

## Effects of thermal treatment on colour and texture of *Typha latifolia* L.\*\*

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**A b s t r a c t.** Through the analysis of the residual activity of peroxidase (POD), chromatic aberration, shear intensity and shear power, the effects of different thermal treatment times at 100°C on the POD, surface colour and texture of *Typha latifolia* L. were evaluated. The results showed that the activity of POD decreased with the increasing thermal treatment time at 100°C. The regeneration amount of POD increased first for some time and then started to decrease with the treatment time. Thermal treatment times 1.0 and 1.5 min at 100°C exhibited maximum regeneration of POD for the samples stored at 20 and 37°C, respectively. The sample had acceptable texture and surface colour when they were treated at 100°C for 4 min because the POD in the sample was inactivated to an acceptable level.

**K e y w o r d s:** colour, texture, regeneration of POD, pre-treatment, vegetable processing

### INTRODUCTION

*Typha latifolia* L., which is called ‘pucai’ vegetable in China, belongs to *Typha* genus. This vegetable is distributed all over the world. *Typha latifolia* L. is also growing in Poland, and its name is ‘pałka szerokolistna’. It is used for industrial purposes. In English it is also called ‘Bulrush, Common Bulrush, Broadleaf Cattail, Common Cattail, Great Reedmace’. In China, *Typha latifolia* L. is mostly grown in the lakes, canals or ponds in the south of the Yangtze River. The young stem of *Typha latifolia* L. is used as edible vegetable in traditional Chinese cuisines, while in other nations or regions it is often used as water-growth plant for landscaping and purification of water or as herb (Ke, 1998). In famous Huai-Yang Cuisine, belonging to Chinese ‘Eight Great Cuisines’, *Typha latifolia* L. is one of the most

important vegetables for some famous dishes in Chinese restaurants and family meals (Zhou and Zhang, 2004). At present, the production of *Typha latifolia* L. reaches to about 20 000 tons each year in Southern China (Huai-an City Statistics Bureau, 2003). Unfortunately, it is very difficult to preserve the colour and texture of fresh *Typha latifolia* L. in cold or room storages (Li and Duan, 2004). Therefore, it is important to develop a preservation technology based on thermal treatment.

Peroxidase (POD) enzyme affects the quality of vegetable products in postharvest storage and processing (Han, 1999). POD is a redox enzyme which can catalyze four different reactions:

- peroxidation,
- oxidation,
- peroxide reaction
- hydroxylation.

The main reaction that POD catalyzes is peroxidation in which the substrates are peroxide and reducing agents (Wang, 1988). The main roles of POD in reducing vegetable quality include: enzymatic browning, development of off-flavour especially in low acid vegetables during storage, and lipid oxidation. In the processing of *Typha latifolia* L., severe enzymatic browning and off-flavour would take place if the activity of POD in the products is not prohibited. Therefore, inactivation of POD in vegetables is necessary for the retention of product quality. In processing vegetables, thermal treatment is a very important and effective method for enzyme inactivation (Machado and Saraiva, 2002; Shitermathe, 1990). Yamatoto *et al.* (1962) reported that the inactivation of POD is first-order and biphasic reaction over the temperature range of 60-90°C. Adams

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(1997) reported that non-linear first-order kinetics of inactivation is observed when high-purity horseradish POD (HR-POD) is heated under mildly acidic condition. On heating HR-POD at 70°C in acetate buffer at pH 5.6, strong activity regeneration is observed after short heating time whilst weak regeneration is observed after long heating time. The major reason for this behavior is the existence of iso-enzymes with different heat-stability in vegetables (Chilaka *et al.*, 2002). Ramesh *et al.* (2002) reported the results of using pulsed microwave to blanch spinach, carrot, and bell peppers at 95°C, and established the kinetics of POD inactivation. Sheu and Chen (1991) studied blanching vegetable soybeans prior to freezing using lipoxygenase (LIP) and POD (auxiliary index) as indices. Because heat-stable POD is inactivated slowly during thermal treatments, complete inactivation of POD can take a long time and hence may cause significant damage to the texture and colour of the fruit and vegetables. The thermal treatment time cannot be prolonged randomly to inactivate POD. Since *Typha latifolia* L. is stored at room temperature to reduce the cost during processing, enzyme inactivation is essential. However, there exists little information reported on blanching of aquatic vegetables and regeneration of POD associated with quality preservation.

