

## REGENERATION OF PEROXIDASE FROM PINTO BEANS (*Phaseolus vulgaris*)

### REGENERATION OF PEROXIDASE FROM PINTO BEANS (*Phaseolus vulgaris*)

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**ÖZET:** Kısmi olarak inaktive edilmiş taze barbunya peroksidazının (POX) rejenerasyon kinetiği 30° ve 1°C'lerde incelenmiştir. Rejenerasyon, her iki sıcaklıkta da yavaş bir şekilde gerçekleşmiş ve rejenerasyonun çoğunluğu ilk 1 saat içinde tamamlanmıştır. Beklenildiği gibi 30°C'deki rejenerasyon, 1°C'deki rejenerasyondan daha hızlı gerçekleşmiştir. Ayrıca, 30°C'de gerçekleşen yüzde rejenerasyon ile 65°C'deki ısıtma süresi arasında, ters gelişen bir ilişki olduğu belirlenmiştir.

**Anahtar Kelimeler:** *Peroksidaz, rejenerasyon, barbunya*

**ABSTRACT:** The regeneration kinetics of partially inactivated peroxidase (POX) from fresh pinto beans were investigated at 30°C and 1°C. At both temperatures, the regeneration progressed slowly and most of the regeneration completed in 1 h. As expected, the regeneration at 30°C occurred faster than the regeneration at 1°C. In addition, an inverse relationship was observed between the percent regeneration at 30°C and heating time at 65°C.

**Key Words:** *Peroxidase, regeneration, pinto beans*

#### INTRODUCTION

Peroxidase (POX, donor:hydrogen peroxide oxidoreductase, E.C. 1.11.1.7) has conventionally been used as a blanching indicator in the processing of vegetables. This is because POX is one of the most heat stable and widely distributed enzymes in the plant kingdom. Moreover, there is an empirical relationship between the prevention of off-flavor development in frozen vegetables and inactivation of POX (GUYER and HOLMQUIST, 1954; BURNETTE, 1977; WILLIAMS et al., 1986; LOPEZ and BURGOS, 1995). Furthermore, the detection of POX and measurement of its activity can easily be done in almost every fruit and vegetable.

After blanching, POX can regain some of its activity and this may lead to undesirable changes on the quality of frozen vegetables (HEMADA and KLEIN, 1991; LEE and PENNESI, 1984; LEE et al., 1984; MCLLELLAN and ROBINSON, 1987; MILLER et al., 1990). The regeneration of POX from various fruits and vegetables has extensively been studied, including carrot, spinach and apricot (GIBRIEL et al., 1978), horseradish (LU and WHITAKER, 1974), asparagus (RODRIGO et al., 1996) and peas (PINSENT, 1962). However, no data for the regeneration of POX from pinto beans has been reported in the literature. This study was undertaken to determine the regeneration of POX from fresh pinto beans.

#### MATERIALS AND METHODS

##### Materials

Fresh pinto beans, purchased from a local market in Ankara, were shelled, washed, drained, packed in polyethylene bags, frozen and stored at -35°C until used for enzyme extraction.

##### Preparation of acetone powder

Acetone powder, prepared according to the method of COSETENG and LEE (1987), was used as the enzyme source for this study. Enzyme extraction, activity measurements and heat inactivation experiments were carried out in 0.01 M Na-phosphate buffer, pH 6.8.

### Extraction of POX

Acetone powder (2-3g) was suspended in 200 mL buffer containing 1g of polyamide as phenolic scavenger. The slurry was continuously mixed with a magnetic stirrer for 1.5 h at +2°C and filtered through eight layers of muslin cloth to remove the solid particles. The filtrate was centrifuged immediately at 7000 x g for 20 min. The resulting supernatant containing the soluble POX enzyme was used as the enzyme source for this study.

### POX activity

Enzyme activity was determined spectrophotometrically by the method of HEIL et al. (1988) using guaiacol as substrate.

### Heat inactivation experiments

The heat inactivation experiments were performed according to the method of YEMENİCİOĞLU et al. (1997) at 65°C.

### Regeneration

The regeneration study was carried out by incubating the partially inactivated enzyme solutions at 30°C and 1°C for various times.

