Journal of Applied Sciences

ISSN 1812-5654

science alert

ANSInet
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Journal of Applied Sciences 14 (21): 2819-2824, 2014
ISSN 1812-5654 / DOI: 10.3923/jas.2014.2819.2824
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Red Cheese Production from Soymilk by *Monascus purpureus* and *Lactobacillus casei*

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Abstract: Cheese is a dairy product with high nutrition and usually made from cows, sheep and goat milk. In this study, cheeses production from soymilk by using *Lactobacillus casei* (L), *Monascus purpureus* (M) and combination of *L. casei* and *M. purpureus* (LM) were investigated. The result found that protein coagulation of soymilk could be performed by direct inoculated with *Lactobacillus casei* and combination of *L. casei* and *M. purpureus* which *L. casei* produced lactic acid to decrease pH to pI of protein in soymilk. While the curd was not occur in the soymilk inoculated with only *M. purpureus*. Red cheese was produced by adding LM to the cultured soymilk at 8 weeks of cultivation time compared with using M for protein coagulation. The growth of *M. purpureus* changed chemical compositions of the red cheese from both M and LM especially fat and protein contents. Fat content dramatically increased from 15.84±0.18-18.97±0.58% during ripening contrary to cheese using *L. casei* fermentation for protein coagulation without *M. purpureus* adjunction. Adhesiveness of red cheese M and LM increased from 12.58±0.26-0.17±0.70% and 19.36±0.75-6.99±0.63%, respectively. In contrast, protein content was decreased from 44.56±0.55-0.09±0.47% during ripening of cheese L. Red cheese M and LM decreased from 45.24±0.44-5.34±0.57% and 43.97±0.64-1.20±0.52%, respectively. In the sensory evaluation, the red cheese from soymilk had accepted more than that from cow's milk and blue cheese in aspect of odour.

Key words: Red cheese, *Monascus*, coagulation, soymilk, *Lactobacillus*, lactic acid

INTRODUCTION

Cheese is a diary product with high nutrition and usually made from cows, sheep and goat milk (Omotosho *et al*., 2011; Hamid and Abdelrahman, 2012). The nutrition in cheeses was different according to type of milk (El-Bakry, 2012; Oseni and Ekperigin, 2013; Meraz-Torres and Hernandez-Sanchez, 2012). However, high fat content in cheeses product are cause of health problem for consumer. To reduce the risk, soy protein was consider as a highly food nutrition material since it lack in cholesterol and rich in lecithin and isoflavones (Hwang *et al*., 2009). Fermented soy cheeses or sufu and tofu were popular in Asian country especially China.

Various microorganisms involved in cheese making processes such as lactic acid bacteria, fungi and yeast. Lactic Acid Bacteria (LAB) play an important role during milk coagulation in cheese making process due to the production of lactic acid. Lactic acid affect the milk coagulation when the pH reached to pI of protein. Lactic acid bacteria such as *Lactobacillus*, *Bifidobacterium* and *Pediococcus* cause a rapid acidification of milk by production of organic acid, especially lactic acid and others (acetic acid, ethanol, aroma compounds). Various species of *Lactobacillus* was isolated from dairy products (cheese and yoghurt) in Iran and found major genus of lactic acid bacteria (Forouhandeh *et al*., 2010). Non-Starter Lactic Acid Bacteria (NSLAB) was also widely distribute in traditional cheese of Iran and showed an important role in flavor development of cheese (Abdi *et al*., 2006). Various kinds of fungi were also used for cheese production for better odor and flavor. The soluble extract of Moa-tofu fermented with *Mucor* sp. had a high antioxidant activity (Hang and Zhao, 2011). *Mucor* sp. was used in the production of Moa-tofu, traditional soybean food in China. It was found that *Mucor* sp. has

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a potential to be use in the cheese ripening process of semi-hard cheese (Zhang *et al*., 2013). The application was done by surface smear and found the change in protein fraction and modification of cheese texture to more compact uniform. *Penicillium roqueforti* was used to produce blue mold cheeses. Its flavor was enhanced from proteolysis and lipolysis activity of fungi to produce short chain fatty acid that make a typical flavor of blue mold cheese when compared to other cheeses (Vandamme, 2003; Walker and Mills, 2014). Beside of *P. roqueforti*, a non-pathogenic fungus *Monascus purpureus* was frequently used in the fermented product in oriental area such as China, Taiwan and Japan. This microorganism provides aroma color and bioactive compound such as monacolin K (Chen and Hu, 2005) that can reduce chloresterol level (Manzoni and Rollini, 2002). *Monascus* sp. Fermented Soybean (MFS) having a novel product with containing high level of antioxidant and Angiotensis I Converting Enzyme (AEC) inhibitory activity (Pyo and Lee, 2007). Whole soymilk prepared from MFS was found to contain high level of cholesterol synthesis inhibitor (monacolin K), isoflavone aglycone content and antioxidant activity compare to unfermented soybean (Lim *et al*., 2010).

