# Activation of Polyphenoloxidase in Pear Fruits by High Pressure Treatment

Masashi Asaka, Yoshio Aoyama, Ritsuko Nakanishi and Rikimaru Hayashi *

After high pressure treatment, color of slices of Bartlett pears, apples and potatoes clearly darkened, while no color change was observed in unpressurized specimens.

Polyphenoloxidase in pear fruits was activated by high pressure treatment as well as SDS treatment. Thus activated polyphenoloxidase showed same optimum pH at 6.5. The activated, partially purified enzyme showed the apparent *Km* of 22.7 mM. While a pH-activity curve of non-pressurized extract showed only one peak around pH 4, and this activity was not increased after high pressure treatment. However, pressurization or SDS treatment caused little or no activation of polyphenoloxidase in homogenates of apples, bananas, potatoes and sweet potatoes, and homogenates of leaves of peas, cabbages, lettuces, tea, and celery.

After pressurization, slices of Bartlett pears packed in film bags were kept at 5℃ for 1 month. No color change was observed in slices packed in laminated oxygen barrier film bags without microbial deterioration, while the color of slices packed in polyethylene film bags darkened.

**Key words:** high pressure, browning, *Pyrus communis*, pear, polyphenoloxidase, activation, oxygen barrier.

During the course of study on the application of high pressure treatment to food science and technology[1-8], it has been observed that the color of fruits and vegetables including pears, apples, potatoes and sweet potatoes rapidly darkens after high pressure treatment (see refs. 6 and 8). Browning is mainly caused by polyphenoloxidase (*ortho*-diphenol: oxygen oxidoreductase, EC 1.10.3.1), and decreases marketability of the product.

The enzyme has been extensively studied[9,10]. Multiple polyphenoloxidases in pear fruits have been reported: Bartlett pears contain two enzymes showing optimum pH at 4.0[11], and acetone powder of the fruits contains another enzyme showing optimum pH at 6.2[12]. Japanese pears have three enzymes with optimum pH at 4, 4.2 and 7-7.5[13].

Kenten showed that enzyme in a water extract from broad bean leaves was activated by an acid or a base shock[14], or by treatment with anionic detergents such as sodium dodecyl sulfate (SDS)[15]. The enzymes from sugar beets, spinaches, grapes and broad beans were activated by treatment with urea, proteases, or fatty

* *The Research Institute for Food Science, Kyoto University, Uji, Kyoto 611, Japan*

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acids[9]. The enzyme of d'Anjou pears seemed to be activated by SDS[16].

To investigate the cause of unfavorable browning on the high pressure processing of fruits and vegetables, effects of high pressure treatment on polyphenoloxidase in Bartlett pear fruits (*Pyrus communis*) have been studied.

## Materials and Methods

### 1. Materials.

Bartlett pear fruits were obtained from the orchard of this institute. Other fresh fruits and vegetables were purchased from a retail market in Uji city. Laminated oxygen barrier film bags were obtained from Toyo Seikan Co. Ltd.

### 2. High pressure treatment.

1) High pressure treatment of slices. Slices of Bartlett pears, apples and potatoes were sealed *in vacuo* in polyethylene film bags and laminated film bags. The sealed bags were treated with hydrostatic pressure at 400 MPa and 25℃ for 10 min by a high pressure processor (Mitsubishi Heavy Industries, type MFP-7000).
2) High pressure treatment of homogenates and cell-free extracts. Homogenates and cell-free extracts filled in polyethylene bottles were treated with hydrostatic pressure at 100-600 MPa and 25℃ for 10 min by a pressure generator (Hikari Koatsu, type KP5B)[7].

### 3. Preparation of polyphenoloxidase.

All steps were carried out at 5℃. Slices of fruits and vegetables were homogenized with an equal volume of distilled water, and filtrated through 4 sheets of cotton cloth. The homogenate was centrifuged at 10,000×g for 20 min. Ammonium sulfate was added to the supernatant. The protein fraction precipitating between 0 and 90% saturation with ammonium sulfate was collected by centrifugation, and dissolved in a small volume of 0.1 M phosphate buffer (pH 7) and dialyzed overnight against the same buffer. After centrifugation, obtained supernatant was used as partially purified polyphenoloxidase.

