

Effect of Extraction Method on Yield and Quality of *Citrus depressa* Juice

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Certain qualitative properties of *Citrus depressa* juice extracted by belt-press, centrifugal, and screw-press extractors were investigated to evaluate the methods for extracting *Citrus depressa* juice currently used in Japan. Among the three methods, juice yield was highest with screw-pressing. The migration rate of polymethoxyflavonoids (PMF) to the juice was also highest with screw-pressing. Screw-pressed juice had different physicochemical properties from the other two types of juices, and it was evaluated as being unsuitable for drinking following a sensory test. *Citrus depressa* juice processed by belt-press and centrifugal extractors had lower juice yield and a lower migration rate of PMF. *Citrus depressa* juice processed by centrifugal extractors was evaluated by a sensory test as being the best of the three types of juices for drinking. PMF and vitamin C found in *Citrus depressa* juice were largely retained during filtering, sterilizing, and bottling after being extracted with these three methods.

Keywords: *Citrus depressa*, juice, extractor, belt-press, centrifuge, screw-press

Introduction

Citrus depressa is a citrus fruit, known as “*Shiikuwasha*” in Japan. While details of its place of origin and history are unknown, *Citrus depressa* is known to grow naturally in the Okinawa region of Japan, and its fruit has long been used for food and as a detergent for textiles. Recently, the health-promoting functionality of some components of *Citrus depressa*, such as nobiletin and tangeletin, referred to as polymethoxyflavonoids (PMF), has been discovered (Mimaki *et al.*, 2000; Yoshimizu *et al.*, 2004), and demand for *Citrus depressa* is increasing. These days, the greater part of the total harvest is used for processing, mainly the extraction of juice. *Citrus depressa* juice is too sour and bitter to drink directly, so it is usually drunk after being distilled by water or having some sweeteners added.

An in-line extractor, a belt-press extractor, or a centrifugal extractor has been used to produce citrus juice such as satsuma mandarin (*Citrus unshu* Mark.) or yuzu (*Citrus junos* Sieb. ex Tanaka) juice (Watanabe *et al.*, 1982; Ohta *et al.*, 1983). *Citrus depressa* is too small to be processed by an in-line extractor. In the Okinawa region, belt-press, centrifugal, and screw-press extractors are currently used to produce *Citrus depressa* juice. A belt-press extractor extracts juice by pressing whole fruit with a rolling belt, and a centrifugal extractor produces juice by cutting and smashing whole fruit by centrifuging in a centrifugal

separator equipped with a cutter in the bottom area (Ohta *et al.*, 1983). A screw-press extractor extracts juice by crushing and pressing whole fruit with a rolling screw (Ohta *et al.*, 1982). In this study, we aimed to produce high-quality juice from *Citrus depressa* fruit, to evaluate the performance of the different kinds of extractors by tracing vitamin C and PMF during juice processing, and to compare certain qualitative properties of the final juices, using two lots of *Citrus depressa* fruits.

Materials and Methods

Materials Fruit of *Citrus depressa* (‘Kugani’ family) was harvested in November and December 2005 in the Nago area of Okinawa, Japan. Fruit samples weighed 18 to 22 g in November and 30 to 33 g in December. The fruit was harvested one to two days before it was used for extracting juice and stored at room temperature. The fruit was washed with water just before the extraction.

The flow of processing for *Citrus depressa* juice and schematic drawings of belt-press, centrifugal, and screw-press extractors are shown in Figs. 1 and 2.

Extraction Belt-pressing was performed by a B-45 belt-press extractor (Thank You Foods, Kakogawa, Japan) whose electricity consumption was 0.4 kW. Whole fruit of *Citrus depressa* (100 kg) were pressed with rotating belts (clearance, 6 mm) at 400 kg/h to make single-strength juice (B-J1) and residue (B-R1). Centrifuging was performed by an H-122 centrifugal separator (Kokusan, Tokyo, Japan) whose electricity consumption was 1 kW. Whole fruit of *Citrus depressa* (100 kg) were squeezed by

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centrifuging ($1200\times g$, 3 min) at 50 fruits/batch (about 1 kg/min) to make single-strength juice (C-J1) and residue (C-R1). Screw-pressing was performed by an SRE-230L single screw-press extractor (Shinwa Engineering, Ishinomaki, Japan) whose electricity consumption was 1.5 kW, and whose L/D (length of screw)/(diameter of screw) was 6.3. Cut fruit of *Citrus depressa* (300 kg) were squeezed by screw-pressing at 1.5 t/h to make single-strength juice (S-J1) and residue (S-R1).

