The development of ripened cheese containing lactic acid bacteria: The effect on chemical composition, acid production and sensory value

Tridjoko W. Murti

Faculty of Animal Science Universitas Gadjah Mada

ABSTRACT: This research was performed to evaluate the effect of different numbers of lactic acid bacteria cultures on chemestry of ripened farm house type cheese. The study was to investigate the effete of supplementation of 1, 2, 3 and four kinds of lactic acid bacteria on ripened cheeses. Cheese has developed using rennet which has strength 1:15.000, auditioned by *L.casei*- containing culture (P1), *S. thermophilus-L. bulgaricus* (P2), *S.thermophilus-L.bulgaricus- L.casei (P3)* and *S. thermophilus-L. bulgaricus-L.acidophilus* and *Bifidobacterium ssp* (P4). The results showed that the addition of LAB have improved the cheese result, crude protein and lactic acid content, while ripening time till 3 month have raised pH, crude protein, fat, and lactic acid especially for P3 and P4. The use of LAB has changed the flavour score especially sweetness, acidity and bitterness. The ripening has improved the flavour score and the note of trained panellist on flavour.

Key words: Ripened Cheese, LAB Culture, Chemical Composition, Flavor

INTRODUCTION

Rapid growth in Indonesia's economy has been associated with a transformation of its food consumption pattern, which was mainly based on rice, starchy roots, fish and vegetables, to a wider variety including wheat and livestock products. As demand for agricultural products rises, imports have become an important source of food supplies and intermediate inputs such as feed for the poultry industry and cotton for the textile industry (Bond et al. 2007). Milk is a valuable source of nutritional substances that for the case of Indonesia are largely imported from Australia, New Zealand, USA and Europe: it is considered as the most complete of food. Through the centuries, man has wisely learnt how to use this food source in two ways:

- As a foodstuff: for direct consumption (milk as a drink);
- transformed: as an element from which it is possible to obtain dairy products (cheese, butter, yoghurt, etc.)

The chemical composition of milk depends on various factors: species, breed, age of the animal, feeding, health condition, environmental factors and lactation period. Water, proteins, fat, sugars, mineral salts, vitamins and enzymes are the substances we usually find in milk.

Table 1. Average chemical composition of milk of different species (For 100 g of fresh milk)

Species	Water	Protein	Fat	Lactose	Mineral salts
Bufallo	82.2	4.8	7.5	4.7	0.8
Goat	86.5	309	4.3	5.8	0.8
Ewe	80.9	6	7.5	5.4	1.1
Cow	87.5	3.2	3.7	4.6	1

Cheese is a food made from milk, usually the milk of cows, buffalo, goats, or sheep, by coagulation (Callendreli, 2005). The milk is acidified, typically with a bacterial culture, then the addition of the enzyme rennet or a substitute (e.g. acetic acid or vinegar) causes coagulation, to give "curds and whey".[1] Some cheeses also have molds, either on the outer rind (similar to a fruit peel) or throughout (Wikipedia). More than 300 varieties (e.g., American, Cheddar, Mozzarella, Colby) of cheese are available in various flavors and forms (e.g., chunks, slices, cubes, shredded, grated/crumbled, string/stick, spreads), and packages to meet consumers needs (Fox & McSweeney, 2004). The popularity of cheese is attributed to its great taste, the availability of new and different varieties, convenience and versatility of use, and its nutritional value.

Cheese is the fresh or ripened product obtained from the acid, rennet or mixed coagulation of whole or partially skimmed milk. The process that transforms milk into cheese is called a technological process. Each cheese, and more in general, each dairy product is produced following a specific technological process.

In general, Cheese making have three main steps:

a. Coagulation
b. Pressing or whey syneresys
c. Ripening

The important step of cheese making included two of 3 steps i.e.: coagulation milk protein by rennet helped by microbes or not and whey syneresis. The use of certain microbes indicates that there is a fermentation process that releases some volatile odors (Murti, et al, 2005). Some of microbes involves in fermented milks come from bacteria, or yeast. Genus bacteria generally used in fermented milks, i.e.: Lactobacillus (*L. delbrueckii subs. bulgaricus, L. acidophilus, L. helveticus, L. brevis, L. fermenteum*, and *L. kefir*), Streptococcus (S. *thermophilus*), Leuconostoc (Ln. *mesenteroides*, and *Ln lactis*), Pediococcus (*P.acidilactici*, and *P. pentosaceus*), Acetobacter (*A.aceti*), and Bifidobacterium (*B. breve, B. adult, B. infantis, longum, B. bifidum*, and *B.pesudolongum*). While for yeast included *Torulaspora delbrueckii*, *Kluyveromyces marxianus subsp. Marxianus*, *Candida kefir*, and *Saccharomyces cereviseae*). Some of these bacteria produce mainly lactic acid and known as lactic acid bacteria (Murti, 2005a).

