



Antioxidant Properties of Soy Milk Fermented with *Lactobacillus paracasei* KUMBB005

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ABSTRACT

In addition to be natural, nutritious and safe, epidemiological studies have shown that traditional fermented soybean products are remarkable sources of bioactive phytochemicals offering potent antioxidant and anticarcinogenic effects. Among the phytochemicals isoflavones, saponins, phytosterols and tocopherols, most of the research focused on isoflavones for antioxidant properties. Thus fermented soy milk with *Lactobacillus paracasei* KUMBB005 a test probiotic strain was evaluated for its Total Phenol Content (TPC) and its antioxidative potential by DPPH activity and FRAP assay. TPC was found to be 300 mg/g, DPPH reduction radical scavenging activity was 27% and FRAP assay has also increased, revealing that, soy milk fermented with our test probiotic strain has credible antioxidative potential.

Keywords: Soymilk, *Lactobacillus paracasei* KUMBB005, TPC, DPPH, FRAP.

INTRODUCTION

Oxidative damage plays a drastic pathological role in humans resulting in complications such as cancer, emphysema, cirrhosis, atherosclerosis, arthritis and accelerated aging^{1, 2}. Oxidative damage is caused by an imbalance between the generation of Reactive Oxygen Species (ROS) and the activity of the body's antioxidant defenses. ROS such as the superoxide anion radical, hydroxyl radical, singlet oxygen and hydrogen peroxide are known to generate both by normal cellular metabolism and by exogenous factors². Therefore antioxidants are believed to play a very important role in the body defense system against ROS. Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have limited use as they are suspected to be carcinogenic. The search for natural antioxidants as alternatives to synthetic ones is a subject of great interest nowadays³. Many kinds of natural compounds have shown antioxidant activity against different kinds of ROS. As an intensely investigated topic, more and more attention has been directed towards soybeans and their products that contain various amounts of phytochemicals (isoflavones, saponins, phytic acid, phytosterols, Kunitz and Bowman-Birk trypsin inhibitors and phenolic acids) shows functional, antioxidants and radical scavenging properties. These effects have partly been ascribed to the higher antioxidant properties of fermented soybean compounds especially isoflavones particularly aglycones such as daidzein and genistein exert the antioxidant potential and found to execute anticancer activity against various cancers^{4, 5}. In addition to be natural, nutritious and safe, epidemiological studies have shown that traditional fermented soybean products exhibit potent anticarcinogenic effects. The observation highlighted that an effective intake of isoflavones from the soy-based foods depends mainly on the aglycone content in

preventing chronic disease. Several reports have underlined the release of antioxidant compounds during fermentation process by enzymatic conversion of the conjugated isoflavones into the respective bioactive aglycones^{6, 7}. Based on these premises, the aim of this study was to determine the Total Phenol Content (TPC) and antioxidant profile of the product from soy milk fermented with *Lactobacillus paracasei* KUMBB005.

MATERIALS AND METHODS

Preparation of soy milk

Soy milk was prepared according to previously described by Sumarna (2010)⁸. The soybeans 250g was soaked in water for 12 h at room temperature. After decanting the water, the soaked soybeans were ground in a blender for 5 min, the extract was then filtered through cheese cloth. The soy milk was then autoclaved at 121° C for 45 min.

Fermentation of soymilk with *Lactobacillus paracasei* KUMBB005

The sterile soymilk 250 ml in 500 ml conical flask was inoculated with active culture of *Lactobacillus paracasei* KUMBB005 (5%v/v) and incubated at 37°C for 48 h. After fermentation the soy milk was freeze dried for extraction.

Extraction of isoflavone

The extraction of crude isoflavone from soymilk were performed by Ping et al (2012)⁹ with slight modification. The freeze-dried samples 10 g were extracted with 20 ml of 80% ethanol in a flask and were stirred for 1 h at 60°C. The extracted solutions were centrifuged at 5000× g for 10 min, and the supernatants were dried by evaporation. Following the evaporation of aqueous ethanol, the insoluble residue was dissolved in 10 ml of 80% ethanol. The extract obtained was used for further antioxidant activity.



