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# Anti-α-glucosidase activity of Chinese traditionally fermented soybean (douchi)

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**Abstract**

Anti-α-glucosidase activity of aqueous douchi extracts was investigated in this study. Thirty-one douchi samples collected from different parts of China exerted various degree of inhibitory activity against rat intestinal α-glucosidase. Among them, three samples, sourcing from Hunan, Sichuan and Jiangxi province, respectively, showed a significant higher anti-α-glucosidase activities than other samples ($p < 0.05$). Moreover, three fungal strains, namely *Aspergillus oryzae*, *Actinomucor elegans* and *Rhizopus arrhizus* were then used to prepare douchi in our laboratory. The α-glucosidase inhibitory activities of all soybeans increased slightly and no apparent differences were found in anti-α-glucosidase activity among the soybeans at the end of pre-fermentation. For maturation, different salt levels (5.0%, 7.5%, 10.0% and 12.5%) were then added to the douchi qu resulted from pre-fermentation. The anti-α-glucosidase activity of douchi qu fermented with *A. oryzae* were higher than those of *A. elegans* and *R. arrhizus* and the highest anti-α-glucosidase activities was observed in douchi qu fermented with *A. orzyzae* at 5.0% and 7.5% salt levels. The results indicated that *A. oryzae* could utilize cooked black soybean to generate certain α-glucosidase inhibitor more effectively than *A. elegans* and *R. arrhizus*.


*Keywords:* Anti-α-glucosidase; Douchi; Fermented soybean; α-Glucosidase inhibitor; Anti-hyperglycemic

## 1. Introduction

Diabetes mellitus has become a common disease not only in developed countries but also in developing countries due to the changes in people's lifestyle and dietary habits (Horton, 1995). α-Glucosidase inhibitor is usually used to prevent or medically treat type II diabetes (Non-insulin dependent mellitus, NIDDM) (Alain, 1998; Floris et al., 2005; Holman, 1998; Patricia, Steven, Jennifer, & Bryan, 2005). These inhibitors combine with intestine α-glucosidase and block the uptake of postprandial blood glucose (Holman, 1998). Although powerful synthetic α-glucosidase inhibitors (i.e. voglibose) are available, they usually can cause hepatic disorders and other negative gastrointestinal symptoms (Murai et al., 2002). Hence, natural α-glucosidase inhibitors from food sources have become an attractive therapeutic approach for treating post-prandial hyperglycemia (Gallaher & Schneeman, 1986; Heacock, Hertzler, Williams, & Wolf, 2005; Kyung & Moo, 2000; Murai et al., 2002; Ye, Shen, & Xie, 2002).

Soybean and its products have been appreciated by consumers as health foods due to their valuable nutritional and medicinal attributes. In particular, the intake of soybean foods has been associated with the prevention and treatment of chronic diseases, such as cardiovascular disease and cancers. Douchi (or touchi) is a popular fermented soybean product among Chinese community worldwide. Many historical medical books have described douchi being able to prevent and cure diseases. Zhang Zhongjing of the Han Dynasty recorded that soup cooked with cape jasmine and douchi was useful to relieve tiredness, weakness, insomnia, and poor appetite (Li, Yin, Zhang, Zhang,

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& Zhao, 2002). Recent studies show that fermented soybeans products possess anti-diabetic properties (Fujita, Yamagami, & Ohshima, 2001; Fujita, Yamagami, & Ohshima, 2003; McCue, Kwon, & Shetty, 2005). The douchi extract, for example, elicited significant anti-hyperglycemic effect at a minimum effective dose of 0.3 g in human (Fujita et al., 2003). Moreover, the douchi extract demonstrates excellent anti-hyperglycemic effect without causing any side effects such as diarrhea, retching and flatulence, which are commonly encountered with the use of currently available α-glucosidase inhibitory therapeutic drugs (Fujita et al., 2003).

