Phytochemistry, Vol. 26, No. 2, pp. 349–351, 1987.
Printed in Great Britain.

0031–9422/87 $3.00 + 0.00
Pergamon Journals Ltd.

LIPOXYGENASE–LIPASE SYSTEM IN OAT HULLS IN RELATION TO DORMANCY

MARYSE LASCORZ and ROGER DRAPRON

Laboratoire de Technologie Alimentaire, Institut National de la Recherche Agronomique, 91305 Massy, France

(*Revised received* 29 *April* 1986)

Key Word Index—*Avena sativa*; Gramineae; oat; lipoxygenase and lipase activities; free fatty acids; linoleic acid; dormancy; dry storage.

Abstract—Lipoxygenase (LOX) and lipase activities were found in white or black coloured hulls from two varieties of oat seeds with different germination characteristics. These activities were quite similar for the two varieties both after harvesting and after 14 weeks of dry storage representing the required time for obtaining *ca* 100% germination with these seeds. The hulls contained a significant quantity of free fatty acids (FFA) of which *ca* 30% was linoleic acid (18:2), a privileged substrate of LOX. The water content of the hulls was 12% after harvesting and *ca* 8% after dry storage thus allowing lipase and LOX activities. Lipase activity was shown by an increase in the content of FFA. The fluidity of 18:2 at storage temperature made possible its transformation into hydroperoxides by LOX as revealed by the decrease in the amount of FFA. Thus, the two oat seed varieties were very similar, despite their highly different germinative capacities.

INTRODUCTION

Cereal seeds often have some difficulty in germinating after harvesting. This phenomenon, called dormancy, is more or less important depending on species, variety, location and year of harvesting. The main reason seems to be maintenance of the embryo in a state of hypoxia under the seed layers. After dry storage of the seeds [1], called dry postmaturation [2], the germination inhibition is suppressed, i.e. the dormancy is heat labile [3]. As oxidative systems appear to be able to affect the phenomenon, we were interested in lipoxygenase (LOX). This enzyme (EC 1.13.11.12) is widely present among plants [4]; it catalyses the hydroperoxidation of polyunsaturated fatty acids and its privileged substrate linoleic acid (18:2) is fluid at room temperature. Garssen *et al.* in 1971 [5] supposed that LOX activity could keep a low pressure of O_2 under the seed layers at some physiological stages. Later, Rudrapal and Basu [6] showed that lipid peroxidation might produce a loss of germinative capacity.

RESULTS AND DISCUSSION

Germination tests were carried out with two varieties of spring sown oat, Sirene with black and Gambo with white hulls. Variation in germination rate at 25° is shown in Fig. 1. The germination percentage obtained after 7 days according to temperature is shown in Fig. 2. Isolated embryos germinated easily; there was 100% germination after 3 days. On the contrary, naked groats showed 50% germination for Gambo and 75% for Sirene after 7 days and hulled groats 15 and 70% germination, respectively, after 7 days. Thus, dormancy seems partly to result from the hulls (difference of germination between the naked and the hulled groats) and from the pericarp layers (difference of germination between the embryos and the naked groats). The role of the latter is more marked for Gambo than for Sirene. Likewise, the degree of dormancy at 25° was higher for Gambo than for Sirene. Accordingly,

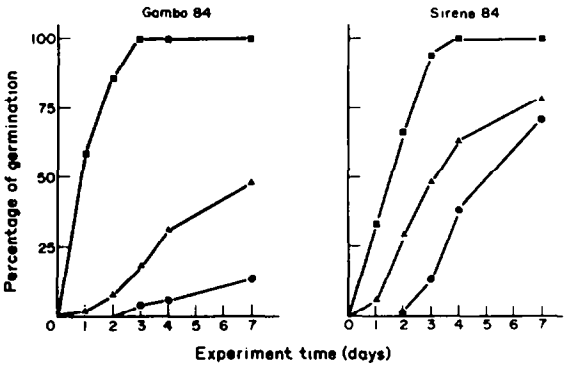

Fig. 1. Oat seed Sirene and Gambo (harvest 1984) percentage of germination at 25°: (■) embryos; (▲) naked groats; (●) hulled groats.