Table 2. Types of fermentation in lactic acid bacteria

Genus	Fermentation type	Main products	Lactate isomers
Streptococcus	Homo	Lactate	L
Pediococcus	Homo	Lactate	L, DL
Lactobacillus:			
-obligate homo	Homo	Lactate	L, D or DL
-Facultative hetero	Homo	Lactate	L, D or DL
	Hetero	Lactate, Acetate	L, D or DL
Obligate hetero	Hetero	Lactate, Acetate, CO2	L, D or DL
Leuconostoc	Hetero	Lactate, Acetate, CO2	D
Bifidobacterium	Hetero	Lactate, acetate	L

Dellaglio et al., 1994 in MUrti, 2005b

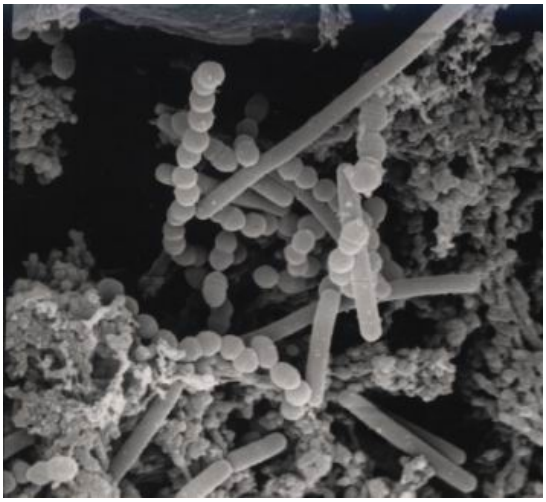

Figure 1. Bacteria in yoghurt (*S. thermophilus* and *L. delbrueckii subsp. bulgaricus*) (T.W. Murti, 2005)

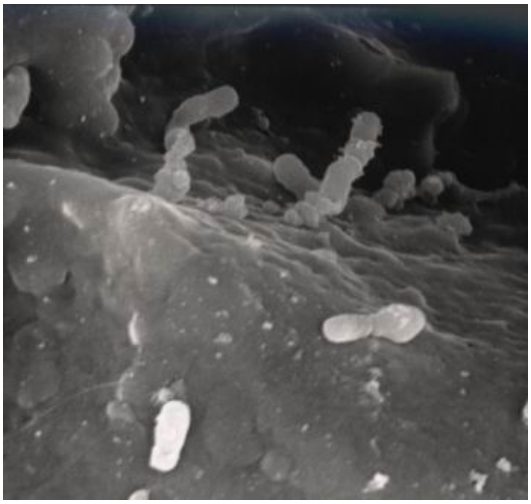

Figure 2. *Bifidobacterium pseudolongum* in yoghurt (T.W. Murti, 2005)

The leading factors in the cheese-making process are the lactic bacteria. They are necessary for the lactic fermentation: they transform lactose (the milk sugar) into lactic acid; this acidification process

helps the rennet's work, the draining of the whey after the curd has been broken and the cheese maturation. Lactic bacteria are indispensable for the production of buffalo Mozzarella cheese. Many lactic acid bacteria produced mostly (only) lactic acid (homofermentation), while other produces also acetic acid, CO2 and ethanol (heterofermentation). These lactic acid bacteria could be divided also into mesophilic lactic acid bacteria and thermopiles lactic acid bacteria depended on their temperature of growth. Some of lactic acid bacteria are considered as probiotic bacteria, which give health benefits for human being. The use of LAB will influence the flavor score and note of cheese (Lee et al., 1990ab, Murti and Cahyadi, 2007). The breakdown of lactose by LAB will act as the precursor of flavor as diacetil, acetoine and butan-2-3 diol. These substances are becoming specific flavor of cheddar cheese and fresh cheeses (Ortigosa *et al*., 2001).

