NOTE

Localization of Lipase Activity in Oat Grains and Milled Oat Fractions[1]

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ABSTRACT

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Lipase activity was measured in oat grains by monitoring release of labeled fatty acids from glycerol tri[1-^{14}C]oleate. In oats germinated for 24 hr, only traces of activity were in the embryo; almost all of the lipase was in the other tissue. Fine dental drills and abrasive disks were used to selectively remove the embryo, embryo plus endosperm, and bran. Most of the lipase (80%) was in the outer bran layers. Analysis of four milled fractions, ie, bran, first shorts, second shorts, and flour from dehulled oats showed that the bran fraction and the first shorts had highest lipase activity. The percent distribution of total lipase in oats at 12% moisture in the four fractions was 69.2, 5.1, 24.7, and 1.0, respectively. In freeze-dried oats at 7% moisture, it was 83.3, 7.7, 6.2, and 2.8, respectively. The bran fraction accounted for 65% of the dry weight.

Many dry, ungerminated plant seeds have little or no lipase activity (triglyceride acylhydrolase, EC 3.1.1.3). Activity normally appears only during germination (Huang and Moreau 1978, Tavener and Laidman 1972). In some seeds, including faba bean (Dundas et al 1978) and oats (Hutchinson and Martin 1952), lipase is present in dry grains. Commercial oat-milling processes include heat treatments (about 110° C for 2 hr) to inactivate the enzymes, but the potential for lipolysis leading to fatty acid rancidity or off-flavors in damaged seeds or in untreated oat flour or oat fractions is still high (Dundas et al 1978, Hutchinson and Martin 1952, Widhe and Onselius 1949). Interest has been shown in utilizing oat protein (Youngs 1974), lipid (Sahasrabudhe 1979), starch (Paton 1981), and gums (Wood et al 1978) for food or industrial use. Since the products of lipolysis would interfere with oat fractionation, interest in oat lipids (Sahasrabudhe 1979) and in oat lipase (Matlashewski et al 1982) has been renewed.

Earlier studies on oats showed that lipase was present in the outer layers of the kernels and could be eliminated by a technique of wet-scrubbing (Martin and Peers 1953), or by soaking whole groats in 1*N* HCl (Youngs 1978). We investigated in greater detail the localization of lipase activity in oat kernels and the distribution of lipase activity in four milled fractions obtained from an Ottawa Research Station experimental mill.

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MATERIALS AND METHODS

Oats (*Avena sativa* L. cv. Hinoat) were obtained from the Ottawa Research Station, Agriculture Canada, and stored with hulls intact at −20° C until required.

Germination. Oat grains (50 per sample) were dehulled by hand and allowed to germinate for 24 hr on moist Whatman no. 1 filter paper in the dark at 25° C. Embryos (scutellum and embryonic axis) were removed by dissection, and both parts were freeze-dried and ground finely in a mortar and pestle with liquid N_2. The resulting powder was defatted by Soxhlet lipid extraction for 1 hr with petroleum ether at 40–60° C (Matlashewski et al 1982).

Dissection of Dry Grain. In samples of hand-dehulled oat grains, ball-headed, dental surgical drill bits (1.5 mm in diameter) were used to remove embryo only, and embryo plus endosperm, leaving a shell of bran (aleurone and pericarp). Dental surgical abrasive disks were used to remove the bran, leaving the endosperm. Treated kernels (50 per treatment) were ground in liquid N_2 in a mortar and pestle, and the powder defatted.

Milling. Dry, ungerminated oats were dehulled in an impact dehuller (Ottawa Research Station) and stored at −20° C until required. Samples (200 g) were milled with the Ottawa Research Station experimental mill (Kemp et al 1961). Two wire mesh screens (24 wire, 0.869-mm mesh opening, and 75 × 75, 0.244-mm mesh) and one silk screen (10XX silk, 0.140-mm mesh) separated the milled material into four fractions. These were: particles larger than 0.869 mm in diameter; particles 0.244–0.869 mm; particles 0.140–0.244; and particles smaller than 0.140 mm. With milled wheat samples, these fractions are called bran, first shorts, second shorts, and flour, respectively. With oats, the distribution of bran and endosperm tissue among these four fractions was different. For simplicity, the terms bran fraction, shorts, and flour fraction were retained, but the composition of each may be different from the equivalent fraction from milled wheat.

