カボス果汁のアンジオテンシンⅠ変換酵素およびリパーゼ阻害活性

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Angiotensin I-Converting Enzyme and Lipase Inhibitory Activities of Kabosu Juice（*Citrus sphaerocarpa* Hort. ex Tanaka）

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（2015年11月27日受理）

Abstract

Two juices prepared from green and yellow fruits of kabosu were evaluated for their inhibitory activities against angiotensin I-converting enzyme (ACE) and lipase. Green kabosu juice was found to have significantly inhibitory activities against ACE and lipase. But yellow kabosu juice showed lesser inhibitory activities than those of green kabosu juice. This is the first report that describes the lipase inhibitory activity of green kabosu juice. Furthermore, this study suggested that green kabosu juice might play an important role in the prevention of lifestyle related diseases and could be used as a potential nutraceutical.

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, antibacterial, and antiviral activities (Kim *et al*., 2001; Nakagawa *et al*., 2006a). From a viewpoint of health promotion by dietary habits, these activities in citrus fruits and juices are so important to prevent lifestyle related diseases. However, few studies have focused on the biological activity of sour orange such as kabosu (Kawaii *et al*., 1999).

Kabosu (*Citrus sphaerocarpa* Hort. ex Tanaka) fruit has pleasant and fresh odors and has been used as raw materials for vinegar, seasonings, jams and juices in Japan. Annual production of this fruit is about 5200 metric tons in Japan (Yamauchi *et al*., 1991; Nogata *et al*., 2006). This fruit contains a number of nutrients such as citric acid, ascorbic acid, minerals, and flavonoids, and this fruit has been known as healthy food. However, there is little information on the health promoting activity of kabosu fruit except for the antioxidant activity and the inhibitory activities of tyrosinase and blood cholesterol elevation (Matsuura *et al*., 2006; Miyake *et al*., 2007; Ogawa and Mochizuki, 2003).

Angiotensin I-converting enzyme (ACE) inhibitor could be valuable for hypertension therapy. ACE is so important to regulate blood pressure, and it catalyzes the conversion of the inactive angiotensin I to a potent vasoconstrictor, angiotensin II. (Matsui *et al*., 1992; Miyake *et al*., 1998) 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 hyperlipidemia. 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).

The occurrence of health-promoting components in

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some citrus juices had already been reported. However, up to now, there are few 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 information on dietary agents. In this study, we investigated ACE and lipase inhibitory activities of green and yellow kabosu juices, and showed green kabosu juice as a potential nutraceutical.

Materials and Methods

Materials

Two kinds of kabosu fruit were investigated in this study, since surface color of kabosu fruits gradually turned from green to yellow with fruit development. Green kabosu fruits were collected at Oita Prefecture in Japan in September. Each fruit sample (100 g fresh weight) was washed and cut a width of 10 mm after dividing a fruit into eight equal parts. 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 rpm, 30 min) with a Hitachi himac CT 6D high speed refrigerated centrifuge (Tokyo, Japan). The clear supernatant was stored at -50 ℃ until the measurement of total soluble phenol content and the ACE and lipase inhibitory activities. The same procedure was carried out for yellow kabosu fruits collected at Oita Prefecture in Japan in December.

Chemicals

Caffeic acid, captopril, dimethylsulfoxide, (-)-epigallocatechin gallate (EGCG), Folin-Ciocalteu' s phenol reagent, hippuryl-L-histidyl-L-leucine (Hip-His-Leu), tetrahydrofuran, and 2,4,6-trinitrobenzene sulfonate (TNBS) were purchased from Wako Pure Chemical Industries, Ltd, Osaka, Japan. 4-Methylumbelliferyl (4-MU) oleate, rabbit lung angiotensin converting enzyme (ACE), and porcine pancreas lipase were purchased from Sigma-Aldrich Co., St. Louis, USA. Chemicals used as positive control were dissolved in 5% dimethylsulfoxide at a concentration of 0.1 mM.

Total soluble phenol content in kabosu juices

Total soluble phenol analysis was carried out according to the method of Camacho-Cristobal *et al.* (2002). 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 U-1800 spectrophotometer (Tokyo, Japan) using caffeic acid as the standard.

Inhibition of ACE by kabosu juices

The assay of ACE inhibitory activity was done according to the method of Matsui *et al.* (1992). A mixture containing 0.03 mL of sample solution in 5% dimethylsulfoxide, 0.1 mL of 0.06 U/mL ACE, and 0.25 mL of 7.6 mM Hip-His-Leu in borate buffer (pH 8.3) containing 200 mM NaCl was incubated at 37 ℃ for 30 min. The reaction was stopped by addition of 0.25 mL of 0.5 M HCl, and the stopped solution was then adjusted to pH 9.12 by adding 0.2 mL of Kolthoff buffer [0.1 M Na_2HPO_4 – 1.0 M NaOH (1 : 2)], and followed by 0.025 mL of 0.1 M TNBS in 0.1 M Na_2HPO_4. After the mixture was incubated at 37℃ for 20 min, 4.5 mL of 4 mM Na_2SO_3 in 0.2 M NaH_2PO_4 was added to the mixture and the absorbance was measured at 416 nm with a Hitachi U-1800 spectrophotometer (Tokyo, Japan). The percentage inhibition of ACE 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 abs is the absorbance of the reaction mixture used distilled water instead of sample solution and blank abs is the absorbance of the reaction mixture used buffer instead of enzyme solution. Captopril was used as a positive control (Nakagawa *et al.*, 2006b).