## RESULTS AND DISCUSSION

The regeneration of POX from fresh pinto beans at 1° and 30°C progressed slowly and most of the regeneration completed in 1 h (Fig 1 and Fig 2, respectively). At both temperatures, almost the same amount of regeneration (approximately 10%) occurred for 5 min heated samples. However, regeneration of 10 and 15 min heated samples was higher at 30°C than at 1°C. Thus, it appears that little amount of POX regeneration may occur in case of rapid chilling of blanched vegetables. Similar to our results, RODRIGO et al. (1997) noted that the regeneration of POX from horseradish at 4°C occurred more slowly than that of at 25°C. In contrast, GIBRIEL et al. (1978) reported that the regeneration of POX from carrots was higher at -5°C than that of at 18°C. We believe that higher regeneration at -5°C may be due to the release of latent enzymes as a result of freezing and subsequent thawing.

As seen in Fig. 1 and Fig. 2, the more the inactivation of POX was achieved at 65°C, the lower the percent regeneration occurred. This indicates the presence of a relationship between the percent regeneration and heating time. To obtain this relationship, the logarithm of the percent POX regeneration at 30°C in 1 h vs heating time at 65°C was plotted (Fig. 3). The semi-log equation of this relationship is given by the following equation:

$$\log Y = -0.0449415 x + 1.09342 \quad (r = -0.98965)$$

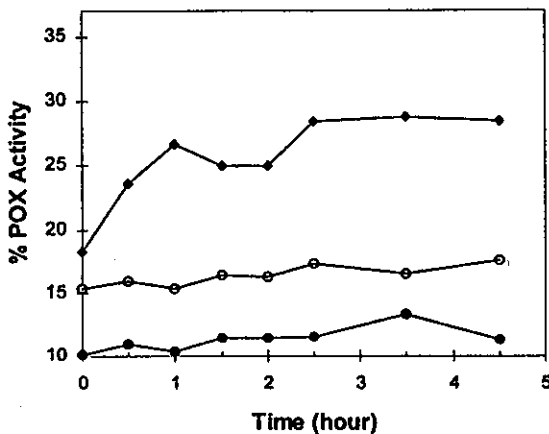


Fig. 1. Regeneration of POX from fresh pinto beans at 1°C. Heated at 65°C for: (●) 5 min (○) 10 min (●) 15 min.

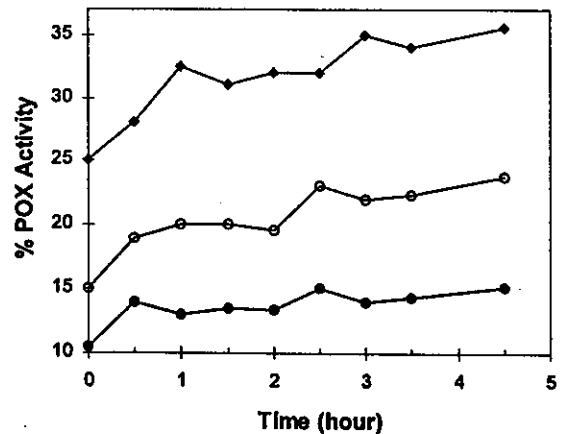


Fig.2. Regeneration of POX from fresh pinto beans at 30°C. Heated at 65°C for: (●) 5 min (○) 10 min (●) 15 min.

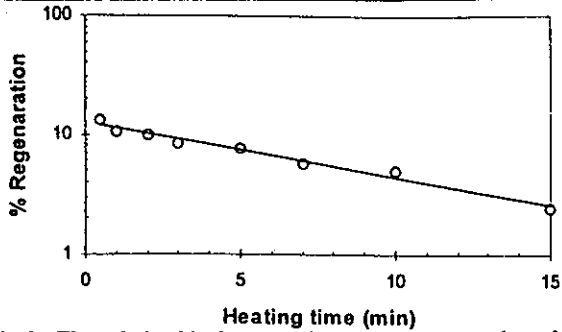


Fig. 3. The relationship between the percent regeneration of POX from fresh pinto beans 30°C. and heating time 65°C.

As expected, an inverse relationship was observed between the logarithm of percent regeneration and heating time:

$$\log (\% \text{ regeneration}) \propto [\text{heating time}]^{-1}$$

This indicates that POX regeneration may be prevented or at least reduced by increasing the blanching time. However, this increase should be carefully regulated to avoid overblanching and wasting of energy.

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