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A Research Note Peroxidase of Kiwifruit

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ABSTRACT

Peroxidase from kiwifruit (*Actinidia chinensis*) was extracted, precipitated with ammonium sulfate, and purified by DEAE-cellulose chromatography. Only one band with peroxidase activity with an estimated molecular weight of 40,00–42,000 daltons was found by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

INTRODUCTION

KIWIFRUIT (*Actinidia chinensis*) is native to China. In 1906 Alexander Allison (Zuccherelli, 1987) imported seeds from China to New Zealand and the plants produced their first fruits in 1910. In 1940 a crop was started in the Tauranga area, where today 90% of the New Zealand production is grown.

Beutel et al. (1976) have determined the composition of kiwifruit including Brix, minerals, vitamins, ash, and proteins among other parameters. Kiwifruit is important in nutrition due to the high ascorbic acid and fiber levels. The former could explain, as Okuse et al. (1981) point out, why kiwifruit undergoes less browning than other typical fruits.

Further studies of Kiwifruit characteristics have been done by Park and Luh (1981) who found four catecholase isoenzymes of 15000, 20000, 25000, and 45000 daltons molecular weight and confirmed that the active site was histidine. Grison and Pilet (1985) found that there was a relationship between peroxidase (POD) and polyphenoloxidase (PPO) and that the PPO activity could be due to POD activity.

The purpose of this study was to purify and characterize the peroxidases in kiwifruit.

MATERIALS & METHODS

THE KIWIFRUIT used in this experiment was a Hayward cultivar from New Zealand. The flesh was blended (1:1 w/v) in an omni-mixer for 30 sec with 50 mM Tris-HCl, pH 7.8 buffer, and the extract centrifuged at 20000 × g for 10 min. The supernatant (200 mL) was treated with ammonium sulfate (351g/L) and the resulting precipitate dissolved in small portions of 50 mM Tris-HCl, pH 7.8 buffer and dialyzed against the same buffer. The extract was partially purified with a DEAE-cellulose column using 50 mM Tris-HCl, pH 7.8 buffer, in a gradient from 0 to 1M NaCl and the fractions with peroxidase activity were pooled. All steps were carried out at 4°C.

Peroxidase activity

(POD) was determined spectrophotometrically at 460 nm using o-dianisidine as chromogenic indicator (Srivastava et al., 1983). The total reaction volume was 3 mL containing: 2.7 mL 50 mM sodium

Fig. 1—Electrophoresis of crude protein extract and purified peroxidase of kiwifruit. Molecular weights of: A—kiwifruit PO isoenzyme; B—kiwifruit proteins from crude extract; S—standard proteins.

acetate, pH 6 buffer; 0.1 mL 0.5% hydrogen peroxide; 0.1 mL enzyme extract and the addition of 0.1 mL 0.25% O-dianisidine (w/v). The POD activity was expressed in units of peroxidase per mg protein. A unit of peroxidase was defined as an increase of 0.001 unit of absorbance per min.

Protein content

Protein was determined using the Bio-Rad protein assay method (Bradford, 1976) and bovine serum albumin as standard.

Electrophoresis

SDS-PAGE (sodium dodecyl sulfate, polyacrylamide gel electrophoresis) was performed according to Laemmli (1970). Ten percent acrylamide/bisacrylamide was used in a slab gel system. Electrophoresis was carried out at 4°C using 25 mA/gel current for 4–5 hr running time. The electrode buffer was glycine-Tris pH 8.3. Samples of 100 µL (7.15 µg protein, with activity of 24.88 units POD/mg protein) were layered on the top of the gel. For activity staining, the gels were soaked for 30 min at 20°C in 50 mM sodium acetate, pH 6 buffer. They were then treated with 0.1% hydrogen peroxide and 0.25% o-dianisidine. Appearance of a dark brown band indicated peroxidase activity. For protein staining, the gels were fixed in a solution of 0.1% Coomassie Blue R-250, in 10% acetic acid and 25% isopropanol and 65% water. The gels were destained in a solution of 7% acetic acid and 10% methanol in water.

