

HEAT SENSITIVITY, OPTIMUM pH AND CHANGES IN ACTIVITY OF SORGHUM PEROXIDASE DURING MALTING AND MASHING

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Peroxidase activity was demonstrated in dry and germinating sorghum seeds. The specific activity increased about 14-fold during malting for a 96-hour period. On the average about 41% of peroxidase activity was located in the endosperm, and the remaining 56% in the acrospire and rootlet of sorghum malt. The crude enzyme extract retained 77%, 17.5% and 7.6% of activity after heating at 60°, 70° and 80°C, respectively. More than 50% of the peroxidase activity in the finished malt survived mashing at 65°C. Optimum activity was recorded at pH 5.5 which falls within the observed pH range of sorghum worts. The level of residual peroxidase activity in the wort differed with sorghum species.

Key Words: *Sorghum, malting, mashing, peroxidase*

INTRODUCTION

Peroxidase (E.C. 1.11.7) catalyses the reductive destruction of hydrogen peroxide and thus forms part of the defense system of living organisms against oxygen radical-mediated peroxidation of unsaturated lipids.⁷

Lipid peroxidation is undesirable in malting and brewing because the resulting products, namely, the hydroperoxides, and their decomposition products, the aldehydes¹⁸, affect the availability of wort nutrients, may interfere with yeast metabolism^{3,13} and participate in reactions which affect the flavour and colloidal-stability of beer^{2,5,6,10}.

A knowledge of the properties of peroxidase and other oxygen-scavenging enzymes in relation to malting and brewing will be useful in the control of lipid oxidation in brewing¹. This has been investigated in barley⁶, and to our knowledge, not yet in sorghum which has a higher content of unsaturated lipids than barley¹⁷. This is the impetus for this investigation into the heat sensitivity, optimum pH and changes in activity of sorghum peroxidase during malting and mashing.

EXPERIMENTAL

Improved Nigerian sorghum varieties (SK 5912 and KSV 8) used for the experiment were obtained through the seed service of Federal Department of Agriculture, Zaria, Nigeria.

Malting and mashing: Sorghum grains were steeped for 24 hr at room temperature (30°C) and malted in dark cupboards⁹. Kilning was done at 48°C for 24 hr and laboratory scale mashing was carried out by recommended methods⁸.

Enzyme extraction and assay: Sorghum (50 grains) was ground with a pestle and mortar. Thereafter the ground grains were extracted for 1 hr at 4°C with 100 ml of 0.1 M sodium phosphate buffer, pH 6.0. The homogenate was centrifuged at 5,000 rpm for 15 minutes, and the resulting supernatant was used as the crude enzyme extract. Peroxidase activity was assayed as described by Mclellan and Robinson¹⁴.

Heat inactivation of peroxidase: Aliquots of the crude enzyme were placed in pyrex test tubes of uniform thickness (1 mm) and subjected to various temperatures (60°C, 70°C, 80°C and 85°C) for periods of time up to 15 minutes. Thereafter, the heated extracts were cooled rapidly in ice and the residual peroxidase activity determined within 1 hr to avoid the regeneration of peroxidase activity¹⁴.

Protein determination: Protein content of enzyme extract was estimated by the folin-lowry method¹¹ using bovine serum albumin as standard.

Reagents: O-dianisidine was obtained from BDH, England.

All other reagents were of analytical grade and were obtained from standard sources. Except where otherwise stated, all preparations were made in deionised water.

RESULTS

Peroxidase activity during malting: The specific activity of peroxidase increased by about 14-fold during the germination of both sorghum varieties (Table I). Peroxidase activity was retained after kilning at 48°C for 24 hours.

