

# Isoflavone Changes During Fermentation of Kerandang (*Canavalia virosa*) Milk using *Lactobacillus plantarum-pentosus* and its Anticancer Activity

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## ABSTRACT

Kerandang (*Canavalia virosa*) seeds contain bioactive compounds such as isoflavone whose beneficial effects need to be explored. The intake of isoflavone genistein and daidzein has been shown to provide protection against reactive oxygen species, cardiovascular disease and cancer disease among other benefits. The objective of this research was to investigate the isoflavone changes during fermentation of Kerandang milk and to evaluate its anticancer activity on colon cancer 26 cells. Kerandang milk was fermented using *L. plantarum-pentosus* T14 and *L. plantarum-pentosus* T35 for 24 hr at 37 °C. The *L. plantarum-pentosus* T14 and *L. plantarum-pentosus* T35 growth rates, total lactic acid, pH and isoflavone content were analyzed. The viability of colon cancer 26 cells was assayed using the WST-8 method. The results indicated that a change occurred from isoflavone glucoside to isoflavone aglycone during fermentation using *L. plantarum-pentosus* T14 and *L. plantarum-pentosus* T35. The methanol extract of isoflavone from Kerandang milk fermented by *L. plantarum-pentosus* T14 and *L. plantarum-pentosus* T35 had anti cancer activity as indicated by the decreased viability of colon cancer 26 cells. The methanol extract of isoflavones from Kerandang milk fermented using *L. plantarum-pentosus* T14 has greater anti cancer activity than the methanol extract of isoflavones from Kerandang milk fermented using *L. plantarum-pentosus* T35 and methanol extract of Kerandang milk. In conclusion, fermentation of kerandang milk with lactic acid bacteria can increase its functional value, such as anticancer activity.

**Keywords:** Kerandang (*Canavalia virosa*) milk, fermentation, isoflavon, colon cancer 26 cell, cell viability

## INTRODUCTION

Kerandang (*Canavalia virosa*) is classified as a legume and can be found in Africa, South America, Australia, India, and in the Philippines and Indonesia (Smartt, 1985; Gaydou

*et al.*, 1992; Eke *et al.*, 2007). This plant grows and produces pods and seeds with the advantages of being able to grow on nutrient-poor soils and being resistant to drought, so that in the dry season, this plant can survive on sandy beachland (Eke *et al.*, 2007). Kerandang crops grow and produce seed on

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beaches in the Bantul and Kulon Progo regencies, Yogyakarta Special Region, Indonesia (Djaafar *et al.*, 2010).

Product or fermented beverage from the fermentation of lactic acid bacteria associated with milk was initially intended as a fermented beverage however, various types of beans have also been used as a basic ingredient of fermented beverages, with the most common production involving the extraction of the juice of the beans with a certain ratio of water and beans, followed by fermentation using lactic acid bacteria (Giyarto *et al.*, 2011).

Some research on fermented legume milk using lactic acid bacteria has investigated reducing the content of oligosaccharides (which can cause flatulence in legume materials) and improving the content of isoflavone (a glycone that has the functional value in legume products). For example, Mital and Steinkraus (1975) have shown that fermentation of soymilk with *Lactobacillus celebiosis* NRRL-B-1840, *L. fermentii* NRRL-B-585, *L. plantarum* and *Streptococcus thermophilus* B246 can reduce the content of raffinose and stachyose. Soymilk fermentation using lactic acid bacteria can increase the concentration of the isoflavone aglycone because lactic acid bacteria produce  $\beta$ -glucosidase that can hydrolyze the  $\beta$ -glycosidic bond into isoflavone glucoside (Tsangalis *et al.*, 2002; Pyo *et al.*, 2005a; Pyo *et al.*, 2005b; Otieno *et al.*, 2005). Soymilk fermented by *Lactobacillus* (*L. plantarum* ASCC 276, *L. fermentum* VRI-003, *L. casei* ASCC 290 and *L. acidophilus* ATCC 4962) can improve the concentration of the isoflavone aglycone after 24 hr fermentation at 37 °C with an average cell viability of  $8.5 \times 10^{10}$  colony forming units (CFU).g<sup>-1</sup> (Tang *et al.*, 2007).

