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Heat-acid coagulation of market-returned UHT milk using various coagulants and calcium chloride

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Abstract

Curdling of the market-returned ultra-high temperature (UHT) fresh milk by heat-direct acid coagulation was investigated. By using three acidic coagulants, the most suitable processing conditions (temperature of heat treatment: pH) for each coagulant were acetic acid (80°C: 4.7), citric acid (70°C: 4.7) and lactic acid (70°C: 5.0), respectively. In addition, pH had significant effect ($p \leq 0.05$) on Hunter L and b values regardless of types of the coagulating acid; i.e. the higher the pH, the higher L but the lower b values. An increase in coagulation temperature resulted in lower L but higher a and b values of the curds made with citric and lactic acid. When the most appropriate conditions previously identified for each acid were compared, acetic acid was chosen as most suitable for heat-acid coagulation of the aged UHT milk. Calcium chloride was also necessary for curd preparation and the most suitable level was 0.02% (by weight of the milk).

Introduction

UHT milk, although normally being sterilized by indirect heating, deteriorates to certain extent especially when kept for extended storage time. Heat treatment can alter native characteristics of casein micelles by enlarging their sizes due to denaturation and agglomeration or by migration of calcium phosphate (Singh and Wanngana, 2001). In addition, partial denaturation of whey proteins by heat and formation of complex structures with caseins result in reduced efficiency necessary for formation of lattice structure of the caseins (Bylund, 1995). Regarding these occurrences, coagulation of casein micelles into cheese curds can be impaired in spite of the fact that the native caseins are easily destabilized either by treatment with proteolytic enzymes or by acidification (Dalgleish and Corredig, 2012). Contrastingly, coprecipitates of casein and whey proteins could be made from sheep milk using the best percentage of added calcium chloride at 0.2% (Al-saadi and Deeth, 2011). Heat treatment is necessary for coprecipitate production since it causes denaturation of whey proteins and their interaction with caseins, particularly between κ-casein and β-lactoglobulin through disulfide bonds (Jang and Swaisgood, 1990). In comparison with casein, whey proteins are more stable in the presence of ionic calcium salts, but sensitive to heat (Modler, 1985a,b). Naturally, κ-casein is not calcium sensitive and, due to the dominating localization of κ-casein at the surface of the micelles, the solubility of calcium κ-caseinate prevails over the insolubility of the other two (αs and β) caseins in the micelles and the whole micelle is soluble as a colloid (Fox, 1995). However, destabilization of native casein micelles can be achieved by many different means or agents.

Milk coagulation is one of the important steps for manufacturing various types of cheeses and other products such as yoghurts. Curdling of milk proteins occurs by three major means including enzyme coagulation, acid coagulation and heat-acid coagulation. Whey proteins are the most susceptible to alteration of their native structures during heat-acid coagulation. Immunoglobulins precipitate first at the lower temperature, followed by β-lactoglobulin which unfolds to expose its sulfhydryl (-SH) groups. Upon heat treatment and an addition of acid, stability of both whey proteins and casein micelles is altered resulting in formation of whey proteins-casein complexes via sulphur bridges (-S-S-; disulphide bonds) (Bylund, 1995; Swaisgood, 2008). Thus, coagulation of caseins in this way also concomitantly combines whey proteins within the curds. Chandan (1991) stated that various types of coagulants, used in production of cheeses made by heat-acid coagulation, include such examples as i) sour buttermilk in Armavir cheese

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(West Caucasus), ii) lime or lemon juice, lactic acid, citric acid, calcium lactate, yoghurt and acid whey in Chhana (India) and iii) vinegar in Ricotta (Italy), Ziger (Germany) and Hudelziger (Switzerland). In Malaysia, dadih is a unique traditional Malay dairy dessert which is made from milk to which whey (obtained by fermenting milk overnight with asam gelugur; Garcinia atroviridis) has been added to acidify it to a pH just above the isoelectric point of casein, before addition of sugar and salt (Chye *et al*., 2012). It appears useful to examine the development of processes in order to obtain products resembling traditional cheeses or processed cheeses without the use of microbial fermentation or enzymes and there has been a beginning in the direction leading to production of processed cheeses using directly acidified, non-renneted cheeses (Pal, 2002).

In Thailand, UHT milk is popular among the Thai consumers due to its convenience, long storage life and no refrigeration chain being needed for distribution all over the whole country. Total annual production of UHT milk is approximately 165 million L. However, the unsold UHT milk is often returned to the manufacturer a few (2-3) months before the expiry date. The cost of production is high relative to the sale price; therefore, the profit for this product is small. In addition, the market-returned milk will bring about a problem in managing the unsold good. If this market-returned UHT milk, which still possesses coagulable and valuable proteins, is further processed into a product such as processed cheese block by heat coagulation and direct acidification, both the manufacturers and the consumers can obtain advantages from this kind of modification. In addition, the effects of different types of coagulant in curdling of aged UHT milk have not been studied so far. Therefore, this research was conducted to investigate chemical and physical properties of the market-returned UHT milk, determine effects of pH, kind of acid and temperature on various properties of the cheese curds and evaluate effects of different levels of $CaCl_2$ on curd's characteristics.

