African Journal of Microbiology Research Vol. 6(2), pp. 426-430, 16 January, 2012
Available online at http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMR11.1249
ISSN 1996-0808 ©2012 Academic Journals

Full Length Research Paper

Nano study of antioxidant activities of fermented soy whey prepared with lactic acid bacteria and kefir

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Accepted 12 December, 2011

This study aims were to test antioxidative activity of supernatant from fermented soy whey to find out their nutraceutical potential. Fermented soy whey was prepared using *Lactobacillus plantarum*, *Streptococcus thermophilus* and Kefir, fermented at room temperature (25-26°C) for 24 and 48 h. Antioxidative properties were assessed by DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity and reducing power. All fermented soy whey extracts exhibited a potentially antioxidant activities, yet extract kefir soy whey with 48 h incubation gave significant highest antioxidant activity compared to others. The results suggest that fermented soy whey formulations could provide a functional food alternative to milk-based fermented products.

Key words: Soy whey, fermentation, LAB, Kefir, antioxidant.

INTRODUCTION

Soybeans are rich in nutrients and bioactive substances (Friedman et al., 1991). Soybean is related with the reduction of several diseases, such as heart illness (Lovati et al., 2000), hypertension (Rivas et al., 2002), breast cancer (Messina and Wood, 2008) and reduction of cholesterol (Tovar-Palacio et al., 1998).

Soybean whey is the liquid waste of tofu production, which until now is still a fairly disturbing environmental pollutant. This whey contains about 4.3 mg protein/ml and has a high biological oxygen demand (BOD) of 13,730 ppm after a 5-day incubation. Because of its high BOD, whey presents a serious waste disposal problem. Proteins that do not coagulated become the largest component in the whey out of 226.06 to 434.78 mg/L (Tay, 1994). Small protein includes 2S and 7S fractions of soybean whey have good foaming capacity and high solubility (Kajiyama et al., 1995). Besides proteins, other important components are left in the whey is isoflavones (Wang et al., 1998). Isoflavones in soybeans has been recognized as a major functional compound to prevent chronic diseases such as cancer and arteriosclerosis.

Biochemical modification by microorganism contributes directly into many advantageous properties of products (Chukeatirote et al., 2006). Some extensive studies have revealed the high antioxidant activity of fermented soybeans (Chaiyasut and Kumar, 2010; Hu et al., 2004; Yang et al., 2000). Lactic acid bacteria (LAB) are widely used in food industry commonly known to have health-promoting attributes. Amadou et al. (2010) reported the antioxidative capacity of peptides isolated from *Lactobacillus plantarum* Lp6 fermented soybean protein meal and these peptides fractions exhibited strong antioxidative capacity. Heat-killed cells (HKC) and cytoplasmic fraction (CF) prepared from *L. plantarum* NTU 102 give strong evidence on health promoting capability including antioxidant activities and antiproliferative activities against breast and colon cancer cell lines *in vitro* (Liu and Pan, 2010). Milk Fermented by *Streptococcus thermophilus* showed antimutagenic activity (Bodana and Rao, 1990). Antioxidant activity of Kefir product from soymilk (Liu et al., 2005) and rice milk (Sirirat and Jelena, 2010) were reported high.

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Soy protein can decomposed into simple peptides either enzymatically or fermentative. Microbial biotransformation of isoflavone has been widely reported and generated aglycone that is considered to have a better bioactive capacity. However, the study of soy whey as a raw material production of bioactive components is limited. The objective of this study was to investigate the antioxidative capacities of extract soybean whey fermented with *L. plantarum, S. thermophilus* and Kefir. The work will be beneficial toward waste treatment technology alternative as well as nutritional and medicinal utilization of this soy whey.

MATERIALS AND METHODS

The soy whey used in these experiments was obtained from the local tofu industry in Ungaran, Semarang, Indonesia. *L. plantarum*, *S. thermophilus* for the experiment were obtained from Culture Collection of the School of Technology and Natural Science (STIH), Bandung Institute of Technology (Bandung); while Kefir grain from local industry around Bandung. A, a-Diphenyl-b-picrylhydrazyl (DPPH) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents and solvents used were of the highest grade available.

Soy whey fermentation for preparation of bioactive materials

The strain of *L. plantarum* and *S. thermophilus* were inoculated in sterile LB broth at 37°C for 36 h. The 1% (v/v) inoculums were then re-suspended in sterile soybean whey. Kefir soy whey was manufactured by sterilized (121°C for 15 min) whey and inoculated with 1% (w/v) kefir grains. All the cultures were incubated at room temperature (25-26°C) for 24 and 48 h. Metabolite extracts were collected by centrifugation at 4°C at 5000 rpm for 15 min, then lyophilized and keeps in cold temperature until further used.

