Effects of Genetic Variants of κ-casein and β-lactoglobulin and Heat Treatment on Coagulating Properties of Milk

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ABSTRACT : Fifty-two Holstein cows with different phenotypes of κ-casein (κ-CN) and β-lactoglobulin (β-LG) were selected to provide weekly milk samples for heating at 30, 70, 75 and 80°C for 2 min. Coagulating properties of heated milk samples measured as rennet clotting time, rate of curd firming and curd firmness at cutting were determined by a Formagraph. Milk samples were analysed for fat and casein. Least squares analyses of data, after adjustments were made for effect of milk casein and fat contents, indicated that although an increase in heating temperatures resulted in less desirable coagulating properties, the effect of milk types was inherent irrespective of heating temperatures. The shortest rennet clotting time (6.06 min), fastest rate of curd firming (5.61 min) and firmest curd (38.05 mm) were obtained from milk with the B variant for κ-CN and B variant for β -LG when preheated at 30°C. It appears that milk bearing κ-CN B is more resistant to heat perturbation. All milk samples having the κ-casein AA (milk types AA/AA, AA/AB, AA/BB) did not have a measurable K20 value when preheated at 70°C. This effect was observed for κ-casein AB (milk types AB/AA, AB/AB, AB/BB) at 75°C and κ-casein BB (milk types BB/AA, BB/AB, BB/BB) at 80°C. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 8 : 1212-1217)***

Key Words : Genetic Variants, κ-casein, β-lactoglobulin, Heat Treatment, Coagulating Properties, Rennet Clotting Time, Rate of Curd Firming and Curd Firmness

INTRODUCTION

It has been well documented that the heating of milk prior to renneting renders it difficult to coagulate by normal renneting procedures. High heat treatment of milk has a deleterious effect on the enzymatic and non enzymatic stages of coagulation. The enzymatic stage of rennet coagulation is retarded in such milk because of interaction between heat-denatured β-lactoglobulin (β-LG) and κ-casein (κ-CN), which reduces the sensitivity of κ-CN to rennet (Wheelock and Kirk, 1974). High heat treatment also shifts the equilibrium of Ca^{2-} to the casein micelles and away from the milk serum, thereby diminishing the Ca^{2-} dependent non enzymatic stage of milk coagulation (van Hooydonk et al., 1987). As a result, several researchers (van Hooydonk et al., 1987; Singh et al., 1988; Imafidon and Farkye, 1993) have made much effort to facilitate rennet coagulability by means of pH adjustment techniques. However, there has been no consideration to improve it through the selection of genetic variants of milk proteins. Imafidon et al. (1991) found that polymorphic combinations of β-LG BB or AB with κ-CN AA produced the most stable system to heat perturbation but those of β-LG AA with κ-CN AA or BB yielded the least. Such results would be of commercial interest to cheese makers if these are applicable to cheese milk preheated under commercial conditions.

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To the best of our knowledge, research is almost non existent on the combined effect of genetic variants of milk proteins and heat treatments on coagulating properties of milk. Previously, we reported on the effects of genetic variants of κ-CN and β-LG and heat treatment on cheese yielding capacity (Choi and Ng-Kwai-Hang, 1998) and cheese and whey compositions (Choi and Ng-Kwai-Hang, 2002). This has prompted us to design an experiment to investigate the effects of genetic variants of κ-CN and β-LG in different combinations on rennet clotting time, rate of curd firming and curd firmness at cutting for milk samples preheated at 30, 70, 75 and 80°C for 2 min.

MATERIALS AND METHODS

Origin of milk sample, heat treatments and chemical analyses

Throughout this study, procedures for milk collection, heat treatments of milk samples, and analyses of components in the milk samples were the same as previously described (Choi and Ng-Kwai-Hang, 1998). In summary, milk samples were collected from 9 groups of cows with the following 9 combinations for the phenotypes of κ-CN and β-LG: AA/AA, AA/AB, AA/BB, AB/AA, AB/AB, AB/BB, BB/AA, BB/AB, and BB/BB. In addition, milk containing a mixture of different phenotypes for the two proteins was sampled from the bulk tank of the college farm. The 10 milk samples were preheated at 30, 70, 75 and 80°C prior to the determination of coagulating properties

Table 1. Overall means and standard deviations for coagulating properties for all milk samples included in the study

Preheating temperatures (°C)	Coagulating properties	Mean	SD	Range	N
30	RCT	8.02	3.67	6.03-9.24	193
	K20	8.87	3.42	5.35-13.37	172
	A30	30.52	7.28	24.25-39.08	193
70	RCT	9.74	3.98	8.12-12.43	207
	K20	9.21	3.03	6.66-12.14	103
	A30	24.63	8.55	17.10-35.01	207
75	RCT	11.85	4.49	9.66-14.15	197
	K20	9.83	2.86	8.50-10.67	25
	A30	17.88	8.73	8.98-30.48	197
80	RCT	13.47	5.87	11.25-16.22	153
	K20	ND[1]	-	-	0
	A30	6.58	5.89	2.42-15.52	153

[1] ND = no data available

(Marziali and Ng-Kwai-Hang, 1986).

