Peroxidases of Selected Fruits and Vegetables and the Possible Use of Ascorbic Acid as an Antioxidant

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Abstract. The various isozymes of peroxidase of a range of vegetables and kiwifruit were compared using sodium dodecyl sulfate polyacrylamide gel electrophoresis followed by specific activity staining. Peroxidase isozymes were determined in potato (*Solanum tuberosum* L.), carrot (*Daucus carota* L.), tomato (*Lycopersicon esculentum* Mill.), kiwifruit [*Actinidia deliciosa* (A. Chev.) C.F. Liang et A.R. Ferguson], cauliflower [*Brassica oleracea* (Botrytis group)], green beans (*Phaseolus vulgaris* L.), and horseradish (*Armoracia rusticana* Gaertn, Mey Scherb.). There was only one isozyme in cauliflower (70 kDa), two in kiwifruit (45-43 kDa), and a range of isozymes (120-36 kDa) in horseradish. Ascorbic acid inhibited peroxidase activity in the extracts.

The role peroxidases play in the living plant is not completely understood, although they have been associated with cell wall biosynthesis, response to injury, disease resistance, and wound repair.

Peroxidases consist of a family of isozymes (Shanon et al., 1966) that catalyze the same or similar reactions. All of these enzymes contain identical heme groups but differ in the precise composition of the glycoprotein (Gaspar et al., 1982). Peroxidases are found in almost all vegetables; horseradish peroxidase is the most extensively studied. Many major isozymes have been found using electrophoresis (Hoyle, 1977).

The molecular weights of the peroxidases produced by cultivated tobacco (*Nicotiana tabacum* L.) cells (103, 90, 67, and 46 kDa) are similar to those produced by potato tuber (105, 94, 56.5, and 48.5 kDa). The similarity of peroxidases in potato and tobacco, both solanaceous plants, reflects the genetic similar-

ity between these related plants (Decedue et al., 1984). Peroxidases normally increase in activity and number during ripening. Thomas et al. (1981) found five isozymes in tomato fruit.

Our studies on peroxidase were focused on the isoperoxidase found in some fruit and vegetable extracts. These studies are of interest to the food industry, since the activity of this enzyme may influence fruit and vegetable quality.

Potato tubers, carrot roots, tomato fruit, kiwifruit, cauliflower heads, and green bean pods were obtained from the local market. Horseradish peroxidase was purchased from Sigma, Madrid. The extracts were prepared from three sample specimens, and duplicates were used to measure peroxidase activity and protein content.

A sample of each type of produce was blended in 50 mM tris-HCl pH 7.8 buffer at a 1:2 ratio (w/v). The extract was centrifuged at 10,000× g for 10 min at 4°C to remove debris. The clarified supernatant was used to determine the various isozymes using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and to measure peroxidase activity using spectrophotometry. Ascorbic acid was used as a natural antioxidant.

Peroxidase activity. Peroxidase activity was measured with a spectrophotometer at 460 nm

using *o*-dianisidine as a chromogenic indicator (Préstamo, 1989). Peroxidase activity was expressed in units of peroxidase per milligram protein. A unit of peroxidase activity was defined as an increment of 0.001 of absorbance/min.

Antioxidant experiment. In the peroxidase activity reaction, 2 to 25 µl ascorbic acid (15 mg·ml⁻¹) was substituted by buffer following the same reaction to determine peroxidase activity. Ascorbic acid was added until peroxidase was inhibited completely.

Ascorbic-acid content. The ascorbic acid content of the fruits and vegetables was determined following the AOAC method (1984).

Electrophoresis. SDS-PAGE was performed according to Laemmli (1970). Acrylamide/bisacrylamide (12% w/v) was used in a mini-slab gel system. Electrophoresis was carried out at 4°C using 175 V for 90 min. The electrode buffer was glycine-tris (pH 8.3). Samples of 15 µl (4 to 12 µg protein) were loaded into each well at the top of the gel. For activity staining, gels were soaked for 30 min at 20°C in 50 mM sodium acetate buffer, pH 6. Gels were then treated with 0.1% hydrogen peroxide and 0.25% *o*-dianisidine. The appearance of dark brown bands was caused by peroxidase activity and indicated the presence of a peroxidase isoenzyme in the gel.

