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Vinegar extraction from unripe shikuwasa (*Citrus depressa* L.), an Okinawan citrus fruit

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Abstract:
Introduction. Nakamoto Seedless, a variety of shikuwasa (*Citrus depressa* L.) in Okinawa, can be used to produce vinegar extracts because it has no seeds causing bitter taste. However, Nakamono Seedless is hardly cultivated commercially in Okinawa. This research was aimed to develop vinegar extract from Ogimi Kugani, another major variety of shikuwasa, and compare its characteristics with those of extracts from Nakamoto Seedless.
Study objects and methods. The study featured vinegar extracts from the whole shikuwasa of Nakamoto Seedless (20% fruit) and Ogimi Kunagi (5, 10, and 20% of fruit) varieties. The fruit was harvested in June, July, and August. We tested the samples for limonin and polymethoxyflavones content and sensory attributes, especially bitterness.
Results and discussion. Vinegar extracts with 20% of Ogimi Kugani harvested in June and July tasted bitter compared to those from Nakamoto Seedless harvested in August, but extracts from Ogimi Kugani harvested in August were not bitter. In addition, 5 and 10% vinegar extracts from Ogimi Kugani harvested in June had lower bitterness. The vinegar extracts from both shikuwasa varieties contained polymethoxyflavones – bioactive compounds – and similar flavor.
Conclusion. The whole shiluwasa fruit can be used to produce vinegar drinks, Ponzu soy sauce, salad dressings, etc.

Keywords: Okinawan citrus, Ogimi Kugani, Nakamoto Seedless, vinegar extract, polymethoxyflavones, sinensetin, nobiletin, tangeretin

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INTRODUCTION

Shikuwasa (*Citrus depressa* L.) is a common citrus fruit in Okinawa, the southernmost prefecture of Japan. Shikuwasa is very sour and also called Hirami lemon or thin-skinned flat lemon. In Okinawan dialect, shikuwasa means "to eat vinegar", however it does not contain vinegar, and the name refers to its strong sour taste. This native Okinawan citrus fruit became popular all over Japan and is used to garnish dishes as well as to make juices and jams.

As it grows, shikuwasa changes color from dark green to yellow or orange. Unripe (green) fruit is extremely sour and is used to make condiments. Ripe (yellow) shikuwasa has better taste from December to the end of January. Shikuwasa harvested from August to September is usually used as an acidifying agent in vinegar substitution. Fruit harvested from September to December is used in juice production and from December to January can be eaten in the raw form.

Shikuwasa is mainly cultivated in the northern part of Okinawa. In 2017, 3398 t of shikuwasa were produced, with the fruiting area of shikuwasa being 363 ha. Shikuwasa contains high levels of nutritional components, including polymethoxyflavones [1], which are well known for their biological activities. Polymethoxyflavons demonstrate anti-tumor, anti-inflammation, and anti-hyperglycemic properties [1–4]. Therefore, extracting polymethoxyflavones and other functional components from shikuwasa can contribute to healthy food products.

Among known varieties of shikuwasa in Okinawa, Ogimi Kugani (hereinafter Kugani) is the most popular. Kugani is a commercially available superior landrace which is widely used in the food industry, fresh or processed [5]. Nakamoto Seedless (hereinafter Seedless) is a seedless variety of shikuwasa that is becoming popular, due to its characteristics, which are similar to those of Kugani [6].

Citrus tree cultivation involves thinning young fruits to improve fruit size and quality. Thinning is performed from June to July because shikuwasa is not typically used during this period. Thinned fruits as well as normally harvested fruits unsuitable for sale, for example bruised fruits, should be effectively utilized to obtain processed food products such as vinegar extracts.

To produce a vinegar extract with low bitterness, Hirose *et al*. used only the peels of residual substances from Kugani, and Hanagasaki *et al*. used residual substances from Tankan (*Citrus tankan* Hayata) [7, 8]. Using this technology, Ponzu sauce (Japanese citrus vinegar soy sauce) production is already being commercialized in Okinawa. In addition, the whole Seedless fruit harvested from June to August is useful to produce vinegar extract because it has no seeds, contains low levels of limonin, and has low bitterness [9]. In spite of the fact that vinegar extract from the whole Kugani fruit is expected to be bitter because it typically contains 14 seeds per fruit, the potential use of the whole unripe Kugani should be explored to avoid wastes [6].

