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Antioxidant Activity and Lipase and Alpha-glucosidase Inhibitory Activities of Yuzu Juice (*Citrus junos Tanaka*)

Atsumi SHIMADA

ユズ果汁の抗酸化活性およびリパーゼ、α-グルコシダーゼ阻害活性

島　田　淳　巳

Abstract

Yuzu (*Citrus junos* Tanaka) juices prepared from green and yellow ripened fruits were evaluated for their antioxidant activities and inhibitory activities against lipase and alpha-glucosidase. Green yuzu juice was found to have significantly antioxidant effect and the inhibitory activities against lipase and alpha-glucosidase. But yellow ripened yuzu juice showed lesser inhibitory activities than those of green yuzu juice. This study suggested that green yuzu juice might play an important role in the prevention of lifestyle related diseases and could be used as a potential nutraceutical.

***Key words*:** Yuzu, *Citrus junos*, Antioxidant, Lipase, Alpha-glucosidase

Introduction

Citrus fruits are rich sources of various health-promoting substances and have been used for the treatment of lifestyle related diseases such as diabetes mellitus, dyslipidemia, and hypertension. Their effectiveness has been attributed to the presence of biologically active flavonoids (Kawaii *et al*., 1999). Flavonoids in citrus fruits and juices show antiallergy, anti-inflammatory, analgesic, antioxidant, anticarcinogenic, antitumor, antibacterial, antiviral, and hypolipidemic activities (Kim *et al*., 2001; Nakagawa *et al*., 2006). From a viewpoint of health promotion by dietary habits, these activities in the fruits and juices are so important to prevent lifestyle related diseases. (Kawaii *et al*., 1999).

Yuzu (*Citrus junos* Tanaka) fruits are used as one kind of yellow sour orange similar to lemon fruits. The average annual production of the fruit is about 25000 metric tons in Japan. Yuzu fruits have pleasant and fresh odors and have been used as raw materials for vinegar, seasonings, jams and juices in Japan. Yuzu contains a number of nutrients such as citric acid, ascorbic acid, minerals, and flavonoids, and it has been known as a healthy food (Tajima *et al*., 1990; Kawaii *et al*., 2000; Kumazawa *et al*., 2007).

Natural antioxidants contained in dietary plants may play an important role in the prevention of carcinogenesis and in extending the life span of animals and may offer effective protection against peroxidative damage in living systems (Miyake *et al*., 1998). Superoxide dismutase (SOD), free radical scavenging enzyme, is one of the first line of cellular defence against oxidative injury. Obesity is a strong risk factor for various diseases, such as hypertension, hyperlipidemia, arteriosclerosis, and diabetes. An effective way to prevent obesity is to inhibit fat absorption. Inhibitors which limit the intestinal absorption of dietary fat could be used as medication for treatment of hyperlipidaemia. Pancreatic lipase is the key enzyme for dietary fat absorption, and an inhibitor for pancreatic lipase can be used for the alteration of fat absorption (Kawaguchi *et al*., 1997). Alpha-glucosidase inhibitors could retard the use of dietary carbohydrates to suppress postprandial hyperglycemia, since alpha-glucosidase is key enzyme that catalyzes the final step in the digestive process of carbohydrates (Kim *et al*., 2000). It is important to search for those effects in daily food such as vegetables, fruit, seeds, and spices from the viewpoint of utility and safety. The occurrence of health-promoting components in some citrus juices has already been reported. However, up to now, there are no comparative studies about health-promoting activities of citrus juices. Hence, comparative studies of citrus juices under the same conditions and assays could be considered an effective approach to provide important comparable information on dietary agents. In this study, we investigated the biological activities of green and yellow ripened yuzu juices, and showed green yuzu juice as a potential nutraceutical.

