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The Quality of High Pressure-Induced and Heat-Induced Hyuganatsu Marmalade

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Abstract: Hyuganatsu is a typical Japanese citrus with desirous smell and edible albedo. The objectives of this study are to establish a method for softening hyuganatsu peel without heating and a process for making a high pressure-induced (HP-) marmalade and to compare with heat-induced (H-) marmalade and citrus shaddock family marmalade. Firmness of peel was (greatest to least) pressurized at 500 MPa for 30 min, soaked in citric acid solution at pH 2.7 for 24 h, and boiled for 10 min, respectively. The cell walls of flavedo and albedo did not loosen after pressurization. However, after soaking or heating, the middle lamella of albedo separated. When flavedo was soaked at pH 2.7, 9.3% of pectin was extracted. The amount of naringin was the least in hyuganatsu < pummelo < grapefruit), and juice sacs < flavedo ≤ segment walls < albedo, respectively. Thus, marmalade was processed. Albedo, segment walls and juice sacs of hyuganatsu were homogenized with citric acid solution (pH 2.7) and mixed with sliced flavedo. Then it was soaked for 24 h at pH 2.7. Sucrose was then added (final sugar 50%), vacuum packed, then pressurized for 30 min at 500 MPa or boiled for 10 min, respectively. There was no significant difference in sensory evaluation between HP- and H-marmalade. However, the color, transparency and total evaluation of HP-marmalade were better than H-marmalade. Consequently, total evaluation of hyuganatsu-HP-marmalade was rated highly. So, hyuganatsu was considered to be more suitable for marmalade because it was more palatable than the others.

Key words: Hyuganatsu, pectin, high pressure, processing, marmalade, texture, structure.

1. Introduction

In Japan, many kinds of citrus fruit that may be relatively unknown in other countries are cultivated and have a unique flavor. Among them, hyuganatsu (*Citrus tamurana* Hort. ex Tanaka), locally named konatsu (in Kochi prefecture) or new summer orange (in Shizuoka and Ehime prefectures), has been regarded as a citrus fruit with potential commercial value. The name comes from Hyuga, the ancient name of Miyazaki prefecture in Kyushu where it originated. "Natsu" means summer. Hyuganatsu was found in Miyazaki city in the 1820s, after which it became widely cultivated throughout the region. It is thought to be either a mutated yuzu or perhaps more likely a chance hybrid between yuzu and pummelo. This fruit is mainly grown in Japan's Miyazaki and Kochi prefectures. Most citrus fruit in Japan is usually harvested by the end of the year in order to avoid chilling injuries and maintain its commercial quality. Hyuganatsu fruit is however, commonly left on the trees until the following spring because of its resistance to winter's severe cold [1]. Thus, hyuganatsu is popular in Japan from early spring to summer.

Hyuganatsu has a light yellow color and round shape with a diameter of 8-10 cm and a weight of 180-200 g. It has a pleasant aroma, a sweet and a slightly sour taste, and is juicy. The white pith (albedo) between the flesh and peel is very thick but not bitter and is eaten together with the flesh. Thus, citrus hyuganatsu is

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desirable because of its flavor, taste and nutrients. Its oil is higher in trans-β-farnesene, 1-carvone than other citrus fruits, and has a higher number of ketones [1]. The taste is very fragrant.

High pressure processing is a new method of avoiding off-flavor and thus attracted the attention of the food industry in Japan [2]. Therefore, high pressure-induced (HP-) food products such as jam, fruit sauce, juice, uncooked ham, rice cakes containing herbs (yomogi mochi) and cooked at-high-pressure processed rice have since been marketed in Japan. High pressure is able to produce jam without heating [3], because pressurization accelerates hydrogen bonds between pectin molecules [2]. Heat-induced (H-) jam and marmalade (products by conventional thermal treatment) have some faults such as off-flavor and deterioration of nutrients and especially color. However, high pressure is able to produce jam without heating. Thus, HP-jam was patented by Meidi-ya Ltd. in Japan in 1990 [4], and pressure-induced jam, such as strawberries, blue berries and apples have since been marketed using this method. However, pectin is added to this pressure-induced jam. Citrus possesses sufficient pectin and acid to form jelly/marmalade. Therefore, a new method for forming jelly without the addition of pectin was investigated using citrus yuzu.

