Physicochemical and Rheological Properties of Acid-Induced Milk Proteins Gel

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Abstract: ***The spontaneous syneresis of acid gels was studied. We investigated the effect of temperature and acid concentration on the degree of syneresis, rheological properties and microstructure of curds. Milk was coagulated with citric acid solution, syneresis extent and curd yield were determination afterward the whey was drained and weighed. Rheometer was used to analyze the viscoelasticity of the curd and SEM (Scanning Electron Microscopy) was used for the curd microstructure. The syneresis extent was influenced by the heat treatment of the milk before its coagulation and by the acid concentration. In addition, the interaction effect of the two factors showed that the combination 2.5%, 80 had higher syneresis degree and the combinations 5%, 90℃; 7.5%, 90℃ and 10%, 90℃ recorded the highest curd yield. The density and the porosity of the casein matrix decreased with the increase of the temperature. The viscoelasticity of the curds was also affected by the temperature and the acid concentration. These results can be used to manufacture heat combined acid type cheeses.***

Keywords: syneresis, citric acid, curd, viscoelasticity, microstructure, yield

1. Introduction

Gelation of the proteins in milk is the basis for the manufacture of cheese and fermented milk products. Various approaches can be used to destabilize the milk proteins for cheese production[1]. The utilization of rennet is the common way to destabilize the milk proteins for cheese making, which consists of an enzymatic hydrolysis of κ-casein with a concomitant drop of pH, which causes a dissolution of the colloidal calcium and aggregation of casein micelles. Contrary to the use of organic acid which process, consists of the release of colloidal calcium phosphate (CCP) from casein micelles and net-negative charges lost with decreasing the pH, thereby reducing the electrostatic and steric repulsion and favor aggregation[2]. Some authors have investigated on acid gel (GDL); however, these works focused on yogurt manufactureconditions. Many cheese varieties are produced by direct acidification (direct acidified cheese) of high heat treated milk, whereby the whey proteins are denatured and are recovered in the cheese as casein-whey protein complex[3]. Citric acid is an organic acid extract from lemon juice (Citrus limon) which makes it available and accessible. Previous research haves shown that citric acid has been can be used to make many types of cheese such as Paneer cheese[4], Ricotta cheese[5, 6],Wara cheese[7]. The extent of whey separation from milk gel remains important for the final cheese characteristics (moisture, texture, rheology, structure). This phenomenon can be affected by several factors including temperature of pretreatment, coagulant strength, pH so on. In addition, cheese yield is determinant for profitability in cheese-manufacturing plants.
The objectives of the current study were therefore to investigate 1) the extend of spontaneous syneresis of citric acid induced gel at different heat temperatures and different concentrations of citric acid 2) evaluate the curd yield 3) study the rheological and microstructural properties of the curds.

2. Materials and Methods

2.1 Materials

Raw milk was obtained from Beijing SanyuanLuhe Dairy Farm and stored at 4 ℃. Citric acid powder was obtained from Sinopharm Chemical Reagent Co., Ltd (China).

2.2 Coagulation, whey separation, spontaneous

Cow milk (70 mL) were put in 100 mL beaker and heated in a water bath (DK-S24 SUMSUNG Instrument. Korea) to 70 ℃, 80 ℃ and 90 ℃ respectively for five minutes. Citric acid was diluted with distilled water (g/mL) then 4mL of each solution were separately added to the milk. The samples were stirred for 30 seconds and allowed to stand in the water bath for 15 min. The apparatus used for whey draining consisted of a conical funnel containing a metallic net with 1 mm mesh size. The content of the beaker was reversed over the metallic net. The whey was allowed to drain during 10 min at room temperature then collected in a graduated tube (100 mL).

2.3 Syneresis and yield evaluation

The whey collected in the glass tube and the resultant curd were weighed using a precision balance. The syneresis degree was the ration between the collected whey and the originated milk weight. The curd yield was estimated as the ration of the collected curd and the weight of the milk. The curd yield and the extent of spontaneous syneresis were calculated following.

$$SE = \frac{Wf}{Mv} \times 100$$

(E1)

$$CY = \frac{Cw}{Mw} \times 100$$

(E1)

SE: Syneresis extent, Wf: Final whey volume, Mv: milk volume

CY: Curd yield, Cw: Curd weight, Mw: Milk weight, E: Equation

2.4 Rheological analysis of milk Curds

The viscoelastic properties of the curds were measured according to the method described by Ramirez Lobato, Espinosa and Eduardo[8] with slight modifications using a Physica MCR 301 Rheometer (Anton Paar Graz, Auatralia) at 35 ℃. A probe (P50) with a circular plate 30 mm diameter was used. Curd samples (~ 1.5 g) were carefully placed in the measuring system and left to rest for 30 min for structure recovery. Afterwards, amplitude sweeps were carried out to characterize the viscoelastic linear region (LVR) of the samples; frequency sweep was performed from 0.1 to 100 Hz at 0.1% of strain. The storage modulus (G′) and the loss modulus (G″) were determined. Measurements were carried out the same day after whey draining and each sample were measured in triplicate.

