Effects of Genetic Variants of κ-casein and β-lactoglobulin and Heat Treatment of Milk on Cheese and Whey Compositions

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ABSTRACT : Milk samples with different phenotype combination of κ-casein and β-lactoglobulin and different preheating temperatures of 30, 70, 75 and 80°C were used for cheesemaking under laboratory conditions. For the 853 batches of cheese, mean composition was 59.64% total solids, 30.24% fat and 23.66% protein, and the whey contained 6.93% total solids, 0.30% fat and 0.87% protein. Least squares analysis of the data indicated that heating temperature of the milk and κ-CN/β-LG phenotypes had significant effects on cheese and whey compositions. The total solids, fat and protein contents of cheese were negatively correlated with preheating temperatures of milk. Cheese from BB/BB phenotype milk had the highest and those from AA/AA phenotype milk had the lowest concentrations of total solids, fat and protein. Mean recoveries of milk components in the cheese were 53.71% of total solids, 87.15% of fat, and 80.32% of protein. For the 10 different types of milk, maximum recoveries of milk components in cheese occurred with preheating temperature of 70°C or 75°C and lowest recoveries occurred at 80°C. The whey averaged 6.94% total solids, 0.30% fat and 0.87% protein. Losses of milk components in the whey were lowest for milk preheated at 80°C and for milk containing the BB/BB phenotype. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 5 : 732-739)***

Key Words : Genetic Variants, κ-Casein, β-Lactoglobulin, Heat Treatment, Cheese Composition, Whey Composition

INTRODUCTION

The two most important cheese characteristics that are of interests to the cheesemaker are its yield and quality which is dependent on its composition. Both yield and quality of cheese are affected by composition of milk (Lou and Ng-Kwai-Hang, 1992a, 1992b) and genetic variants of milk proteins (Marziali and Ng-Kwai-Hang, 1986a, 1986b; Ng-Kwai-Hang and Grosclaude, 2002). Several attempts have been made to increase cheese yield (Lau et al., 1990; Marshall, 1986) by the inclusion of non-casein proteins into the cheese. This has been achieved by the processing of milk prior to cheesemaking and includes ultrafiltration and heat processing (Zall and Chen, 1986), pH cycling (Singh et al., 1988), denaturation of whey proteins (Lawrence, 1993) and modification of the normal cheesemaking procedure (Marshall, 1986). The increase in cheese yield by several of the above methods is accompanied by changes in cheese and whey compositions. In a previous report, we (Choi and Ng-Kwai-Hang, 1998) reported on the effects of heating temperatures and genetic variants of κ-casein (κ-CN) and β-lactoglobulin (β-LG) on cheese yield. The present study investigates, under laboratory conditions, the effects of genetic variants of milk proteins and heat treatments on cheese and whey compositions and incorporation of milk components into cheese.

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MATERIALS AND METHODS

Origin of milk sample, heat treatment and cheesemaking

Throughout this study, procedures for milk collection, heat treatment of milk samples prior to cheesemaking, cheesemaking techniques under laboratory conditions, collection and weighing of milk, cheese and whey samples, analysing components in the original milk and the resulting cheese and whey were the same as previously described (Choi and Ng-Kwai-Hang, 1998).

Briefly, 52 lactating Holstein cows were selected to provide milk for the nine possible combinations of three κ-CN and three β-LG phenotypes. These nine different phenotype combinations will henceforth be denoted as AA/AA, AA/AB, AA/BB, AB/AA, AB/AB, AB/BB, BB/AA, BB/AB, and BB/BB to indicate the phenotypes of κ-CN and β-LG, respectively. In addition, milk containing a mixture of different phenotypes for the two proteins from the bulk tank of the Macdonald Campus farm served as controls in cheesemaking experiments. The milk samples were preheated at 30, 70, 75 and 80°C prior to cheesemaking under standard laboratory conditions (Marziali and Ng-Kwai-Hang, 1986a). After overnight pressing, the cheese curds were weighed, grated, hermetically sealed in plastic bags, and stored frozen at -10°C pending chemical analysis. Subsamples of the initial milk used and corresponding whey samples were analysed in duplicate for total solids, fat and protein.