The douchi processing is mainly made up of fungal solid-state fermentation (pre-fermentation), followed by salting and maturation (post-fermentation). Based on the microorganisms used, douchi products can be classified into *Aspergillus*-type (i.e. Liuyang douchi and Yangjiang douchi etc.), *Mucor*-type (i.e. Yongchuan douchi), *Rhizopus*-type (i.e Indian tempe) and *Bacterial* type (i.e. Qianxi douchi, Babao douchi & Japanese natto) (Liang, Cheng, & Ma, 2004; Niu & Ma, 2005). Salt plays multiple roles in the fermentation of various soybean products. In sufu, for instance, besides imparting salty taste to the final product, salt is important in controlling microbial growth and enzymatic activities as well (Han, Wang, Rombouts, & Nout, 2003). On the other hand, salts supplemented to douchi during post-fermentation lowers the antioxidative activity of douchi (Zou, Wang, Cheng, Li, & Tatsumi, 2006). Fifty percent salt added in douchi could deduce to the loss of 61% isoflavone in raw soybean during the fermentation (Wang et al., 2007). Although microorganisms and salts are important in endowing unique sensory properties and enhancing the nutritional values of the fermented soy products, their roles in generating α-glucosidase inhibitors have not been investigated. In this study, we analyzed the α-glucosidase inhibitory activity of 31 douchi samples collected from various parts of China. The anti-α-glucosidase activity of douchi samples prepared using different fungal strains (namely, *A. oryzae*, *A. elegans* and *R. arrhizus*) at different salt levels (5.0–12.5%, w/w) were also studied. The first objective of this study is to investigate the anti-α-glucosdiase activity of Chinese douchi. The second is to investigate the factors which are related to the anti-α-glucosdiase activity of douchi and sufu.

## 2. Materials and methods

### 2.1. Materials

Thirty-one douchi samples were purchased directly from douchi manufacturers from different parts of China (Table 1). Fungal strains, namely *Aspergillus oryzae* 3.951, *Actinomucor elegans* 3.118 and *Rhizopus arrhizus* 3.078 were kindly provided by the Institute of Microbiology, Chinese Academy of Sciences (Beijing, China). Intestinal acetone powders of rat and 4-nitrophenyl α-D-glucopyranoside (4-NPG) were purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals were of analytical grade.

### 2.2. Fungal strains and culture conditions

Fungal strains of *A. oryzae* 3.951, *A. elegans* 3.118 and *R. arrhizus* 3.078 were grown individually on malt extract agar (pH 4.0) at 28 °C for 2–3 days. Then, 25 ml sterile distilled water was added to each of the 3-day-old fungus grown on malt-extract agar plates and the plates were scraped aseptically with inoculating loop. The resulted spore suspensions were then allowed to propagate on a sterilized substrate consisting of wheat bran (50.0 g), wheat flour (10.0 g) and sterilized distilled water (50 ml) in an incubator at 28 °C for three days. After the complete growth of the organisms, spores of the three fungal strains were harvested by adding sterilized water to the fermenting substrate, shaking the flask and filtration to make homogenous spore suspensions. The number of spores in each suspension was enumerated using a thrombocytometer.

### 2.3. Douchi preparation

The preparation of douchi is illustrated in Fig. 1. Black soybeans were washed, soaked in water (1:3, w/w) at 25 ± 2 °C for 8–10 h, and steamed at 121 °C for 30 min in a retort (YMQ.L31.400, Beijing Jiangtai Medical Instrument Co., Beijing, China). After cooling to 30 °C, the cooked soybeans were inoculated either with the spores of *A. oryzae*, *A. elegans* or *R. arrhizus* ($10^6$ spores per gram of cooked soybean) and fermented in an incubator (LTI-601SD, Tokyo Rikakikai Co., Tokyo, Japan) for 60 h at 28 °C and 90% relative humidity. Semi-finished products were called douchi qu (koji). For post-fermentation treatment on douchi, different levels of salt (5–12.5%, w/w) were added to the douchi qu and the douchi qu was allow to age for one month.

### 2.4. Preparation of aqueous extract of douchi

Douchi samples were minced and lyophilized with a freeze-dryer (EYELA Co., Tokyo, Japan). Exactly 4.000 g of each sample was mixed with 40 ml distilled water, homogenized (20 000 rpm, 2 min) and centrifuged (3800 rpm, 10 min). The resulted supernatant of each sample was collected and freeze-dried again. Finally, the powder was diluted with 10 ml distilled water, filtered and stored at 4 °C until use.

### 2.5. Measurement of α-glucosidase inhibitory activity

The inhibitory activity of douchi extracts against rat α-glucosidase was determined by measuring the formation of 4-nitrophenol by α-glucosidase after the reaction with 4-nitrophenyl α-D-glucopyranoside (4-PNP) as described by Yamaki and Mori (2006). The inhibitory activity of