Effects of High Hydrostatic Pressure on Distribution Dynamics of Free Amino Acids in Water Soaked Brown Rice Grain

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Abstract. High hydrostatic pressure (HP) with approximately below 400 MPa can induce a transformation of food materials to an alternative form, where membrane systems are damaged but certain enzymes are still active. HP treatment of water soaked brown rice grain could modify the mass transfer inside and apparent activities of enzymes, resulting in HP-dependent change of distribution of free amino acids. Thus, the distribution of free amino acids in brown rice grain during preservation after HP treatment was analyzed. Just after HP treatment at 200 MPa for 10 min, the distribution of free amino acids was not apparently different from that of untreated control. In contrast, after 1 to 4 days preservation at 25°C, amino acids, such as Ala, Glu, Gly, Asp and Val, showed higher concentrations than those in control. This result suggested that HP treatment induced proteolysis to produce free amino acids. However, Gln, Thr and Cys, showed no apparent difference, suggesting that conversion of certain amino acids produced by proteolysis occurred. Moreover, the concentration of γ-aminobutyric acid (GABA) in HP-treated sample was higher than that in untreated control. These results suggested that HP treatment induced alteration of distribution of free amino acids of rice grains via proteolysis and certain amino acids metabolism pathways.

1. Introduction
High hydrostatic pressure (HP) processing is one of non-thermal technologies for microbial inactivation, which can inactivate microorganisms with preventing alteration in the flavour and nutrient contents of foods. Recently, HP processing has been considered that it can potentially be used not only for microbial inactivation but also for alternation of food materials.

In general, lipid phase transitions are the most pressure sensitive of all biological systems, which cause destruction of lipid membranes under high pressure conditions approximately above 100 MPa at an ambient temperature [1]. Proteins are stable against pressure compared with lipids. Monomeric proteins become denatured above 400 MPa. Moderate pressures, of 200 to 300 MPa, are also known to dissociate oligomeric proteins into their monomers. However, activities of certain enzymes, such as amylases, are reportedly stable under high pressure treatment below 600 MPa at 20°C [2].
These findings suggest that HP processing with approximately 100 and 400 MPa can induce a transformation of food materials to an alternative form, where membrane systems are damaged but certain enzymes are still active. In the alternative form of food materials, mass transfer inside can be accelerated and certain biochemical reactions can proceed. We propose the “high-pressure induced transformation (Hi-Pit)” as an alternative use of HP in food processing. Kinefuchi et al. observed a high accumulation of \( \gamma \)-aminobutyric acid (GABA) in brown rice during water soaking after HP treatment at 400 MPa [3]. Saldo et al. found that HP treatment at 400 MPa accelerated ripening of goat’s milk cheese [4]. Ueno et al. reported that turnip root treated over 200 MPa turned green-blue color during subsequent storage [5]. These findings could be explained by the Hi-Pit effect that HP enhanced the apparent activities of enzymes, glutamate decarboxylase, proteases and enzymes for pigment synthesis, respectively.

Rice (\textit{Oryza sativa} L.) is an important crop especially in Asia. In Japan, water soaking has been traditionally practiced prior to rice cooking. The change in contents during water soaking could be caused by enzymes in rice grain responsible for proteolysis and amino acid metabolisms. HP treatment of water soaked rice grain could modify the mass transfer inside and apparent activities of enzymes, resulting in HP-dependent change of distribution of free amino acids. The distribution of free amino acids plays an important role in the tastes and nutrition. In this study, we analyzed the effects of HP on distribution dynamics of free amino acids in water soaked rice grain.

2. Materials and Methods

2.1. Sample preparation for HP treatment

Brown rice (\textit{Oryza sativa} L. ssp. Japonica, cultivar Koshihikari) was harvested in 2006 and purchased at a local rice store. A 20 g of brown rice grains was put in a polyethylene bag with 30 ml of distilled water containing sodium azide (Wako Pure Chemical Industries, Osaka, Japan) at a concentration of 0.2% (w/v) and sealed by a vacuum sealer FCB270 (Fuji Impulse, Toyonaka, Japan). The sealed grains were stored at 25ºC for 22 hours to make grains swollen enough.

2.2. HP treatment and subsequent preservation

Each pack of the soaked brown rice grains was put into the stainless-steel vessel (60 mm in diameter, 180 mm in height) of the HP apparatus (WIP, Kobe Steel, Kobe, Japan), which was filled with distilled water as a hydraulic fluid. The piston with copper section pressurized the packed grains at 200 MPa for 10 min; the temperature was maintained at 25ºC during compression. After high-pressure treatment, the grains were washed by distilled water and swept with paper towel to remove water or glutamate solution on surface. Approximately 4 g of the grains were separately packed in 5 polyethylene bags, sealed, and preserved for 0, 1, 2, 3 and 4 d at 25ºC. For untreated control, the incubated brown rice grain samples without HP treatment were also prepared.

2.3. Free amino acid analysis

After preservation at 25ºC, 2 g of the grains were homogenized with 18 ml of distilled water in a mortar. The extract was centrifuged at 6000 \( \times \) g for 3 min. The supernatant was centrifuged again at 6000 \( \times \) g for 3 min. The resulting supernatant was applied for free amino acid analysis. The free amino acids in the supernatant were extracted by a solid phase extraction and were derivatized by using EZ:faast amino acid analysis kit (Phenomenex, Torrance, USA) followed by detection through gas chromatography [6]. A GC-14A gas chromatograph equipped with a flame ionization detector (FID) (GC-14A, Shimadzu, Kyoto, Japan) was used with a capillary column supplied in the EZ: faast kit.

