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Process development for utilization of fermented tofu whey as a source of tofu coagulant and antioxidants

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ABSTRACT : Tofu whey is the liquid waste in tofu production industries, contains valuable compounds such as non-digestible oligosaccharides (NDO), which promote the growth of beneficial lactic acid bacteria in the colon and is currently discarded by the food industry. Tofu whey was reported that it was used as a growth medium for the production of lactic starters and substituting for expensive basal medium for the production of L- Lactic acid by lactic acid bacteria. In this study, fermented tofu whey (TW), a by-product of tofu industry was investigated for the preparation of tofu, micro flora and chemical changes of TW during tofu whey fermentation. The gel properties of tofu coagulated with fermented TW were also studied. During the fermentation stages the change of lactic acid bacteria (LAB) was found. The pH value, protein, carbohydrate, organic acid changes during the production of fermented tofu whey were studied. It was found that the pH value of acidic whey had a significant effect on coagulation properties of TW tofu. The microbiological findings in this study have clearly demonstrated the presence of the high counts of LAB investigated and a large amount L-lactic acid produced by LAB must have act as the main tofu coagulant.

KEY WORDS : Tofu whey, Antioxidant, Coagulant, Fermentation, LAB

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Tofu whey is the liquid waste in tofu production industries, is currently discarded by the food industry. However, it contains valuable compounds such as non-digestible oligosaccharides (NDO), which promote the growth of beneficial lactic acid bacteria in the colon, and are, therefore, recognized as prebiotics. Acidic fermentation of NDO in the caesium appears to be related with an increase in mineral absorption. The consumption of soy foods is increasing due to the benefits reported on nutrition and health. In this way, soybean is associated with the reduction of several diseases, such as certain cancers and heart illness On the other hand, the role played by soybeans in the reduction of cholesterol or in the prevention of obesity is firmly established. Soybean proteins offer special benefits not found in other foods and are being used to replace some dairy foods, or in diet. During the process of making tofu whey is usually discarded, but could be used as a good source of carbohydrates and proteins. 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). This suggests that tofu whey has so many nutrients that it can be used as a natural medium for the microorganism growth, especially lactic acid bacteria. Tofu whey was reported that it was used as a growth medium for the production of lactic starters and substituting for expensive basal medium for the production of L- lactic acid by lactica acid bacteri. 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 *et al.*, 2010; Hu *et al.*, 2004; Yang *et al.*, 2007). Lactic acid bacteria (LAB) are widely used in food industry commonly known to have health-promoting attributes. Isoflavones in soybeans has been recognized as a major functional compound to prevent chronic diseases such as cancer and arteriosclerosis.

Tofu, the most widely accepted soy food, is a versatile protein-rich food and has traditionally been used as a source of protein or meat substitute in Eastern diets (Baik and Mittal, 2003).For the basic tofu production, soymilk is coagulated with salts or acids (coagulants) either alone or in combination to produce a protein gel which traps water, soy lipids, and other constituents in the matrix.Coagulation of soymilk is the most important step in the tofu-making process. Calcium sulphate, calcium chloride, glucono-*d*-lactone (GDL), magnesium sulphate and magnesium chloride are many of the different tofu coagulants used on an industrial scale for the preparation of tofu (Prabhakaran *et al*., 2005). The effects of types of coagulants on viscoelastic properties of tofu were investigated (Molamma *et al*., 2006).The effect of different coagulants on the function and properties of tofu was also investigated (Cheng *et al*., 2005; Utsumi and Kinsella, 1985).

Tofu wastes deteriorate very quickly because of their high water content (80%) and their high content of nutritious substances for bacteria (Molamma *et al*., 2006). Although, tofu wastes contain high quantities of beneficial nutrients, most tofu by-products are used as animal feeds, fertilizer or simply disposed of. Effective utilization of wastes from the food industry is an important subject in order to reduce emissions into the environment. The tofu whey refuse is highly perishable and needs a quick treatment for effective utilization. Thus food wastes can be converted into valuable products such as soy beverage, coagulants and antioxidant sources. In the present study, we tried to develop a process to convert the tofu whey refuse into fermented tofu whey as source of coagulants and antioxidants.