Materials and Methods

UHT milk raw material

Alteration of chemical and physical properties of the aged UHT milk (supplied by the major local UHT milk producer, i.e. Foremost Friesland PLC., Bangkok, Thailand) kept at 30 + 1°C (average ambient temperature) was studied by Subhimaros *et al.* (2003). It was revealed that the market-returned samples, sterilized at ≥ 133°C for ≥ 1 s and retrieved within 3 months before the expiry date (the time span after the production date being approximately 6 months for locally produced milk), were still suitable for protein coagulation. Therefore, these samples; hereafter interchangeably called the aged UHT milk or the UHT milk, were homogeneously blended and used for analyses and determination of their chemical and physical properties so that they would be used as raw material in this study.

Verifying suitable conditions for protein coagulation

In this first major step of the whole study, effects of varying temperature and pH; i.e. each type of acid being used separately, on chemical and physical properties of the cheese curds were identified. The milk sample (400 g) was added with $CaCl_2.2H_2O$ (0.02% w/w, food grade), heated in a water bath (WB-14, Memmert, Schwabach, Germany) at 70, 80, and 90 + 1°C, respectively, for 5 min. Sequentially, each type of acid namely acetic, citric and lactic acid, each being analytical reagent, at 10% (w/v) was separately added into the preheated milk so as to adjust the pH values to 4.7, 5.0 and 5.3, respectively. The samples were thoroughly agitated and allowed to set in a water bath for 15 min. Each mixture of curd and whey was passed through cotton cloth resting on a separatory funnel and let stand still for 1 h to separate cheese curd from whey. The collected curds from each sample were analyzed for % yield, protein, fat and moisture content (Marshall, 1993) and for the Hunter color values (L, for lightness; a, + for redness and – for greenness; b, + for yellowness and – for blueness) using a Minolta Chromameter (CR 300 series) (Minolta Co., Osaka, Japan) and a white ceramic plate for standardizing the instrument. Protein recovery was also calculated and expressed as a percentage.

The experiment was a symmetric factorial (3 x 3) in completely randomized design (CRD) with 2 replicates and the means were separated at a significance level of 0.05 using Duncan's New Multiple Range Test (DNMRT) (Cochran and Cox, 1992). Whenever the main effects of temperature and/or pH were significant, i.e. the interaction effects being not significant, the means were further analyzed to show the effects of corresponding variables.

Selection of the most suitable acid

Once the appropriate temperature and pH could be verified for each acid, curdling of the UHT milk was re-conducted using the same procedure as described previously. The collected curds prepared separately by acetic, citric and lactic acid were analyzed and measured for exactly the same parameters given in the last experiment. However, the experimental design was a CRD with four replicates and the means were compared at the same significance level.

Effect of calcium chloride

From the previous part of this research, calcium chloride was used at a fixed level of 0.02%. An experiment was conducted at this stage to verify whether the amount of this precipitating salt could be reduced. Therefore, the salt levels set to 0.0, 0.01 and 0.02% (by weight of the milk) were used in milk coagulation. The collected curds were appraised only for % yield, protein, fat and moisture content. Protein recovery was also calculated and reported. The experiment was a CRD with four replicates and the means were compared using the same statistical test and significance level.

Results and Discussion

Properties of the UHT milk

Preliminary study showed that the retrieved UHT milk samples were still suitable for further manufacture into the processed cheese products owing to their normal properties. These were: no evidence of superficial coagulum, creamy white color, ordinary odor of freshly processed UHT milk, and no observed gelation or fat separation (Subhimaros *et al*., 2003). The samples were composed of 88.67% water, 3.03% protein, 2.87% fat, 4.71% carbohydrate and 0.72% ash. Protein and fat content of the experimental aged UHT-milk sample was lower than those values of the local UHT milk samples in Lahore, Pakistan, whose values (after shaking) did not change and were between 3.30-3.70% and 3.50-3.80%, respectively, after 12-week storage. Its mean pH value (6.45) was lower than those values of the same samples, which were in the range of 6.75-6.85 (1 week), but higher than the values (6.17-6.20) of some samples stored for 12 weeks (Hassan *et al*., 2009). In addition, the alcohol test (with 68% ethanol) revealed no curd or precipitate showing that the stability of proteins was still maintained. This alcohol test (Dahlberg and Garner, 1959) is of practical value in determining the susceptibility of milk to coagulation by heat. It is mainly used to detect milk which has a tendency to curdle during the sterilizing process.

Heat coagulation and direct acidification

From the statistical analysis results, it could be ascertained that sometimes both, the main effects and the interaction effects of temperature and pH, on each chemical and physical property were concurrently significant, and sometimes only the main effects were significant. The results are given in Table 1 (interaction effects of both parameters) and Table 2 (main effects), respectively.