Total phenol content

Total phenol contents (TPC), of the extracts were determined by method as developed by Slinkard and Singleton (1977)¹⁰. Gallic acid is used as a standard; stock solution of gallic acid was prepared by dissolving 0.5 g gallic acid in 10 ml of ethanol in a 100 ml volumetric flask and diluting to volume with double distilled water. To prepare a calibration curve 0, 1, 2, 3, 5 and 10 ml of gallic acid stock solution were added into 100 ml volumetric flask separately and then diluted to volume with double distilled water. The resultant solutions contained concentrations of 0, 50, 100, 150, 250 and 500 mg/l gallic acid¹¹. The sample 40 μ l was pipetted into separate cuvette and 3.16 ml of double distilled water was added. Folin–Ciocalteu's reagent 200 μ l was added and mixed well. After 8 min, 600 μ l of 20% sodium carbonate solution was mixed thoroughly. The solution was allowed to stand at 40° C for 30 min and absorbance of each solution was noted at 765 nm against the blank (without phenolic solution).

2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The effect of DPPH radical was measured as described by Nagai et al (2012)². The assay mixture contained 0.03 ml of 1.0 mM of DPPH radical solution in ethanol, 0.24 ml of 99% of ethanol and 0.03 ml of sample solution. The mixture was rapidly mixed and after 30 min of incubation the absorbance of the solution was measured at 517 nm. Ascorbic acid (0.1 and 1.0 mM) was used as standard and distilled water was used as negative control. Analysis was performed in triplicate and the average values are reported. Inhibition of free radical DPPH was calculated according to the formula

$$\% \text{ of inhibition} = (\text{Abs of control} - \text{Abs of sample} / \text{Abs of control}) \times 100$$

Ferric reducing antioxidant power FRAP assay

The ferric reducing antioxidant power of fermented soy extract was measured according to the method developed by Benzie and Strain (1996)¹² and Chaiyasut et al (2010)¹³ with slight modification. FRAP solution was freshly prepared by mixing 25 ml of sodium acetate buffer (pH 3.6, 300 mM), 2.5 ml of 10 mM TPTZ solution in 40 mM HCl solution and 2.5 ml ferric chloride (20mM) solution. Samples at different concentrations (100, 200, 300, 400 and 500 μ l/ml) were then added to 3 ml of FRAP reagent and the reaction mixture was incubated at 37° C for 30 min. The increase in absorbance at 593 nm was measured and the results were compared with standard ferrous sulphate. Analysis was performed in triplicate and the average values are reported.

RESULTS AND DISCUSSION

Total phenol content

Phenolic compounds are powerful antioxidants, due to hydroxyl groups they act as free radical terminators. Their

bioactivities may be related to their abilities to chelate metals, inhibit lipoxygenase and scavenge free radicals. As a chemical structure of phenolic compounds is responsible for their antioxidant activity, measurement of TPC could be allied to antioxidant properties¹⁴. The amount of total phenol was determined with the Folin–Ciocalteu reagent which is sensitive to reducing compounds including polyphenols, thereby producing a blue colour upon reaction¹⁵. Thus TPC can be determined spectrophotometrically at 765 nm. Gallic acid was used as a standard compound and the total phenols were expressed as mg/g Gallic Acid Equivalent (GAE) using the standard curve equation: $y=0.005x + 0.159$ $R^2= 0.996$ as shown in Figure 1, Where y is absorbance at 765 nm and x is TPC¹⁶. Soy milk fermented with *Lactobacillus paracasei* KUMBB005 was pooled with 80% of ethanol and centrifuged, the supernatant was dried and the residue was dissolved in 10 ml of 80% ethanol. The phenolic content in the ethanolic extract of *Lactobacillus paracasei* KUMBB005 fermented soymilk was found to be 300 mg/g. Dajanta et al (2012)¹⁷ studied Thua Nao extracts and reported that the high contents of phenolics were responsible for the higher free radical scavenging effects and total antioxidant activities. Yao et al (2010)¹⁸ stated that a high level of TPC is responsible for existence of high levels of isoflavones exerted by microbial fermentation. As related to previous reports the present study showed that the fermented soy with *Lactobacillus paracasei* KUMBB005 contain high amount of phenolic compounds and could be able to exhibit the greatest antioxidant activity by radical scavenging activity and ferric reducing power.

