Running title: Quality of ginger after high pressure treatment
The effect of high-pressure treatment on the quality of grated ginger

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Abstract

A high hydrostatic pressure was applied to grated ginger in order to inactivate quality degrading enzymes non-thermally. The effects of high-pressure treatment on the flavor and color of grated ginger were investigated just after the treatment and during storage.

After the high-pressure treatment (400 MPa, 5 min), geraniol dehydrogenase (GeDH) was inactivated to less than 5%, but the activity of polyphenol oxidase (PPO) remained 37%. As a comparison, heat treatment (100°C, 10 min) inactivated GeDH to 43% and PPO to about 10%. In the storage, the transformation from geranial, neral and citronellal to their corresponding alcohols correlated with the residual GeDH activity in the grated ginger. In the high-pressure-treated sample, terpene aldehydes almost disappeared without the formation of corresponding alcohols. Browning was not observed just after the high-pressure treatment, but had finished in the thermally-treated sample. Color change during storage was proportional to the residual activity of PPO.

Key words: ginger; flavor; high-pressure treatment; polyphenol oxidase; geraniol dehydrogenase

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Introduction

To extend the shelf life of food products, one or combination of processings such as freezing, drying, pasteurization, and sterilization are often used. Traditional treatments for food preservation, however, adversely affect their quality. Food scientists and the food industry are therefore searching for novel methods that may destroy undesired microorganisms with less adverse effects on product quality. One of the solutions may be high-pressure processing. Currently this method is successfully applied on a commercial scale for pasteurization of a whole range of food products, for example, fruit juices, oysters and ham.[1)] At ambient temperatures, application of pressures in the range of 400-600 MPa inactivate vegetative microorganisms and reduce the activity of enzymes retaining small molecules responsible for flavor, taste and color.[2)]

Zingiber officinale Roscoe, commonly known as ginger, is widely used as a spice, flavoring agent and herbal medicine, and is also employed in the perfume industry. In Japan, grated ginger products are commercially available for convenient use. Ginger has its unique flavor properties with the combination of pungent and volatile flavorful stimuli. The pungency is provided by non-volatile phenolic compounds, whereas the essential oil, especially geranial gives ginger its characteristic aroma.[3)] Therefore, geraniol dehydrogenase (GeDH, E.C.1.1.1.183) plays an important role in the formation of ginger odor. It has been reported that the conversion of geraniol to geranial is catalyzed by $NADP^+$-dependent GeDH.[4-6)]

Natural phenolic compounds in fruits and vegetables in the presence of polyphenol oxidase (PPO) are oxidized to *o*-quinone that subsequently polymerizes non-enzymatically to brown pigments.[7,8)] This browning process leads also to a reduction in nutritional quality.[7, 9, 10)] Any papers about browning enzymes of ginger are not seen, but it is certain that browning of grated ginger causes quality deterioration.

It is necessary to reduce activities of enzymes in the grated ginger products in order to stabilize its flavor and color. In this paper, we attended the application of high-pressure treatment for the preservation of the freshness of grated ginger products. The objective of this paper is to evaluate the effects of high-pressure treatment on flavor and color during the

processing and storage.

Materials and methods

Sample preparation Fresh rhizomes of ginger were purchased from the local market in Japan. The rhizomes of ginger were washed to remove soil, peeled and grated. The grated ginger was packed in Teflon-tube (10 mL).

High-pressure treatment The plastic tubes containing the samples were pressurized by using a prototype pressurization apparatus (Kobe steel Ltd, Kobe, Japan) to 400 MPa at room temperature for 5 min. For comparison, untreated and thermally-treated samples were prepared. Thermal treatment was achieved in a thermostatic bath at 100 °C. After thermal treatment for 10min, the sample tubes were cooled in a water-ice bath. These samples were stored at 12 or 20 °C in a dark.

Analysis of volatile compounds Distilled water (50 ml) was added to 5 g of grated ginger sample, and extracted with 30 mL of diethylether under NaCl saturation. The ether solution was separated and dried over anhydrous sodium sulfate. The ether concentrate was applied to GC and GC-MS analysis.