### 4. Determination of browning.

Surface color of slices was measured using a spectro colorimeter (Nippon Denshoku, type Z-Σ90). Browning was expressed as changes of $L$, $a$ and $b$ value.

### 5. Assay for polyphenoloxidase activity.

Polyphenoloxidase was assayed in 0.1 M sodium phosphate, pH 6.5, containing 25 mM pyrocatechol at 30℃ as described by Moore and Flurkey[17]. The activity was measured by color development by absorbance at 410 nm[17] (Shimadzu, UV-Visible Spectrophotometer, type UV-160A) or with oxygen uptake by an oxygen electrode (Rank Brothers, Rank Oxygen Electrode)[11]. One unit of the activity was defined as a change in one absorbance unit per min at 410 nm or one μmole oxygen uptake per min.

### 6. Protein determination.

The protein concentration of extracts was determined according to the method of Lowry *et al*[18].

## Results and Discussion

### 1. The effect of high pressure treatment on browning of fruits and vegetables.

After pressure was released, the bags were opened. Slices of Bartlett pears, apples, and potatoes were kept at room temperature. After high pressure treatment, Hunter's $L$ value rapidly decreased in slices of pears and apples, and slowly

decreased in slices of potatoes (**Fig. 1**). Hunter's *a* value increased in all of these slices (**Fig. 2**). While no color change was observed in unpressurized specimens.

These results meant that browning was accelerated by high pressure treatment. Since it was well-known that browning of fruits and vegetables was caused by polyphenoloxidase, the effect of high pressure treatment on the activity of polyphenoloxidase was examined.

**2. Activation of polyphenoloxidase in pear fruits by high pressure treatment.**

Activity of polyphenoloxidase in the extract obtained from pressurized pear slices was 5 times higher than the activity in the extract from unpressurized pears. No more increase of the activity was observed

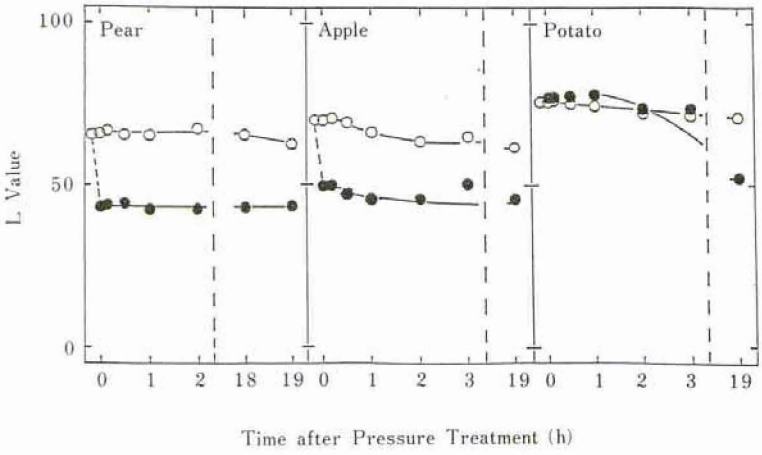


**Fig.1.** Change in Hunter's *L* value after high pressure treatment of slices of pears, apples and potatoes.

After pressurization, samples were kept at room temperature.
●; pressurized at 400 MPa and 25°C for 10 min. ○; unpressurized.