The POD inactivated after heat-treatment can be partially restored when stored at room temperature. This is a unique phenomenon of regeneration of POD and an important characteristic of POD. Chilaka *et al.* (2002) reported that regeneration of POD activity after thermal inactivation is common in plants and attributed this to quality deterioration of partially blanched vegetables during storage. Reports on regeneration of polyphenoloxidases are rare (Ke, 1998). Based on our previous study, the blanching for *Typha latifolia* L. in short time had little effect on the nutrient attributes such as vitamin C (Zhou and Zhang, 2004).

The objectives of this study, therefore, were to establish effective blanching conditions for *Typha latifolia* L. to preserve the colour and texture of the blanched material based on residual POD activity. The effects of storage conditions on the regeneration of POD were also evaluated to make appropriate recommendations to the producers.

#### MATERIALS AND METHODS

Fresh cut young stem of *Typha latifolia* L. (cv. '90-10', 20 kg a batch) was harvested from a local farm near Huaian city, Jiangsu Province, China. As soon as the samples arrived at the laboratory, bruised and unwanted parts were removed and only the tender parts were selected and stored in a refrigerator at 4°C before treatments, which is a favorite storage temperature in the local processing factories. The blanching experiments were carried out in 24 h.

Two hundred grams of raw material (cut to 1-3 mm slices) were mixed with the 400 ml phosphate buffer (0.05M, pH 6.0-7.0, containing 1.0M sodium chloride).

They were grounded in a triturator and filtered through muslin cloth. The filtrate was centrifuged at 5000 r.p.m. for 10 min at 4°C and then the solution was stored in the refrigerator.

In most research on blanching of vegetables, the inactivation temperatures were chosen in the range of 60–90°C (Chong, 1997; Han, 1999), which are different from that used for *Typha latifolia* L. processing. To preserve the quality of *Typha latifolia* L. with shorter time, 100°C blanching was used in commercial processes. In experiment, this was done by using water bath following a practical processing procedure. Thermal inactivation was performed by placing test glass tubes (15 mm diameter and 50 mm height), containing 5 ml of the sample solution, in a thermostatic water bath (WKW-01, Shanghai Experimental Instrument Factory Co., Shanghai, China) previously equilibrated at 60°C, for 1 min (Chong, 1997) and then transferred to another thermostatic water bath at 100°C and was held for 0.5, 1, 1.5, 2, 3, 5, 7, and 10 min followed by rapid cooling in an ice-water mixture. The temperature of blanching was monitored by a thermocouple measurement device (WDJ-01, Shanghai HUAGUANG Device Co., Shanghai, China). Each treatment was repeated three times. After thermal treatments, the samples were packaged with vacuum using 25 µm PVC films, and then placed in a constant temperature chamber (501, Shanghai Experimental Instrument Factory Co., Shanghai, China) at either 20 or 37°C.

In order to study the regeneration of POD from *Typha latifolia* L. during storage, the solutions obtained from the heat-treated samples at 100°C for different times were stored under 20 and 37°C in two separate sets simultaneously. The relative activity of POD was measured after 2-day storage.

The activity of POD was measured according to the detailed procedures described in Wang (1988). Specifically, the increase in optical density upon addition of an aliquot of 0.1 ml of enzyme solution to 2.9 ml of substrate mixture solution was measured at 430 nm at 25°C using a UV-752 spectrophotometer (Lengguang Technological Company, Shanghai, China), every 15 s over 2 min. The initial reaction rate ( $\Delta OD \text{ min}^{-1}$ ) was used to express enzyme activity and calculated by linear regression of the measured value of absorbance increased over 2 min. The substrate mixture solution was composed of 0.1 ml hydrogen peroxide 0.3% (mass), 0.2 ml o-benzene diamine 1% (mass) in alcohol solution, and 2.6 ml phosphate buffer with the concentration of 0.1 mol pH 7.0. The enzyme solution, without thermal treatment, was diluted 10 times to determine the activity of POD. The relative activity of PODs in thermally treated samples was represented as the ratio against the initial activity of the POD from raw material (Wang, 1988).

