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Heat Inactivation of a Soluble Peroxidase Extract from Acetone Powder of Kohlrabi and its Reactivation after Heat Treatment

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ABSTRACT

Heat inactivation in the temperature range 70–95°C and subsequent reactivation at ambient temperature of soluble peroxidase extracts from acetone powder of kohlrabi were studied by activity measurements and isoelectric focusing in polyacrylamide gel. Between 70 and 80°C, inactivation proved not to be a simple first-order process. This showed the presence of enzyme fractions of different heat stability in the extract. At 90°C and 95°C, monophasic first-order curves were obtained which represented the inactivation of the heat stable fraction. Reactivation was most marked in its initial phase and its extent decreased with increasing temperatures of heat treatment. The progress curves of reactivation showed two or three consecutive logarithmic phases, depending on the temperature of heat treatment. This phenomenon was interpreted as resulting from selective reactivation of isoenzymes as supported by zymograms.

INTRODUCTION

Undesirable changes, such as off-flavour formation or bleaching of chlorophyll or carotenoid pigments, occurring during the storage of

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canned, quick-frozen or dried vegetables are attributed to the action of some oxidative enzymes (catalase, peroxidase and lipoxygenase) (Zoueil & Esselen, 1959; Yamamoto *et al.*, 1962; Blain *et al.*, 1968; Blain, 1970; Ben-Aziz *et al.*, 1971*a*, *b*; Eriksson, 1973; Burnette, 1977; Kanner *et al.*, 1977; Schaller & Vámos-Vigyázó, 1978; Lee & Hammes, 1979; Yamauchi *et al.*, 1980). The majority of the enzymes present in vegetable tissues are inactivated during the blanching process. However, peroxidase (POD), considered to be the most heat stable enzyme of plant origin, has been reported by a number of authors to partially regain its activity after heat treatment (Schwimmer, 1944; Zoueil & Esselen, 1959; Winter, 1969; Adams, 1978; Naveh *et al.*, 1982; Ongley & Adams, 1982). Irreversible inactivation of POD would require severe blanching conditions for most vegetables. These would adversely affect both the nutritive value and the hedonic properties of the processed products (Esselen & Anderson, 1956; Zoueil & Esselen, 1959; Böttcher, 1975; Lathrop & Leung, 1980). Consequently, a great number of communications have been devoted to studying the response of POD to heat treatment (Lee & Wagenknecht, 1958; Eriksson & Vallentin, 1973; Böttcher, 1975; Duden *et al.*, 1975; Ling & Lund, 1978; Paulus & Duden, 1981; McLellan & Robinson, 1984).

Heat inactivation of POD has been established to follow mono- or biphasic first-order kinetics, depending on the source of the enzyme and the temperature of heat treatment (Resende *et al.*, 1969; Winter, 1969, 1971; Clochard & Guern, 1973; Duden *et al.*, 1975; Ling & Lund, 1978). Sometimes a third transition phase could also be observed (Yamamoto *et al.*, 1962; Naveh *et al.*, 1982). Reactivation of peroxidase after heat treatment has been much less studied and the reports on its kinetics are contradictory. Schwimmer (1944), Lu & Whitaker (1974) and Naveh *et al.* (1982) found reactivation to proceed at the highest rate during the first hours following heat treatment; according to Adams (1978) the process consists of two first-order phases, while yet other workers consider it to be too complex to fit the usual kinetic equations (Joffe & Ball, 1962).

In a preliminary study we observed differences in POD behaviour in the homogenates of various vegetables kept for 10 min at temperatures between 55 °C and 110 °C (Mihályi & Vámos-Vigyázó, 1975). Reactivation was found to occur in the homogenates of cauliflower, horseradish and kohlrabi. As initial reactivation was most marked in the latter, this was selected for further study.

MATERIAL AND METHODS

The enzyme source

Kohlrabi (*Brassica oleracea* var. Gongyloides) was purchased from a co-operative farm in a suburb of Budapest. The cultivar Kék szalonna (Blue Lard) was used throughout the study.