Measurement of DPPH radical-scavenging activity

The fermented soybean whey effect toward DPPH radicals was measured according to the method of Shimada et al. (1992). Various concentrations extract samples (3 ml, 1.25-5 mg/ml) were separately mixed with 1 ml of a methanolic solution containing DPPH radicals 0.1 mM. The mixture was shaken vigorously and left to stand for 30 min in the dark, and the mixture's absorbance was then measured at 517 nm. The tests were performed triplicate. The scavenge DPPH radicals was calculated as (%) = [1-(absorbance of sample at 517 nm)/(absorbance of control at 517 nm)]×100.

Measurement of reducing power

The reducing power of fermented soybean whey was determined as described by Oyaizu (1986). Different concentrations of sample in distilled water (1.25 to 5 mg/ml) were mixed with equal volume of 0.2 M phosphate buffer (pH 6.6) and 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min. Subsequently, 2.5 ml of trichloroacetic acid (10%) was added and the mixture was centrifuged at 5000 rpm for 10 min. The 4 ml of supernatant was mixed with 0.5 ml of 0.1% ferric chloride then left for 10 min. The tests were performed triplicate. The absorbance at 700 nm was measured. Increased absorbance indicated increased reducing power.

RESULTS AND DISCUSSION

Study of antioxidant potential of fermented soy whey at room temperature and incubation time 24 and 48 h, based on preliminary studies have been done before. In early studies, soy whey was fermented above room temperature with an incubation time of less than 24 h and showed antioxidant activity but very low (data not shown). Soy whey fermentation extracts using *L. plantarum* at 24 and 48 h, hereinafter referred to as LP24 and LP48. A similar naming applied to other samples, extracts from *S. thermophilus* and Kefir, respectively named ST24, ST48, KF24 and KF48.

DPPH radical-scavenging activity

DPPH radicals are organic compounds containing nitrogen are unstable (radical) with a strong absorbance at 520 nm and dark purple. After reacting with antioxidant compounds, DPPH will be reduced and the decrease in color intensity. Decrease in color intensity was caused by a reduction in conjugated double bond in DPPH. This can occur when a single electron capture by antioxidants, resulting in the absence of opportunity electrons resonate (Molyneux, 2004). This stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples (Ebrahimzadeh et al., 2008).

Figure 1 illustrates the percentage of inhibition of fermented extracts of soy whey with all cultures and conditions against DPPH radical. In general, all extracts showed that the percentage of inhibition increased with increasing concentration of the samples. The same pattern also appears for each extract with the same microbial cultures, which 48 h incubation gave a higher antioxidant activity than 24 h. However, fermented soy whey using kefir for 48 h showed the highest activity almost at all concentrations. Even at the lowest concentration (1.25 mg / ml) KF48 has been able to provide over 60% inhibition. Especially KF48 at 5.0 mg/ml exhibits an excellent DPPH·radical-scavenging activity, nearly 90%. KF48 showed a significantly stronger DPPH scavenging activity comparing to LP24, LP48 and ST24 ($p < 0.05$), moderate to KF24 and ST48 is not very significant ($p > 0.05$). The results revealed that KF48 possibly contained some potential bioactive compounds, which were electron donors and effectively react with free radicals to convert them to more stable products and terminate the radical chain reaction. While KF24 and ST48 are still highly potential having bioactive components that can be studied further.

Reducing power

Reductive capabilities of the fermented soy whey extracts showed in Figure 2. The Fe^{3+}-Fe^{2+} transformation in the presence of all extracts were used for measurements of