In order to develop the health benefit food as an alternative choice for consumer, the new product should be invented. Therefore, the aim of this study was to produce new fermented soymilk products "red soy-cheese" from *Monascus purpureus* with coagulation of lactic acid from *Lactobacillus casei*. Moreover, the sensory evaluation of red cheese from soymilk was also investigated comparing to that from cow's milk and blue cheese.

MATERIALS AND METHODS

Study area: This study was conducted from September 2013 to May 2014 at Faculty of Science and Technology, Phranakhon Rajabhat University and Department of Microbiology, Faculty of Science, King Mongkut's University of Technology Thonburi, Thailand.

Preparation of soymilk: One kilogram of dry beans was soaked in 3 L of hot water for 14 h. The husks of rehydrated beans were removed and then undergo wet grinding with food processor. Water was added to give the ratio of water to beans on a weight basis 5:1. The resulting slurry of soy bean was grinded with food processor prior to filtration through cheesecloth and adjusted the filtrate to design final volume and then boil for 15 min. Transfer sterile soymilk to stainless steel container with a close-fitting lid. The designed amount soymilk per piece of cheese was 5 L.

Preparation of starter culture: The cultures of *Lactobacillus casei* and *Monascus purpureus* were obtained from stock culture of Department of Microbiology, Faculty of Science, King Mongkut University of Technology Thonburi (KMUTT). *L. casei* was culture in MRS broth and incubated at 37°C for 24 h with shaking condition. *M. purpureus* was cultivated on Potato Dextrose Agar (PDA) at room temperature (30±2°C). Starter was prepared by inoculated 20 mL of overnight culture of *L. casei* into 200 mL of steriled soymilk and incubated at 37°C for 24 h. Total count of *L. casei* was determined by stained film method and adjust to 10^6 CFU mL^{-1} before used. Spore suspension of *M. purpureus* was prepared from 20-day olds culture by added 20 mL of steriled 0.1% Tween 80 and scratch to remove spore mass. Spore suspension was filtrated through cotton wool. Total spore count was evaluated by using haemacytometer and adjusts to10^6 spores mL^{-1} before used.

Optimum condition for soy-protein coagulation: Cultures of *L. casei* and spore suspension of *M. purpureus* were separated and added to each 200 mL-5 L sterile soymilk with amount of 10^6 CFU mL^{-1} or 10^6 spores mL^{-1}, stirred the milk and incubated at 40°C. The coagulation of combination of *L. casei* and *M. purpureus* was also done in the same procedure. Two-hundred millileter of soymilk was sampling every 2 h until 16 h of incubation. The pH value and yield of coagulation were determined. Curd that obtained from only *M. purpureus*, citric acid was used for soy protein coagulation.

Odor analysis and effect of flavor agent: Three milliliters of synthetic flavor agents that are cream-vanilla flavor were added to soymilk at the beginning of coagulation processes with combination of *L. casei* and *M. purpureus* as mention above. The cultured milk was incubated at 40°C for 10 h and filtered through cheesecloth. Soy protein curd was added by 3% (w/w) of NaCl prior to put into piece mould and pressed with force of 22 g cm^{-2} for 16 h. The odor of soymilk coagulant was tested for satisfactory of consumer according to the Ranking test method (Stone and Sidel, 1978). The control experiments were done with lack of synthetic flavor agents and addidtion of 3% whey.

pH value, percentage of yield and chemical analysis of soy cheese: Soy cheese was produced as mention above with addition of 1.5% of cream-vanilla flavor prior to added 24 h culture of *L. casei* with 10^6 CFU mL^{-1} for soy cheese production. The combination of *L. casei* and spore suspension with 10^6 spores mL^{-1} of *M. purpureus* for red soy cheese production. The culture soymilk then mixed well and incubated at 40°C for 10 h followed by filtration through. Soy protein curd was added with 3% (w/w) of NaCl and put into piece mould and pressed with force of 22 g cm^{-2} for 16 h. Cheeses were ripening at 10°C for 8 weeks and samples were taken 2, 4 and 8 weeks for chemical analysis such as protein content, total fat content and moisture content.

pH value of cheese was determined according to AOAC (1995) procedure. One gram of cheese was mixed with water in a ratio of 1:10, shaken well and stands for 1 h before determined with pH meter.

