

CHAPTER VI

RHEOLOGICAL PROPERTIES OF SKIM AND GOAT MILK GEL BY MILK CLOTTING ENZYME FROM *P. acidilactici* SH AND *L. paracasei* CF1

6.1 INTRODUCTION

The physical and textural characteristics of the milk gel are dependent on milk type and their composition (Vladimir et al., 2014). Some of the common physical properties of soft gel products are firmness, creaminess, viscosity, chalkiness, and syneresis (Tamime & Robinson, 1999). Casein is the main protein component of milk accounting for about 80% of the total protein and plays a major role in the rheological properties of milk gels (Van-Vliet et al., 2004) and protein composition determines the texture characterization of gel (Rita et al., 2012). Enzymes are commonly used to degrade the protein structure to enhance the products (Corredig & Dalgleish, 1997). Degradation of casein micelles in milk can be realized by various proteolytic enzymes obtained from animal, plant and microbial sources (Pezeshki et al., 2011) resulting in coagulation of milk protein gel. Coagulation of milk by enzyme is divided into two stages; firstly enzymatic hydrolysis of κ -casein to *para*-casein, and secondly casein micelle aggregation (Lucey, 2002). Gel texture in dairy product is considered as one of the most important factor used to determine the type of products (Wang & ying, 2012). The acceptance of a gel product by consumers depends on it is appearance, flavor and texture therefor; analysis of the rheological properties of dairy products is useful in determining the structure of the product (Jaros et al., 2007).

The pH and temperature are important factors that determine the rheological and textural property of the final milk protein gel. The micelles of casein are stable at the neutral pH which enhances the gel storage or shelf life (Anwer et al., 2013). Temperature greatly has influence on the structural properties of a final gel by affecting the enzyme reaction rate. Heat treatment of milk changes the stability of milk component (Horne, 1998). Increasing the temperature up 40°C enhances the strength of hydrophobic bond and calcium ion which is more strongly attached to the micelle. Further increase in the temperature to 90°C induces the κ -casein and β -lactoglobulin in whey to make complex compound due to denaturation of whey protein (Sodini et al., 2006). Lowering the pH of the gel has been found to increase the rate of syneresis (Hansen et al., 2010). Daviau et al., (2000) reported that dropping the pH of milk from 6.7 to 5.8 leads to faster rennet coagulation and faster syneresis.

The texture and sensory properties of milk gels are greatly influenced by the total solids content of the milk. Several researchers reported the relationship between enrichment of milk with milk powder for yogurt making that influence the rheological and physical properties of yogurt (Harwalkar & Kalab, 1986; Becker & Puan 1989). Casein network before and during gel formation were reported responsible for whey expulsion and some rheological properties (Lucey, 2001) in various cheese-making because of its effect on cheese quality and yield of gel (Weber, 1987). Milk curd formed with less whey expulsion would have a longer storage time (Serra et al., 2007). Syneresis is affected by temperature, pH, enzyme concentration and processing time by promoting protein matrix contraction (Mateo et al., 2009). Castillo et al. (2006)

reported that syneresis can occur by three possibilities: change in solubility, rearrangement and shrinkage of casein particles.

Flavor is one of the important for sensory properties which is vital in the selection and acceptance of dairy products. Fermentation and processing of dairy product such as yogurt and soft cheese influence the flavor and sensory attributes of final product. The flavor compounds in cheese are derived from the milk protein breakdown by indigenous enzyme such as plasmin or animal, plant and microbial enzyme which added to the milk or microorganism which, involved in the degradation of milk proteins, fat and carbohydrates (Forde & Fitzgerald, 2000).

Dadih is a non-fermented milk product traditionally, made by adding the leaves of *kesenai* (*Streblus asper*) to the milk followed by steaming after and as result a soft curd product called dadih is obtained. Dadih has a characteristic of custard-like dessert well known in Malaysia and traditionally made from buffalo milk. Hafiz, (2012) reported that MCE from LAB isolated from buffalo milk was able to clot skim milk. It is expected that the extracellular proteinase produced from LAB can be served as substitute for rennet and produce gel-like cheese.

Therefore, the purpose of this study was to evaluate the rheological properties of skim and goat milk gels made by enzyme produced from *P. acidilactici* SH and *L. paracasei* CF1. The effect of increasing total solids to both milk on gel properties and sensory properties of dadih was also determined.