When high methoxyl pectin is extracted by soaking in 0.01 N HCl solution (pH 2.0) at 35 °C, vegetables are softened due to the removal of Ca^{2+} [5-7]. This extraction method of pectin [5-7] was used for softening the peel of yuzu by using citric acid instead of HCl. However, yuzu marmalade which was processed after soaking at pH 2.0 was slightly sour, especially more sour in yuzu marmalade with 50% sucrose than that with 60% sucrose [8]. Therefore, yuzu was soaked in 0.06% citric acid solution (pH 2.7), then yuzu marmalade with 50% sucrose was processed [9]. When soaked at pH 2.7, flavedo did not become softer than when it was soaked at pH 2.0 [10]. Yuzu marmalade was made [9], because the optimum pH value for most standard preserves and jellies is in the range of 2.6-3.4. Too much acid causes excessive syneresis, while too little acid (resulting in pH values which are too high) impairs proper jellification or prevents setting of altogether [11]. Thus, pH 2.7 is more suitable for the gelation of pectin (jelly-forming) and gives a more desirous viscosity for marmalade.

Thus, this process is applicable in citrus hyuganatsu which is one of the citrus shaddock families. In this paper, hyuganatsu will be soaked in 0.06% citric acid solution (pH 2.7), then hyuganatsu marmalade with 50% sucrose will be processed. Hyuganatsu will be compared with pummelo and grapefruit which are also citrus shaddock families. Then it will be judged that whether hyuganatsu is suitable for HP-marmalade or not.

The objectives of this study are to establish a method for softening hyuganatsu peel without heating and a process for making a HP-marmalade to compare with H-marmalade and other kinds of citrus shaddock family marmalade.

2. Materials and Methods

2.1 Sample Preparation

Marketed hyuganatsu (*Citrus tamurana* Hort. ex Tanaka, cultivated in Kochi, Japan, obtained in May), pummelo (*Citrus grandis* Osbeck, cultivated in Kochi, Japan, obtained in April) and grapefruit (*Citrus paradisi* Macf, imported from California, USA, obtained in June) were used for the experiment and shown in Figs. 1a-1c. The surface of the peel is called flavedo (an epicarp) and is yellow. The inside of flavedo is called albedo (a mesocarp). It is white and spongy. An endocarp consists of several segments of juice sacs which are surrounded by a segment wall [9]. Hyuganatsu, pummelo and grapefruit were divided into flavedo, albedo, segment walls and juice sacs, respectively, with weight percentages shown in Figs. 1d-1f.

The schematic flowchart for processing marmalade is shown in Fig. 2. For processing marmalade, half of hyuganatsu (Fig. 1a), a quarter of pummelo (Fig. 1b)

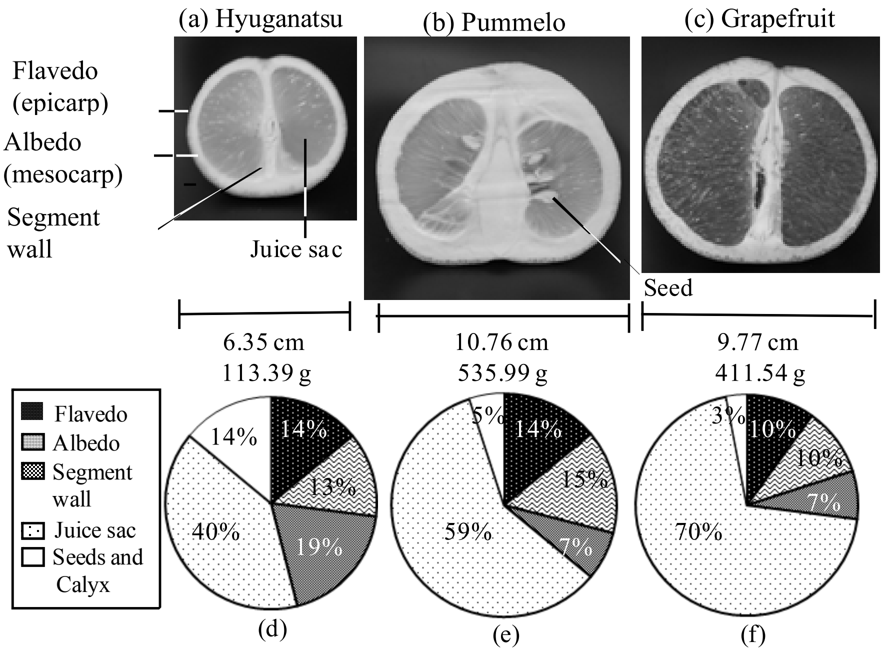


Fig. 1 The structure and weight percentage of flavedo, albedo, segment walls and juice sacs of hyuganatsu, pummelo and grapefruit.