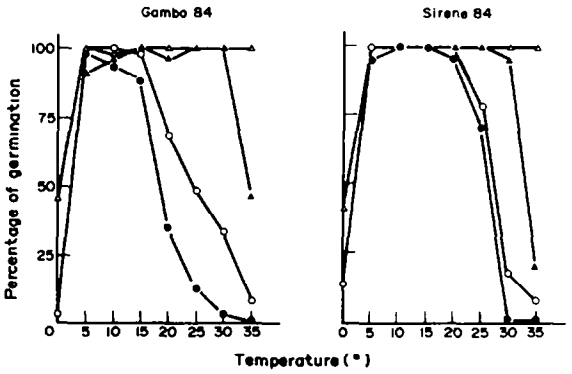

Fig. 2. Oat seed Sirene and Gambo (harvest 1984) percentage of germination after 7 days: (▲) naked and (●) hulled groats at dormant stage; (△) naked and (○) hulled groats after 14 weeks of dry storage at ±25°.

for these two varieties dormancy occurred from a temperature of 20°. After 14 weeks of dry storage, the hulled and naked groats germinated as well as the embryos.

Dormant Sirene and Gambo seed hulls contain hexane-soluble lipids (0.30 and 0.43% dry wt, respectively). These values were slightly lower after 14 weeks of dry storage, i.e. 0.24 and 0.34% dry wt for Sirene and Gambo, respectively. These 'free lipids' contain triacylglycerols, 1,2- and 1,3-diacylglycerols, monoacylglycerols, free fatty acids (FFA) and polar lipids. The composition and amount of the free lipid fatty acids are given in Fig. 3. It appears that 18:2 constitutes *ca* 30% of these acids. Figure 4 shows the composition and amount of FFA; 18:2 comprised *ca* 25%.

Dry storage led to a loss of 18:2 for the variety Sirene only (Fig. 5). This observation is complicated by the possible action of a lipase, which may release some fatty acids from the acylglycerols. Activity of lipase can take place at very low water content [7, 8], as well as that of LOX [9]. The water contents of Sirene and Gambo seeds are 12–13% dry wt in the seeds after harvesting and 9–7% dry wt after storage. A large lipase activity was found in the hulls. It was about the same for the two varieties, in both the dormant and non-dormant stages. LOX activity in the hulls was also similar for the two varieties after harvesting and remained unchanged after dry storage. Water content, lipase and LOX activities are shown in Table 1.

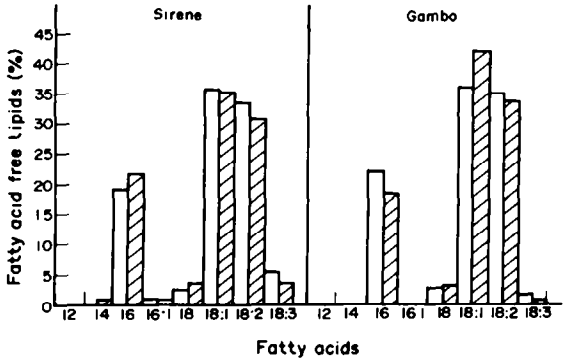

Fig. 3. Oat seed hull (harvest 1984) fatty acid free lipid composition and amount: open, at dormant stage; shaded, after 14 weeks of dry storage at ±25°.

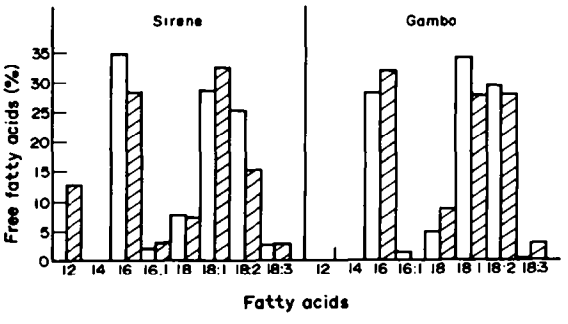

Fig. 4. Oat seed hulls (harvest 1984) free fatty acid composition and amount: open, at dormant stage; shaded, after 14 weeks of dry storage at ±25°.

