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Buckwheat Lipoxygenase: Inactivation by Gamma-Irradiation

H. Michael Henderson,[a] N. A. Michael Eskin[b]

[a]Department of Food Science, [b]Department of Foods and Nutrition, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2

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Joseph Borsa

Radiation Applications Research Branch, Atomic Energy of Canada Ltd, Pinawa, Manitoba, Canada R0E 1L0

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ABSTRACT

Lipoxygenase (LOX) activity was detected in buckwheat extracts, and was shown to have a pH optimum of 6·5. LOX activity was not affected during 6 days of germination. The specific activity increased when ground buckwheat seeds were extracted with 0·01% or 0·1% solutions of calcium chloride or magnesium chloride, compared to extractions with distilled water or 1·0% solutions of either of these salts. Increase in specific activity was greater in the presence of calcium ions compared to magnesium ions. Gamma-irradiation of buckwheat seeds up to 4 kGy resulted in a decrease in LOX activity to a level of approximately 22% of the untreated control, but increased to approximately 43% of the control at a dose of 6 kGy. These results suggest that gamma-irradiation reduces LOX activity, but is not likely to completely prevent lipid oxidation in buckwheat.

INTRODUCTION

Buckwheat (*Fagopyrum esculentum* Moench.), family Polygonaceae, classified as a pseudo-cereal, is a crop adapted to cool, moist climates and to

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a growing season of 80–90 days (Anon., 1977). It is mainly cultivated in France, Poland, the USSR, Japan and the north-eastern United States, and is used both for human consumption and for livestock feed (Schery, 1972). In Canada, the total area devoted to the production of buckwheat in 1988 was approximately 20 200 ha (G. Mazza, pers. comm.), with two-thirds of the Canadian production regularly exported to Japan, where buckwheat is marketed as flour for the manufacture of noodles, and as groats for several products (Mazza & Campbell, 1985). The buckwheat grains are hard, small, angular three-sided seeds with dark brown hulls, and the approximate analysis of the dark buckwheat flour (12% moisture) is (in g/100 g edible portion): protein, 11·7; lipid, 2·5; total carbohydrate, 72·0 (total fibre 1·6); ash, 1·8 (Watt & Merrill, 1975).

Starch is the major component of buckwheat. The quality of buckwheat deteriorates quite rapidly during storage, and it seemed possible that, although low, the lipid content could be implicated in flavour deterioration of buckwheat flour, as has been suggested by Aoki *et al.* (1981). A major contributing factor could be lipid oxidation, which may, in part, be catalyzed by lipid-degrading enzymes. The pro-oxidant lipoxygenase (linoleate: oxygen oxidoreductase, EC 1.13.11.12) is an enzyme occurring in a wide variety of plants, including peas (Chen & Whitaker, 1986), tomatoes (Zamora *et al.*, 1987), haricot beans (Kermasha & Metche, 1986), fababeans (Eskin & Henderson, 1974*a*,*b*) and lentils and lupins (Eskin & Henderson, 1977), and catalyses the oxidation, in the presence of molecular oxygen, of *cis*,*cis*-1,4-pentadiene systems in unsaturated fatty acids to conjugated *cis*,*trans*-hydroperoxides (Eskin *et al.*, 1977).

This paper reports the presence of lipoxygenase (LOX) activity in buckwheat seeds, and examines the efficacy of gamma-irradiation in inhibiting LOX activity, with the objective being to control flavour deterioration in buckwheat flour, particularly during prolonged storage.

MATERIALS AND METHODS

Source material

Buckwheat varieties Manor and Mancan were provided by Dr Clayton Campbell, Agriculture Research Station, Morden, Manitoba, Canada. The seeds from the Manor variety were harvested in 1987, while the Mancan variety was harvested in 1988. All seeds were stored at −18°C until required. Pea (cv. Century) flour was obtained from Woodstone Foods, Portage la Prairie, Manitoba, Canada.