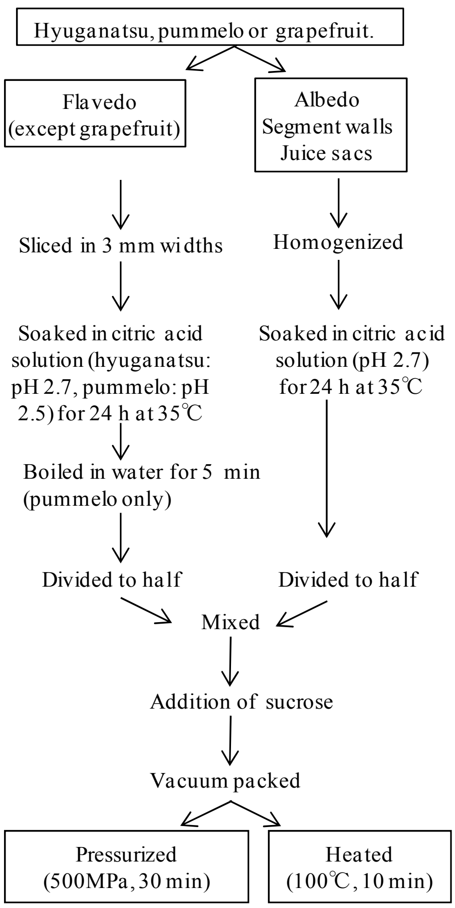


Fig. 2 The schematic flowchart for the processing of marmalades.

and a quarter of grapefruit (Fig. 1c) were used for one experiment, respectively. Flavedo was sliced in 3 mm widths. Flavedo of hyuganatsu (8.06 g) was soaked in 0.06% citric acid solution (pH 2.7, 16.12 g) for 24 h at 35 °C. That of pummelo (19.26 g) was soaked in 0.3% citric acid solution (pH 2.5, 38.52 g) for 24 h at 35 °C, then boiled in water for 5 min, because it was very hard. Flavedo of grapefruit was not used, because it was very bitter.

Albedo, segment walls and juice sacs (hyuganatsu: 7.15, 10.78 and 22.99 g; pummelo: 20.45, 9.11 and 78.02 g; grapefruit: 10.52, 7.42 and 71.50 g, respectively) were homogenized with 0.06% citric acid solution (pH 2.7, half amount of albedo, segment walls and juice sacs: 20.46 g for hyuganatsu; 53.79 g for pummelo; 44.72 g for grapefruit), and adjusted to pH 2.7 using citric acid, then they were soaked for 24 h at 35 °C. They were mixed with flavedo then sucrose (Nacalai Tesque, Inc., Kyoto) was added (final sugar 50%). However, sugar content of the juice was subtracted from the added sucrose content. The sugar content (brix) of juice and the hyuganatsu marmalade were measured by a digital refractometer (PR-100 or

PR-200, Atago Ltd., Tokyo).

The samples were then vacuum packed, and pressurized for 30 min at 500 MPa at room temperature (about 20 °C) using a Dr. Chef high pressure food processor (Kobe Steel Ltd., Kobe, Japan) or boiled in hot water (850 mL) for 10 min, respectively.

2.2 Texture Measurement of Flavedo (Hyuganatsu)

Changes in texture of hyuganatsu samples during soaking, pressurizing or heating, and texture of HP- or H-marmalade were measured by a creepmeter (Rheoner, RE-33005, Yamaden Ltd., Tokyo, Japan). The yellow surface of the flavedo was punctured at 1 mm/s by a plunger (cylindrical shape: 2 mm diameter, 22 mm long) using a loadcell of 2 kg. The rupture stress and rupture strain (the mean of 10 measurements) were indicated.

2.3 Structure Measurement of Peel (Hyuganatsu)

The minute structures of the hyuganatsu samples (flavedo and albedo) were observed using a cryo-scanning electron microscope (S-4500, Hitachi Ltd., Tokyo, Japan). Samples were cut and dehydrated with 40% and 50% ethanol. The specimen was contained in a metal holder and quickly frozen by immersing in liquid nitrogen (LN_2). The frozen specimen was transferred to a cold stage of the cryo-system for scanning electron microscopy and cut with a knife (-150 °C). After etching at -85 °C, the surface was coated with gold then observed at -120 °C under low acceleration voltage (1 kV).

2.4 Extraction and Determination of Pectin from Four Parts of Hyuganatsu

The raw and soaked samples were cut into tiny pieces, then 99.5% ethanol (four times amount of sample) was added. This was homogenized three times for 2 min using an excel-auto homogenizer (EO-9, Nihon-Seiki, Tokyo) then poured into a weighted glass-filter (3G2). It was washed successively using 80% ethanol until there was no sugar-reaction by the phenol-sulfuric acid method [12], 90% ethanol and acetone, then dried at 20 °C. The alcohol-insoluble-solids (AIS) were prepared by this method.

Pectic substances from AIS were extracted into three reagents, successively: solutions were distilled water (at 20 °C, 12 h × 2), 0.4% sodium hexametaphosphate solution (at 20 °C, 2 h × 2) and 0.05 N HCl solution (at 85 °C, 1 h) [14]. These fractions were designated as water-soluble pectin (WSP), sodium hexametaphosphate-soluble pectin (PSP) and HCl-soluble pectin (HSP), respectively. The amount of galacturonic acid in these fractions was determined by the carbazole method [15].