6.2 MATERIALS AND METHODS

6.2.1 Preparation of Soft Gel from Goat and Skim Milk Using of Enzyme

Produced by *P. acidilactici* SH and *L. paracasei* CF1

6.2.2 Samples Preparation

Fresh goat milk was purchased from a local farm in Nilai, Negeri Sembilan, Malaysia. (Total solid 9.4% using a pocket refractometer (Pal-2 Atago® Tokyo), and pH 6.5). The total solid was increased to 11.5 °Brix by the addition of goat milk powder. Skim milk powder (OXOID LTD., BASINGSTOKE HAMPSHIRE ENGLAND) was reconstituted to 12.5 g was dissolved in 100 ml of distilled water. Both samples were pasteurized at 70°C for 15 min and rapidly cooled to room temperature. Calcium chloride 0.00665 g (6mM w/v) was then added to the goat and skim milk samples. The curd MCE from *P. acidilactici* SH and *L. paracasei* CF1 were prepared as described in chapter 3. Different concentrations of enzymes (5, 10 and 15%, v/v) were added to the skim milk and goat milk mixture. The mixture was thoroughly stirred and held at 50°C for 10 min for gel formation then the samples were cooled at 4°C for further analysis.

6.2.3 Texture Analysis

A texture profile analysis (TPA) was performed with the analytical method of goat and skim milk gels were analysis using a texture analyzer, TA-XT2 model using an Expert Software version 1.05 (Stable Micro Systems, Surrey, UK) with slightly

modified from Kumar and Mishra (2004). The gel samples were prepared in cubes 2 cm height x 3 cm radius the cubes were placed in the center of the plate of texture analyzer. The test conditions were at room temperature. A probe of aluminium cylinder P36R with a diameter of 35 mm was used. A compression strain of 60% was used with a 5 kg load cell at a speed of 5 mm/s. The texture variables, hardness (expressed as g), gumminess (expressed as g), chewiness (expressed as N*mm), cohesiveness (a dimensional) and springiness (expressed as mm) the samples were measured in triplicates.

6.2.4 Determination of Syneresis

Syneresis of the milk gel was determined as described by Chye et al. (2012) with modification was determined by placing No. 1 Whatman filter paper in a Buchner funnel placed in an Erlenmeyer flask and attached to a vacuum pump (Fisher Scientific, FB 70155). A total of 20 g of the curd samples was then spread evenly on the filter paper and vacuum-filtered for 10 min. The collected residue was weighed and percent syneresis was calculated by following formula.

$$\text{Syneresis (\%)} = \frac{V1 - V2}{V1} \times 100$$

Where: V1 = weight of sample before vacuum; V2 = weight of sample after vacuum

6.2.5 Goats Milk Dadih

6.2.5.1 Preparation of Dadih from Goat and Skim Milk Using 10% of Enzyme Produced by *P. acidilactici* SH

The Total solid (TS) of goat milk and was 8.1 °Brix total solid of original goat milk and was increased to 11.5 °Brix by adding goat milk powder to the fresh goat milk and TS was checked using a pocket refractometer (Pal-2 Atago® Tokyo). Preliminary studies using 5%, 10% and 15% sugar concentration in making dadih showed that using 10% sugar concentration gave the best result hence, 10% sugar concentration was chosen to for dadih production (10% w/v). 0.1% salt was also added to the milk mixture. The mixture was pasteurized at 70°C for 15 min, cooled to room temperature and calcium chloride 0.00665 g (6mM) was added. A 10% (v/v) of MCE produced by *P. acidilactici* SH was added to the mixture. The sample was kept at 50°C for 10 min and then steamed for 5 min followed by cooling at room temperature and store at 4°C for further analysis.

Dadih made with skim milk was similarly prepared for comparison. Skim milk powder (12.5 g) was weighed and dissolves in 100 ml of water with TS reading 12.8 °Brix. The mixture was pasteurized at 70°C for 15 min cooled to room temperature and calcium chloride 0.00665 g (6mM) was added. A 10% (v/v) of MCE produced by *P. acidilactici* SH was added to the mixture. The sample was kept at 50°C for 10 min and then steamed for 5 min followed by cooling at room temperature and store at 4°C for further analysis.