Capillary GC analysis was carried out on a Shimadzu GC-14A gas chromatoguraph (Shimadzu Co., Kyoto, Japan) equipped with a flame ionization detecter (FID) and fused silica capillary column DB-WAX (60 m×0.25 mm i.d., 0.25mm film thickness; J&W Scientific). Helium gas was used as carrier gas in a linear flow rate of 33 cm/sec. Injector temperature was 230 °C. The column temperature was programmed from 50 to 230 °C at 4 °C /min and the final temperature was held for 20 min. Each compound was identified by comparing its retention index (Kovats index) and mass spectrum with those of the authentic compound.

Volatile identification was achieved on a GC (Hewlett-Packard 5890 Series II)-mass spectrometry (MS; Jeol Automass 50). Analytical conditions were as follows: temperature program, 50-230 °C, 4 °C /min, isothermal at 230 °C, 20 min; injector temperature, 230 °C; helium carrier gas, 35 cm/sec; ion source temperature, 150 °C; ionization voltage, 70 eV.

GC-Olfactometry (GC-O) was carried out on GC-14A equipped with sniffing system OP 275 (GL Sciences Inc., Tokyo, Japan). The operating conditions of GC-O were the same

as GC analysis.

Color analysis The color of the ginger samples was measured with a tristimulus colorimeter (Chroma Meter, type-CR-100, Minolta, Japan) using the CIELAB uniform color scale described by L*, a*, and b* parameters.[11] The total color change (ΔE^*_{ab}) was estimated by the expression: $\Delta E^*_{ab} = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$.

Enzymatic activity

***Geraniol dehydrogenase* (GeDH)** A crude enzyme solution was prepared from grated ginger. The grated ginger of 1 g was homogenized twice for 30 s in 25 ml of Tris-HCl buffer (pH 7.2, 4°C) containing 15% glycerol, 2 mM dithioerythritol, and 10 mM mercaptoethanol.[5] The homogenate was centrifuged at 20,000×g (20 min×2) at 4 °C, and the resulting supernatant was used in the assay for GeDH activity.

The measurement of its alcohol dehydrogenase activity was done by a modification of the method of Sangwan *et al.*,[4] and Sekiwa *et al.*[5] Geraniol was used as a substrate after being dissolved in DMSO. Geraniol (2 mM) and NADP (0.65 mM) were dissolved in 3.5 mL of a 0.1 M glycine-NaOH buffer (pH 9). The reaction was started by adding 0.5 mL of the crude enzyme solution to above substrate mixture. A blank was prepared by adding DMSO instead of substrate solution. The change in absorbance at 340 nm at 20°C (Temperature controlled cell holder, TCC240A, Shimadzu Co.), indicating the reduction of NADP, was traced for 10 min (UV-visible spectrophotometer, UV-1600, Shimadzu Co.) and calculated the slope from the linear portion of the curve. The residual activities in thermal and pressure treatment were calculated based on the slope of the line in untreated sample (control).

***Polyphenol oxidase* (PPO)** To prepare a homogenate, 4 g of grated ginger was placed in a glass homogenizer with 50 mM sodium phosphate buffer (12 mL, pH6.5). After the homogenization for 10 min, the homogenate was filtered and centrifuged (10,000×g) at 5 °C for 20 min. The supernatant was used as polyphenol oxidase (PPO) extract. PPO activity was assayed spectrophotometrically. The reaction mixture contained 1 mL of the PPO extract and 0.4 mL of a substrate solution of 0.1 mM caffeic acid in ethanol. The change in absorbance at 480 nm at 25 °C was traced for 5 min. One unit of the enzyme

activity is defined as an increment of 0.1 in absorbance per min at the maximum rate of increase.

Results and discussion

Analysis of volatile compounds The volatile concentrates were obtained from untreated, high-pressure-treated and thermally-treated samples of grated ginger by extraction with diethyl ether. Volatile compounds in the ginger samples were listed in Table 1. Thirty-two volatile compounds including 26 mono-terpenes and 4 sesquiterpenes were definitely or tentatively identified by comparison of mass spectra and Kovats index (KI). The major volatile components of this ginger sample were β-bisabolene, β-sesquiphellandrene, geranial, α-farnesene, β-phellandrene, camphene, 1,8-cineole and neral. In the thermally-treated ginger sample, zingerone was significantly increased (Table 1).