2.5 Color Measurement of Flavedo

The color (L-, a- and b-values) of flavedo of hyuganatsu (raw, soaked at pH 2.7 for 24 h, pressurized at 500 MPa for 30 min or heated for 10 min) and color of flavedo and jelly (without flavedo) in HP- or H-marmalade were measured by using a spectrophotometer ZE-6000 (NDK, Osaka, Japan).

2.6 Rheology Measurement of Marmalade

The steady-flow viscosity, thixotropy and dynamic-viscoelasticity of jelly in marmalade (hyuganatsu, pummelo and grapefruit) were measured at 25 °C by using a Rheosol-G3000 (UBM Ltd., Kyoto, Japan). Steady-flow viscosity of six kinds of marmalades was compared.

2.7 Extraction and Determination of Naringin from Four Parts of the Citrus Shaddock Family and Marmalade

The raw and treated samples (1.0-6.0 g) were cut into tiny pieces, then distilled water (10 mL) was added. The extracts, by soaking for 3 min, were strained through a glass-filter (3G2). The amount of naringin was determined by the Iwata and Ogata method [16]. The diethylene glycol (10 mL) and 1 N NaOH (1 mL) were added to the extracts (1 mL), they were incubated for 30 min at 30 °C, then the absorbances at 420 nm were determined.

2.8 Sensory Evaluation of Marmalades

Sensory evaluation of hyuganatsu marmalades was performed using a five point scale (-2 to 2). The color (bad to excellent), transparency (opaque to transparent), flavor (smell) of fruit (weak to strong), mouth feel (rough to smooth), texture of jam (soft to firm, not like to like), sweetness, sourness and bitterness (weak to strong, not like to like), and total evaluation (bad to excellent) were all compared. Samples were evaluated by 25 students and teachers (20-64 years old).

3. Results and Discussion

3.1 The Weight Percentage of Flavedo, Albedo, Segment Walls and Juice Sacs

The structure and weight percentage of flavedo, albedo, segment walls and juice sacs of hyuganatsu, pummelo and grapefruit are shown in Figs. 1a-1c and Figs. 1d-1f, respectively. The weight of a fruit, sugar content in juice and the pH value of juice were as follows: hyuganatsu (113 ± 4 g, 14.7% ± 0.4% sugar, pH 3.33 ± 0.25), pummelo (536 ± 43 g, 11.5% ± 0.4% sugar, pH 3.37 ± 0.13) and grapefruit (412 ± 66 g, 10.4% sugar, pH 3.22), respectively. The size of fruit was (biggest to smallest): pummelo > grapefruit > hyuganatsu. The percentage of juice sacs was (greatest to least): grapefruit > pummelo > hyuganatsu, and that of segment walls + albedo was reversed: hyuganatsu > pummelo > grapefruit.

3.2 Changes in Texture of Flavedo (Hyuganatsu) during Soaking, Pressurizing or Heating and Processing Marmalade

Changes in stress-strain curves (Fig. 3a), rupture stress (Fig. 3b) and strain (Fig. 3c) of flavedo (hyuganatsu) during soaking in citric acid solutions, pressurizing or heating, and rupture stress and strain of HP- or H-marmalade are shown in Fig. 3.

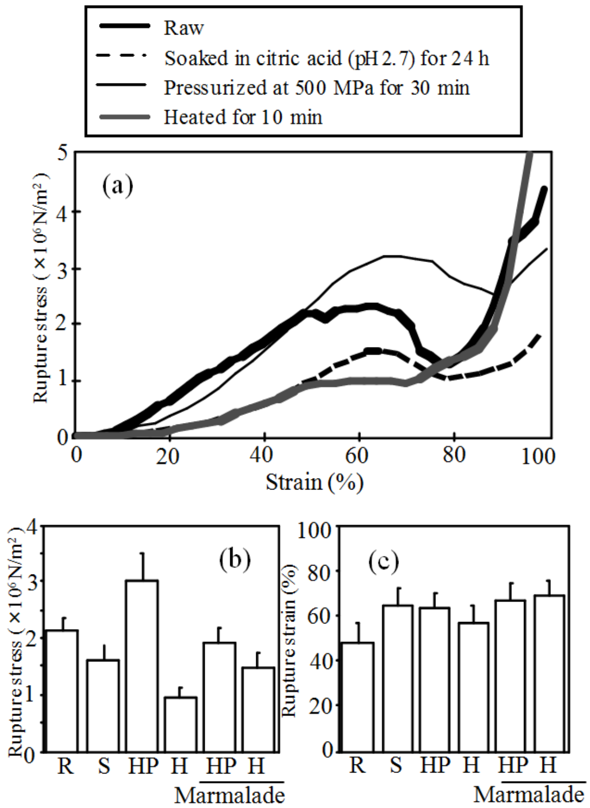


Fig. 3 Typical stress-strain curves, rupture stress and strain of flavedo of hyuganatsu after soaking pressurizing or heating and HP- or H-marmalade.