Legumes contain isoflavones which provide a potential protective effect against many diseases and conditions including cancer, cardiovascular disease, osteoporosis and menopause symptoms (Thompson *et al.*, 2006; Messina and Wood, 2008; Xu *et al.*, 2010).

Albulescu and Popovici (2007) in their discussion on isoflavonoids noted that they are a subclass of the more ubiquitous flavonoids, which are naturally occurring polyphenols. Isoflavonoids differ from other classes of flavonoids by their greater structural variability, their frequent presence in plants in their free form rather than as a glycoside and by the greater frequency of isoprenoid substitution. They are divided into subclasses depending on the oxidation level of the central pyran ring. Isoflavones are the most abundant of the subclasses of isoflavonoids but they have a very limited distribution in nature, and soybeans, red clover, peas, nuts, grain products and certain herbs, are the main natural dietary source of these compounds. Until now, 12 different soybean isoflavone isomers have been reported. The primary isoflavones in soybeans are the glucosides (genistin, daidzin, glycitin, 6''-O-malonylgenistin and 6''-O-acetyldaidzin) and their aglycones, (genistein, daidzein and glycitein). Genistein, one of the best known and studied isoflavones, has been isolated from soybeans and has been the focus of scientific research since 1966. Daidzein is also classified as a phytoestrogen since it is a plant-derived non steroidal compound that possesses estrogen-like biological activity, with weak estrogenic and weak anti estrogenic effects. Genistein glycosides are the most abundant and daidzein glycosides are the second most abundant isoflavones in soybeans and soy foods, while unfermented soy foods, such as tofu, contain daidzein, mainly in its glycoside forms. (Albulescu and Popovici, 2007). Fermented soy foods, such as tempeh, soybean milk and miso, contain significant levels of aglycone (Murphy *et al.*, 1999; Pyo *et al.*, 2005a; Pyo *et al.*, 2005b; Tang *et al.*, 2007).

Kerandang has been known to contain genistin, daizin, genistein and daizein (Djaafar *et al.*, 2013). Isoflavones are in the phytoestrogen group and have estrogen-like properties, but their potential is less than that of estrogen (Albulescu and Popovici, 2007). Isoflavones have structural

polyphenols that act as antioxidants, among other capabilities as reducing agents, hydrogen donors, chelating agents, and single oxygen quenchers (Albulescu and Popovici, 2007; Chen and Anderson, 2002; Mustafa *et al.*, 2010). The anti carcinogenic activity of isoflavones can be effected through the inhibition of cancer cell proliferation and apoptosis (programmed cell death induced in the cancer cells) (Han *et al.*, 2007; Bronikowska *et al.*, 2010). The objectives of the current research were to investigate the isoflavone change during fermentation of Kerandang milk using *L. plantarum-pentosus* and to evaluate its anticancer activity on colon cancer 26 cells.

## MATERIALS AND METHODS

### Kerandang beans

Kerandang beans were obtained from wild plants growing on sandy beachland in Bugel village, Panjatan district, Kulon Progo regency, Yogyakarta, Indonesia. Harvesting was conducted by picking old brown pods from the trees. Beans were removed from the pods and the beans were dried to 10% water content and the seed epidermis was removed mechanically using an abrasive peeler to produce yellowish, clean, peeled beans. Lactic acid bacteria cultures

Two strains of lactic acid bacteria (*L. plantarum-pentosus* T14 and *L. plantarum-pentosus* T35) were obtained from the Food Nutrition Culture Collection, Gadjah Mada University, Yogyakarta, Indonesia. Culture stock was kept in 10% glycerol and 10% skim milk (ratio 1:1) in sterile 1.5 mL polyethylene screw cap tubes at -40 °C. The strains were rejuvenated in De Mann Rogosa Sharpe (MRS) broth (Oxoid, Jakarta, Indonesia) at 37 °C for 24 hr.

