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Effect of *Monascus* aged vinegar on isoflavone conversion in soy germ by soaking treatment

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ABSTRACT

Soy germ rich in isoflavones has attracted much attention for health-promoting characteristics. An effective approach via *Monascus* aged vinegar soaking was adopted to enhance the aglycone amount. The profiles and interconversion of soy germ isoflavones via *Monascus* aged vinegar soaking were investigated, and the distribution in vinegars were also explored. The aglycones were dramatically increased by 40.76 times. Concomitantly, β-glycosides and malonylglycosides were significantly decreased. The proportion of aglycones presented a sharp increase with the endogenous β-glucosidase activity at the initial 4 h incubation. There appeared to be correlations between β-glucosidase activity and the hydrolysis of conjugated isoflavones. The results demonstrated that the reactions of decarboxylation, de-esterification and de-glycosylation were involved in the *Monascus* aged vinegar soaking, supporting synergistic effects of enzymolysis by endogenous β-glucosidase from soy germ and acid hydrolysis of vinegars. Soaking by vinegar is a promising pathway for preparing aglycone-rich soy germ.

1. Introduction

Soybean (*Glycine max* Merrill) was originally cultivated in China 5000 years ago. It has been widely consumed in Asian countries (e.g., China, India, Japan and Korea) for thousands of years. Nowadays, soy products are not only consumed by Asian populations, but also encouraged for western diets. This phenomenon is mainly due to their nutritional properties and the presence of health-promoting functional ingredients in soy products such as isoflavone, a well-known phytoestrogen (Phommalth, Jeong, Kim, Dhakal, & Hwang, 2008). Isoflavone has been reported to be 1–5 mg/g in dry-soybean (Murphy et al., 1999). Isoflavone contents vary in various parts of soybeans, and its contents in the soy germ are about 6–10 times higher than that in the cotyledon (Murphy, Barua, & Hauck, 2002). Therefore, soy germ, the richest source of soy isoflavones, can be utilized as a health promoting ingredient in food supplement markets (Schryver, 2002).

Isoflavones are known for their biological activities including estrogenic, antifungal, antioxidant activities (Matsuura, Sasaki, & Murao, 1995). Soy isoflavones consist of 15 chemical forms, and are classified into two groups, the aglycones (daidzein, genistein, and glycitein) and β-glucoside conjugates. The succinyl-β-glucosides derivatives are only found in some soybean fermented products, such as natto (*Bacillus subtilis*). In unprocessed soybean or soybean germ, malonylglycoside contents were the highest, followed by β-glycosides, aglycones, and acetylglycosides (Hsieh, Kao, & Chen, 2005). Concentrations of acetylglycosides, β-glycosides, and aglycones tend to increase during the commercial processing (Charron, Allen, Johnson, Pantalone, & Sams, 2005; Coward, Smith, Kirk, & Barnes, 1998; Franke et al., 1999; Yu, Liu, Qiu, & Wang, 2007). Chemical forms of isoflavones can affect their stability during various processing conditions, as well as their bioavailability. The aglycones are structurally similar to the mammalian estrogen and, therefore, mimic the function of estradiol in the human body, whereas the β-glucosides have less estrogenic activity (Brouns, 2002; Izumi et al., 2000; Setchell, 1998). Additionally, the β-glucosides absorbed more slower and in less amounts than their aglycones by humans due to their hydrophilic

Abbreviations: A-daidzin, 6-O-acetyldaidzin; A-glycitin, 6-O-acetylglycitin; A-genistin, 6-O-acetylgenistin; M-daidzin, 6-O-malonyldaidzin; M-glycitin, 6-O-malonylglycitin; M-genistin, 6-O-malonylgenistin; RP-HPLC, reversed-phase high performance liquid chromatography; TFA, trifluoroacetic acid; p-NPG, p-nitrophenyl-β-D-glucopyranoside; UV, ultraviolet; p-NP, para-nitrophenol.

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nature and higher molecular weight (Izumi et al., 2000). In particular, isoflavone aglycones have been associated with the prevention and treatment of hormone-dependent disorders based on epidemiological (Messina, Persky, Setchell, & Barnes, 1994) and small-scale human clinical studies (Kurzer, 2000). Therefore, it would greatly increase isoflavone bioactivities and bioavailability if β-glucosides forms were converted to aglycone forms.

Therefore, the enrichment of isoflavone aglycones in soybean foods before consumption attracts growing attention. Several methods for the transformation of isoflavone glycosides to isoflavone aglycones, including water soaking, acid hydrolysis, fermentation, heat, and enzymatic transformation, have been attempted (Chen, Lo, Su, Chou, & Cheng, 2012; Chien, Hsieh, Kao, & Chen, 2005; Hubert, Berger, Nepveu, Paul, & Daydé, 2008; Liu, Zhang, Wu, Wang, & Wang, 2013; Toda, Sakamoto, Takayanagi, & Yokotsuka, 2000). The acidic hydrolysis method has advantages, low cost, simple technology and high hydrolytic percentage. But it also produces side reactions which causes high purification cost, and generates environmental pollution (Lee, Seo, & Oh, 2013). Fortunately, vinegar soaking approach would get out of this trouble.