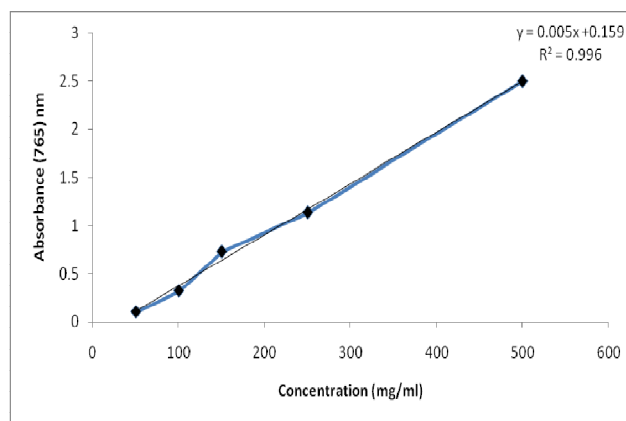


Figure 1: Standard curve of gallic acids

DPPH radical scavenging activity

DPPH is one of the stable free radicals which is of violet color, accepts an electron or hydrogen atom from the antioxidant compounds and is converted into a colorless or somewhat yellow diamagnetic DPPH molecule¹¹. As the data presented in Figure 2, the inhibition of DPPH absorption for the ethanolic extracts of fermented soy milk by *Lactobacillus paracasei* KUMBB005 with IC₅₀ value of 27 mg/ml showed effective activity than that of standard IC₅₀ value of 20 mg/ml. Previous study by Hubert et al (2007)⁶ states that after fermentation, soy germ

extracts exerted a more potent scavenging effect towards O_2 . In the study conducted by Pyo et al (2005)¹⁹ it is reported that the soybean fermented with *B. thermophilum* KFRI 00748 extract showed highest radical scavenging. Earlier Lee et al (2005)²⁰ reported that soybeans fermented with *Monascus* MFS 31499 and MFS-31527 showed effective antioxidant activity and scavenging ability on DPPH. Related to the earlier studies, the present study reports that the DPPH radical scavenging activity of the fermented soy milk extracts were effective than that of standard ascorbic acid. From this it can be predicted that increase in the concentration of isoflavones aglycones during fermentation may increase superoxide scavenging activity. Therefore the extract has the ability to serve as a good antioxidant by inhibiting free radicals.

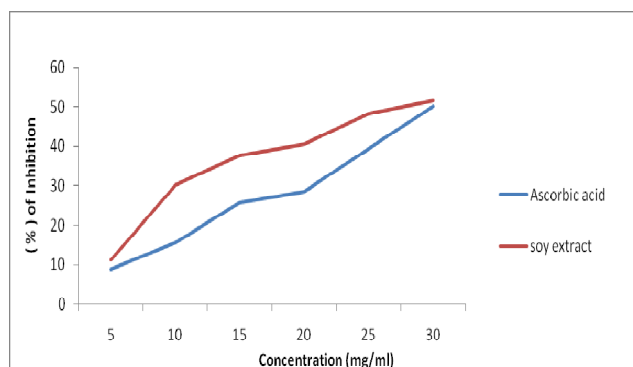


Figure 2: DPPH radical scavenging activity of soy extract fermented with *Lactobacillus paracasei* KUMBB005

