

Effects of High-pressure Treatment on Mume Fruit (*Prunus mume*)

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ABSTRACT. Physical and physiological changes in mume (*Prunus mume* Sieb. et Zucc.) subjected to a 10-minute hydrostatic high-pressure treatment at 5, 10, 50, 100, 150, and 200 MPa were investigated. Mume fruit exhibited substantial injury at pressures >5 MPa. All treatments induced color changes, which became more apparent at pressures >100 MPa. Fruit subjected to pressures ≥ 100 MPa deteriorated and were rendered commercially unacceptable. After transfer to atmospheric pressure all treated fruit exhibited lower CO₂ evolution rates compared with control fruit. Only fruit subjected to 5 MPa exhibited an increase (27%) in titratable acidity. Ethylene production rate in mume fruit was very high, but consistently and dramatically decreased after treatment, regardless of the pressure applied. The decline in ethylene production was associated with a decrease in ACC oxidase activity. Chemical names used: 1-aminocyclopropane-1-carboxylic acid (ACC).

High-pressure treatment (HPT) is a nonchemical treatment that has been reported to inactivate microorganisms through membrane disruption (Hoover et al., 1989; Como et al., 1997). HPT mainly affects noncovalent bonds (Hayashi, 1989), in contrast to high temperature treatments which can destroy covalent bonds in vitamins, amino acids, and other substances related to freshness. For example, Horie et al. (1991) reported that strawberry jam prepared by high pressure retained 95% of its initial ascorbic acid concentration and was preferred by a taste panel over a heat-processed jam. Presently, there is little information on the effect of high pressure on the physiology of fresh fruit.

Mume (*Prunus mume*) fruit produces large amounts of ethylene, which limits its storage or marketable life (Zhang et al., 1991). One of the unique characteristics of HPT is that it influences enzyme activation or inactivation (Morild, 1981). This paper reports some HPT-induced changes in mume fruit, especially from the physiological viewpoint.

Materials and Methods

PLANT MATERIALS AND HPT. 'Kosyusaisyo' mume harvested from the experimental field of the Tokyo University of Agriculture were used in the experiments. Before treatment, the samples were vacuum-sealed in polyethylene bags. Hydrostatic pressure was generated by a high-pressure unit (ITP-70, IHI, Tokyo, Japan). Once loaded, the vessel was filled with the pressure-transmitting medium, composed mostly of propylene glycol. The compression was direct, piston type. After being automatically closed, the vessel was driven by the pump against the piston to generate the pressure, at a preset level. The force would act normally on any surface of a sample. The temperature was controlled by an external cooling unit, with the initial coolant temperature at -1 °C. Fruit were subjected to HPT at 5, 10, 50, 100, 150, and 200 MPa, for a duration of 10 min. The time required for compression and decompression was within 30 s.

During HPT, the temperature within the treatment chamber increased several degrees, but did not exceed 10 °C.

QUALITY EVALUATION. Peel color was measured with a colorimeter (ZE-2000; Nippon Denshoku Industries Co., Ltd., Tokyo, Japan) immediately after treatment. Values of L*, a*, and b* could be read on the instrument. Chroma and hue angle were calculated from a* and b* (McGuire, 1992). All of the data were compared using Duncan's multiple range test for significance ($P < 0.05$). For titratable acidity the macerated tissue was titrated with 0.1 mol·L⁻¹ NaOH.

GAS MEASUREMENTS. Two mume fruit were placed in 55-mL glass jars immediately after the pressure treatment. Jars were sealed and incubated at 25 °C for 1 h. Separate 0.5- and 1-mL gas samples were taken for CO₂ and C₂H₄ measurement, respectively. Carbon dioxide was measured with a gas chromatograph (GC-8A, Shimadzu Co., Ltd., Tokyo, Japan) equipped with a thermal conductivity detector and ethylene was measured with a gas chromatograph (GC-9A, Shimadzu) equipped with a flame ionization detector. Each jar of two fruit was considered a replication and each datum point represents the mean and standard error of three replications.