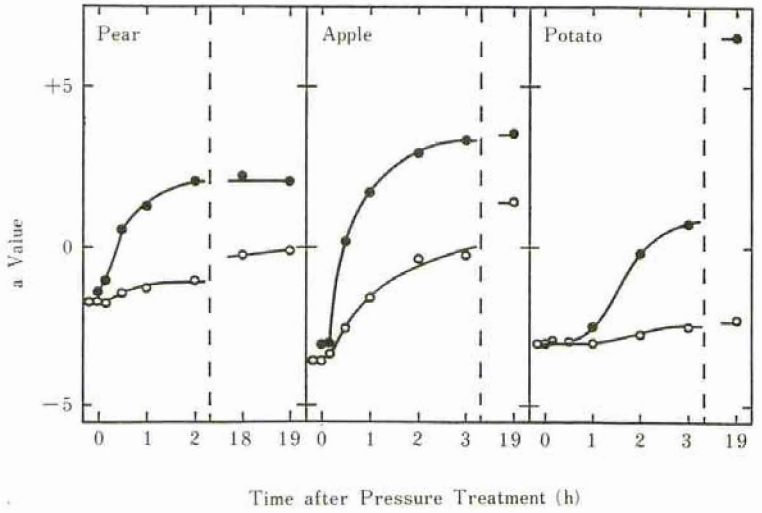


**Fig.2.** Change in Hunter's *a* value after high pressure treatment of slices of pears, apples and potatoes.

After pressurization, samples were kept at room temperature.
●; pressurized at 400 MPa and 25°C for 10 min. ○; unpressurized.

by a repeated pressurization of the former extract at 400 MPa (**Table 1**). This observation shows that polyphenoloxidase of the pears itself is activated by the pressure treatment as confirmed by experiments described below.

Activity of polyphenoloxidase in the cell-free extract was increased by the pressure treatment at 200 MPa or higher, being highest at 400 and 500 MPa (**Fig. 3**); the activation was confirmed by two activity measurements with an absorbance increase at 410 nm and with oxygen uptake. The activation was brought about rapidly (within 5 min) by the pressure treatment at 400 MPa (**Fig. 4**). This activation was observed in partially purified polyphenoloxidase (data not shown). The activated enzyme showed optimum pH at 6.5 (**Fig. 5**) and the apparent *Km* of 22.7 mM (**Fig. 6**).

Similar phenomena of the activation of polyphenoloxidase have been reported: the enzyme in a water extract from broad bean

**Table 1.** Comparison with polyphenoloxidase activity in pressurized and non-pressurized pear fruits.

| | Non-pressurized slices (units/g fresh weight) | Pressurized slices (units/g fresh weight) |
|---|---|---|
| Non-pressurized extract | 2.77 | 11.41 |
| Pressurized extract | 33.66 | 12.53 |

The cell-free extracts of pear fruits were prepared from slices that were pressuried at 400 MPa and 25°C for 10 min or non-pressurized. Then, these extracts were pressurized at 400 MPa and 25°C for 10 min.

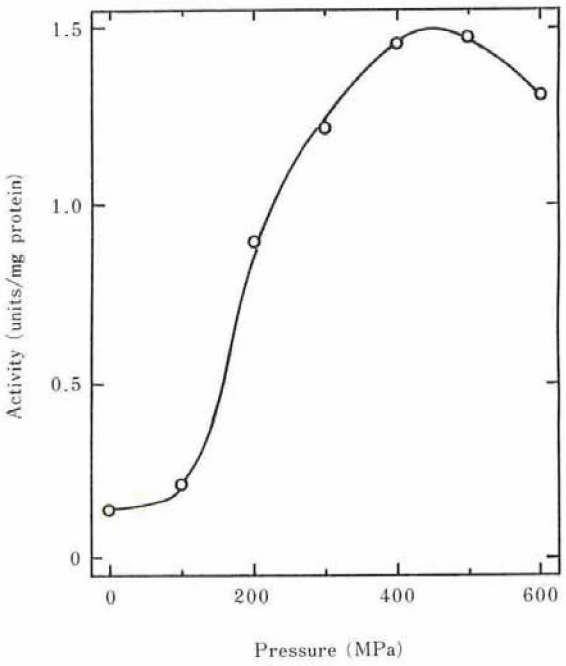


**Fig.3.** Activation of polyphenoloxidase from Bartlett pears by high pressure treatment.

The extract of pears was treated with various pressure at 25°C for 10 min. The enzyme assay was performed in 3.0mℓ of 0.1 M sodium phosphate buffer, pH 6.5, containing 25 mM pyrocatechol at 30°C. One unit of the activity was defined as a change in one absorbance per min at 410 nm.