3. Results and Discussion

3.1. Free amino acid distribution just after HP treatment
The free amino acid concentration in rice sample just after HP treatment at 200 MPa for 10 min was measured and compared with the untreated control (Table 1). Gln showed the highest concentration of 1.3 μmol/g-rice. Ile, Phe, Lys and Trp were not detected. For non-protein amino acids, γ-aminobutyric acid (GABA) and hydroxyproline (Hyp) were detected. The GC-FID analysis in this study could not distinguish GABA and Ser, because these two compounds showed an identical retention time. The value of GABA (Ser) was determined using standard curve of GABA. GABA was detected by CE/MS analysis at a concentration roughly 10-fold higher than that of Ser (data not shown). Hydroxylisine (Hly) and cystine (C-C) were not detected. To evaluate the effect of HP treatment on the amino acid distribution, the concentration of each amino acid in HP treated- and untreated samples were shown in a scatter plot (Figure 1A). Most amino acids were distributed on a line with a slope of 1.0. This result indicated that the HP treatment showed no apparent effect on the free amino acid distribution in brown rice.

| Table 1 Free amino acid concentration of water soaked brown rice grains just after HP treatment [μmol / g-rice] |
|--------------------------------------------------|---------|---------|
| HP (200 MPa, 10 min) | Untreated control |
| Ala | 0.588 ± 0.008 | 0.589 ± 0.006 |
| Gly | 0.124 ± 0.005 | 0.103 ± 0.005 |
| Val | 0.172 ± 0.002 | 0.224 ± 0.006 |
| Leu | 0.074 ± 0.003 | ND |
| Ile | ND | ND |
| Thr | 0.465 ± 0.007 | 0.487 ± 0.010 |
| GABA (Ser)* | 0.453 ± 0.030 | 0.456 ± 0.041 |
| Pro | 0.089 ± 0.001 | 0.084 ± 0.008 |
| Asp | 0.264 ± 0.008 | 0.313 ± 0.012 |
| Met | 0.219 ± 0.003 | 0.213 ± 0.006 |
| Hyp | 0.432 ± 0.003 | 0.473 ± 0.010 |
| Glu | 0.133 ± 0.003 | ND |
| Phe | ND | ND |
| Lys | ND | ND |
| His | ND | 0.108 ± 0.014 |
| Tyr | 0.205 ± 0.003 | 0.190 ± 0.020 |
| Trp | ND | ND |
| C-C | ND | ND |
| Cys | 0.217 ± 0.006 | 0.180 ± 0.005 |
| Gln | 1.318 ± 0.012 | 1.474 ± 0.008 |

Values are averages of three experiments ± standard deviations.

* The value was determined using a standard curve of GABA.
ND, not detected.

3.2 Free amino acid distribution dynamics during preservation

HP-treated and untreated rice samples were preserved at 25°C for 4 days after HP treatment. Using amino acids concentration in samples preserved for 1 to 4 days, the scatter plots were made (Figure 1). The plots of amino acids distributed in a line with a slope more than 1.0. Ala, Gly, Glu, Val, Leu, Ile, Pro, Asp, Glu, Phe and Lys in HP-treated samples showed higher concentrations than those in untreated controls. These amino acids are rich in the total amino acids, containing those in proteins, in brown rice. The result suggested that HP treatment improved the proteolysis and that the amino acids of high concentrations were released from degraded proteins. In contrast, Gln, Thr, Met, Tyr and Cys in HP-treated and untreated samples did not show apparent differences in concentrations throughout the preservation period. Thr, Met, Tyr and Cys were relatively poor in the total amino acids in brown rice. So, it was suggested that even an improved proteolysis by HP treatment could not cause significant increase in concentrations of these amino acids. Gln, which showed no increase effect by HP treatment, is one of rich amino acids in the total amino acids in brown rice. HP treatment possibly improved proteolysis, as well as conversion of Gln to other compounds.

Two non-protein amino acids, GABA and Hyp, were detected in the HP-treated and untreated samples of 0 d preservation. These two amino acids showed different dynamics during preservation.
GABA showed increased concentration in HP-treated samples. In contrast, Hyp did not apparently increase by HP treatment. GABA and Hyp are produced from Glu and Pro by glutamate decarboxylase (GAD) and prolyl 4-hydroxylase, respectively. The concentration of Glu in the total amino acids in brown rice is higher than that of Pro. The substrate supply by HP-promoted proteolysis could be different between Glu and Pro. Saikusa et al. analyzed the distribution of free amino acids in rice grain during water soaking and showed increase in the contents of GABA during water soaking [7]. So, content and/or activity of GAD in brown rice would increase by water soaking. Moreover, the reported GAD is a monomeric protein [8], whereas prolyl 4-hydroxylase is an oligomeric protein [9]. The HP effect on the structures and function of the two enzymes may be different. These assumptions could explain the different response to the HP treatment between GABA and Hyp.

In conclusion, our results suggested that high pressure treatment induced alteration of distribution of free amino acids of rice via proteolysis and certain amino acids metabolism pathways.

**Figure 1.** Scatter plots of free amino acids concentration between untreated and HP treated brown rice after 0 d (A), 1 d (B), 2 d (C), 3 d (D) and 4 d (E) preservation. For each amino acid, a point having the concentrations in untreated (horizontal axis) and HP-treated (vertical axis) samples was displayed.

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