Sterilizing and bottling Each single-strength juice (B-J1, C-J1, and S-J1) was filtered with a stainless-steel filter (40 mesh). The filtered juices (B-J2, C-J2, and S-J2) were passed through a copper tube ($\phi 0.5\text{ cm} \times 8\text{ m}$), which was heated in boiling water, at a flow rate of 250 mL/min to sterilize the juices. The sterilized juices (B-J3, C-J3, and S-J3) were immediately poured into glass bottles (250 mL). The bottles were stoppered with caps, set upside down for 20 min at room temperature, and then cooled in water for 20 min.

Sampling Samples of the B-J1~3, C-J1~3, S-J1~3, B-R1, C-R1, and S-R1 were taken and stored at -20°C until PMF was measured. Fifteen milliliters each of B-J1~3, C-J1~3, and S-J1~3 were immediately mixed with a 5% methaphosphoric acid aqueous solution (15 mL) and stored at -20°C until the vitamin C was measured. Ten grams of each B-R1, C-R1, and S-R1 was immediately mixed with a 5% methaphosphoric acid aqueous solution (15 mL) and stored at -20°C until the vitamin C was measured. B-J4,

C-J4, and S-J4 were stored at 5°C for a week before the vitamin C and PMF were measured.

Evaluation of the solid-liquid separating ratio The solid-liquid separation ratio of each extraction was calculated from the water contents of the solid (residue) and liquid (juice) parts determined by freeze-drying (initial temperature, -20°C ; final temperature and pressure, 20°C and 0.8 Pa).

Vitamin C measurement Vitamin C was measured following the method of Yamaguchi *et al.* (2001). Juice samples mixed with a 5% methaphosphoric acid aqueous solution were directly used for the test. A test solution from residue sample was prepared as follows. Each residue sample in a 5% methaphosphoric acid aqueous solution was homogenized, the homogenate (1 g) was diluted by a 5% methaphosphoric acid aqueous solution (4 mL), the solution was centrifuged ($3000\times g$, 10 min), the supernatant was filtered with a Cosmonice filter W (pore size, $0.45\mu\text{m}$; Nacalai Tesque, Kyoto, Japan), and the resulting filtrate was used as the test solution. As for *Citrus depressa* fruits (material), a test solution was prepared as well as a residue sample, using a 5% methaphosphoric acid aqueous solution (30 mL) for one whole fruit in the first step.

PMF Measurement Each sample was freeze-dried using a 20-85ATNNS freeze-dryer (Takara ATM, Tokyo, Japan). Dried juice samples (from 10 mL juice) were extracted by 70% ethanol (10 mL) two times, and the extract was brought up to 25 mL with 70% ethanol. The dried residue samples were milled by an IDS-50 blender (Sunbeam Products, Boca Raton, USA) to make a uniform powder. Dried residue powder (100 mg) was extracted three times by 70% ethanol (2 mL), and the extracted solution was brought up to 10 mL with 70% ethanol. Each extract was analyzed by HPLC 600E multi-solvent flow system (Waters, Milford, USA.) equipped with a Cosmosil 5C18-MS column (4.6 mm i.d. \times 150 mm, Nacalai Tesque, Kyoto, Japan). The mobile phase was composed of 10 mM phosphoric acid/acetonitrile (90: 10) (solvent A) and acetonitrile (solvent B) and programmed to mix 20 to 100% solvent B to solvent A for 15 min at a flow rate of 1 mL/min. Nobiletin, tangeletin, and 5-demethylnobiletin were detected at 280 nm.

Brix scale measurement The Brix scale was measured by an Rx-5000a Brix meter (Atago, Tokyo, Japan) at 20°C .

Acidity measurement Acidity was measured by titra-

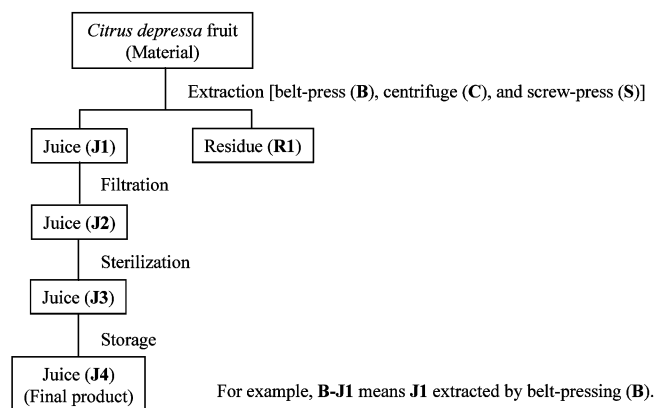


Fig. 1. Processing flow for *Citrus depressa* juice.

