Chapter 24
Use of Pressure Activation in Food Quality Improvement

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Abstract Beside intensive studies on inactivation microorganisms by high hydrostatic pressure (HP) for food storage, pressure effects on property of food materials have also been studied based on knowledge in pressure effect on biomolecules. Pressure effects on biological membranes and mass transfer in cellular biological materials and on enzyme activity would give an idea that HP treatment can introduce two types of activations into food materials: improved mass transfer and enzyme activity. Studies focusing on these pressure activations on food materials were then reviewed. Rice flour with an exclusively fine mean particle size and small starch damage was obtained due to improved water absorption properties and/or enzyme activity by HP. HP treatment increased of free amino acids and γ-aminobutyric acid (GABA) in rice and soybeans due to improved proteolysis and amino acid metabolism. Improvement of antioxidant activity and alteration of polyphenolic-compounds composition in food materials were also demonstrated by HP treatment. The HP-induced activations on food materials could contribute towards processing technologies for food quality improvement.

Keywords Biological membranes • Mass transfer • Enzyme activity • Food quality improvement

24.1 Overview

Since the earliest experiments for inactivation of *Escherichia coli* and *Staphylococcus aureus* under 2,900 atm at 25 °C by Roger H. in 1895, a number of studies have been accumulated on inactivation of microorganisms by high hydrostatic pressure (HP) (ZoBell 1970). This phenomenon was also expected to apply pressure as non-thermal techniques for preservation and extension of shelf life of foods and agro products. In 1899, Hite demonstrated the preservation period of milk could

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K. Akasaka, H. Matsuki (eds.), *High Pressure Bioscience*,
Subcellular Biochemistry 72, DOI 10.1007/978-94-017-9918-8_24

be extended by HP treatment at 650 MPa for 10 min (Hite 1899). Hite et al. then demonstrated that HP treatment could also extend preservation period of vegetables in 1914 (Hite et al. 1914). Their results indicated that among spoilage microorganisms yeast showed to be more sensitive against pressure than bacterial strains. Bacterial strains, which showed relatively pressure resistance, cannot grow under acidic conditions. Thus, anti-spoilage effect of HP treatment was concluded. However, some pressure-resistant bacteria, such as spores of *Bacillus* sp., have been demonstrated to remain after HP treatment. So, the foodstuffs contaminated by the bacterial spores are sterilized with the combined pressure and thermal treatment (Wuytack and Michiels 2001; Aoyama et al. 2005).

Beside those studies on HP inactivation of microorganisms for food storage, pressure effects on physical property of food have also been studied. In 1914, Bridgman applied 500–600 MPa on egg and demonstrated that egg yolk and egg white coagulated by HP treatment but no effect on egg shell (Bridgman 1914). He indicated that pressure caused denaturation of the egg proteins by a different manner from heat treatment. Payens and Heremans (1969) reported the influence of pressure on β-casein molecule in milk. Macfarlane (1973) demonstrated meat could be softened by pressure under certain conditions. In Japan, knowledge concerning effects of HP on organisms and biomaterials has been accumulated since around 1950s (Murakami 1970). After Hayashi proposed advanced applications of HP on food processing (Hayashi 1987), research and development for establishment of HP food processing technologies was accelerated. His idea was that use of pressure (100–1,000 MPa) instead of heat treatment can allow manufacturing high quality foods without the change in the flavor and nutrient component in comparison with the heated one.

In general, HP treatment above approximately 100 MPa at ambient temperature on a cellular biological material has led to damage of biological membranes caused by phase transition of the lipid bilayers (Meersman and Heremans 2008). When biological membranes are damaged, mass transfer inside and between cells can be promoted. On the other hands, proteins are relatively resistant against pressure compared with lipids. Pressures at approximately 200–300 MPa are known to dissociate protein oligomers to their monomers. Monomeric proteins are denatured between 400 and 800 MPa. Certain enzymes were reportedly still active even under 600 MPa (Knorr et al. 2006). The pressure tolerance of enzymes indicates that HP treatment at approximately 100–400 MPa could increase apparent enzyme activities by improved association between enzymes and their substrates due to improved mass transfer by damage of biological membranes. Moreover, conformational equilibrium shift of proteins under HP might also increase enzyme activities.