R: raw; S: soaked in citric acid for 24 h at pH 2.7; HP: pressurized at 500 MPa for 30 min; H: heated for 10 min in boiling water.

The rupture stress of flavedo soaked for 24 h at pH 2.7 decreased slightly due to the removal of Ca^{2+}. By pressurization at 500 MPa for 30 min, the rupture stress of flavedo increased, since pectin does not degrade through trans-elimination by pressurization. Heated flavedo became softer than raw flavedo. Heating at above pH 5, pectin degrades by trans-elimination [20]. However, the pH value of flavedo was acidic, thus, pectin might degrade by hydrolysis.

The rupture stress of flavedo in HP-marmalade decreased slightly more than that of raw flavedo. The firmness of flavedo in H-marmalade was softer than HP-marmalade. Heating for 10 min in a vacuum pack at pH 2.7 might accelerate the degradation of pectin by hydrolysis, consequently flavedo became soft. However, the flavedo in H-marmalade was firmer than heated flavedo. It might be dehydrated probably with sucrose.

3.3 Changes in Structure of Peel (Hyuganatsu) during Soaking, Pressurizing or Heating

The minute structures of flavedo and albedo of hyuganatsu are shown in Fig. 4. The magnifications used to observe the changes in structures during soaking, pressurizing and heating were ×1,000 (cells, Figs. $4a_1$-$4a_4$ and Figs. $4b_1$-$4b_4$) and ×20,000 (cell wall, Figs. $4c_1$-$4c_4$, Figs. $4d_1$-$4d_4$), respectively. The size of cells of the outer part (surface) of flavedo was smaller than that of the inner part (Figs. $4a_1$-$4a_4$). Round cells with thick/thin cell walls were also observed. The structures of albedo became more spongy as the albedo moved inward.

When flavedo and albedo of hyuganatsu were soaked for 24 h at pH 2.7, both cell walls became loose (Figs. $4c_2$ and $4d_2$) and the middle lamella of albedo separated (Fig. $4d_2$). After heating, the middle lamella of albedo separated (Fig. $4d_4$) and the cell walls of flavedo became slightly loose (Fig. $4c_4$). However, they did not loosen after pressurization (Figs. $4c_3$ and $4d_3$).

In a previous paper [10], after soaking for 24 h at pH 2.7 the cell walls of yuzu flavedo did not loosen, but the albedo became loose. The middle lamella of flavedo of yuzu separated after soaking for 24 h at pH 2.0, and the cell walls of albedo became loose after 3 h and melted after 24 h of soaking at pH 2.5 [10].

3.4 Changes in Pectin of Hyuganatsu

Pectic substances in hyuganatsu were extracted into three reagents: WSP, which was extracted in cold water, contained water-soluble pectin and water-soluble pectate; PSP, which was extracted in sodium hexamethaphosphate solution, contained pectinate and