The aim of the present study was to develop vinegar extract from the whole unripe Kugani fruit and compare the characteristics of this extract with those of Seedless vinegar extracts to improve the profitability of the fruit.

STUDY OBJECTS AND METHODS

Shikuwasa (Nakamoto Seedless and Ogimi Kugani varieties) has been cultivated in Okinawa Agricultural Research Center (26º37' N, 127º59' E; 40 masl) since 2005. The Seedless and Kugani fruit under study were harvested in June 8, July 6, and August 3 (2018), respectively.

The objectives of the study were vinegar extracts made from Seedless (20% fruit) and Kugani (5, 10, and 20%). The fruit weight was defined by averaging the weights of 70~450 fruits, equal to approximately 1 kg. The fruit diameter was determined by averaging the diameters of 20 fruits. Moisture content was determined by calculating the weight difference before and after freeze-drying.

Production of vinegar extract. As for shikuwasa of Kugani variety harvested in different months, the fruit was broken down using a Bamix M200 mixer (Bamix, Switzerland). Afterwards, a 20% sample was prepared by stirring 10 g of the fruit and 40 mL of spirit vinegar (Kraft heinz, USA). Stirring was performed with a 4 cm stirrer bar at 2.0×g for 10, 30, 60, and 90 min.

Similarly, we prepared 20% Seedless vinegar samples. To compare vinegar extracts from Kugani and Seedless harvested in July and August, the samples were stirred for 60 min. The mixed samples were centrifuged at 1190×g for 20 min and filtered using No. 2 qualitative filter paper (Advantec Co. Ltd., Japan).

To compare vinegar extracts from shikuwasa harvested in June, we used 20% Seedless as well as 5 and 10% Kugani vinegar samples. The Kugani samples were obtained by mixing 2.5 or 5 g of Kugani fruit with 47.5 and 45 mL of vinegar and stirring for 60 min.

Freeze-dried powder process. To analyze polymethoxyflavone and limonin levels in shikuwasa, the samples were freeze-dried using an FD-550 freeze-drier (Tokyo rikakikai Co. Ltd., Japan). After drying, the samples were crushed in an IFM-800 mill (Iwatani Corp, Japan) using a 1.4 mm mesh.

Titratable acidity determination. A volume of 10 mL of the vinegar extracts was mixed with 100 mL of pure water and titrated with 0.5 mol/L NaOH until pH 8.2 ± 0.3 using a pH meter. We applied acid-base titration [10].

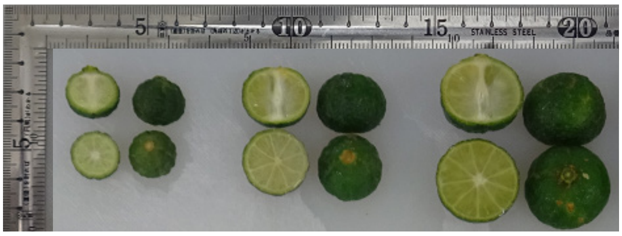
Polymethoxyflavone analysis. Polymethoxyflavone extraction was performed as described by Ichinokiyama *et al.* [11]. To extract polymethoxyflavones from shikuwasa, 100 mg of freeze-dried powder samples with 1 mL of methanol:DMSO (1:1) were subjected to ultrasonic wave for 10 min (M1800-J, Japan) and centrifuged at 2000×g for 2 min. We performed it three times. Extract solutions were obtained by diluting to a volume of up to 5 mL.

To obtain polymethoxyflavones from vinegar extracts, 1 mL of the vinegar extracts with 1 mL of ethanol were subjected to ultrasonic wave for 30 min. The insoluble component was removed by centrifugation at 2000×g for 2 min. The solutions were prepared for HPLC, namely filtered using a 0.20 μm hydrophilic PTFE (Advantec Co. Ltd.).