These findings could lead us to an idea that HP treatment can introduce two types of activations into food materials: improved mass transfer and enzyme activity. In this chapter, studies focusing on pressure activations on food materials towards processing technologies for food quality improvement were reviewed.

24.2 Pressure Effects on Biomembranes and Mass Transfer in Cellular Biological Materials

Based on the phase transition of lipid bilayer by HP above 100 MPa at ambient temperature, HP was assumed to damage the cell structure in the plant tissue, especially affecting the plasma membrane (Kato and Hayashi 1999). Transmission electron microscopy revealed that HP at more than 100 MPa affected cellular membrane systems, especially in nuclear membrane, in yeast *Saccharomyces cerevisiae* (Osumi 1990; Shimada et al. 1993). The dielectric property analysis was also successfully used to evaluate damage of cellular membrane systems. The Cole-Cole arc decreased with increase in hydrostatic pressure applied for onion (Ueno et al. 2009a), turnip root (Ueno et al. 2009b), Japanese radish (Ueno et al. 2009c) and soybean (Ueno et al. 2010).

Damages in internal cell structures and membranes of food materials could lead to changes in food texture and physicochemical properties (Tangwongchai et al. 2000; Islam et al. 2003), as well as improved mass transfer of materials within cells and/or tissues. Then, drying, absorption, extraction and so on are expected and reported to accelerate (Eshtiaghi et al. 1994). The rate of mass transfer depends on the damage of internal cell structure. For example, the drying rate of Japanese radish treated by HP at 400 MPa (ambient temperature) for 5 min was faster than that of the untreated product, slower than those treated by both freeze-thaw and heat, and close to that treated by chloroform vapor (Ueno et al. 2009c). The water absorption of rice grains soaked in distilled water also improved under HP of 100–300 MPa at 40 °C (Fig. 24.1) (Kido et al. 2013). The improved mass transfer caused by HP could lead to improvement of association between certain enzymes and their substrates in cells and/or tissues of food materials. Thus HP treatment would have a potential to initiate and enhance certain enzymatic reactions in food materials, if the enzymes are still active during and after HP treatment.

24.3 Pressure Effects on Enzyme Activity

When HP applied for a protein, its conformational equilibrium between native and denatured states (N $\leftrightarrows$ D) would shift towards a state with the smallest volume according to Le Chatelier's principle. The conformational change by pressure is considered to mainly due to decrease in volume of cavities in the protein and to the shift of the hydration state (Fourme et al. 2012). This reversible change of the population of proteins with each conformational state could cause to the change of apparent activity of an enzyme by HP. Kido et al. (2013) analyzed pectinase (Sumizyme PTE; Shin Nihon Chemical) activity using pectin as a substrate under pressure at 100–300 MPa (Fig. 24.2). The initial reaction rate increased from 0.1 to 200 MPa with increase in the pressure level, but decreased from 200 to 300 MPa. The maximum initial reaction rate was obtained at 200 MPa. These