Chemical analyses

Milk, whey and cheese samples were analysed for total solids by oven drying to constant weight and for fat by the gravimetric method of Roese-Gottlieb after extraction with an alcohol-ether mixture according to the Association of Official Analytical Chemist procedure (AOAC, 1980). Prior to solvent extraction, the cheese samples were dissolved by boiling with a mixture of concentrated HCl and 10% ammonium hydroxide. Protein in cheese and whey samples was determined by the Kjeldahl method according to standard procedure for dairy products (AOAC, 1980). Total protein in milk samples was analysed by the amino black dye-binding procedure outlined by Foss Electric (A/S N Foss Electric, 1978) and modified by Ng-Kwai-Hang and Hayes (1982).

Statistical analyses

The effects of heat treatments and genetic variants of κ-CN and β-LG on composition of cheese and whey were analysed by least squares procedures (SAS, 1988). The preheating temperatures of milk were divided into four classes which corresponded to 30, 70, 75 and 80°C, respectively. There were ten categories for milk types which included the nine combinations for κ-CN/β-LG phenotypes and one for the bulk tank mixed phenotype milk. Milk concentrations in fat and casein were included in the model as covariates to test statistical significance since these two components are known to significantly affect cheese and whey compositions.

RESULTS AND DISCUSSION

Variability in cheese and whey composition

The overall unadjusted means and standard deviations for cheese and whey components derived from 853 batches of cheese made under laboratory conditions are presented in table 1. Total solids in cheese varied from 52.17 to 64.29% (i.e. moisture of 35.71 to 47.83%). Fat in cheese, with an overall mean of 30.24% and standard deviation of 4.75%, was more variable than protein in cheese which had an overall mean of 23.66% and a standard deviation of 2.77%. The range in values were 25.42 to 34.53% and 20.36 to 26.26% for fat and protein contents of cheese, respectively. The values obtained for the cheese composition were within the ranges of Cheddar type cheese made under different sets of conditions and reported by other researchers (Marziali and Ng-Kwai-Hang, 1986b; Barbano et al., 1991; Lau et al., 1990; Imafidon and Farkye, 1994; Tong et al., 1994). More than 80% of the cheese produced had a lower than targeted value of 63% for total solids. Values for moisture in non-fat substances (MNFS), with a mean of 57.73% and a range of 52.93 to 63.97% were higher than other reported values (Barbano and Sherbon, 1984; Gilles and Lawrence, 1985). It has been suggested that MNFS should not exceed 56% for a good quality cheddar cheese (Lawrence and Gilles, 1987). Relatively high values for MNFS were obtained in this study because of the high moisture in cheese as a result of incorporation of more milk protein into the cheese. The fat in dry matter (FDM) content of cheese with a mean of 50.48% was less than the reported values of 52 to 56% by other workers (Banks et al., 1984, Lawrence et al., 1984) but is within the minimal Canadian legal limit of 49%. Protein in dry matter of cheese (PDM) with an overall mean of 39.84% and a range of 36.17 to 42.53%, was less variable than FDM and was within the ranges of previously reported values (Barbano and Sherbon, 1984; Ng-Kwai-Hang et al., 1988). A high PDM will result in a cheese that appears drier than what its moisture content would indicate.

Table 1. Overall means and standard deviations for cheese and whey compositions for all samples included in this study

Product and component	Mean	Standard deviation
Cheese		
Total solids	59.64	4.92
Fat	30.24	4.75
Protein	23.66	2.77
Salt	1.44	0.17
Moisture in non-fat substances	57.73	4.44
Fat in dry matter	50.48	5.40
Protein in dry matter	39.84	5.07
Whey		
Total solids	6.93	0.39
Fat	0.30	0.16
Protein	0.87	0.20

Table 1 also gives a summary of the composition of whey corresponding to the 853 batches of cheese made under laboratory conditions. Total solids in whey had an overall mean of 6.93% and a standard deviation of 0.39%. Lactose which is not incorporated into cheese would account for most of the total solids in whey. Fat and protein in whey with means of 0.30 and 0.87%, respectively, were lower than values reported in other studies (Barbano and Sherbon, 1984; Marziali and Ng-Kwai-Hang, 1986b; Lau et al., 1990; Ng-Kwai-Hang et al., 1988). This difference in composition is mainly due to the fact that in this study attempts were made to increase protein incorporation into cheese by preheating the cheese milk.