Sinensetin (Wako Pure Chemical Corp., Japan), as well as nobiletin, and tangeretin (Sigma-Aldrich, Japan) were dissolved in ethanol to a concentration of 0.1 mg/mL and used as standards. Quantitative analysis of each polymethoxyflavone was performed as described by Hirose *et al.* [7]. The sample solutions (5 μL) were injected onto a Union UK-C18 HPLC column (3×100 mm, 0.4 mL/min flow rate, Japan) at 40ºC. The solvent was acetonitrile/water/trifluorasetic acid (50/50/0.05). Analyses were performed at 340 nm using an LC-20A UV detector (Shimadzu corp., Japan). Amounts of polymethoxyflavones were calculated as the sum of sinensetin, nobiletin, and tangeretin.

Limonoid analysis. To extract limonoid from shikuwasa, 100 mg of the freeze-dried sample was mixed with 2 mL of acetic acid and 5 mL of ethyl acetate and then vortexed for 1 min. To obtain limonoid from vinegar extracts, 0.4 mL of the vinegar extracts was mixed with 1 mL of ethyl acetate and then vortexed for 1 min. Both ethyl acetate phases were collected after centrifugation at 2000×g for 2 min. These steps were repeated three times, and the supernatant was completely evaporated under reduced pressure.

Extract solutions were obtained by reconstituting the powdered samples with 2 and 0.4 mL of acetonitrile for shikuwasa and vinegar extract, respectively. The



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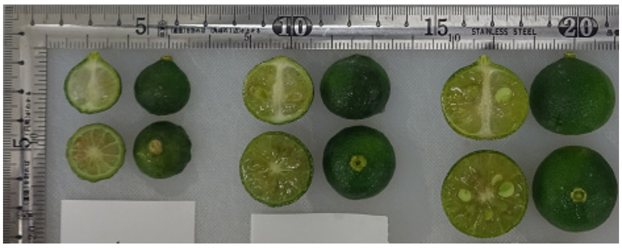

b

Figure 1 Nakamoto Seedless (a) and Ogimi Kugani (b) harvested from June to August (from left to right)

Table 1 Characteristics of Nakamoto Seedless and Ogimi Kugani harvested in different months

Harvesting date	Variety	Fruit weight, g	Fruit diameter, cm	Moisture content, %
June 8, 2018	Nakamoto Seedless	2.27	1.67	75.9
	Ogimi Kugani	3.51	1.92	80.4
July 6, 2018	Nakamoto Seedless	5.48	2.18	82.0
	Ogimi Kugani	8.81	2.58	82.0
August 3, 2018	Nakamoto Seedless	10.07	2.81	83.6
	Ogimi Kugani	15.31	3.24	82.9

solutions were prepared for HPLC by filtering. Limonin and nomilin (Wako Pure Chemical Corp., Japan) were each dissolved in acetonitrile to a concentration of 0.1 mg/mL and used as standards.

Quantitative analysis of limonin and nomilin was performed as described by Hirose *et al*. [7]. A volume of 2 μL of the sample solutions was injected into Cadenza CD-C18 HPLC column (0.4 mL/min, 40ºC). The solvent was acetonitrile/water/formic acid (40/60/0.1). The analyses were carried out at a wavelength of 210 nm using an LC-20A UV detector (Shimadzu corp., Japan).

Sensory evaluation. Vinegar extracts were diluted 10 times with distilled water, and 4% (w/w) caster sugar was added. Six men and six women aged 20–50 evaluated aroma, bitterness, green smell, and overall acceptability of the samples using a 5-point scale. For the shikuwasa aroma, 5 represented the strongest shikuwasa aroma and 1 represented the weakest aroma or the strongest vinegar aroma. For bitterness, 5 represented the lowest bitterness and 1 represented the strongest bitterness. For green smell, 5 represented the lowest green smell and 1 represented the strongest green smell. For overall acceptability, 5 represented "like" and 1 represented "dislike".