Determination of coagulating properties

A Formagraph manufactured by Foss Electric (Foss, Hellerup, Denmark) was used for the determination of the coagulating properties of the 10 types of milk preheated at 4 different temperatures. The procedures (Foss, 1980) described by the manufacturer were followed. The heating module of the Formagraph was filled with distilled water and calibrated to maintain a temperature of 37±0.5°C for approximately 30 min. A 1.6% solution of Hansen's adult bovine extract plus porcine pepsin was made in distilled water. A 10 mL sample of milk was introduced into each well of the heating block and allowed to reach a temperature of 37°C. Then 200 μL of the 1.6% enzyme solution were added and well mixed. The pendulums were turned on and from the trace paper, rennet clotting time (RCT), rate of firming (K20), and curd firmness after 30 min (A30) were determined.

Statistical analyses

Effects of genetic variants of κ-CN and β-LG and milk composition on coagulating properties of milk (RCT, K20 and A30) were analyzed for each of the 4 preheating temperatures by ordinary least squares procedure using General Linear Models procedures of SAS (1988). Model was fitted to the data which included the 10 types of milk based on the κ-CN/β-LG combinations, as fixed effects. Milk concentrations in fat and total casein were included in the model as covariates to test the significance and obtain regression coefficients for these parameters. In this way adjustments were made for previously reported (Marziali and Ng-Kwai-Hang, 1986) effects of milk composition.

RESULTS AND DISCUSSION

Variability in coagulating properties of milk

The unadjusted means and standard deviations for the coagulating properties of 750 milk samples preheated at 30, 70, 75 and 80°C and measured by the Formagraph are presented in Table 1. The relatively high standard deviations for those parameters within each of the temperature treatment groups are an indication of variability due mainly to milk types (Ng-Kwai-Hang and Grosclaude, 2002) and milk composition (Marziali and Ng-Kwai-Hang, 1986; Politis and Ng-Kwai-Hang, 1988). The mixed milk bulk tank samples averaged 9.24±3.55, 9.75±2.74 and 27.77±5.58 for RCT, K20 and A30, respectively. These values were close to those obtained for the AA/BB milk type since the bulk tank contained a high proportion of this specific milk type. The values shown in Table 1 were within the ranges reported by several researchers (Marziali and Ng-Kwai-Hang, 1986; Politis and Ng-Kwai-Hang, 1988; Imafidon and Farkye, 1993; Macheboeuf et al., 1993; Montilla et al., 1995) for experiments under different conditions. The shortest coagulation time, fastest rate of firming and firmest curds were associated with the B variant of κ-casein and β-lactoglobulin (milk type BB/BB) whereas longest coagulation time, slowest rate of firming and softest curds were associated with the AA/AA milk type. Upon preheating the milk to 70°C, the values for RCT and K20 increased and those for A30 decreased and all of the 104 samples of the κ-CN AA containing milk (types AA/AA, AA/AB, AA/BB) did not give measurable K20 values. Preheating the milk to 75°C further increased the coagulation time to 11.85 min and decreased the curd firmness to 17.88 mm. Of the 10 milk types analysed only those containing κ-CN BB (types BB/AA, BB/AB, BB/BB) gave measurable K20 values. When preheated to 80°C, none of the 153 samples for the 10 milk types produced recordable K20 values. Preheating milk at 80°C further increased the RCT which averaged 13.47 and decreased A30 to 6.58. Milk type AA/BB, when preheated at this higher temperature failed to coagulate and hence no measurable A30 was available. The preheating of milk at

Table 2. Analysis of variance for the effects of milk types and milk composition on coagulating properties of milk preheated at different temperatures