The molecular weight standards used in these studies were phosphorylase *b* (94 kDa), albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (20 kDa), and *a*-lactalbumin (14.4 kDa). Total protein concentration was measured by dye binding (Bradford, 1976).

Among all the fresh produce assayed, kiwifruit had the highest ascorbic acid concentration (Table 1). Kiwifruit and potatoes had the lowest peroxidase activity, while green beans had the highest (Fig. 1). When determining proper blanching time, researchers should take this activity level into account. Interestingly, fruits and vegetables with lower peroxidase activity are more suitable for freezing than canning.

Table 1. Ascorbic acid content in selected fruits and vegetables.

Product	Ascorbic acid ^a (mg/100 g fresh wt)
Potato	32.50
Green beans	21.25
Cauliflower	50.00
Carrot	8.50
Tomato	15.50
Kiwifruit	84.00

^aMean of duplicate values.

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There seems to be a relationship among some of the major peroxidase isozymes (Fig. 2). Four of the extracts contain a 70 kDa band, three extracts contain a 55 and 43 kDa band, and two contain 40-36 kDa bands. Cauliflower, an inflorescence, contains only one isozyme (70 kDa); kiwi, a fruit, two isozymes (45-43 kDa); and horseradish, a root, contains many isozymes. The rest of the extracts have a range of isozymes between 120 and 36 kDa.

The lowest molecular weight isozyme visualized was at 36 kDa. This isozyme was absent in fresh potato tuber; however, its level increased in extracts from wounded potato tuber tissue aged for 1 to 8 days (Roberts et al., 1988). This isozyme is also present in horseradish and carrots.

Peroxidase is involved in tomato ripening (Rothan and Nicolas, 1989). Only one peroxidase (62-58 kDa) was present in tomato fruit

at the green stage, whereas more isozymes appeared as ripening progressed (data not shown).

We also determined the effectiveness of ascorbic acid, a natural antioxidant, inactivation of peroxidase. Addition of antioxidants has become increasingly popular as a means of increasing the shelf life of food. Recently, however, the safety of synthetic antioxidants has been questioned. Ascorbic acid has been used as a peroxidase inhibitor (Shinkle and Jones, 1988).

Between 2 and 170 μ M ascorbic acid was required to inhibit peroxidase activity completely in all assayed extracts (Fig. 1). Kiwifruit needed the lowest, while green beans required the highest ascorbic acid concentration. As more ascorbic acid was added, the peroxidase activity reaction was delayed 75 to 120 min.

Peroxidase activity does not depend only on the number of isozymes present, since there is only one isozyme in cauliflower extract that exhibited high activity and two isozymes in kiwifruit that showed the lowest peroxidase activity. The K_m and V_{max} for these isoenzymes must also be taken into account.

Based on our observations, we postulate that products with high peroxidase activity must be blanched or treated with antioxidant to decrease isozyme activity. Those fruits and vegetables that exhibit low peroxidase activity and mainly contain low-molecular-weight isozymes need not be blanched, however, they can be treated with antioxidants if needed. This relationship may explain, in part, why carrots can be stored at -18C as a frozen product in acceptable condition for 9 months without blanching and antioxidant addition (Espinosa et al., 1984). In the case of kiwifruit, similar results were obtained (Cano et al., 1989).

In addition, using ascorbic acid as an antioxidant is not only completely safe for human consumption but could enhance the vitamin C content of certain fruits and vegetables.

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Fig. 1. Peroxidase activity and micromoles of ascorbic acid needed to inactivate peroxidase in various fruits and vegetables.

Fig. 2. Peroxidase isozymes of various fruit and vegetable extracts. Std = standard; Cau = cauliflower; Car = carrot; Tom = tomato; Pot = potato; Kiwi = kiwifruit; G.b. = green beans; Hr = horseradish.

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