RESULTS AND DISCUSSION

Figure 1 demonstrates shikuwasa (Nakamoto Seedless and Ogimi Kugani varieties) harvested from June to August. The weight, diameter, and moisture of both fruit varieties were increasing during the time (Table 1). The characteristics of Seedless were lower than those of Kugani.

Changes in chemical components of Seedless and Kugani with time are shown in Table 2, changes in chemical components of both extracts with time are shown in Tables 3 and 4. In both varieties, the content of polymethoxyflavones was the highest in June and decreased with time ($P < 0.01$ for Seedless and $P < 0.001$ for Kugani). Comparing polymethoxyflvones content in both varieties, there was no significant

Table 2 Chemical components of Nakamoto Seedless and Ogimi Kugani harvested in different months

Component	Nakamoto Seedless			Ogimi Kugani		
	June	July	August	June	July	August
Polymethoxyflavones, mg/g d.w.	19.18 ± 1.46^{a}	12.33 ± 0.06^{b}	10.81 ± 0.14^{b*}	17.89 ± 0.66^{a}	12.44 ± 0.08^{b}	7.87 ± 0.04^{c}
Sinensetin	1.50 ± 0.12^{a}	1.04 ± 0.01^{b*}	0.90 ± 0.02^{b*}	1.21 ± 0.05^{a}	0.90 ± 0.01^{b}	0.56 ± 0.01^{c}
Nobiletin	11.01 ± 0.89^{a}	7.31 ± 0.04^{b}	6.22 ± 0.08^{b*}	11.03 ± 0.42^{a}	7.94 ± 0.05^{b*}	4.81 ± 0.03^{c}
Tangeretin	6.66 ± 0.47^{a}	3.98 ± 0.02^{b*}	3.69 ± 0.05^{b*}	5.65 ± 0.20^{a}	3.60 ± 0.03^{b}	2.50 ± 0.02^{c}
Limonin, mg/g d.w.	0.29 ± 0.04	0.18 ± 0.04	0.28 ± 0.03	1.55 ± 0.09^{a*}	0.94 ± 0.03^{b*}	0.68 ± 0.05^{c*}
Nomilin, mg/g d.w.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Data represent mean ± SE (n = 3)

Different letters among the same variety indicate significant differences by Tukey–Kramer's test ($P < 0.05$)

Asterisks indicate high significance by Tukey–Kramer's test ($P < 0.05$), compared with the other variety in the same month

Polymethoxyflavones represent the sum of sinensetin, nobiletin, and tangeretin

n.d. – not detected

Table 3 Characteristics of vinegar extracts from Nakamoto Seedless and Ogimi Kugani harvested in June

Parameter	Nakamoto Seedless	Ogimi Kugani		
Material/vinegar (w/w)	20%	20%	10%	5%
Vinegar volume, mL	31.5 ± 0.4[c]	31.9 ± 0.2[c]	38.4 ± 0[b]	42.2 ± 0.2[a]
Titratable acidity, %	4.70 ± 0.10[c]	4.82 ± 0[b]	4.87 ± 0.10[b]	4.99 ± 0.10[a]
Polymethoxyflavones, mg/100 mL	33.48 ± 0.13 (25.6)[a]	27.14 ± 0.30 (26.6)[b]	13.01 ± 0.19 (12.7)[c]	7.76 ± 0.02 (7.6)[d]
Sinensetin	2.89 ± 0.04 (38.2)[a]	2.20 ± 0.03 (44.0)[b]	1.10 ± 0.02 (22.1)[c]	0.63 ± 0.01 (12.7)[d]
Nobiletin	22.80 ± 0.10 (40.9)[a]	19.15 ± 0.21 (42.1)[b]	8.63 ± 0.13 (19.0)[c]	5.03 ± 0.01 (11.1)[d]
Tangeretin	7.78 ± 0.02 (23.1)[a]	5.80 ± 0.07 (24.9)[b]	3.27 ± 0.05 (14.0)[c]	2.09 ± 0.01 (9.0)[d]
Limonin, mg/100 mL	0.79 ± 0.04 (45.7)[c]	2.73 ± 0.09 (42.9)[a]	2.55 ± 0.02 (40.1)[a]	1.31 ± 0.05 (20.1)[b]
Shikuwasa aroma	2.75 ± 0.21	3.17 ± 0.20	2.83 ± 0.26	2.58 ± 0.25
Green smell	4.08 ± 0.33	3.75 ± 0.24	3.75 ± 0.36	4.25 ± 0.34
Bitterness	3.67 ± 0.30[a]	2.42 ± 0.33[b]	3.42 ± 0.28[ab]	4.25 ± 0.27[a]
Overall acceptability	3.83 ± 0.20[a]	2.83 ± 0.24[b]	3.50 ± 0.19[ab]	3.67 ± 0.14[a]