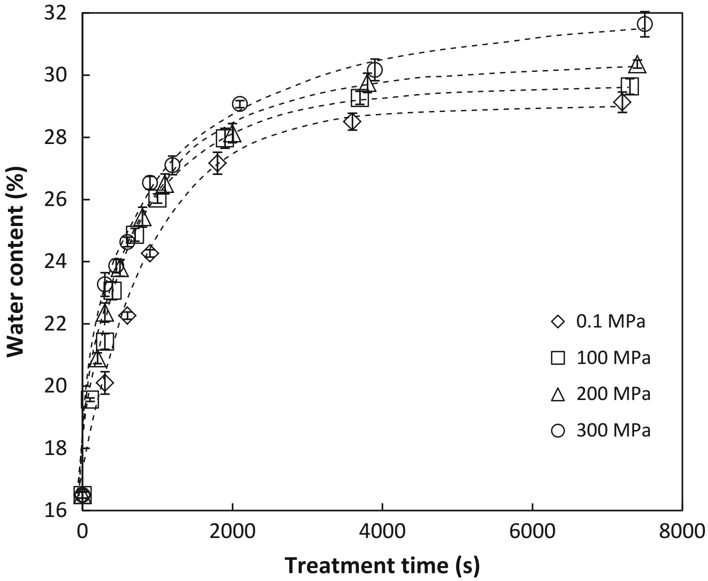

Fig. 24.1 Water absorption curves of rice grains soaked in distilled water under 0.1 MPa (atmospheric pressure; *diamonds*), 100 MPa (*squares*), 200 MPa (*triangles*) or 300 MPa (*circles*) at 40 °C. Error bars represent the standard deviations from at least three experiments. *Dotted lines* represent approximated curves based on the water absorption equation (Kido et al. 2013)

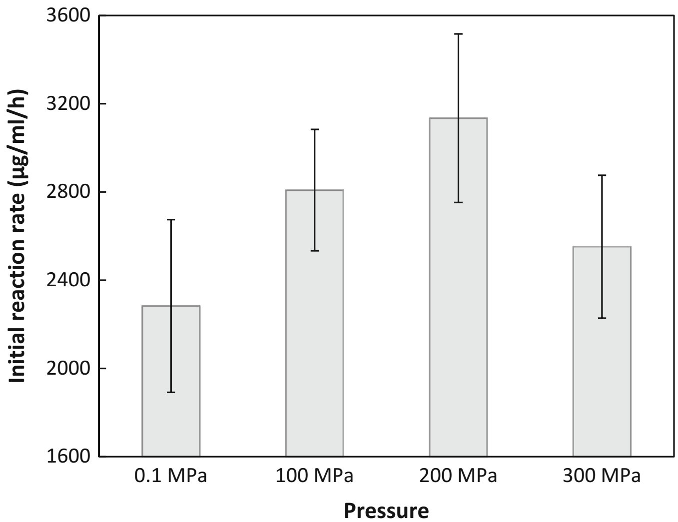

Fig. 24.2 Initial reaction rate of pectinase (Sumizyme PTE) using pectin as the substrate under HP treatment. Error bars represent the standard deviations from at least three experiments

results suggest that pressure effect on the conformational states of the pectinase gives the apparent optimum activity under pressure condition at 200 MPa, and that the conformational equilibrium shifts to the denatured state under a high-pressure levels such as 300 MPa. Although the pressure resistance against conformational equilibrium shift to their denatured states varies depending on the proteins, certain enzymes, such as α- and β-amylase and β-glucanase, were reportedly still active even over 600 MPa at ambient temperature (Knorr et al. 2006). HP roughly 100–600 MPa could not only suppress but also improve the activity of enzymes due to the conformational equilibrium shift.

Interestingly, Asaka and Hayashi (1991) applied HP treatment of 100–600 MPa (25 °C) for 10 min on the crude extract containing polyphenol oxidase in pear fruits, and subsequently analyzed its activity under atmospheric pressure at 30 °C. As the results, the activity of polyphenol oxidase increased with increase in the pressure level applied with the maximum activity at 400 and 500 MPa. HP treatment at 600 MPa relatively decreased the activity than that at the 400 and 500 MPa. The results indicate that the improvement of enzymatic activity of the polyphenol oxidase by the HP treatment for 10 min still remain even after depressurization. The conformation shift caused by pressure is considered as a reversible process. Depressurization makes the proteins with the denatured state to irreversible hydrophobic aggregation, which is pressure-gelatinization of proteins. The results by Asaka and Hayashi provided a possibility that certain enzymes are activated by irreversible conformation shift during high-pressure treatment. These results allow us to have an insight that HP can be used for improved enzymatic reactions in food materials.