Radiation treatment

Buckwheat samples (cv. Mancan) were cleaned and dried or tempered, as required, to 12% moisture content. Seed samples (100 g) were then placed in sealed brown paper bags for cobalt-60 gamma-ray treatment. Irradiation was carried out in an AECL Gamma-Cell 220, in the Whiteshell Research Laboratories at Pinawa, Manitoba, Canada. The dose rate was 15·5 Gy per second, the temperature of the sample in the irradiation chamber was approximately 30°C, and the maximum duration of irradiation was 25·6 min. Samples were irradiated to doses of 0, 2, 4 and 6 kGy, and, following irradiation, were stored at −18°C until analyzed.

Germination

Dry buckwheat seeds were germinated over a 6-day period with samples removed at 0, 2, 4 and 6 days following germination as described previously (Eskin & Wiebe, 1983).

Protein determination

Protein was determined by the method of Lowry *et al.* (1951) using crystalline bovine serum albumin as the standard.

Enzyme preparation

Whole buckwheat seeds (7·5 g) were soaked overnight in distilled water at room temperature. The soaked seeds were blotted dry, and then ground in a pestle and mortar into as homogeneous a mass as possible. Then ground seeds were immediately extracted with 50 ml of distilled water, or with an aqueous solution of calcium chloride or magnesium chloride, at room temperature, by homogenizing in a Waring blender for 1 min. The homogenate was allowed to stand for 15 min at room temperature, and the decanted liquid was centrifuged at 12 000*g* for 20 min at 2–4°C. The supernatant liquid (crude extract) was retained for enzyme studies.

Lipoxygenase assay

Lipoxygenase activity was determined by monitoring substrate oxidation polarographically with an oxygen electrode at 30°C, using a biological oxygen monitor. Three millilitres of air-saturated substrate solution (2·0 mM linoleic acid dispersed with 0·028% Tween 20 in Tris-maleate buffer, 0·2M, pH 6·5) was placed in a reaction chamber and allowed to equilibrate for

3 min. Fifty microlitres of the enzyme solution was added, and the oxygen uptake recorded continuously.

Specific activity was calculated as mmol oxygen uptake/min/mg protein. At zero reaction time, all substrate solutions were 100% saturated with air. At 30°C, the concentration of oxygen in a 100% air-saturated aqueous solution is 0·23 mM (Yamaguchi *et al.*, 1969). All assays were performed in triplicate. The reaction rate was calculated from the initial portion of the oxygen uptake progress curve.

RESULTS AND DISCUSSION

Lipoxygenase (LOX) activity was demonstrated for the first time in buckwheat (cv. Manor) crude extracts, at a low level (0·0061 mM O_2 min^{-1} mg^{-1} protein) compared to LOX activities for other plant sources determined under similar experimental conditions. These values for lentils (cv. Denjasta) and lupins (cv. Blanca) are 0·380 and 0·056, respectively (Eskin & Henderson, 1977), and 0·546 for fababeans (cv. Ackerperle) (Eskin & Henderson, 1974*a*,*b*). In this study, LOX activity for pea (cv. Century) flour was found to have a corresponding value of 0·342. The rather low LOX activity detected in buckwheat, however, could still be significant during prolonged storage by contributing to flavour deterioration.

Buckwheat LOX was active within a pH range of 6·0–7·0, with a pH optimum of 6·5. A similar pH optimum was obtained, in this study, for pea flour LOX. The majority of plant LOXs appear to exhibit optimum activities at, or close to, 6·5–7·0 (Eskin & Henderson, 1974*a*, 1977; Eskin *et al.*, 1977; Chen & Whitaker, 1986; Kermasha & Metche, 1986; Zamora *et al.*, 1987).