Preparation of soluble enzyme extracts

The peeled root was diced with a stainless steel knife, distilled water was added until the solids content of the mixture reached about 4% (w/v), then this was homogenized in an Ultra-Turrax homogenizer (Janke & Kunkel, Ika-Werk, Stauffen i. Br., GFR) and allowed to stand overnight at 5 °C. After filtration, the enzyme protein was precipitated from the filtrate with three volumes of −20 °C acetone and allowed to stand overnight at 5 °C. Subsequently, the precipitate was washed several times with −20 °C acetone and dried in air at ambient temperature. Fifty grams per litre water extracts of the acetone precipitate were used in the experiments.

Heat inactivation

In order to minimize the heating time, the enzyme solution was injected into a test tube containing a 40-fold volume of distilled water preheated to the desired temperature (Ling & Lund, 1978). The temperature of the enzyme solution thus diluted was recorded with the aid of a thermocouple. At the end of the heating period, the test tube containing the solution was immediately dropped into an ice-water bath.

Heat inactivation experiments were performed for 0–35 min at temperatures of 70, 75, 80, 90 and 95 °C. (Preliminary experiments showed no measurable change in activity after heat treatments below 70 °C; Vámos-Vigyázó *et al.*, 1980). '0'-time stands for instantaneous cooling of the solution after injection. The increases in thermal loads caused by the heating and cooling periods were calculated from the thermocouple records. Heating times were found to be negligible. The method of Shapton *et al.* (1971) was used to calculate the increments of residence times for the different keeping temperatures from the time–temperature records of the cooling period. The calculated increments

ranged from 0·1 s (70 °C) to 1·6 s (80 °C) and were taken into account only with the '0'-min heat treatments. Heat inactivation experiments were carried out in duplicate.

Reactivation

The heat treated enzyme solutions were kept at 22 ± 2 °C for 24 h. According to preliminary experiments carried out with tissue homogenates (Vámos-Vigyázó *et al.*, 1979) this was sufficient time for maximum reactivation. During the reactivation period samples were withdrawn at intervals for activity measurements.

Measurement of enzyme activity

POD activity was assayed in triplicate, essentially according to the method of Mihályi & Vámos-Vigyázó (1975). As reactivation started immediately after cooling of the enzyme solutions, the time from the end of the cooling period to the beginning of the activity measurement was standardized at 1·5 min. The activities of heat treated and reactivated samples were expressed as a percentage of the value found in the untreated sample.

Isoelectric focusing

Isoelectric focusing was performed in polyacrylamide gel rods, using a pH gradient of 3·5–10 (Ampholine, Pharmacia, Uppsala). The run took between one-and-a-half and two hours. The active enzyme fractions were stained by immersion, for 10 min at 38 °C, in a solution containing 0·125 g *o*-dianisidine dissolved in 70 cm^3 of 60 % (v/v) ethanol, 14 cm^3, pH 5·0, acetate buffer and 0·2 cm^3 of 30 % (v/v) H_2O_2 (added immediately before use) in 100 cm^3 (Pozsár, 1979).

RESULTS AND DISCUSSION

Heat inactivation

The logarithms (log_{10}) of residual POD activities obtained after heat treatments at different temperatures are plotted versus durations in Fig. 1.