Heat inactivation of sorghum peroxidase: Sorghum peroxidase retained about 77% of its activity after heating at 60°C for 15 minutes. At 70°C and 80°C, 17.5% and 7.5% of peroxidase activity was retained for the same period of time, respectively. At 85°C only about 5% of peroxidase activity was

TABLE I. Peroxidase activity during malting of sorghum varieties, KSV 8 and SK 5912*

Stage	KSV 8		SK 5912	
	Total units	Specific activity (units/mg protein)	Total units	Specific activity (unit/mg mg protein)
Sorghum	1.33	0.5	1.07	0.49
End of steep	3.53	1.4	2.62	1.0
End of germination	23.67	6.9	23.3	6.9
Finished malt	20.3	7.0	18.17	7.0

*Values represent means of three replicate experiments.

TABLE II. Location of peroxidase in sorghum malt*

Location	Specific activity (units/mg protein)	Total units	Percentage of total activity
KSV 8			
Endosperm	0.33	16.3	44
Acrospire and Rootlet	0.41	20.5	56
SK 5912			
Endosperm	0.28	13.8	39
Acrospire and Rootlet	0.43	21.7	61

*Values represent means of three replicate experiments. Peroxidase was isolated from 72 hours old malts.

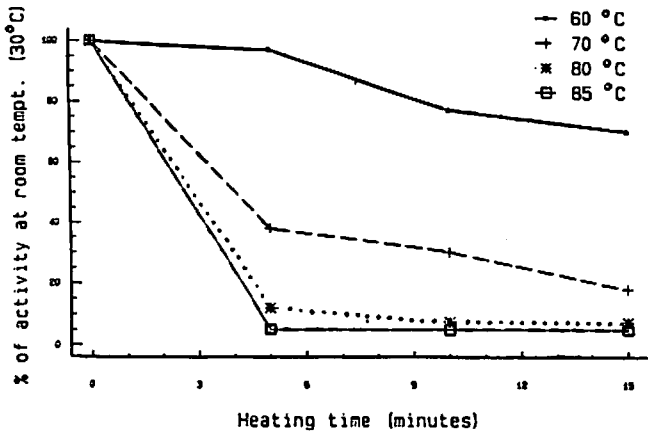


FIG. 1. Heat inactivation of sorghum peroxidase.

detected after 5 minutes (Figure 1). At higher temperatures (90°C and above) peroxidase activity was not detected within 5 minutes.

pH optimum of sorghum peroxidase: Sorghum peroxidase exhibited optimum activity at pH 5.5. Enzyme activity fell more sharply on the alkaline side than on the acidic side of pH 5.5.

Distribution of peroxidase activity: Between 39% and 44% of peroxidase activity was detected in the endosperm, while between 56% and 61% of activity was detected in the acrospire and rootlet put together.

Effect of mashing on peroxidase activity: Sorghum peroxidase retained about 50% of its activity after mashing at 65°C for 1 hour.

DISCUSSION

The increase in peroxidase activity observed during malting has been observed with barley⁶. The malting phase of brewing has been suggested as a likely stage for the successful control of lipid oxidation⁴. Lipid oxidation at this stage is both enzymatic and non-enzymatic¹⁰. The increase in peroxidase activity is thus desirable.

The ability of sorghum peroxidase to survive the heat treatment at kilning and mashing means that the oxygen-scavenging role is guaranteed in the wort. The pH profile of peroxidase activity (Figure 2) shows that reasonable activity will occur at the pH of wort.

The heat resistance of peroxidase, as observed here has been demonstrated in peroxidase from different plant sources^{6,15,19}. Peroxidase activity was more heat-resistant in the intact wort than in crude extracts of the enzyme. Although the pattern of heat inactivation of peroxidase from different sorghum varieties was similar, it is possible that they differ in their heat sensitivities. In assays with diluted worts as enzyme source, wort of KSV 8 and SK 5912 varieties retained 70% and 60% of peroxidase activities in the finished malt, respectively. Since significant amounts of the products of lipid oxidation form during mashing¹⁶ it will be of advantage in brewing to use sorghum varieties with more heat resistant peroxidases. Another heat-related property of peroxidase which could be of importance is its ability to regenerate some activity after heat inactivation^{15,12}. Such a property would allow the regeneration of peroxidase activity after heat inactivations that occur during brewing. The introduction of such a peroxidase preparation, for example cabbage peroxidase¹⁴, would help scavenge oxygen

