Lipase Activity in Oats During Grain Maturation and Germination[1]

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ABSTRACT

Lipase activity (glycerol tri[1-^{14}C]oleate hydrolysis) was assayed in developing oat (*Avena sativa* L.) grains from 2 to 44 days postanthesis. In three cultivars (Hinoat, Sentinel, OA 424-1), activity was detected throughout kernel development. On a dry weight basis, a first peak of lipase activity was found during the first nine days postanthesis in all three cultivars. Sharp drops in activity per gram, reflecting rapid changes in kernel dry weight, followed. A second peak occurred at 23 days (Hinoat) and at 30 days (Sentinel, and OA 424-1). Lipase activity per 100 kernels increased as the grains matured, and reached maximum levels between 23 (Hinoat) and 30 days (Sentinel and OA 424-1). Peaks of activity coincided with maximum fresh and dry weights. Activity declined as the grains dried to harvest maturity. Final activities were 11.1 (Hinoat), 14.8 (Sentinel), and 3.4 (OA 424-1) μmol/min per 100 grains; final levels per gram of dry weight were 5.0, 7.6, and 1.1 μmol/min g, respectively. OA 424-1 had significantly lower levels. In Hinoat grains, classified into six groups of uniform developmental stage, lipase activity per 100 kernels was maximum in late dough, but was moderately high in milky and early dough stages. During germination of Hinoat, a 60% increase in activity occurred in the first 12 hr following the start of imbibition. Activity in the embryo, negligible at 24 hr, increased during the next three days. Activity in the rest of the kernel decreased gradually.

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Lipases catalyze the hydrolysis of triglycerides, diglycerides, and, in some cases, monoglycerides. Lipolytic enzymes have been described for several plants. Lipase is barely detectable in the mature seeds of many cereals (Tavener and Laidman 1972, Widhe and Onselius 1949) and oilseeds (Huang and Moreau 1978, Theimer and Rosnitschek 1978), and appears only after germination has started (Huang and Moreau 1978, Lin and Huang 1983, Tavener and Laidman 1972, Theimer and Rosnitschek 1978). In other plants, such as rice (Shastry and Rao 1976), oats (Martin and Peers 1953), peanut (Sanders and Pattee 1975), and faba bean (Dundas et al 1978), lipase activity is found in the ungerminated seeds and is consequently of considerable commercial importance. After bruising (Sahasrabudhe 1982, Welch 1977) during shelling or dehulling, or in untreated flour from these seeds, the enzymes can become active, causing fatty acid (hydrolytic) rancidity or soapy flavors (Dundas et al 1978, Widhe and Onselius 1949).

Lipase activity was observed in oat kernels at the "milky" stage of development and in mature grains (Martin and Peers 1953). Detailed information on lipase activity throughout oat caryopsis development is not available. In wheat (Tavener and Laidman 1972) and rapeseed (Theimer and Rosnitschek 1978), lipase activity, initially undetectable, increases after the start of germination. Widhe and Onselius (1949) reported no difference in lipase activity between ungerminated and four-day germinated oats. In contrast, Janecke (1951) described an increase in lipase as germination proceeded. Neither study compared activity in embryo and de-embryonated grains. Recent interest has been shown in utilization of oat protein (Youngs 1974), lipid (Sahasrabudhe 1979), starch (Paton 1981), and gums (Wood et al 1978). Because the products of lipolysis (fatty acids, mono-, diglycerides) would interfere with oat fractionation, and heat treatment to inactivate lipase could also alter properties of the kernel components (starch damage, protein denaturation), interest in oat lipase has been renewed (Matlashewski et al 1982). Fractionated oat kernels could be used at maturity or earlier in kernel development and also after sprouting (germination). Detailed information of lipolytic activity during kernel development and germination is therefore required.

This paper describes the changes in lipase activity in three oat cultivars during grain maturation as related to the stage of development and to lipid and nitrogen content of the caryopses. Lipase activity was also followed in the embryo and in the rest of the grain during germination.