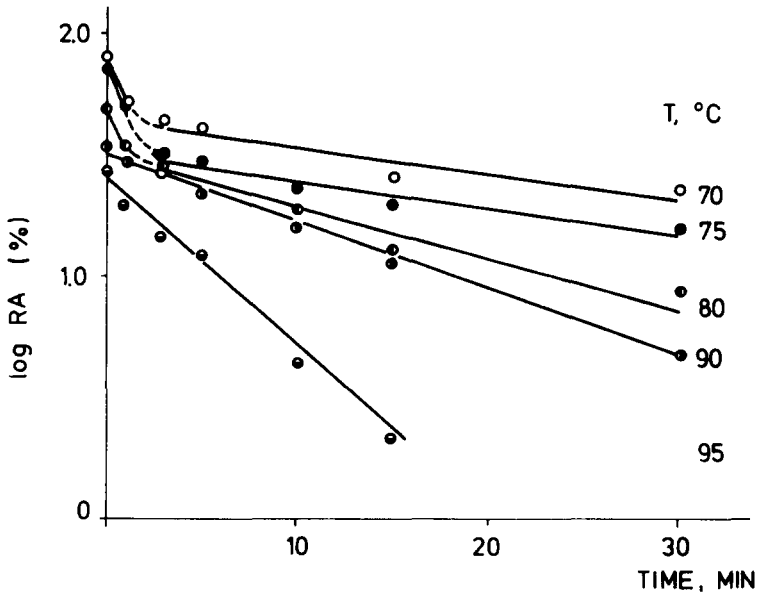

Fig. 1. Relationship between the logarithm of residual enzyme activity and duration of heat treatment in soluble peroxidase extracts of kohlrabi acetone powder. *RA* = residual activity related to the value found in the untreated sample; *T* = temperature of heat treatment.

The curves obtained at the three lower temperatures show the process to consist of more than one phase. Even at these temperatures inactivation of the heat labile enzyme fraction started within a few seconds (at '0' time). At the two higher temperatures (90 °C and 95 °C), inactivation of the heat labile fraction was instantaneous. The curves show the inactivation of the thermostable fraction. This was found to be a first-order reaction. The values of *D* (decimal destruction time), calculated for the thermostable fraction, were, in order of increasing temperature: 60, 43, 24, 18·4 and 5·7 min. The increase in temperature required to reduce *D* by one order of magnitude (z) was 25 °C.

Reactivation

The increase in activity observed in the enzyme extracts during 24 h at ambient temperature, following 15-min heat treatments at different temperatures, is shown in Fig. 2.

Reactivation started immediately after heat treatment and was the

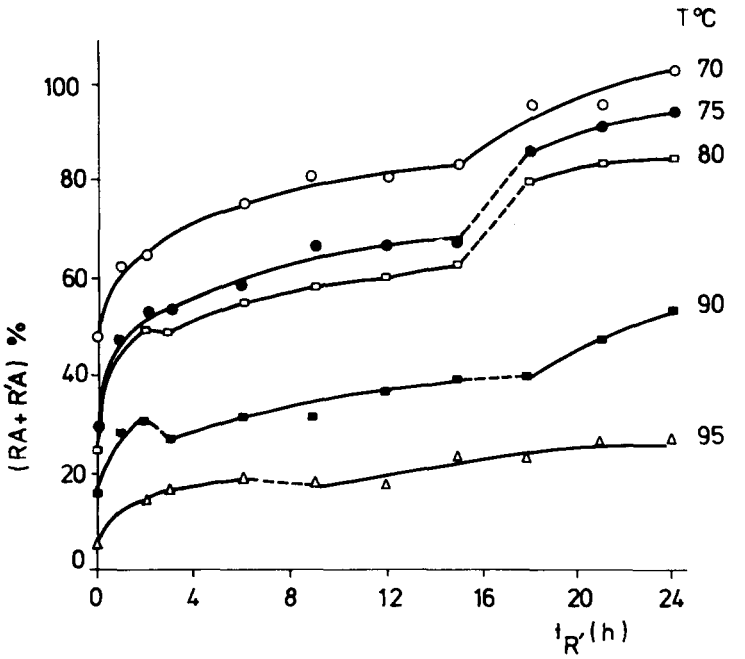

Fig. 2. Reactivation of peroxidase in the soluble extracts of kohlrabi acetone powder after heat treatment. $(RA + R'A)$ = residual + regenerated activity as related to the value found in the untreated sample; $t_{R'}$ = reactivation time; T = temperature of 15-min heat treatment.

more pronounced the lower the treatment temperature. Regeneration was found to proceed in several phases. The whole 24-h process, but also the individual phases, best fitted logarithmic equations: the sum of residual and regenerated activity was a linear function of the logarithm of time. In the range of heat treatments between 70 and 90 °C, the best fits were obtained by assuming a triphasic process; for the sample treated at 95 °C, the best fit was biphasic. It follows from the logarithmic character of the curves that reactivation was most pronounced during the initial period.

Changes in isoenzyme composition

Figure 3 shows the POD isoenzyme composition of the untreated kohlrabi extract of a sample kept at 90 °C for 15 min and of the same sample allowed to stand after cooling for 2 h and 24 h, respectively, at room temperature.