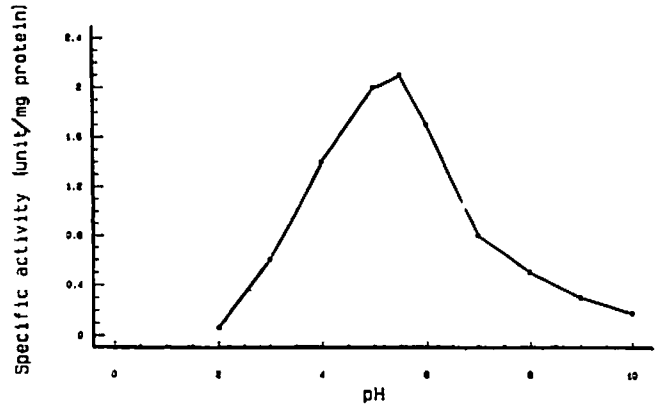


FIG. 2. Effect of pH on activity of sorghum peroxidase. Buffers used were 0.1 M acetate, pH 3-5; 0.1 M phosphate, pH 6-8; 0.1 M borate, pH 9-10.

radicals and thus limit the rate of lipid oxidation in the later stages of brewing. The possibility of regenerating the activity of heat-inactivated sorghum peroxidase is currently being investigated in our laboratory.

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REFERENCES

- Bamforth, C. W. *Journal of the Institute of Brewing*, 1983, **89**, 420-423.
- Bamforth, C. W. *The Brewer*, 1986, February, 48-51.
- Bamforth, C. W., Muller, R. E. & Walker, M. D. *Journal of American Society of Brewing Chemists*, 1993, **51**, 79-88.
- Baxter, D. E. *Journal of the Institute of Brewing*, 1982, **88**, 390-396.
- Brown, J. W. *Ferment*, 1989, **2**, 51-53.
- Clarkson, S. P., Large, P. J. & Bamforth, C. W. *Journal of the Institute of Brewing*, 1992, **98**, 111-115.
- Floyd, R. A. *FASEB Journal*, 1990, **4**, 2587-2597.
- Institute of Brewing Recommended Methods of Analysis, Institute of Brewing, London, 1977.
- Jayatissa, P. M., Pathrana, R. A. & Sivayogasunderam, K. *Journal of the Institute of Brewing*, 1980, **86**, 18-20.
- Kobayashi, N., Kaneda, H., Kano, Y. & Koshino, S. *Journal of the Institute of Brewing*, 1993, **99**, 143-146.
- Lowry, O. H., Rosebrough, N. J., Farry, A. L. & Randall, R. J. *Journal of Biological Chemistry*, 1951, **193**, 265.
- Lu, A. & Whitaker, J. R. *Journal of Food Science*, 1974, **39**, 1173-1177.
- Matsushita, S. *Journal of Agricultural and Food Chemistry*, 1975, **23**, 150-154.
- McLellan, K. M. & Robinson, D. S. *Food Chemistry*, 1980, **7**, 252-266.
- McLellan, K. M. & Robinson, D. S. *Food Chemistry*, 1987, **26**, 97-107.
- Meerssche, J. V., Blockmans, C., Deureux, A. & Masschelein, C. A. *European Brewery Convention Proceedings of the European 19th Congress, London*, 1983, 19.
- Palmer, G. H., Etokakpan, O. U. & Igyer, M. A. *MIRCEN Journal*, 1989, **5**, 265-275.
- St Angelo, A. J. & Ory, R. L. *Journal of Agricultural and Food Chemistry*, 1975, **23**, 150-154.
- Williams, D., Lim, M. H., Chen, A. O., Parghorn, R. M. & Whitaker, J. R. *Food Technology*, 1986, **40**, 130-141.