Significant interactions of pH and temperature were not found for % fat and color values of cheese curds prepared by acetic acid (Table 1) but on % yield, % protein, % protein recovery and % moisture ($p < 0.05$). It was evident that cheese curds prepared by adjusting pH to 5.3 and temperature to 70°C had the lowest % yield since this direct acidification pH is the furthest value away from the isoelectric point (pI) of caseins (at pH 4.6). In addition, gelation of proteins at a temperature of 70°C could hardly occur and the milk treated at 90°C starts to form gel at pH 5.36 and the gelation phenomenon completes at pH 5.17 (Fox and Mulvihill, 1990). Ramasubramanian *et al*. (2012) studied the effect of preheating on Ca^{2+}-induced coagulation of whole milk at 70°C by comparing the coagulums obtained after (i): no preheating. (ii) heating at 90°C for 10 minutes; and (iii) UHT treatment at 140°C for 6 seconds. The objective of the preheat treatment was to cause denaturation of the whey proteins and promote their attachment to the casein micelles prior to coagulation. It was found that the sample, which had not been preheated, produced whey with the highest protein content, while the preheated (90°C/ 10 min) sample produced whey with the least protein after coagulation. Despite expectation that UHT pretreatment would cause extensive denaturation of whey proteins and their attachment to casein micelles, the whey resulting from Ca^{2+}-induced coagulation of the UHT-treated milk still had a significantly larger amount of protein in comparison with that obtained in another preheated (90°C/ 10 min) sample. Thus, the lowest %yield, as resulted from the lowest protein recovery, was obtained for the curds prepared by these two parameters (pH 5.3/ temperature of 70°C, which were not as low enough as the pI of casein (4.6) and not as high enough as 90°C suitable for promoting the denaturation of β-lactoglobulin and its attachment to κ-casein on the surface of casein micelles. Under such these unsuitable conditions used, both caseins (in micelle) and β-lactoglobulin may be involved in less network formation during coagulation of the UHT milk by Ca^{2+}. Thus, the lowest %yield was obtained for the curds prepared by adjusting these two parameters (pH 5.3/ 70°C).

Protein content of the curds made at pH 4.7 and 80°C with acetic acid was the highest. This could be explained considering that acidification at this pH was mostly close to the pI of caseins resulting in complete structural rearrangement and coagulation of casein micelles. At pH 4.5, coprecipitate of sheep milk (without preheating) was mainly composed of caseins

Table 1. Chemical and physical properties of the cheese curds prepared at different pH and temperature using various coagulants and 0.02% $CaCl_2$

