Preparation of Isoflavone glucosides from Soy germ and β-Glucosidase from *Bacillus coagulans* PR03 for Isoflavone aglycones Production

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ABSTRACT

This research optimized isoflavone aglycones production from soy germ. The processes included isoflavone glucosides extraction from soy germ to use as a precursor for isoflavone aglycones production. β-glucosidase was produced from B. coagulans PR03 and used to convert glucosides form to aglycones. For the β-glucosidase production using Plackett and Burman Design (n=8) and 2^2 factorial experiment with central composite design, the suitable medium containing peptone (2.00%), beef extract (14.84%), glucose (2.00%) and magnesium sulphate (0.10%) with pH 7.96 was used and incubated at 30 ^oC. The resulting β-glucosidase activity was 4.01 mU/ml. However, the isoflavone glucosides was extracted by use of soy germ in 80% ethanol at a ratio of 1:5 with high power ultrasonication technique at 80 ^oC for 160 min using a completely randomized design. The extracted isoflavone glucosides contained daidzin, genistin and glycitin of 307.47, 214.84 and 73.63 mg/100 g. (dry basis), respectively. Finally, isoflavone aglycones production

was optimized using 2^2 factorial experiment with central composite design, the total isoflavone glucosides (595.95 mg/100 g. dry basis) was convert to isoflavone aglycones (112.90 µg/ml) by β-glucosidase at 37.5 ^oC for 120 hours.

Keywords: Isoflavone aglycones, Isoflavone glucosides, Soy germ, β-glucosidase, *B. coagulans* PR03

INTRODUCTION

The significance of healthy food consumption has become more prominent as many consumers are gaining more awareness of good eating habit such as eating more fruits and vegetables. It is known that bioactive compounds in those foods can reduce the risk of many severe diseases such as cardiovascular diseases, diabetes and cancers (Crozier et al., 2009). One of the most acknowledged bioactive compounds is isoflavone. It is a secondary metabolite from the plant with a limited taxonomic distribution such as soybean (*Glycine max*). Isoflavones have been associated with biological properties such as estrogenic and anti-estrogenic activities against hypocholesterolemia (Murphy, 1982), atherosclerosis, osteoporosis, anti-carcinogenic activity against breast cancer (Ohta et al., 2002) and reduced hot flush in postmenopausal women (Han et al., 2002). It can bind with estrogen receptors, and it is known as phytoestrogen (Sarkar et al., 2002). Suggested dosage of isoflavone consumption is approximately 18-20 mg for one serving size (4 time to 80 mg/day) (Barnes et al., 1995).

The isoflavones in soybean are generally in forms of glucosides (daidzin, genistin, glycitin) more than aglycones (daidzein, genistein and glycitein). The whole soybean seed contains only 2% soy germ, and the highest quantity of isoflavones is found in the soy germ. Isoflavones found in soy germ are 5-6 times higher than the other parts, for instance, seed coat and cotyledon (Nahas et al., 2004). There is an investigation of isoflavone and antioxidant activity in cotyledon, seed coat and soy germ from four varieties of soybean. It was found that the total isoflavones in cotyledon, seed coat and soy germ were 2.73-9.71, 5.56-16.94 and 27.76-81.43 mg/ fresh weight, respectively (Yue et al., 2009).

Isoflavone is a flavonoid substance that is soluble in various polar solvents. Wiriyacharee et al. (2012) studied the extraction of isoflavone from soy germ with different solvents: water, ethanol, methanol, acetone and acetonitrile. It was found that the isoflavone glucosides was efficiently extracted with polar solvents from aqueous acetone and acetonitrile. It can be seen that isoflavones is well soluble. Moreover, high power ultrasonic technique allows polar solvents to easily penetrate extracted materials by destroying the plant cell membrane. The technique can reduce the time of the extraction with