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THE INFLUENCE OF GDL CONCENTRATION ON MILK pH CHANGE DURING ACID COAGULATION

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Abstract: Skim milk powder was reconstituted to obtain milk A (with 8.01% TS). Milk A was standardized with 3% of skim milk powder and 3% of demineralized whey powder (DWP), respectively, to obtain milk B (with 11.15% TS) and milk C (with 11.10% TS). Milk samples were heat treated at 85ºC/10 min, 90ºC/10 min and 95ºC/10 min, respectively. Untreated milk was used as control.

Acidification was carried out at 25ºC, 35ºC and 45ºC during 240 min with GDL (glucono-δ-lactone), namely with the amount of 0.5%, 0.75%, 1.0%, 1.25%, 1.5%, 1.75%, 2.0% and 3.0% of GDL, respectively.

The results showed that all investigated factors, explicitly GDL concentration, acidification temperature and applied heat treatment of milk as well as added DWP influence the change of pH during acidification.

Milk samples standardized with DWP had smaller buffer capacity and faster change of pH than samples standardized with skim milk powder.

Only at acidification temperature of 25ºC, added DWP did not influence the change of milk buffer capacity regardless of the change of casein:whey protein ratio. Under this acidification condition, both milk samples standardized with skim milk powder and DWP had similar final pH values.

Key words: acidification, skim milk powder, demineralized whey powder, GDL, acidification temperature, heat treatment.

Introduction

The fermentation of milk with lactic acid bacteria (production of fermented products: yogurt and acid-curd cheese varieties) and acidification with organic

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and inorganic acids (production of casein and coprecipitates) are regularly used for acidic coagulation of milk (Denin Djurdjević, 2001, Guinee et al., 1993, Jovanović, 2001, Maćej, 1983, Maćej et al., 2001).

During the last 20 years, milk acidification with glucono-δ-lactone has been commonly used for investigating changes that occur on casein micelles during gel formation. The structure of casein micelle undergoes intensive change during acidic coagulation of milk as discussed elsewhere (Maćej et al., 2001, Denin Djurdjević, 2001, Roefs, 1986, Bringe and Kinsela, 1990, Heertje et al., 1985, Dalgleish and Law, 1988). The most important changes that occur in casein micelle are: 1) Physicochemical changes of casein micelle (Guinee et al., 1993, Heertje et al., 1985, Roefs, 1986); 2) Dissociation of colloidal calcium phosphate (CCP) (van Hooydonk et al., 1986, Roefs, 1986, Dalgleish and Law, 1989, Bringe and Kinsela, 1990, Holt and Horne, 1996, Parnell-Clunies et al., 1988, Walstra, 1990); 3) Dissociation of casein and factors that influence dissociation such as hydrophobic effect, solubilization of calcium and phosphate from casein micelle, izoelectric precipitation of casein (Guinee et al., 1993, van Hooydonk et al., 1986, Heertje et al., 1985, Bringe and Kinsela, 1990, Roefs, 1986, Dalgleish and Law, 1988, Singh et al., 1996); 4) Voluminosity changes during milk pH value reduction (van Hooydonk et al., 1986, Guinee et al., 1993, Heertje et al., 1985, Roefs, 1986); 5) Reduction of zeta-potential as influenced by milk pH value (van Hooydonk et al., 1986, Guinee et al., 1993, Heertje et al., 1985, Darling and Dickson, 1979); 6) Changes of casein micelle hydration (Guinee et al., 1993, Parnell-Clunies et al., 1988, Djordjević, 1987, Torado de la Fuente and Alais, 1975); 7) Changes of casein micelle size (Roefs, 1986, Holt and Horne, 1996, Goddard and Augustin, 1995); 8) Structural changes of casein micelle during acidification and gel formation (Heertje et al., 1985, Parnell-Clunies et al., 1988, de Kruif and Roefs, 1996, Parker and Dalgleish, 1977).

Since there are few information in available literature, the aim of this work was to investigate the influence of milk dry matter content and protein composition as well as applied heat treatments and acidification temperature on milk pH value change during acidification with GDL.

Material and Method

Skim milk powder was reconstituted to obtain milk A (with 8.01% TS). Milk A was standardized with 3% of skim milk powder and 3% of demineralized whey powder (DWP), respectively, to obtain milk B (with 11.15% TS) and milk C (with 11.10% TS). Milk samples were heat treated at 85ºC/10 min, 90ºC/10 min and 95ºC/10 min, respectively. Untreated milk was used as control.