Acid	pH	Temperature (°C)	% Yield	% Protein (dry basis)	% Fat^{ns} (dry basis)	% Moisture	% Protein recovery
Acetic	4.7	70	$7.41^{a}\pm0.36$	$42.16^{b}\pm0.72$	20.48 ± 0.82	$64.02^{cd}\pm2.70$	$39.28^{a}\pm5.37$
		80	$7.23^{a}\pm0.11$	$44.70^{a}\pm0.09$	20.03 ± 1.13	$64.11^{bcd}\pm5.15$	$38.31^{ab}\pm2.00$
		90	$7.42^{a}\pm0.22$	$40.98^{bc}\pm0.73$	20.03 ± 1.09	$60.61^{d}\pm0.74$	$39.53^{a}\pm1.21$
	5.0	70	$7.63^{a}\pm0.33$	$39.47^{c}\pm1.06$	20.89 ± 0.59	$65.59^{bcd}\pm4.10$	$34.09^{ab}\pm1.70$
		80	$7.36^{a}\pm0.06$	$41.28^{bc}\pm1.02$	20.03 ± 1.13	$66.11^{bcd}\pm1.98$	$33.94^{ab}\pm0.85$
		90	$7.31^{a}\pm0.01$	$42.42^{b}\pm1.41$	20.03 ± 1.09	$65.82^{bcd}\pm2.71$	$36.97^{ab}\pm1.48$
	5.3	70	$4.90^{b}\pm0.07$	$29.03^{d}\pm1.83$	19.04 ± 1.38	$85.85^{a}\pm1.76$	$6.61^{c}\pm0.51$
		80	$7.49^{a}\pm0.18$	$40.00^{bc}\pm0.40$	20.22 ± 0.03	$68.35^{bc}\pm3.55$	$31.25^{b}\pm2.46$
		90	$7.52^{a}\pm0.08$	$41.51^{bc}\pm0.20$	19.19 ± 0.49	$69.93^{b}\pm2.00$	$31.02^{b}\pm2.56$
Citric	4.7	70	$7.61^{a}\pm0.10$	$41.96^{a}\pm0.38$	21.14 ± 0.13	$64.89^{cde}\pm0.62$	$37.01^{ab}\pm0.81$
		80	$7.32^{a}\pm0.17$	$41.78^{ab}\pm0.36$	21.14 ± 0.04	$61.45^{de}\pm0.27$	$38.92^{ab}\pm0.28$
		90	$7.32^{a}\pm0.44$	$41.88^{ab}\pm0.56$	20.81 ± 0.62	$59.96^{e}\pm1.70$	$40.47^{a}\pm0.17$
	5.0	70	$7.78^{a}\pm0.17$	$39.58^{c}\pm0.29$	20.66 ± 0.69	$70.05^{b}\pm3.45$	$30.49^{c}\pm4.40$
		80	$7.50^{a}\pm0.05$	$40.61^{abc}\pm0.27$	21.03 ± 0.34	$63.03^{cde}\pm0.27$	$37.13^{ab}\pm0.23$
		90	$7.33^{a}\pm0.22$	$40.71^{abc}\pm0.52$	20.38 ± 0.78	$62.47^{cde}\pm0.30$	$36.96^{ab}\pm1.30$
	5.3	70	$2.60^{b}\pm0.06$	$24.18^{d}\pm0.81$	19.63 ± 0.02	$87.44^{a}\pm0.03$	$2.61^{d}\pm0.15$
		80	$7.61^{a}\pm0.48$	$39.31^{c}\pm0.81$	21.28 ± 0.24	$68.84^{bc}\pm6.19$	$30.61^{c}\pm4.81$
		90	$7.72^{a}\pm0.04$	$39.94^{bc}\pm1.88$	20.89 ± 0.66	$66.77^{bcd}\pm2.67$	$33.77^{bc}\pm1.30$
Lactic	4.7	70	$7.46^{a}\pm0.68$	$40.11^{c}\pm0.19$	21.08 ± 0.42	$61.95^{cd}\pm1.76$	$37.51^{a}\pm1.87$
		80	$7.60^{a}\pm0.39$	$41.87^{bc}\pm0.39$	20.98 ± 0.08	$62.11^{cd}\pm0.19$	$39.80^{a}\pm1.87$
		90	$7.15^{a}\pm0.60$	$42.68^{b}\pm0.74$	20.12 ± 1.07	$63.45^{bcd}\pm0.53$	$36.85^{a}\pm4.29$
	5.0	70	$7.53^{a}\pm0.46$	$45.27^{a}\pm1.46$	20.41 ± 2.28	$66.85^{b}\pm3.70$	$37.50^{a}\pm4.63$
		80	$7.31^{a}\pm0.04$	$40.92^{bc}\pm0.64$	20.90 ± 0.44	$64.48^{bcd}\pm0.24$	$35.09^{a}\pm1.00$
		90	$7.38^{a}\pm0.61$	$41.87^{bc}\pm0.70$	19.86 ± 1.41	$60.41^{d}\pm1.64$	$40.50^{a}\pm5.71$
	5.3	70	$5.82^{b}\pm0.10$	$27.83^{d}\pm1.64$	18.12 ± 0.60	$85.21^{a}\pm2.46$	$7.87^{b}\pm0.98$
		80	$7.66^{a}\pm0.17$	$40.13^{c}\pm0.13$	19.30 ± 0.82	$64.72^{bcd}\pm1.86$	$35.78^{a}\pm1.23$
		90	$7.41^{a}\pm0.34$	$40.28^{c}\pm0.05$	18.93 ± 1.51	$65.74^{bc}\pm1.51$	$33.69^{a}\pm0.03$

^{a,b} Means ± SD with different superscripts in the same column are significantly different ($p \leq 0.05$) considering each coagulating acid separately
^{ns} not significantly different

and the amount of whey proteins (α-lactalbumin; α-La and β-lactoglobulin; β-Lg) in the coprecipitate increased with increasing pH of heat treatment (Al-saadi and Deeth, 2011). These authors also studied effect of heating temperature and found that, at 65°C, casein only was precipitated, as revealed by polyacrylamide gel electrophoresis, and heating at this temperature enhanced the binding of calcium to casein but was not enough to denature whey proteins. At 75°C, whey proteins started to precipitate with casein, and at 85-95°C, the coprecipitates contained the most whey proteins, especially α-La and β-Lg owing to complex formation between denatured β-Lg and κ-casein (Reddy and Kinsella, 1990) and α-La (Shalabi and Wheelock, 1976). Although whey proteins in the aged UHT milk used in this study would have been denatured, the remaining undenatured ones could take part in complex formation of the two types of proteins, which in turn resulted in the highest protein content in the curd prepared at pH 4.7 and 80°C. However, too high temperature (90°C) might result in excess formation of this complex, thus hindering aggregation of denatured casein micelles (Harwalkar and Kalab, 1981). Moisture content of the cheese curds was generally varied by alteration of pH and temperature, i.e. the sample treated at pH 4.7 and 90°C having the lowest value. At pH value close to the pI, cheese curds possess high hydrophobicity and tend to repel water and, therefore, heating the milk to a higher temperature (90°C) should accelerate syneresis and whey separation (Fox and Mulvihill, 1990). Numerous researchers also concluded that heat-acid cogulation of milk by varying pH and temperature was feasible (Rosenau *et al*., 1975; Rosenau *et al*., 1978; Rosenau, 1984; Pal, 2002).