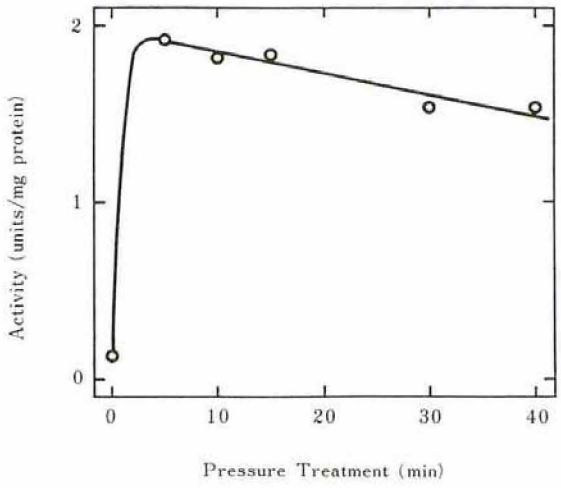


**Fig.4.** Time course of polyphenoloxidase activation by pressurization.

The extract was pressurized at 400MPa and 25°C.
See Fig.3 for the experimental details.

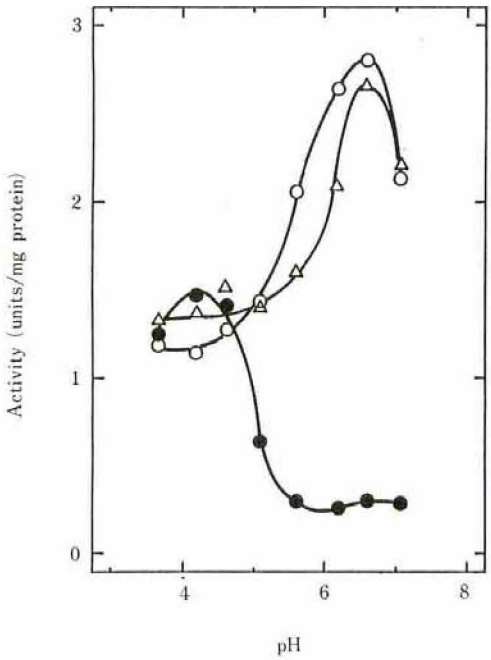


**Fig.5.** The pH-activity curve of polyphenoloxidase in the cell-free extract of Bartlett pears.

See Fig.3 for the experimental details.
○; pressurized at 400 MPa and 25°C for 10 min. ●; unpressurized.
△; treated with 0.2% SDS for 5 min.

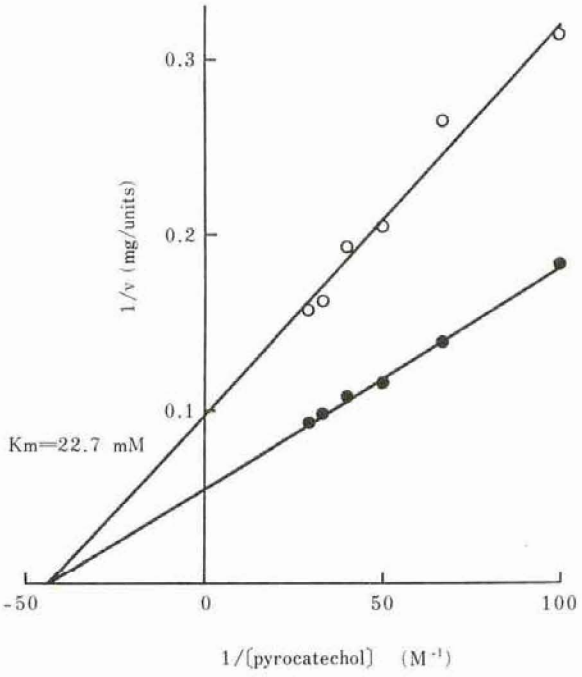


**Fig.6.** Kinetics of activated polyphenoloxidase.

Partially purified polyphenoloxidase was treated at 500 MPa and 25°C for 10 min or with 0.1% SDS.
See Fig. 3 for the experimental details.
○; pressurized at 400 MPa and 25°C for 10 min. ●; treated with 0.1% SDS for 5 min.