Ferric reducing antioxidant power (FRAP) assay

FRAP is a colorimetric method assayed based on the reduction of a ferric tripyridyltriazine (TPTZ) complex to its ferrous form²¹. Antioxidant compounds which act as a reducing agent exerts their effects by donating hydrogen atom to ferric complex and thus break the radical chain reaction. In the present study antioxidant capacity of fermented soy with *Lactobacillus paracasei* KUMBB005 and the standard ferrous sulphate were studied. The ethanol extracts of soy milk fermented with *Lactobacillus paracasei* KUMBB005 showed increased ferric reducing power with the increased concentration as standard antioxidants. Yao et al (2010)¹⁸ has reported that the black soy bean fermented with *Bacillus* sp., exhibited higher antioxidant activity compared to *Aspergillus* sp., and Yeast. Previously Yadav et al (2012)³ has reported that the fermented product showed enhanced FRAP reducing power compared to unfermented one. The result was similar to studies conducted by Dajanta et al (2013)²² and Chonkeeree et al (2013)²³ that fermentation of black and yellow soybeans with *Bacillus subtilis* showed increased ferric reducing power compared to unfermented soybeans. Comparable to previous reports the data present in Figure 3 proved the absorbance of fermented soy with *Lactobacillus paracasei* KUMBB005 has increased ferric reducing power as the concentration of sample and standard increase. It gives an idea that the reducing power was based on the reduction of ferric

cyanide complex to the ferrous form in the presence of antioxidant. As reported by early studies it is estimated that higher phenolic content results in higher the Fe^{2+} ion chelating activity. Therefore the soymilk fermented with *Lactobacillus paracasei* KUMBB005 could support antioxidant effect.

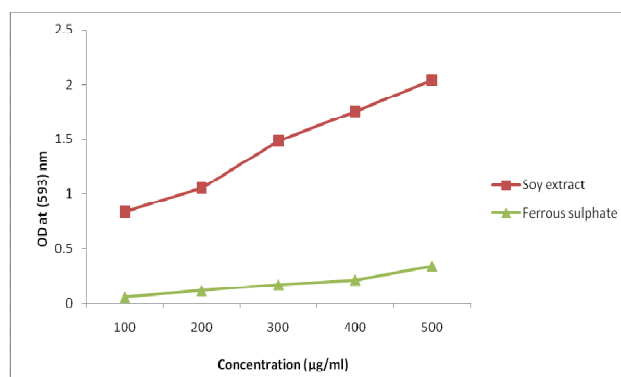


Figure 3: Ferric reducing antioxidant power assay soy extract fermented with *Lactobacillus paracasei* KUMBB005

CONCLUSION

The present study focused on antioxidative potential of fermented soymilk with *Lactobacillus paracasei* KUMBB005, the fermented extract showed the TPC of 300mg/g, the DPPH radical scavenging activity with IC_{50} of 27 mg/ml and FRAP showed increased ferric reducing power with increasing concentration of sample. From this it is hypothesized that the fermentation of soymilk with probiotic bacterial culture could catalyzes the release of isoflavones into aglycones and thereby enhance the phenolic content. Based on the antioxidant potential of the extract from soymilk fermented with our test strain *Lactobacillus paracasei* KUMBB005, need to be investigated for its potential health promoting activity.

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REFERENCES

1. Abubakr MAS, Hassan Z, Muftah M, Imdakim A, Sharifah NRSA, Antioxidant activity of lactic acid bacteria (LAB) fermented skim milk as determined by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and ferrous chelating activity (FCA), African Journal of Microbiology Research, 6, 2012, 6358-6364.
2. Nagai T, Tanoue Y, Kai N, Suzuki N, *In Vitro* antioxidative activity and antihypertensive activity of soy sauce cake derived from the manufacturing of Japanese style fermented soy sauce, Food and Nutrition Sciences, 3, 2012, 1118-1127.
3. Yadav D, Vij S, Hati S, Singh BP, Dhanday M, Dahiya M, Vandna, Evaluation of total antioxidant activity of soy yoghurt, Indian Journal of Dairy Science, 65, 2012, 220-224.