Similar results were obtained for the cheese curds prepared by the same levels of pH and temperature using citric and lactic acid. Percentage yield of the sample treated with either citric acid or lactic acid at pH 5.3 and 70°C was the lowest. Protein content of the curds made by citric acid at pH 4.7 and 70°C was the highest but not significantly different from the samples prepared at 80°C and 90°C. This might be due to the fact that heating the milk up to 70°C was sufficient to cause formation of β-lactoglobulin-κ-casein complexes. The lactic acid-curd made at the temperature of 70°C and pH of 5.0 had the highest protein content. This pH level was different from the value of 4.7 (suitable for acetic and citric acid). Values of pKa for acetic, citric and lactic acid are 4.8,3-4.1-4.8 and 3.9, respectively (Salaün *et al*., 2005). According to these pKa's, it might be possible the rate of pH reduction in the UHT milk was in the following order: acetic > citric > lactic acid due to easiness of each relevant acid to dissociate. Thus, it seemed that acetic acid seemed to readily help coagulate more caseins and whey proteins into the coagulum and followed by citric acid if the coagulating pH was closer enough to the pI of casein. At pH 4.7, acetic acid should totally dissociate to give protons resulting in the fast drop of pH and its resultant carboxylic groups (-COO^-) could possibly form ionic bonds with added Ca^{2+} and only the remaining Ca^{2+} could further form salt bridges between casein micelles, which also associated whey proteins (α-La and β-Lg). As for citric acid, it has three pKa values, two of which are lower than the pI of casein. Thus, only one carboxyl group should dissociate while the other two groups should not. Thus, its pH drop should not be as fast as that of acetic acid. In addition, the molar concentration of acetic acid used (as calculated from the same 10% w/v solution of each acid) is the highest since its molecular weight is the lowest. Consequently, apart from being the most readily dissociated acid, its concentration might also be substantially adequate to coagulate both caseins and whey proteins. This was observed in the acetic acid-treated sample whose percentages of protein in the coagulum were generally higher. UHT milk was thermally treated at a higher temperature than 133°C for not less than 1 s (Office for Standards of Industrial Products, 1987). The acidity of milk increases with temperature, partially as a result of changes in the buffer capacity of the milk salts and the expulsion of CO_2 on heating. The thermal decomposition of lactose, as a partial source of acidity in heated milk, has been well documented (Parry, 1974). Moreover, direct addition of lactic acid during heat-acid coagulation should increase its amount only in the lactic acid-treated sample. Thus, the milk is even more buffered through additional accumulation of lactic acid and lactates, in comparison with those samples prepared with the other two acids. This greater buffering capacity of lactic acid should have altered the coagulation of casein micelles at the ordinary pI (at pH 4.6) shifting away the appropriate coagulating pH with lactic acid.

It was interesting to note that citric acid, in comparison with acetic and lactic acid, also yielded the cheese curds with the lowest protein content (26.38%) but the highest moisture content (63.69%) if the UHT milk was treated at pH 5.0 together with an addition of $CaCl_2$ (200 ppm) and rennet as found in our previous work. Additionally, it could be proved that heat-acid coagulation is more effective in recovering proteins in contrast to acid-enzyme coagulation, i.e. the protein contents of the curds prepared by the former process being 39.47-42.42%, 39.58-40.71% and 40.92-45.27% if prepared with acetic, citric and lactic acid, respectively. However, the curds prepared by the latter process (acid-enzyme coagulation) had the protein contents of 35.03, 26.38 and 34.95% for the three corresponding acids (Subhimaros *et al.*, 2003). Protein recovery was higher if the curds were prepared by heat-acid coagulation when compared to the enzyme (rennet) coagulation (Parnell-Clunies *et al.*, 1985) due to copious formation of whey proteins-casein complexes appropriately bound to denatured casein micelles (Bylund, 1995; Swaisgood, 2008). Generally, higher protein content and recovery were obtained at pH of 4.7, 4.7 and 5.0 for acetic, citric and lactic acid, respectively. Except for caseins in Latin American White (LAW or Queso Blanco) cheese, whey proteins such as β-Lg, α-La and others were also present since they were made insoluble and precipitated at low pH and high temperature conditions. β- Lg and α-La are major whey proteins which have very high protein efficiency ratio (PER) at 3.2 and are exceptionally nutritious for human beings (Torres and Chandan, 1981). This justification should strongly support an advantage of heat-acid coagulation of UHT milk. In addition, there was a tendency that (protein + fat) content of the cheese curds increased when moisture content decreased. Although not being ultimately analyzed in the processed cheese, this result was in accordance with the works of Dimitreli and Thomareis (2004) and Dimitreli *et al.* (2005).

