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Improving the antioxidant functionality of *Citrus junos* Tanaka (yuzu) fruit juice by underwater shockwave pretreatment

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ABSTRACT

Citrus junos Tanaka (yuzu) has a strong characteristic aroma, and hence, yuzu juice is used in a number of Japanese foods. We herein evaluated the functional compounds of yuzu juice to investigate whether underwater shockwave pretreatment affects its functionality. Employing the shockwave pretreatment at an increased discharge and energy of 3.5 kV and 4.9 kJ, respectively, resulted in an increase in the flavanone glycoside content and oxygen radical absorbance capacity (ORAC). The ORAC value of yuzu juice cultivated in Rikuzentakata increased approximately 1.7 times upon underwater shockwave pretreatment. The treatment method proposed herein exhibited reliable and good performance for the extraction of functional and antioxidant chemicals in yuzu fruits, and was comparable with traditional squeezing methods. The high applicability and reliability of this technique for improving the antioxidant functionality of yuzu fruit juice was demonstrated, confirming the potential for application to a wide range of food extraction processes.

1. Introduction

Citrus fruits are important commercial crops that are widely cultivated in the regions between the tropical and temperate zones. *Citrus junos* Tanaka (yuzu), a sour fruit, is cultivated mainly in Japan and Korea. In Japan, the annual production amounted to approximately 25,000 tons in 2009. Yuzu produced in Rikuzentakata (Iwate prefecture) is known as "Northern Limit Yuzu" (NLY) and has a pleasant aroma. As NLY is produced in small quantities, it tends to be expensive. In addition, Rikuzentakata sustained great damage during the Great Tohoku Earthquake and Tsunami in 2011, and so NLY is currently being promoted as part of the reconstruction plan.

Yuzu is industrially used in the production of sweets, beverages, cosmetics, perfumery, and aromatherapy (Sawamura, 2010). Almost all parts of the yuzu fruit are employed, including its peel, juice, and seeds. Compared with other citrus fruits, yuzu has a strong characteristic aroma, and is well known for the pleasant fragrance of its outer rind. Citrus limonoids, such as limonin and nomilin, are responsible for the bitter taste of citrus fruits, and are characterized by their substituted furan moiety. Yuzu juice is therefore commonly used in Japanese cooking. Important bioactive components present in yuzu fruits include vitamin C, β-carotene, flavonoids, limonoids, and dietary fiber. The predominant citrus fruit-derived flavonoids are glycosides, which also function as antioxidants (Yoo, Lee, Park, Lee, & Hwang, 2004; Zou, Xi, Hu, Nie, & Zhou, 2016). Many biochemical studies have indicated that antioxidants inhibit or delay the oxidation of other molecules by interrupting the initiation or propagation of the oxidizing chain reaction (Valko et al., 2007). In addition, their metabolites possess significant protective biological activities, including anticancer, antiviral, and anti-inflammatory properties (Da Silva Emim, Oliveira, & Lapa, 1994; Manthey, Guthrie, & Grohmann, 2001). In particular, antihypertensive, diuretic, analgesic, and hypolipidemic activities are mainly attributed to the presence of citrus flavonoids, as discussed in a number of studies (Galati et al., 1994, 1996; Monforte et al., 1995). Animal studies have also confirmed that citrus limonoids and their derivatives have important biological activities, including the reduction of chemically induced tumorigenesis and inhibition of oral carcinogenesis (Lam, Zhang, & Hasegawa, 1994; Miller et al., 1994), and may be used as chemopreventive agents (Jayaprakasha, Murthy, Uckoo, & Patil, 2013; Shimada, 2015; Silalahi, 2002; Yoshimizu et al., 2004). Therefore, a high intake of these biological compounds in citrus fruits may reduce the risk of a range of diseases.

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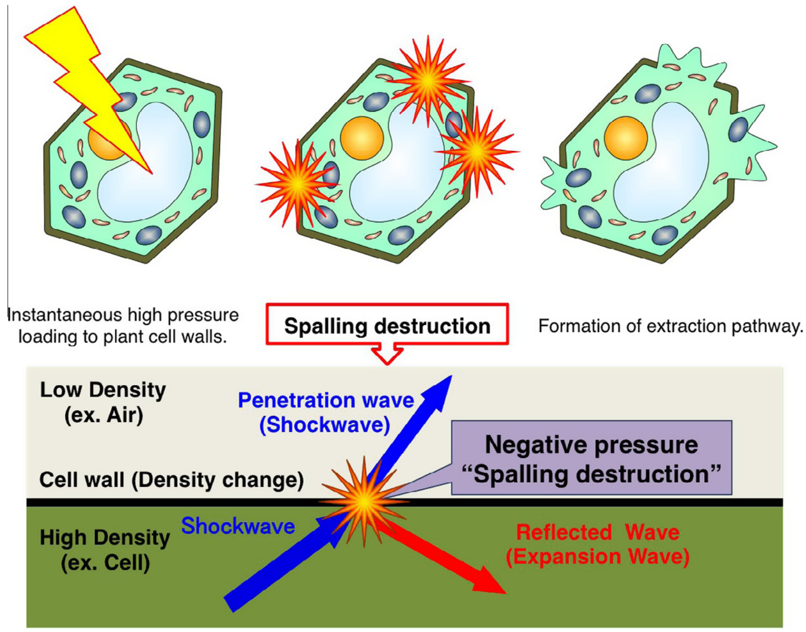