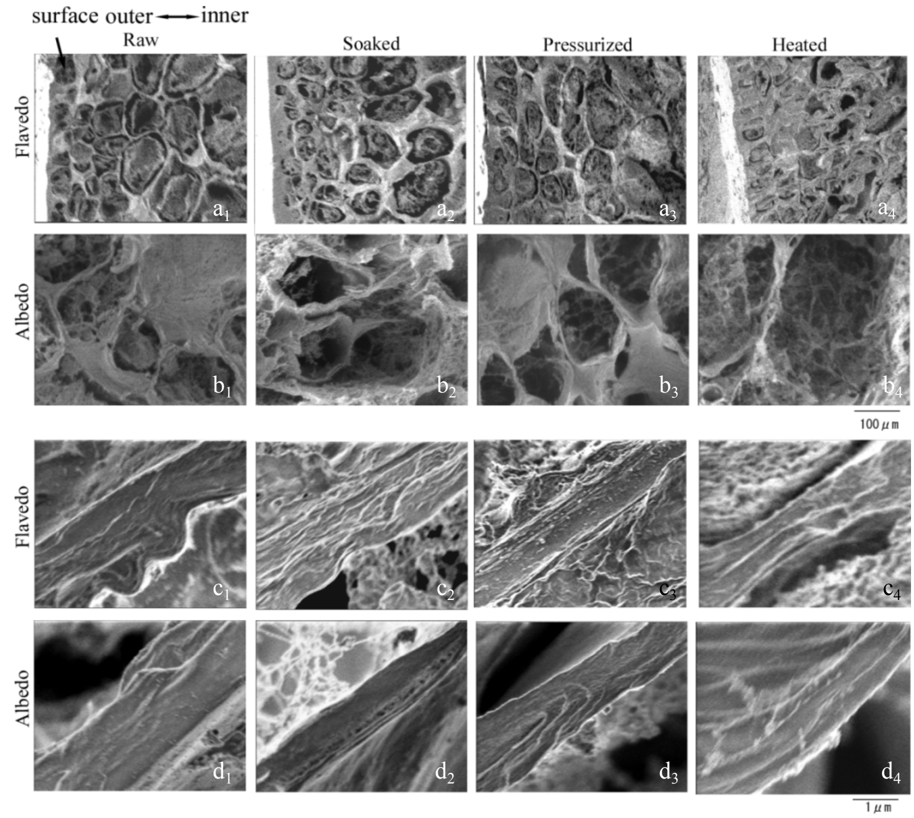


Fig. 4 Changes in cryo-scanning electron micrographs of flavedo and albedo of hyuganatsu during soaking, pressurizing and heating.

Soaked: soaked in citric acid solution for 24 h at pH 2.7; pressurized: pressurized at 500 MPa for 30 min; heated: heated for 10 min in boiling water.

water-insoluble pectate; HSP, which was extracted in 0.05 N HCl solution and was protopectin [14].

Pectin composition of flavedo, albedo, segment walls and juice sacs of raw hyuganatsu and changes in pectin composition of flavedo by soaking in citric acid solution are shown in Fig. 5. The amount of pectin was the greatest in albedo ≥ flavedo ≥ segment walls > juice sacs, respectively; however, it was almost the same. Conversely, juice sacs contained an extremely small amount of pectin. The amount of pectin in albedo of hyuganatsu was smaller than that of yuzu [14].

The percentage of HSP was greatest in all parts (except juice sacs) compared to PSP > WSP, respectively. The percentage of WSP in hyuganatsu was smaller than that of PSP, HSP and also raw peach fruit [21]. It suggests that hyuganatsu contains a smaller amount of low molecular weight-pectin than peach.

When flavedo of hyuganatsu was soaked in citric acid (pH 2.7) for 24 h, only 9.3% of pectin was extracted. However, about 30% pectin of flavedo of yuzu was extracted by soaking for 24 h at pH 2.0 written in a previous paper [8, 10]. Also, more pectin was extracted when flavedo was soaked at pH 2.0 rather than at pH 2.7, due to the acceleration of the removal of Ca^{2+} [10]. Thus, rupture stress of hyuganatsu decreased slightly because the pH value was 2.7.

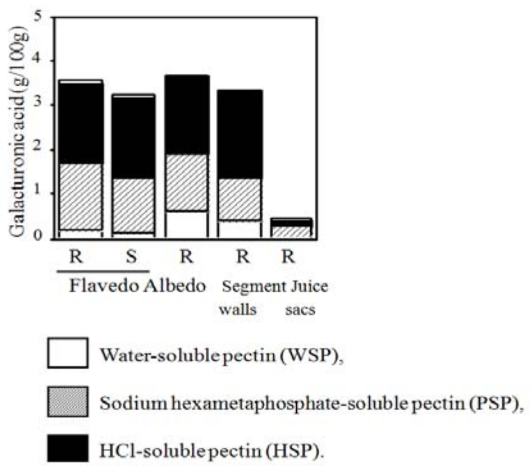


Fig. 5 Pectin composition of raw flavedo, albedo, segment walls and juice sacs of hyuganatsu and changes in pectin composition of flavedo by soaking in citric acid solution.
R: raw; S: soaked in citric acid for 24 h at pH 2.7.

3.5 Changes in Color of Marmalade

The L-value (+ brightness, Fig. 6a), a-value (+ red, - green, Fig. 6b) and b-value (+ yellow, - blue, Fig. 6c) of flavedo of hyuganatsu during soaking, pressurization or heating and marmalades are shown in Fig. 6. L-, a- and b-values decreased when flavedo was soaked, pressurized or heated and also that of peel and jelly in marmalades decreased. However, L- and b-values of HP-marmalade were higher than that of H-marmalade, and more similar to that of raw flavedo. Thus, peel and jelly of HP-marmalade preserved its natural color.

3.6 Changes in Rheology of Marmalades

The steady-flow viscosity of marmalades (citrus shaddock family) is shown in Fig 7. The viscosity was slightly higher in pummelo (Fig. 7b) > hyuganatsu (Fig. 7a) > grapefruit (Fig. 7c), respectively. However, a great difference in viscosity between HP- and H-marmalade was not found.