### Cancer cell line

Colon cancer 26 cells were obtained from the Animal Cell Technology Laboratory, Faculty of Agriculture, Ehime University, Matsuyama, Japan.

### Preparation of Kerandang milk

Preparation of Kerandang milk was conducted as described by Chun *et al.*, (2007), with modifications. Whole beans were washed and soaked for 3 hr in water. The ratio of dry beans to water was 1:6 (weight per volume, w/v). The water was decanted and the beans were washed. The swollen beans were ground with hot water (90 °C) using a blender (Waring®; East Windsor, NJ, USA) for 2 min at high speed. The ratio of dry beans to water used for grinding was 1:10 (w/v). The slurry was filtered through a double-layer of cheese cloth. The resultant Kerandang milk was dispensed in 100 mL screw cap bottles and then pasteurized for 20 min at 80 °C.

### Fermentation of Kerandang milk

The test inocula were prepared by transferring the cultures from the MRS broth medium into Kerandang milk and sub culturing in the same medium twice, with incubation at 37 °C for 20–24 hr. samples of 150 mL of Kerandang milk were inoculated with a single culture (0.2%, volume per volume, v/v) and then incubated at 37 °C for 24 hr. Each bottle were taken out and sampled aseptically at 6 hr intervals during fermentation. Samples were directly analyzed for pH using a pH meter, total acid using titratable acidity method (Association of Official Analytical Chemists, 2006) and lactic acid bacteria cell growth using the plate count method on MRS agar (Case and Johnson, 1984).

### Determination of pH

The pH of the aliquots withdrawn every 6 hr during the fermentation was monitored using a microprocessor pH meter (Orion 3 Start; Thermo Scientific; Waltham, MA, USA) at 27 °C after calibrating with fresh standard buffers at pH 4.0 and 7.0.

### Determination of total acid and lactic acid bacteria cell growth

Titratable acidity was determined by

the method of Case and Johnson (1984) using titration with a 0.1 N NaOH solution expressed as the percentage of lactic acid. The cell number was measured in triplicate using the pour plate method (Case and Johnson, 1984) with lactobacilli MRS media (Oxoid; Jakarta, Indonesia) city, state if applicable, country). A sample of 1 mL was serially diluted with 0.85% NaCl solution and then 100  $\mu$ L of diluted sample was placed on a sterile plate. MRS medium with 1.5% agar and 0.8% CaCO<sub>3</sub> was poured onto the plate and mixed carefully. After incubation at 37 °C for 24 hr, single colonies were counted.

### Ultra performance liquid chromatography analysis of isoflavone

Extraction quantification of glucosides and aglycones from fermented Kerandang milk was performed according to the methods of Tsangalis *et al.* (2002) and Pyo *et al.* (2005a). A supernatant from lactic acid fermentation of the Kerandang milk was filtered through a bond elute C-18 (VARIAN) and then eluted with 2 mL of 80% methanol. The insoluble residue was separated by centrifugation (Centrifuge 5804 R) at 4,000 revolutions per minute (rpm) at 4 °C for 10 min and filtered through Millex-HV PVDF 0.45  $\mu$ m prior to transferring to Ultra performance liquid chromatography (UPLC) vials.

Transformation of isoflavone was carried out using liquid chromatography with a quaternary pump, a diode array ultraviolet visible (UV-Vis) detector and a vacuum degasser. The UPLC ACQUITY™ PDA-ELS system (Waters Cooperation, Milford, MA, USA) was equipped with a PDA e $\lambda$  Detector, Binary Solvent Manager, Sample Manager and an Acquity UPLC® BEH C18 1.7 mm (2.1  $\times$  100 mm) reverse-phased column which was set thermostatically at 25 °C. It was used to separate the isoflavone isomers. UPLC linear gradation was used to isolate the isoflavones for detection composed of 10% (v/v) acetonitrile and 0.1% (v/v) formic acid in water (solvent A) and 100% acetonitrile containing 0.1% formic