Notwithstanding the detailed discussion given previously, it was found in our study that %yield, %protein of the curd and especially %protein recovery were very much lower than those values reported in the literatures. Vattula *et al*. (1979) recovered 96% of cow milk protein by heating skim milk at 85oC and adding 0.2% calcium chloride. Al-saadi and Deeth (2011), in production of coprecipitate from sheep milk, revealed the best percentage of added calcium chloride was 0.2%, which resulted in a recovery of 97.5% of milk proteins. In a study on heat-induced coagulation of whole cow milk by high levels (20-200 mM) of calcium chloride, Ramasubramanian *et al.* (2012) found that UHT-treatment at 140°C for 6 seconds was less effective than preheating milk at 90°C for 10 minutes, and a maximum of 98% of the protein in milk preheated as such was coagulated by 50 mM added calcium chloride at 70°C with holding for 5 minutes. In our study, added calcium chloride was used at a maximum of only 0.02% (one-tenth of the reported optimum value). At 0.025% addition of calcium chloride, the recovery of milk proteins was only 50% (Al-saadi and Deeth, 2011). Heat

Table 2. Main effect of pH and temperature on physicochemical properties of the cheese curds

Coagulant	Effect of pH				Effect of Temperature			
	pH	L	b	% Fat (dry basis)	Temperature (°C)	L	a	b
Acetic	4.7	86.46^{b}±0.76	+15.17^{a}±0.82	20.18^{ns}±1.01				
	5.0	87.05^{b}±1.26	+14.38^{a}±1.25	20.32^{ns}±0.94				
	5.3	88.60^{a}±0.62	+12.52^{b}±0.45	19.48^{ns}±0.63				
Citric	4.7	86.45^{b}±1.08	+14.93^{a}±0.93	21.03^{ns}±0.26	70	88.13^{a}±0.87	-1.16^{b}±0.30	+13.25^{b}±1.06
	5.0	87.04^{b}±0.92	+14.56^{a}±1.03	20.69^{ns}±0.60	80	87.67^{a}±1.37	-0.92^{b}±0.27	+14.05^{b}±0.95
	5.3	88.52^{a}±1.20	+13.03^{b}±1.14	20.60^{ns}±0.31	90	86.21^{b}±1.07	-0.68^{a}±0.20	+15.22^{a}±1.14
Lactic	4.7	86.44^{b}±0.73	+15.11^{a}±0.82	20.73^{a}±0.70	70	88.16^{a}±1.26	-0.99^{b}±0.26	+13.29^{b}±1.37
	5.0	87.33^{ab}±0.95	+15.21^{a}±2.64	20.39^{a}±1.30	80	87.38^{ab}±0.80	-0.92^{b}±0.10	+13.86^{b}±1.02
	5.3	88.43^{a}±1.25	+12.92^{b}±1.22	18.60^{b}±0.98	90	86.66^{b}±1.34	-0.65^{a}±0.24	+16.08^{a}±2.24

^{a,b} Means ± SD with different superscripts in the same column are significantly different ($p \leq 0.05$) considering each coagulating acid or temperature separately

^{ns} not significantly different

coagulation of milk proteins is influenced by both a decrease in pH during heating (Rose, 1961) and a high concentration of calcium ions in milk (Fox, 1981). It was postulated that the level of added calcium used in this study was not substantially adequate to recover the high proportion of proteins into the curd. Moreover, it is possible that UHT treatment led to the denaturation and attachment of both α-La and β-Lg to casein micelles, which inhibited the aggregation of the casein micelles and thus reduced their sensitivity to coagulation by calcium (Ramasubramanian *et al.*, 2012). This would in turn affect the content of protein in the coagulum.

Cheese curds prepared by coagulation at pH values closer to the pI had significantly lower lightness but higher yellowness values (Table 2), irrespective of the type of coagulants. Lightness of food product is related to its moisture content resulting in its capacity to reflect light, i.e. the higher the moisture content, the higher the lightness. Therefore, the samples prepared at pH 4.7, which had the lowest moisture contents, should have lower L values. Milk is obviously an adequate source of vitamin A which is one type of carotenoid, being classified as yellow (Swaisgood, 2008). After heat-acid coagulation, carotenoids, which are hydrophobic in nature, could be retained more in those curds possessing high hydrophobicity. Thus, the curds made by adjusting pH closer to the pI, which were made more hydrophobic (Fox and Mulvihill, 1990), still entrapped more carotenoids resulting in higher yellowness. Main effect of varying pH on fat content of the curds prepared only by lactic acid was observed. As discussed previously, lactic acid and lactate salts can act as buffering agents and counteract with pH change in milk and concurrently result in higher hydrophobicity of the curds when compared to the other two coagulants. In addition, the curds prepared using this acid but with pH closer to the pI had higher fat content (20.39-20.73%) due to the same reason of hydrophobicity as compared to the sample prepared at pH 5.3 which had only 18.60% fat.