Percent yield of cheese was determined by using the following equatuion:

$$\text{Yield of cheese (\%)} = \frac{X_2}{X_1} \times 100$$

Where:

X1 = Volume (mL) of soymilk used

X2 = Weight (g) of protein coagulant (after fermentation and whey was removed)

Moisture content of cheese was determined according to AOAC (1995) procedure by incubated 10 g of samples at 105°C for 3 h.

Total protein was determined with Kjedahl method (AOAC, 1995).

Fat content of samples were evaluated by soxhlet method which modified from AOAC (2000) procedure.

Sensory evaluation: Thirty volunteers were established for sensory test of soy cheese, red soy cheese with different coagulation. The panel was asked to evaluated samples of 8 week old ripening cheeses for appearance, color, odor, texture and overall acceptability by using 5-points hedonic scale (5, excellent; 4, good; 3, average; 2, poor; 1, very poor) compare with blue cheese and red cow's cheese from co-fermentation of *L. casei* and *M. purpureus*. The attribute mean score was calculated (Seleet *et al*., 2014).

Statistical analysis: Anova and Duncan's multiple range tests were used to determine significance.

RESULTS AND DISCUSSION

Protein coagulation by lactic acid from *L. casei*, *M. purpureus* and combination: By addition of *L. casei* culture to soymilk at room temperature, the pH of the mixture was reduced and protein was coagulated. The appearance of curd was found when the pH dropped to 5.32 with a yield of 75.62% at 24 h cultivation. The increasing temperature to 40°C could stimulate activity of *L. casei* to produce high level of lactic acid resulting to protein coagulation with a yield of 67.98% after 8 h cultivation. This indicated that temperature is one factor that effected to increased yields of cheese.

When *M. purpureus* was added to soymilk and cultivated at 40°C for 24 h, the result showed that the pH value of that mixture did not significantly change and no curd appearance (Table 1). *M. purpureus* produced less organic acid in the cultivation so it has low efficiency to coagulated soymilk protein. The results agree with the report of Rosenblitt *et al*. (2000) that *M. purpureus* could produced acetic acid when culture in solid and liquid medium containing glucose. Moreover, the fungus was able to produce protease in order to degrade protein in soymilk to short peptide resulting to decrease coagulation of protein (Tseng *et al*., 2000).

Protein coagulation by lactic acid from *L. casei* was also compared with *M. purpureus* when cultivated at 40°C. During the growth of *L. casei* in soymilk, the pH of soymilk gradually decreased and the curd occurred on ward 8 h of fermentation with yield of 67.98% (Table 1). The maximal yield (87.67%) was obtained at 14 h cultivation whereas longer cultivation up to 16 h resulted to decrease the yield.

The coagulation of protein from soymilk using combination of lactic acid from *L. casei* and *M. purpureus*

Table 1: pH and protein coagulation by *L. casei*, *M. purpureus* and combination of both at temperature of 40°C

	M. purpureus		*L. casei*		Combination	
Time (h)	pH	Yield (%)	pH	Yield (%)	pH	Yield (%)
0	6.58	-	6.51	-	6.51	-
2	6.51	-	6.42	-	6.51	-
4	6.48	-	6.03	-	6.37	-
6	6.48	-	5.42	-	5.27	77.54
8	6.47	-	4.90	67.98	4.77	80.76
10	6.47	-	4.75	78.11	4.52	88.28
12	6.45	-	4.62	82.55	4.44	81.42
14	6.45	-	4.55	87.67	4.42	70.83
16	ND	ND	4.50	81.04	ND	ND