This research was performed to evaluate the effect of different numbers of lactic acid bacteria cultures on chemestry of ripened farm house type cheese (3 month of ripening time) supplemented by culture of lactic acid bacteria in commercial fermented milk containing *L. casei*- (P1), yoghurt bacteria-containing fermented milk (P2), mixed bacteria in P1 and P2 (P3) as well as yoghurt bacteria supplemented by *L. acidophilus* and *Bifidobacterium sp* becoming mixed culture of 4 bacteria (P4).

MATERIALS AND METHODS

The experiments have conducted at the Laboratory of Dairy Science and Milk Industry (ISO 17025:2005) at the Faculty of Animal Science, Universitas Gadjah Mada at Yogyakarta- Indonesia.

Materials

Milk was collected from dairy cows managed under local condition at Centre of Milk Production (UPT Perah) of the Faculty of Animal Science. While rennet used in these experiments has been made from kid of sheep aged 3 week, slaughtered following Islamic ritual, and calculated having force 1: 15.000. The culture of fermented milk was obtained from commercial sources in supermarket and label declared contain *L. casei*, yoghurt bacteria: *Streptococcus thermophilu*s and *Lactobacillus delbrueckii* subs *bulgaricus*, and yoghurt bacteria contain Bifidobacteria and *L. acidophilus*.

Methods

Farm house cheese type has been developed using goat rennet as described by Murti (2004). The development of cheese was

- Chemical analysis were to detect crude protein, fat, pH, acidity, as well as organic acids

Milk was pasteurized at 76 ^{0} C (10 minute) after homogenization at 3.000 rpm, cooled to fermentation temperature at 33 ^{0} C, separated into 5 parts (control and treated cheeses with culture of 1 LAB/ P1 (containing *L. casei*), culture of 2 kind LAB (containing *Streptococcus thermophilu*s and *Lactobacillus delbrueckii* subsp *bulgaricus*,) 3 kind of LAB (containing *L. casei*, *Streptococcus thermophilu*s and *Lactobacillus delbrueckii* subsp *bulgaricus*) as well 4 kind of LAB (*Streptococcus thermophilu*s and *Lactobacillus delbrueckii* subsp *bulgaricus*, *L. acidophilus and Bifidobacterium sp)* and then incubated during 45 minute at 39 ^{0} C, except for P1 at 30 ^{0} C.

The treated cheeses and control has been removed from incubator to be added rennet at 1: 15.000 force, followed incubation during 40 hours. The curd has been separated from its whey by cutting the curd, followed addition warm sterilized wáter at 38 ^{0} C, filtered using cheese cloth during 24 hours. The products obtained were then pressed using 0.0013 Pa forcés, followed by addition of salt (300 g at 1 liter of wáter), packaged using aluminium foil and ripened at refrigeration during 3 month.

Physical and Chemical Analysis

- Physical analysis, the cheeses were observed its value on cheese rendements, water content, and pH. Water content was calculated after placed it at oven temperture 135 ^{0} C during 2 hours. While pH has performed using digital pH meter after calibration using buffer solution at pH 4,0 and 7,0.

- Protein content has been measured using Kjeldahl method, while fat has been measured using Soxhlet . Dry matter rendement was calculated as cheese weight X dry maytter content of cheese

Organic Acid Analysis

Cheeses samples (2 g) were prepared at 70 mL aquabidestilata, stirred magnetically during 1 minute, incubated at 50 ^{0} C . at the room temperature, sample then filtered using milipore paper 0,2 mmeter before injected to HPLC column Aminex HPX-87 H, between 20- 80 mM(Murti, 2005b). Flow rate wa kept at 1 ml/ minute at 40 ^{0} C and recorded at wave length of 340 nm.

Flavor Analysus

Cheese intensity basic taste solution (sweetness, acid-taste, bitternes, saltiness) Hav made as in tabel I: It was then appreciated by 9 students acted as entrained panelist to taste these basic taste score at 5 scale of taste score (0= none, 1= little X, 2 =moderate X, 3= taste X, and 4= very taste x). While for the consumers perception, it was detected using 5 scale of flavor note (none, 1= little, 2= moderately like, 3= like and 4 = well like) as Afnor method (1980) in Murti (2005b) or Hui (1993)

Data Analysis

Protein, Fat, and pH was analyzed using Randomized design one way analysis (Astuti, 1980) fasilitated by SPSS 10 program.. Flavor score and note whis are non parametric data were analyzed descriptivel to be compared to the standard solution.