Fermented whey is usually made from tofu whey, naturally fermented by its normal micro biota. Traditionally, fermented tofu whey was maintained by inoculating fresh tofu whey with a small volume of *Lactobacillus* culture. The processing method for producing the fermented tofu whey based tofu is similar with the tofu made using chemical coagulant. In the manufacture of tofu, soymilk is coagulated by heating, in combination with coagulants (salts, or acid and enzymes). Yield and quality of tofu have been reported to be influenced by soybean varieties, soybean quality, processing conditions and coagulants (Oboh, 2006), Cai *et al*., 1997). The coagulation of soymilk relies on the complex interrelationship between type of soybean, soymilk cooking temperature, volume, solid content, pH, coagulant type, amount and time (Cai and Chang, 1998). Coagulation of soymilk is a complex interaction of several variables (Hou and Chang, 2004). Poysa and Woodrow (2006) reported that different coagulants produce tofu with different textural and flavour properties. CS creates a bridge by which the soy proteins in the milk can aggregate. It may also interact with proteins to enhance the cross-linking of polymers (Beddows and Wong, 1987). The combined heat- and calcium-induced mechanisms work to produce the tofu. The resulting tofu product is affected by such things as pH, concentration of coagulate, and the rate at which the product is stirred (Beddows and Wong, 1987).

The objective of this study was to investigate the possible future standardized processing method of fermented tofu whey production and utilization as source of tofu coagulant and antioxidants. The work will be beneficial toward waste treatment technology alternative as well as nutritional and medicinal utilization of this tofu whey. This study could provide a better fundamental basis for developing starter cultures that can be used to produce safe tofu with reproducible quality.

METHODOLOGY

Raw material :

Soybean (Variety JS 335) was obtained from CIAE farm. It was stored at room temperature with 8±9% moisture before tofu processing. Magnesium chloride was purchased from local food ingredient suppliers.

Preparation of soymilk form soybean:

One hundred grams of soybeans were first rinsed and soaked in 500 ml of deionized water for 4 h at room temperature. Hydrated soybeans were drained, rinsed and ground in a waring blender for 2 min on high speed with hot water (1:6 soybean and water ratio). The slurry was boiled for 15 min at 90-94°C and filtered to remove the coarse material (okara) from the soymilk slurry. The volume of the final soymilk was set to 500ml using deionized water. In each experiment, the soymilk was freshly prepared daily.

Fermentation of acidic whey :

1000 ml of fresh tofu whey was transferred in container, cooled at ambient temperature. Then a small amount of already fermented tofu whey was added and stirred. The fermented tofu whey can be stored at 4°C for one week. After mixing, the fermentation process was kept for 72 h at 37^0 C. Aliquots were collected every 5 h of fermentation before 24 h fermentation and every 10 h of fermentation after 24 h fermentation for pH measurement. Other aliquots were also collected in sterile container for microbiological analysis. The tofu whey sample of 18 h fermentation was generally used as coagulant in the manufacture.

Preparation of tofu using chemical coagulants:

500 ml of freshly prepared soymilk (20°C) was transferred to a glass container and maintained in a 90°C

water bath for 15 min. The hot soymilk was removed from the water bath and transferred to a beaker. The coagulant used in the study was magnesium chloride at 80 mM.

The curd was then transferred into a laboratory-designed tofu box (steel mould) lined with miracloth for molding and tofu whey removal. The cloth was folded over the top of the curd and pressing was achieved with a weight (the weight covered the entire top surface of the folded cloth of curd while pressing), for 1 hours. The tofu was then unwrapped from the miracloth and the weight of the fresh tofu brick was recorded. Textural analysis was conducted promptly.

Preparation of tofu by using fermented tofu whey:

500 ml of freshly prepared soymilk (20°C) was transferred to a glass container and maintained in a 90°C water bath for 15 min. The hot soymilk was removed from the water bath and transferred to a beaker and then cooled to 85 °C. The fermented whey solution was slowly poured into the container, while stirring slowly and stop adding fermented whey until the soymilk-coagulant occurred. The volume of the fermented whey used was about 135 ml. The soymilk-coagulant suspensions were allowed to stand undisturbed for a period of 15 min to ensure that coagulation has occurred (Tripathi *et al*., 2011). The curd was then transferred into a laboratory-designed tofu box (steel mould) lined with miracloth for molding and tofu whey removal. The cloth was folded over the top of the curd and pressing was achieved with a weight (the weight covered the entire top surface of the folded cloth of curd while pressing), for 1 hours. The tofu was then unwrapped from the miracloth and the weight of the fresh tofu brick recorded. Textural analysis was conducted promptly. Tofu was stored in water under refrigerated conditions.After pressing, tofu and tofu whey were weighed separately. The tofu was transferred into a plastic bag and stored in a refrigerator for further analysis.