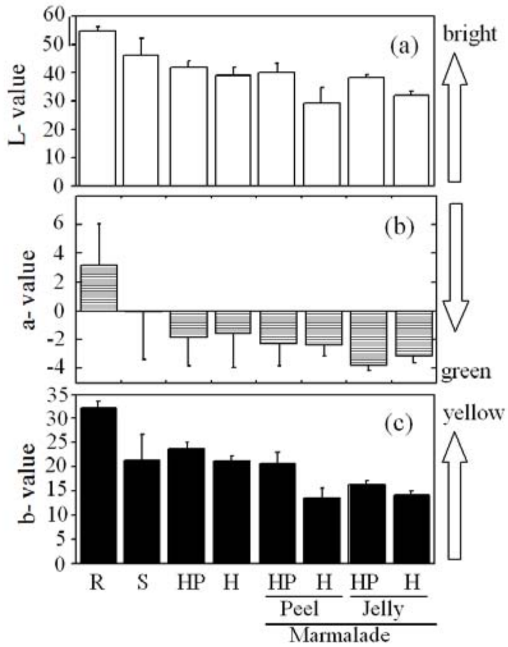
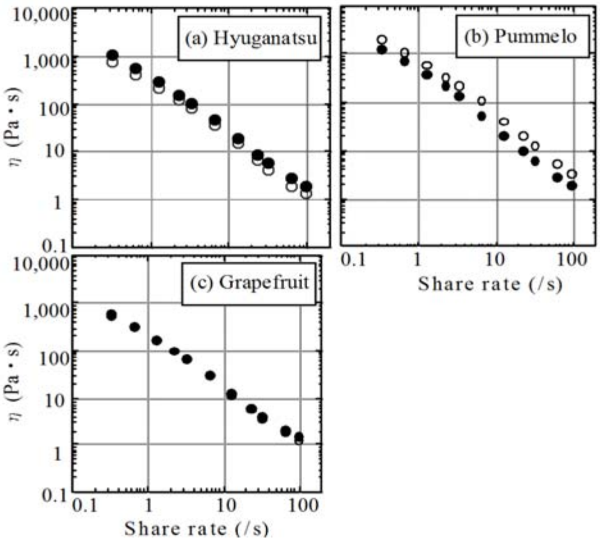


Fig. 6 Changes in L-, a- and b-values of flavedo of hyuganatsu during soaking, pressurization or heating and marmalades.
R: raw; S: soaked in citric acid for 24 h at pH 2.7; HP: pressurized at 500 MPa for 30 min; H: heated for 10 min in boiling water.



○ HP-marmalade, ● H-marmalade.

Fig. 7 Steady-flow viscosity of high pressure-induced (HP-) and heat-induced (H-) marmalades.

Since the thixotropy and dynamic-viscoelasticity showed a similar tendency, they are not shown.

3.7 The Amount of Naringin in Citrus Shaddock Family and Marmalades

The amount of naringin in the citrus shaddock family and marmalades is shown in Fig. 8. The amount of naringin was the greatest in grapefruit (Fig. 8c) > pummelo (Fig. 8b) > hyuganatsu (Fig. 8a), and albedo > segment walls ≥ flavedo > juice sacs, respectively. That of marmalades showed a similar tendency (Figs. 8d and 8e). The amount of naringin in hyuganatsu was the least. Therefore, Japanese eat hyuganatsu with albedo. Hyuganatsu is suitable for marmalade because it was more palatable than the others.

3.8 Sensory Evaluation of Marmalades

Sensory evaluation of hyuganatsu marmalade is shown in Fig. 9. There was no significant difference in sensory evaluation between HP- and H-marmalade. However, the color, transparency and total evaluation of HP-marmalade were comparatively better than H-marmalade. The color was maintained by pressurization. Also, the bitterness of HP-marmalade was weaker than H-marmalade. It was suggested that the bitterness was restrained by pressurization [17]. Flavor (smell) of fruit of HP-marmalade was weaker and texture of the jelly was softer than H-marmalade. However, for a total evaluation, HP-marmalade of hyuganatsu was higher rated (about four points/five points).