Maximum shear force is the greatest force with which the machine cuts the *Typha latifolia* L., expressed in gram force (g). Shear power is the total power the machine applied on each sample divided by the depth of cut made expressed in g s mm<sup>-1</sup> (Zhang *et al.*, 2001). The maximum shear force

and shear power were used to evaluate the extent of damage of thermal treatment on the texture of *Typha latifolia* L. The smaller the maximum shear force and shear power, the larger the extent of damage of thermal treatment on the texture. To select the appropriate condition to effectively inactivate the POD and retain good texture, *Typha latifolia* L. was cut to 10 cm long and heat-treated at 100°C for 1, 2, 3, 4, 5, and 10 min, then cooled for measurement. Shear force and shear power of *Typha latifolia* L. were determined using a texture analyzer (TA.XT2i, Stable Micro Systems Ltd., Surrey, UK). The cross-head speed was set at 1 mm s<sup>-1</sup> during shearing. Every measurement was repeated three times.

*Typha latifolia* L. contains significant amount of pigments. Most those pigments are heat-unstable and cause the change of product colour. Chlorophyll is the main pigment in the vegetable and the change of structure determines the colour of vegetable. The conversion of chlorophyll to pheophytin during heat-treatments can cause the colour of vegetable to change from green to dark green or brown (Derek, 1975). To study the effect of heat treatments on the colour change of *Typha latifolia* L., the raw material and the samples heated in water at 100°C for different times and stored at 20°C for 2 days were used for comparison.

The surface colour of *Typha latifolia* L. for raw material and thermally treated samples was measured using a spectrophotometer (TCP II, Shanghai Optical Instrument Company, Shanghai, China) with a white background. The instrument was a tristimulus colorimeter which measures four specific wavelengths in the visible range, specified by the Commission Internationale de l'Éclairage (CIE) (Zhang *et al.*, 2003). The L-, a-, and b-values are the three dimensions of the measured colour which give specific colour value of the material. The L-value represents the light-dark spectrum with a range of 0 (black) to 100 (white), the a-value represents the green-red spectrum with a range of -60 (green) to +60 (red) while the b-value represents the blue-yellow spectrum with a range of -60 (blue) to +60 (yellow). During the experiment, the external surface colour of the 3 randomly selected caudexes of *Typha latifolia* L. was measured for every sample.

Thermally treated enzyme solutions were placed at 37 and 20°C for 2 days. The activity of POD was measured every 24 h.

In the production of vegetables the o-phenylenediamine test is often used to test inactivation situation of POD (Adams, 1997). After heat treatment of *Typha latifolia* L. at 100°C for 1, 2, 3, 4, and 10 min, samples were cooled immediately, then cut into pieces and placed in 1% o-phenylenediamine solution in alcohol. No colour change in solution indicates the inactivation of POD.

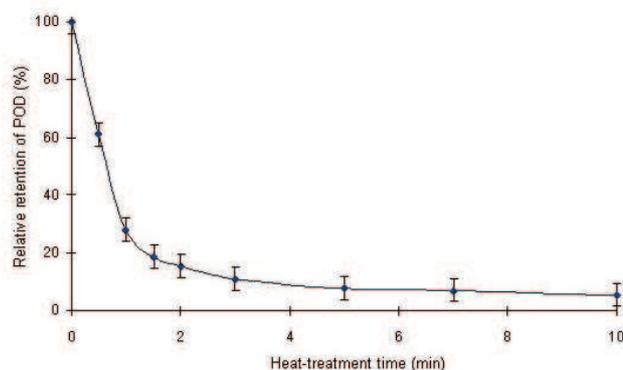
The data were statistically analyzed using ANOVA of SPSS 17.0. Differences among the mean values were analyzed using one-way analysis of variance with the application of Duncan tests. Mean values were considered significantly different for  $p \leq 0.05$ .