An analysis of variance showed that preheating temperatures of milk and phenotype κ-CN and β-LG affect significantly ($p<0.05$) total solids, fat, and protein in cheese (table 2) and whey (table 3).

Table 2. Analysis of variance for the effects of milk types and milk composition on cheese composition for milk heated at 30, 70, 75 and 80°C

Preheating temperatures (°C)	Source	df	Sum of squares		
			Total solids	Fat	Protein
30	Milk types	9	134.61	205.63	13.46
	Fat in milk, %	1	270.97**	1439.18**	414.77**
	Casein in milk, %	1	1.81	119.23**	181.20**
	Residual	177	2809.60	2490.76	963.48
70	Milk types	9	115.38*	60.10	33.72
	Fat in milk, %	1	569.25**	2111.01**	587.59**
	Casein in milk, %	1	65.68**	330.15**	169.99**
	Residual	198	1196.97	1212.50	885.04
75	Milk types	9	191.81**	143.49*	22.05
	Fat in milk, %	1	257.43**	1229.86**	423.89**
	Casein in milk, %	1	91.72**	554.97**	257.53**
	Residual	189	1382.32	1233.66	663.10
80	Milk types	9	406.60**	224.08**	78.16**
	Fat in milk, %	1	199.11**	1064.84**	286.23**
	Casein in milk, %	1	379.71**	809.46**	87.08**
	Residual	177	1970.05	1127.54	497.91

* p<0.05, ** p<0.01.

Table 3. Analysis of variance for the effects of milk types and milk composition on whey composition for milk heated at 30, 70, 75 and 80°C

Preheating temperatures (°C)	Source	df	Sum of squares		
			Total solids	Fat	Protein
30	Milk types	9	2.15**	0.34	0.76**
	Fat in milk, %	1	1.44**	0.26**	0.05
	Casein in milk, %	1	1.32**	0.00	0.88**
	Residual	181	13.92	4.84	2.99
70	Milk types	9	1.45	0.29	0.60**
	Fat in milk, %	1	1.32**	0.35**	0.05*
	Casein in milk, %	1	2.85**	0.08*	0.85**
	Residual	198	32.50	3.44	2.46
75	Milk types	9	1.93**	0.11	0.43**
	Fat in milk, %	1	0.99**	0.22**	0.01
	Casein in milk, %	1	1.77**	0.06*	1.12**
	Residual	189	13.91	2.13	1.46
80	Milk types	9	1.05	0.31*	0.17**
	Fat in milk, %	1	0.62**	0.30**	0.01
	Casein in milk, %	1	1.48**	0.04	0.29**
	Residual	178	15.21	2.53	1.05

* p<0.05, ** p<0.01.

Effects of preheating temperatures and phenotypes of κ-CN/β-LG on cheese composition

Figure 1 shows the least squares means of total solids, fat and protein in cheese after corrections were made for fat and casein contents of milk. For all the combinations of κ-CN/β-LG phenotypes, total solids in cheese decreased as preheating temperature of milk was increased from 30°C to 80°C. In all cases, the drops in total solids were highest from 75°C to 80°C than from 30°C to 75°C. The B variant of κ-casein and B variant of β-lactoglobulin were associated with high total solids in cheese. The combination of BB/BB phenotype contained 64.29, 62.92, 61.94 and 58.01% total solids in the cheese when the milk was preheated at 30°C, 70°C, 75°C and 80°C, respectively compared to values of 61.59, 60.72, 58.27 and 52.17%, for AA/AA phenotype milk preheated at the corresponding four temperatures. The