MATERIALS AND METHODS

The three oat (*Avena sativa* L.) cultivars used in this study were chosen for their range in yields and in protein and lipid contents. Hinoat has very high protein (over 20%) and about 5% lipid, but is low yielding; Sentinel has high protein (17–19%) and low lipid (3–4%), and is quite high yielding; OA 424-1 has medium protein (16%) and low lipid (3–4%), but is high yielding.[5] Values for protein (N × 6.25) and lipid are given on percent groat dry weight basis. Triplicate four-row plots (3 m long, 30.5 cm between rows) were sown in a randomized block design at the Ottawa Research Station

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field plots in May of 1980. The two central rows were used.

Hinoat was also grown in the greenhouse (16-hr photoperiod; 26° C, light; 16° C, dark). The seeds were germinated in vermiculite, and single plants (leaves 8–10 cm long) transferred to 13-cm pots of soil–sand–vermiculite (1:1:1). The pots were fertilized at weekly intervals with a commercial preparation (Green Cross Supergro) containing N:P:K (20:20:20) and micronutrients. Heading and maturation (in days from seeding) were the same for greenhouse and field-grown Hinoat. Final groat weight and nitrogen contents were similar; dry weight of groats was 2.55 g per 100 grains (greenhouse-grown) and 2.35 g per 100 grains (field material); nitrogen content was 100 and 97 mg per 100 grains, respectively. Values were similar to previously published values for field-grown Hinoat (Sahasrabudhe 1979).

Samples consisting of 15 panicles were collected at intervals from 2 to 44 days postanthesis. Glumes and hulls were removed by hand, samples of the developing kernels (caryopses) taken for fresh and dry weights, and the remaining kernels freeze-dried and stored at −20° C until required. For lipase determinations, 50–100 freeze-dried kernels were ground to a fine powder in liquid nitrogen with a mortar and pestle. The samples were defatted by Soxhlet lipid extraction in petroleum ether (40–60° C) for 1 hr (Matlashewski et al 1982).

At any one harvest date, kernels at two or three stages of development were present on each panicle. Six stages were distinguished: stage 1, kernels 2–3 mm long, green, anthers still attached; stage 2, kernels 3–4 mm, obovate, green, milky; stage 3, 8–10 mm, oval, green, early dough; stage 4, 8–10 mm, becoming yellow, dough; stage 5, completely yellow, hard; stage 6, light-brown, dry. To measure lipase activity at each stage of development, samples (50–100 kernels) were selected at uniform stages of development from greenhouse-grown Hinoat.

Lipase activity was assayed by measuring rate of release of ^{14}C-labeled fatty acids from glycerol tri[1-^{14}C]oleate. The method followed was similar to one used for bacterial lipase (Nantel and Proulx 1973), castor bean lipase (Borgstrom and Ory 1970), and rapeseed lipase (Rosnitschek and Theimer 1980), and was adapted for oat flour suspensions and extracts (Matlashewski et al 1982). Details of the assay procedure, fatty acid extraction and separation, and determination of radioactivity were described in an earlier paper (Matlashewski et al 1982). Samples of 50–100 kernels were used for each analysis. Data are expressed per 100 grains and per gram of dry weight where appropriate.

Dehulled oats (Hinoat) were germinated on moist Whatman No. 1 filter paper in the dark at 25° C for up to four days. Samples taken earlier than 24 hr were kept whole. In samples after 24 hr, the developing embryo (embryonic axis plus scutellum) was dissected from the rest of the kernel. Both parts—embryo and bran-endosperm—were freeze-dried, ground, and defatted. Samples of 50–100 kernels were assayed for lipase activity.

Lipid content of the developing grains was quantified after extraction in chloroform–methanol (2:1) as described by Sahasrabudhe (1979). Nitrogen was determined by micro-Kjeldahl analysis (AOAC 1974).