The human nose was combined with the GC as a detector for identifying the odors of effluents from a capillary column (GC-Olfactometry).[12] The freshly grated ginger sample had a strong lemon-like, floral and green odor. Connell and Jordan (1971) reported that freshly harvested Queensland-grown ginger rhizomes possess a "citrus-like" aroma, and the compounds with this citrus-like odor were identified as geranial and neral.[13] As shown in Table 1, the odor of geranial and neral was described as a strong citrus-like, especially lemon-like odor in fresh ginger. Linalool, nerol and geraniol have a floral odor. Nishimura (1995) reported that these terpene alcohols were the important odorants in fresh ginger.[14] Hexanal had a green odor and citronellal had a fresh citrus-like odor in Japanese Kabosu[15], and they could contribute to the freshness of the ginger odor.

The flavor profile of grated ginger was slightly changed by thermal treatment. In particular, zingerone was increased during thermal treatment. It could give a sense of cinnamonic odor in the thermally-treated sample (Table 1). It has been considered that zingerone was transformed from gingerol.[16] On the other hand, in the high-pressure-treated sample, zingerone was found only in a trace amount.

In brief, there were only small differences in odor qualities of untreated, high-pressure-treated, and thermally-treated samples before storage.

Table 1. Volatile compounds in grated ginger and the concentration after thermal or pressure treatment

peak	compound	KI[a]	aroma attributes[c]	concentration (mg/kg)[d]		
				untreated	pressure	thermally
1	α-pinene	1014	woody, cypress like	1.86	1.40	2.28
2	camphene	1051	incense	5.47	4.24	6.83
3	hexanal	1062	green	0.93	0.87	0.89
4	β-pinene	1090	citrus	0.12	0.06	0.16
5	β-myrcene	1137	citrus	0.87	0.76	1.14
6	α-phellandrene	1145	sweet, incense	0.35	0.23	0.41
7	D-limonene	1182	lemon, citrus	1.28	0.99	1.54
8	β-phellandrene	1195	rubbery	8.66	6.40	9.27
9	1,8-cineole	1199	woody, citrus	4.71	4.24	6.34
10	2-heptanol	1305	wet	0.64	0.70	0.81
11	acetic acid	1447	sour, pungent	0.47	0.52	0.41
12	citronellal	1477	yuzu, citrus	0.12	0.06	0.08
13	decanal	1502	orange, citrus	0.35	0.41	0.33
14	α-cubebene	1506	citrus	0.87	0.52	0.57
15	linalool	1539	citrus, floral	0.35	0.41	0.57
16	bergamotene	1557	iris, citrus	0.29	0.23	0.33
17	2-undecanone	1597	wet	tr[b]	tr[b]	tr[b]
18	*trans*-sabinene hydrate	1605	green	0.70	0.58	0.81
19	β-farnesene	1656	citrus	1.51	1.10	1.46
20	neral	1677	lemon, citrus	5.17	5.06	12.85
21	α-terpineol	1689	woody	1.86	1.40	2.11
22	borneol	1698	musty	3.66	2.73	3.98
23	α-zingiberene	1715	orange	2.56	4.65	8.94
24	β-bisabolene	1721	floral	214.42	112.67	126.75
25	geranial	1724	lemon, citrus	34.65	42.15	49.59
26	α-farnesene	1733	citrus, floral	22.09	14.19	20.57
27	citronellol	1749	floral	tr[b]	0.17	0.33
28	β-sesquiphellandrene	1771	citrus	56.80	41.22	52.36
29	nerol	1789	floral	tr[b]	tr[b]	0.08
30	geraniol	1836	floral	0.99	0.81	1.06
31	zingerone	2224	cinnamonic	3.55	0.00	8.54
32	geranic acid	2306	herbal, green	0.35	0.47	0.57

[a] Kovats indices calculated for DB-WAX capillary column.