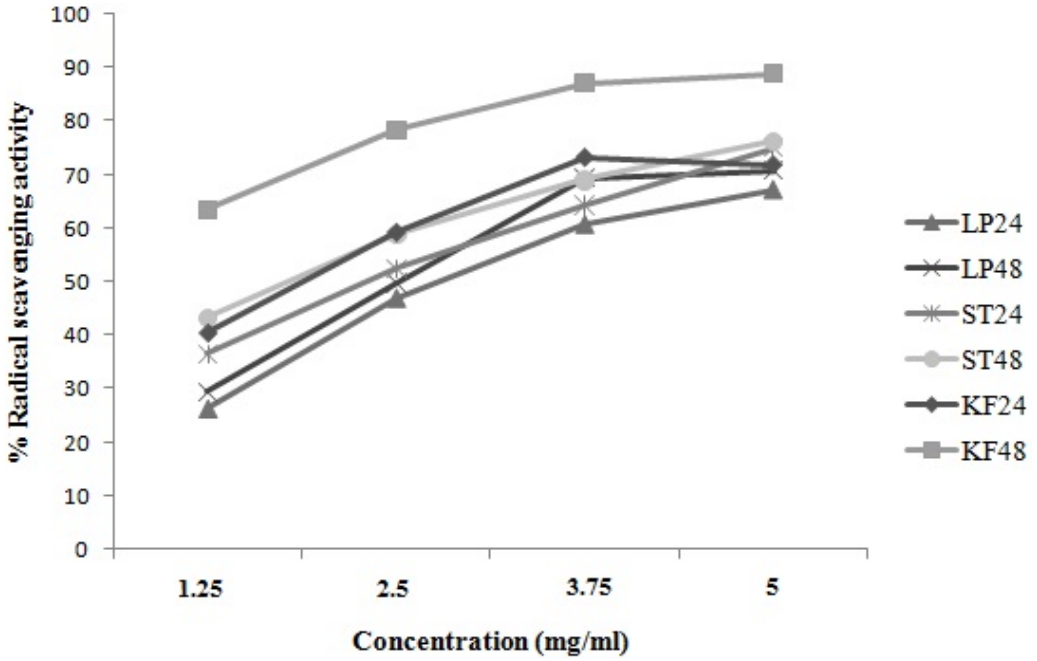

Figure 1. DPPH scavenging effect of fermented soy whey at different concentrations.

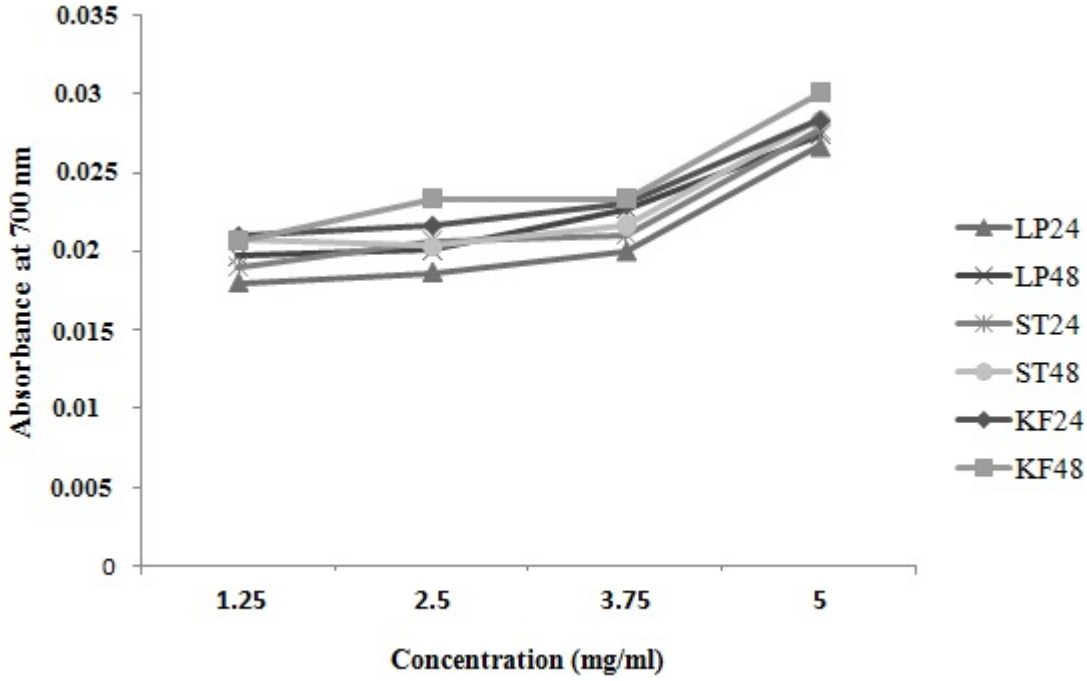

Figure 2. Reducing power of fermented soy whey at different concentrations.

the reductive ability, according to the method of Oyaizu (1986). The reducing capacity of a compound may provide as a significant indicator of its potential antioxidant activity (Meir et al., 1995). The antioxidant activity of assumed antioxidants has been attributed to various mechanisms, among which are the prevention of chain initiation, the binding of transition metal ion catalysts, decomposition of peroxides, the prevention of continued hydrogen abstraction, the reductive capacity, and radical scavenging (Diplock, 1997). Like the DPPH

scavenging activity, the reducing power of all extracts increased with increasing concentration. However, all fermented soy whey extracts did not show a significant different activity between samples at all concentration and conditions ($p > 0.05$). The reducing capacity of these extracts relatively low with absorbance mostly under 0.03. Only KF48 gave absorbance 0.03 at 5.0 mg/ml. Although the reducing power of a substance may be an indicator of its potential antioxidant activity, there is not necessarily a linear correlation between these two activities (Yildirim et al., 2001).