Material and Methods

Materials Green yuzu fruits were collected at Oita prefecture in Japan in October. The fruits were frozen in a freezer and stored at -50 ℃ until further analysis. Each fruit sample (100 g fresh weight) was washed and cut into 10 mm lengths. The sample was homogenized in distilled water with an Iwatani mixer grinder (Osaka, Japan) at the medium-speed setting for 30 s, and the resulting homogenate was filtered through four layers of cheesecloth. The volume of the filtrate was made up to 100 ml with distilled water, and the aqueous solution was centrifuged (4 ℃, 12,000 X g, 30 min) with a Hitachi (Tokyo, Japan) himac CT 6D high speed refrigerated centrifuge. The clear supernatant was used for the measurement of the biological activity. The same procedure was carried out for yellow ripened yuzu fruits collected at Oita prefecture in Japan in December.

Chemicals Acarbose, caffeic acid, (-)-epigallocatechin gallate (EGCG), Folin-Ciocalteu's phenol reagent, *p*-nitrophenyl-alpha-D-glucopyranoside (NPG), superoxide dismutase kit SOD Test Wako, tetrahydrofurane were purchased from Wako Pure Chemical Industries, Ltd, Osaka, Japan. Alpha-glucosidase from *Saccharomyces cerevisiae,* 4-methylumbelliferyl (4-MU) oleate and porcine pancreas lipase were purchased from Sigma-Aldrich Co., St. Louis, USA.

Total soluble phenol content of yuzu juices Total soluble phenol analysis was carried out according to the method of Camacho-Cristobal *et al* (2002). The water extracts (10 mg) was

dissolved in distilled water and the volume of the sample solution was made up to 50 mL with distilled water. The sample solution (0.5 mL) was mixed with 2.5 mL of 10% Folin-Ciocalteu's phenol reagent. After 3 min, 2.5 mL of 10% Na_2CO_3 was added. After the mixture was incubated for 1h at room temperature, the absorbance was measured at 765 nm with a Hitachi (Tokyo, Japan) U-1800 spectrophotometer using caffeic acid as the standard.

Superoxide radical scavenging assay The superoxide radical scavenging activity was determined by using a superoxide dismutase kit SOD Test Wako (Yamaguchi *et al*., 2006). Briefly, superoxide radicals were generated by the xanthine/xanthine oxidase system, and reduced nitro blue tetrazolium to water-soluble diformazan which exhibited an absorption maximum at 560 nm. The superoxide scavenging activity of the sample is determined by measuring the inhibition rate of diformazan production. The sample solution (0.15 mL) was mixed with 1.5 mL of color-producing solution and 1.5 mL of enzyme solution. The reaction mixture was incubated at 37 ℃ for 20 min, and the absorbance was measured at 560 nm with a Hitachi (Tokyo, Japan) U-1800 spectrophotometer. The superoxide radical scavenging activity was calculated according to the following equation: the scavenging activity (%) = [(Control Abs $-$ Control $_{blank}$ Abs) $-$ (Sample Abs $-$ Sample $_{blank}$ Abs)]/ (Control Abs $-$ Control $_{blank}$ Abs) X 100, where control is the activity of the enzyme with distilled water instead of sample solution and blank is the activity without the enzyme. EGCG was used as positive control.

Lipase inhibitory assay The assay of lipase inhibitory activity was done according to the method of Kawaguchi *et al* (1997). The sample solution (0.02 mL) mixed with 0.1 ml of lipase (13 mg) in McIlvaine buffer (pH 6.8; 1 L) and 0.2 mL of 0.1 mM 4-MU oleate in the same buffer contained 5% tetrahydrofurane. After incubation at 37 ℃ for 20 min, 1mL of 0.1 M HCl and 2 mL of 0.1 M sodium citrate were added. The amount of 4-methylumbelliferone released by the lipase was fluorometrically measured at an emission wavelength of 450 nm and an excitation of 360 nm with a Hitachi (Tokyo, Japan) F-2500 fluorescence spectrophotometer. The percentage inhibition of the lipase activity was calculated according to the following equation: the inhibitory activity (%) = [(Control Abs $-$ Control $_{blank}$ Abs) $-$ (Sample Abs $-$ Sample $_{blank}$ Abs)]/ (Control Abs $-$ Control $_{blank}$ Abs) X 100, where control is the activity of the enzyme with distilled water instead of sample solution and blank is the activity without the enzyme. EGCG was used as positive control.