acid (solvent B). The pump was set at a flow rate of 0.4 mL.min<sup>-1</sup>. After injection of a 25  $\mu$ L sample or isoflavone standard into the column, solvent A was set at 100% for 1 min, reduced to 60% over 5 min and finally 100% for 1 min prior to the next injection. The diode array UV-Vis detector was set at a wavelength of 260 nm to detect the isoflavone glucosides and aglycones. Mixed standards containing all isoflavone glucosides and aglycones were used for quantification of isoflavone. A single standard was also prepared for peak identification.

### Cell line maintenance

The colon cancer 26 cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS). Each 2 d, the cell lines were maintained; after incubation, the DMEM was discarded and each cell was washed with 5 mL phosphate buffer saline (PBS). Each colon 26 cell sample was treated with 1 mL of 0.25% trypsin- ethylenediaminetetraacetic acid (EDTA) for 1–2 min in an incubator (37°C; 5% CO<sub>2</sub>) and then 1 mL FBS was added. Cell suspension was collected, added with 3.5 ml DMEM supplemented with 10% FBS and centrifuged (KUBOTA 4000; Fujioka, Japan) at 1,000 rpm for 5 min. After centrifuging, the supernatant was removed and suspended with DMEM medium. A sample of 2 mL cell suspension was added to a Petri dish containing 10% FBS-DMEM and then placed in an incubator (CO<sub>2</sub> Incubator; Sanyo Electric Co. Ltd.; Moriguchi, Japan) at 37 °C and 5% CO<sub>2</sub> for 2 d.

### Cancer cell viability assay

Cancer cell viability was determined using the WST-8 assay according to the methods of Tomimori *et al.* (2012). Each sample at 16  $\mu$ g-mL<sup>-1</sup>; 32  $\mu$ g-mL<sup>-1</sup> and 65  $\mu$ g-mL<sup>-1</sup> concentration was prepared using the medium solution containing 20% FBS-2DMEM. Cells were plated at 2  $\times$  10<sup>4</sup> cells. mL<sup>-1</sup>. The cells was added into 66-well

plates (MICROTEST™ Tissue Culture Plate, 96 well; BD Falcon™; Franklin Lakes, NJ, USA) at 100 µL per well. The plates were placed in the incubator for 3 hr at 37 °C and 5% CO<sub>2</sub>. Then, each prepared sample was added at 100 µL per well and incubated at 37 °C and 5% CO<sub>2</sub> for 2 d. Then, 20 µL WST-8 solution was added and each plate was incubated for a further 1 hr at 37 °C and 5% CO<sub>2</sub>. Absorbance at 450 nm was measured using an automated micro plate reader (Model 680; Biorad; Hercules, CA, USA). The cell viability in untreated control cultures was considered 100%, and the cell viability of each treated group was compared relative to this value. The cell viability was determined using Equation 1:

Cell viability (%) =

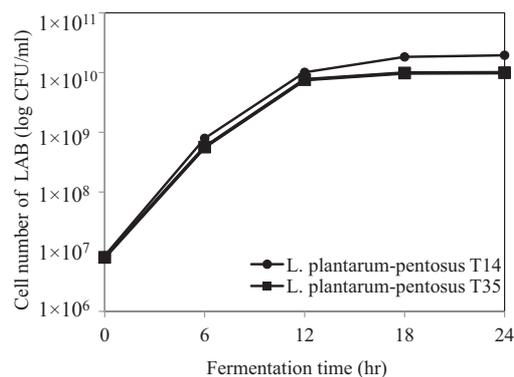
$$\frac{[(OD_{450}control - OD_{450}control\ blank) - (OD_{450}sample - OD_{450}sample\ blank)]}{(OD_{450}control - OD_{450}control\ blank)} \times 100$$

where OD<sub>450</sub> is wavelength absorbance at 450 nm, control is 10% FBS-DMEM and aquadest solution, control blank is 10% FBS-DMEM without cell and sample blank is 10% FBS-DMEM and sample without cells.