6.2.6 Statistical Analysis

All data were analyzed using MINITAB Version 16 and a General Linear model of one-way ANOVA and using Turkey's Pairwise Comparison. The variables were two types of milk (goat and skim milk) and 3 different concentrations of MCE (5, 10 and 15%). All measurable milk clotting activity at ($P \leq 0.01$) data were measured for statistical analysis.

6.3 RESULTS

Addition of the enzyme at different concentrations to skim milk and goat milk showed different rheological properties as described below.

6.3.1 Texture Analysis

6.3.1.1 Hardness (g)

The concentration of the MCE produced by *P. acidilactici* SH significantly ($p < 0.01$) affected the hardness of gel observed in goat and skim milk. The hardness of goat milk gel (g) was 5.80, 13.81 and 8.30 for 5%, 10% and 15% enzyme concentration, respectively. However, skim milk gel recorded a higher hardness values compared to goat milk with values of 6.42, 14.38, and 8.77 (g) at 5%, 10% and 15% enzyme concentration, respectively (Table 12). It was observed at using enzyme more 10% tend to destroy the texture of the milk gel.

Table: 12 Hardness (g) Analyses of Goat and Skim Milk Gel Produced by *P. acidilactici* SH Enzyme *

Enzyme concentration % (v/v)	Goat milk	Skim milk
05	05.80±0.35 ^c	06.42±0.99 ^b
10	13.81±0.98 ^a	14.38±1.01 ^a
15	08.30±0.64 ^b	08.77±1.19 ^b

*(a-c) Means in the same column followed by different letters were significantly different (P<0.05).

Although a slightly softer gel was produced by MCE from *L. paracasei* CF1 (Table 13), a similar pattern was observed as for MCE *P. acidilactici* SH. Hardness values were 3.63 and 10.39 (g) for goat milk gel and 5.33 and 11.75 (g) for skim milk gel at 5 and 10% enzyme concentration. The gel strength of goat and skim milk was reduced at 15% enzyme produced by *L. paracasei* CF, similar to that observed for *P. acidilactici* SH.

Table: 13 Hardness Analyses of Goat and Skim Milk Gel Produced by *L. paracasei* CF1 Enzyme*

Enzyme concentration %(v/v)	Goat milk	Skim milk
05	03.63±0.513 ^c	05.33±0.31 ^c
10	10.39±0.442 ^a	11.75±0.59 ^a
15	06.40±0.426 ^b	07.49±0.50 ^b

*(a-c) Means in the same column followed by different letters were significantly different (P<0.05).

6.3.1.2 Gumminess (g)

Gumminess of the goat milk and skim milk gel was significantly (p<0.05) affected by enzyme concentrations. Slightly higher values in gel gumminess were observed for MCE *P. acidilactici* SH than *L. paracasei* CF1 at the enzyme concentration used (Table 14 and 15). Gumminess of goat milk gel was 4.23, 6.71 and 4.14 at 5%, 10% and 15%, respectively. A slightly higher gumminess values were observed for skim

milk gel that were 5.21%, 6.78%, and 5.33 for 5%, 10% and 15% enzyme concentration, respectively. A decline in gumminess was observed with further increase in enzyme concentration to 15% in both milk types.

Table: 14 Gumminess (g) Analyses of Goat and Skim Milk Gel Produced by *P. acidilactici* SH Enzyme*

Enzyme concentration %	Goat milk	Skim milk
5.0	4.23±0.51 ^b	5.21±0.39 ^b
10	6.71±0.82 ^a	6.78±0.26 ^a
15	4.14±0.67 ^b	5.33±0.69 ^b

*(a-b) Means in the same column followed by different letters were significantly different (P<0.05).

Table: 15 Gumminess (g) Analyses of Goat and Skim Milk Gel Produced by *L. paracasei* CF1 Enzyme*

Enzyme concentration %	Goat milk	Skim milk
5.0	3.33±0.33 ^b	4.15±0.29 ^c
10	4.56±0.39 ^a	5.37±0.42 ^a
15	3.53±0.20 ^b	4.35±0.35 ^b

*(a-c) Means in the same column followed by different letters were significantly different (P<0.05).