Extraction of ground buckwheat seeds with 0·01% or 0·1% solutions of calcium chloride or magnesium chloride resulted in an increase in specific activity compared to the corresponding extractions with distilled water (Table 1). Extraction with 1·0% solutions of either salt, however, increased the specific activity only slightly. Control experiments, with enzyme omitted, indicated that the divalent ions themselves brought about a slight degree of nonenzymic oxidation of linoleic acid. The value for nonenzymic oxidation effects was therefore subtracted from the enzymic results. Extraction of buckwheat in salt solutions, particularly at the two higher concentrations, resulted in lower protein concentrations recovered in the supernatant after centrifugation, compared to the corresponding extractions with distilled water, indicating that 'salting out' effects may be partly responsible for the results obtained. The increases in LOX activity observed may also be due, in part, to activation by divalent metal ions (Restrepo *et al.*, 1973; Eskin &

TABLE 1
Effect of Calcium and Magnesium Ions (means and standard deviations) on Lipoxygenase from Buckwheat (cv. Manor)

Extracting solution (%)[a]	*LOX specific activity*[b]
Distilled water	0·59 ± 0·06*
$MgCl_2.6H_2O$, 0·01	1·08 ± 0·05*
$MgCl_2.6H_2O$, 0·1	1·28 ± 0·68*
$MgCl_2.6H_2O$, 1·0[1]	0·72 ± 0·16*
$CaCl_2.2H_2O$, 0·01	2·42 ± 0·50**
$CaCl_2.2H_2O$, 0·1	2·60 ± 0·69**
$CaCl_2.2H_2O$, 1·0[2]	0·65 ± 0·15*

[a] [1]0·049M; [2]0·068M.
[b] mmol oxygen uptake min^{-1} mg^{-1} protein ($\times 10^2$), at 30°C. All values represent the means of four samples tested. Values with different superscripts are significantly ($p < 0·05$) different.

Henderson, 1974*a*). The greater increase in specific activity in the presence of calcium ions compared to magnesium ions may be explained by the higher ionic strength of the calcium chloride solutions (ionic strengths of the 1·0% calcium chloride and 1·0% magnesium chloride solutions are 0·20 and 0·15, respectively), or by the enzymic preferential cofactor requirement for calcium. However, the slight increase in specific activity in the presence of 1·0% solutions of either salt was less than expected, and this may have been partly due to a possible combination between linoleic acid with the divalent ions forming insoluble soaps, in which state linoleic acid would be unavailable as a substrate for LOX activity, to the extent of almost cancelling out the activation effect on the enzyme by metal ions.

Germination of buckwheat seeds up to 6 days did not result in any significant increase in LOX activity.

Gamma-irradiation of intact buckwheat seeds (cv. Mancan) up to 4·0 kGy resulted in a decrease in LOX activity to 21·9% of the original untreated control activity (Fig. 1), suggesting that irradiation up to this level could be used to control flavour deterioration (lipid oxidation) in stored buckwheat flour. Irradiation at a dosage level of 6·0 kGy, however, almost doubled LOX activity, compared to the level at 4·0 kGy, to 42·7% of the control activity. This increase in activity suggests that irradiation at 6·0 kGy may have increased the extractability of the enzyme, compared to lower dosage levels. In a related study, Farkas and Goldblith (1962) found that a higher (calculated) dose of 62 kGy resulted in a residual activity of 33% soybean

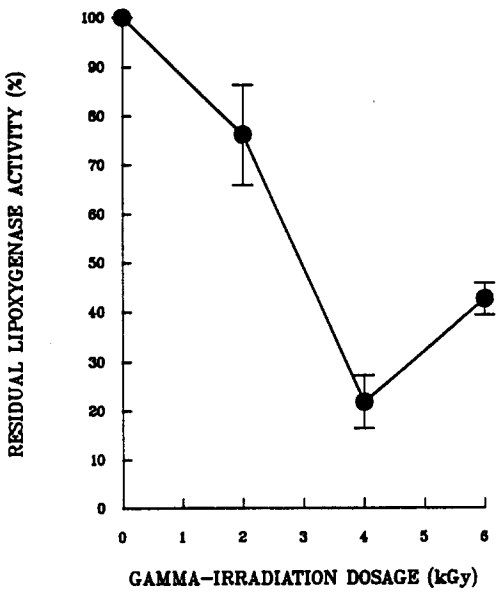

Fig. 1. Effect of gamma-irradiation on lipoxygenase from buckwheat (cv. Mancan). The ground buckwheat was extracted into 0·01% $CaCl_2 . 2H_2O$. Each result represents the mean of two samples tested.