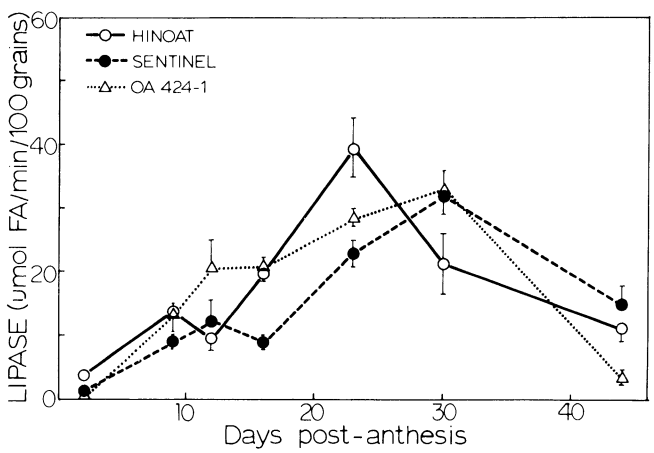

Fig. 1. Changes in lipase activity in developing grains of three field-grown oat cultivars.

TABLE I
Dry Weight and Moisture Content (percent of fresh weight) Determined in the Caryopses of the Oat Cultivar During Kernel Maturation from Two to 44 Days Postanthesis

	Hinoat		Sentinel		OA 424-1	
Days Post-Anthesis	Dry Wt[a]	Moisture (%)	Dry Wt[a]	Moisture (%)	Dry Wt[a]	Moisture (%)
2	0.04	85.4	0.06	62.2	0.06	72.2
9	0.56	67.2	0.19	81.6	0.37	76.1
12	0.68	66.9	0.50	70.2	0.67	71.0
16	1.48	51.3	0.78	64.6	1.30	60.0
23	2.30	30.4	1.85	47.8	2.53	47.3
30	2.22	12.1	1.95	33.3	3.04	33.6
44	2.23	15.1	1.95	18.8	3.19	19.3

[a]Grams per 100 grains.

RESULTS AND DISCUSSION

Lipase activity, measured by the release of fatty acids from glycerol tri[1-^{14}C]oleate, was detected in kernels from field-grown oats throughout kernel development. Total activity per 100 kernels increased during development to a maximum and then decreased as the grains completed maturation. The three cultivars showed similar changes (Fig. 1). In the earliest maturing cultivar, Hinoat, the maximum lipase activity (39 μmol of fatty acid [FA] released per minute per 100 grains) was detected at 23 days postanthesis; in Sentinel and OA 424-1, maxima (31.8 μmol/min and 32.6 μmol/min, respectively) were at 30 days postanthesis. In all three cultivars, the peaks occurred when the grains had just reached maximum dry weight; moisture content was 30–35% (Table I). Lipase was still present in the harvest-dry grains, although at much reduced levels. Final levels for Hinoat, Sentinel, and OA 424-1 were 11.1, 14.8, and 3.4 μmol/min per 100 grains, respectively, with OA 424-1 significantly lower ($P = 0.05$).

Expressed on a dry weight basis, lipase had two peaks of activity. The first peak, the largest, occurred within the first nine days after anthesis. Activities were 105.4 (Hinoat), 45.6 (Sentinel), and 35.4 (OA 424-1) μmol of FA/min/g of dry weight. A sharp drop in activities (to low levels, days 15–20) then occurred in all three cultivars. A second, smaller peak occurred at 23 days postanthesis (Hinoat, 17.0 μmol/min/g) and at 30 days (Sentinel, 16.3 μmol/min/g; OA 424-1, 10.73 μmol/min/g). Changes in lipase activity were similar for all three cultivars. Activities then declined to final levels in the harvest-dry grains of 5.0 ± 0.8 (Hinoat), 7.6 ± 1.5 (Sentinel), and 1.1 ± 0.2 (OA 424-1) μmol/min/g of dry weight. Activities for OA 424-1 were again significantly lower ($P = 0.05$). These large changes in lipase activity (expressed per unit dry weight) that occurred in the early stages of development (up to day 20) probably reflect the rapid changes in starch and protein contents of the kernels during this period (Peterson et al 1975).