Preheating temperatures (°C)	Source	RCT		K20		A30	
		df	SS	df	SS	df	SS
30	Milk types	9	133.24	9	813.79**	9	2,066.24**
	Fat in milk, %	1	8.22	1	26.32	1	22.55
	Casein in milk, %	1	37.11	1	73.36*	1	875.46**
	Residual	181	2,501.53	160	2157.14	181	10,075.43
70	Milk types	9	341.83*	6	381.69**	9	4,870.27**
	Fat in milk, %	1	42.92	1	0.08	1	244.79
	Casein in milk, %	1	13.89	1	6.33	1	1,002.85**
	Residual	195	3,294.52	94	1040.28	195	14,001.42
75	Milk types	9	244.10	2	2.81	9	5,060.56**
	Fat in milk, %	1	2.58	1	8.40	1	7.08
	Casein in milk, %	1	3.95	1	7.43	1	739.06**
	Residual	185	3,905.56	20	206.73	185	12,974.22
80	Milk types	8	308.09	-	ND	8	1,275.69**
	Fat in milk, %	1	130.62	-	ND	1	2.52
	Casein in milk, %	1	13.61	-	ND	1	173.63*
	Residual	142	5,364.31	-	ND	142	4,397.54

* $p<0.05$, ** $p<0.01$, ND = No data available.

temperatures above 30°C interfered with the rennet coagulation process as a consequence of several factors as explained by Wheelock and Kirk (1974), van Hooydonk et al. (1987), Imafidon and Farkye (1993) and Singh et al. (1988). Results presented in Table 1 indicate that there are interactions of genetic variants of κ-CN and β-LG with the effects of heat treatment on coagulating properties of milk.

Effect of milk composition and preheating temperatures on coagulating properties

The samples used in the present study averaged 4.03± 0.77% fat and 2.71±0.44% casein which varied considerably depending on the 10 different milk types as previously reported by Choi and Ng-Kwai-Hang (1998). Standard deviations in Table 1 reflect the variations in coagulating properties of milk due to casein and mineral composition (Grandison et al., 1984; Okigbo et al., 1985a, 1985b; Storry et al., 1983) somatic cell counts (Politis and Ng-Kwai-Hang, 1988), glycosylation of κ-CN (Robitaille et al., 1993), genetic variants of milk proteins (Ng-Kwai-Hang and Grosclaude, 2002). Milk types with B variant of κ-CN and β-LG contained more fat and casein than those with the A variant. An analysis of variance showed that preheating temperatures and casein content of milk significantly ($p<0.01$) affected the curd firmness of all the 10 milk types.

Rennet clotting time was significantly influenced ($p<0.01$) by heat treatment for milk containing the following κ-CN/β-LG combination: AA/AB, AA/BB, AB/AB and the mixed bulk tank milk. Fat content in AB/AB milk type and casein content in BB/AB milk type also significantly ($p<0.05$) affected RCT. The rate of curd firming (K20) was affected by the preheating temperature of the milk and in the extreme of cases, no values for this parameter were obtained. Milk containing the A variant of κ-CN was the most sensitive to heat treatment. No values for K20 were obtained for types AA/AA, AA/AB and AA/BB milk with preheating temperature of 70°C whereas for the κ-CN AB group (AB/AA, AB/AB, AB/BB) and the BB group (BB/AA, BB/AB, BB/BB), failure to produce K20 values occurred at preheating temperatures of 75°C and 80°C, respectively.

Effect of phenotypes of κ-CN/β-LG on coagulating properties

An analysis of variance to test the effects of milk types within each of the preheating temperature group and including fat and casein contents as covariates were performed and the results summarised in Table 2. Rennet clotting time was significantly affected ($p<0.05$) by milk types at preheating temperature of 70°C and not at 30, 75 and 80°C. In an earlier work (Marziali and Ng-Kwai-Hang, 1986) with non-heated milk and considering phenotypes of κ-CN and β-LG separately, it was reported that genetic variants of κ-CN did not affect RCT. This was in contrast to observations made by Kirchmeier et al. (1982), Mariani et al. (1979), Schaar (1984). For unheated milk, the three parameters of coagulating properties (RCT, K20, A30) have been reported on several occasions (Ng-Kwai-Hang and Grosclaude, 2002) to be influenced by phenotypes of β-LG. At a preheating temperature of 30°C, all the 10 milk types produced measurable K20 values which varied significantly ($p<0.01$) depending on the milk type and casein concentration. When milk was preheated at 70°C, the 3 milk types containing κ-CN AA failed to produce measurable K20 values. The remaining 7 milk types had significantly ($p<0.01$) different rates of curd firming. With

