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EXTRACTION OF A SALT-INSOLUBLE α-GLUCOSIDASE FROM *SORGHUM VULGARE* GRAIN*

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Key Word Index—*Sorghum vulgare*; millet; birdproof; Gramineae; malt; α-glucosidase; amylase; enzyme extraction.

Abstract—Sorghum grain α-glucosidase may be either insoluble in sodium chloride under alkaline conditions, or partially soluble, depending upon the sorghum variety. A good correlation was found between the degree of sodium chloride insolubility of the grain α-glucosidase and the degree of water-insolubility of the malt amylases. Peptone, in the presence of sodium chloride, was effective in liberating sodium chloride insoluble α-glucosidase from grain. Similarly, the water-insoluble amylases of malt were solubilized by peptone in water. Maximum liberation of grain α-glucosidase was only achieved, however, by using a combination of 8 M urea, 0·1 M sulphite, 5% peptone and 1% Triton. It is suggested that the insolubility in both enzymes is caused by insoluble tannin-enzyme complexes.

INTRODUCTION

NOVELLIE,[1] during studies on the amylases of sorghum malts, found that in certain varieties of sorghum, particularly the "birdproof" varieties, the amylases could not be extracted, or were poorly extracted, by water. Nevertheless these amylases were active in the insoluble state, and could be completely extracted by a 2% solution of peptone.[2] Peptone had no effect on the extraction and determination of the water-soluble amylases found in normal types of sorghum malts. During the course of studies on the α-glucosidase activity of sorghum grain a similar phenomenon was found. Although α-glucosidase is completely water insoluble in all varieties of sorghum grain, it may normally be released to a limited extent by the use of sodium chloride under alkaline conditions.[3] In the case of certain varieties, however, no liberation was achieved, although a suspension of milled grain was still able to produce glucose from maltose.

In this paper, methods are presented for the liberation of α-glucosidase from these varieties of grain having the NaCl-insoluble enzyme, and a correlation is made between this phenomenon and that of the insoluble amylases of birdproof sorghum *malt*. A positive correlation would yield a useful diagnostic tool for the identification of "birdproof" varieties of sorghum grain without recourse to malting and subsequent determination of diastatic power.

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[1] NOVELLIE, L. (1959) *J. Sci. Food Agric.* **8**, 441.
[2] NOVELLIE, L. (1960) *J. Sci. Food Agric.* **7**, 408.
[3] WATSON, T. G. and NOVELLIE, L. (1974) *Phytochemistry* **13**. In Press.

RESULTS AND DISCUSSION

The variety of sorghum grain used in the present investigation, unlike that described previously,[3] did not liberate α-glucosidase activity upon treatment with 1 M NaCl at pH 9·3. Enzyme activity was found, however, by the direct incubation of milled grain with buffered maltose solution. For the extraction of the water-insoluble amylases from "birdproof" varieties of sorghum malt, Novellie[2] found peptone and Triton reagents, in aqueous solution, to be particularly effective. The use of 5% peptone, 1% Triton X205 or 2% PVP did not release α-glucosidase activity, but were effective however, if used in conjunction with 1 M NaCl (Table 1), although in the case of PVP, extraction was low at neutral pH and increased under both acidic and alkaline conditions Papain, caffeine and to a lesser extent histidine were also effective in the presence of NaCl. Of these reagents only papain and to a certain extent Triton also enhanced the extraction of α-glucosidase from the normal sorghum variety described previously[3] (Table 1).

TABLE 1. EXTRACTION OF α-GLUCOSIDASE FROM THE GRAINS OF NORMAL AND "BIRDPROOF" *Sorghum vulgare* VARIETIES

Additions to extracting solution of 1 M NaCl, pH 9·3	Enzyme activity (nmol/min/mg) Normal grain	"Birdproof" grain
None	0·75	Nil
Peptone, 5%	0·79	0·60
Papain, 5%	1·43	0·85
Triton X205, 0·2%	—	0·68
1·0%	0·92	0·71
5·0%	—	0·71
Histidine, 5%, pH 7·0	0·60	0·36
Caffeine, 1%, pH 7·0	0·76	0·57
PVP, 2%, pH 5·0	—	0·52
6·0	—	0·48
7·0	—	0·22
9·3	0·79	0·52
Papain, 5% + Triton X205, 1%	—	1·24
Papain, 5% + peptone, 5%	—	0·83
Papain, 5% + PVP 2%	1·27	0·83
Peptone, 5% + Triton X205, 1%	—	0·70
Direct assay; no extraction	3·38	1·72

Extractions carried out at 30° for 1 hr.

As in the case of normal sorghum grain, further extraction of α-glucosidase was achieved using a combination of 8 M urea and 0·1 M Na_2SO_3 (Table 2). However, the improvement was small, but could be enhanced by the addition of Triton X205, peptone or a combination of the two. The most effective combination was found to be 8 M urea, 0·1 M Na_2SO_3, 1% Triton X205 and 5% peptone extracted for two hr at 0°, giving a 76% improvement in extraction over urea plus Na_2SO_3 alone. Peptone was not effective in improving the extraction from the normal sorghum grain and Triton caused only a 12% improvement compared with a 52% improvement for the "birdproof" grain. Under optimum conditions, extraction exceeded the total enzyme content of the grain as determined by the direct method, and the total enzyme recovered, that is, extract plus residue, was 85% higher. Either not all enzyme sites are available to substrate during direct analysis, or the enzyme becomes more active in the soluble state.