Dehulled oats had 12% moisture content; oat samples were also freeze-dried to 7% moisture content before being milled. Material recovered in each fraction was weighed and percent recovery in the four fractions calculated. Samples of each fraction were further ground in liquid N_2 and defatted as above.

Lipase Activity. Samples of defatted powder were suspended in 0.05 *M* Tris-HCl buffer, pH 7.5, containing 1% Triton-X-100, 0.2% benzene, and 10 m*M* glycerol tri[1-^{14}C]oleate (0.5 μCi/mmol) in a ratio of 1 g of powder per 10 ml of buffer. Lipase activity was measured by following the release of ^{14}C-oleic acid (labeled in the 1-carbon position) as previously described (Matlashewski et al 1982).

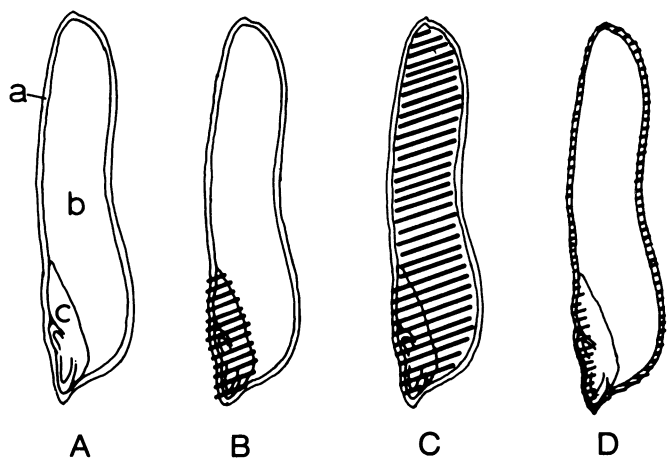

Fig. 1. Selective removal of parts of the oat kernel. **A,** intact kernel, longitudinal cross section: a = bran, b = endosperm, and c = embryo (germ); **B,** removal of embryo; **C,** removal of embryo plus endosperm; **D,** removal of bran. Shaded areas are portions removed.

RESULTS AND DISCUSSION

Lipase activity in defatted flour suspensions prepared from ungerminated whole oat kernels was 7.77 ± 0.32 μmol fatty acid hydrolyzed per minute per 100 kernels. After 24 hr of germination, the kernel had absorbed water and swelled. The embryo (embryonic axis and scutellum) was then easily removed from the rest of the grain. Values for lipase of 0.37 ± 0.13 μmol/min per 100 kernels for the embryo and 8.33 ± 0.61 μmol/min per 100 kernels for the remaining kernel tissue indicated that little activity was in the embryo at 24 hr of germination; of the total lipase activity (8.33 + 0.37 μmol/min per 100 kernels), 85% was therefore in the remaining kernel tissue. Lipase activity of the whole kernel at 24 hr was higher (8.70 μmol/min per 100 kernels) than that in the dry, ungerminated grain (7.77). Janecke (1951) reported an increase of lipase activity in whole oats during early germination.

When embryo alone or embryo plus endosperm was removed (Fig. 1B,C), there was little change in total lipase activity of the remainder of the kernel compared with the intact control (Fig. 2). These data confirm that little lipase activity occurred in the embryo in the dry, ungerminated state and show that little lipase was present in the endosperm removed by this treatment. When the outer layers of oat caryopses were removed by abrasion (Fig. 1D), the remaining endosperm showed little lipase activity, confirming that most of the lipase was in the bran layer.

Earlier studies showed that lipase was present in the outer layers of the oat kernel (Hutchinson et al 1951) and could be removed by a wet-scrubbing technique (Martin and Peers 1953). In these studies, however, the embryo and bran were not separated from the remaining kernel tissue, although the germ half had lower lipase activity than the distal half.