Determination of physical characteristics of tofu whey:

Soluble solids/brix (%):

Total solid contents of tofu whey was measured by using the lab hand refractometer (Model ATAGO N1, brix 0-32%, made in Japan). With samples equilibrated at 20°C prior to taking measurement, 1 ml of each sample was poured on refractometer prism and readings were taken. Values were expressed as brix percentage.

pH measurement:

The pH measurements of tofu whey were conducted by using a hand held pH/mV/temperature meter (Model pH 323, Ser.-Nr. 63260002, WTW 82362 Weilheim, Germany) attached to a stainless steel pH/temperature probe. Prior to taking measurements, the instrument was calibrated with distilled water pH 7.

Measurement of total solid contents (TDS):

The TDS analysis of tofu whey was performed by conductivity meter (H12300, microprocessor conductivity meter, Hanna instrument Inc., Woonsocket, Romania, USA).

Microbiological analysis of acidic whey :

Total bacterial count (TBC):

Serial diluted fermented tofu whey was plated in duplicates on plate count agar, the total bacteria were enumerated after incubation at 37°C for 48 h. Results were expressed as log cfu/ml of fermented tofu whey.

Lactic acid bacteria (LAB) :

Lactic acid bacteria (LAB) were determined in pour-plates on MRS culture media. MRS plates were incubated at 37°C for 48 h. Results were expressed as log cfu/ml of fermented tofu whey.

Chemical analysis of fermented tofu whey:

pH:

Changes in pH of acidic whey were recorded with a pH meter (F-23, Horiba, Japan). Three measurements were made at each sample and averaged.

Protein content:

The crude protein concentration of acidic whey was estimated according to the method of Lowry *et al.* (1951) using bovine serum albumin as a standard. The absorbance employed at 660 nm.

Total soluble sugar content:

The total soluble sugar concentration of the acidic whey was determined according to the total soluble sugar assay of food as described by Nielsen and Yang (2002). The mixture solution of 0.2 ml diluted samples with 0.8 ml distilled water and 5.0 ml grace alkone reagent was incubated at 100°C for 10 min. The reaction was terminated by cooling rapidly with cooling water at room temperature for 10 min and the sugar concentration was determined from absorbance at 620 nm using glucose as a standard.

Proximate analysis:

Chemical composition of the soybean, soymilk and tofu was done. The crude moisture, protein and fat content were determined by vacuum oven method, Kjeldahl method using a protein conversion factor of 6.25 and Soxhlet extraction method, respectively.

Determination of antioxidant activity (2, 2- diphenyl-1-picrylhydrazyl (DPPH) inhibition):

DPPH is a stable free radical in a methanolic solution.

In its oxidized form, the DPPH radical has an absorbance maximum centered at about 520 nm (Molyneux, 2004). Taken 0.5 g dried sample and added 4 ml methanol for extraction for overnight in the test tube. After extraction filtered it using Whatman no. 1 filter paper and maintained 5 ml using methanol. Taken 0.2 ml extracted sample in the test tube and 3.8 ml DPPH reagent was added and shaken well. The samples were allowed incubating at room temperature for 30 min. The absorbance of the DPPH solution was measured at 517 nm against blank and control (methanol + DPPH reagent).

Calculation:

The radical scavenging activity was measured as the decrease in the absorbance of DPPH using the following equation:

$$\text{Scavenging effect (\% inhibition)} = \frac{(Ao - Ae)}{Ao} \times 100$$

where; Ao = Absorbance without extract and Ae = Absorbance with extract.