24.4 Super-Fine Rice Flour Production by Enzymatic Treatment with High Hydrostatic Pressure

Rice flour is used for the production of a number of foods, particularly in Asian countries. In response to the recent expansion of rice-flour use, new and improved rice-flour production methods should be developed in order to meet this rise in demand. In general, rice flours with fine particle size and low starch damage are suitable for processing rice-flour-based bread and Western-style sweets, i.e. as a substitute for wheat flour. Particle size can be controlled by pulverization conditions. However, particle size and starch damage tend to have a trade-off relationship; fine rice flour obtained through harder pulverization tends to show higher starch damage. Pectinase used in the enzyme-treated milling method (Morohashi et al. 1998) only mildly damages the tissue structure of rice grains before subsequent mild wet pulverization, which leads to the production of fine rice flour with low starch damage. Based on the HP effect that improved enzyme activity and mass transfer, we applied HP for the enzymatic treatment to improve the enzyme-treated milling method (Kido et al. 2013; Homma et al. 2013).

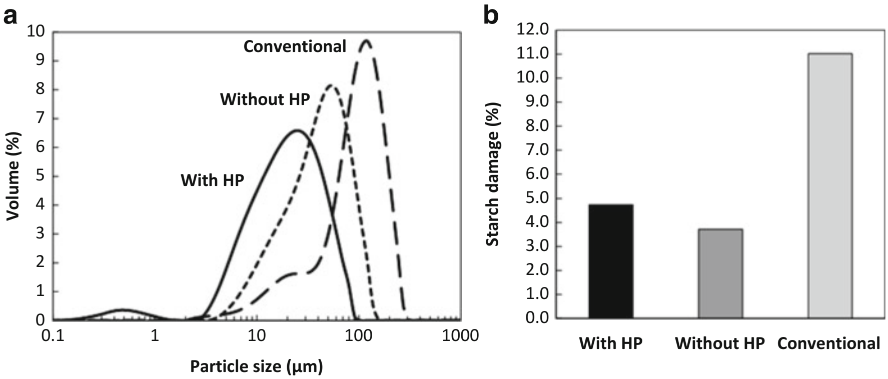

Fig. 24.3 Volume-based particle size distribution (**a**) and starch damage (**b**) of rice flour manufactured by conventional, enzyme-treated milling method without HP and enzyme-treated milling method with HP at 200 MPa (40 °C)

The apparent pectinase activity showed the highest under HP at 200 MPa. The water absorption ability also showed to be improved under HP. We therefore construct a process, which consists of HP treatment at 200 MPa (40 °C) for 1 h and subsequent wet pulverization. Using this process, rice flour with an exclusively fine mean particle size less than 20 μm and starch damage less than 5 % was obtained (Fig. 24.3). The resulting super-fine rice flour has a superior processing property suitable for bread, pasta, noodles and Western-style sweets. A pressure level of 200 MPa would give pectinase the optimal conformational equilibrium for activity and/or optimal association with pectin. The improved water absorption would contribute to tissue damage in the rice grain, thus reducing particle size in the resulting rice flour. The production process strongly correlates to improved water absorption properties and/or enzyme activity as a result of HP treatment.