Data represent mean ± SE, n = 3 (except sensory evaluation)

Different letters among each sample indicate significant differences by Tukey–Kramer's test ($P < 0.05$)

Polymethoxyflavones represent the sum of sinensetin, nobiletin, and tangeretin

Values in parentheses indicate the recovery ratio of fruit

Table 4 Characteristics of vinegar extracts from Nakamoto Seedless and Ogimi Kugani harvested in July and August

Parameter	July		August	
	Nakamoto Seedless	Ogimi Kugani	Nakamoto Seedless	Ogimi Kugani
Material/vinegar (w/w)	20%			
Vinegar volume, mL	29.8 ± 1.1[b]	36.4 ± 1.1[a]	21.8 ± 0.6[b]	27.5 ± 0.4[a]
Titratable acidity, %	5.04 ± 0.10[a]	4.66 ± 0[b]	5.01 ± 0.10[a]	4.78 ± 0[b]
Polymethoxyflavones, mg/100 mL	19.60 ± 0.51 (31.7)	21.42 ± 1.22 (35.7)	17.95 ± 0.06 (48.9)[a]	15.47 ± 0.12 (55.7)[b]
Sinensetin	1.71 ± 0.05 (43.9)	1.74 ± 0.10 (55.1)	1.46 ± 0.01 (47.8)[a]	1.04 ± 0.01 (52.7)[b]
Nobiletin	13.32 ± 0.34 (48.7)	15.11 ± 0.87 (54.3)	12.12 ± 0.04 (57.5)[a]	10.32 ± 0.08 (60.8)[b]
Tangeretin	4.56 ± 0.11 (30.7)	4.57 ± 0.26 (36.2)	4.37 ± 0.02 (34.9)[a]	4.10 ± 0.04 (46.5)[b]
Limonin, mg/100 mL	0.58 ± 0.01 (70.9)[b]	1.26 ± 0.15 (27.3)[a]	0.92 ± 0.03 (78.8)[b]	2.85 ± 0.02 (98.2)[a]
Shikuwasa aroma	2.54 ± 0.18	3.00 ± 0.27	2.46 ± 0.33	2.85 ± 0.35
Green smell	4.08 ± 0.34	3.69 ± 0.30	4.31 ± 0.27	4.65 ± 0.18
Bitterness	3.92 ± 0.23[a]	3.15 ± 0.27[b]	4.31 ± 0.28	4.31 ± 0.22
Overall acceptability	3.85 ± 0.15	3.54 ± 0.14	3.62 ± 0.28	3.54 ± 0.22

Data represent mean ± SE, n = 3 (except sensory evaluation)

Different letters among each sample from the same harvest date indicate significant differences by Tukey–Kramer's test ($P < 0.05$)

Polymethoxyflavones represent the sum of sinensetin, nobiletin, and tangeretin

Values in parentheses indicate the recovery ratio of fruit

difference in June, but in August it was significantly higher in Nakamoto Seedless compared with Kugani (Table 2). Additionally, polymethoxyflavones in the vinegar extracts from both varieties significantly decreased with time (Seedless: $P < 0.001$, Kugani: $P < 0.001$) (Tables 3, 4). However, the recovery ratio of polymethoxyflavones from material increased with time. Hence, earlier harvested fruit contain higher amounts of polymethoxyflavones, which possess bioactive properties.