6.3.1.3 Chewiness

Chewiness of goat and skim milk gel was significantly ($p < 0.01$) affected by enzyme concentrations. Gel chewiness recorded considerably higher values for enzyme *P. acidilactici* SH than *L. paracasei* CF1 at 5%, 10% and 15% enzyme concentration (Table 16 and 17). Chewiness of goat milk gel for MCE *P. acidilactici* SH was 4.70, 6.40 and 3.80 at 5%, 10% and 15%, respectively. A slightly higher chewiness values were observed for skim milk gel that were 5.01%, 6.97%, and 5.76 for 5%, 10% and

15% enzyme concentration, respectively. A decline in chewiness values was observed with further increase in enzyme concentration to 15% in both milk types.

A similar trend was observed in the chewiness of goat and skim milk gel using MCE *L. paracasei* CF1. The chewiness of skim milk gel was slightly higher with values of 4.17, 5.69, and 4.32 at 5%, 10% and 15% enzyme concentration respectively. Chewiness of goat milk was 3.01, 4.82, and 3.31 at 5%, 10%, and 15% enzyme concentration respectively. Similarly, a decline in the chewiness value was observed with further increase in the enzyme concentration to 15% in both goat and skim milk gels.

Table: 16 Chewiness Analyses of Goat and Skim Milk Gel Produced by *P. acidilactici* SH Enzyme*

Enzyme concentration %	Goat milk	Skim milk
5.0	4.70±0.45 ^b	5.01±0.29 ^c
10	6.40±0.52 ^a	6.97±0.30 ^a
15	3.80±0.20 ^b	5.76±0.11 ^b

*(a-c) Means in the same column followed by different letters were significantly different (P<0.05).

Table: 17 Chewiness Analyses of Goat and Skim Milk Gel Produced by *L. paracasei* CF1 Enzyme*

Enzyme concentration %	Goat milk	Skim milk
5.0	3.01±0.29 ^b	4.17±0.15 ^b
10	4.82±0.30 ^a	5.69±0.20 ^a
15	3.31±0.33 ^b	4.32±0.26 ^b

*(a-b) Means in the same column followed by different letters were significantly different (P<0.05).

6.3.2 Determination of Syneresis

6.3.2.1 Syneresis of Milk Gel Produced by 10% (w/v) *P. acidilactici* SH MCE

During Storage at 4 °C for Four Weeks

A significant ($p < 0.01$) increase in the percentage of whey was observed with increase in storage period (Table 18). Syneresis of milk gel was not observed immediately after its formation but increase to 5% for of goat milk gel and 7% for skim milk recorded after one day storage at 4°C. A slight increase in whey percentage was observed after 7 d of in both skim milk (6%) and goat milk (10%). Longer storage time (>7 d) gel syneresis increased in both skim and goat milk gel until 28 d of storage. The whey percentages in skim milk gel after 14, 21, and 28 days were 8%, 12% and 28%, respectively. However, the amount of whey released from skim milk gel was higher than those of goat milk gel (Table 18).

Table 18: Percentage (%) Syneresis of Goat and Skim Milk Gel at 4°C using 10% of *P. acidilactici* SH Enzyme*

Days	Syneresis %	
	Goat milk gel	Skim milk gel
1.0	07±0.40 ^{eg}	05.0±1.00 ^h
7.0	10±0.20 ^e	06.0±0.30 ^{hg}
14	13±0.47 ^d	08.0±0.38 ^{ef}
21	19±0.45 ^c	12.0±0.52 ^d
28	47±1.06 ^a	30.0±0.79 ^b

*No significant different between values with the same letter

6.3.2.2 Syneresis Milk Gel Produced by 10% *L. paracasei* CF1 MCE During Storage at 4 °C for Four Weeks

The syneresis of milk gel produced by *L. paracasei* CF1 MCE for both goat and skim milk gel was more noticeable compared to that of milk gel produced by *P. acidilactici* SH MCE. The results show a significant ($p < 0.01$) increase in syneresis after 7 d storage. After one day of storage, the amount of whey increased in both goat and skim milk gel to 10% and 12%, respectively. Similar trend was observed in the syneresis at 7, 14, 21 and 28 d. The percentage syneresis for goat milk gel during these periods was 13, 16, 21 and 42%, respectively. Higher percentage of whey released was observed for skim milk gel which was 16, 24, 42 and 59% (Table 19). However, it was observed that the percentage syneresis of goat milk using *L. paracasei* CF1 MCE was consistently lower during the storage period.