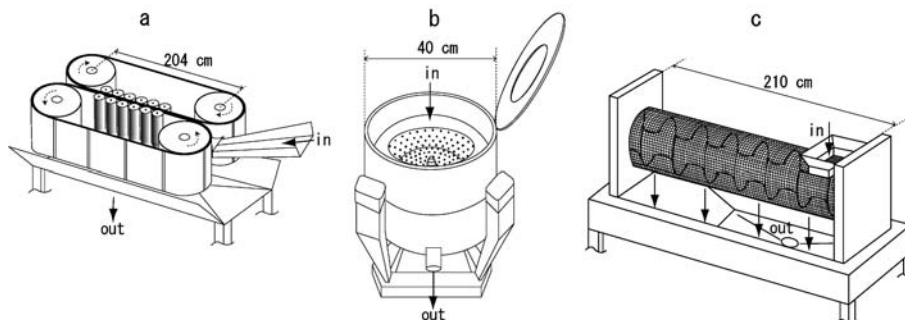


Fig. 2. Schematic drawings of belt-press, centrifugal, and screw-press extractors. a: Belt-press extractor, b: Centrifugal extractor, c: Screw-press extractor.

tion of a 0.1 N NaOH aqueous solution and was calculated as the equivalent of citric acid.

Pulp content measurement Pulp content was measured by the centrifuging method (Ohta *et al.*, 1983).

Ash content measurement Ash content was measured by the direct ashing method.

Color tone measurement Color tone (L, a, and b values) was measured with a CT-310 colorimeter (Minolta, Tokyo, Japan).

Sensory test Twenty female panelists (average age, 23.8 years) evaluated the sourness, sweetness, bitterness, odor, aftertaste, and overall palatability of juices of *Citrus depressa* (B-J4, C-J4, and S-J4). These items were subjectively evaluated on a scale from -3 to +3.

Results and Discussion

The solid-liquid separation ratios for *Citrus depressa* fruits harvested in November and December 2005 after extraction by three types of extractors (belt-press, centrifugal-type, and screw-press) are listed in Table 1. Screw-pressing produced the highest juice yield (liquid percentage), followed by centrifuging and belt-pressing. For all extraction methods, the juice yield in December was higher than in November, which may be because *Citrus depressa* fruits harvested in December are riper than in November. Generally, when fruits ripen, their tissues are softened by solubilization of pectin by pectin methyl-esterase and polygalacturonase (Yoshioka, 1992). The tissue of *Citrus depressa* fruits harvested in December

may have had more water-soluble substances than in November, resulting in a higher juice yield.

Little change was observed in the vitamin C content of *Citrus depressa* fruit during juice processing by belt-press, centrifugal, and screw-press extraction after evaluating the total output (juice and residue) and considering the solid-liquid separation ratio (Fig. 3). Vitamin C effectively migrated to the juice part during these three extraction methods. During filtering, sterilizing, and bottling, a small amount of vitamin C was lost after each extraction method using *Citrus depressa* fruits harvested in November, but not in December. Ifuku *et al.* (1975) reported that a small amount of vitamin C was lost during heating of satsuma mandarin juice extracted by in-line and chopper-pulper juice extractor. Sanchez-Moreno *et al.* (2003) reported that the vitamin C content in orange juice hardly changed during high-pressure treatment and storage at 4°C.

PMF of *Citrus depressa* also rarely changed during juice processing using these three methods, after evaluating the total output. The distribution ratios of PMF in juice and residue parts differed depending on the extraction method (Fig. 4). The difference in the PMF content of the juice and residue parts was more significant than the difference in vitamin C. It is assumed that the higher migration rate of PMF to juice by screw-press extraction than by belt-press or centrifugal extraction is related to the fact that screw-press extraction crushes the whole fruit with the peel, thus including more PMF than with the flesh only (Nogata *et al.*, 2006), whereas belt-press and centrifugal extraction mainly crush the flesh, with the peel remaining unbroken. This effect, depending on the extraction method, was more significant in November than in December, because *Citrus depressa* fruits harvested in November include more PMF than those harvested in December.

Table 2 lists some physicochemical properties of *Citrus depressa* juices (B-J4, C-J4, and S-J4) extracted from fruit harvested in November and in December. The Brix scale corresponds to sugar concentration, which relates to a sweetness, and the acidity relates to a sourness. Both are important factors in the taste of fruit juice. The Brix scale

Table 1. Solid-liquid separation ratios obtained from three types of extraction from *Citrus depressa* fruit harvested in November and December 2005.