The following analyses of milk samples were performed:

- Dry matter content determination - AOAC method 16.032
- Determination of total nitrogen content by Kjeldahl method - FIL/IDF 20B: 1993

Acidification was carried out at 25ºC, 35ºC and 45ºC during 240 min. Different concentrations of GDL (glucono-δ-lactone): 0.5%, 0.75%, 1.0%, 1.25%, 1.5%, 1.75%, 2.0% and 3.0%, respectively, were used for acidification. During acidification the change of pH value was monitored with pH-meter Sentron 1001.

Results and Discussion

Milk quality parameters are shown in table 1.

Tab. 1. - Milk quality parameters

Sample	HT	Investigated parameters								
		DM (%)			N (%)			N in DM (%)		
		Calculated parameters								
		8	Sd	Cv	8	Sd	Cv	8	Sd	Cv
A	Untreated	8.01	0.1306	1.63	0.4105	0.0071	1.73	5.13	0.1466	2.86
	85ºC/10'	8.33	0.3165	3.80	0.3967	0.0169	4.26	4.78	0.3666	7.68
	90ºC/10'	8.56	0.4621	5.40	0.3905	0.0159	4.08	4.58	0.4126	9.01
	95ºC/10'	8.81	0.9419	10.69	0.4021	0.0279	6.94	4.63	0.7679	16.60
B	Untreated	11.15	0.0786	0.70	0.5605	0.0015	0.26	5.03	0.0311	0.62
	85ºC/10'	11.34	0.0488	0.43	0.5565	0.0053	0.96	4.91	0.0544	1.11
	90ºC/10'	11.49	0.1303	1.13	0.5535	0.0112	2.02	4.82	0.1508	3.13
	95ºC/10'	11.49	0.1155	1.01	0.5524	0.0110	1.99	4.81	0.1191	2.48
C	Untreated	11.10	0.1482	1.34	0.4990	0.0410	8.22	4.50	0.3971	8.83
	85ºC/10'	11.21	0.0784	0.70	0.4646	0.0044	0.94	4.14	0.0458	1.10
	90ºC/10'	11.23	0.1187	1.06	0.4705	0.0045	0.95	4.19	0.0697	1.66
	95ºC/10'	11.38	0.1409	1.24	0.4703	0.0074	1.58	4.13	0.0769	1.86

The results shown in table 1. indicate that during heat treatment dry matter content of milk increased as a result of evaporation during heat treatment. On the other hand, nitrogen matter content of milk decreased during heat treatment, which agrees with the results of Fetahagić et al., 2001, Denin Djurdjević, 2001, Jovanović, 2001, Maćej, 1983, 1989, and Maćej and Jovanović, 1998, 2000. Nevertheless, we did not find linear relationship between the temperature of applied heat treatment and decrease of nitrogen matter content. A milk sample that was standardized with DWP had the lowest nitrogen matter content in dry matter (DM), which agrees well with the results of Denin Djurdjević, 2001.

The influence of GDL concentration on pH change during acidification

The influence of applied heat treatment, acidification temperature and GDL concentration on the change of pH value of milks A, B and C, respectively, is shown in Figs. 1, 2 and 3.

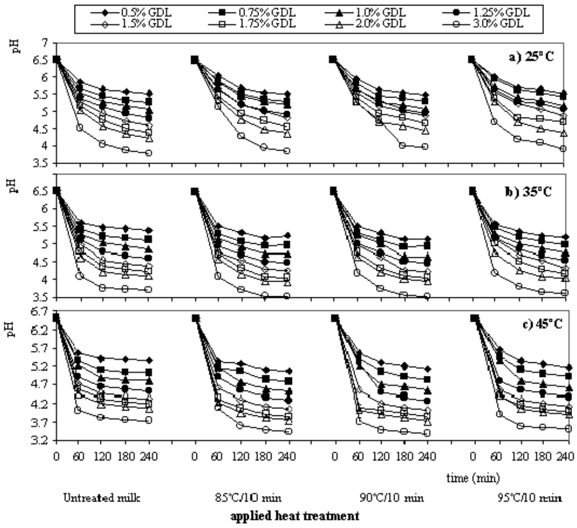

Fig. 1. - Change of pH value during acidification of milk A at: a) 25ºC, b) 35ºC and c) 45ºC