Limonin content did not show a decreasing trend in Seedless, but decreased in Kugani with time ($P = 0.217$ for Seedless and $P < 0.001$ for Kugani). Limonin level was significantly higher in the Kugani than in Seedless variety. Nomilin was not detected in both varieties. In general, the Seedless vinegar extract contained lower concentrations of limonin than the extract from Kugani.

Changes in polymethoxyflavone and limonin levels during the stirring process are demonstrated in Figs. 2 and 3.

The amount of polymethoxyflavones in vinegar extracts from Kugani harvested from June to August significantly increased ($P < 0.001$ in June, $P < 0.01$ in July, and $P < 0.001$ in August) during the stirring process (Fig. 2). Limonin levels in the extracts significantly decreased in July but increased in August

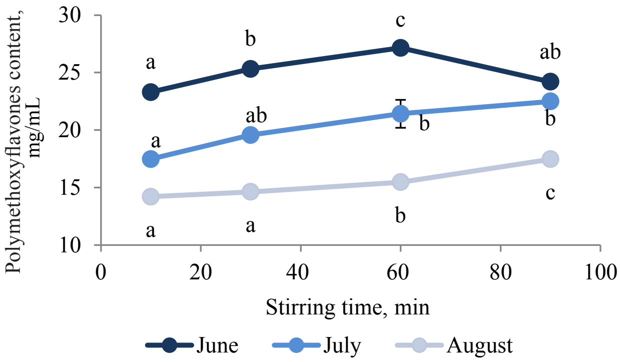

Figure 2 Levels of polymethoxyflavones in Ogimi Kugani vinegar extracts depending on harvest month and stirring time

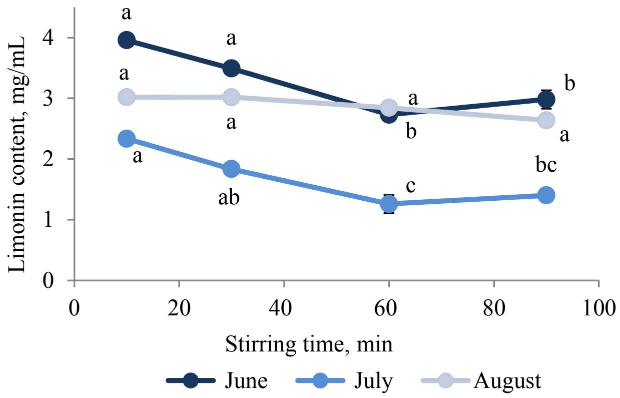

Figure 3 Levels of limonin in Ogimi Kugani vinegar extracts depending on harvest month and stirring time

($P < 0.001$ in June, $P < 0.001$ in July, and $P < 0.05$ in August) during the process (Fig. 2).

Limonin levels in the vinegar extract from Kugani harvested in August were not changed during the stirring time, whereas those in the extract from the fruit harvested in June and July significantly decreased (Fig. 3).

The stirring time of 10–60 min decreased but that of 60–90 min slightly increased the limonin concentration in the vinegar extracts from Kugani harvested in June and July. Thus, 60 min was selected as the optimal stirring time, and this time we used to compare the limonin level in Kugani extracts with that in Seedless.

We also analyzed 5 and 10% vinegar extracts from Kugani harvested in June. The obtained vinegar volumes and titratable acidity significantly increased with the decreasing fruit concentration. As for polymethoxyflavones and limonin, their levels depended directly on the fruit amount (Table 3).

Comparison of the vinegar extracts from Seedless and Kugani are shown in Tables 3 and 4. There was no significant difference in vinegar volume in 20% extracts from Seedless and Kugani harvested in June. However, the 5 and 10% Kugani extracts demonstrated significantly higher vinegar volume than the Seedless extracts in all the months.

The titratable acidity of the 5% Kugani vinegar extract was the highest in June, whereas that of the Seedless extract was the lowest (Table 3). In July and August, the Seedless extract showed higher titratable acidity compared to Kugani (Table 4). Total polymethoxyflavone levels in the vinegar extract from Seedless in June were the highest, while the 5% Kugani extract contained the lowest amount of polymethoxyflavones.