Inhibition of lipase by kabosu juices

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), 0.2 mL of 0.1 mM 4-MU oleate in the same buffer contained 5% tetrahydrofuran. 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 F-2500 fluorescence spectrophotometer (Tokyo, Japan). 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. EGCG was used as a positive control. (Kusano *et al*., 2008)

Statistical analysis

The data were expressed as the means ± S.E. (n = 3). Duncan's multiple-range test was used to assess the statistical significance ($p < 0.05$).

Results

Total soluble phenol content in kabosu juices

Two juices prepared from green and yellow kabosu fruits were analyzed for total soluble phenol content (Fig. 1). Total soluble phenol content in green and yellow kabosu fruits were 151 mg/100 g and 153 mg/100 g, respectively. Green and yellow kabosu juices contained similar total soluble phenol content.

Inhibitory activities of kabosu juices for ACE

ACE inhibitory activities of kabosu juices were shown in Fig. 2. Green and yellow kabosu juices inhibited ACE to 72% and 60% of control value, respectively. The ACE inhibitory activity of green kabosu juice was higher than that of yellow kabosu juice, but was lower than that of 0.1 mM captopril, a potent ACE inhibitor (Nakagawa *et al*., 2006b).

Inhibitory activities of kabosu juices for lipase

Lipase inhibitory activities of kabosu juices were shown in Fig. 2. Green and yellow kabosu juices inhibited lipase to 77% and 11% of control value, respectively. The lipase inhibitory activity of green kabosu juice was higher than that of yellow kabosu juice, and also was higher than that of 0.1 mM EGCG, a positive control (Kusano *et al*., 2008).

Discussion

Two juices from green and yellow ripened kabosu fruits were evaluated for their inhibitory activities against ACE and lipase. Green kabosu juice exhibited to be a potent effective juice, since green kabosu juice was found to have significantly inhibitory activities against ACE and lipase. Green kabosu juice mentioned above showed higher activities than those of yellow kabosu juice, although green kabosu juice contained similar content of total soluble phenol to yellow one. Kabosu juice contains bitter constituents such as hesperidin, narirutin, naringin, neohesperidin, and rutin (Nogata *et al*., 2006), but we have no information about the

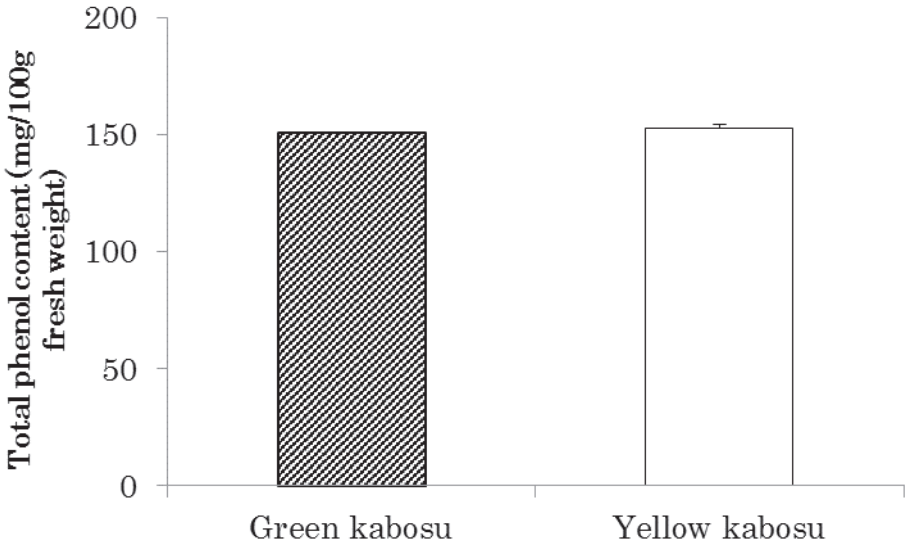

Fig. 1. Total soluble phenol content in green and yellow kabosu juices. Bars indicate the mean ± S. E. (n=3).