[b] trace

[c] Perceived at sniffing-port on GC-O.

[d] Amount of volatile compounds calculated on the basis of chromatographic areas using cyclohexanol as internal standard.

Changes of volatile compounds during storage

Although citrus-like flavor is one of the most important attributes of ginger, the attribute is apt to decrease during storage. We therefore investigated the effect of the GeDH activity on the interconversion between terpene alcohols and terpene aldehydes.

Figure 1 indicates the residual activity of GeDH in the grated ginger after thermal and high-pressure treatment. The activity decreased to 43% of the original activity by the thermal treatment at 100 °C for 10 min. On the other hand, the activity decreased to less than 5% by the high-pressure treatment at 400 MPa for 5 min. As a result, GeDH in ginger was found to be very stable to high temperature but extremely unstable to high hydrostatic pressure.

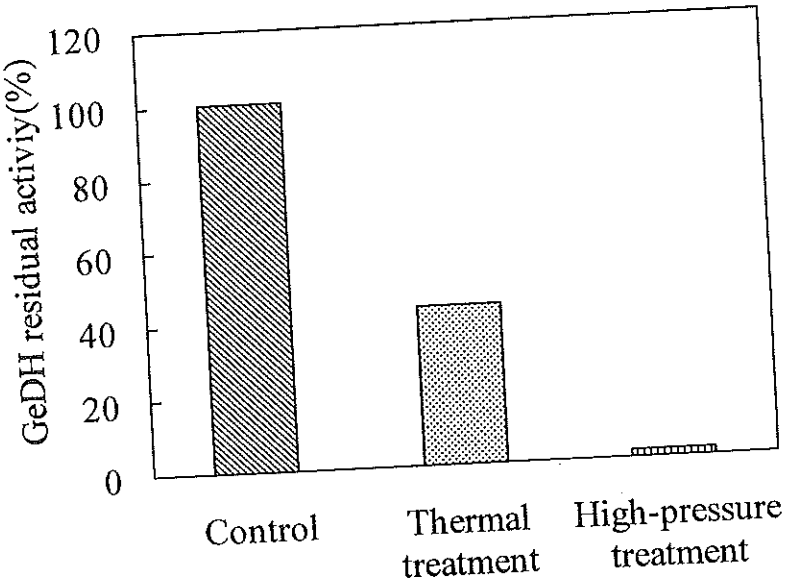
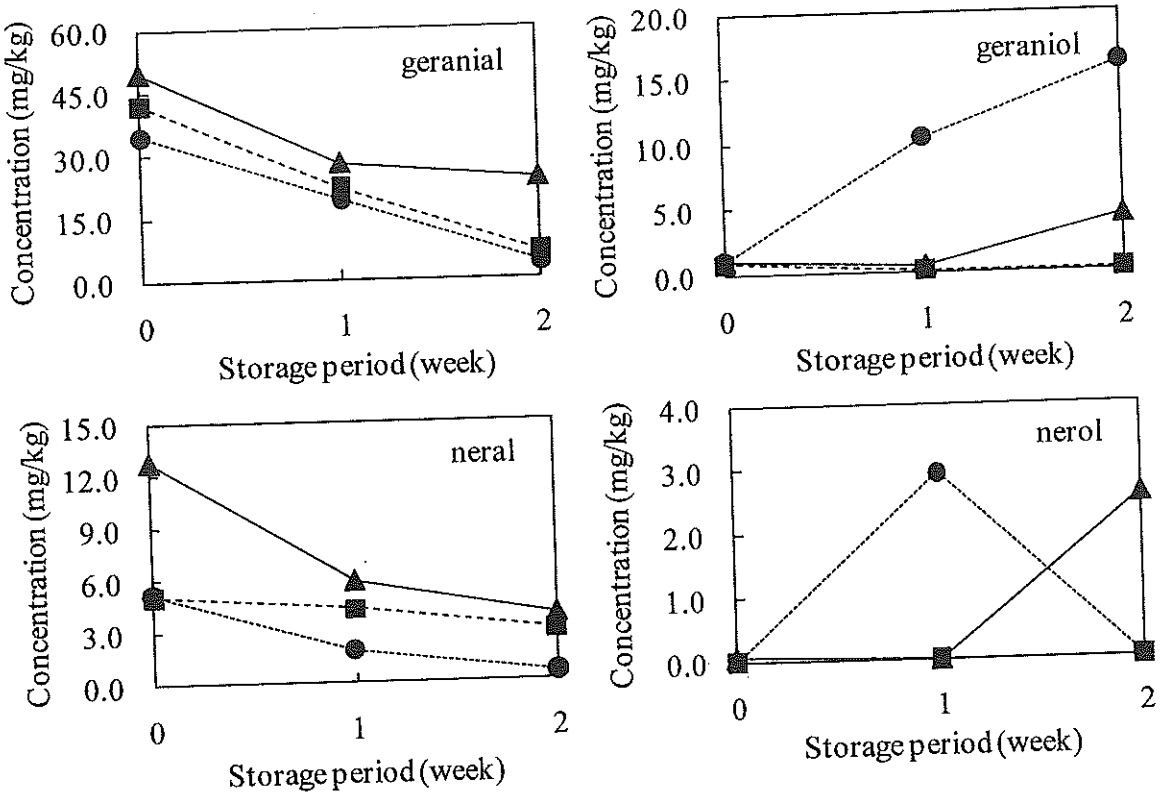