For the ACC assay, 1.2 to 1.3 g of pulp tissue was homogenized with two times its volume in 95% ethanol in a mortar with a pestle after gas measurements. The homogenate was centrifuged at 10,000 g_n for 15 min at 2 °C. The supernatant was concentrated to dryness with nitrogen gas at 45 °C. The dry residue was dissolved in 0.85 mL water and an aliquot was assayed for ACC according to the method described by Lizada and Yang (1979). One micromole of HgCl₂ was added to extract in a 15 × 105 mm test tube. The tube was sealed with a rubber serum cap and kept in ice. About 0.1 mL of a cold mixture of 5% NaOCl and saturated NaOH (2:1, v/v) was injected into the test tube through the rubber cap. The mixture was agitated for 20 s on a Vortex. After incubation for 3 min in ice, the tube was again agitated and a 1-mL gas sample was withdrawn for ethylene determination.

ACC oxidase activity was determined in vitro by measuring the conversion of added ACC to ethylene. A 1.2- to 1.3-g sample of pulp tissue was homogenized with 5 times its volume in 0.1 mol·L⁻¹ Tris-HCl buffer, pH 7.2, containing 30% (w/v) glycerol, 10 mmol·L⁻¹ ascorbate, 5 mmol·L⁻¹ dithiothreitol at 2 °C in a mortar with a pestle. The homogenate was centrifuged at 12,000 g_n for 20 min. The enzyme reaction was initiated with the addition of 0.5 mL enzyme

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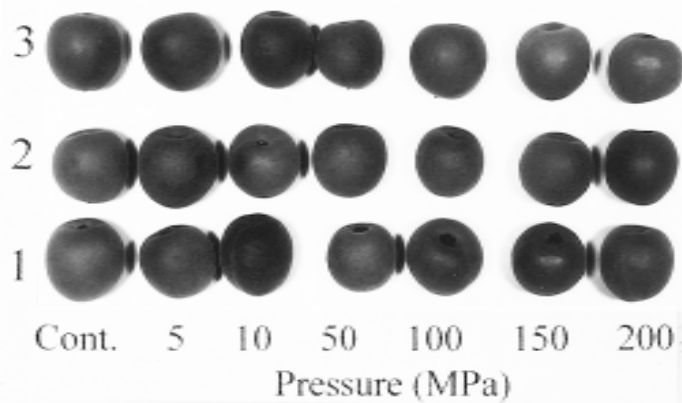


Fig. 1. Mume fruit after high-pressure treatment at control (Cont.), 5, 10, 50, 100, 150, and 200 MPa for 10 min. The three fruit per pressure are replications.

Table 1. Color changes as described by L^* , chroma, and hue angle of peel after high-pressure treatment at control, 5, 10, 50, 100, 150, and 200 MPa for a duration of 10 min.

Pressure (MPa)	L^*	Chroma	Hue angle
Control	45.84 a ^z	26.48 a	108.14 a
5	35.72 b	18.38 b	101.12 a
10	34.77 b	17.55 b	107.33 a
50	36.45 b	17.13 b	103.33 a
100	37.61 b	17.02 b	89.97 b
150	36.36 b	16.26 b	87.92 b
200	37.13 b	14.92 b	91.05 b

^zValues have same letter within columns for each color parameter are not significantly different at 5% level (Duncan's multiple range test).

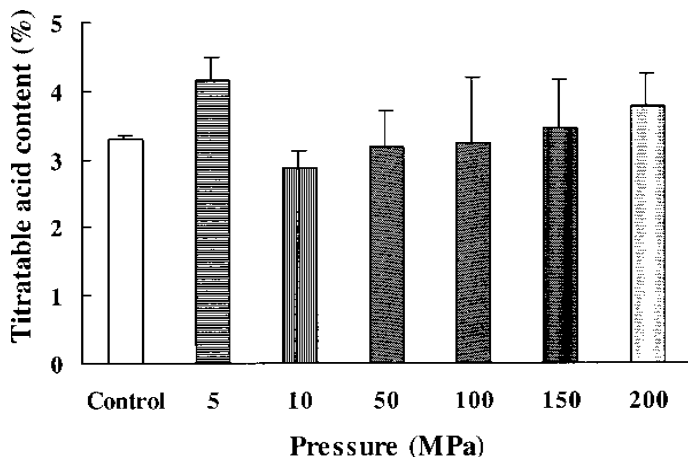


Fig. 2. Titratable acid content of fruit after high-pressure treatment at control, 5, 10, 50, 100, 150, and 200 MPa for 10 min. Error bars represent SE of the means ($n = 3$ fruit).

extract in the assay medium consisting of 100 $\text{mmol}\cdot\text{L}^{-1}$ Tris-HCl buffer, pH 7.2, 30% glycerol, 1 $\text{mmol}\cdot\text{L}^{-1}$ ACC, 10 $\text{mmol}\cdot\text{L}^{-1}$ sodium ascorbate, 0.05 $\text{mmol}\cdot\text{L}^{-1}$ FeSO_4 , and 10 $\text{mmol}\cdot\text{L}^{-1}$ NaHCO_3 in a total volume of 2 mL including enzyme.