Alpha-glucosidase inhibitory assay The assay of alpha-glucosidase inhibitory activity was done according to the modified method of Yamaki *et al*. (2006). The sample solution (0.02 ml) was mixed with 0.02 ml of 750 U/ml alpha-glucosidase in 0.05 M potassium phosphate buffer (pH 6.7) and 0.02 ml of NPG (9 mg) in the same buffer (10 ml). After the mixture was incubated at 37 ℃ for 1 h, the reaction was stopped by addition of 5ml of 0.36 M Na_2CO_3 and the absorbance was measured at 405 nm with a Hitachi (Tokyo, Japan) U-1800 spectrophotometer. The percentage inhibition of the alpha-glucosidase activity was calculated according to the following equation: the inhibitory activity (%) = [(Control Abs $-$ Control $_{blank}$ Abs) $-$ (Sample Abs $-$ Sample $_{blank}$ Abs)]/ (Control Abs $-$ Control $_{blank}$ Abs) X 100, where control is the activity

of the enzyme with distilled water instead of sample solution and blank is the activity without the enzyme. Acarbose was used as positive control.

Results

Total soluble phenol of yuzu juices Two juices prepared from green and yellow yuzu fruits were analyzed for total soluble phenol (Fig. 1). Average weights of total soluble phenol content in green and yellow yuzu fruits, respectively, were 245mg/100 g, and 159 mg/100 g. Green yuzu juice contained higher total soluble phenol content than yellow yuzu juice.

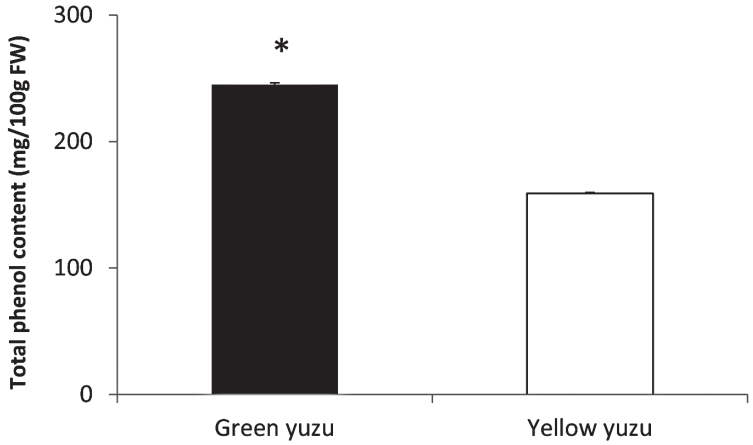

Fig. 1. Total soluble phenol of two juices from green and yellow ripened yuzu fruits. Bars indicate standard error of the mean (n=3). Asterisk indicates statistical probability, $p<0.05$.

Antioxidant activity of yuzu juices The superoxide radical scavenging activities of two juices were shown in Fig. 2-A. Two juices prepared from green and yellow yuzu fruits, respectively, showed superoxide radical scavenging activities by 87%, and 68% of the control value. The superoxide radical scavenging activities of green yuzu juice showed higher than that of yellow ripened yuzu juice, and the activities of two juices showed higher than that of 0.1 mM EGCG used as a positive control.

Lipase inhibitory activity of yuzu juices Citrus flavonoids and tea catechins such as hesperidin, (-)-epigallocatechin gallate, (-)-epigallocatechin-3,5-digallate, and oolonghomobisflavan A have been reported as lipase inhibitors (Kawaguchi et *al*., 1997; Nakai *et al*., 2005). Lipase inhibitory activities of yuzu juices were shown in Fig. 2-B. Two juices prepared from green and yellow yuzu fruits, respectively, showed lipase inhibitory activities by 76%, and 31% of the control value. The lipase inhibitory activities of green yuzu juice showed higher than that of yellow ripened yuzu juice, and the activity of green yuzu juice showed higher than that of 0.1 mM EGCG used as a positive control (Kusano *et al*., 2008).