ND: No detection

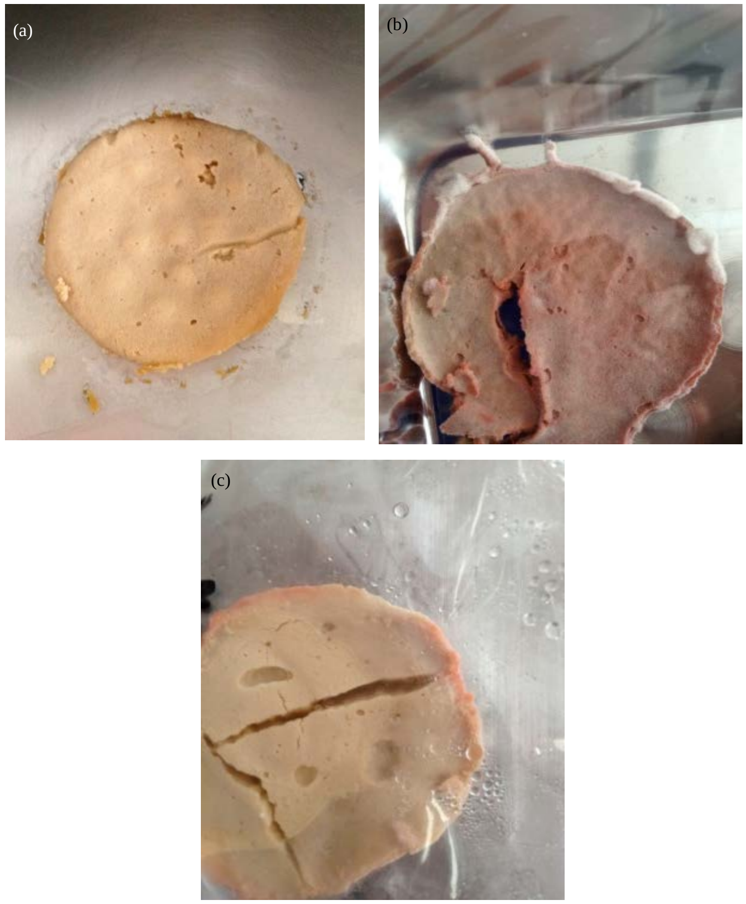

Fig. 1(a-c): Characteristics cheese producing *Lactobacillus casei*, (a) *Monascus purpureus*, (b) Their combination and (c) When incubated for 8 weeks

were also investigated at 40°C. The results showed that the repulsive electrostatic forces of protein in soymilk reduced and then the protein coagulated. Appearance of curd after whey removal looks likely rough was found at 6-hour cultivation with yield of 77.54% (Fig. 1). The maximal yield (88.28%) was found at 10 h cultivation whereas, longer cultivation to resulted to decrease the yield. This result indicated that using combination condition can reduce time of protein coagulation for 4 h with higher yield comparing to only lactic acid from *L. casei*. This may be due to *M. purpureus* utilized sucrose in soymilk to glucose then *L. casei* produced lactic acid using that glucose as substrate (Tseng *et al*., 2000; Donkor *et al*., 2007; Chooklin *et al*., 2011).

Cheese production and red cheese evaluation: Fermented soymilk with *L. casei* and *M. purpureus* had intense smell of beans. To reduce stench odor, artificial flavor may be used to add in to the curd. From the preliminary study, the result showed that the cream-vanilla is the best flavor comparing to pandan and milk-butter flavoring. The cream-vanilla is used to make red soy cheese.

Cheeses producing by *L. casei* (L), *M. purpureus* using citric acid for protein coagulation (M) and *L. casei* collaborate with *M. purpureus* fermentation (LM) were investigated. During ripening at 10°C, *M. purpureus* grew in cheese using citric acid for protein coagulation (M) faster than in cheese using *L. casei* collaborate with *M. purpureus* fermentation (LM) (Fig. 1). This may be *L. casei* can create certain compounds capable of inhibiting the growth of the fungus *M. purpureus*.

During ripening, the chemical compositions of 3 kinds of cheese were changed. Proteins of those cheeses were reduced while fat content increasing significantly. Amount of protein in cheese with *M. purpureus* was reduced to approximately 10%, while

Table 2: Chemical composition and texture analysis of cheeses during the ripening

Sample and ripening time (week)	Moisture content (%)	Fat content (%)	Protein (%)
L			
0	66.58 ± 1.52^{c}	15.84 ± 0.18^{b}	44.56 ± 0.55^{e}
2	66.13 ± 2.31^{c}	15.92 ± 0.04^{b}	44.08 ± 0.40^{e}
4	65.00 ± 0.69^{c}	16.40 ± 0.53^{b}	43.38 ± 1.62^{e}
8	60.85 ± 0.34^{a}	18.97 ± 0.58^{c}	40.09 ± 0.47^{d}
LM			
0	66.02 ± 0.32^{c}	19.36 ± 0.75^{c}	43.97 ± 0.64^{f}
2	65.93 ± 1.90^{c}	20.07 ± 0.59^{d}	43.12 ± 0.40^{f}
4	65.51 ± 0.67^{c}	20.24 ± 0.29^{d}	42.63 ± 0.21^{e}
8	64.53 ± 0.07^{b}	26.99 ± 0.63^{c}	41.20 ± 0.52^{d}
M			
0	66.72 ± 1.41^{c}	12.58 ± 0.26^{a}	45.24 ± 0.44^{f}
2	66.18 ± 1.91^{c}	12.83 ± 0.86^{a}	43.31 ± 0.53^{e}
4	65.19 ± 1.89^{c}	13.10 ± 0.34^{a}	41.77 ± 0.48^{d}
8	63.58 ± 0.44^{a}	20.17 ± 0.70^{d}	35.34 ± 0.57^{a}