Results

QUALITY EVALUATION. The visual quality of mume fruit was negatively affected by HPT (Fig. 1). Fruit subjected to as low as 5

MPa pressure exhibited accelerated peel color change, with the effect on peel color becoming more pronounced in fruit subjected to pressures ≥ 100 MPa. Treated fruit subjected to pressure ≥ 100 MPa exhibited shriveling and underwent palpable softening. Fruit subjected to HPT below 100 MPa were judged to be at the limit of marketability, while those subjected to ≥ 100 MPa were unmarketable.

Discoloration, i.e., browning of treated fruit, was indicated by a decline in lightness (L^* value) combined with the marked decline in hue angle from 108° to $<100^\circ$ in fruit treated at ≥ 100 MPa (Table 1). The decrease in chroma with increasing pressures further indicated a change from a vivid to a dull color. Titratable acidity increased by 27% in fruit subjected to 5 MPa compared with the control treatment, but exhibited little change in all other treatments (Fig. 2).

After incubation at 25 $^\circ\text{C}$, the peel of control fruit and those subjected to 5 MPa changed in color from green to yellow. Beyond this initial color change following decompression, visual comparison indicated a subsequent retardation of peel color development in fruit subjected to 5 MPa.

GAS MEASUREMENTS. All fruit subjected to HPT exhibited lower CO_2 evolution rates relative to control fruit (Fig. 3). The most pronounced inhibition was observed in fruit subjected to ≥ 100 MPa. At the lower pressures, the fruit subjected to 5 MPa showed the greatest (34%) depression of CO_2 evolution.

Ethylene production in control fruit was high and variable (Fig. 4 and Table 2). The variability might be due to differences in physiological maturity, which were not apparent in the freshly harvested fruit. The most dramatic and consistent effect of HPT was the suppression of ethylene production (Fig. 4), regardless of the pressure applied.

ACC AND ACC OXIDASE ASSAY. The suppression of ethylene production was accompanied by a 75% reduction in ACC oxidase activity (Table 2). The level of the ethylene precursor, ACC was not affected by HPT.

Discussion

Following decompression, mume fruit exhibited substantial changes throughout the pressure range used. Color changes were evident even in fruit subjected to pressures <100 MPa. Abnormal ripening was most evident in fruit subjected to pressures ≥ 100 MPa, which led to browning and softening, rendering treated fruit commercially unacceptable.

The possibility of inactivating microorganisms in mume by HPT appears remote, as the pressure required for the destruction of bacteria is ≈ 300 MPa (Hoover, 1993), well above the threshold for injury observed in the present study. It also appears unlikely that HPT could control postharvest diseases of fungal origin. Como et al. (1997) reported that pressures >175 MPa are required to damage *Colletotrichum gloeosporioides* (Penz.) Sacc., the pathogen causing anthracnose. If similar pressures are required to control other fungal pathogens, then HPT may be utilized for processed but not fresh fruit.

In contrast, the physiological response of mume to 5 MPa presents an interesting possibility. The suppression of both respiration and ethylene production, as well as the generally normal, but delayed ripening of mume subjected to this pressure has the potential of extending the postharvest life with HPT. These fruit apparently recovered from HPT-induced changes. Enzymes are known to show differential responses to HPT (Hoover, 1993; Knorr, 1993), with marked effects on activities usually being observed in the pressure range of 100 to 300 MPa (Jaenicke, 1981).

