CHARACTERIZATION OF WASTE WATER FROM TOFU INDUSTRY

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ABSTRACT

Tofu is one of the more accepted soy products in the world, a very popular food among the Asian population and is gaining popularity among Indonesian as well, due to the associated health benefits and economically available in the market. During tofu processing, generates solid waste, which has been utilized as feedstuff, functional food etc, and liquid waste which has not been utilized yet and when dispose to the environment can cause bad odour and pollution to the surface and ground water. More over waste water of tofu (whey) from home industry still contains valuable materials such as isoflavones which are dominated by daidzein and genistein. Genistein has been shown effective in preventing of osteoporosis, and daidzein important in preventing of cancer, improving of hormone steroid metabolism, reducing cholesterol and also protect of liver cell from toxin. The objectives of this study were to isolation and characterization of whey chemical compound for further biotransformation. Waste water collected from tofu industry at Serpong,Tangerang, Indonesia. Characterization of whey chemical compound using Fourier transform infrared spectroscopy (FTIR) and high performance liquid chromatography (HPLC) resulted daidzein and genistein derivates.

Keywords: Whey, pollution, isoflavones, daidzein, genistein, FTIR, HPLC

INTRODUCTION

Tofu is one of the more accepted soy products in the world, a very popular food among the Asian population and is gaining popularity among Indonesian as well, due to the associated health benefits and economically available in the market. During tofu processing, generates solid waste, which has been utilized as feedstuff, functional food etc, and liquid waste which has not been utilized yet and when dispose to the environment can cause bad odour and pollution to the surface and ground water. More over waste water of tofu (whey) from home industry still contains valuable materials such as isoflavones which are dominated by daidzein and genistein.

Isoflavones are one of the six main subclasses of flavonoids and the only one which contains a rearranged C15 skeleton based on 3-phenylchroman. The interesting biological properties described for isoflavonoids include antioxidant, antimicrobial, anti-inflammatory, estrogenic, and cancer hemoprotectant activities. Genistein and daidzein have antioxidant properties, leading to the inhibition of lipid peroxidation in a liposomal system, and are quenchers of singlet oxygen [1].

Isoflavones are part of the diphenol compounds, called phytoestrogens, which are structurally and functionally similar to estradiol, the human estrogen but much less potent. Because of this similarity, isoflavones were suggested to have preventive effects for many kinds of hormone-dependent diseases [2]. In addition, isoflavones may have a role decreasing the risk of cardiovascular diseases [3], by reducing the level total cholesterol as well as low density lipoprotein (LDL) cholesterol [4].

Isoflavones occur naturally in plants and mostly in soybeans. Twelve forms of isoflavones are known in soybeans and soy products, including 3 free forms, as an aglucons (namely, genistein, daidzein, and glycitein) and 3 conjugated forms to each aglucon, called glucosides (glycosides). The conjugated forms have an additional glucose moiety, which could be free of other groups (ß-glucosides: namely, genistin, daidzin, and glycitin) or could be bound to either an acetyl group (ß-O-acetylglucosides) or a malonyl group (ß-O-malonylglucosides) [5].

![Figure 1. Structures of isoflavones aglicon.](image)
Because there is indication that the isoflavones have a health claims as reviewed by Kurzer and Xu, 1997[6], include reduction in postmenopausal symptoms and risks of osteoporosis in women. Study the probability of utilization of whey as a cheap and abundant material for biotransformation of isoflavones is important. In the present study, characterization of its chemical compounds was conducted using spectroscopic methods such as Fourier transform infrared spectroscopy (FTIR) and high performance liquid chromatography (HPLC).

MATERIALS AND METHODS

Material

Waste water of tofu collected from several tofu industries at Serpong, Tangerang Indonesia.

Isolation of isoflavones from whey

Sample prep preparation

Isolation of isoflavones from whey was performed by filtering and fixating. Whey (3 liter) were filtered and fixated into Amberlite XAD4 column. The residue was washed with distillation water (3 liter), and then the column was eluted with methanol (2.5 liter). The eluat was evaporated and added with hot water. The aqueous phase was extracted with chloroform and evaporated into crude.

Determination of eluent

The crude from chloroform fraction was added with methanol and spotted to Silica Gel 60 F254 plate; put the plate to the chamber using n-hexane and ethyl acetate as mobile phase. Take the plate out and visualized using UV light in the wavelength 254 nm and 366 nm [7].

Column chromatography

Silica gel (23.0775 g) was used as stationary phase, mixed with n-hexane, and put into column. Then the crude (0.1689) from chloroform fraction was poured into column, eluted with n-hexane, collected, and evaporated (Fraction I). After the band in the column was not moved, the sample was eluted with n-hexane: ethyl acetate 3:1 (Fraction II); n-hexane: ethyl acetate 1:1 (Fraction III); n-hexane: ethyl acetate 1:3 (Fraction IV). Each fraction was collected and evaporated, then analyzed by thin-layer chromatography (TLC). The fraction which has similar retention factor (Rf) was collected together.