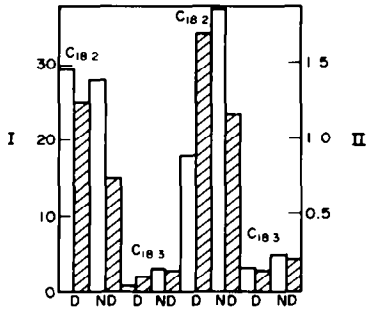

Fig. 5. Oat seed hulls (harvest 1984) free linoleic and linolenic acid amount in percentages of fatty acid free lipids (I) and of free lipids (II): shaded, Sirene and open Gambo varieties, (D) at dormant stage and (ND) at non-dormant stage (after 14 weeks of dry storage at ±25°).

Table 1. Water content, lipase and LOX activities in oat seed hulls Sirene and Gambo from the harvesting year 1984 at dormant (D) and non-dormant (ND) stages

	Sirene		Gambo	
	D	ND	D	ND
Water content (% dry wt)	12.2	9.4	13.2	6.7
Lipase activity ($mg_{18:1/g}$ dry wt)	15.9	14.5	18.0	17.5
LOX activity (nKatal/g dry wt)	40.0	37.5	36.4	35.5

During three years of experiments with Sirene and two years with Gambo, variations in the amounts of free lipids and FFA were observed (Table 2). This observation agrees with the finding that lipid concentration and fatty acid composition can be influenced by the year of harvesting, its location and variety [10, 12]. In our study, the FFA contents of 18:2 were 20.1, 25.2 and 15.0%, respectively, for Sirene in 1983, 1984 and 1985 and 29.5 and 16.7% for Gambo in 1984 and 1985. In the same way, LOX activities were about the same for each year, i.e. 41.7, 40.0 and 42.5 nKatals/g dry wt, respectively, for Sirene in 1983, 1984 and 1985. For Gambo, it was 36.4 nKatals/g dry wt for 1984 and 1985. Dormancy was quite similar from one year to another for Sirene, but quite different from 1984 to 1985 for Gambo (Fig. 6).

There were no large differences between oat seed varieties Sirene and Gambo, both in terms of free lipid fatty acid composition, FFA content and LOX and lipase activities. However, the varieties showed differences in hull colour and germination capacity under optimal conditions. There may be a LOX activity during storage, but as lipase can be active at low water contents and as unsaturated fatty acids are liquid at room temperature, this phenomenon could not easily be observed. During catalysis of hydroperoxide formation by LOX, lipase releases some other fatty acids which can be used by LOX. The hulls—considered as dead tissues—contain enzymes and their substrates. These reactions may not take place *in vivo*, but nevertheless the differences observed *in vitro* after 14 weeks of dry storage result from some of these reactions.

In spite of the presence of LOX, lipase and their substrates, it was not possible to establish a correlation between lipid oxidation and breaking dormancy.

Table 2. Free lipid and free fatty acid contents of oat seed hulls Sirene and Gambo for three harvesting years (1983–1985)

	Sirene Harvesting years			Gambo Harvesting years	
	1983	1984	1985	1984	1985
Free lipids (% dry wt)	0.18	0.30	0.17	0.43	0.25
Free fatty acids (% dry wt)	0.007	0.020	0.014	0.014	0.022

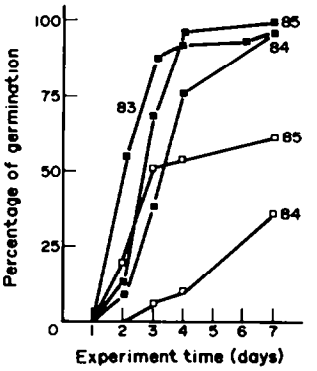

Fig. 6. Oat hulled groat percentage of germination at 20°: (■) Sirene variety from harvesting years 1983, 1984 and 1985; (□) Gambo variety from harvesting years 1984 and 1985.

Nevertheless, the existence of these enzymes in the hulls was demonstrated.

EXPERIMENTAL

Germination tests. These were performed twice in Petri dishes, on wet cotton wool, with 25 seeds in each dish, in the dark and for 7 days. It was considered that a seed germinated as soon as the radicle penetrated through the coat.

Sampling. Hulls were removed by hand, ground in liquid N_2 using a ball grinder and then lyophilized.