Instrumental texture profile analysis (TPA) of tofu samples:

Texture profile analysis uses mechanical parameters of texture, which imitate the action of jaws, and the texture analyser is programmed to compress a bite-size piece twice in a reciprocating motion. Samples of 2x2 cm dia. cylinders of tofu, cut from the main block with a cork borer, then trimmed to length by cutting with fine wires set in a frame 2 cm apart were used for texture measurement. A two-cycle compression test was used, in which the probe touched the sample and compressed it to 75% of the brick height (15 mm) at a cross-head speed 18 mm/min, returned and repeated the test using the same parameters and recorded the fracturability, hardness, cohesiveness, gumminess, springiness, chewiness and resilience as forces (Mangaraj *et al*., 2005).

RESULTS AND DISCUSSION

The experimental findings obtained from the present study have been discussed in following heads:

Microbial changes :

Soybeans are rich in proteins, lipids, carbohydrates, minerals and vitamins. Carbohydrates in soybeans are soluble. In the preparation of tofu, soymilk is coagulated by heating, in combination with salts, acid or enzymes (Kohyama *et al*., 1995) and then the soymilk-coagulant is pressed to produce a lot of tofu-whey, soluble carbohydrates, a small amount of protein and other nutritional factors could be lost in tofu whey, which is a good culture medium for various bacteria to grow. Fig. 1 presents the micro flora changes of acidic whey, where, as mentioned before, a small amount of already fermented tofu whey was added into fresh tofu whey, which are naturally fermented at temperature (37°C) for 72 h. The initial counts of TBC, and LAB were found to be around 10^4cfu/ml, but coli forms and fungi were not detectable in fresh tofu whey. Tofu whey's initial temperature was around 65°C only some high temperature-tolerant bacteria could have survived. After the fresh tofu whey was cooled to room temperature, a small amount of already fermented tofu whey, which contained a large amount of LAB, was added to the pan with fresh tofu whey. The counting of TBC and LAB have shown that the growth rate of LAB was similar with that of TBC; the viable counts of LAB were significantly higher than that of other bacteria during 24 h fermentation of tofu whey. LAB shows faster growth in the tofu whey, attaining populations of about 10^7cfu/ml after 6 h. The strains attained maximum populations of about 10^9cfu/ml after 20-24 h fermentation. This implies that LAB was the dominating strains in the acidic whey. When the tofu whey was fermented for 24–48 h, there was a reduction in the viable counts of LAB, but a slight increase in TBC. Tofu whey was naturally fermented at room temperature, so it was easily contaminated by coli forms and number of coli forms rapidly increased to 10^3cfu/ml after 6 h fermentation, but decrease was detected after 18–36 h. This indicates that the dominating strains of the tofu whey inhibited the growth of coli forms at low pH. Whereas the counts of coli forms rapidly increased 10^3cfu/ml after 48–72 h because of the increase in pH value of the tofu whey. The number of yeasts and moulds were not detectable during 0–36 h fermentation, but later changed in a similar way with coli forms. This implies tofu-whey spoilage as pH increased.

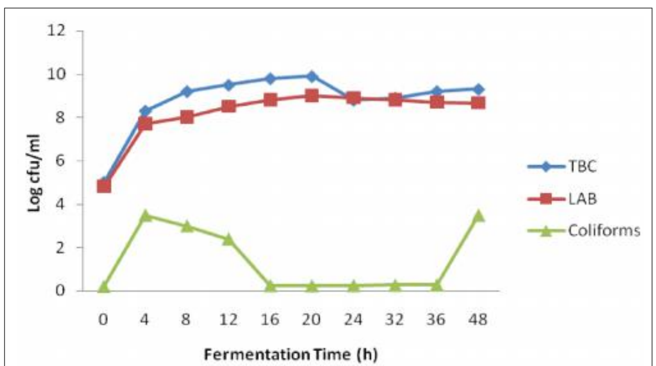

Fig. 1 : Microbial change during tofu whey fermentation

pH of tofu-whey :

The initial pH value of fresh tofu whey was 6.5, pH changes of tofu whey added already fermented whey (TWA) rapidly decreased during 16–18 h fermentation (Fig. 2). The pH of tofu whey down up to 3.9. This indicates that fermented tofu whey contains a large of LAB, which can be used as starter cultures rapidly propagating in sufficient culture medium, fresh tofu-whey. It is presumably due to the number of acid producing bacteria increased after 24 h produces acids thus lower pH. The number of total fungal increased because of assimilating organic acid and the number of spoilage bacterium increased because of proteolysis leading to high pH. Therefore, the fermented tofu whey after 18–20 h fermentation has been used as tofu coagulant because at this low pH they contain a very small number of spoilage bacterium.