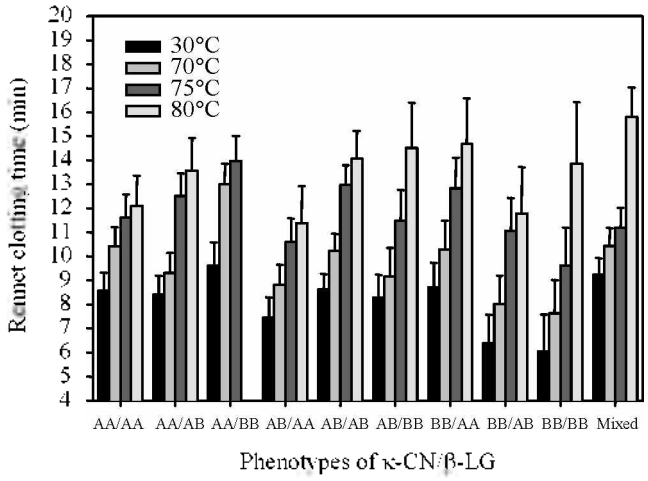

Figure 1. Least squares means and standard errors for effect of milk types on rennet clotting time at 30, 70, 75 and 80°C.

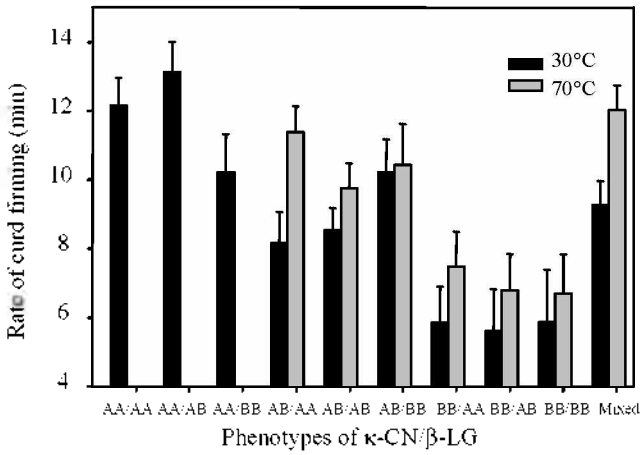

Figure 2. Least squares means and standard errors for effect of milk types on rate of curd firming at 30, 70 and 75°C.

preheating to 75°C, only 3 out of the 10 milk types had measurable K20 values which were not significantly different. The effect of milk type on K20 at preheating temperature of 80°C could not be determined because no values for this parameter were obtained for this group of heat treated milk. Casein content and milk types significantly ($p<0.01$) affected the curd firmness for all the preheating temperature under study.

The least squares means of RCT, K20 and A30 after adjustments were made for fat and casein contents of milk are shown in Figures 1, 2 and 3 respectively. For all milk types, RCT increased with raising preheating temperatures from 30 to 80°C. The magnitude of this increase depended on the milk type. For example, BB/BB milk had an RCT of 6.06 and 13.87 min at the two preheating temperatures resulting in a differential of 13.87-6.06=7.81 min compared to a value of 3.51 min (12.10-8.59) for the AA/AA milk. At

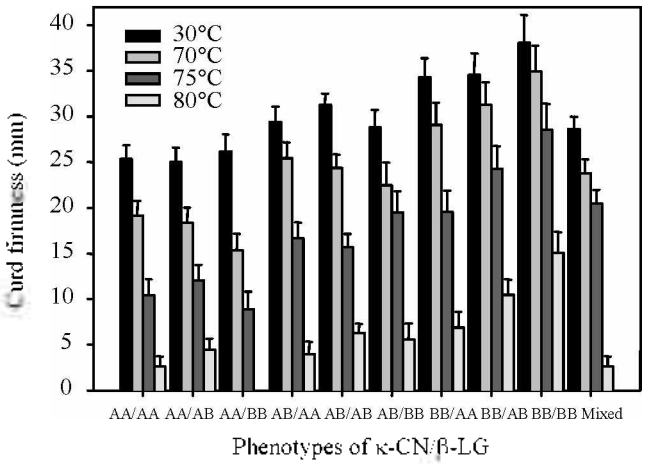

Figure 3. Least squares means and standard errors for effect of milk types on curd firmness at 30, 70, 75 and 80°C.