Alteration of coagulation temperature had significant effects on color values of the curds prepared only with citric and lactic acid (Table 2). These results could be explained by greater syneresis (water repulsion) at higher temperature resulting in less water and more fat entrapped in the curds. Therefore, the curds obtained at higher temperatures should have lower L but higher b values. In addition, preparation of the curds at higher temperatures could enhance the Maillard reaction, which is accelerated by an increase in temperature (Gordon and Kalan, 1974), making such curds to have higher yellowness and redness values.

From the physicochemical results obtained and especially with the protein content and recovery, the following conditions were chosen for heat-acid coagulation of the UHT milk: 80°C / pH 4.7, 70°C / pH 4.7 and 70°C/ pH 5.0 for acetic, citric and lactic acid, respectively. Thus, these aforementioned combinations of temperature and pH would be used for the next step of the research.

The most suitable acid for coagulation of the UHT milk

Appropriate conditions (temperature and pH) for heat-acid coagulation of the UHT milk were differently verified for each coagulant (acetic, citric and lactic acid). These conditions were used again to re-prepare the cheese curds, with the concentration of $CaCl_2$ being fixed at 0.02%, and the properties of the curds were analyzed.

It appears that % yield, the fat content, the protein

Table 3. Color values of the cheese curds prepared by various coagulants

Coagulant	L	a[ns]	b
Acetic	86.81[b]±0.31	+0.86±0.05	+14.44[a]±0.53
Citric	86.83[b]±0.47	+0.79±0.09	+14.78[a]±0.82
Lactic	88.24[a]±1.05	+0.80±0.24	+12.98[b]±0.81

[a,b] Means ± SD with different superscripts in the same column are significantly different ($p \leq 0.05$)
[ns] not significantly different

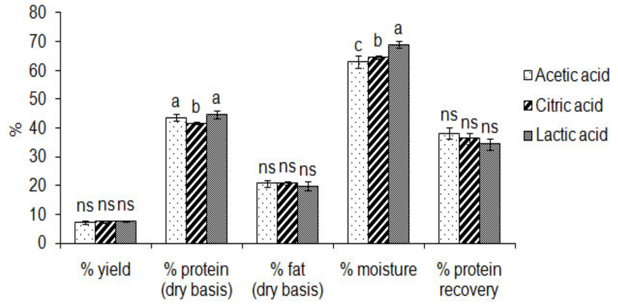

Figure 1 Yield and chemical properties of the cheese curds prepared using various coagulants after addition of 0.02% $CaCl_2$

recovery and redness were not affected but the protein content, moisture content, lightness and yellowness were significantly ($p < 0.05$) affected by the type of acid (Figure 1 and Table 3). The cheese curds made with citric acid had the lowest protein content when compared to those made with acetic acid and lactic acid. This could be explained by the rationale of pKa values as discussed previously. Also, the effects of the decreased rate of pH effect with citric and lactic acid occurs over pH ranges corresponding with the pKa values (Resch *et al*., 2005) and is consistent with the fact that buffering capacity of a system is greatest at pH values near its pKa (Zumdahl, 1993). However, if pH change of food system occurs successively and slowly, protein-protein interaction can be even more enhanced resulting in greater development of protein mass (Parnell-Clunies *et al*., 1985). Thus, the curds made with acetic and lactic acid had higher protein content than citric acid. Moreover, higher protein content in the former two samples (43.61% and 44.73% for acetic and lactic acid, but 41.74% for citric acid) might be due to more whey proteins bound to the precipitated casein micelles. As quoted by Resch *et al*. (2005), the Hofmeister or lyotropic series of anions influences stabilization of the structure of proteins and other macromolecules and the series for common anions is $SO_4^{2-} > HPO_4^{2-} >$ acetate$^-$ > citrate$^-$ > Cl^- > NO_3^- > I^- > SCN-. Anions on the left-hand side of this series, usually referred to as kosmotropes, increase the surface tension of water, enhance hydrophobic bonding and decrease the solubility of non-polar molecules (salting-out). Meanwhile anions on the right-hand side, referred to as chaotropes, show weaker interactions with water than with themselves, decrease hydrophobic interactions within the protein, have less effect on surface tension, and increase the solubility of nonpolar molecules (salting in). Moisture content of lactic acid curds was the highest since this sample was acidified at pH 5.0, which was not close to the pI of caseins and, therefore, protein gel should attain more hydrophilic portions and bind more water molecules. It was confirmed once again that heat-acid coagulation at higher temperature could help expel more water from the cheese curds, i.e. result in better syneresis, and acetic acid curds which were prepared at 80°C had lower moisture content than the samples of citric and lactic acid prepared at 70°C. Lactic acid sample had the highest lightness due to the best reflection of light given by its highest moisture level. On the contrary, its yellowness was the lowest owing to the lowest amount of fat (Table 3).