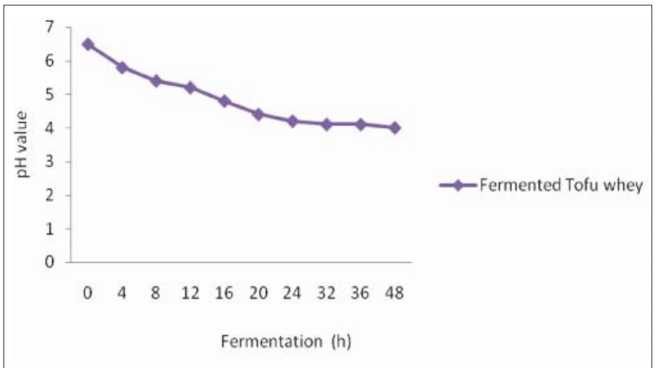

Fig. 2 : **Effect of fermentation on pH of tofu whey**

Effect of fermentation on protein changes and total soluble sugar :

The main soluble carbohydrates in the tofu whey were glucose, fructose, sucrose, raffinose, and stachyose. The total soluble sugar and crude protein changes are shown in Fig. 3 and 4 for the 28 h fermentation of tofu whey.

The initial total soluble sugar, and crude protein contents in tofu whey were about 5.57 and 7.81 mg/ml, respectively. The decrease in total soluble sugar content in tofu whey was found after 18 h which was supposed to

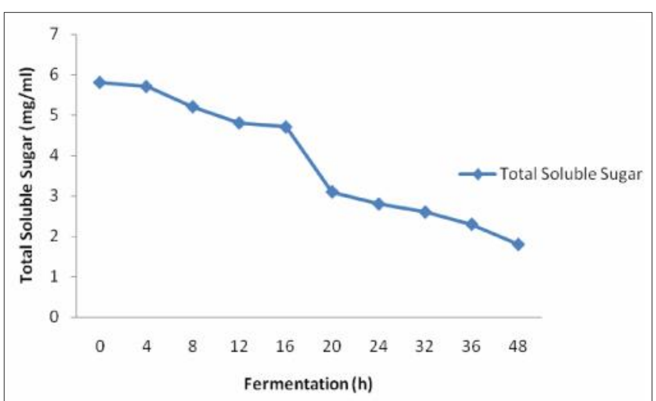

Fig. 3 : **Effect of fermentation on total soluble sugar**

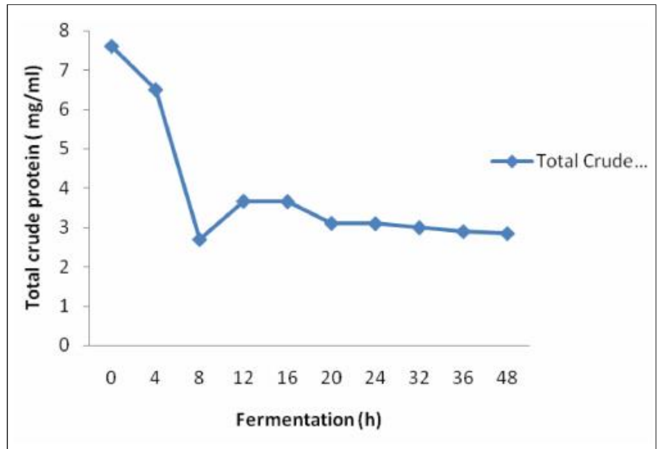

Fig. 4 : **Effect of fermentation on total crude protein**

consumed by bacteria after 48 h fermentation. Stachyose and raffinose in tofu whey were metabolized to produce a small amount of *a*-galactoses but a great amount of RS was found to be metabolized as well, the levels of RS and TSS, therefore, rapidly decreased after 18 h fermentation. The total crude protein changes of tofu whey decreased rapidly before 8 h, and then decreased slowly to about 3.25 mg/ml. These results suggest that the large amount of micro flora proteinase was produced to hydrolyze protein in tofu whey to supply the rapid growth of micro flora before 8 h fermentation.