## RESULTS AND DISCUSSION

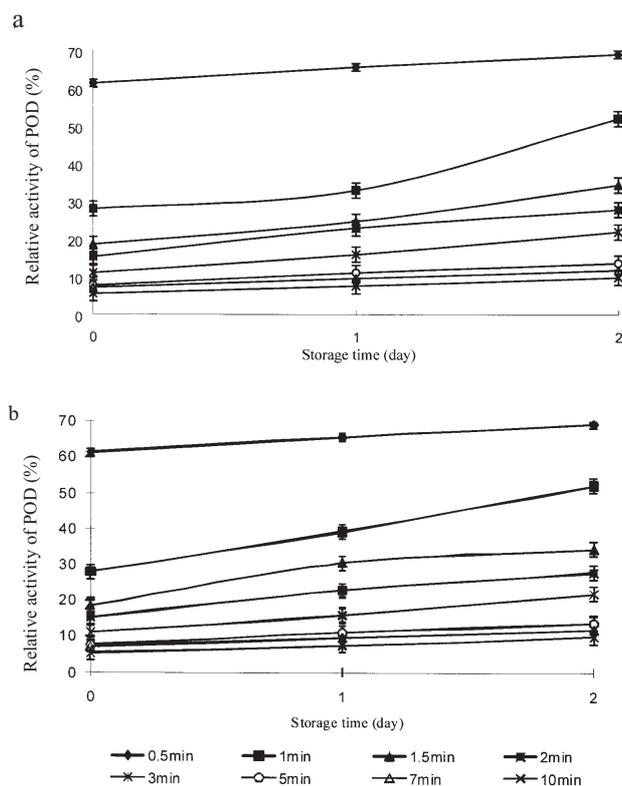
The retention of POD activity in the sample solution was shown in Fig. 1. When *Typha latifolia* L. was heated at 100°C, the retention of POD activity decreased sharply from the initial level of raw material (100%) to 27.92% in the heat-treated sample within 1 min. While further heating from 1.5 to 10 min, the retention of POD activity decreased slowly. This biphasic kinetics of thermal inactivation of POD has been reported in several studies (Adams, 1997; Morales-Blancas *et al.*, 2002; Soysal and Soylemez, 2005). It appears from Fig. 1 that the heat-treatment time of 1-1.5 min at 100°C was a transitional point from quick drop to slow drop. Similar observations were reported for several vegetables by Chong (1997).

It is also obvious from Fig. 1 that the POD in *Typha latifolia* L. cannot be reduced to 5 percent even after treating for 10 min at 100°C, which cannot completely inactivate the POD in *Typha latifolia* L. Without further treatment, the residual POD could catalyze the browning reaction and cause the deterioration of colour.

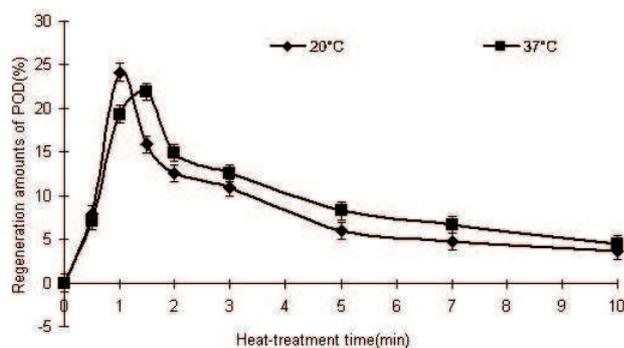
The effect of the storage conditions on the relative activity of POD samples treated at 100°C for selected durations from 0.5 to 10 min is shown in Fig. 2. Generally the POD values of heat-treated samples increased with storage time. The POD activity values at 20 and at 37°C on the 1st day were similar, but the values at 20°C were slightly higher than that at 37°C on the 2nd day. Increased activity of POD stored after 2 days over that of POD without storage could be the result of regeneration of POD in the heated samples. The regeneration of POD activity is shown in Fig. 3 for the two storage temperatures of 20 and 37°C. The amount of POD regeneration during storage increased first and then decreased with increasing treatment time. It reached the maximum value when the heating times were 1 and 1.5 min for the samples stored at 20 and 37°C, respectively. Moreover,



**Fig. 1.** Inactivation of POD when subjected to heat treatments at 100°C in 0.05 M phosphate buffer, pH 7.0 without storage. Mean  $\pm$  SD – n = 3.