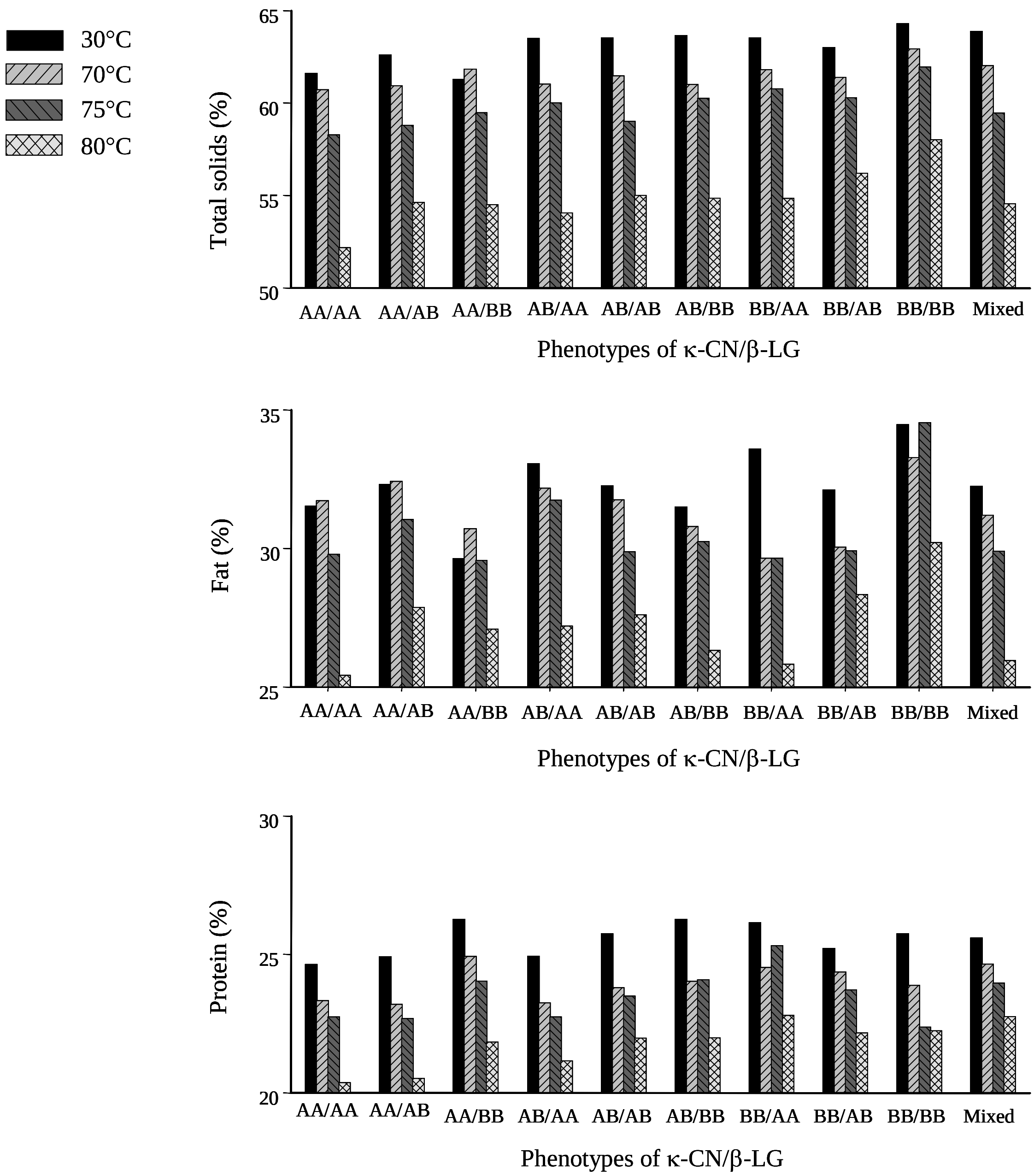

Figure 1. Effects of heating temperatures and phenotypes of κ-casein and β-lactoglobulin on total solids, fat and protein in cheese

