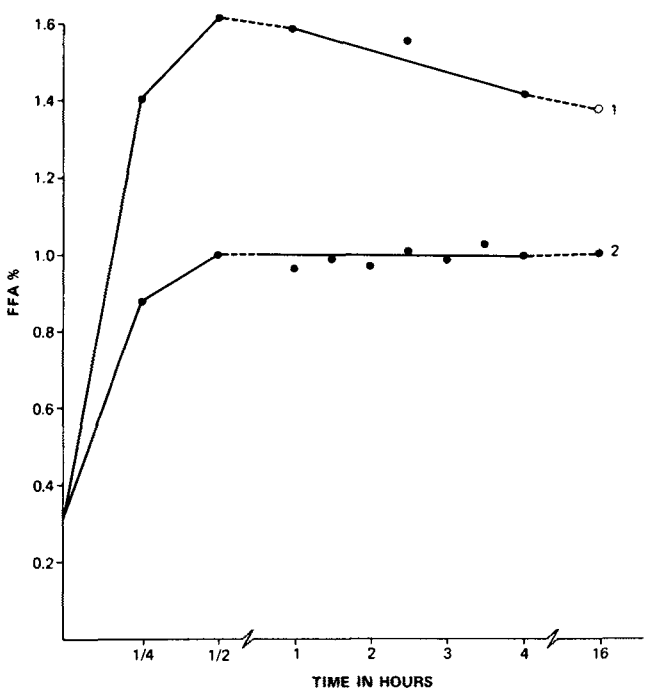

FIG. 1. Development of FFA during incubation at pH 7.5 up to 16 hr. 1, freshly harvested sample; 2, two-year-old sample.

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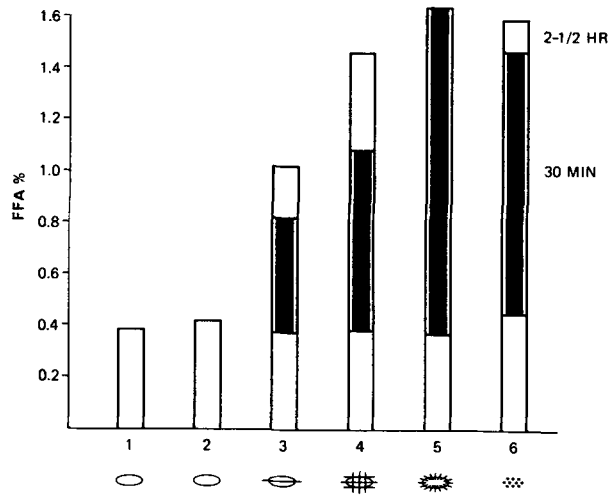

FIG. 2. Effect of cell fracture on the lipase activity in oat. 1, intact whole groat; 2, whole undamaged groat soaked in buffer at 38 C for 30 min; 3, groat spliced in 2 halves at the crease; 4, groat cut in segments; 5, groat mascerated; 6, powdered sample. Lower open bar represents initial FFA content, solid black bar represents development of FFA in 30 min, top open bar represents further development of FFA up to 2.5 hr.

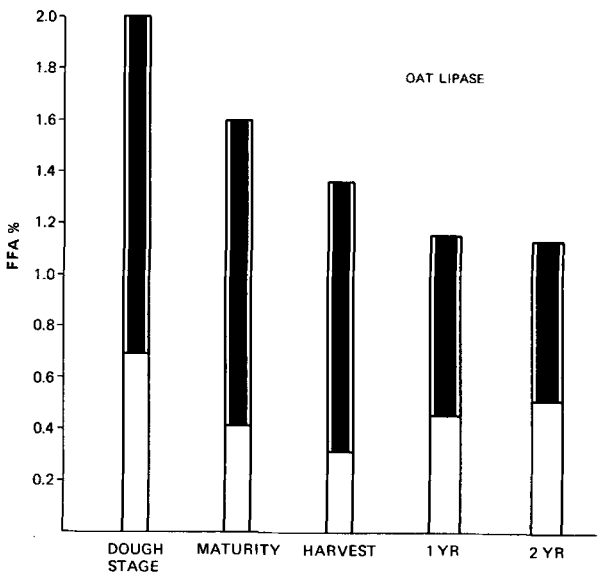

FIG. 3. Lipase activity in HINOAT oat during development and storage. Open bar represents initial FFA content. Solid black bar represents development of FFA during 30 min of incubation.

The lipase in oat is localized in the aleurone layer of the grain whereas the lipid is dispersed in the sub-aleurone layers and the endosperm. A complete cell fracture was essential for the lipase to act. The importance of cell fracture was demonstrated by determining the development of FFA during 30 min of incubation in undamaged, intact grains and in those sliced or crushed. Sample 1 (Fig. 2) was an intact whole grain. Sample 2 was an intact whole grain soaked in buffer and incubated for 30 min. No development of FFA was noted. Sample 3, sliced in half at the crease, showed an increase of 450 mg of FFA/100 g sample. Sample 4, cut into segments by 3 horizontal and 3 vertical cuts, shows an increase of 700 mg whereas sample 5, crushed with a glass rod to completely mascerate the grain, shows an increase of 1,230 mg. Sample 6 was powdered separately in a laboratory coffee grinder. The upper open bar (samples 3, 4 and 6) represents the increase in FFA over 2 hr following the initial 30 min of incubation. No further increase was noted in sample 5 up to 2.5 hr.

Figure 3 shows the variation in lipase activity in HINOAT oat during development and storage. The dough stage, about 2 weeks preharvest, showed highest lipase activity of 1,300 units, which was not significantly altered up to harvest with 1,180 units at maturity. Freshly harvested sample showed a lipase activity of 1,000 units. The stored samples showed about 45-50% loss of lipase activity in 2 years.

Variation in the FFA content in individual grains within a sample of oat was determined by analyzing separately 8-15 groats. The mean values (mg/100 g) with standard deviation of 2 different samples of oat were 321:1 ± 41.5 and 455.4 ± 28.9. The FFA determinations were made on undamaged oat groats selected by examination under a microscope, as damaged samples invariably had higher values of FFA. Similarly, the values in FFA content of 2 samples following incubation as described were 1312.6 ± 66.2 and 1929 ± 102.3. Because of variation among individual grains within the sample, it was necessary to take 2 halves of 2 grains separately, one for control and the other for determination of FFA development following incubation. The 2 halves, either sliced longitudinally at the crease or into proximal and distal parts in relation to the germ, showed similar FFA contents and lipase activity, indicating an even distribution of FFA and the lipase enzyme. Localization of the lipase in the outer aleurone layers of the grain (7) was confirmed by scraping the outer layers of individual grains using a dentist's drill (Heathco) and by carefully removing the inner endosperm using the same tool. Ninety percent of the lipase activity was found in the outer aleurone layers as calculated on the basis of 100 g initial sample weight. The endosperm showed less than 10% of the total activity.

The method has a potential application in determining the extent of lipid deterioration and keeping quality of cereal and oilseed products, and in genetic development of oats with reduced lipase activity.

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