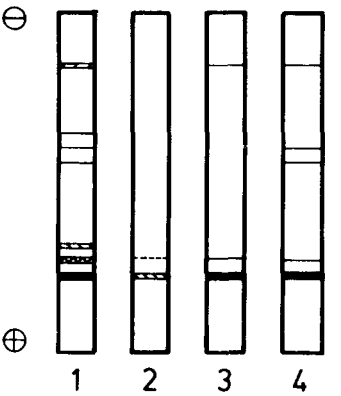

Fig. 3. Heat inactivation and reactivation of peroxidase isoenzymes in the soluble extracts of kohlrabi acetone powder. Zymograms of: 1—untreated extract; 2—extract kept for 15 min at 90 °C; 3—extract 2 after 2 h at room temperature; 4—extract 2 after 24 h at room temperature. For details of electrophoretic separation see text.

The zymograms show the selective heat inactivation of the isoenzymes. Of the seven active bands found in the fresh kohlrabi extract, only two proved resistant to the 15-min treatment at 90 °C. These were the fractions of the most acidic isoelectric points. After 2 h at ambient temperature these gained somewhat in strength and a third band reappeared. After 24 h five bands could be observed.

CONCLUSIONS

Heat inactivation

The logarithmic inactivation curves obtained are similar to those reported by Yamamoto *et al.* (1962) for sweet corn, by Ling & Lund (1978) for horseradish and by Naveh *et al.* (1982) for corn-on-the-cob. The central curvilinear phase was considered by Yamamoto *et al.* (1962) as transient and might be due to simultaneous inactivation of the heat labile and heat stable enzyme fractions. In our experience the appearance of the third phase depends, to a great extent, on experimental conditions. At 90 °C and above, the heat inactivation of POD from a number of sources has been found to proceed according to monophasic first-order kinetics (Joffe & Ball, 1962; Yamamoto *et al.*, 1962; Duden *et al.*, 1975; Park & Fricker, 1977). This is in agreement with our findings.

As shown by the z value, kohlrabi POD is among the most heat

resistant peroxidases found in vegetables (Vámos-Vigyázó, 1981). The z values of the the heat stable fraction of the horseradish enzyme were established as 27·9 and 31·4°, respectively, according to the degree of purity of the preparation (Adams, 1978); those of spring and autumn spinach homogenates were established as 33 and 45°C, respectively (Duden *et al.*, 1975). Green beans, green peas and cauliflower were found to have z values of 15·3, 9·9 and 13·6°C, respectively (Jankow, 1963; Varoquaux *et al.*, 1975; Ramaswamy & Ranganna, 1981).

Reactivation and isoenzyme composition

The conflicting findings published in the literature on the kinetics of POD reactivation after heat inactivation might be—at least partly—due to the different thermal properties and behaviour of peroxidases from different sources. Similarly, the kohlrabi POD reactivation rate was also found to be highest in the first 2 or 4 h after heat inactivation of the enzyme of green peas, green beans and horseradish (Schwimmer, 1944; Lu & Whitaker, 1974; Varoquaux *et al.*, 1975). The progress curves of reactivation, as published by Schwimmer (1944) and Naveh *et al.* (1982), were similar to those found by us for kohlrabi. However, no attempts have been made so far to describe them by mathematical equations. Neither were these curves interpreted by the authors mentioned as consisting of several phases. The individual phases of reactivation might be due to different reaction rates and extents of the individual isoenzymes, as shown in Fig. 3. The POD isoenzymes of green beans were found to have different degrees of heat resistance, the acidic ones being more heat stable than the basic ones (Lee, 1981). This is in agreement with the results presented here. In contrast to the findings of Ongley & Adams (1982) no new isoenzymes could be detected after reactivation. It should be noted that these authors performed their experiments in a range of higher temperatures (90–150°C) and with POD from other sources.

The extent of reactivation (as related to residual activity) was found by several authors to be highest after heat treatments carried out at an intermediate temperature (Park & Fricker, 1977; Adams, 1978). However, as already stated, no data are available on the relationship between the initial rate of reactivation and the temperature of heat treatment. Naveh *et al.* (1982) found a linear relationship between maximum reactivation and residual activity, as obtained directly after heat treatment. This could not be confirmed by our results.

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