Fourier transform infrared spectroscopy

The functional groups of compound were characterized using Fourier Transform Infrared spectroscopy. Infrared spectra were obtained on Shimadzu IRPrestidge-21/FTIR-8000 spectrophotometer by transmittance sampling technique. Samples were measured with spacer of 0.01 mm ~ 0.02 mm thickness on a KBR cell plate and then placed in the sample holder. Apodization was performed by Happ-Genzel method with number of scans: 45, resolution: 4.0 and wavelength range: 500 ~ 4000 cm⁻¹. The chromatogram was then analyzed to obtain data of functional groups.

High performance liquid chromatography (HPLC) analysis

Chemical compound were characterized using Hitachi L-7100 pump, Tokyo, Japan. The samples 20 µL were injected into HPLC system for analysis. A reversed phase bondapack C-18 column was run with gradient solvent system. UV-Vis detector (L-7420) set at 260 nm was used for separation and subsequent detection of daidzein and genistein of whey. The mobile phases were consisted of two variation: mobile phase A – water: acetic acid (97%: 3%) and B - methanol: acetic acid (97%: 3%). The flow rate was set at 1.0 ml/min. Standard daidzein and genistein from Sigma Chemical Co., St. Louis., were run simultaneously for quantitative and qualitative analysis.

RESULT AND DISCUSSION

Isolation of Isoflavones

The result of isolation of isoflavones revealed in 0.0023 g fraction I, 0.0354 g fraction II, 0.0627 g fraction III and 0.0284 g fraction IV.

From color test of fraction III resulted in yellow spot (Rf = 0.72) which means the fraction contain isoflavones. Blue and purple spot in the color test using NH3 means the sample contain isoflavones group (Markham, 1988). Based on the data, fraction III which has the highest recovery (0.0627) and showed positive test in the color test, and further analysis was done using HPLC.

FTIR Analysis

FTIR was performed to identify the chemical structure of compounds. The basic structure of compounds can be determined by the spectral locations of their infrared absorption. From FTIR analysis, it is shown that the compound contained hydroxyl (O-H), carbonyl(C=O), ether (C-O-C) and aromatic double bond which are functional groups of isoflavones compound. The sample gives the following spectrum as shown in Figure 2.

Analysis of FTIR frequencies is shown in Table 1. The sample shows a strong and broad absorption at 3600-3200 cm⁻¹, this bond corresponds to the hydroxyl groups (-OH). A broad band between this region is likely due to the hydrogen-bonded O-H stretching mode of a phenol. C-H aromatic stretching groups are also shown at 3072 cm⁻¹. This indicates the presence of an
aromatic compound in the sample. In the lower region, an aromatic pattern is observed in the 1500-1650 cm$^{-1}$ region. A strong absorption at 1613 cm$^{-1}$ is characteristic for carbonyl groups (C=O). Several peaks are also shown in this region indicating C=C aromatic groups. In the fingerprint region (region below 1500 cm$^{-1}$), the sample gave absorption at 828 cm$^{-1}$. This is due to the C-H aromatic bending mode. Other peaks are observed at 1042-1190 cm$^{-1}$ region indicating the C-O groups.

With this preliminary analysis, it is predicted that the whey sample contains isoflavon compounds, genistein and daidzein. Although prediction of isoflavones from waste water of tofu industry using FTIR is quite good. To clarify more information about those chemical compounds, further analysis was performed by HPLC analysis as follows.

**HPLC Analysis**

Characterization of isoflavones: both daidzein and genistein of whey from waste water tofu industry were analysed by HPLC and identified by comparing retention times of the standards. From HPLC analysis both chloroform and ethyl acetate fraction showed contains of daidzein and genistein. Retention time (Rt) of sample in chloroform fraction is 35.98 and 38.73 (Table 2), however, in ethyl acetate fraction Rt of daidzein and genistein are 36.02 min. and 38.74 min. respectively, the result is a similar with Rt standard of daidzein (36.02 min.) and genistein (38.74 min.) as reported by Sudiyani et al., 2006 [8]. HPLC chromatograms of standard solution of daidzein, genistein and chloroform fraction is shown in Figure 3.

**CONCLUSION**

FTIR and HPLC were successfully applied for characterization of waste water from tofu industry. According to the results, apparently prediction of isoflavones from waste water of tofu industry using FTIR is quite good. Retention time (Rt) of sample in ethyl acetate fraction is 36.02 min. and 38.74 min.
shown a similar with Rt standard of daidzein and genestein. Characterization of chemical compounds from tofu industry is of fundamental importance for future research on utilization of whey as a cheap and abundant material for biotransformation of isoflavones.

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