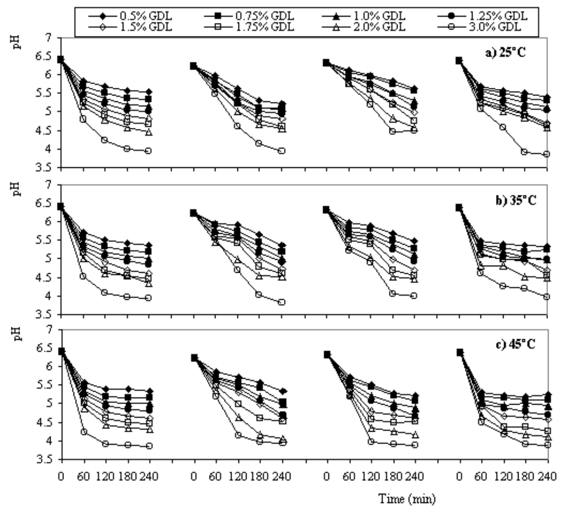

Fig. 2. - Change of pH value during acidification of milk B at: a) 25ºC, b) 35ºC and c) 45ºC

The pH value of milk decreased faster when concentration of GDL was increased, as Figs. 1, 2 and 3 show. These results agree with those of Dybowska and Fujio, 1996, who concluded that higher concentration of GDL accelerated the onset time of gelation and the rate of network formation.

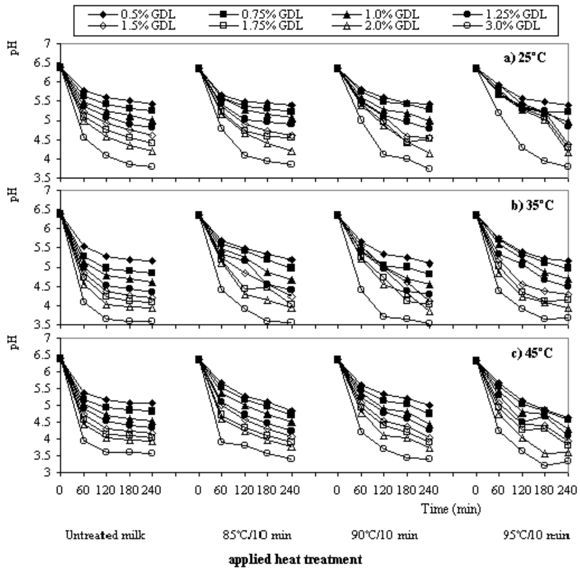

Fig. 3. - Change of pH value during acidification of milk C at: a) 25ºC, b) 35ºC and c) 45ºC

The pH value of untreated milk samples decreased faster during acidification at 25ºC than pH of heat-treated samples. These results agree with the conclusion of Horne, 1999, who reported that heat treatment of milk alters the rate at which bonds between casein micelles are formed as well as the mechanism of micelles interactions during protein network formation.

The influence of acidification temperature and applied heat treatment on pH value after 60 min of acidification with GDL is shown in table 2.

Tab. 2. - Influence of applied heat treatment, GDL concentration and acidification temperature on pH value change after 60 min

Milk sample	GDL (%)	Applied heat treatment of milk											
		Untreated			85ºC/10'			90ºC/10'			95ºC/10'		
		Acidification temperature											
		25ºC	35ºC	45ºC	25ºC	35ºC	45ºC	25ºC	35ºC	45ºC	25ºC	35ºC	45ºC
A	1.5	5.28	4.96	4.72	5.67	4.78	4.60	5.54	4.98	4.54	5.57	5.17	4.55
	2.0	5.04	4.63	4.45	5.36	4.59	4.24	5.29	4.65	4.03	5.29	4.77	4.44
	3.0	4.51	4.09	4.02	5.13	4.09	4.09	5.27	4.19	3.72	4.68	4.19	3.91
B	1.5	5.33	5.24	5.12	5.78	5.60	5.56	5.92	5.58	5.44	5.31	5.12	4.95
	2.0	5.15	5.00	4.87	5.61	5.43	5.36	5.77	5.31	5.25	5.23	4.81	4.67
	3.0	4.80	4.53	4.25	5.46	5.55	5.18	5.75	5.20	5.18	5.05	4.61	4.50
C	1.5	5.22	4.86	4.74	5.35	5.24	4.98	5.47	5.52	5.05	5.90	5.17	5.06
	2.0	4.99	4.55	4.45	5.16	5.13	4.60	5.37	5.20	4.77	5.67	4.85	4.76
	3.0	4.57	4.08	3.93	4.78	4.41	3.90	5.00	4.42	4.21	5.18	4.39	4.25

As expected, during the first 60 min, the reduction of pH value was greater at higher acidification temperature, since the rate at which GDL hydrolyzes increases at higher temperatures. At 25ºC, heat treated milk samples had higher pH value after 60 min than untreated milk samples, regardless of the content and composition of dry matter (as Figs. 1, 2 and 3 show), which indicates a greater buffer capacity of heat treated milk to GDL. According to Kim and Kinsella, 1989, gelation of heat-treated samples starts at higher pH values, which shows that heat treatment increases the rate of gel formation.