leaves was activated by an acid or a base shock[14], or by treatment with anionic detergents, such as SDS[15]. The enzymes from sugar beets, spinaches, grapes and broad beans were activated by treatment with urea, proteases, or fatty acids[9]. The enzyme of d'Anjou pears seemed to be activated by SDS[16]. This enzyme in Bartlett pears was also activated by 0.1 or 0.2% SDS to show an optimum pH at 6.5 (**Fig. 5**) and the apparent *Km* of 22.7 mM (**Fig. 6**).

Considering these reports, we concluded that this enzyme in Bartlett pears exists in a latent state *in situ* and is activated by SDS as well as high pressure treatment.

Slices of potatoes and apples also darkened after high pressure treatment. Therefore, further experiments were made to find whether plant polyphenoloxidase were generally activated by pressure or SDS treatment. However, pressurization at 300 or 500 MPa or SDS (0.1 or 0.2%) treatment caused little or no activation of the enzyme in homogenates of apples, bananas, potatoes and sweet potatoes, and homogenates of leaves of peas, cabbages, lettuces, tea and celery, while the enzyme in the homogenate of La France pears was activated as in Bartlett pears (**Fig. 7, 8**).

Multiple polyphenoloxidases have been reported: Bartlett pears contained two enzymes showing optimum pH at 4.0[11], and acetone powder of the fruits contained another enzyme showing optimum pH at 6.2[12]. Japanese pears had three enzymes with optimum pH at 4, 4.2 and 7-7.5[13]. In this experiment, a pH-activity curve of non-pressurized extract showed only one