Extraction method	Nov.	Dec.
Belt-press	62 : 38	58 : 42
Centrifuge	59 : 41	45 : 55
Screw-press	51 : 49	35 : 65

Solid: liquid (w/w).

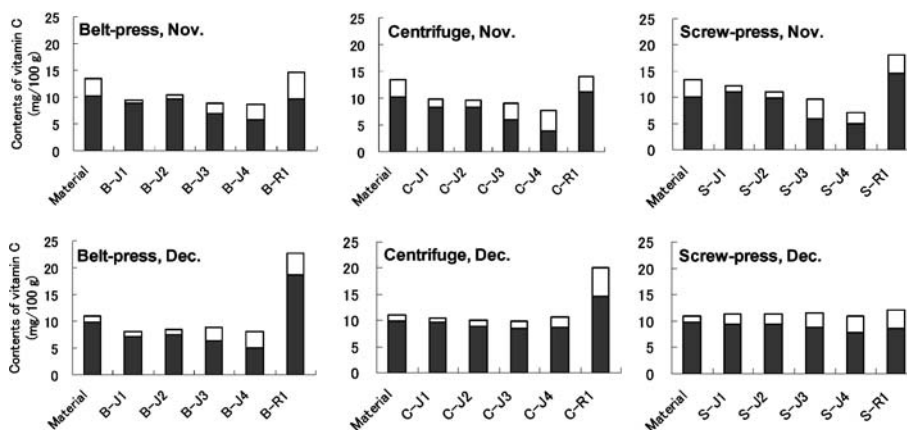


Fig. 3. Vitamin C content of material fruit, juices and residue obtained from three types of extraction (belt-press, centrifuge, and screw-press) from *Citrus depressa* harvested in November and December 2005.

■: Reduced vitamin C; □: Oxidized vitamin C.

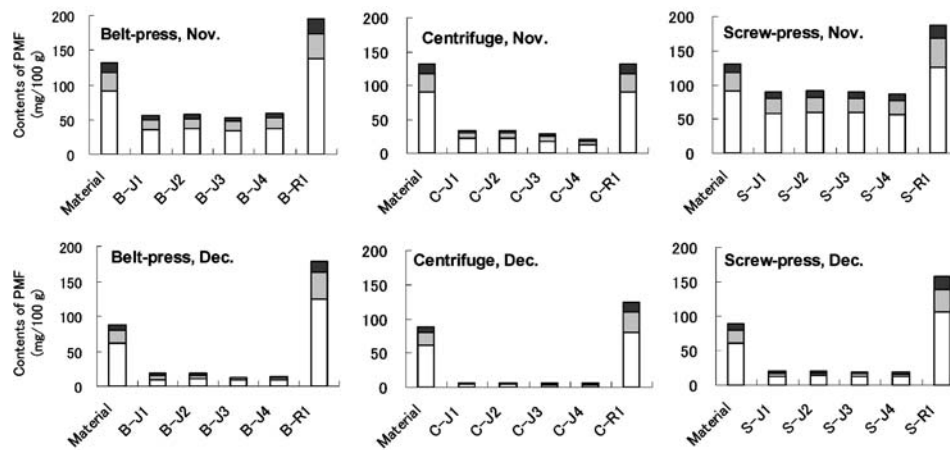


Fig. 4. PMF content of material fruit, juices and residue obtained from three types of extraction (belt-press, centrifugal, and screw-press) from *Citrus depressa* fruit harvested in November and December 2005.

■: 5-Demethylnobiletin; ■: Tangeletin; □: Nobiletin.

Table 2. Physicochemical properties of *Citrus depressa* juices (B-J4, C-J4, and S-J4) processed from fruit harvested in November and December 2005.