The character of casein micelles influences forces that control gelation, therefore, changes on casein micelle depend on both temperature and pH value. Hydrophobic interactions, which play the most significant role in gelation of casein micelle, are favored at higher temperatures (Goddard and Augustin, 1995). Hydrophobic interactions are weaker while repulsive forces are enhanced at lower temperature, so repulsive forces have to be reduced to a greater extent during acidification before intermicellar attractions can dominate. This could explain variations in pH value at the onset of gelation at different temperatures (Bringe and Kinsela, 1990).

The increase of acidification temperature from 25ºC to 35ºC had a greater influence on the rate of pH decrease than increase from 35ºC to 45ºC did. This agrees with the results of Deane and Hammond, 1960, who reported that an increase of acidification temperature from 20ºC to 35ºC reduces coagulation time by 75-80%. Kim and Kinsella, 1989, concluded that the increase of acidification temperature leads to the decrease of coagulation time, while the greatest reduction of coagulation time is noticed when temperature is increased from 45ºC to 50ºC. Gelation started after 180 min, 128 min, 68 min, 33 min and 16 min, respectively, at acidification temperatures of 35ºC, 40ºC, 45ºC, 50ºC and 55ºC, which indicates that higher temperatures increase the rate of GDL-induced acidic coagulation. According to Banon and Hardy, 1991, the first signs of coagulation of samples coagulated at 15ºC and 20ºC occur at pH 5.0 and 5.1, respectively. Dybowska and Fujio, 1996, found that gelation started after 145 min, 110 min and 50 min (namely, at pH 4.90, 4.95 and 5.18), respectively, when coagulation temperature was increased from 30ºC to 35ºC and then to 40ºC.

Untreated milk samples A and C had similar pH values after 60 min of acidification regardless of the applied acidification temperature, which indicates that casein has the most important role in maintaining buffer capacity of raw milk. On the other hand, pH drop of heat-treated samples vary depending on GDL concentration, acidification temperature and applied heat treatment.

Heat-treated samples B and C show irregular drop of pH during acidification, which indicates a greater buffer capacity and exchanged pattern of coagulation, as Figs. 2 and 3 show. Milk samples B heat-treated at 95ºC/10 min had the most pronounced irregular decrease of pH during acidification, particularly at 25ºC. The increase of acidification temperature to 35ºC or 45ºC reduced irregularity and to a great extent improved the pattern of pH change.

The influence of applied acidification temperature and concentration of GDL on final pH value after 240 min is shown in table 3.

T a b . 3. - Influence of applied heat treatment, GDL concentration and acidification temperature on pH value change after 60 min

Milk sample	GDL (%)	Applied heat treatment of milk											
		Untreated			85ºC/10'			90ºC/10'			95ºC/10'		
		Acidification temperature											
		25ºC	35ºC	45ºC	25ºC	35ºC	45ºC	25ºC	35ºC	45ºC	25ºC	35ºC	45ºC
A	1.5	4.58	4.39	4.33	4.82	4.27	4.06	4.89	4.23	4.02	4.86	4.32	4.12
	2.0	4.23	4.10	4.08	4.36	3.94	3.75	4.45	3.95	3.72	4.37	4.05	3.90
	3.0	3.77	3.71	3.74	3.83	3.53	3.42	3.96	3.53	3.38	3.89	3.62	3.51
B	1.5	4.83	4.62	4.63	4.81	4.74	4.62	4.98	4.71	4.61	4.72	4.72	4.59
	2.0	4.48	4.36	4.32	4.57	4.54	4.06	4.59	4.46	4.17	4.59	4.49	4.13
	3.0	3.94	3.93	3.86	3.95	3.82	3.95	4.51	4.01	3.88	3.84	3.97	3.88
C	1.5	4.62	4.21	4.17	4.61	4.24	4.08	4.55	4.12	4.03	4.39	4.28	3.94
	2.0	4.22	3.93	3.94	4.22	3.95	3.78	4.16	3.84	3.74	4.19	3.95	3.61
	3.0	3.78	3.58	3.57	3.86	3.55	3.42	3.75	3.54	3.41	3.80	3.69	3.32