a preheating temperature of 30°C, shortest RCT were observed with types BB/BB (6.06 min) and BB/AB (6.40 min). The AA/BB and mixed bulk tank milk had the longest RCT with values of 9.63 and 9.25 min, respectively. When milk was preheated to 70 and 75°C, BB/BB type had the shortest (7.64 and 9.63 min) and AA/BB type had the longest (12.99 and 13.96 min) RCT. Preheating milk to 80°C changed the pattern of RCT according to milk type with failure of the AA/BB milk to coagulate and type AB/AA showing shortest RCT of 11.25 min. This was partly due to insufficient amount of rennet used to allow for measurement of coagulating properties by the Formagraph. Because the same concentration of rennet was used for all milk types, this may suggest that milk with κ-CN AA and β-LG BB was more insensitive to rennet coagulation than the other types after preheating to 80°C. The heating of milk results in denaturation of whey proteins which complex with the casein micelles and this sterically hinders the aggregation of rennet converted micelles (McMahon et al., 1993; van Hooydonk et al., 1987).

With the failure of all samples preheated at 80°C and 7 out of 10 milk types preheated at 75°C to produce K20 values, it was not possible to show the effect of milk types on K20 values for those two preheating temperatures in Figure 2. Similar results were observed by Imafidon and Farkye (1993) and Montilla et al. (1995) who could not measure K20 when bulk tank milks were heated at temperatures of 70°C and above. It is interesting to note that milk with κ-CN BB (types BB/AA, BB/AB, BB/BB) could still produce recordable K20 values after preheating to 75°C whereas the other 7 milk types did not have K20 values at this preheating temperature. The B variant of κ-CN has the ability, in some ways, to protect β-LG from heat denaturation as reported by Dannenberg and Kessler (1988) and Imafidon et al. (1991). Fastest rate of firming in

samples preheated at 30 and 70°C were associated with the B variant of κ-CN irrespective of the β-LG type. The average K20 values for κ-CN BB containing milk were 5.40 and 6.94 at preheating temperatures of 30 and 70°C, respectively. Corresponding values for κ-CN AB containing milk were 8.94 and 10.51. The mixed bulk tank milk samples showed the same trends of K20 as the κ-CN AB containing milk. At a preheating temperature of 30°C, κ-CN AA milk averaged 12.0 for K20 which was not measurable with preheating at 70°C.

Figure 3 showed that curd firmness decreased with increasing preheating temperature for all milk types. Firmest curds were obtained with milk types containing the B variant of κ-CN. The values ranged from 36.04 to 39.08 mm for BB/AA and BB/BB milk types, respectively with milk preheated at 30°C. At a preheating temperature of 80°C, these values dropped to 7.67 and 15.52 mm. The presence of κ-CN A in the milk was associated with softer curds. With preheating at 30°C, A30 for κ-CN A milk ranged from 24.78 mm for type AA/AA to 27.68 for type AA/BB milk. The increase of preheating temperature to 80°C caused the value for AA/AA type to drop to 2.71 mm whereas AA/BB type failed to coagulate and hence no measurable A30 value was obtained. In fact the latter milk type - heat treatment combination (AA/BB at 80°C) was the only one of the total of 40, that failed to coagulate. In general, within a specific type of κ-CN, the B variant of β-LG produced a firmer curd than the A variant. The opposite trends were observed when comparing type AA/AA with type AA/BB milk since the latter had softer curd than the former at preheating temperatures of 70, 75 and 80°C.

Faster rate of curd firming was correlated with firmer curds. These results are consistent with those of Schaar (1984), Marziali and Ng-Kwai-Hang (1986), Pagnacco and Caroli, Ng-Kwai-Hang et al. (1989), van den Berg et al. (1992), and Horne et al. (1994). In general, shorter RCT was correlated with faster K20 (Ng-Kwai-Hang et al., 1989). However, Okigbo et al. (1985c) pointed out that shorter RCT did not always produce faster rate of curd firming. This is well illustrated with milk preheated to 70°C where type AB/AB had a longer coagulation time than type AA/AB (10.23 v/s 9.32 min.). The former milk type had a K20 of 9.5 min. whereas the latter milk type did not produce a K20 value. In the statistical analysis, adjustments were made for casein content in milk because caseins will affect the overall structure and properties of the micelles which influence coagulating properties of milk (Marziali and Ng-Kwai-Hang, 1986). It has been reported (Horne et al., 1994) that κ-CN B is a less effective stabilizer of the micelle than κ-CN A and this might explain in part some of the results obtained in this study. In terms of relationships with cheese yields, milk containing the B variant of κ-CN and β-LG would have an advantage because they are associated with faster rate of firming and firmer curds, irrespective of the heat treatment. These assumptions were confirmed by our earlier report (Choi and Ng-Kwai-Hang, 1998) on cheesemaking.

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