2.5 Microstructure of milk curds after whey separation

SEM was used to examine the changes in curds microstructure.Samples were prepared according to the previous study of Kuo & Gunasekaran[9] with modifications. Curd samples approximately 2×2×10 mm, were cut using razor blade and immediately fixed in 2.8 g/100 g glutaraldehyde in a 0.05 mol/L sodium phosphate buffer (pH 7.4) for 3 h at 7 ℃. Samples were post fixed with 1% osmium acid for 2 h. The samples were rinsed three times with distilled water, 3 min / time. The fixed curd samples were dehydrated in a graded ethanol series of a 30, 50, 60, 70, 80, 90, 95 and 100% for 15 min in each. Samples were dried using a Leica CPD 030 CO_2 critical point dryer. The dried, fractured curd samples were mounted on aluminium SEM stubs using a carbon-based tape and coated with gold in a DC sputter coater (HITACHI MC1000) (The coating with gold increases the conductivity of biological samples and improves the sample surface secondary electron emissivity, improve the signal to noise ratio and make clear the image. In addition because the prevention of thermal damage of the sample).

2.6 Statistical analysis

The data were presented as mean SE of three values, statistical difference between treatments (heat temperature and coagulants concentrations) was assessed using Least Squares Meansas well as the syneresis extent and curd yield, using JMP for Windows.

3. Results and Discussion

3.1 Effect of heat temperature and citric acid concentrations on the syneresis extent

The extent of syneresis was compared between the different heat temperatures (70 ℃, 80 ℃ and 90 ℃). There was a significant difference (P< 0001) between the extents of syneresis at all the temperature treatment of the milk. 70 ℃ recorded the higher syneresis extent than 80 ℃ and 90 ℃**(Table 1)**. The treatment temperature has affected the syneresis extent and as seen in the **Table 1,** the syneresis extent decreased with the increase of the temperature. Lucey[1] reported that high-heat treatment causes considerable whey protein denaturation; β-lactoglobulin becomes mostly attached to the κ-casein of casein micelle. Thus more high is the temperature more the whey proteins are denatured which will probably reduce the drainage of the whey. The acid concentration affected significantly the syneresis extent. The syneresis extent decreased with the increase of the concentration. 2.5% recorded the highest syneresis extent compared with the others concentration followed by 5% and 10% recorded the lowest syneresis extent**(Table 2)**. This may be attributed to the coagulation pH level during the whey separation. The high acid concentration (10%) may resulted in a considerable decrease of the pH, the isoelectric points of the whey proteins was approached allowing the aggregation the whey protein (both those associated with the casein micelles and those in the serum[10]. An interaction was found between the factors (treatment temperature and acid concentration). The treatment combinations 2.5, 80 and 2.5,70 recorded the highest syneresis extent unlike to 10, 90 which recorded the lowest syneresis extent**(Table 3).**

Table 1: Syneresis extent as function of the treatment temperature

Temperature (℃)	Syneresis extent (%)	SE
70	84.86^{a}	0.36
80	81.62^{b}	0.36
90	78.58^{c}	0.36

Values are means of three replications. Levels not connected by same letter are significantly different. SE: Standard Error

Table 2: Syneresis extent as function of the coagulant concentration

Concentrations (%)	Syneresis extent (%)	SE
2.5	86.25^{a}	0.42
5	81.81^{b}	0.42
7.5	79.73^{c}	0.42
10	78.95^{c}	0.42

Values are means of three replications. Levels not connected by same letter are significantly different. SE: Standard Error

Table 3: Interaction between temperature and acid concentration on the syneresis extent

Acid conc. (%)	Temp. (℃)	Syneresis (%)	SE
	70	86.42^{ab}	0.73
2.5	80	87.09^{a}	0.73
	90	85.23^{ab}	0.73
	70	85.85^{ab}	0.73
5	80	82.82^{b}	0.73
	90	76.76^{c}	0.73
	70	83.83^{ab}	0.73
7.5	80	78.29^{c}	0.73
	90	77.11^{c}	073
	70	83.32^{b}	0.73
10	80	78.29^{c}	0.73
	90	75.24^{c}	0.73
P-value		0.0001	

Values are means of three replications. Levels not connected by same letter are significantly different. SE: Standard Error

3.2 Effect of heat temperature and citric acid concentrations on curds yield

The comparison of the curd yield between heat temperature (70 ℃, 80 ℃ and 90 ℃) is shown in **Table 4.** There was a significant difference curd yield at all the treatment temperatures and the yield increased with the temperature. The increase of the curd yield was probably due to the binding of the denatured whey proteins to the k-casein due to the high heat treatment[3]. In addition the increase of the curd yield was probably due to the increase of the curd moisture due to the high degree of whey protein denaturation. Heat treatment of milk at ~ 110 ℃ for 60 seconds increased protein recovery in curd by 10%[11].