	Nov.			Dec.		
	B-J4	C-J4	S-J4	B-J4	C-J4	S-J4
Brix scale (%)	11.7	11.6	11.5	10.8	10.9	10.6
Acidity (%)	5.7	5.7	4.5	3.5	3.4	2.8
Pulp content (%)	0.2	1.2	2.7	0.8	3.0	5.8
Ash content (%)	1.7	1.4	0.7	0.5	1.0	0.5
Color value (L)	18.8	24.3	8.5	18.9	19.5	11.6
(a)	-1.7	-0.2	3.1	-1.0	-0.1	2.5
(b)	31.0	37.4	14.4	31.5	32.3	19.7

was slightly higher in November than in December, and the acidity was higher in November than in December for the three types of juices. In the sensory test, for all juices, sweetness was rated higher in December than in November, while sourness had an opposite trend (Fig. 5). This means that sweetness in the November juice was masked by a stronger sour taste. It is assumed that December juice was evaluated to have better sweetness than November juice by sensory test, not because the sugar content increased but because fewer acids were present in the December juice. The sugar-acid rate (Brix scale)/(acid percentage as citric acid) was 2.0 to 2.6 in the November juice and 3.1 to 3.8 in the December juice, which is quite low compared to other fruit juices (Sugawara *et al.*, 1979). Owada has reported that the minimum sugar-acid rate of Satsuma mandarin juice that is acceptable to consumers is more than 12.5 (Owada *et al.*, 1978). In the sensory test, B-J4 and S-J4 were evaluated below C-J4 for sourness (Fig. 5), but the acidity of C-J4 was not the lowest (Table 2). This indicates that sourness may not be caused exclusively by acids, and that its intensity may change according to the condition of any coexistent substances in the juice, rather than that the acidity of C-J4 was preferred. The pulp content was higher in December than November

for all juices, and screw-press extraction migrated more pulp to the juice (Table 2). Rega *et al.* reported that pulp in orange juice had the effect of retaining aroma compounds and modified the rheological properties of the juice matrix (Rega *et al.*, 2004). S-J4 exhibited different properties in color tone from B-J4 and C-J4, lower “L” and “a” values and a higher “b” value (Table 2), meaning a lower luminance and yellow tone and a stronger red tone. C-J4 received a significantly higher score than B-J4 and S-J4 in all parts of the sensory test (Fig. 5), but there was not always a conspicuous difference in the related physicochemical properties.

Citrus depressa juice is too sour and bitter to drink straight. The principle components of bitterness in citrus fruits are glucosides of flavonoid and limonoid, which are contained in higher quantities in peel and segment walls, as well as in earlier stages of growing (Fukutani *et al.*, 1983; Ohta *et al.*, 1995). In screw-pressing, these bitter components may migrate in larger quantities into the juice part, because the method crushes whole fruit tissue. In the sensory test, bitterness was evaluated to be worst in screw-pressed juice (Fig. 5). Yield and physicochemical properties of belt-pressed juice were closer to those of centrifuged juice (Tables 1, 2), but the results of the sensory test were closer to that of screw-pressed juice (Fig. 5). It indicates that some components that have a negative influence on the sense of taste, including the bitter components, are contained in higher quantities in belt-pressed and screw-pressed juices. Belt-press extraction may apply a higher pressure to peel and segment wall parts, where the bitter components are stored, than centrifugal extraction. Screw-pressed juice was evaluated to be worst in almost all aspects in the sensory test (Fig. 5). However, screw-pressed juice had much more PMF compared with other juices (Fig. 4), which can be a great advantage for *Citrus depressa* products. This indicates that screw-pressing, while not suitable for extracting juice for drinking from *Citrus depressa*, may be useful for extracting PMF from peel. It is reasonable to make use of these merits in the processing and utilization of *Citrus depressa*. For

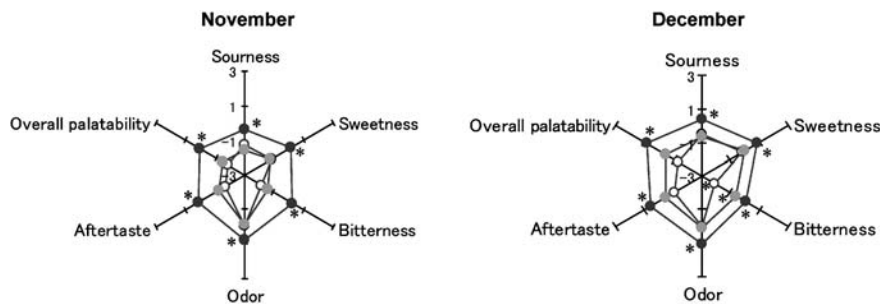


Fig. 5. Results of sensory test of *Citrus depressa* juices produced using different extraction methods.

●: Centrifugal-type, ●: Belt-press, ○: Screw-press.

*: significantly different from the other two juices for each item in the same month ($P < 0.05$).

example, juice may be made by belt-pressing, with the residue from the belt-pressing subsequently screw-pressed, resulting in a PMF-rich fraction utilizable as a material in healthful foods or other commodities. This kind of multi-step utilization of *Citrus depressa* is currently under investigation.

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