24.5 Increase of Free Amino Acids and γ-aminobutyric Acid in Rice and Soybeans by Hydrostatic Pressure

Especially in Asian countries, rice is one of the most important crops. A number of researches and developments have been conducted for improvement of nutrition values and functions of rice. We investigated the possibility that improvement of proteolysis and amino acid-metabolism in brown rice by HP treatment (Shigematsu et al. 2010). Brown rice grains were soaked in water and subjected to HP treatment at 200 MPa (20 °C) for 10 min. After the treatment, brown rice grains were swept with paper towel to remove water and stored at 25 °C for 4 days. The free amino acid distribution of HP- treated samples, just after HP treatment, showed no apparent difference from that of untreated control without HP treatment. However,

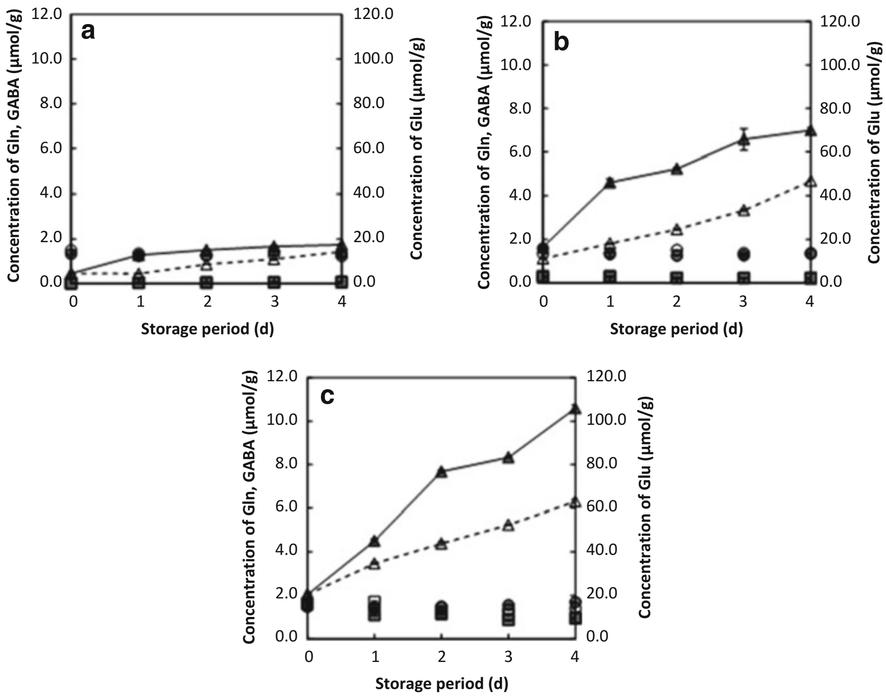

Fig. 24.4 Time course of GABA (*triangles*), Glu (*boxes*), and Gln (*circles*) concentrations in HP-treated (*closed symbols*) and HP-untreated (*open symbols*) samples, which had been soaked in Glu concentration of 0 (**a**), 0.01 (**b**), 0.05 g ml^{-1} (**c**) during storage. Error bars representing the standard deviations from at least three experiments

during 4 days storage at 25 °C after HP treatment, certain amino acids including γ-aminobutyric acid (GABA) in the HP-treated samples increased with time and showed higher concentrations than those in untreated samples (Fig. 24.4a). To investigate the feasibility for use of HP-treated brown rice grains as a bioreactor producing GABA, glutamic acid (Glu) was supplied into brown rice grains during water soaking and subjected to HP treatment. The GABA concentrations during storage increased with the increase in the Glu concentrations in the soaking solutions (Fig. 24.4b, c). The initial GABA production rate was accelerated by HP treatment.

In soybeans, which contain a large amount of proteins, the effect of HP on the free amino acids was analysed (Ueno et al. 2010). Water-soaked soybeans were subjected to HP treatment at 200 MPa (20 °C) for 10 min. After the treatment, soybeans were swept with paper towel to remove water and stored at 25 °C for 4 days. As in the brown rice, during 4 days storage at 25 °C after HP treatment, certain amino acids including GABA in the HP-treated soybean samples increased with time and showed higher concentrations than those in untreated samples. These results provide feasibility for a novel use of HP technology to alter the metabolic pathways of food materials, such as proteolysis and amino acid metabolism in brown rice grains and soybeans, and to accumulate useful metabolites.