To make cheese, milk is curdled using an agent called *rennet* present in the tissue of the calf stomach. Rennet (*chymosin*) is a proteolytic enzyme and its role is to destabilize casein micelles by cutting the liaison between Phe $_{105}$-Met $_{106}$ of the Casein (-CN) and allow them to coagulate. They break down -casein present on the surfaces of casein micelles in milk. Deprived of the protective action of k-casein, casein micelles form a gel. When examined by electron microscopy (Kalab, 2000), they form a thin matrix consisting of their clusters and short chains, encapsulating fat globules. Void spaces in the matrix are filled with the liquid milk serum called *whey* which is a solution of lactose, minerals, and vitamins, and a suspension of whey proteins. The removal of the whey makes the casein matrix more compact and also brings the fat globules closer together. The subsequent steps in the cheese manufacture are aimed at separating the curd from the whey and ripening it, and the result of ripened cheese was presented at Table 3.

Table 3. Physical performance of ripened cheese

	P0	P1	P2	P3	P4
Rendement, g/l	72,17^a	80,27^b	81,73^b	88,18^c	74,77^a
Water content, %					
-early ripening	58,7 ^{a}	58,9 ^{a}	60,9 ^{a}	59,3 ^{a}	58,9 ^{a}
-ripening 3 month	54,7 ^{b}	57,1^a	61,1^a	60,6^a	57,4 ^{a}
pH					
- early ripening	3,81 ^{b}	3,62 ^{c}	3,39 ^{d}	3,7^b	4,29 ^{a}
- ripening 3 month	3,84 ^{b}	3,78 ^{b}	3,59 ^{c}	3,79^b	4,52 ^{a}

^{a,b,c,d} different superscript at diifferent row indicated significantly different (P< 0,05)
^{a,b,c,d} different superscript on column (P< 0,05)

The result have indicated that the rendement of product were higher in treatments than in control. It was suggested that the cheese supplemented by 1 to 4 type of lactic acid bacteria have entrapped some water in the space among the matrix, although they were not significantly different as compared to those of control without LAB culture supplementation, especially at early time of ripening in 4-6^o C.

The matrix build by fermentation of LAB before and during ripening time have been observed by many authors (Chassaing et al., 1990; Ong, 2007). But, except for the control, there was not significantly difference of water content between treatment after 3 month of ripening time. Water

content of ripened cheese after 3 month were relatively higher in the cultures containing LAB than control. The high water content of products have led to be considered as soft cheeses (Murti, 2005b). In general, the water content of cheeses during ripening will reduce (Ong, 2007)

Mostly of the product have presented the value of pH lower than control except for the P4 either at early or after 3 month of ripening time. The use of LAB have influenced the reduction of pH. The presence of some probiotic LAB at P4 may have enhanced the production of other acid than lactic acid, acetic acid lead to higher pH. All of the product have presented the value of pH after 3 month of ripening higher than before ripening time. It has indicated that during ripening, casein breakdown by proteolysis would produced NH3 leading to rise the pH (Walstra et al., 1999)

Chemical Composition

Chemical composition of the cheeses treated by supplementation of LAB, have been presented in Table 4. The use of some probiotic LAB will influence proteolytic and the production of organic acids in different ways (Ong, et al, 2007a). Kato et al (1989) have also indicated that amino acids and peptides released during ripening of cheeses will fortly influence cheese flavors. Protein content of the products treated were higher along the use of more LAB, except for P4.It was possible that the use of LAB have caused the breakdown of protein to build bacteria cell which contain also protein. The higher content of cheeses protein after ripening time due probably to the higher content of cheese dry matter. The more the use of LAB, the higher content of protein either before or after ripening time.
Fat content of treated cheeses were not significantly different as compare to those of control. Lactic acid bacteria used in these experiment have known null or weak capacity of lipolysis, lead to undifferent results.

Lactic acid presents indicated the results of metabolism in LAB, in which before producing lactic acid, LAB produce pyruvic acid. Product P1 and P4 have presented more pyruvic acid than control, P2 and P3. It has demonstrated that lower pyruvic acid were equal to higher lactic acid inP2 and P3.