Fig.1. Residual activity of GeDH after thermal and high-pressure treatment.

The change in absorbance at 340 nm, indicating the reduction of NADP, was traced for 10 min and calculated the slope from the linear portion of the curve. The residual activities in thermal and pressure treatment were calculated based on the slope of the line in untreated sample (control).

The changes in the amounts of geranial and geraniol during storage are shown in Fig.2. In the untreated and high-pressure-treated samples, we confirmed the striking decrease in geranial. On the other hand, the amounts of geraniol significantly increased in the

untreated samples, but did not increase in the high-pressure-treated samples. Furthermore, we confirmed the same behavior on the transformation from citronellal to citronellol. Decreasing in neral was the almost same as geranial, but nerol showed unusual change in its quantity. The quantitatively non-balanced interconversion between neral and nerol could be attributed to the isomerization between geranial and neral, and/or geraniol and nerol. It was also able to consider that these terpene aldehydes or alcohols transformed to low volatile compounds such as carboxylic acids or diols. It seems reasonable to assume these interconversions, since geranial and citronellal was almost disappeared without any increment in corresponding alcohols in the high-pressure-treated sample.



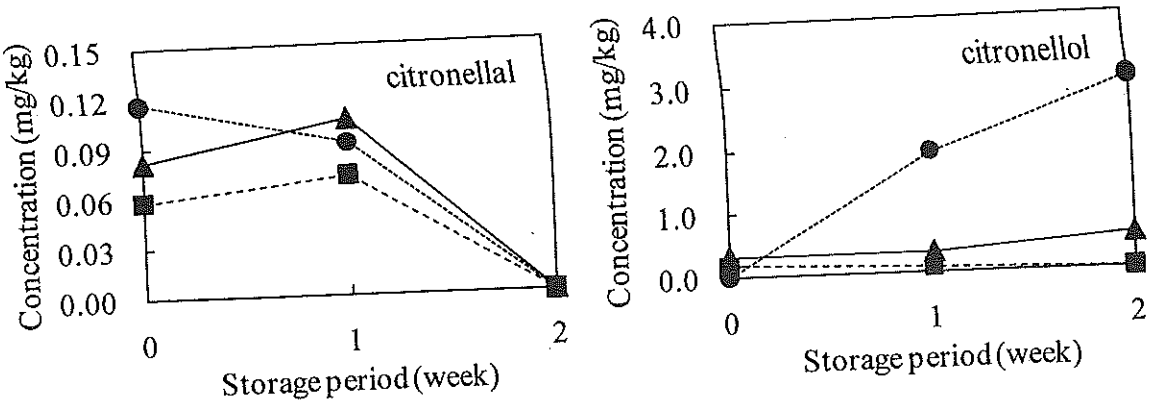


Fig.2. Changes in the volatile compounds of untreated, thermally-treated, or high-pressure-treated ginger during storage at 20 °C.