ACC serves as an intermediate in ethylene biosynthesis, the following pathway of ethylene biosynthesis in plant tissue, includ-

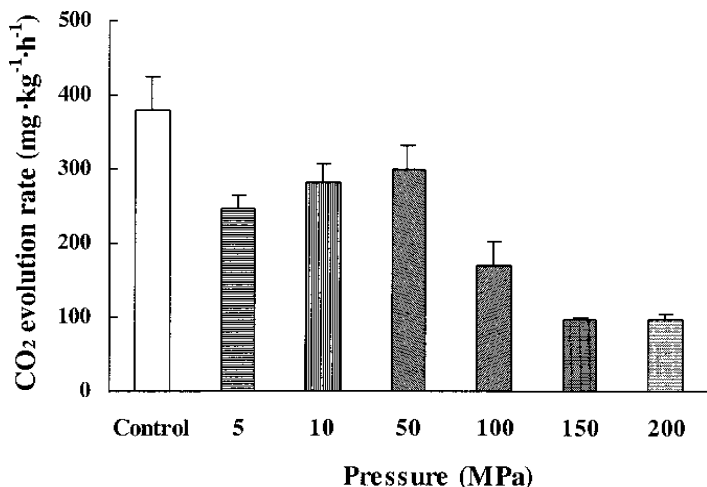


Fig. 3. Carbon dioxide evolution rate of fruit after high-pressure treatment at control, 5, 10, 50, 100, 150, and 200 MPa for 10 min. Two fruit were placed in jars immediately after treatment. Each jar of two fruit was considered a replication and each datum represents the mean and SE of three replications.

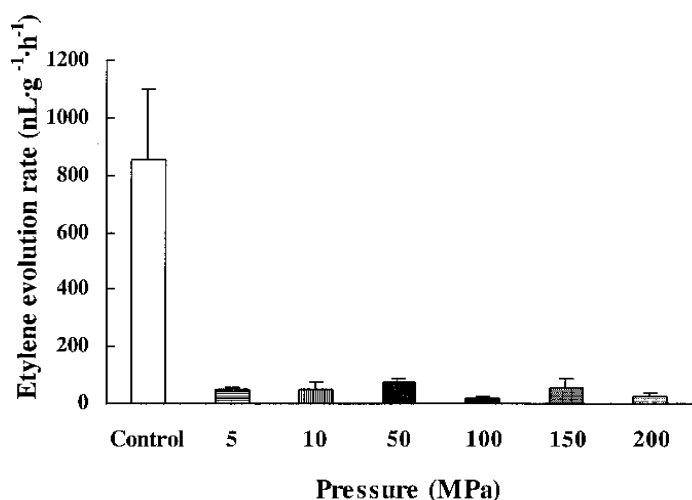


Fig. 4. Ethylene evolution rate of fruit after high-pressure treatment at control, 5, 10, 50, 100, 150, and 200 MPa for 10 min. Two fruit were placed in jars immediately after treatment. Each jar of two fruit was considered a replication and each datum represents the mean and SE of three replications.

Table 2. Effect of high-pressure treatment on ethylene evolution rate, ACC level, and ACC oxidase activity (expressed as ethylene evolution rate).^z

Pressure (MPa)	C ₂ H ₄ (nL·g ⁻¹ ·h ⁻¹)	ACC (nmol·g ⁻¹)	ACC oxidase (nL·g ⁻¹ ·h ⁻¹)
Control	238 ± 24.8	31.8 ± 1.95	65.7 ± 7.02
5	79 ± 6.0	34.1 ± 9.73	16.4 ± 0.16

^zEthylene evolution rate, ACC level, and ACC oxidase activity were measured immediately after high-pressure treatment at 5 MPa. Values represent mean ± SE for three replications.

ing mume fruit, has been established: methionine→S-adenosylmethionine→ACC→ethylene (Yang and Adams, 1980). The activity of ACC oxidase that catalyzes the conversion of ACC to ethylene increases during ripening in mume (Zhang et al., 1991). The inhibition of ethylene production in fruit subjected to 5 MPa can be attributed, in part, to the inhibitory effect of HPT on ACC oxidase. The persistence of the inhibition of ethylene production even after decompression indicates an irreversible alteration of this enzyme.

Based on fruit appearance, it might be surmised that the physiological status of fruit subjected to 5 MPa is different from that of fruit subjected to pressure ≥100 MPa. Even after decompression, fruit subjected to pressures ≥100 MPa showed a dramatic decline in CO₂ evolution rates, possibly indicating irreversible damage as a consequence of HPT. This observation, coupled with the extensive change in gross morphology, indicates complete tissue disintegration.

Except for 5 MPa, the pressures applied and evaluated in this study are above the threshold for irreversible tissue damage. HPT at 10 and 100 MPa, respectively, involve applying pressures two and three orders of magnitude greater than atmospheric. From a physiological and practical standpoint, the evaluation of pressures <5 MPa should provide more insights into the effect of HPT on mume and other fruit.

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