Table 19: Percentage (%) Syneresis of Goat and Skim Milk Gel at 4 °C using 10% of *L. paracasei* CF1 Enzyme*

Days	Syneresis %	
	Goat milk gel	Skim milk gel
1.0	10±1.00 ^e	12±1.52 ^e
7.0	13±1.60 ^{de}	16±1.25 ^d
14	16±1.15 ^d	24±1.45 ^c
21	21±1.04 ^c	42±0.81 ^b
28	42±1.06 ^b	59±1.48 ^a

*No significant different between values with the same letter

Based on the results obtained for the texture profile analysis, it was observed that using 10% *P. acidilactici* SH MCE produced good texture profile for both goat and skim milk gel with low amount of whey released. Hence, this concentration was used in producing dadih from both goat and skim milk.

6.3.3 Texture Profile Analysis of Dadih Using 10% of (w/v) *P. acidilactici* SH Milk Clotting Enzyme

Dadiah was prepared by mixing milk with 10% of sugar 0.1% of salt, 0.0065g of CaCl_2 and powdered milk (goat or skim) to increase the total solid to 15°Brix and 10% *P. acidilactici* SH MCE as outlined in the method. The texture profile of skim and goat milk dadiah is shown in Table 20. It was observed that dadiah from goat milk was softer in texture and higher in gumminess than skim milk but of no significant difference between the dadiah samples. However, the chewiness of dadiah was significantly different between dadiah made from goat milk and skim milk with values of 6.82 and 4.3, respectively.

Table: 20 Texture Profiles of Skim Milk and Goat Milk Dadiah using 10 % of the Enzyme produced by *P. acidilactici* SH*

Texture profile analysis	Goat milk dadiah	Skim milk dadiah
Hardness (g)	16.81±1.24 ^a	19.22±1.24 ^a
Gumminess (g)	07.92±1.34 ^b	07.45±1.25 ^b
Chewiness	06.82±0.81 ^b	04.43±1.12 ^c

(a-c) Means in the same column followed by different letters were significantly different ($P < 0.05$)

6.3.4 Syneresis of Dadiah during storage

Percentage of syneresis increased with storage time and was significantly different ($p < 0.01$) between the dadiah samples (Table 21). After 1 d of storage, the percentage of syneresis was 3.7% for goat milk and 4% for skim milk. A rapid increase in percentage syneresis was observed for dadiah made using skim milk than goat milk

after 7 d storage. After 28 d of storage, the percentage syneresis was significantly lower for goat milk (24%) compared to skim milk dadih (27%).

Table: 21 Percent (%) Syneresis of Skim and Goat Milk Dadih at 4°C using 10% of Enzyme produced by *P. acidilactici* SH *

Days	Syneresis %	
	Goat milk dadih	Skim milk dadih
1.0	3.0±0.35 ^g	4.00±0.20 ^{fg}
7.0	5.0±0.25 ^f	7.00±0.60 ^e
14	7.0±0.45 ^e	11.0±0.40 ^d
21	10.0±0.35 ^d	13.6±0.58 ^c
28	24.0±0.35 ^a	27.0±0.40 ^b

*No significant different between values with the same letter

6.4 DISCUSSION

Rheological and physical properties are important characteristics of milk gel that help in determining the quality and overall sensory acceptability of the product. The texture and sensory properties of milk gels are greatly influenced by many factors such as total solids (Lee & Lucy, 2010), type of milk, heat treatment and the chemical composition of milk e.g. (protein content, fat, carbohydrates, pH and endogenous enzymes (Gutierrez-Mendez et al., 2013)). The breakdown of κ -casein layer on the micelle surface by enzyme leads to aggregation of casein and gel formation (Muller-Buschbaum et al., 2007). The casein network structure is also affected by composition of milk and technological conditions of coagulation (Gunasekaran & Ak 2003; Phadungath, 2005). In this present study it was observed that factors such as the milk type, source of microbial enzyme and enzyme concentration affected the gel properties. The concentration of *P. acidilactici* SH MCE and *L. paracasei* CF1 MCE significantly ($p < 0.01$) affected the textural properties of the curd. Using different

concentration of MCE affected the amount of κ -casein hydrolysis that may be due to changes in the balance between the rates of the enzymatic and aggregation reactions (Lomholt & Qvist, 1997). Increase of enzyme concentration influenced the textural properties because reaction of enzyme is faster and leading to a higher degree of κ -casein hydrolysis before aggregation and, also affected the casein micelles leading to less caseinomacropptide formation on the surface at a given degree of aggregation or gel formation (Karlsson et al., 2007; Sbodio & Revelli, 2012; Sathya et al., 2013).