Tofu yield and textural properties :

Table 1 shows the chemical composition of the soybean and tofu coagulated with different coagulants. The tofu preparation was processed from the same soybean and the same batch of soymilk. A significant ($P < 0.05$) increase and decrease of protein and fat, respectively, was observed when soybean was processed into tofu. Similar trend of significant increase and decrease in the content of protein and fat, respectively, was observed when the soymilk was coagulated into tofu irrespective of the source of coagulant. After the addition of the various coagulants into the soymilk, a quick formation of curd was observed with separation of whey. It is supposed that the coagulants allow the release of fats during processing, probably suggesting that the processing method considerably decreases the fat-binding capacity of protein. The moisture content of tofu samples varied from 73.6% in chemical coagulant tofu to 76.5 % in fermented tofu whey coagulated tofu. The variation in the moisture content of tofu prepared with different coagulants is probably due to the differences in gel network within the tofu particles that is influenced by different ions and its ionic strengths towards the water holding capacity of soy protein gels.

The textural properties of tofu play an important role in terms of quality and consumer acceptability. As shown in

Table 1 : Proximate analysis of soybean and tofu

Varieties/ Tofu	Moisture (%)	Fat (%)	Ash (%)	Protein (%)	Carbohydrate (%)
Variety JS 335	6.17	17.07	5.67	34.54	36.52
By chemical coagulants	72.27	7.69	4.58	7.31	7.56
By fermented tofu whey	75.18	5.82	3.92	9.75	5.33

Table 2 : Texture analysis of Tofu from chemical coagulants and fermented tofu whey

Tofu	Hardness (g)	Springiness	Cohesiveness	Chewiness (g)
Chemical coagulant ($MgCl_2$, 80 mM 90^0 C)	855	0.68	0.33	196
Fermented Tofu whey	272	0.83	0.39	122

Table 3 : Analysis of tofu whey

Tofu preparation methods	Optimal coagulating conditions	pH	Solid contents (%)	Conductivity (mS)	TDS (g/l)	Transmittance (%)
By chemical coagulant	$MgCl_2$ (80 mM 90^0 C)	5.63	4.8	7.46	2.45	97.5
By fermented Tofu whey	135 ml at 90^0 C	5.87	3.2	4.77	2.72	97

Table 2, the tofu texture varied depending on the nature of coagulants. The hardness of the tofu prepared with chemical coagulants higher than that of fermented whey tofu. The pH of fermented tofu whey decreased with increasing time and affect the textural properties of tofu. The low pH of fermented tofu whey is useful in preparing a tofu with harder textural properties. Cohesiveness is a measure of the degree of difficulty to break down the internal structure of the tofu. The cohesiveness of fermented tofu whey tofu was found to differ with chemical coagulant tofu. Springiness represents the extent of recovery of tofu height after pressing (Yang *et al.*, 2007). The springiness of the fermented tofu whey tofu samples differ significantly with chemical coagulant tofu. The springiness of fermented tofu whey tofu was higher than that of the chemical coagulant tofu. The gumminess and chewiness of the fermented tofu whey tofu increased with increase in fermentation time.

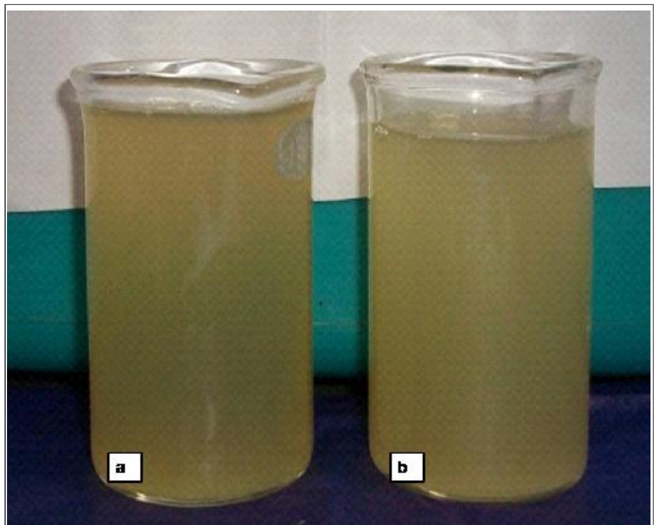

Fig. 5 : Tofu whey prepared by a) $MgCl_2$ b) Fermented tofu whey

Tofu whey analysis after tofu preparation:

