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Changes in chemical composition and peroxidase activity of turnip (*Brassica rapa)* during processing and frozen storage

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Absract

Chemical composition content and peroxidase enzyme activity of raw, canned and frozen turnip roots were determined. The peroxidase activity determined in the roots of raw turnip was 134x10^5 EU/100 g fresh weight and 170x10^5 EU/g protein. There were significant changes in the activities of peroxidase of canned and frozen turnip roots, as well as in the contents of protein. The peroxidase activity of canned turnip roots was practically undetectable while there was residual activity after blanching and freezing. Peroxidase is one of the most heat-stable enzymes in plants. This study shows that heat treatment of 3 min at 95°C is preferred for POD activity inactivating compared with other heat treatment. In addition, conserving process inactivated of POD activity, too.

Key words: Turnip, vegetable, peroxidase, storage.

Introduction

Peroxidases (EC.1.11.1.7 hydrogen donor oxidoreductase) are widely distributed and have been isolated from many higher plants, animal tissues, yeasts and various microorganisms. The wide distribution of this enzyme suggests that it could be of great biological importance. However, the role of peroxidase in metabolism is not still clear, due to the large number of reactions it catalyzes and the number of its isozymic species [1]. In plants they participate in the lignification process [2] and in the mechanism of defence in physically damaged or infected tissues [3]. Their formation is probably related to the acquisition of host cell resistance mechanisms [4].

Besides being an important factor in the physiology of plants, these enzymes are of great interest in food technology because of their influence on the quality, particularly taste, smell and color of raw and processed fruits and vegetables [5]. Peroxidase causes undesirable changes when it combines with hydrogen peroxide to produce phytotoxic free radicals which, in turn, react with food components such as ascorbic acid, carotenoids and fatty acids leading to the loss of some nutrients and the development of cold damage symptoms [6].

One commonly used method to avoid changes in quality due to enzymatic activity is blanching technique which also extracts gases from the plant tissues. Peroxidase is one of the most heat-resistant of plant enzymes, so it is used as an indicator of the adequateness of the blanching process [7, 8]. However, problems, i.e. overblanching, may arise from so much heating to inactivate it completely [9, 10]. Peroxidase inactivation depends on the temperature of the blanching water, the size of the vegetable and the nature of the enzyme present [11-14].

Turnip is a cool season root crop and it has a size of small apple. The crops rely on honeybees for their pollination. Turnip is grown for its root and foliage. The entire root is roughly conical and the taproot is thin and 10 cm or more in length. It is grown from seed as an annual. If grown for the root, the crop is planted between early spring and the first week in June, depending on the desired harvest date 60 days after planting. Roots are usually harvested when 3 inches or less in diameter. For turnip greens, the crop is planted in the spring for an early crop, or is seeded in September for a fall crop. The turnip leaves are usually light green, thin and hairy. If the turnip leaves are eaten they resemble mustard greens. The greens are ready for harvest 50 days after planting. The aboveground part of the turnip develops from the stem but is fused with the root. Turnip varieties differ in their color and the shape of their root. There are both white and yellow fleshed varieties in turnips although the white fleshed is the most common. All turnip is for the fresh market and all is sold locally and to direct market.

Materials and Methods

Turnip roots, procured from the local market, were cut into small pieces and blanched at 75°C for 5 min, or at 95°C for 3 min and unblanched as a control. One part of blanched turnips was put inside 250 and 500 ml jars filled with 2% NaCl solutions and autoclaved at 115°C for 30 min, and other parts were frozen at -24°C. Same process was applied to the control groups. Canned turnips were stored at ambient temperature and frozen turnips were stored at -18°C for 30 days. Chemical compositions and POD activities of all samples were analyzed by periods of tenth, twentieth and thirtieth day intervals.

Preparation of turnip homogenate: Samples were powdered with liquid nitrogen. A 15 g sample of the turnips was homogenized in 25 ml of 0.3 M phosphate buffer (pH 7.0). The homogenate was centrifuged at 27,000´g for 20 min at 4°C. The supernatant was collected and stored at 4°C until use [12, 13]. It was used as a crude enzyme extract for the POD and protein analyses. Protein content was determined according to the method of Bradford [14] using bovine serum albumin as a standard.

POD activity assay: The POD activity was determined spectrophotometrically according to the method described by Angelini *et al.* [15]. The assay mixture contained 0.975 ml of 0.05 M phosphate buffer (pH 5.5), 1 ml of 40 mM guaiacol and 1 ml of 22.5 mM H_2O_2, and finally 0.025 ml of the enzyme extract was added to the cuvette. Changes in the absorbance at 470 nm were measured for 3 min using a UV-VIS spectrophotometer (CHEOBIS s.r.l.). POD activity was expressed as EU/g protein and EU/ 100 g weight.