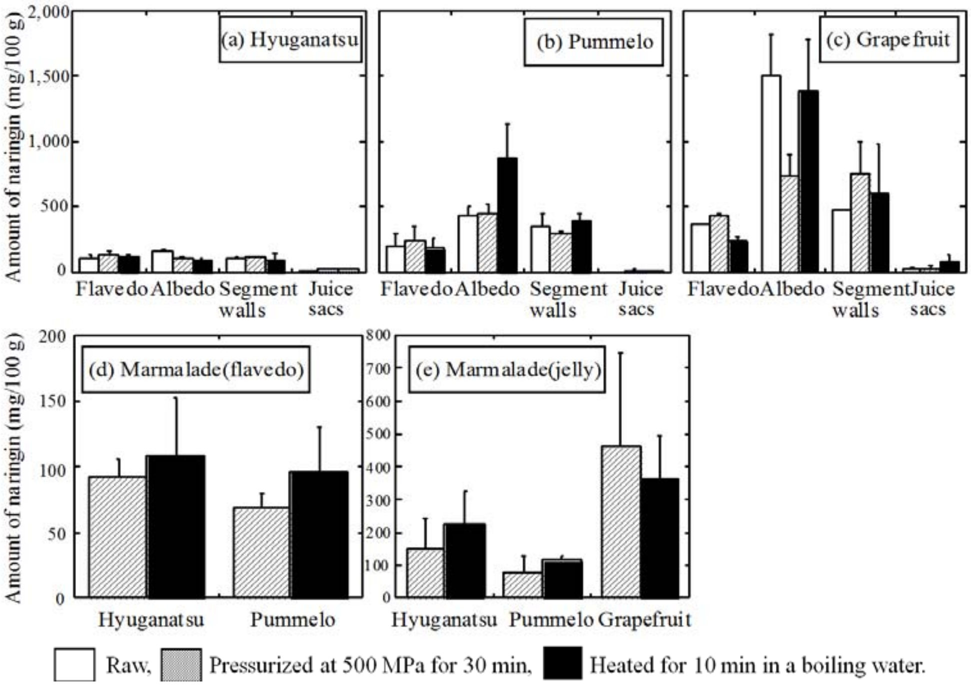


Fig. 8 The amount of naringin in hyuganatsu, pummelo, grapefruit and marmalades.
Pressurized: pressurized at 500 MPa for 30 min; heated: heated for 10 min in boiling water.

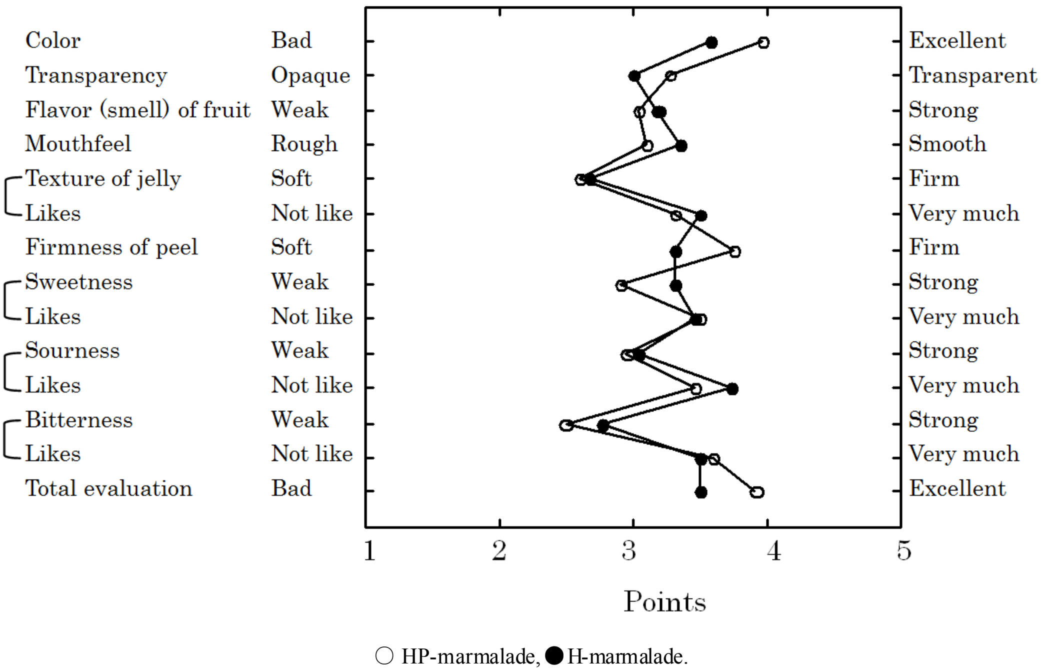


Fig. 9 Sensory evaluation of hyuganatsu marmalade.

4. Conclusions

Hyuganatsu is a typical Japanese citrus with desirous smell and edible albedo. Firmness of peel (flavedo) of hyuganatsu was (greatest to least): pressurized for 30 min at 500 MPa > soaked in citric acid solution for 24 h at pH 2.7 > boiled for 10 min, respectively. The cell walls of flavedo and albedo did not loosen after pressurization. However, after soaking or heating, the middle lamella of albedo separated. Sliced flavedo and homoginized albedo, segment walls and juice sacs of hyuganatsu were soaked in citric acid solution (pH 2.7), then the marmalades with 50% sucrose were processed. A great difference in viscosity between HP- and H-marmalade was not found. The amount of naringin was the least in hyuganatsu (hyuganatsu < pummelo < grapefruit). HP-marmalade was evaluated better than H-marmalade by a sensory test. Thus, the total evaluation of hyuganatsu HP-marmalade was highly rated. Hyuganatsu was considered to be more suitable for marmalade because it was more palatable than other marmalades (pummelo and grapefruit).

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