Fig. 1. Schematic of the mechanism of spalling destruction by underwater shockwave treatment.

Shockwaves propagate in plant media at speeds exceeding the speed of sound, dividing into a penetration wave and a reflected wave upon a change in density. The penetration wave propagates to the low-density side as a shockwave, while the reflected wave becomes an expansion wave and propagates to the high-density side. Underwater shockwaves, which cause instantaneous high pressure, penetrate the entire plant cell and selectively destroy plant cell walls by spalling destruction (Fig. 1).

In shockwave-treated plants, multiple cracks are generated on the tracheid, resulting in the pit membrane flaking off the tracheid through spalling destruction. These cracks act as a permeation pathway, increasing the extraction ratio of essential oils in steam distillation processes (Kuraya, Miyafuji, Takemoto, & Itoh, 2014). For example, Bousseta et al. (2009, 2012) investigated the effects of pulsed electric fields and high voltage electrical discharges on the efficiency of the aqueous extraction of total soluble matter and polyphenols from grape pomaces. A large difference was observed between the total amount of polyphenols in the untreated system and that in the high voltage electrical discharge treated system. The effect of instantaneous high pressure in the conventional mixer blending extraction method gave increased yields of the tomato saponin, esculeoside A (Manabe et al., 2011), while improving the extraction efficiency of lipophilic gingerols and shogaols from ginger (Maehara, Watanabe, Takemoto, & Itoh, 2011). Moreover, the remaining lignocellulose cell walls were destroyed with high levels of disintegration, thus allowing access to the oil remaining in the vacuoles of the *Jatropha curcas* L. seeds. During development of a device for pressure shockwave pretreatment, high yields of these oils were achieved (Maroušek et al., 2013a, 2013b). These results indicated that the implementation of underwater shockwave treatment as a preprocessing step is useful for the extraction of functional components from biomass.

We herein introduce a novel application of this pretreatment process, aimed at improving the antioxidant functionality of *Citrus junos* Tanaka (yuzu) fruit juice. We also evaluate the functional compound contents in yuzu fruit juice to investigate whether such a pretreatment method affects its characteristics.

2. Materials and methods

2.1. Materials

Yuzu fruits grown at Rikuzentakata (NLY) and Kochi (KY) (Kochi yuzu was grown in Kami city and was of the Kumon variety) were provided by the Iwate Agricultural Research Center. All fruits were fully ripe and firm when harvested in November 2013. For both fruits, the juice was extracted by hand pressing using a hand-operated citrus juicer (Nanyo LLC, Tokushima, Japan), and the yuzu juice examined was a mixture of 10 and 4 fruit samples from KY and NLY, respectively. The juice extraction percentage was calculated based on the average weight of the fruits and the weight of the juice. The juice samples were filtered through a nonwoven fabric net, centrifuged at 10,000 rpm for 10 min, and stored at −30 °C prior to analysis.

2.2. Chemicals

Formic acid and ethanol, as analytical grade reagents, as well as sodium dihydrogen phosphate dehydrate, as a special grade reagent, were obtained from Nacalai Tesque, Inc. (Kyoto, Japan). The structures of the major flavanone glycosides in yuzu juice are shown in Fig. 2. The flavanone glycosides hesperidin (HSP), neohesperidin (NHSP), naringin (NRG), and narirutin (NRT), the limonoids limonin (Lim) and nomilin (Nom), as analytical grade reagents, ascorbic acid and fluorescein disodium, as a special grade reagent, were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) as a standard for oxygen radical absorbance capacity (ORAC) assays, 2,2′-azobis(2-methyl propionamidine), dihydrochloride (AAPH), and acetonitrile were obtained as analytical grade reagents from Sigma-Aldrich, Inc. (St. Louis, MO, USA). Distilled and deionized water was used in the preparation of all solutions.

2.3. Calibration standards and sample preparation

Stock solutions (100 µg/mL) of HSP, NHSP, NRG, NRT, Lim, and Nom were prepared in 95.5% ethanol and stored at −30 °C.