## RESULTS

### Growth of lactic acid bacteria in Kerandang milk

Changes in the viable cell number of the two strains of lactic acid bacteria in Kerandang milk during fermentation at 37 °C are shown in Figure 1. In general, *L. plantarum-pentosus* T14 and *L. plantarum-pentosus* T35 showed relatively good growth in Kerandang milk, with the initial cell growth rate varying slightly depending on the species. Initial cell populations of  $1 \times 10^6$ – $1 \times 10^7$  CFU.mL<sup>-1</sup> rapidly increased and reached  $1 \times 10^9$ – $1 \times 10^{10}$  CFU.mL<sup>-1</sup> in all Kerandang milk samples after 6–12 hr fermentation and remained constant after 24 h fermentation. *L. plantarum-pentosus* T14 had higher cell numbers ( $1 \times 10^6$ – $1 \times 10^7$   $10^{10}$  CFU.mL<sup>-1</sup>) than *L. plantarum-pentosus* T35 ( $1 \times 10^9$  CFU.mL<sup>-1</sup>) at 24 hr fermentation.



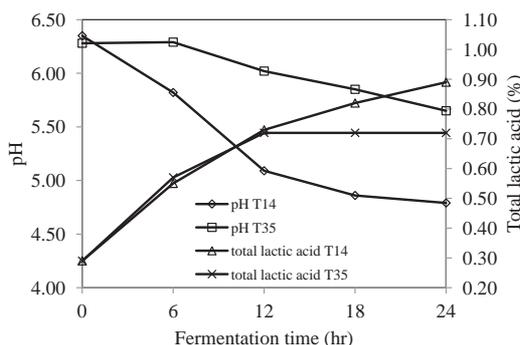
**Figure 1** Growth of *L. plantarum-pentosus* T14 and *L. plantarum-pentosus* T35 in Kerandang milk during fermentation at 37 °C (LAB = Lactic acid bacteria; CFU = Colony forming units.)

This suggests that differences in the strains and the sources of strains have different growth capabilities on the same medium. *L. plantarum-pentosus* T14 isolated from tempeh (soy fermented from Indonesia) was better able to adapt to grow in Kerandang milk than *L. plantarum-pentosus* T35 isolated from bamboo shoot pickle. Mital and Steinkraus (1975) reported that the strains *L. acidophilus* ATCC No. 4356, *L. cellobiosus* NRRL-B-1840 and *L. plantarum* B-246 ( $1 \times 10^9$  CFU.mL<sup>-1</sup>) attained higher maximum populations in soymilk than *L. bulgaricus* (Marshall) ( $1 \times 10^6$  CFU.mL<sup>-1</sup>). In addition, Chun *et al.* (2007) reported that after 9 to 12 hr of fermentation, the cell population was highest in soymilk inoculated with *L. paraplantarum* KM or *E. durans* KH than in samples inoculated with *S. salivarius* HM or *W.confusa* JY.

### Acid production and pH decline

Acid production expressed as lactic acid and the change in pH during fermentation of Kerandang milk at 37°C is shown in Figure 2. Total lactic acid increased during fermentation followed by a pH decrease. The decrease in the pH of *L. plantarum-pentosus* T14 was more

rapid during the first 12 hr of fermentation but with *L. plantarum-pentosus* T35 it was slower. The total lactic acid increase in Kerandang milk fermented using *L. plantarum-pentosus* T14 was greater than in *L. plantarum-pentosus* T35. In addition to the pH change, the pH reduction in fermentation using *L. plantarum-pentosus* T14 was greater than for *L. plantarum-pentosus* T35. These results corresponded with the bacterial growth and cell populations, wherein the cell number of *L. plantarum-pentosus* T14 was greater than for *L. plantarum-pentosus* T35 (Figure 1). In accordance with Sumarna (2008), organic acid production, pH decline and other metabolic activities occurred during the first 12 to 24 hr of incubation in soymilk, which corresponded to the exponential phase of the growth.