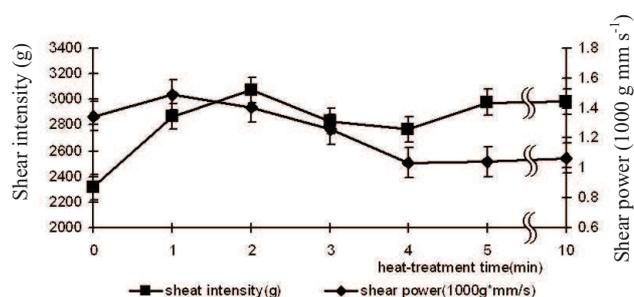


**Fig. 2.** Activity change of POD during storage at: a – 20, b – 37°C. Explanations as in Fig. 1.



**Fig. 3.** Effect of heat-treatment time at 100°C and storage temperatures on the regeneration of POD (storage time: 2 days). Explanations as in Fig. 1.

the maximum regeneration amount of POD in the treated samples stored at 20°C was larger than that at 37°C. It is interesting to note that the regenerated POD for the samples stored at 20°C was larger than that stored at 37°C when the heating time was shorter than 1 min. The opposite is true when heating time was longer than 1.5 min. According to Wang (1998), the POD regeneration can be caused by both heat-labile and heat resistant fractions. Therefore, when the heating time was shorter than 1 min, the regeneration was



**Fig. 4.** Effect of heat-treatment at 100°C on the shear force (intensity) and shear power (time: 2 days). Explanations as in Fig. 1.

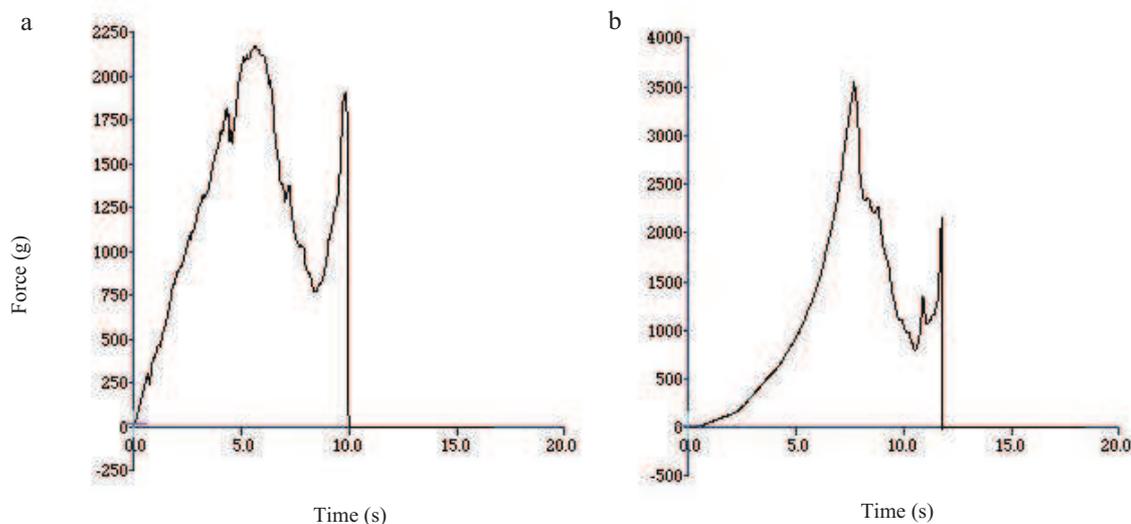
mainly caused by the heat-labile fraction of POD; while the regeneration for heat-treating time longer than 1.5 min was caused principally by the heat-resistant fraction of POD.