Chemical composition analysis: Total dry matter, pH and titratable acidity [16] and crude cellulose [17] were determined.

Statistical analysis: Analysis of variance of the data was evaluated by the SPSS (SPSS for windows, Release 9.0.0 1998). Duncan's Multiple Range Test was employed to determine the statistical significance of the differences between the means.

Results and Discussion

In this study main objective was to determine the POD activity under different conditions, but also to determine general components such as dry matter, crude cellulose, titratable acidity and pH owing to importance of nutrition and protection technology. We estimated that the changes of this components were not important for the short storage periods. For this reason, the analysis of these components was performed initially and at the end of the storage periods (Table 1).

Table 2 shows the changes of protein and POD activities in blanched and unblanched turnip roots during canned and frozen storage. There was no reactivation in POD enzymes during canned storage. Blanching at 95°C for 3 min completely inactivated turnip roots POD and any residual activity was determined in these samples during frozen storage. POD activities in unblanched and at 75°C blanched frozen turnip roots were stable during 30 days of frozen storage (Figs 1 and 2). Complete inactivation of enzymes can be easily achieved either using higher temperatures or increasing the time of thermal process. In this case, properties of the products such as color, texture, flavor, aroma and nutritive quality can be adversely affected. For maximum retention of quality, clearly the need is for sufficient heat treatment to stabilize the product against quality deterioration but at the same time, to minimize quality loss as a direct result of heating [6, 11, 18].

Blanching caused decrease in protein quantity because of the heat label constituent. Proteins have been reported to decrease by heat, pH, NaCl etc.[19, 20]. Protein amounts and POD activity increased in samples frozen after blanching at 75°C and solely frozen samples. In the tenth and twentieth days, they started to decrease.

There was reactivation both raw and 75°C blanched samples frozen during first 10 days. Because of unsufficient protein denaturation, there is regeneration with increasing activity especially during first ten days [11-21]. Reactivation, protein content and POD activity might reach to initial rate after processing. Our different findings cannot be explained.

In blanched but not conserved samples POD activity was not inactivated completely. The neutral POD regained activity rapidly, within 10 min after the heated enzyme was cooled and incubated at room temperature. The increase in activity was very slow and remained almost unchanged during prolonged incubation. This is in the same trend as observed for the protein. Heat-treated PODs from several plant sources have shown an ability to recover their activity while being stored at ambient temperature after heat treatment. The ability of POD to reactivate after it is denatured by heat varies with treatment conditions and the species of vegetable, and may differ between isoenzymes of the same species. A reactivation to 16% of the original activity was observed in green asparagus extract [10] and Brussels sprouts and cabbage [22] after 2.5 h of incubation at 30°C following heating at 75°C. Reactivation has also been observed in purified POD. A 15-30% restoration of original POD activity was noted for the cationic isoenzyme of green peas after several hours of incubation at 25°C following a 50-60°C heat treatment, but there was no regain after a 70°C heat treatment [23]. Nonlinear heat inactivation of POD may occur

Table 1. Composition of turnip roots, initial and 30th day.

Blanching Temperature °C	Process	Dry Matter	Crude Cellulose	Titratation acidity	pH
	Raw	10.1	1.52	0.14	5.76
75	Canned	10.0	1.51	0.13	5.73
	Frozen	10.0	1.55	0.13	5.73
95	Canned	10.0	1.50	0.12	5.70
	Frozen	10.0	1.50	0.13	5.71

Table 2. Protein and POD activity of raw (canned and frozen) and canned and frozen after different blanching temperatures in turnip roots at different storage times (fw).

Blanching Temperature, °C	Process	Storage Times (day)	Protein, g/100g	EU/100 g	EU/g protein
	Raw	0	0.79	$134x10^5$	$170x10^5$
	Canned	10	0.55	0.00	0.00
		20	0.50	0.00	0.00
		30	0.49	0.00	0.00
	Raw	0	0.79	$134x10^5$	$169x10^5$
	Frozen	10	0.94	$205x10^5$	$218x10^5$
		20	0.88	$192x10^5$	$218x10^5$
		30	0.77	$107x10^5$	$138x10^5$
75 (5 min)	Canned	0	0.39	$828x10^4$	$212x10^5$
		10	0.21	0.00	0.00
		20	0.21	0.00	0.00
		30	0.20	0.00	0.00
	Frozen	0	0.39	$828x10^4$	$212x10^5$
		10	0.41	$915x10^4$	$223x10^5$
		20	0.37	$754x10^4$	$203x10^5$
		30	0.34	$542x10^4$	$159x10^5$
95 (3 min)	Canned	0	0.24	$115x10^4$	$479x10^4$
		10	0.21	0.00	0.00
		20	0.21	0.00	0.00
		30	0.20	0.00	0.00
	Frozen	0	0.24	$115x10^4$	$479x10^4$
		10	0.21	0.00	0.00
		20	0.21	0.00	0.00
		30	0.20	0.00	0.00