When comparing five coagulants (hydrochloric, citric, lactic, acetic and tartaric acid), Chandan *et al*. (1979) reported that LAW cheese made with hydrochloric, acetic and lactic acid contained more than 53% moisture; a value being unacceptable due to adverse effect on cheese body, texture, and slice-ability. However, since acetic acid imparted a slight vinegar flavor to the cheese and tartaric acid was relatively more expensive, citric acid was selected as a coagulating acid for their further work. Also, a number of research articles have reported alternative techniques for successful preparation of cheeses by using various coagulants in place of animal rennet (Fernández-Salguero *et al*., 2003; Prados *et al*., 2007; Raheem *et al.*, 2007; Tejada *et al*., 2008; Bornaz *et al*., 2010). As for the present study, no attempt was made to use an enzyme preparation, no matter from animal or plant origins, for curdling the milk. This study was focused solely upon the possibility of direct acidification (by various acids) enhanced by addition of calcium chloride and heat treatment so as to prepare the curds from almost expired UHT milk. This was envisaged by a reason of drastic reduction of the cost otherwise incurred in processed cheese production, in that, there is no need to incorporate micro-organisms or enzyme systems. Also, the need for ageing under refrigeration is eliminated, producing additional saving (Pal, 2002). Although the protein content of the acetic acid-treated sample was lower (but not significantly) than that of lactic acid sample, its moisture content was significantly lower.

Table 4. Curd yield and composition of acetic acid-treated sample as influenced by calcium chloride content

$CaCl_2$ (% by wt.)	% Yield[ns]	% Protein (dry basis)	% Fat[ns] (dry basis)	% Moisture	% Protein recovery[ns]
0.00	8.17±0.14	40.66[b]±0.77	21.23±0.56	62.09[b]±0.70	41.54 ± 0.77
0.01	8.04±0.30	40.91[b]±1.11	20.87±0.47	62.90[b]±0.79	40.27 ± 1.50
0.02	8.11±0.13	43.35[a]±1.41	21.15±0.51	64.21[a]±0.80	41.48 ± 1.29

[a,b] Means ± SD with different superscripts in the same column are significantly different ($p \leq 0.05$)
[ns] not significantly different

The cheese curds were initially prepared for use in a processed cheese block, which should not have too high moisture content in order not to be easily spoiled by the microorganisms. Furthermore, non-rennet directly acidified processed cheese products can be made to offer a range of flavors, of which a number of natural and synthetic choices are available. Thus, acetic acid was chosen as the most suitable coagulant. However, Kumar *et al.* (2013) concluded, on the basis of sensory and textural characteristics, that citric acid was found the most appropriate for making chhana as well as rasogolla from admixture of buffalo and buttermilk.

Curd yield and its composition as affected by calcium chloride content

Calcium chloride, at 0.0, 0.01 or 0.02%, respectively, was added to the UHT milk sample to prepare the cheese curds with the previously chosen temperature and pH suitable for acetic acid. The results are given in Table 4.

It is obvious that the highest protein content (43.35%) and the highest moisture content (64.21%) of the curds were obtained with the highest $CaCl_2$ content. According to the heat coagulation mechanism theories of van Boekel (1993) and Singh (2004), at pH < 6.4 and/or high calcium ion concentration, coagulation of milk proteins can occur because of decreased electrostatic repulsions between casein micelles (because of the decreasing pH) or the formation of calcium-salt bridges between casein particles inducing flocculation of micelles (due to high calcium ions). Consequently, when the pH of milk decreases after calcium addition, micellar aggregation may result from a reduction in electrostatic repulsions between micelles and/or formation of intermicellar calcium bridges. During heating, the whey proteins of the aged UHT milk, although having been substantially denatured after this kind of sterilization, could possibly still attach to the κ-casein on the micelle surface, and when Ca^{2+} was added to milk, micellar aggregation incorporated the attached whey proteins also. Thus, the highest level (0.02%) of added Ca^{2+} should result in the highest protein content in the curds. Furthermore, addition of calcium chloride could result in large and coarse gel matrix with good adsorption of water (Fox and Mulvihill, 1990). Therefore, the curds with the highest protein content also had the highest moisture content.

Conclusions

The results of this study show that the aged UHT milk processed from fresh milk and retrieved within three months before the expiry date, was still suitable for heat-acid coagulation. Comparison between pH, temperature and type of acid used for direct acidification shows that acetic acid (10% w/v) was the most suitable one for preparation of the cheese curds by adjusting pH of the milk to 4.7 after heating it up to 80°C. Calcium chloride at 0.02% (by weight of the milk) also improved curd preparation, although this addition level might not be adequate. Therefore, further study is needed to identify its optimum value for maximum coagulation of the aged UHT milk.

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