Molecular weight

SDS-PAGE was performed using the following molecular weight standards: phosphorylaseb (94,000 daltons); albumin (67,000 dal-

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Table 1—Peroxidase activity and protein content of kiwifruit

Sample	A/min/mL ^a x ± σ	µg protein/mL x ± σ	Units POD/mg protein ^b x ± σ
Crude extract	2.64 ± 0.16	518 ± 58	4.94 ± 0.23
Purified extract	2.43 ± 0.47	113 ± 2	21.54 ± 2.33

^a A = Absorbance at 460 nm

^b PO = Peroxidase activity

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COLOR/COMP OF GERMINATED A MARANTH SEEDS. . .

Table 1—Effect of germination on hypocotyl length, composition, reactive lysine and *in vitro* protein digestibility of amaranth seeds^a

Time of incubation (hr)	Water activity (a _w)	Hypocotyl length (cm)	Crude protein (%)	True protein (%)	Fat (%)	Crude fiber (%)	Ash (%)	Reactive lysine (g/100g protein)	<i>In vitro</i> protein digestibility ^b (%)
0	0.92 ^c		15.5	14.4	8.0	3.9	3.4	5.8	80.6
12	0.92	0.3	16.2	13.6	7.1	3.9	3.4	5.5	78.0
24	0.93	0.6	16.2	13.6	5.3	4.2	—	5.6	77.0
36	0.93	1.4	20.0	15.8	4.9	4.1	—	5.7	74.9
48	0.93	2.6	20.8	16.8	4.5	4.6	3.2	5.7	79.2
72	0.93	3.1	21.9	17.6	3.3	4.7	3.2	4.9	79.2
LSD (0.05)	0.00	—	1.4	0.8	0.5	0.4	0.2	0.4	0.4

^a Values of composition expressed on a dry weight basis.

^b Casein *in vitro* digestibility was 91.4%.

^c Soaked sample.

Table 2—Changes in Hunter color during germination of amaranth seeds

Time of incubation (hr)	Hunter parameters			
	L	a	b	ΔE
0	63.0	0.8	10.3	30.6
12	62.4	0.7	11.2	31.6
24	60.5	0.9	11.2	33.4
36	54.8	1.6	12.5	39.2
48	53.1	5.2	12.7	40.6
72	48.5	3.7	12.7	45.2
LSD (0.05)	5.9	1.2	0.7	0.7

increase in ΔE. In other words, sprouting decreased lightness of samples and enhanced red and, to a lesser degree, yellow colors. Interestingly, this suggests that the amaranth variety used here synthesized amaranthin pigments. The germinated flour showed a pinky color, resembling strawberry-based products, which could be attractive for food formulations.

In summary, a high percentage of amaranth germination was attained in a short time. Germinated samples showed high contents of true protein and available lysine. Also, protein digestibility was relatively high. The final color appeared adequate for various food applications.

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tons); ovalbumin (43,000 daltons); carbonic anhydrase (30,000 daltons); trypsin inhibitor (20,000 daltons); and α-lactalbumin (14,400 daltons).

RESULTS

THE RESULTS of kiwifruit POD activity in the crude extract and after purification with a DEAE-cellulose column are shown in Table 1.

Before determining POD activity it was necessary to dialyze the extract against pH 7.8 Tris-HCl buffer to remove the high ascorbic acid, which interfered with the peroxidase activity. Determination after purification of the crude extract by dialysis showed that the POD activity had increased over four-fold.

The results of electrophoresis are shown in Fig. 1. Only one band of 40,000–42,000 M.W. obtained by staining the separated band from the crude and purified extracts was found (column A).

In column B, (Fig. 1) the most representative kiwifruit protein bands from the crude extract are shown. These protein

bands can characterize the kiwifruit. More studies are needed to identify these proteins.

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