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Effect of High Pressure on Activity of Some Oxidizing Enzymes

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The inactivation effects of high pressure treatment on some oxidizing enzymes were investigated, compared with thermal inactivation. Glucose oxidase and ascorbate oxidase were inactivated irreversiblly above 300 MPa and the inactivation was followed by first-order reaction. The activation volume of inactivation of these enzymes was determined from the pressure dependence on the rate constants. Tyrosinase and superoxide dismutase were stable against high pressure. but the former was thermolabile and the latter thermostable. Thus, these enzymes are classified into three types: thermostable and pressure-stable enzyme (superoxide dismutase), thermolabile and pressure-stable enzyme (tyrosinase). thermolabile and pressure-labile enzymes (glucose oxidase and ascorbate oxidase). Thermal treatment is more effective than high pressure treatment for irreversible enzyme inactivation.

Key words: high pressure, enzyme, inactivation, glucose oxidase, ascorbate oxidase, superoxide dismutase, tyrosinase.

The residual enzyme activity in food processing seems to be one of the major problems. The effects of high pressure treatment on enzyme activity were different among enzymes (1)—(3). Although many studies have been performed about hydrolizing enzymes, there are only few studies about the oxidizing enzymes related to food quality. So we studied the effects of high pressure treatment on the activity of some oxidizing enzymes. The inactivation of the enzymes was investigated kinetically as compared with thermal inactivation.

Materials and Methods

1. Materials

The enzymes were purchased from Wako Pure Chemical Industries, Ltd. and used without purification: glucose oxidase from *Aspergillus nigar*. ascorbate oxidase from cucumber, tyrosinase from mushroom, superoxide dismutase from bovine erythrocyte. High pressure treatments were performed with high pressure test machine MFP-7000 (Mitsubishi Heavy Industies, Ltd).

2. Methods

Enzyme activity was determined as follows (4)—(6): glucose oxidase, colorimetry with glucose and *o*-dianisidine and peroxidase; ascorbate oxidase, oxygen uptake by oxygen electrode: tyrosinase, colorimetry with tyrosine: superoxide dismutase, colorimetry with xanthine-xanthine oxidase/cytochrome c.

Results and Discussion

The inactivation curves of glucose oxidase and ascorbate oxidase at different pressures are shown in Fig. 1. The plot indicates that the inactivaton is first-order reaction. As pressure increases, the inactivation velocity of these enzymes increases. However, the extrapolation to 0 min does not show 100% of enzyme activity, showing very short time treatment of pressurization and depressurization could cause inactivation of the enzyme. The

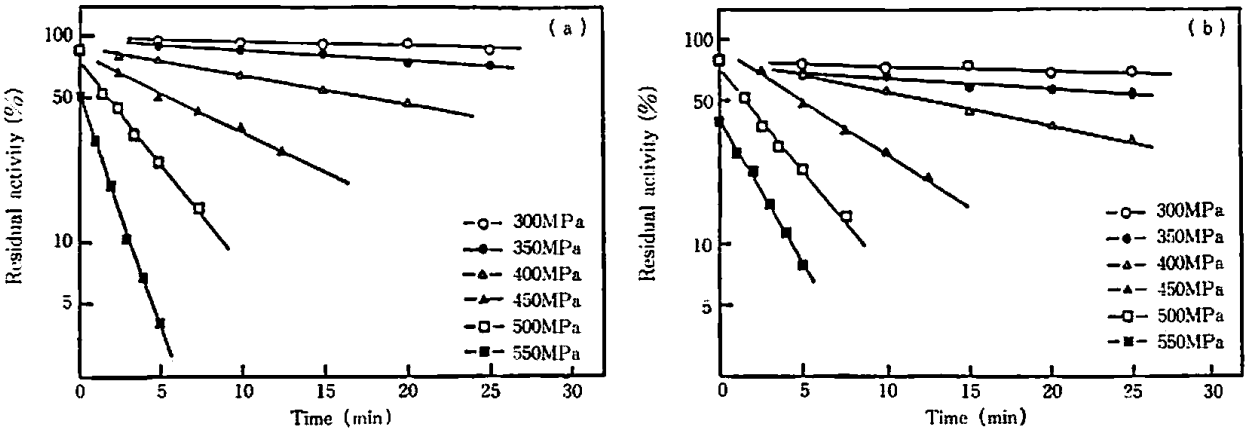

Fig. 1. Inactivation of glucose oxidase(a) and ascorbate oxidase(b) by high pressure treatment at 20 ℃.

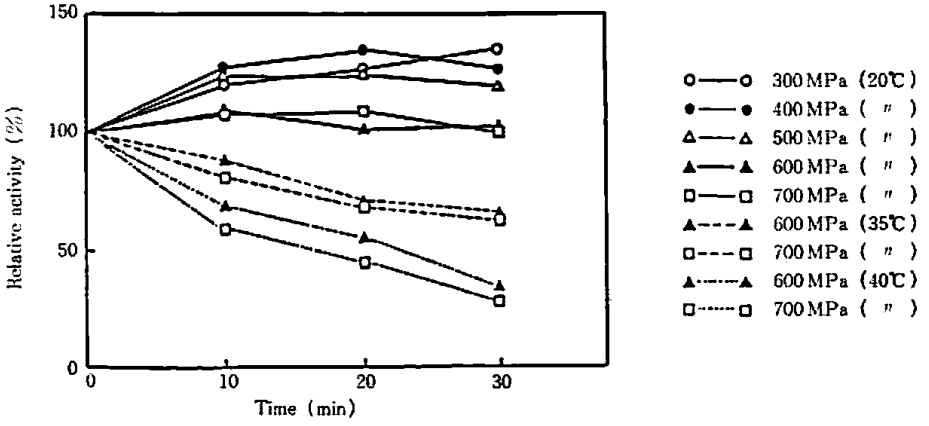

Fig. 2. Effects of high pressure treatment on tyrosinase.

Table 1. Effects of pressure and temperature on the inactivation of the oxidizing enzymes and activation volume ($\Delta V^{\neq}$) and activation energy (Ea)

	$P_{50/10m}$[a] (MPa)	$T_{50/10m}$[a] (℃)	$\Delta V^{\neq}$ (ml/mol)	Ea (kcal/mol)	P_{T10}[b] (MPa)
Glucose oxidase	440	56	−50	56	80
Ascorbate oxidase	410	56	−38	46	145

[a] The pressure or temperature which caused loss of half of the activity in 10 min.

[b] Pressure increase for the inactivation equivalent to temperature increase of 10℃.

activation volumes for inactivation of these enzymes were determined from the pressure dependence upon the rate constants (Table 1). Tyrosinase was stable against high pressure treatment (Fig. 2), but unstable against heat treatment (data not shown). Superoxide dismutase was stable against both high pressure and heat treatment.

These studies indicate that enzymes are classified into three types : 1) thermostable and pressure-stable, 2) thermolabile and pressure-stable, 3) thermolabile and pressure-labile.

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