patterns for fat and protein in cheese according to phenotypes of κ-casein and β-lactoglobulin and preheating temperatures of cheese milk were similar to those observed for total solids i.e. highest fat and protein in cheese derived from BB/BB milk preheated at 30°C and lowest fat and protein in cheese from AA/AA milk preheated at 80°C. Cheese made from BB/BB milk contained 34.46, 33.38, 34.53 and 30.21% fat and 25.73, 23.87, 22.37 and 22.24% protein when preheating temperatures were 30, 70, 75 and 80°C, respectively. Corresponding values for cheese made from AA/AA milk were 31.53, 31.71, 29.79 and 25.42% fat; 24.64, 23.32, 22.73 and 20.36% protein. The relationships between total solids, fat and protein in cheese were expected because more than 90% of cheese total solids are due to fat and protein. The higher concentration of total solids, fat and protein in cheese associated with the B variant of κ-casein and β-lactoglobulin concur with the results of Marziali and Ng-Kwai-Hang (1986b). In our previous study (Choi and Ng-Kwai-Hang, 1998), it was reported that cheese yield was increased when milk was heated at 75°C prior to cheesemaking. The increase in cheese yield was attributed to inclusion of additional

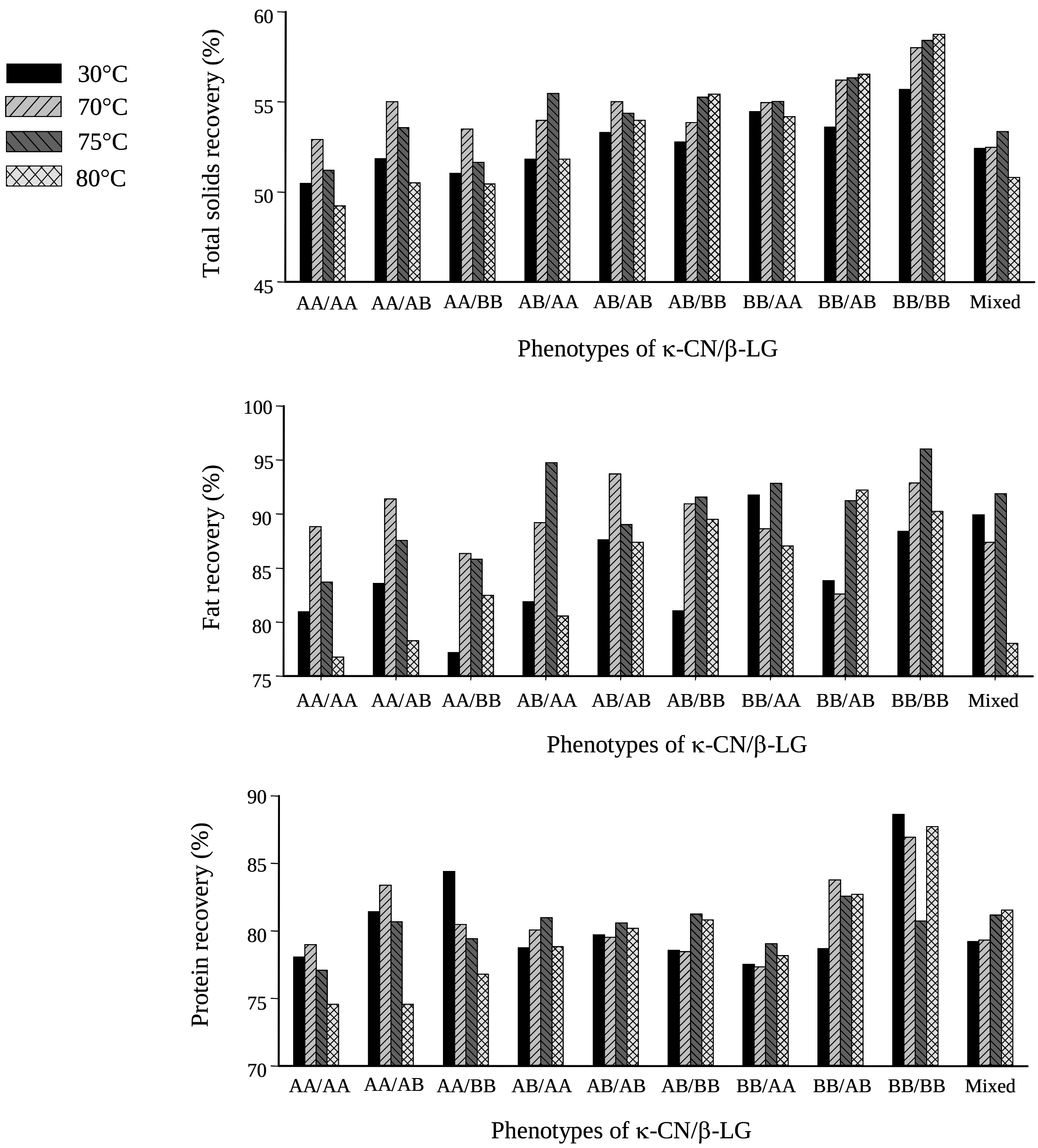

Figure 2. Effects of heating temperatures and phenotypes of κ-casein and β-lactoglobulin on recovery of milk total solids, fat and protein in cheese

protein in the cheese. Higher protein in cheese is associated with higher moisture content i.e. lower total solids in cheese. Higher moisture is accompanied by lower fat in cheese. In cheese, protein is associated with more than its weight of water and this would explain lower protein and higher moisture in cheese with increase of preheating temperature of milk. The heating of milk at temperatures of 70, 75 and 80°C would result in the formation of complex between κ-casein and whey proteins, especially, β-lactoglobulin and α-lactalbumin. The amount of complexes formed is positively correlated with heating temperatures and is incorporated in the cheese fraction.

Effects of preheating temperatures and phenotypes of κ-CN/β-LG on recovery of milk components in cheese

Because the composition of milk will influence the amounts of total solids, fat and protein incorporated into cheese, results were also expressed as percentages of those components recovered in cheese from 100 g of milk. The recovery data presented in figure 2 illustrate the effects of heat treatment and κ-CN/β-LG phenotypes on recoveries of milk components in cheese. Overall means were 53.71,