LOX in the presence of 20% pea solids, suggesting that gamma-irradiation, even at this dosage level, does not completely inhibit LOX. In this study, gamma-irradiation dosage levels of 2·0, 4·0 and 6·0 kGy were shown to reduce LOX activity, but are not likely to completely prevent lipid oxidation in buckwheat.

REFERENCES

Anon. (1977). *Principles and Practices of Commercial Farming* (5th edn). Faculty of Agriculture, University of Manitoba, Winnipeg, Canada, pp. 123–4.

Aoki, M., Koizumi, N., Ogawa, G. & Yoshizaki, T. (1981). Flavours of buckwheat. I. Identification of the volatile components of buckwheat flour and their distributions in milling fractions. *Nippon Shokuhin Kogyo Gakkaishi*, **28**, 476–81.

Chen, A. O. & Whitaker, J. R. (1986). Purification and characterization of a lipoxygenase from immature English peas. *J. Agric. Food Chem.*, **34**, 203–11.

Eskin, N. A. M. & Henderson, H. M. (1974*a*). Lipoxygenase in *Vicia faba minor*. *Phytochem.*, **13**, 2713–16.

Eskin, N. A. M. & Henderson, H. M. (1974*b*). A study of lipoxygenase from small faba beans. *Proc. IV Int. Congr. Food Sci. and Technol.*, **1**, 263–9.

Eskin, N. A. M. & Henderson, H. M. (1977). A study of lipoxygenase from lentils and lupins. *Ann. Technol. Agric.*, **26**, 139–49.

Eskin, N. A. M. & Wiebe, S. (1983). Changes in phytase activity and phytate during germination of two fababean cultivars. *J. Food Sci.*, **48**, 270–1.

Eskin, N. A. M., Grossman, S. & Pinsky, A. (1977). The biochemistry of lipoxygenase in relation to food quality. *CRC Crit. Rev. Food Sci. and Nutr.*, **9**, 1–40.

Farkas, D. F. & Goldblith, S. A. (1962). Studies on the kinetics of lipoxidase inactivation using thermal and ionizing energy. *J. Food Sci.*, **27**, 262–76.

Kermasha, S. & Metche, M. (1986). Characterization of seed lipoxygenase of *Phaseolus vulgaris* cv. Haricot. *J. Food Sci.*, **51**, 1224–7.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193**, 265–75.

Mazza, G. & Campbell, C. G. (1985). Influence of water activity and temperature on dehulling of buckwheat. *Cereal Chem.*, **62**, 31–4.

Restrepo, F., Snyder, H. E. & Zimmerman, G. L. (1973). Calcium activation of soybean lipoxygenase. *J. Food Sci.*, **38**, 779–82.

Schery, R. W. (1972). *Plants for Man* (2nd edn). Prentice-Hall, Englewood Cliffs, NJ, pp. 447–8.

Watt, B. K. & Merrill, A. L. (1975). Composition of foods: Raw, processed, prepared. Agriculture Handbook No. 8, US Department of Agriculture, Washington, DC, p. 18.

Yamaguchi, M., Henderson, H. M., Hwang, P. M. & Campbell, J. D. (1969). Effect of oxygen concentration on *o*-diphenol oxidase activity. *Anal. Biochem.*, **32**, 178–82.

Zamora, R., Olías, J. M. & Mesías, J. L. (1987). Purification and characterization of tomato lipoxygenase. *Phytochem.*, **26**, 345–7.