Furthermore, there was a similar tendency for each polymethoxyflavone, namely sinensetin, nobiletin, and tangeretin. In July, there was no significant difference in each polymethoxyflavone between the Seedless and Kugani vinegar extracts. In August, the Seedless vinegar extract demonstrated higher level of these polymethoxyflavones than the Kugani extract.

Sensory evaluation showed no significant difference in aroma and green smell among all the samples. The 20% extract from Seedless fruit had significantly higher bitterness than the 20% Kugani sample in July, but there was no significant difference in August (Table 4). As for June, there was no significant difference between the 20% Seedless extract and the 5 and 10% Kugani samples (Table 3).

Overall acceptability of the Seedless vinegar extracts was significantly higher than that of the 20% Kugani samples, but there was no significant difference between the 5 and 10% Kugani extracts in June. Overall acceptability of the Seedless and Kugani vinegar extracts in July and August showed no significant differences.

As mentioned above, limonin levels in the Kugani vinegar extract decreased during the stirring process in June and July but did not change in August (Fig. 3). This implies that limonin was protected from volatilizing or other enzymatic reactions during the stirring process. This can also explain the increasing level of some components in Kugani harvested from July to August. In addition, it may also be associated with the fact that the vinegar extract from Kugani harvested in August was not bitter despite a high content of limonin, which is a bitter compound present in the vinegar extract.

Hirose *et al*. reported that vinegar extract from waste peel of shikuwasa harvested in October and obtained by the direct pulverizing method tasted bitter and contained high levels of limonin [7]. Dea *et al*. found that increasing levels of sucrose or citric acid decreased the perception of bitterness induced by limonin in orange juice [12].

In our study, sucrose or citric acid contained in Kugani fruit juice could have masked the bitter taste of limonin in the vinegar extract from Kugani harvested in August. Therefore, the whole Kugani fruit harvested after August can be used as a raw material for vinegar extracts production by the stirring method. Not all the vinegar extracts made from Seedless fruit tasted bitter as expected. However, the 20% Kugani extract was bitter, especially in June. Therefore, we developed a method to reduce bitterness of Kugani harvested in June.

There are other methods to diminish bitterness. Hirose *et al*. reported that limonin was not detected in vinegar extract from only Kugani peel [7]. In other words, segment membranes and seeds contain limonin but removing them is laborious and time-consuming. In the present study, the 5 and 10% Kugani extracts did not taste bitter and there was no significant difference in bitterness among them and the Seedless vinegar extract in June. Moreover, there were no significant differences in shikuwasa aroma and green smell. Thus, the flavor of the Kugani (5 and 10%) and Seedless vinegar extracts was considered to be the same.

Incidentally, some locals in Okinawa prefer the bitter taste of shikuwasa products, such as juice and jam. Demand for bitter-tasting vinegar extract can be met by using 20% Kugani fruit harvested in June. Seedless is hardly cultivated commercially in Okinawa. To produce vinegar extracts with lower bitterness, it is possible to use only Kugani, as showed in this study. It is also desirable to effectively use thinned or bruised Seedless and Kugani, which are not suitable for sale.

CONCLUSION

We produced vinegar extracts from the whole unripe fruit of Ogimi Kugani and Nakamoto Seedless harvested from June to August. The stirring method was applied to obtain the extracts. The 20% extract from Kugani harvested in June and July was bitter compared to the Seedless extract. However, the 5 and 10% Kugani samples did not differ in bitterness from the 20% Seedless extract in June

In addition, the 20% extracts from Kugani and Seedless fruit harvested in August showed similar bitterness, in spite of the fact that limonin levels in the Kugani extract were higher than those in the Seedless sample. Both vinegar extracts from Kugani and Seedless contained polymethoxyflavones, which decreased from June to August. The flavor of both vinegar extracts was similar.

The extraction technique applied in this study is easy to use and requires simple equipment, which minimize hygienic problems. Producing vinegar extracts from shikuwasa would allow creating a broad range of products such as Ponzu soy sauce, salad dressings, and fruit vinegar drinks. Moreover, vinegar extracts from shikuwasa can be applied to any type of citrus fruit worldwide.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

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