Kernels from several panicles at each harvest date of the field-grown material were sorted into groups of uniform developmental stage and the percent at each stage noted. At the peaks of lipase activity shown in Fig. 1, the samples used contained kernels that were a mixture of late dough, and physiologically mature kernels (stages 4 and 5). In the greenhouse-grown oats, maximum fresh weight occurred at stages 4 (dough) and 5 (physiological maturity), and maximum dry weight at stages 5 (Fig. 2A). Lipid and nitrogen content increased gradually throughout the development (Fig. 2B). Lipase activity was maximum at stages 4 and 5 (Fig. 2C), confirming the results from the field-grown material (Fig. 1).

Earlier studies on oat lipase reported that lipase was present at the "milky" stage (Martin and Peers 1953). Our results demonstrate in greater detail the changes in lipase activity that occur during grain maturation. The decrease in activity (on both per 100 kernels and per gram, dry weight basis) as the kernels lose moisture may

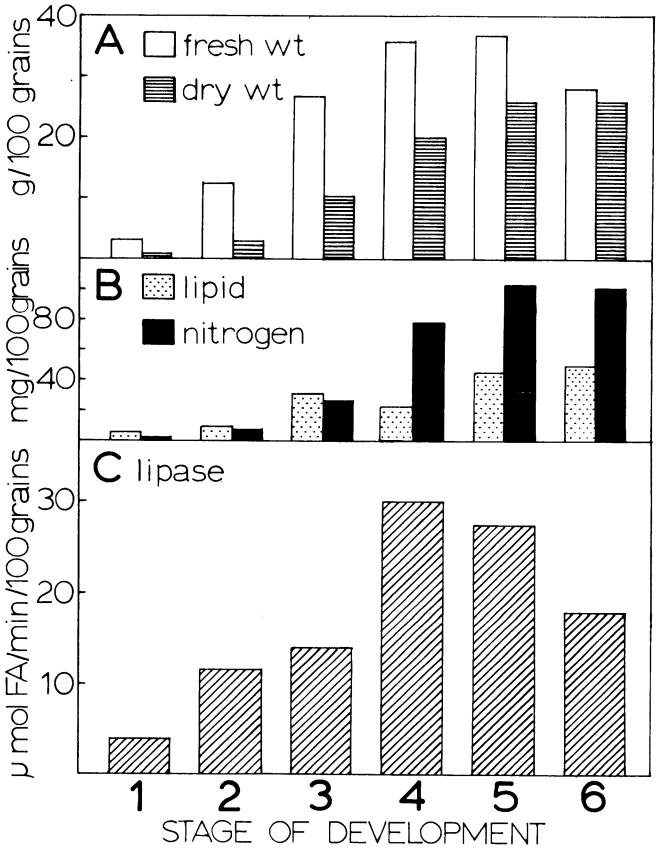

Fig. 2. Changes in Hinoat caryopses at six stages of development. **A,** fresh and dry weights per 100 grains; **B,** lipid and nitrogen content per 100 grains; **C,** lipase activity per 100 grains.

reflect loss of enzyme. On the other hand, it could also be caused by an inhibitory compound, increasing as the grain matures. Naturally occurring lipase inhibitors have been described from soybean (Satouchi et al 1974) and green peppers (Kim et al 1977). Alteration (by cell death and compression) of the outer layers of the caryopsis during grain maturation, however, may affect the extractability (availability) of the enzyme, located near the surface of the kernel (Martin and Peers 1953).