24.6 Improvement of Antioxidant Activity of Food Materials by High Hydrostatic Pressure

Ueno et al. (2009a) applied HP treatments on onion with 200 and 400 MPa (20 °C) for 5 min. During storage at 25 °C after the HP treatment, the antioxidant activity evaluated as DPPH radical scavenging activity increased, whereas no alteration was observed for onion without HP treatment. Flavonoids were extracted from the onion samples and analyzed by high performance liquid chromatography (HPLC). The concentration of quercetin, which is the major flavonoid and antioxidant in onion, in the onion with HP treated increased during the storage. As the results, HP treatment could improve the antioxidant activity of food materials via alteration of antioxidants composition possibly caused by internal enzymatic reactions triggered by HP.

Then we analyzed effect of HP and subsequent preservation on the antioxidant activities of food materials (Shigematsu et al. 2012). Total 51 food materials were subjected to HP treatment at 200 MPa (20 °C) for 10 min and subsequently storage at 25 °C for 2 days. The HP-untreated control samples for each food material were also prepared. The antioxidant activity, evaluated as oxygen radical scavenging capacity (ORAC), of 26 products increased by HP-treatment. In the 26 products, 11 products showed HP-dependent increase of ORAC value during storage after HP-treatment (results of 23 products were shown in Fig. 24.5). Edible part of *Petroselinum crispum* (parsley) showed the highest value of ORAC in the 11 products.

P. crispum was then subjected to HP treatment at 100–600 MPa (20 °C) for 10 min and subsequently storage for 1 h. At each pressure level, the ORAC value was higher than that in the HP untreated control, with the highest value in HP treatment at 300 MPa. The analysis on polyphenolic compounds in *P. crispum*, which was HP-treated at 300–600 MPa (20 °C) for 10 min, showed that the concentration of malonylapiin [apigenin 7-*O*-(6-*O*-malonylglucoside)] decreased, but apiin [apigenin 7-*O*-glucoside] increased during storage for 30 min, resulting in the increase in the malony apiin/apiin ratio (Fig. 24.6). This result suggested that conversion of malonylapiin to apiin was triggered by the HP treatment. The alteration of the apigenin composition would be related to the HP-dependent increase in antioxidant activity in *P. crispum*.

An *in vitro* reconstruction experiment was carried out to evaluate the enzymatic conversion of the apigenins. The methanol extract of pre-boiled parsley and soluble extract of parsley were prepared as the crude extracts containing substrate and enzyme, respectively. After mixing the substrate and enzyme extracts, increase of apiin and decrease of malonylapiin were observed during incubation. In intact cells of parsley, malonylapiin and esterases are separately localized in vacuoles and cytoplasm, respectively (Lechtenberg et al. 2007; Luthria 2008). Our results