Oxidation is an essential reaction in all living organisms. The formation of free radicals and other reactive oxygen species are unavoidable during the oxidative metabolic process. These reactive radicals play a significant function in signal transduction (Hancock et al., 2001). However, excessive free radicals can cause oxidative stress. Oxidative stress participates a major part in the development of chronic and degenerative illness such as cancer, autoimmune disorders, rheumatoid arthritis, cataract, aging, cardiovascular and neurodegenerative diseases (Willcox et al., 2004; Pham-Huy et al., 2008). The human body has some systems to neutralize oxidative stress by producing antioxidants, which are either naturally produced in situ, or supplied through foods and/or supplements. The synthetic antioxidants known have high antioxidative effect, yet its use is restricted because of the potential health hazards, but natural compounds were concern about their safety (Ito et al., 1986). Therefore, the development and use of natural antioxidants as an alternative to synthetic ones is of great interest. Most studies on natural antioxidants derived from edible sources such as vegetables, fruits, grains and mushrooms. Recent efforts to use waste for the production of antioxidants increased. Wei and Chiang (2009) reported that hydrolysates of porcine blood possess antihypertensive and antioxidant activities. Recovery of carotene (Ahmad et al., 2008) and flavonoid extraction from knotwood and bark (Pietarinen et al., 2006) revealed antioxidative compounds.

Based on the above data, duration of fermentation clearly have a significant influence on the increase in antioxidant activity. In all samples, the scavenging capacity of DPPH radicals increased with the length of fermentation time. The same phenomenon was also reported by other researchers to test the antioxidant activity of different substrates and microbes. Chang et al. (2009) ferment soybean for 0, 1, 2, 5 and 10 days with *Rhizopus oligosporus* and tempeh fermented for 10 days exhibited the highest antioxidant activities than the others. Mao-tofu fermented for 3, 5, 7 and 9 days by a strain of *Mucor* sp. showed that longer fermentation time of Mao-tofu gave the extracts a higher extraction yield, higher degree of hydrolysis of the protein fractions and higher antioxidant activity (Hang and Zhao, 2011). Improved protein solubility caused by the decomposition of complex proteins into peptide chains that are shorter and the molecular weight decreases. This is caused by microorganisms that secrete the enzyme activity of protease (Farnworth, 2005). In some research on antioxidants fermented soy milk, it is known that certain bioactive peptides in phyto-milk has antioxidant activity. In the fermentation of soy milk, the presence of ring structures on certain peptides, such as the phenolic ring in the tyrosine or histidine imidazole compounds can stabilize a free radical, because the compound has the potential to donate hydrogen to free radicals (Lv et al., 2009; Elias et al., 2008).

KF48 clearly has higher antioxidant content than other samples. Kefir differs from other milk products, the product of fermentation with a mixed group of microflora confined to a matrix of discrete “kefir grains” (Marshall and Cole, 1985). In the kefir grains, lactic acid bacteria and yeasts are embedded in a slimy polysaccharide matrix named kefiran, thought to be produced by the lactobacilli in the grain (La Riviere et al., 1967). Given that microbial consortia can perform even more complicated tasks and endure more changeable environments than monocultures can, they represent an important new frontier for synthetic biology. Mixed populations can perform functions that are difficult or even impossible for individual strains or species. Balancing two or more tasks so that they are efficiently completed within one organism can pose insuperable challenges in some situations (Brenner et al., 2008). Scavenging effect of KF48 compared to soymilk kefir (Liu et al., 2005) revealed interesting study. DPPH scavenging radical capacity of soymilk also increased with longer fermentation, however there is no significant different between 24 and 32 h incubation.

At that both time, the percentage inhibition of DPPH radical were about 90%. Based on those data, the potential antioxidant produced by kefir with soy whey substrate can be paralleled with the kefir from soy milk. However, more in-depth study is needed on this, given the fermented soy milk at 20°C (Liu et al., 2005) while the soy whey is fermented at a temperature of about 25°C. Besides the protein content of soy milk and whey are different. In this case, the composition of soy milk proteins relatively intact while the soy whey proteins are small proteins (2S and 7S).

Conclusion

On the basis of the results of this study, all extracts exhibited a potentially antioxidant activities. KF48 extracts have significant highest antioxidant activity compared to other that might be used as a rich source of natural antioxidants, as a food supplement or in the pharmaceutical industry. Further investigation of individual compounds, other *in-vitro* bioactive capacity analysis such as antimicrobial, antimutagenic and antihypertensive are needed for all extracts.

ACKNOWLEGEMENTS

This study is supported by Directory General of Higher Education (DGHE), Ministry of National Education Indonesia through Program Academic Recharging 2011 and Islamic Azad University, Iran. Authors are also gratefull to Dr. Tabatabaee from Islamic Azad University, Yazd branch.

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