With the Ottawa experimental mill, oats yielded a bran fraction of 65% (Fig. 3A). Yield of flour (particles less than 0.140 mm) was low, only 3% from 12%-moisture-content groats and 12.5% from 7%-moisture groats. By comparison, a hard red spring wheat such as Neepawa on the same mill yields 70–75% flour (Kemp et al 1961). The large amount of material in the bran fraction was a result of the sticky, lipid-containing endosperm adhering to the bran tissue. Most of the lipase activity, 70–80%, was recovered in the bran

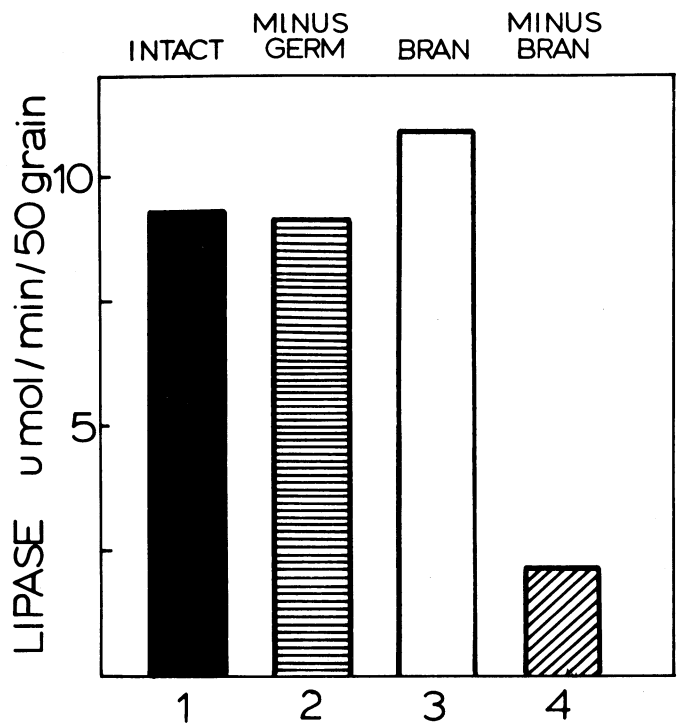

Fig. 2. Lipase activity expressed as μmol free fatty acid release per min per 50 grains in intact grains (1) and treated (2–4). Treatments are those shown in Fig. 1.

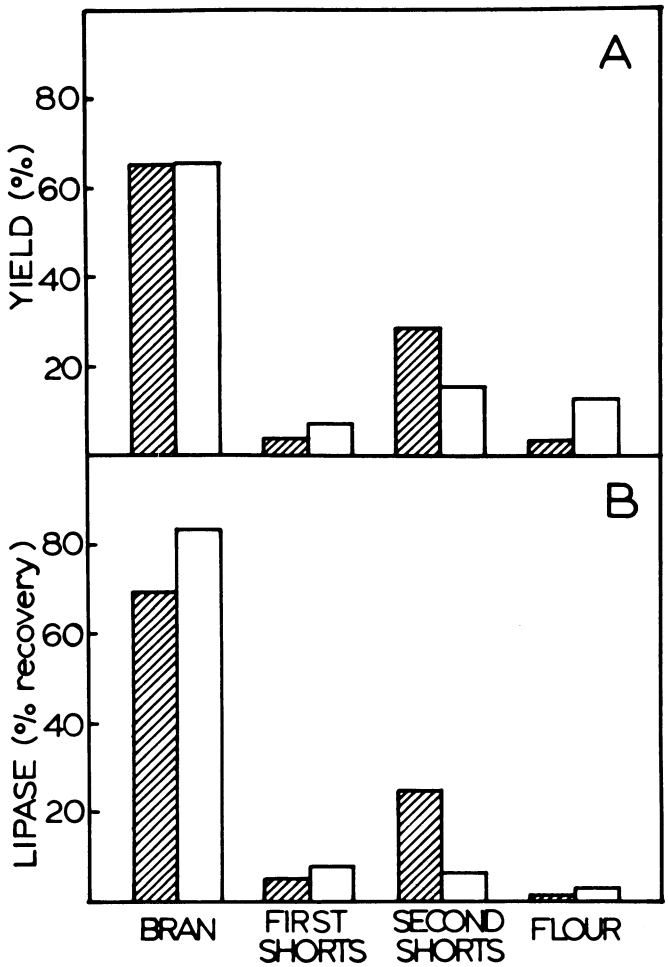

Fig. 3. Recovery of lipase activity in fractions of dehulled oat kernels milled on the Ottawa micro mill. **A,** yield of milled fractions; **B,** percent recovery of lipase. Shaded bars, kernels at 12% moisture; unshaded bars, kernels at 7% moisture.