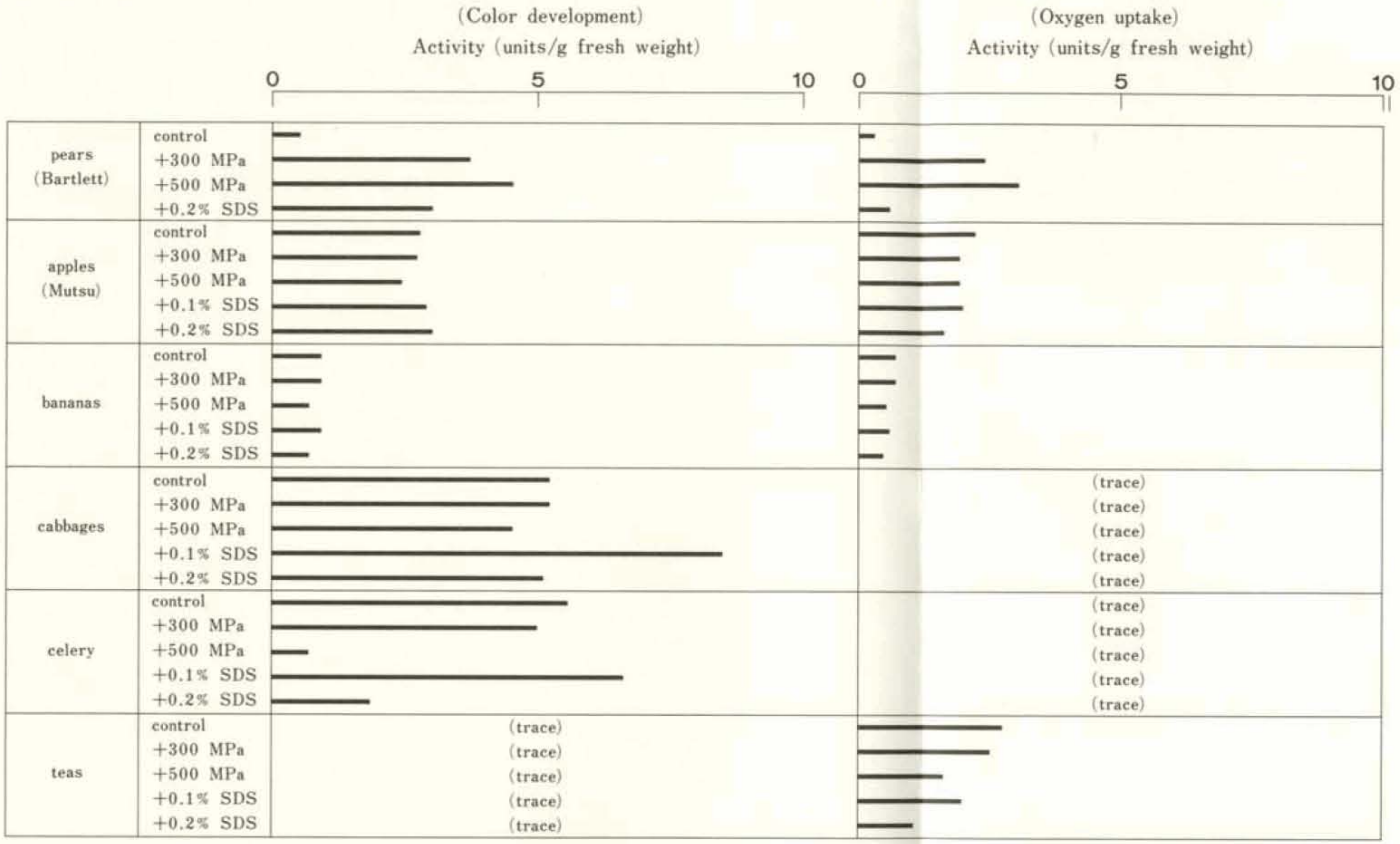


**Fig.7.** Effect of high pressure treatment on the activity of polyphenoloxidase in various plants.

+300 MPa and +500 MPa meant that homogenates of fruits and vegetables were pressurized at 300 and 500 MPa, respectively. +0.1% SDS and +0.2% SDS meant that homogenates of fruits and vegetables were treated with 0.1 and 0.2% SDS, respectively.

Two kinds of assay were used for polyphenoloxidase activity: on color development method, see Fig. 3 for the experimental details. On oxygen uptake method, the enzyme assay was performed in 1.5 mℓ of 0.1 M sodium phosphate buffer, pH 6.5, containing 25 mM pyrocatechol at 30°C. One unit of the activity was defined as a change in one μmole oxygen uptake per min.