**Figure 2** Acid production and change in pH of Kerandang milk during fermentation at 37 °C using *L. plantarum-pentosus* T14 and *L. plantarum-pentosus* T35.

### Isoflavone change in Kerandang milk fermented by *L. plantarum-pentosus* T14 and *L. plantarum-pentosus* T35

Isoflavones form complexes with glucoside conjugates in an inactive form in about 80–95% of isoflavones (Synder and Kwon, 1987; Tsangalis *et al.*, 2002). Based on the results of the analysis of isoflavones using UPLC with four standards—namely, daidzin, genistin, daidzein and genistein (Figure 3) and fermentation of Kerandang milk using *L. plantarum-pentosus* T14 and *L. plantarum-pentosus* T35, they can hydrolyze

isoflavone glucosides (daidzin and genistin) into isoflavone aglycones (daidzein and genistein), but to differing degrees—daidzin hydrolysis using *L. plantarum-pentosus* T14 yielding 56.25% while using *L. plantarum-pentosus* T35 only 42.34% was produced. Similarly, hydrolysis of genistin by *L. plantarum-pentosus* T14 was higher (50.96%) compared to *L. plantarum-pentosus* T35 (22.30%). Figure 3 shows that the increase in the the content of daidzein content using fermentation with *L. plantarum-pentosus* T14 was higher (121.19%) than with using *L. plantarum-pentosus* T35 (56.48%). Likewise, the increase in the genistein content by fermentation with *L. plantarum-pentosus* T14 was higher (174.27%) than with *L. plantarum-pentosus* T35 (161.40%).

According to Chun *et al.* (2007), *L. plantarum* KM can hydrolyze 90% of daidzin and 100% of genistine in fermented soymilk after 6 hr of fermentation. Tsangalis *et al.*, (2002) reported on fermentation of soymilk using *Bifidobacterium animalis*, which transformed the isoflavone genistine into isoflavone genistein (90%), daidzin to daidzein (85%) and glycitin to glycitein (60%).

The isoflavone aglycone has a lower molecular weight than the isoflavone glucoside. Therefore, isoflavone aglycone is more easily digested in the small intestine than the isoflavone glucoside. According to Donkor and Shah (2008), soybean milk fermented using *L. acidophilus* LAFTI L10, *B. lactis* LAFTI B94 and *L. casei* LAFTI L26 at 37 °C for 48 hr will result in isoflavone glucoside hydrolysis into isoflavone aglycone, thus increasing the biological functionality of Kerandang milk.

### Colon cancer 26 cell viability

The anticancer activity of the methanol extracts of isoflavones from Kerandang milk fermented using *L. plantarum-pentosus* T14 and *L. plantarum-pentosus* T35 are presented in Figure 4. Anticancer activity is indicated by the cell viability at various concentrations of the methanol

extract of isoflavone. The high concentration of the methanol extract showed decreased cell viability which indicated there is growth inhibition of colon cancer 26 cells. The anticancer activity of the methanol extract of isoflavones from Kerandang milk fermented using *L. plantarum-pentosus* T14 was greater than the methanol extract of isoflavones from Kerandang milk fermented using *L. plantarum-pentosus* T35 and the methanol extract of isoflavone from Kerandang milk.