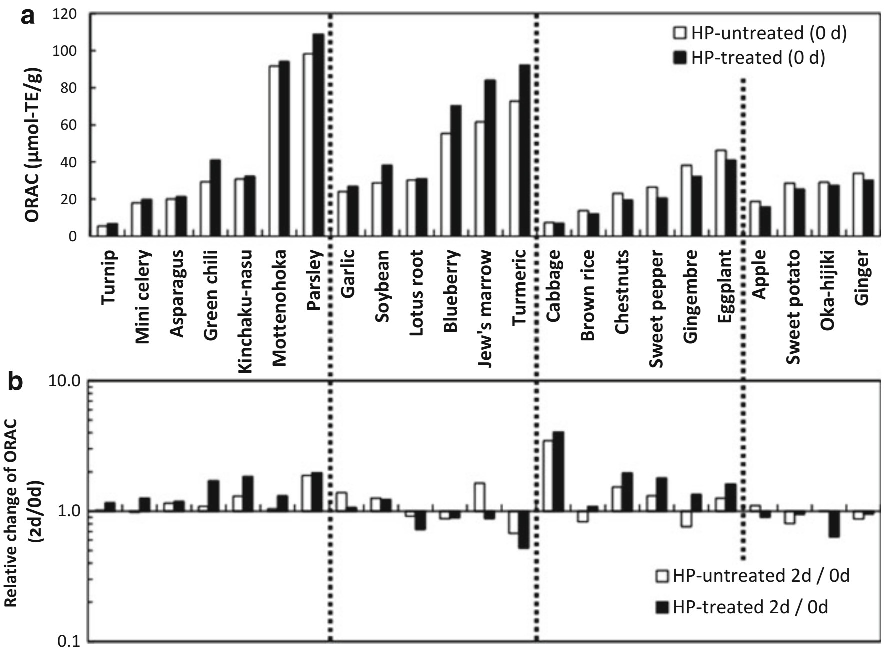

Fig. 24.5 The ORAC values, evaluated as Trolox equivalent, of HP-treated (*closed bars*) and untreated (*open bars*) food materials just after treatment (**a**) and relative change of ORAC value during 2 days storage (**b**)

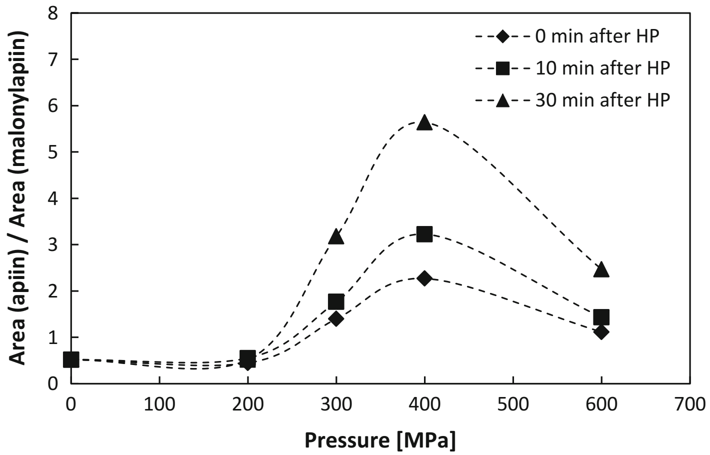

Fig. 24.6 Effect of pressure levels and time after HP treatment on malonylapiin/apiin ratio, evaluated as peak area values in HPLC analyses

suggest that damage of vacuole by HP treatment allowed improved the association between malonylapiin and esterases, which leads the HP dependent conversion of malonylapiin to apiin.

24.7 Summary

Pressure effects on biological membranes and mass transfer in cellular biological materials and on enzyme activity resulted in the two types of activations into food materials by HP treatment approximately at 100–400 MPa: improved mass transfer and enzyme activity. Rice flour with an exclusively fine mean particle size and small starch damage was obtained due to improved water absorption properties and/or enzyme activity by HP at 200 MPa. HP treatments could also cause alteration of biochemical properties or composition of compounds in food materials via the two types of activations. HP treatment at 200 MPa for 10 min increased of free amino acids and γ-aminobutyric acid (GABA) in rice and soybeans during subsequent storage under atmospheric pressure, which would due to proteolysis and amino acid metabolism by improved mass transfer and apparent activities of internal enzymes. In this case, HP caused alteration of cells in food materials, where biological membrane systems were damaged but certain enzymes remain still active. This HP-induced transformation of food materials or "Hi-Pit (Ueno et al. 2009a)" could thus change the component composition via progress of internal enzymatic reactions during subsequent storage after HP treatment. Improvement of antioxidant activity and alteration of polyphenolic-compounds composition in food materials by HP treatment would be also explained by "Hi-Pit" theory.

The HP-induced activations on food materials could be applied for alteration of physiochemical properties, as well as biochemical properties of the food materials. For example, accumulation of useful compounds via improved enzymatic reactions, activated by HP, should contribute towards processing technologies for food quality improvement.

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