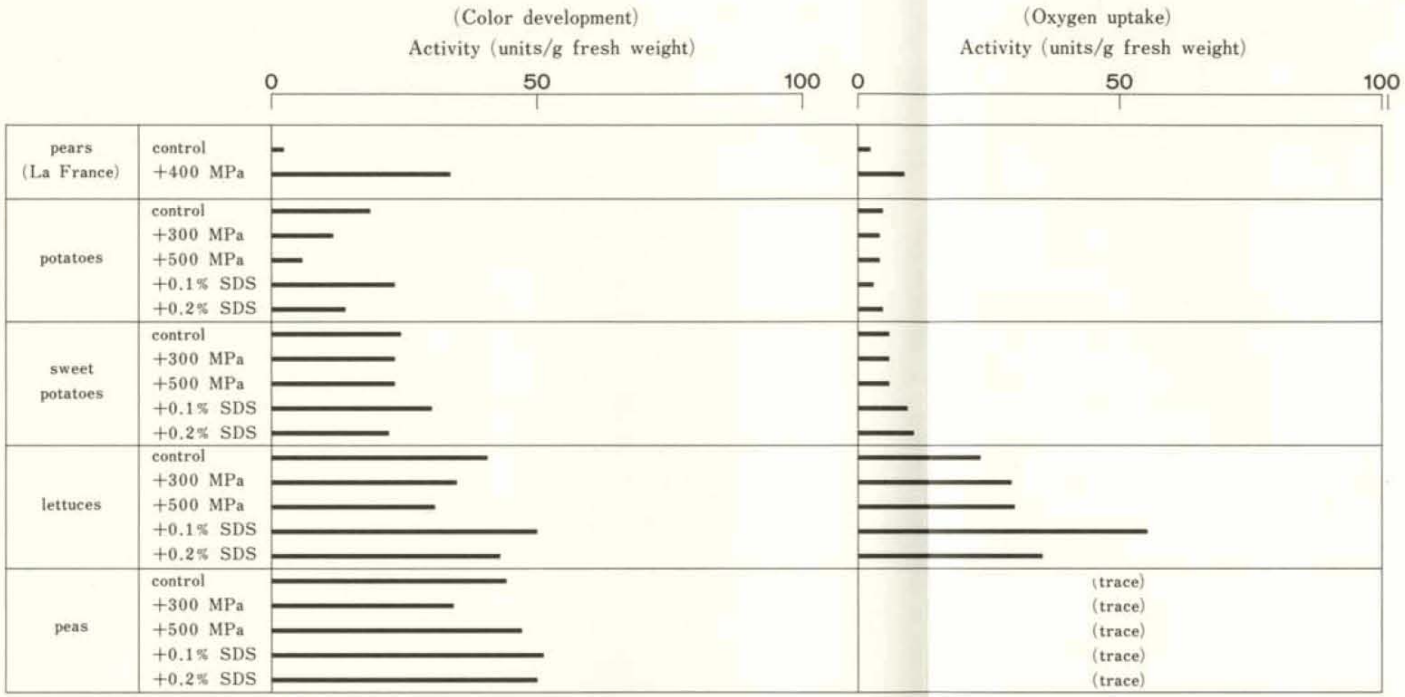


**Fig.8.** Effect of high pressure treatment on the activity of polyphenoloxidase in various plants.

See Fig. 7 for the experimental details.

Table 2. Effect of oxygen barrier property of plastic film bags on the prevention of browning of pressurized Bartlett pears.

| | Hunter's value | | |
|---|---|---|---|
| | *L* | *a* | *b* |
| Slices before pressurization* | 65.94 | -3.01 | 18.87 |
| Slices packed in laminated film bags** | 63.37 | -2.87 | 18.06 |
| Slices packed in polyethylene film bags*** | 41.73 | 6.63 | 11.98 |

The slices were pressurized at 400 MPa and 30°C for 10 min and the color was determined after 1 month at 5°C.

* The color was determined before pressurization.

** Oxygen permeability of laminated film (biaxial oriented Nylon 6/Saran/polypropylene): 12 mℓ/m²·day·atm (at 27°C and 65% RH).

*** Oxygen permeability of low-density polyethylene film: 8000 mℓ/m²·day·atm (at 27°C and 65% RH).

peak around pH 4, and this activity was not increased after the pressure treatment (Fig. 5). Thus, at least two enzymes with optimum pH at 4 and 6.5 may be present in Bartlett pears, although it was not clear whether the activity at pH 4 was generated by an acid shock or not[14].

The conclusion that the pressure-activation of polyphenoloxidase in pears is a cause of the rapid browning[6,8] of the pressurized pear slices will be drawn after the kind and number of the enzymes and the common features of the activation in plants are further clarified.

### 3. The effect of oxygen barrier property of plastic film bags on the prevention of browning of pressurized pears.

Since browning of fruits and vegetables was accelerated by high pressure treatment, the effect of oxygen barrier property of plastic film bags on the prevention of browning of pressurized pears was examined. Slices of Bartlett pears were sealed *in vacuo* in polyethylene and laminated film bags, and pressurized at 400 MPa and 30℃ for 10 min. After the pressurization, these slices were kept at 5℃ for 1 month. No color change was observed in the slices packed in laminated oxygen barrier film bags without microbial deterioration, while the color of slices packed in polyethylene bags darkened (Table 2, Fig. 9).

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Fig.9. Effect of oxygen barrier property of plastic film bags on the prevention of browning of pressurized Bartlett pears.
Pear slices sealed *in vacuo* in polyethylene film bag (left) and laminated film bag (right) were pressurized at 400 MPa and 30°C for 10 min (upper). Lower specimens were non-pressurized. After the pressurization, these slices were kept at 5°C for 1 month.