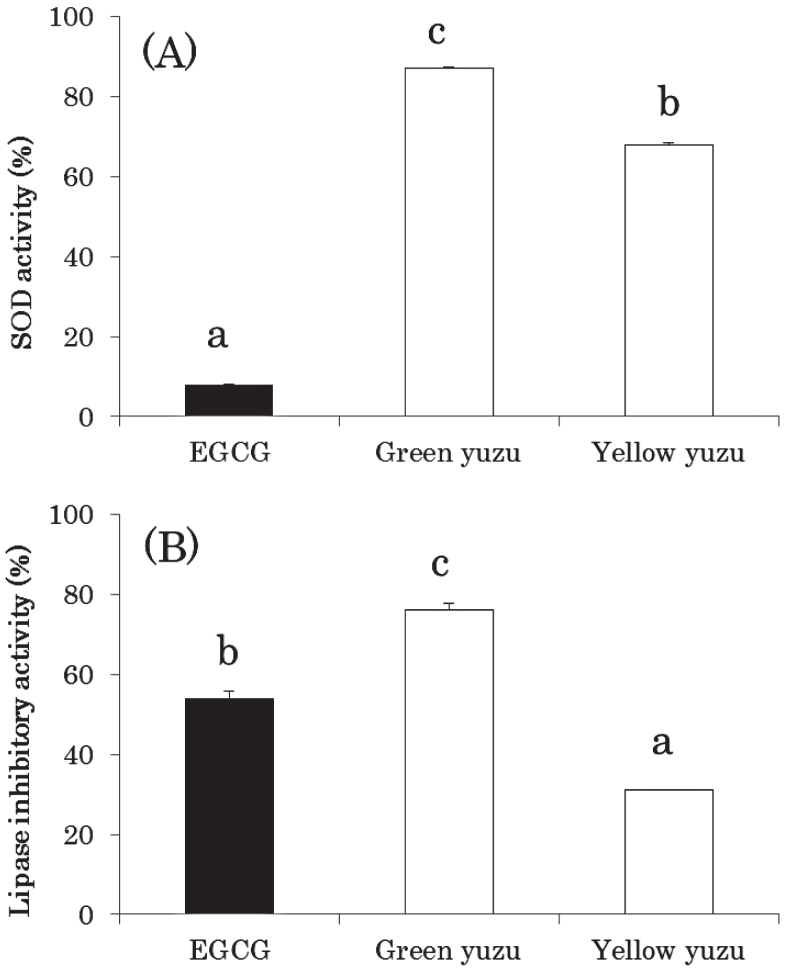

Fig. 2. Effects of two juices from green and yellow ripened yuzu fruits on superoxide radical scavenging activity (A) and lipase inhibitory activity (B). Bars indicate standard error of the mean (n=3). Means with same letter are not significantly different ($P<0.05$, Duncan's multiple-range test).

Alpha-glucosidase inhibitory activity of yuzu juices Alpha-glucosidase inhibitors such as acarbose and miglitol are known to interfere with the carbohydrate-digesting enzymes and delaying glucose absorption. And flavonoids and catechins from citrus and tea have been reported as alpha-glucosidase inhibitors (Watanabe *et al*., 1997; Kim *et al*., 2000). Alpha-glucosidase inhibitory activities of yuzu juices were shown in Fig. 3. Two juices prepared from green and yellow yuzu fruits, respectively, showed alpha-glucosidase inhibitory activities by 40%, and 21% of the control value. The alpha-glucosidase inhibitory activity of green yuzu juice showed higher than that of yellow ripened yuzu juice, but those activities of green and yellow yuzu juices showed lower than that of 0.1 mM acarbose , a potent alpha-glucosidase inhibitor.