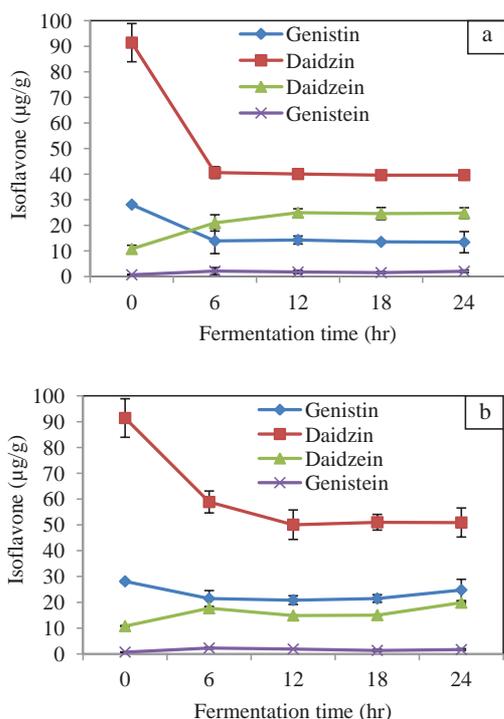
The activity is related to the hydrolysis of isoflavone glucosides into aglycone. Fermentation using lactic acid bacteria causes the hydrolysis of isoflavone glucoside into aglycone and increases the antioxidant activity due to the addition of a hydroxyl group at the C7 atom in ring A of the isoflavone structure (Pyo *et al.*, 2005b). This condition causes aglycone isoflavones to become

more hydrophobic and act as a hydrogen donor in the chain reaction of free radicals (Pyo *et al.*, 2005a; Albuлесcu and Popovici, 2007) and act as an anticancer agent. The total isoflavone aglycone using *L. plantarum-pentosus* T14 for fermentation (50.09  $\mu\text{g}$  per 100 mL) was greater compared to the total isoflavone aglycone from fermentation using *L. plantarum-pentosus* T35 (33.98  $\mu\text{g}$  per 100 mL) and Kerandang milk (21.46  $\mu\text{g}$  per 100 mL). Xu *et al.* (2010) reported that soy milk processed using ultra high temperature (UHT) had a higher isoflavone aglycone content than soy milk that had been traditionally processed and that the isoflavone extract of the UHT-treated soy milk showed antiproliferative activity against leukemia cancer HL-60 cells and prostate cancer DU145 cells .

According to Riboli and Norat (2003), consumption of fruit and vegetables that contain large amounts of polyphenolic compounds, as are found in flavonoids, may prevent diseases associated with oxidative stress such as cancer. The mechanism of the effect of polyphenolic compounds in cellular processes associated with the multiplication of cancer cells is the expression of key proteins in signal transduction pathways (for example, mitogen-activated protein kinase/ or protein aktivator/AP-1 MAPKs), transcription of nuclear factor-kappa B / NF-kB, downstream in the production of genes, modulation of cell cycle regulation and apoptosis induction that affect proliferation and cell apoptosis, immune response and metabolism of carcinogens (Fresco *et al.*, 2006; Nichenametla *et al.*, 2006).

## DISCUSSION

The lactic acid bacteria *L. plantarum-pentosus* T14 and *L. Plantarum-pentosus* T35 are indigenous lactic acid bacteria isolated from tempeh and pickled eggplant in Indonesia. Both strains are able to grow well in Kerandang milk with a source of protein such as soy milk. However *L. plantarum-pentosus* T14 showed greater ability