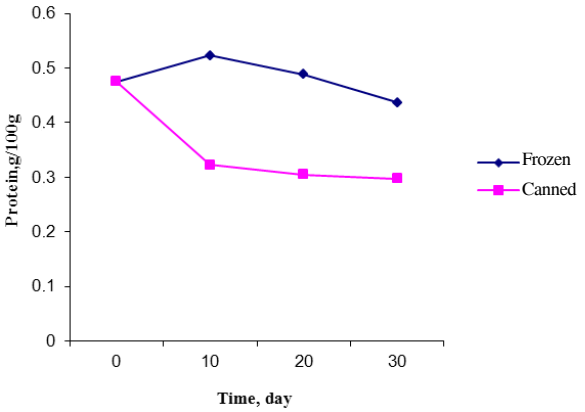

Figure 1. Turnip protein interactions of storage time and storage type.

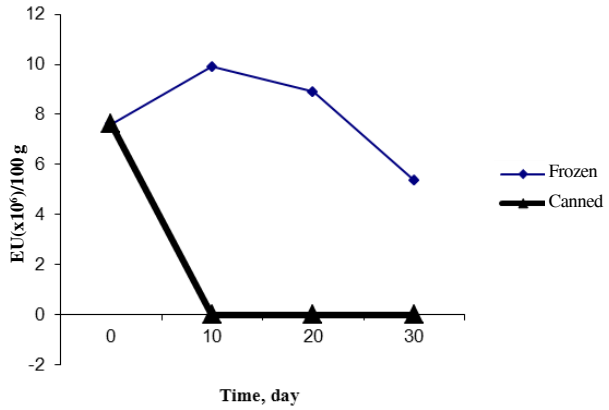

Figure 2. The POD activity of storage time and storage type.

because there are two steps involved, that is, dissociation of heme from the active enzyme and a conformational change in the apoenzyme [24]. Plant extracts contain a mixture of POD isoenzymes with various heat stabilities and often give a nonlinear heat inactivation plot. A nonlinear heat inactivation plot has also been reported for the thermostable isoenzyme A purified from cauliflower[25].

In the tenth day, the increases of both POD activity and protein might be results of enzyme regeneration after denaturation and large amount observed in POD protein portion in the total protein.

A common characteristic of all the peroxidases studied was that reactivation following heat treatment did not occur or did so only to a very low degree when treatments were at low temperatures for long times. The extent of reactivation increased as enzymes were treated at high temperatures for short times. This is in agreement with previous observations that reactivated POD has been found to cause significant deterioration in the quality of various HTST processed foods. Under certain conditions any peroxidase will regenerate after heat treatment. The degree of reactivation will depend on treatment condition and the enzyme itself. The rate of reactivation may be dependent on the amount of intermediate formed in the first reaction or remaining after inactivation. The heating time corresponding to the maximum rate of reactivation was when formation of the intermediate has been completed and only inactivation occurs. Studies have suggested the presence of intermediates during the reactivation process, and the formations of these intermediates were influenced by the heating process. Still more study is needed to elucidate mechanisms involved in the reactivation of peroxidase.

Freezing is one of the most effective methods of preserving the nutritive constituents of raw materials for long time of storage. This method also permits a good preservation of initial sensory traits of the raw material and gives products well suited for further culinary processing. However, frozen vegetables prepared using traditional technology; where blanching is used for protection against enzymes mainly, need additional cooking before consumption. The traditional cooking in water can intensify unfavorable changes in chemical composition, which appeared during the production and storage of frozen vegetables [26].

The results presented in this work showed that there were significant changes in the activities of POD as well as in the contents of protein. There is some concern that the heat treatment required to give complete inactivation may represent an excessive blanch in many cases. However, for some vegetables complete inactivation of peroxidase enzyme is necessary in order to obtain good quality frozen stored products [11].

Conclusions

The results of our study show that the blanching at a temperature of 75°C for 5 min was not sufficient for inactivating POD activity. However, blanching and conserving processes have inactivated POD activity. Furthermore, blanching at 95°C for 3 min has the last critical process point for complete inactivation of turnip roots POD activity. Nevertheless, keep in mind that the POD has maintained its activity in the freezing time and was reactivated with thawing (Fig. 2).

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