All relative regeneration PODs in the treated samples were more than 5% whether stored under 20 or 37°C when the heat-treatment time was greater than 0.5 min. Regeneration of POD decreased rapidly only when heat-treatment was prolonged to 4 min or longer. The residual POD activity in *Typha latifolia* L. heated at 100°C for 10 min reached 9.9% of the raw material when stored at 20°C for 2 days. Similarly, the residual activity of POD heat-treated at 100°C for 3 min reached only 21.9%. If there was no further treatment, the residual POD after heat-treatments could catalyze the browning reaction and cause the deterioration of *Typha latifolia* L.

The effects of increasing heat-treatment time at 100°C on the shear force (intensity) and the shear power are shown in Fig. 4. From 0 to 10 min of the heat-treatment time, the shear force did not change largely, while there was obvious drop of shear force after more than 4 min treatments. There was no obvious difference among the samples with heat-treatment time of 4, 5 and 10 min. This can be attributed to heat transfer in treatments. During the heat-treatment of vegetables, the outside structure was heated first and also became soft first. Shear intensity was the biggest force to cut the texture of *Typha latifolia* L., while the shear power was the average force. It is clear from Fig. 5 that heat-treatment for 4 min did not damage the central part of *Typha latifolia* L., suggesting that the heat-treatment could not damage the *Typha latifolia* L. entirely. Measured shear power confirmed the above observation, and may be a better index to evaluate the effect of heat-treatment on the texture of *Typha latifolia* L.

Figure 5 shows shear forces of the raw material and the sample heated at 100°C for 2 min, respectively. The shear force distribution of the heated sample was sharper than that of the raw material, indicating that the heat-treatment probably damaged the outside part of *Typha latifolia* L.

The colour of *Typha latifolia* L. changed significantly during the heat-treatment (Table 1). From the raw material to the sample heated for 3 min, the *a* value increased,



**Fig. 5.** Shear forces of: a – raw material, b – sample heated at 100°C for 2 min.

**Table 1.** Effect of heat-treatment time at 100°C on the colour of *Typha latifolia* L. after storage at 20°C for 2 days

Treatment time (min)	L	a	b
Raw material	74.13±0.10	-4.88±0.01	17.82±0.13
0.5	65.47±0.11	-5.19±0.02	17.95±0.15
1	67.67±0.20	-8.74±0.10	20.81±0.24
2	68.56±0.43	-8.65±0.03	19.35±0.23
3	69.20±0.31	-8.11±0.04	23.83±0.19
4	64.09±0.12	-6.38±0.06	19.16±0.20
10	64.00±0.13	-6.20±0.03	19.29±0.25

indicating an increase in greenness. Similarly an increment in b value was also observed, indicating an increase in yellowness. It might be caused by removal of the air and diffusion through internal section of the vegetable during heat treatment, which changed clarity and the reflectance of vegetable. It has been reported that when the heat-treatment temperature was higher than 60°C, chloroplast collapses and the chlorophyll were released (Derek, 1975). This contributes to an increasing in greenness and yellowness of vegetables. In contrary, when the heat-treatment time was longer than 4 min, the conversion of chlorophyll to pheophytin resulted in decrease in the greenness and yellowness, and brown colour, which was a symptom of quality deterioration. But there was little colour difference of the processed samples between 4 and 10 min heating.

The result indicated that the sample treated at 100°C for 4 min did not turn red when mixed with o-phenylenediamine solution in alcohol. It implies that treatment at 100 °C for 4 min can inactivate POD basically.

## CONCLUSIONS

1. The peroxidase (POD) in *Typha latifolia* L. can not be decreased to 5% even when treated for 10 min at 100°C.
2. There was slight difference in the POD activity change between two storage temperatures of 20 and 37°C at selected storage times. The POD activity values at these two temperatures in the 1st day were similar, but the values at 20°C were slightly higher than those at 37°C in the 2nd day.
3. The regenerated POD increased first and then decreased with increasing heat time during storage.
4. There were different effects on the shear intensity and the shear power with the increasing heat time. The shear force of the sample heated for 2 min was sharper than that of raw material, indicating that the heat-treatment probably damaged the outside part of *Typha latifolia* L.
5. When the heating time was longer than 4 min, the conversion of chlorophyll to pheophytin caused the greenness and yellowness to decrease, and resulted in brown colour, which was a symptom of quality deterioration.

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