fraction (Fig. 3B), and only 1–3% in the flour fraction. Bran and first shorts had highest activity per gram of material. In 7%-moisture groats, values were 12.4 (bran), 10.8 (first shorts), 4.0 (second shorts), and 2.2 (flour) μmol fatty acids per minute per gram of material. In 12%-moisture groats, these values were 5.6, 7.6, 4.6, and 1.9, respectively.

A small concentration of lipase activity (from 7 to 8 μmol/min in the intact whole groat up to 10–12 μmol/min in the bran fraction) was achieved by milling. However, this type of milling is not useful for separating lipase from other oat fractions (protein, gums, starch) because 65% of the material contained 80% of the lipase. Only 10–20% of the material had low lipase activity.

The data presented here confirm that most of the oat lipase in ungerminated grains occurs in the outer layers (bran) of the kernels. Little or no activity was in the embryo. Milling, as with the Ottawa experimental mill, could not cleanly separate a lipase-containing pure bran fraction from a high-yield flour because much of the endosperm particles adhered to the testa and aleurone layers and were recovered in the bran fraction. However, about 80% of the total lipase was eliminated by removal of the outer bran layers. Conversely, this method could be used to selectively concentrate lipase for industrial use or biochemical characterization.

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LITERATURE CITED

DUNDAS, D. G. A., HENDERSON, H. M., and ESKIN, N. A. M. 1978. Lipase from *Vicia faba* minor. Food Chem. 3:171.

HUANG, A. H. C., and MOREAU, R. A. 1978. Lipases in the storage tissue of peanut and other oil seeds during germination. Planta 141:111.

HUTCHINSON, J. B., and MARTIN, H. F. 1952. The measurement of lipase activity in oat products. J. Sci. Food Agric. 3:312.

HUTCHINSON, J. B., MARTIN, H. F., and MORAN, T. 1951. Location and destruction of lipase in oats. Nature 167:758.

JANECKE, H. 1951. Uber die Haferlipase. Staerke 3:29.

KEMP, J. G., WHITESIDE, A. G. O., MacDONALD, D. C., and MILLER, H. 1961. Ottawa micro flour mill. Cereal Chem. 38:50.

MARTIN, H. F., and PEERS, F. G. 1953. Oat lipase. Biochem. J. 55:523.

MATLASHEWSKI, J. G., URQUHART, A. A., SAHASRABUDHE, M. R., and ALTOSAAR, I. 1982. Lipase activity in oat flour suspensions and soluble extracts. Cereal Chem. 59:418.

PATON, D. 1981. Behavior of Hinoat oat starch in sucrose, salt, and acid solutions. Cereal Chem. 58:35.

SAHASRABUDHE, M. R. 1979. Lipid composition of oats (*Avena sativa* L.). J. Am. Oil Chem. Soc. 56:80.

TAVENER, R. J. A., and LAIDMAN, D. L. 1972. The induction of lipase activity in the germinating wheat grain. Phytochemistry 11:989.

WIDHE, T., and ONSELIUS, T. 1949. Lipolysis in doughs made from various cereal flours. Cereal Chem. 26:393.

WOOD, P. J., SIDDIQUI, I. R., and PATON, D. 1978. Extraction of high-viscosity gums from oats. Cereal Chem. 55:1038.

YOUNGS, V. L. 1974. Extraction of a high-protein layer from the oat groat bran and flour. J. Food Sci. 39:1045.

YOUNGS, V. L. 1978. Oat lipids. Cereal Chem. 55:591.

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