For diagnostic purposes, exhaustive extraction is not required. Extraction with sodium chloride under alkaline conditions and sodium chloride plus 5% peptone should give a clear indication of whether or not the grain belongs to a "normal" or "birdproof" variety.

TABLE 2. ENHANCEMENT BY PEPTONE AND TRITON ON UREA/SULPHITE EXTRACTION OF α-GLUCOSIDASE FROM "BIRDPROOF" GRAIN

Additions to extracting solution of 8 M urea + 0·1 M Na_2SO_3, pH 7·0	Enzyme activity (nmol/min/mg) Extract	Residue	Total	% Enhancement of activity in extract
None	1·18	1·56	2·74	—
Peptone, 5%	1·46	1·37	2·83	34
Triton X205, 1%	1·79	1·27	3·00	52
Triton X205, 1% + peptone, 1%	1·88	1·10	2·98	59
Triton X205, 1% + peptone, 2·5%	1·90	1·08	2·98	61
Triton X205, 1% + peptone, 5%	2·08	1·11	3·19	76

Extractions carried out at 0° for 2 hr.

Although extraction of the birdproof grain with NaCl plus peptone was not complete even after 3 hr (Fig. 1) whereas extraction of normal grain with NaCl reached a maximum after 1 hr, a standard extraction time of 1 hr was selected for both extractions. Under these conditions, good correlation, 96%*, was found between the degree of water insolubility of the amylases of a number of malted sorghum varieties and the enhancement in extractibility of the grain α-glucosidase by peptone (Table 3). Results are the mean values of samples of each grain and malt taken from seven different locations within South Africa. Environment did not play a significant part in determining the degree of enzyme solubility.

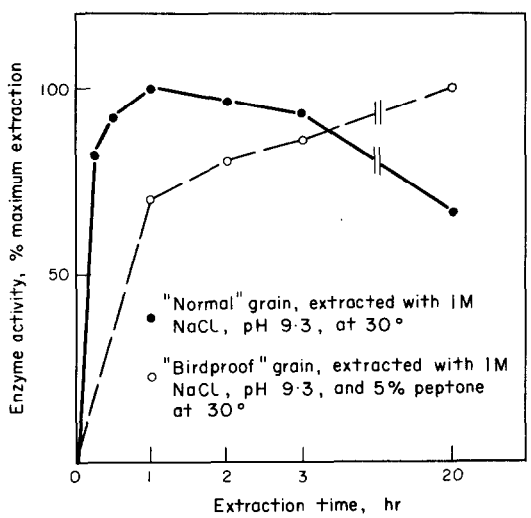

FIG. 1. THE EXTRACTION OF *Sorghum vulgare* GRAIN α-GLUCOSIDASE.

Preliminary experiments have shown "birdproof" sorghum varieties to contain up to ten times higher tannin concentrations than normal varieties. It might, therefore, be speculated that the insolubility of the two enzymes so far studied, α-glucosidase and

* $r^2 \times 100$ Where r = coefficient of linear correlation.

amylase, may be due to insoluble complexes being formed between tannins and the enzymes. Both PVP and Triton are, in fact, known to increase protein extraction by their action on phenolic compounds.[4,5] If tannins are responsible for the insolubility, peptone may be acting by displacement of enzyme from the insoluble tannin complexes. On the other hand, the cumulative effects of peptone and Triton on the urea extractions does suggest a somewhat distinct role for peptone (Table 2).

TABLE 3. EFFECT OF PEPTONE ON THE SOLUBILITY IN WATER OF SORGHUM MALT AMYLASES AND ON THE SOLUBILITY IN SODIUM CHLORIDE OF SORGHUM GRAIN α-GLUCOSIDASE

	Enzyme activities					
	Malt amlyases (Sorghum diastatic units)*		Grain α-glucosidase (nmol/min/mg)		% Solubility	
Cultivar	Water	Peptone 2%	NaCl, 1 M	NaCl, 1 M + peptone 5%	Amylases	α-Glucosidase
DC109	43·5	45·3	0·97	1·06	96	92
DC59	28·7	31·1	0·95	1·10	92	86
NK300	32·9	45·6	0·72	0·87	72	83
Barnard red	56·8	59·1	0·99	1·08	96	92
SSK2	12·8	62·6	0·36	0·82	20	44
SSK52	2·2	46·9	0·08	0·75	5	11
C39	30·8	35·8	0·90	1·05	86	86
VIVA 101	1·3	64·9	0·05	0·74	2	7

* See Ref. 1.

EXPERIMENTAL

Grain. Sorghum; "birdproof" variety SSK2 unless stated otherwise.
Procedures are described in the previous paper.[3]

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