The heating temperature was 100 °C for 10 min in thermal treatment. The pressure treatment condition was 400MPa, room temperature for 5 min. Samples were stored for 2 weeks at 20 °C.
The circle, triangular, square plots show untreatment, thermal and high-pressure treatment, respectively.

PPO inactivation Enzymatic browning is an undesirable reaction leading to quality losses and decreasing of commercial value of horticultural products.[8, 17, 18] Polyphenol oxidase (PPO), metallo-enzyme containing copper as a prosthetic group, is a key enzyme of the browning.[19, 20] These enzymes are ubiquitous in plants, and particularly exist in high amount in apple, pear, litchi, mango, potato, and mushroom.[21, 22] The browning problem by PPO can take place in grated ginger products. However, we couldn't find the paper with reference to enzymatic browning of ginger products. The methods widely used in controlling enzymatic browning, and their advantages/disadvantages are summarized as follows: (1) heat treatment (70-90°C), effective and easy but adversely affects the sensory qualities[10, 23]; (2) control of pH ($\leq$4), convenient but deteriorates the food taste.[24]; (3) use of antibrowning agents, convenient and effective but adversely affects the sensory qualities[25]; (4) exclusion of oxygen (controlled/modified atmosphere, oxygen-impermeable film), safe but temporary and decreases the volatile production[26-30]; (5) use of natural additives (honey, onion extract), safe but relatively ineffective.[31, 32]

In this study, we investigated the effect of high-pressure treatment (400MPa, 5 min)

on the PPO activity in grated ginger, and compared with the activity in the untreated or thermally-treated sample (Fig.3). PPO activity in the thermally-treated sample decreased to about 10 %, while in the high-pressure-treated sample, the activity decreased to 37 %.

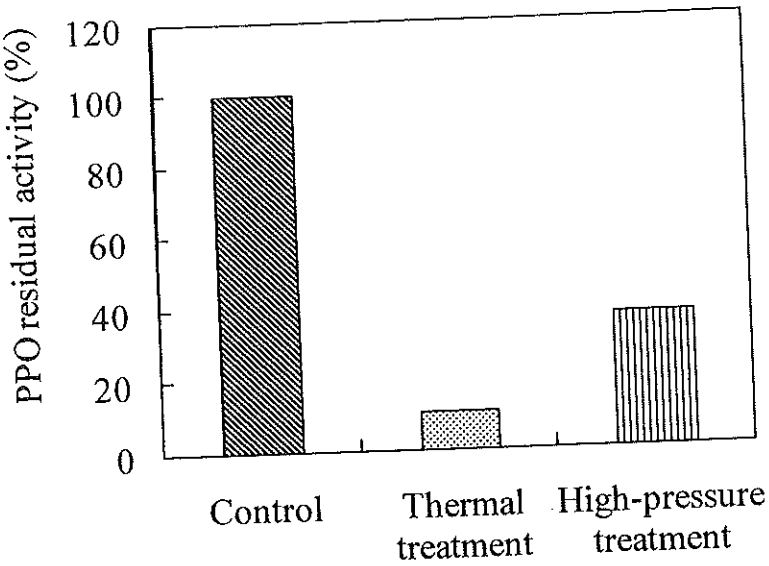


Fig.3. Activity of PPO after thermal treatment and high-pressure treatment.

The heating temperature was 100°C for 10 min in thermal treatment. The pressure treatment condition was 400MPa, room temperature for 5 min. The change in absorbance at 480 nm at 25°C was traced for 5 min. One unit of the enzymatic activity is defined as an increment of 0.1 in absorbance per min at the maximum rate of increase. The enzymatic activity of untreated sample is defined as control.

It was interesting result that the GeDH and PPO showed the different sensitivity to high pressure in spite of the same horticultural product. The effect of high pressure on the PPO activity has been reported about many horticultural products.[33, 34, 35] Asaka et al. (1997) reported that the activity of PPO from pear, Japanese pear, loquat and cherry was increased by pressurization, but the activity of PPO from peach, apricot and Japanese apricot was decreased by pressurization.[36] Thus PPO activity showed different sensitivity to high-pressure treatment. The different sensitivity of GeDH and PPO to high pressure can be depended on such specificity.