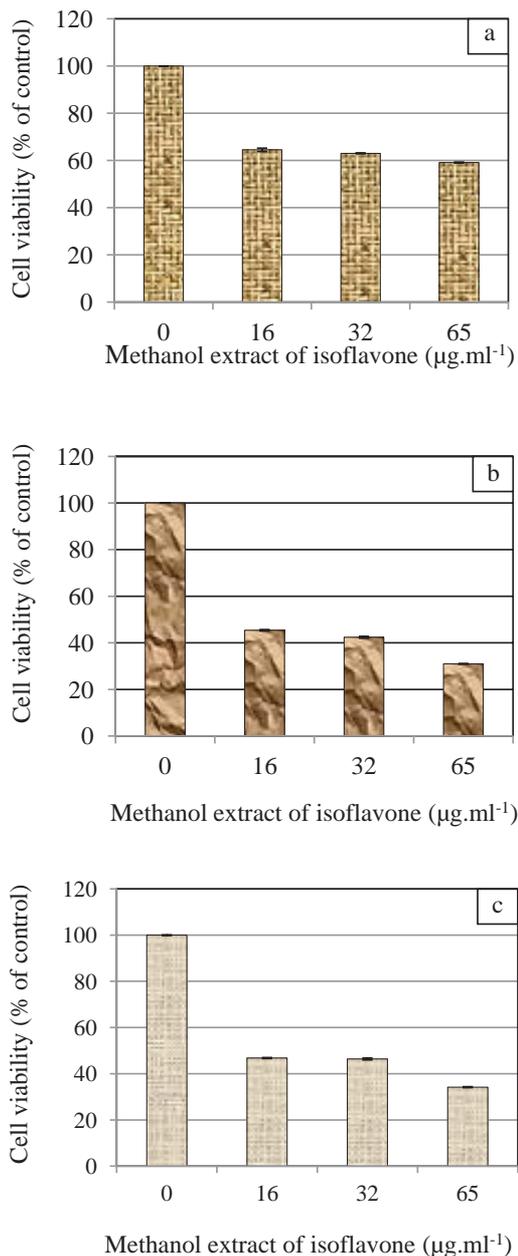
**Figure 3** Isoflavone change during fermentation in Kerandang milk using: (a) *L. plantarum-pentosus* T14 at 37 °C: (b) *L. plantarum-pentosus* T35 at 37 °C. (Vertical error bars indicate mean  $\pm$  SD.)

to grow in Kerandang milk than *L. plantarum-pentosus* T35, although both are included in the one species of *L. plantarum-pentosus*. The growth of both strains was followed by an increase in total lactic acid and a decline in the pH of Kerandang milk.

Kerandang milk contains isoflavones glucosides (daizin and genistin) and isoflavone aglycones (daizein and genistein). Kerandang milk fermentation using *L. plantarum-pentosus* T14 and *L. plantarum-pentosus* T35 can improve the isoflavone aglycone content. This indicates that the lactic acid bacteria produce the  $\beta$ -glucosidase enzyme capable of hydrolyzing the glucoside bond of isoflavone glucosides into isoflavone aglycones. According to Djaafar *et al.* (2003), *L. plantarum-pentosus* T14 produces  $\beta$ -glucosidase with activities of  $558 \pm 9.8$  mU.mL<sup>-1</sup> of culture at 12 hr of fermentation in Kerandang extract media at 37 °C. The increased content of aglycone isoflavones was followed by increased antioxidant and anticancer activity of fermented Kerandang milk. Chun *et al.* (2007) and Tsangalis *et al.* (2002) reported similar results in soy milk fermented using lactic acid bacteria. The methanol extracts of Kerandang milk fermented using *L. plantarum-pentosus* T14 and *L. plantarum-pentosus* T35 have anticancer activity. Thus, Kerandang milk fermented using these two strains of lactic acid bacteria has a functional value as an anticancer agent that requires further clinical research.

## CONCLUSION

*L. plantarum-pentosus* T14 and *L. plantarum-pentosus* T35 cell produced good growth in Kerandang milk. Kerandang milk fermented using *L. plantarum-pentosus* T14 and *L. plantarum-pentosus* T35 contained the isoflavone aglycone. Methanol extract of fermented Kerandang milk had anticancer activity. Kerandang milk fermented using *L. plantarum-*



**Figure 4** Effect of methanol extract of isoflavone on colon cancer 26 cell viability after 2 d incubation: (a) Methanol extract of isoflavone from Kerandang milk; (b) Methanol extract of isoflavone from Kerandang milk fermented using *L. plantarum-pentosus* T14; (c) Methanol extract of isoflavone from Kerandang milk fermented using *L. plantarum-pentosus* T35.

*pentosus* T14 had greater anticancer activity on colon cancer 26 cells than Kerandang milk fermented using *L. plantarum-pentosus* T35. The anticancer activity on the other cancer cell lines of Kerandang milk fermented using *L. plantarum-pentosus* T14 and *L. plantarum-pentosus* T35 requires further investigation.

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