In germinating oats (Hinoat), lipase activity, initially 7.5 µmol FA released per minute per 100 grains (whole groats), increased by about 60% during the first 12 hr of imbibition (Fig. 3). In the embryo, only traces of lipase activity were detected at 24 hr after the start of germination, but activity increased during the four days to about 4–5 µmol FA released per minute per 100 grains. Activity in the rest of the kernel decreased with time from a maximum of about 12 µmol/min to 3.5 µmol/min per 100 grains after four days (Fig. 3).

Widhe and Onselius (1949) reported that lipase activity was the same in ungerminated as in four-day germinated oats. In a more detailed study, Janecke (1951) showed an increase in activity with a peak at 8 hr of germination followed by a decrease; levels after three days of germination were slightly lower than those in the ungerminated grains. Both of these studies used whole grains.

In our study, we separated embryos from the rest of the kernel and showed that all of the changes in lipase activity in the first few hours of germination occurred in the bran-endosperm part. Most (80%) of this lipase is in the outer, bran layers with little activity in the endosperm tissue (Urquhart et al 1983). After 24 hr, lipase increased in the embryo. Lipase activity in whole, ungerminated kernels (7.5 µmol/min per 100 grains) was almost identical with the summed activities of four-day germinated embryos plus bran-endosperm parts (7.2 µmol/min per 100 grains). These results agree with the zero and four-day values of Widhe and Onselius (1949), and changes in total lipase activity were similar to those in the study by Janecke (1951). Increases in lipase activity during early

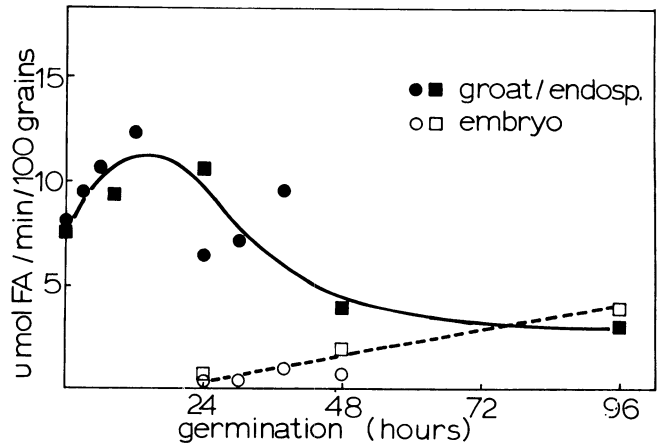

Fig. 3. Lipase activity in embryo and bran-endosperm part of Hinoat kernels during four days of germination at 25°C in the dark. ○, □ = embryo; ●, ■ = whole kernel (0–24 hr) and bran-endosperm (24–96 hr). Circles, experiment 1; squares, experiment 2.

germination are also found in wheat (Tavener and Laidman 1972) and in oilseeds (Lin and Huang 1983).

Although the main function of oat lipase is presumably to hydrolyze storage triglycerides during germination, the high values for lipase at stage 4 (30 µmol/min per 100 grains, Fig. 3) raise the question of a physiological role for the enzyme during grain development. Peanut lipase also increases per seed during development (Sanders and Pattee 1975). Changes in triglyceride composition during seed maturation have been noted in both safflower (Ichihara and Noda 1980) and oats (Brown et al 1970). In safflower, linolenic acid decreases in all lipid classes (including triglycerides) during maturation (Ichihara and Noda 1980); its complete disappearance from mature seeds may involve lipase. In oats, decrease in percent linolenic suggests lipolytic activity, but actual amounts (milligrams of linolenic per grain), calculated from the data of Brown et al (1970), are constant; hence lipase is not implicated. The observed increase in lipase activity during grain development may therefore reflect merely the increasing synthesis of the enzyme and have no physiological role during maturation.

This work provides new, detailed information on the changes in oat lipase activity during grain development and germination. The presence of active oat lipase throughout grain development and at increased levels during germination indicates that, if untreated oat groats, meal, or groat fractions are utilized, lipolytic activity and its products must be considered.

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