Browning development The changes in color during storage were shown in Fig.4. Just after the thermal treatment, ΔE^*_{ab} value was significantly increased, but the change in ΔE^*_{ab} value of pressure-treated sample was not observed. This is a striking characteristic of non-thermal treatment.

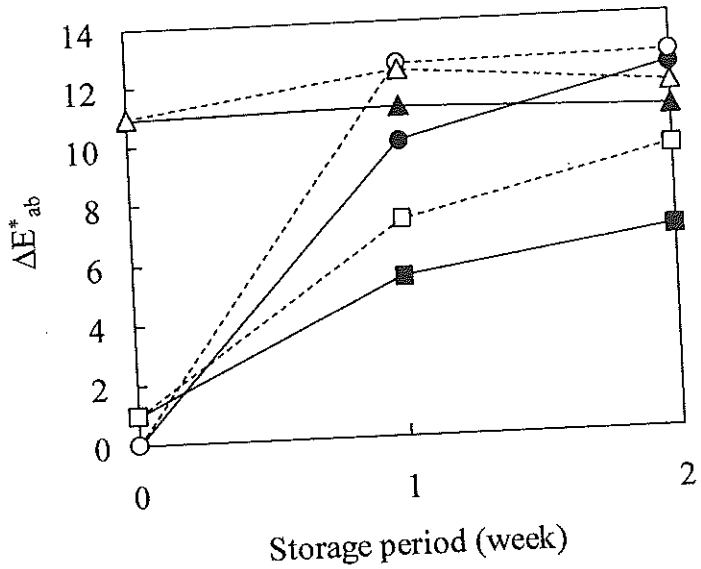


Fig.4. Change in the ΔE^*_{ab} of untreated, thermally-treated and high-pressure-treated ginger during storage at 12°C or 20°C.

The heating temperature was 100°C for 10 min in thermal treatment. The pressure treatment condition was 400MPa, room temperature for 5 min. The samples were stored for 2 weeks at 12 or 20°C in a dark.

The total color change (ΔE^*_{ab}) was estimated by the expression: $\Delta E^*_{ab} = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$. The circle, triangular, square plots show untreatment, thermal and high-pressure treatment, respectively. The filled plots show storage at 12°C, while the open plots show storage at 20°.

During the storage the color of untreated sample turned brownish (data not shown), thus the ΔE^*_{ab} value markedly increased. The ΔE^*_{ab} value of pressure-treated sample also increased during the storage, but the increment was about 60% of untreated sample. This is reflecting their residual PPO activity. As mention before, the residual PPO activity of pressure-treated sample was 37%, thus the browning by polyphenols was suppressed during storage. On the other hand, since the ΔE^*_{ab} value increase just after the thermal treatment,

the ΔE^*_{ab} value became almost constant through the storage. Considering that the residual PPO activity of thermally-treated sample was about 10%, the brownish color had developed during its thermal treatment by not only enzymatic but also non-enzymatic system.

Conclusions

In this study, we investigated the effects of high-pressure treatment on the sensory quality of grated ginger and on the inactivation behavior of the quality-related enzymes in the ginger. The high-pressure treatment (400MPa, 5 min) caused little change in the odor of grated ginger immediately after treatment. During storage, high-pressure treatment can also suppress production of geraniol, nerol and citronellol because of the inactivation of alcohol dehydrogenase.

Immediately after treatments, high-pressure treatment revealed no significant effect on the color development of grated ginger, while thermal treatment caused severe color change. However, browning in high-pressure-treated ginger was developed during storage, which color change (ΔE^*_{ab} value) is about 60% compared to untreated ginger. This may be caused by the remained PPO activity (ca. 37%) in high-pressure-treated ginger.

High-pressure treatment can maintain the quality of ginger up to a certain degree. However in order to produce more high quality horticultural products, further research will be required.

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