Table 4. Chemical composition of ripened cheese

		P 0	P1	P2	P3	P 4
Protein content,%	Early ripening	22,9[a]	24,7[b]	27,3[c]	29,2[d]	27,8[c]
	Ripening 3 mo	32,3[e]	32,8[f]	34,1[g]	36,0[h]	36,4[h]
Lipide,%	Early ripening	49,7[a]	48,8[a]	48,0[a]	51[a]	49[a]
	Ripening 3 mo	57,0[e]	54,3[e]	57,0[e]	55,5[e]	57,1[e]
Pyruvic ac*, ppm	Early ripening	32,05	37,94	32,08	34,75	70,15
	Ripening 3 mo	38,79	31,28	35,4	ND	71,6
Lactic ac*, ppm	Early ripening	249,15	265,8	450,44	502,68	25,38
	Ripening 3 mo	106,16	190	157,75	844,17	517,45

ND=not detected
[a,b,c,d],Different superscripts on rows indícate significantly diferents (P<0,05)
[e,f,g,h], Different superscripts on column sama indicated significantly different (P<0,05)
* not statistically calculated

Flavor

The experiment of flavor was to measure flavor score (the intensity of flavor) and flavor note (consumer acceptability/ likeness of products) has been presented at Table 5.
Overall products have indicated that treated cheese with LAB caused higher score of sweetness, and bitterness. While acid taste of products were lower in cheese containing probiotic cultures as in P1 and P4. The more the cheeses ripened the higher sweetness and conversely the acid taste of products. The bitterness of products, which in cheeses influenced fort the flavor note of cheese as well as acid taste, have been indicated higher along the time of ripeneing and the use of LAB number, except for P4.The use of lactobacilli have been detected realsed some amino acids that contributed to bitterness of products (Puchades et al., 1989). The use of yoghurt culture in P2 have indicated the greatest value of bitterness, probably due to *L. bulgaricus* present which have proteolytic capacity greater than other

Table 5. Flavor score and flavor note of ripened cheese

Product	Ripening month	sweetness	acidity	saltiness	bitterness	likeness
P0	0	1,12	3,12	2,75	2,12	1,41
	1,5	1,25	2,6	2,4	1,62	1,84
	3	1,25	3,1	2,87	2,12	1,56
P1	0	1.25	3.25	3.5	1.6	1.56
	1,5	1,25	2,5	3,4	1,75	ND
	3	1,75	2,1	3,1	2,5	2
P2	0	1,25	3,2	2,8	2,4	1,2
	1,5	1,25	3,2	2,8	2,4	ND
	3	1,4	2,4	2	3,4	1,75
P3	0	1,4	3,7	2,7	2,1	1,3
	1,5	1,6	3,4	3,1	2,5	1,7
	3	1,4	3,2	2,4	2,1	2,0
P4	0	1,4	1,9	2,6	1,75	2,16
	1,5	1,5	2,4	2,75	2,1	2,41
	3	1,6	1,75	2,1	2,1	2,28

Score 0= no.x., 1= litte.x.., 2= moderately.x.., 3 x= sweet/acid/salt/bitter, 4= very.x.... ND= no data
X = kind of taste

LAB .It was known that yoghurt bacteria more proteolytic than probiotic cultures. (Murti and Cahyadi, 2007).

Acceptability of ripened cheeses will reduce after certain time of ripening (Ong, et al., 2007b). During the ripening time, proteolysis will breakdown protein followed by peptidolytic by peptidases of LAB (Swearingen et al., 2001). But, Overall likeness of products indicated that P4 which contains 2 probiotic cultures were better appreciated by entrained panelist than the others, even after 3 months of ripening time at 7-8 ^{0} C as well as control.

CONCLUSIONS

It is concluded that the use of lactic acid bacteria will influence the physical and chemical composition as also indicated by Imm et al.,(2003). The more the use LAB have augmented the rendement of products as well as protein content either at early ripening time or at 3 month of ripening time. The use of LAB did not influenced really the fat content of products.

Pyruvic acid have a relation ship to the production of lactic acid of products. The reduction of pyruvic acid have followed by the augmentation of lactic acid.

The acidity and bitterness of products, which the main flavor of cheese appreciated by consumers were higher in treated cheeses, except for the cheese supplemented by 4 kind of LAB cultures. The kind of substances included amino acids that contributed to bitterness of products should be evaluated in order to improve the acceptability of consumer by developing non bitterness products or even to accelarte ripening time (Trepanier et al., 2002)

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