Free lipid extraction and fatty acid determination. Hulls (5 g) were extd with hexane using a modified Soxhlet apparatus [13] so that the temp. of the solvent did not exceed room temp. After solvent distillation, lipids were weighed.

Free lipid partition and FFA determination. Lipids (with added heptadecanaic acid standard) were analysed by TLC (Kieselgel 60G Merck) using hexane–Et_2O–HCO_2H (60:40:1) containing 0.01% butylhydroxytoluene. The FFA bands were removed, eluted with Et_2O and methylated [14]. They were then analysed by GC.

Assay of lipase activity [15]. Free lipids were extd from ground hulls and then admixed with 5% of olive oil. Samples were then incubated for 72 hr with a water activity of 0.8. The lipids were then extd with Et_2O and the FFA examined as described above.

Preparation of crude extract for LOX determination. Hull powder (100 mg) rinsed with Me_2CO was mixed with 2 ml of Pi buffer 0.05 M pH 6.8 and homogenized for 20 min at 4°. The homogenate was centrifuged at 20000 *g* for 10 min at 4°. The supernatant was used for determination of LOX activity.

Substrate emulsion was prepared according to Surrey but with some modifications [16, 17]. 18:2 (282 mg) was mixed with 250 μl Tween 20, clarified with a few drops of NaOH and adjusted at 100 ml with Pi buffer 0.1 M pH 6.5. The emulsion was stored at 4° in dark for no longer than 4 days, diluted twice and satd with O_2 before use.

Assay of LOX activity. O_2 consumption was determined polarographically at 25° with a Clark O_2 electrode (oxygraph recorder Gilson 5/6). The incubation mixture contained 1.7 ml of substrate emulsion with 50 μl of crude ext. Enzyme activities were calculated from initial rates of O_2 uptake. Initial dissolved O_2 concn was 244 μmoles/l. of substrate emulsion.

Water content was measured by weighing the samples after heating *ca* 100 mg of ground hulls at 130° for 2 hr according to the International Association for Cereal Chemistry Standard for cereal plants [18].

REFERENCES

1. Corbineau, F. and Côme, D. (1980) *C.R. Acad. Sci. Ser. D*, **280**, 547.
2. Côme, D. (1970) *Les obstacles à la germination*, Monographies de Physiologie Végétale, p. 162. Masson, Paris.
3. Chouard, P. (1954) *Dormances et inhibitions des graines et des bourgeons. Préparation au forcage. Thermoperiodisme*, p. 157. C.D.U., Paris.
4. Nicolas, J. and Drapron, R. (1981) *Sci. Alim.* **1**, 91.
5. Garssen, G. J., Vliegenthart, J. F. G. and Boldingh, H. J. (1971) *Biochem. J.* **122**, 327.
6. Rudrapal, A. B. and Basu, N. R. (1982) *Ind. J. Exp. Biol.* **20**, 465.
7. Caillat, J. M. and Drapron, R. (1974) *Ann. Technol. Agric.* **23**, 273.
8. Drapron, R. (1985) In *Properties of Water in Foods* (Simatos, D. and Multon, J. L., eds) p. 171. Martinus Nijhoff, Dordrecht.
9. Brockman, R. and Acker, R. (1977) *Ann. Technol. Agric.* **26**, 167.
10. Beringer, H. (1971) *Z. Pflanzenerneehr. Bodenkd.* **128**, 115.
11. Youngs, V. L. and Püskülcü, H. (1976) *Crop Sci.* **16**, 881.
12. Gullord, M. (1980) *Acta Agric. Scand.* **30**, 216.
13. Genot, C., Drapron, R. and Badilian, B. (1984) *Sci. Alim.* **4**, 631.
14. Schlenk, H. and Gellerman, J. L. (1958) *Analyt. Chem.* **32**, 1412.
15. Drapron, R. and Sclafani, I. (1969) *Ann. Technol. Agric.* **18**, 5.
16. Surrey, K. (1964) *Plant Physiol.* **39**, 65.
17. Nicolas, J., Beaux, Y. and Drapron, R. (1974) *Ann. Technol. Agric.* **23**, 287.
18. I.C.C. (1960) Normes No. 109–110.