Table 3 shows the physic-chemical composition of the tofu whey obtained by coagulated with different coagulants. The pH value of tofu whey obtained after tofu preparation was found to more acidic in chemical coagulant in comparison to tofu whey based preparation. The solid content of tofu whey in chemical coagulant based tofu whey was high and indicates greater loss in comparison to tofu whey based whey. Transmittance value in both conditions was nearly similar (Fig. 5).

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).

Fig. 6 shows the per cent inhibition of fermented extracts of tofu whey against DPPH radical. In general, all extracts showed that the percentage of inhibition increased with increasing fermentation time and gave higher antioxidant activity above 24 h. The results revealed that fermented tofu whey possibly synthesized some potential bioactive compounds, which were electron donors and effectively react with free radicals to convert them to more stable products

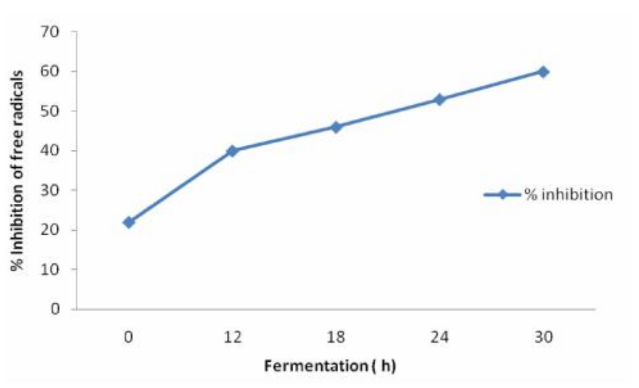

Fig. 6 : DPPH scavenging effect of fermented tofu whey (1.5 mg/ml) at different time (h).

and terminate the radical chain reaction.

Oxidation is an essential reaction in all living organisms during the oxidative metabolic process and resulting in formation of free radicals and other reactive oxygen species. These reactive radicals play a significant function in signal transduction (Hancock *et al*., 2001). Production of 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 (Pham-Huy *et al*., 2008). In human body these free radicals are neutralized by producing antioxidants, which are either naturally produced in situ, or supplied through foods or supplements. Therefore, the use of natural antioxidants as an alternative to synthetic antioxidants is of great interest. Most natural antioxidants derived from edible sources such as vegetables, fruits and grains. Recent efforts to use waste for the production of antioxidants increased.

Based on the results presented in Fig. 6 indicate that fermentation have significant influence on the increase in antioxidant activity. In observation, the scavenging capacity of DPPH radicals increased with increase in fermentation time. 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. The same phenomenon was also reported by other researchers to test the antioxidant activity of different substrates and microbes. 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). 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 (Elias *et al*., 2008). DPPH scavenging radical capacity of soymilk also increased with longer fermentation, however, there is no significant different between 24 and 32 h incubation (Liu *et al*., 2005). The soymilk proteins relatively intact while the soy whey proteins are small proteins (2S and 7S). The protein content and composition of soymilk and whey are different; therefore the antioxidant activity of tofu whey is differing.

Conclusion:

From this study it is inferred that the various species of micro organism occurred in acidic whey during natural fermentation, and the lactic acid bacterial (LAB) was the dominating strains. The LAB attained maximum populations of about 10^9cfu/ml after 20 h fermentation. The fermented tofu whey with pH 4.2 and higher level of lactic acid after 20 h fermentation makes an important contribution as the tofu coagulant in order to make good quality fermented whey tofu. These results suggested that the sources of tofu whey fermented by inoculating with starter cultures selected from fermented tofu whey after 20h fermentation can be used as tofu coagulant to make tofu with the good quality and longer shelf life. It was observed that the extract from fermented tofu whey show a potentially antioxidant activities. The extract at fermentation of 30 h has significant highest antioxidant activity compared to other fermentation time, which indicate as a rich source of natural antioxidants.

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