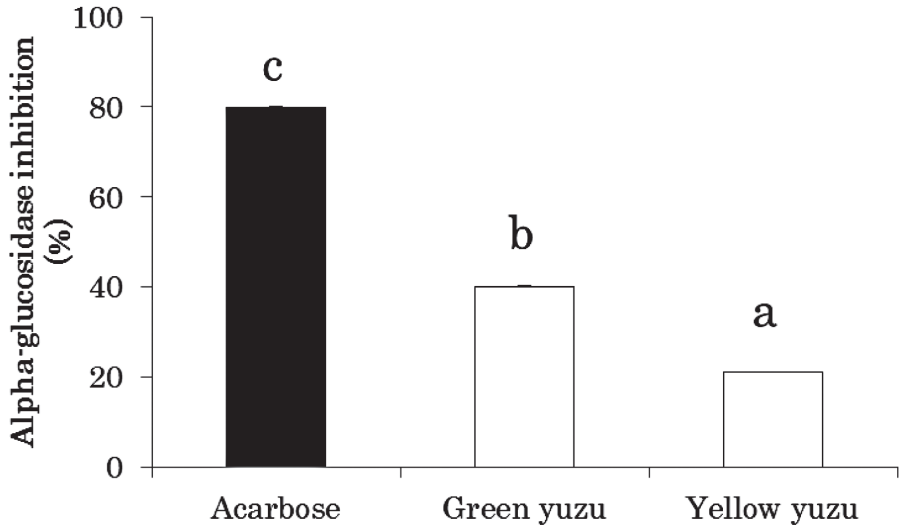

Fig. 3. Alpha-glucosidase inhibitory activities of two juices from green and yellow ripened yuzu fruits. Bars indicate standard error of the mean (n=3). Means with same letter are not significantly different ($P<0.05$, Duncan's multiple-range test).

Discussion

Two juices from green and yellow ripened yuzu fruits were evaluated for their antioxidant effects and the inhibitory activities against lipase and alpha-glucosidase. Green yuzu juice exhibited to be a potent effective juice, since green yuzu juice was found to have significantly antioxidant effects and the inhibitory activities against lipase and alpha-glucosidase. Green yuzu juice contained higher total soluble phenol content than yellow one, and the correlation between those tested biological activities and total soluble phenol content was found in two tested juices. Green yuzu juice contained bitter constituents (hesperidin, naringin, limonin, and nomilin), but yellow yuzu juice scarcely contained hesperidin (Tahata *et al*., 2002) which showed antioxidant activity (Yeh *et al*., 2013), lipase (Kawaguchi *et al*., 1997) and alpha-glucosidase inhibitory activities (Zhang *at al*., 2012).

This study suggested that green yuzu juice might play an important role in the prevention of lifestyle related diseases and could be used as a potential nutraceutical because of its beneficial effects on antioxidant activity and lipase and alpha-glucosidase inhibitory activities. The seasonal variation and the lot-to-lot variation of the soluble phenol content and the tested biological activities of yuzu juice will be examined in near future.

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References

Camacho-Cristobal, J.J., Anzellotti, D., and Gonzalez-Fontes, A. (2002). Changes in phenolic metabolism of tobacco plants during short-term boron deficiency. *Plant Physiol Biochem* **40**, 997-1002.

Kawaguchi, K., Mizuno, T., Aida, K., and Uchino, K. (1997), Hesperidin as an inhibitor of lipases from porcine pancreas and *Pseudomonas*. *Biosci. Biotechnol. Biochem.*, **61**, 102-104.

Kawaii, S., Tomono, Y., Katase, E., Ogawa, K., and Yano, M. (1999), HL-60 differentiating activity and flavonoid content of the readily extractable fraction prepared from *Citrus* juices. *J. Agric. Food Chem.*, **47**, 128-135.

Kawaii, S., Tomono, Y., Katase, E., Ogawa, K., Yano, M., Koizumi, M., Ito, C., and Furukawa, H. (2000), Quantitative study of flavonoids in leaves of *Citrus* plants. *J. Agric. Food Chem.*, **48**, 3865-3871.