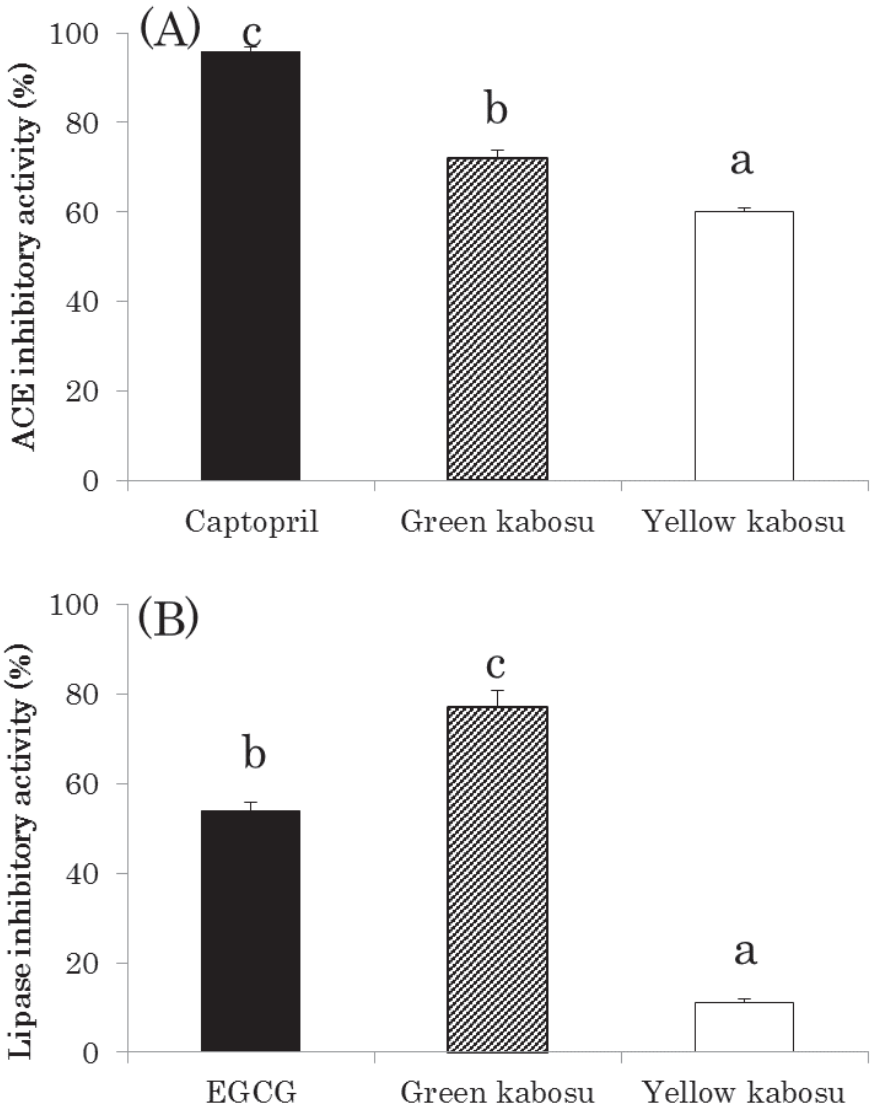

Fig. 2. Effects of green and yellow kabosu juices on ACE inhibitory activity (A) and lipase inhibitory activity (B). Bars indicate the mean ± S. E. (*n*=3). Means with the same letter (a to c) were not significantly different ($p < 0.05$)

differences in the flavonoid composition between green and yellow kabosu juices. Flavanones such as naringenin, hesperetin, naringin and hesperidin did not show ACE inhibitory activity, but flavonols such as quercetin and rutin showed ACE inhibitory activities (Guerrero *et al*., 2012). Hesperidin and neohesperidin showed lipase inhibitory activities, but narirutin and naringin did not show lipase inhibitory activity (Kawaguchi *et al*., 1997). Quercetin and rutin showed lipase inhibitory activities (Habtemariam, 2013). From those results in the references, the differences in ACE and lipase inhibitory activities between green and yellow kabosu juices could be attributed to the flavonoid concentration such as hesperidin, neohesperidin, and rutin. This study suggests that green kabosu juice may play an important role in the prevention of lifestyle related diseases and can be used as a potential nutraceutical because of its beneficial effects on ACE and lipase inhibitory activities. The flavonoid composition in green and yellow kabosu juices and the lot-to-lot variation of the tested biological activities of kabosu juice and the active compounds in kabosu juice will be examined in near future.

Acknowledgements

Kabosu fruits were kindly provided by Mr. Shuji Abe (Yuzu-koshou Honpo Co., Oita, Japan).

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【要約】

緑カボス（未熟果）と黄カボス（成熟果）から得られた果汁を用いて、アンジオテンシンⅠ変換酵素（ACE）とリパーゼ阻害活性について検討した。緑カボスの果汁は強い ACE とリパーゼゼ阻害活性を示すが、黄カボスは緑カボスに比べて強い阻害活性を示さなかった。緑カボス果汁のリパーゼ阻害活性は、今回が初めての報告であり、緑カボス果汁は生活習慣病予防が期待できる食品である。