The results in **Table 5** showed that there was no significant difference between the curd yields at the different citric acid concentrations 5%, 7.5% and 10% except 2.5%, which recorded a low curd yield compared with the previous ones. Similar results was found by Bankar, Raziuddin, and Zanjad[12]. Those authors did not observe a significant increase of Chhana cheese yield of manufactured using citric acid at different concentrations. These results suggested that the amount of acid did not significantly affect the curd yield. The interaction between the factors is shown in the **Table 6.** The treatment combination10%, 90 ℃ recorded the highest curd yield whereas 5%, 70 ℃ recorded the lowest.

Table 4: Curd yield as function of the treatment temperature

Temperature (℃)	Curd yield (%)	SE
70	15.63^{c}	0.32
80	17.46^{b}	0.32
90	19.93^{a}	0.32

Values are means of three replications. Levels not connected by same letter are significantly different. SE: Standard Error

Table 5: Curd yield as function of the acid concentration

Acid concentration (%)	Curd yield (%)	SE
2.5	16.13^{b}	0.37
5	18.05^{a}	0.37
7.5	18.52^{a}	0.37
10	17.98^{a}	0.37

Values are means of three replications. Levels not connected by same letter are significantly different, $p<0.05$, SE: Standard Error

Table 6: Interaction between temperature and acid concentration on the curd yield

Acid conc. (%)	Temp. (℃)	Curd yield (%)	SE
	70	15.79cd	0.64
2.5	80	15.61cd	0.64
	90	16.99cd	0.64
	70	15.29cd	0.64
5	80	17.97abcd	0.64
	90	20.90ab	0.64
	70	16.45^{cd}	0.64
7.5	80	18.52^{ab}	0.64
	90	20.60^{ab}	0.64
	70	14.99^{d}	0.64
10	80	17.72^{bcd}	0.64
	90	21.24^{a}	0.64
P-value		0.0212	

Values are means of three replications. Levels not connected by same letter are significantly different. SE: Standard Error

3.4 Viscoelasticity of milk Curds

Frequency sweeps were used to study viscoelastic behaviour of the rennet and citric acid curds (**Figure 2**). Generally the storage modulus (G') and the loss modulus (G") increased as frequency increased. This similar trend in G' and G'' was observed by Lucey, Munro and Singh[13]. The curd were compared according to the heat temperature and the coagulant concentration. The curd from milk heat at 70 ℃ had a low G' and G'' compared with that from milk heated at 90 ℃ was more elastic and viscous compared with (**Figure 2a, b**). The present results showed that the high temperature had led to a reduction of the viscoelasticity of the curd may be due to the presence of excessive denatured whey proteins. Lucey et al.[14] reported that the low G' of acid casein gels formed at high temperatures was attributed to extensive particle rearrangements during gel formation, resulting in the formation of dense clusters of aggregated particles, which in turn aggregate to form a gel. These results were in disagreement with those of Skelte et al.[15], Guinee and B. O'Brien[16] who reported that high heat treatment of the cheese milk results in an increase of the rigidity of the resultant gel, which coincides with an increase in the elastic modulus. This contradiction can be comprehensible because in our study we measured the viscoelasticity of the resultant curds after whey draining whereas those authors measured the gel viscoelasticity. I addition there is consistency between the results of the effect of interaction of the temperature and the acid concentration (**Table 3**). Those results showed that the combination 5%, 70 ℃ had higher syneresis extent compared with the combination 5%, 90℃, which imply that the curd from the combination 5%, 90℃ had high moisture than the curd from the combination 5, 70. Accordingly the curd from the combination 5%, 90 ℃ was less viscoelastic compared with that from the combination 5%, 70. Regarding the results of **Figure 2c, d**, the curd from milk coagulated with acid at 10% was elastic and viscous compared with that from milk coagulated with 5%, the two curves. As the yield, the acid concentration significantly affects the viscoelasticity of the curd which resulted in a decrease of the viscoelasticity with the increase of the acid concentration. This observation can be attributed to an excessive denaturation of the whey protein due to the high temperature and high concentration of acid.