Vinegar soaked soybeans were authorized as functional food status by China Food and Drug Administration (CFDA) (License WEISHIJIANZI (1997) No. 823) mainly due to the presence of soy isoflavones, particularly aglycone forms. However, little information is available on the changes of isoflavone compositions and the interconversion of soy isoflavones of soy germ during soaking in vinegars. *Yongchun Monascus* aged vinegar, one of the four famous China-style fermented vinegars, was brewed from sticky rice in the traditional way and could be traced back to more than 2000 years ago (the early years of the North Song Dynasty). Various β-glycosidases may be produced during the fermentation process via microorganisms such as acetic acid bacteria (*Acetobacter* sp.), lactic acid bacteria (*Lactobacillus plantarum*), yeast (*Dekkera* sp.), *Monascus purpureus*. Additional, rare report took full advantage of that soy germ was rich in endogenous β-glucosidase. Therefore, the combination of endogenous β-glucosidase and acid hydrolysis may facilitate the transformation of isoflavone forms from glucosides to the aglycones.

Thus, the objectives of this study was to understand the conversion of isoflavone forms of soy germ during the soaking by *Monascus* aged vinegar, investigate the possible mechanism of the transformation and provide a basis for developing functional foods of soy germ.

2. Materials and methods

2.1. Chemicals and reagents

Acetonitrile, methanol, and acetic acid, HPLC grade, were purchased from Fisher Chemical Co. Ltd. (USA). The 12 standards (daidzin, glycitin, genistin, acetyldaidzin, acetylglycitin, acetylgenistin, malonyldaidzin, malonylglycitin, malonylgenistin, daidzein, glycitein and genistein) were purchased from Sigma Chemical Co. Ltd. (St. Louis, MO, USA). HPLC-grade water was prepared with a Milli-Q Water Purification System (Millipore Corporation, Billerica, MA, USA). p-Nitrophenyl-β-D-glucopyranoside (p-NPG) and para-nitrophenol (p-NP) were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). All chemicals were analytical grade.

2.2. Sample collection

Soybean germ (from a soybean variety of Zhongdou-27) was purchased from Jiusan Oil and Fat Chemical Factory in Heilongjiang province of China). The soybean germ was selected again to pick out the cotyledon, seed coat and broken germ, then dried at 40 °C under vacuum condition for 6 h. It was stored at 25 °C in sealed and dry condition for use. Yongchun *Monascus* aged vinegar without sterilization was kindly provided by *Fujian Yongchun Shundetang Food* Co., Ltd. (Fujian province, China).

2.3. Sample treatment

2.3.1. Different treatments on soy germ

Soybean germs were soaked by Yongchun *Monascus* aged vinegar or Recovered Yongchun *Monascus* aged vinegar at 4 times their weight and incubated for 72 h at 25 °C. The soybean germs and the resultant soaking liquor (Yongchun *Monascus* aged vinegar) were then lyophilized, ground to uniformity by a coffee mill, and prepared as samples for isoflavone analysis by high performance liquid chromatography (HPLC).

Soaking in water and cooking were as the controls. Soaking in water: soybean germ was soaked by adding distilled water (natural pH) at 4 times their weight and incubated at 25 °C for 72 h. Recovered vinegar soaking: soybean germ was soaked by the recovered Yongchun *Monascus* aged vinegar (the vinegar has used for soaking soy germ once, and then the resolution was recovered from the last time soking) at 4 times their weight and incubated at 25 °C for 72 h. Cooking: soybean germ was soaked by distilled water and cooked at 121 °C for 30 min. Finally, the soybean germs and soaking liquor were lyophilized to yield soybean germ powder for isoflavone analysis by HPLC.

2.3.2. The ratio of solid to liquid in vinegar soaking

Soybean germ was soaked in different volume of Yongchun *Monascus* aged vinegar (vinegar:soy germ = 1:2, 1:4, 1:6, 1:8, 1:12, respectively, w/w) and incubated at 25 °C for 24 h.

2.3.3. Effects of soaking time on the soy germ

Soybean germ was soaked by adding Yongchun *Monascus* aged vinegar at 4 times (w/w) their weight and incubated at 25 °C for different time (0, 4, 8, 12, 16, 20 and 24 h, respectively). After treatment, the soybean germ was dried, ground to uniformity by a coffee mill, and prepared as samples for isoflavones analysis by HPLC.

2.4. Isoflavone extraction method

The extraction method of soy germ isoflavones was performed according to the procedure described by Rostagno, Palma, and Barroso (2003), with some modifications. In brief, 1 g soybean germ sample was added in 10 mL 80% aqueous methanol and incubated in ultrasonic bath (B.H.A. instruments Co., Ltd., Germany) for 35 min at 20 °C. The ultrasound power density and frequency were set to 150 W and 35 kHz, respectively. The extraction was centrifuged at 8000×*g* for 15 min using SIGMA 1–15 K instrument (BHA instruments Co., Ltd., Germany) and then the supernatant was filtered by 0.45 μm filters for HPLC analysis. Each experiment was repeated at least three times.

2.5. High performance liquid chromatography (HPLC)

The HPLC equipment consisted of an Agilent 1200 series Rapid Resolution LC System (Agilent Technologies, Palo Alto, CA) equipped with an automatic injector and PhotoDiode Array detector (DAD) detector. Data were collected on Agilent Chemstation software.

2.5.1. HPLC determination conditions for isoflavones

The HPLC conditions were adopted to the method developed by Rostagno, Villares, Guillamón, García-Lafuente, and Martinez