Kim, H. K., Jeon, W. K., and Ko, B. S. (2001), Flavanone glycosides from *Citrus junos* and their anti-influenza virus activity. *Planta Med.*, **67**, 548-549.

Kim, J., Kwon, C., and Son, K. H. (2000), Inhibition of alpha-glucosidase and amylase by luteolin, a flavonoid. *Biosci. Biotechnol. Biochem.*, **64**, 2458-2461.

Kumazawa, S., Ikenaga, M., Usui, Y., Kajiya, K., Miwa, S., Endo, J., Chikasawa, C., Suzuki, Y., Suzuki, Y., Shimoi, K., and Nakayama, T. (2007), Comprehensive analysis of polyphenols in fruits consumed in Japan. *Food Sci. Technol. Res.*, **13**, 404-413.

Kusano, R., Andou, H., Fujieda, M., Tanaka, T., Matsuo, Y., and Kouno, I. (2008), Polymer-like polyphenols of black tea and their lipase and amylase inhibitory activities. *Chem. Pharm. Bull.*, **56**, 266-272.

Miyake, Y., Yamamoto, K., Morimitsu, Y., and Osawa, T. (1998), Characteristics of antioxidative flavonoid glycosides in lemon fruit. *Food Sci. Technol. Int. Tokyo*, **4**, 48-53.

Nakagawa, H., Takaishi, Y., Tanaka, N., Tsuchiya, K., Shibata, H., and Higuti, T. (2006), Chemical constituents from the peels of *Citrus sudachi*. *J. Nat. Prod.*, **69**, 1177-1179.

Nakai, M., Fukui, Y., Asami, S., Toyoda-Ono, Y., Iwashita, T., Shiabata, H., Mitsunaga, T., Hashimoto, F., and Kiso, Y. (2005), Inhibitory effects of oolong tea polyphenols on pancreatic lipase *in vitro*. *J. Agric. Food Chem.*, **53**, 4593-4598.

Tahata, K., Inoue, Y., and Nakagawa, K. (2002), The ingredient character of the fraction of yuzu juice and the factor which influence to the ingredient character of yuzu juice. (in Japanese) *Kinki Chugoku Shikoku Nogyo Kenkyu*, **1**, 81-85.

Tajima, K., Tanaka, S., Yamaguchi, T., and Fujita, M. (1990), Analysis of green and yellow yuzu peel oils (*Citrus junos* Tanaka). Novel aldehyde components with remarkably low odor thresholds. *J. Agric. Food Chem.*, **38**, 1544-1548.

Watanabe, J., Kawabata, J., Kurihara, H., and Niki, R. (1997), Isolation and identification of alpha-glucosidase inhibitors from Tochu-cha (*Eucommia ulmoides*). *Biosci. Biotechnol. Biochem.*, **61**,177-178.

Yamaki, K., Mori, Y. (2006), Evaluation of alpha-glucosidase inhibitory activity in colored foods: A trial using slope factors of regression curves. (in Japanese) *Nippon Shokuhin Kagaku Kogaku Kaishi*, **53**, 229-231.

Yamaguchi, S., Sugahara, T., Nakashima, Y., Okada, A., Akiyama, K., Kishida, T., Maruyama, M., and Masuda, T. (2006), Radical and superoxide scavenging activities of matairesinol and oxidized matairesinol. *Biosci. Biotechnol. Biochem.*, **70**, 1934-1940.

Yeh, Y., Hsieh, Y., and Lee, Y. (2013), Effects of yam peel extract against carbon tetrachloride-induced hepatotoxicity in rats. *J. Agric. Food Chem.*, **61**, 7387-7396

Zhang, B., Chen, T., Chen, Z., Wang, M., Zheng, D., Wu, J., Jiang, X., and Li, X. (2012), Synthesis and anti-hyperglycemic activity of hesperidin derivatives. *Bioorg. Med. Chem. Lett.*, **22**, 7194-7197.

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