International Journal of Science and Research (IJSR)
ISSN: 2319-7064
ResearchGate Impact Factor (2018): 0.28 | SJIF (2018): 7.426

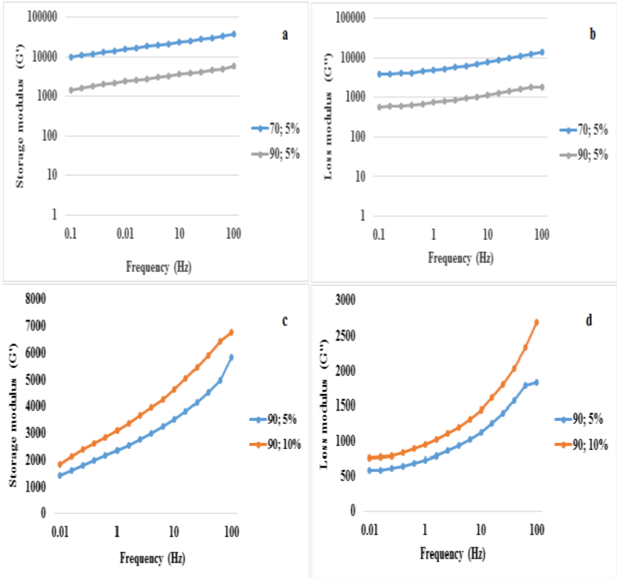

Figure 2: Storage modulus (G') and loss modulus (G'') of citric acid curd CA70; 5%: curd made by milk heated to 70 °C, coagulated with citric acid diluted at 5% with distilled water. CA90; 5%: curd made by milk heated to 90 °C, coagulated with citric acid diluted at 5%, CA90; 10%: curd made by milk heated to 90 °C coagulated with citric acid at 10%

3.5 Microstructure of milk Curds

Scanning electron microscopy (SEM) showed the microstructure of acid curds. As showed by the results in the **Figure 3,** there was a difference in the microstructure of the curds. Generally the curds microstructure was characterized by a protein matrix with casein particles linked together in clusters, chains and strands. However there was a difference between the microstructure of the curd from milk heated to 90 °C and that from milk heated to 70 °C. The difference was observed in the density of the matrix of the two microstructures. **Figure 3a** and 3**b** showed a curd withmore porous microstructure compared with **Figure 3c**. Curd from milk coagulated with acid concentration of 10% presented small sized particles compared to that coagulated with 5% of citric acid coagulant. The curd microstructure confirm the classification made by Phadungath [17] which state that at pH 4.8 and 4.7, casein particles with the previous stage of fusion were followed by a stage of contraction and rearrangement, resulting in new casein particles with spherical shapes. The curds microstructures showed in these results justify the syneresis extent and curd yield results mentioned above in **Table 3,** which results showed higher syneresis extent of the combination 5%, 70 °C compared with the combination 5%, 90 °C and 10%, 90 °C.

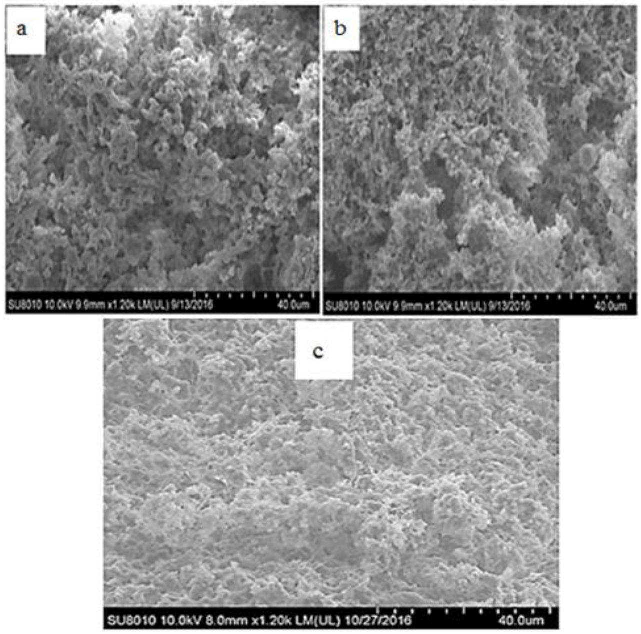

Figure 3: Scanning electron micrograph showing the microstructure of different citric acid curds (a), (b): Acid curds from milk heated to 90 °C, coagulated with citric acid diluted to 5% and 10% with distilled water, (c): Acid curd from milk heated to 70 °C and with citric acid diluted at 5% with distilled water

4. Conclusion

The present study enabled us to understand the phenomenon of syneresis gels obtained by coagulation of cow milk with citric acid and the properties of the curds. Syneresis extent was affected by the heat temperature and acid concentration. In addition, the syneresis extent decreases with the increase of the temperature. In the present study we reported that acid curd yield increase with the increase of the temperature. The syneresis and the curd yield were affected by the interaction between the temperature and the acid concentration. The rheological behaviour of the curd was justified by the microstructure; the less elastic citric acid curd had a porous casein network. From these finding we could suggest that cheese manufacture with citric acid concentrated at 10% after heating the milk to 90 °C will be more profitable. However further studies will be recommended in order to understand the physicochemical, structural, textural properties of cheeses obtained from the conditions used in the present study.

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