$^{a-d}$ mean with different subscript in the same row significantly different ($p>0.05$)

Table 3: Sensory analysis of cheeses ripening at 10°C for 8 weeks

Sample	Appearance	Color	Flavor	Texture	Overall acceptability
LM soy	2.15 ± 0.48^{a}	3.64 ± 1.23^{a}	4.82 ± 0.16^{c}	2.29 ± 0.35^{a}	3.28 ± 0.58^{a}
LM cow	1.89 ± 0.51^{a}	3.48 ± 0.89^{a}	3.15 ± 0.33^{b}	2.34 ± 0.41^{a}	3.15 ± 0.49^{a}
B	4.07 ± 0.28^{b}	3.52 ± 0.82^{a}	1.89 ± 0.27^{a}	2.57 ± 0.37^{a}	3.87 ± 0.23^{a}

$^{a-c}$Mean with different subscript in the same row are significantly different ($p>0.05$), LM soy: Cheese from soymilk fermented with *L. casei* and *M. purpureus* and cream-vanilla, LM cow: Cheese from milk fermented with *L. casei* and *M. purpureus* and cream-vanill, B: (Blue cheese)

the other two kinds of cheese decreased by only 2-4% during the 8 week incubation (Table 2). Proteolysis by *M. purpureus* was better than that of *L. casei* and combination of two species in cheese. Smit *et al*. (2000) reported that various peptides and amino acids obtained from proteolysis during protein coagulation and ripening were caused of flavor. There are several types of peptides in fermented milk products that are biologically active compounds (bioactive peptide) (Pritchard *et al*., 2010).

In cheeses, containing *M. purpureus*, fat content increased by 7.59%, while in cheeses inoculated with combination of both organisms, the fat content increased by approximately 7.63% . Moreover, the cheese without *M. purpureus* had a fat content only 3.13% during the 8 week incubation (Table 2). This result showed that the cheese containing *M. purpureus* having fat content significantly increased during cheese ripening for 8 weeks. Increasing fat content affected to the flavor of the cheese during ripening (Smit *et al*., 2000).

Moisture content of the 3 kind cheeses was reduced during ripeninig process. The cheese with 2 species of microorganisms (LM), moisture content 64.53±0.07%, while with only *M. purpureus* (M) and cheese only *L. casei* (L) moisture content 63.58±0.44 and 60.85± 0.34%, respectively (Table 2).

Sensory assessment: Apparently, consumers did not like both of red cheese from cow's milk and soymilk whereas they prefer blue cheese (Table 3). This might be due to not fully ripening red cheeses during cheese making process and the external appearance does not look uniform. Senses with the color, texture and overall acceptability of those cheeses found that the testers have indifferent feelings to those cheeses (Table 3). The testers are so indifferent to the smell of cow's milk cheeses while they did not like blue cheese. Recognized the smell of cheese from soymilk with vanilla cream flavor was the best. This might be due to remain vanilla cream in cheese during ripening and testers were familiar with the smell (Table 3).

CONCLUSION

Lactobacillus casei can be used for protein coagulation in soymilk because the production of lactic acid during growth and resulted to pH decreased to pI of protein. In contrast, the fungus *M. purpureus* did not promote protein coagulation. However, the fungus continues to grow slowly during the ripening process and turning white to red color of cheese. Protein coagulation at 40°C using combination of *L. casei* and *M. purpureus* was found to be most suitable condition for protein coagulation with high protein yield. Red cheese production using *L. casei* and *M. purpureus* with cream-vanilla was the most satisfied of testers. During ripening of red cheese, *M. purpureus* grew and dramatically changed the chemical composition of cheese. In preference test, panelists preferred red cheese from soymilk more than those from cow's milk and blue cheese for odour. Red cheese from soymilk was a product that is rich in bioactive compounds derived from soymilk, substances producing *L. casei* and *M. purpureus.*

ACKNOWLEDGMENT

The authors thank to Dean of Faculty of Science and Technology for laboratory facilities, Phranakhon Rajabhat University. This study was supported by Institute of Research and Development Phranakhon Rajabhat University, Thailand.

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