Of the samples of untreated milk, samples B had the highest final pH value regardless of applied acidification temperature and used concentration of GDL, as table 3 shows. It again confirms our hypothesis that casein content present in milk controls milk buffer capacity. A greater amount of GDL has to be used to attain certain pH value when protein content of milk is increased without modification of casein:whey protein ratio. On the other hand, milk samples that had modified casein:whey protein ratio due to the addition of DWP (milk C) achieved lower pH values at 35ºC and 45ºC than milk A. At lower acidification temperature (25ºC) modified casein:whey protein ratio did not influence the change of pH value.

According to the above results, it could be concluded that casein has a greater buffer capacity than whey proteins. This means that standardization of milk dry matter intended for the production of fermented milk with DWP leads to the shortening of fermentation duration due to a lower buffer capacity, as the results of D e n i n D j u r d j e v i ć, 2001, and D e n i n D j u r d j e v i ć et al., 2002, showed. Naturally, this question needs further investigation since there are controversial interpretations in available literature.

C o n c l u s i o n

According to all aforementioned, it could be concluded:

The pH value of untreated milk samples decreased faster than pH value of heat treated samples at acidification temperature of 25ºC.

During the first 60 min of acidification, pH value was faster decreased when acidification temperature was increased. After 60 min of acidification, heat-treated milk samples had higher pH value than untreated samples, regardless of dry matter content.

Between 60 and 120 min of acidification, pH value of heat-treated samples decreased faster than pH of untreated samples, which indicates a smaller buffer capacity of heat-treated milk under these acidification conditions (between 60 and 120 min).

The increase of acidification temperature from 25ºC to 35ºC had a greater influence on the rate of pH decrease than increase from 35ºC to 45ºC did.

Untreated milk samples A and C had similar pH values after 60 min of acidification in spite of the applied acidification temperature. However, decreases of pH value in heat-treated milk depend on GDL concentration, acidification temperature and applied heat treatment.

Milk samples B heat-treated at 95ºC/10 min had irregular decrease of pH during acidification, particularly at 25ºC. The increase of acidification temperature to 35ºC or 45ºC improved the pattern of pH reduction.

Untreated milk samples B had the highest final pH value in spite of the applied acidification temperature and used GDL concentration.

A milk sample standardized with demineralized whey powder (milk C) that coagulated at 35ºC or 45ºC had a lower final pH value than milk samples A. At the acidification temperature of 25ºC modified casein:whey protein ratio did not influence final pH value in investigated samples.

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UTICAJ KONCENTRACIJE GDL-A NA PROMENU pH VREDNOSTI MLEKA TOKOM KISELE KOAGULACIJE

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R e z i m e

Obrano mleko u prahu je rekonstituisano da se dobije mleko sa 8.01% SM (mleko A). Mleko A je standardizovano sa 3% obranog mleka u prahu (mleko B sa 11.15% SM) i sa 3% demineralizovane surutke u prahu (mleko C sa 11.10% SM). Uzorci su termički tretirani na 85ºC/10 min, 90ºC/10 min i 95ºC/10 min, respektivno, a kao kontrolno mleko su korišćeni uzorci termički netretiranog mleka.

Acidifikacija je vršena pomoću različitih koncentracija GDL-a (glukono-δ-laktona) i to 0.5%, 0.75%, 1.0%, 1.25%, 1.5%, 1.75%, 2.0% i 3.0%. Acidifikacija je vršena pri temperaturama 25ºC, 35ºC i 45ºC u vremenu od 240 min.

Rezultati su pokazali da promena pH vrednosti zavisi od količine dodatog GDL-a, temperature acidifikacije i primenjenog režima termičke obrade mleka, kao i od dodate demineralizovane surutke u prahu.

Uzorci mleka kojima je dodata demineralizovana surutka u prahu imali su manji puferski kapacitet, što je indukovalo brže sniženje pH vrednosti u odnosu na uzorke kojima je dodato obrano mleko u prahu.

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Jedino pri temperaturi acidifikacije od 25ºC, dodata demineralizovana surutka u prahu nije uticala na promenu puferskog kapaciteta bez obzira na izmenjen odnos kazein:serum proteini. Pri ovim uslovima acidifikacije, uzorci mleka kojima je dodato obrano mleko u prahu i demineralizovana surutka u prahu u istoj količini imali su približno iste krajnje pH vrednosti.

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