Hardness, gumminess and chewiness of milk gel were significantly affected by the enzyme concentration and type of milk. The values for hardness, gumminess and chewiness were higher in gel from skim milk compared to goat milk. Hardness is the force required to achieve a given deformation with a maximum force bite when the sample is placed between the molars (Nateghi et al., 2012). An increase in the enzyme concentration from 5% to 10% for both enzymes *P. acidilacici* SH and *L. paracasei* CF1 resulted in increase in the hardness of skim milk gel. However, at 15% enzyme concentration, the hardness of skim milk and goat milk gel decreased (Table 14, 15). Mahmood and Hurmiz (2012) reported that increasing the total solid concentration of milk led to an increase in gel strength. The high hardness observed in skim milk gel may be caused by the high total solid concentration contained in skim milk compared to goat milk. Rashidi et al. (2015) reported that the hardness low-fat milk curd is greater than high fat curd because of higher casein content. The significantly ($p < 0.01$) higher hardness of skim milk gel may be caused by the low-fat content of skim milk compared to high fat content in goat milk. Koca and Metin, (2004) reported that the protein matrix in high fat cheese causes easy breakage thereby making cheese to

become softer. Sbodio and Revelli (2003); Wium and Qvist (1998) found that increasing the enzyme concentration strongly affected the fractions of casein by making the gel to break easily. Hence, the reduction in the hardness was observed at 15% enzyme concentration from both enzymes used.

An important characteristic of a milk gel is syneresis. Syneresis is considered one of the main problems during storage of the dairy products. Syneresis influences protein losses in whey and thus curd yield. It was observed from this study that the period of storage and type of milk significantly ($p < 0.01$) influenced the amount of whey expulsion during storage period. The amount of whey that was expelled from skim milk gel was higher than that obtained in goat milk gel after four weeks of storage. Mateo et al. (2009) reported that the syneresis is affected by temperature, pH, enzyme concentration and processing condition. Temperature greatly influenced the structural properties of the final gel by affecting the enzyme reaction rate during processing. Guyomarc (2005) reported that the heat treatment of milk induced whey protein aggregates or protein-calcium complexes and retention of the whey proteins into the curd. Denaturation of whey proteins lead to increase in the binding ability with the casein micelle. The association of the whey protein with casein micelles helps in cross-linking the casein particles in the gel network resulting in an increase in the pH at gelation (Lucey et al., 1998). Incubating the milk with the enzyme at 50°C for 10 min allowed milk coagulation and gel formation. The temperature used was optimum for the MCE activity. Gel formation using renin is normally carried at 40 to 45°C. The formation of milk gel is associated with κ -casein via hydrophobic interactions and formed intermolecular disulphide bonds (Haque & Kinsella, 1988). The cross-linkages

between whey protein and casein result in the milk gel structure as in soft cheese and dadih. The temperature used was low since heat treatment of milk above 70°C would cause denaturation of the whey proteins (Haque & Kinsella, 1988). It was observed that the textural properties of enzyme produced by *P. acidilactici* SH at 10% concentration had higher values of hardness, gumminess and chewiness than the gel produced using *L. paracasei* CF1. The results indicate that the syneresis of the goat milk dadih was lower than skim milk dadih during the four weeks storage at 4°C than dadih made using skim milk.

6.5 CONCLUSION

Milk coagulation is the primary step in the development of texture and flavor of milk gel that depends on specific enzymatic degradation of milk compounds. Using 10% enzyme concentration of *P. acidilactici* SH resulted in good gel formation with very low syneresis for both dadih made from goat milk and skim milk. Addition of sugar and salts enhanced the texture and sensory acceptability of both types of dadih. Dadih could be produced using either goat milk or skim milk but goat milk dadih showed better sensory acceptability than skim milk dadih and was more stable during 4 weeks storage at 4°C.