4. Hu SC, Hong K, Song YC, Liu JY, Tan RX, Biotransformation of soybean isoflavones by a marine *Streptomyces* sp. 060524 and cytotoxicity of the products, *World Journal of Microbiology and Biotechnology*, 25, 2009, 115–121.
5. Nam DH, Kim HJ, Lim JS, Kim KH, Park CS, Kim JH, Lim J, Kwon DY, Kim IH, Kim, JS, Simultaneous enhancement of free isoflavone content and antioxidant potential of soybean by fermentation with *Aspergillus oryzae*, *Journal of Food Science*, 76, 2011, 194-200.
6. Hubert J, Berger M, Nepveu F, Paul F, Dayde J, Effects of fermentation on the phytochemical composition and antioxidant properties of soy germ, *Food Chemistry*, 109, 2007, 709-721.
7. Samruan W, Oonsivilai A, Oonsivilai R, Soybean and fermented soybean extract antioxidant activities, *World Academy of Science, Engineering and Technology*, 6, 2012, 1221-1224.
8. Sumarna, Hydrolysis of bioactive isoflavone in soymilk fermented with β -glucosidase producing lactic acid bacteria from local fermented foods of Indonesian, *Malaysian Journal of Microbiology*, 6, 2010, 30-40.
9. Ping SP, Shih SC, Rong CT, King WQ, Effect of isoflavone aglycone content and antioxidant activity in natto by various cultures of *Bacillus subtilis* during the fermentation period, *Journal of Nutrition Food Science*, 2, 2012, 1-5.
10. Slinkard K, Singleton VL, Total phenol analysis: Automation and comparison with manual methods, *American Journal of Enology and Viticulture*, 28, 1977, 49-55.
11. Rehman J, Khan IU, Asghar MN, Antioxidant activity and GC-MS analysis of *Grewia optiva*, *E3 Journal of Biotechnology and Pharmaceutical Research*, 4, 2013, 4-21.
12. Benzie IF, Strain JJ, The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay, *Analytical Biochemistry*, 239, 1996, 70-76.
13. Chaiyasut C, Kumar T, Tipduangta P, Rungsevijitprapa W, Isoflavone content and antioxidant activity of Thai fermented soybean and its capsule formulation, *African Journal of Biotechnology*, 9, 2010, 4120-4126.
14. Mujic, Sertovic E, Jokic S, Saric Z, Alibabic V, Vidovic S, Zivkovic J, Isoflavone content and antioxidant properties of soybean seeds, *Croatian Journal of Food Science and Technology*, 3, 2011, 16-20.
15. Sahu R, Saxena J, Screening of total phenolic and flavonoid content in conventional and non-conventional species of curcuma, *Journal of Pharmacognosy and Phytochemistry*, 2, 2013, 176-179.
16. Maurya S, Singh D, Quantitative analysis of total phenolic content in *Adhatoda vasica* nees extracts, *International Journal of PharmTech Research*, 2, 2010, 2403-2406.
17. Dajanta K, Chukeatirote E, Apichartsrangkoon, A, Nutritional and physicochemical qualities of Thua Nao (Thai Traditional Fermented Soybean), *Chiang Mai Journal of Science*, 39, 2012, 562-574.
18. Yao Q, Xiao-nan J, Dong PH, Comparison of antioxidant activities in black soybean preparations fermented with various microorganisms, *Agricultural Sciences in China*, 9, 2010, 1065-1071.
19. Pyo YH, Lee TC, Lee YC, Enrichment of bioactive isoflavones in soymilk fermented with [beta]-glucosidase-producing lactic acid bacteria, *Food Research International*, 38, 2005, 551-559.
20. Lee CH, Yang L, Xu JZ, Yeung SYV, Huang Y, Chen ZY, Relative antioxidant activity of soybean isoflavones and their glycosides, *Food Chemistry*, 90, 2005, 735-741.
21. Henriquez C, Almonacid S, Chiffelle I, Valenzuela T, Araya M, Cabezas L, Simpson R, Speisky H, Determination of antioxidant capacity, total phenolic content and mineral composition of different fruit tissue of five apple cultivars grown in Chile, *Chilean Journal of Agricultural Research*, 70, 2012, 523-536.
22. Dajanta K, Janpum P, Leksing W, Antioxidant capacities, total phenolics and flavonoids in black and yellow soybeans fermented by *Bacillus subtilis*: A comparative study of Thai fermented soybeans (thua nao), *International Food Research Journal*, 20, 2013, 3125-3132.
23. Chonkeeree A, Chaowarat M, Chumchuere S, Antioxidant activity and total phenolic content of dried fermented soybean products fermented with *Bacillus subtilis* and Lab: Potential for functional food application, *International Proceedings of Chemical, Biological and Environmental*, 58, 2013, 6-21.

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