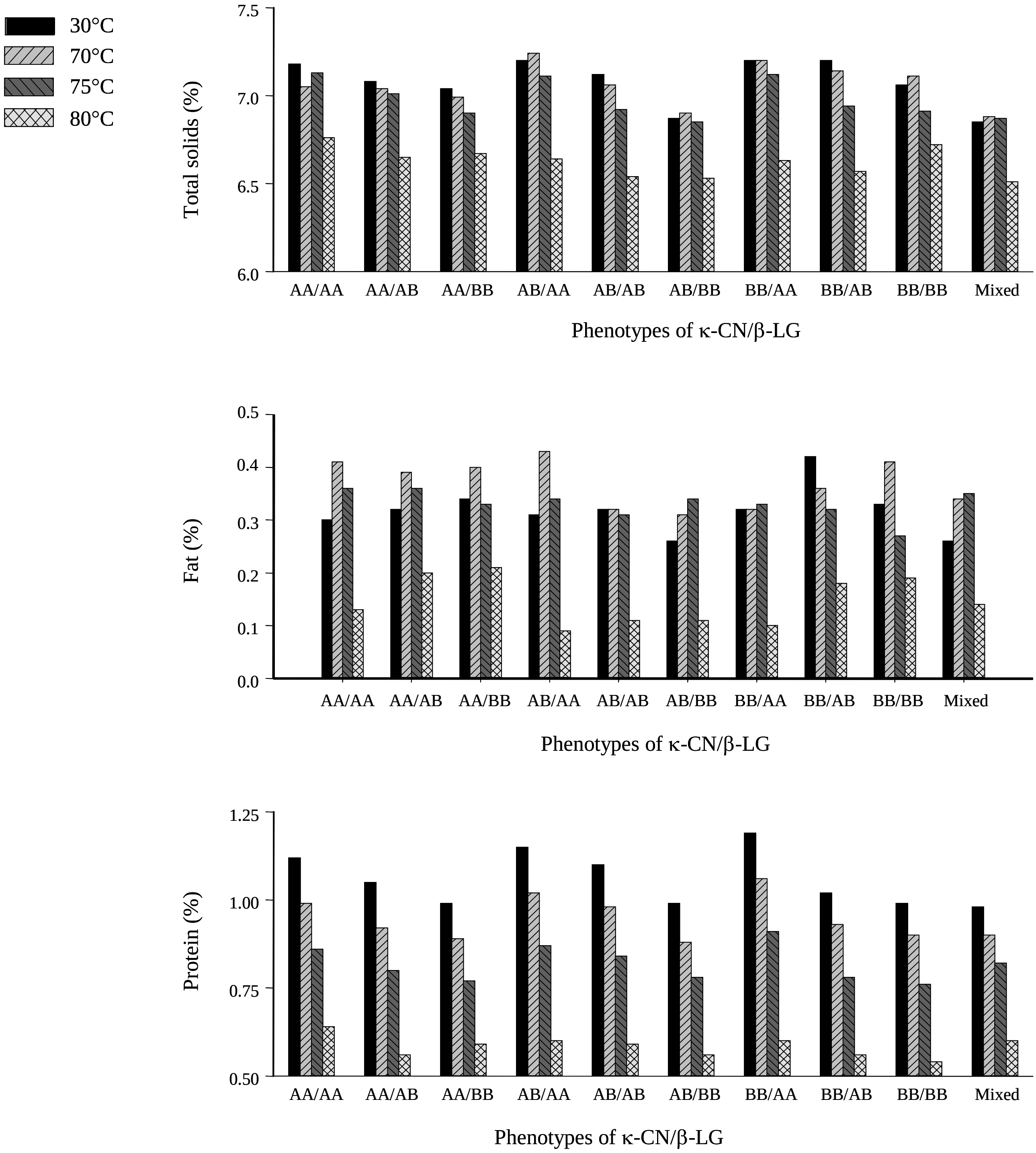

Figure 3. Effects of heating temperatures and phenotypes of κ-casein and β-lactoglobulin on total solids, fat and protein in whey.

87.15 and 80.32% for recoveries of total solids fat and protein in cheese, respectively with ranges of 49.22 to 58.75, 76.75 to 96.02% and 74.54 to 88.62%. Values of 52.42, 89.94 and 79.19% for recovery of total solids, fat and protein respectively for the bulk tank mixed κ-CN/β-LG phenotype milk were in agreement with those obtained in a previous study (Lou and Ng-Kwai-Hang, 1992b). Highest recoveries of total solids in cheese were observed for the phenotype combinations of BB/BB, BB/AB and AB/BB and the recoveries increased as preheating temperature of milk is increased from 30 to 80°C. For example, in BB/BB milk, recoveries of 55.68, 58.01, 58.41 and 58.75% total solids in cheese corresponded to milk preheated at 30, 70, 75 and 80°C, respectively. Milk containing the A variant of κ-casein (i.e. AA/AA, AA/AB, AA/BB phenotype combinations) gave lowest recoveries of cheese total solids which increased when milk was heated 70°C and decreased at heating temperatures of 75 and 80°C. For AA/AA milk, recoveries of total solids in cheese were 50.47, 52.91, 51.19 and 49.22% at preheating temperatures of 30, 70, 75 and 80°C respectively. Higher recoveries of fat and protein in cheese were also observed for the B variant of κ-casein and β-lactoglobulin. Fat recoveries were 80.94, 88.83, 83.70 and 76.75% for AA/AA milk when preheating temperatures were 30, 70, 75 and 80°C, respectively. Corresponding fat recovery values for BB/BB milk were 88.39, 92.87, 96.02 and 90.25%. In the AA/AA milk, lowest recovery of proteins in cheese (74.54%) was achieved at 80°C and highest recover (78.98%) at 70°C. For BB/BB milk the range for protein recovery was from 80.71% at 75°C to 88.62% at 30°C.

Effects of preheating temperatures and phenotypes of κ-CN/β-LG on whey composition

Figure 3 shows the least squares means of total solids, fat and protein in whey according to preheating temperatures of milk and κ-CN/β-LG phenotype combinations. There were no significant ($p<0.05$) difference in total solids content for the different κ-CN/β-LG phenotype milk. For the 30°C preheated milk, total solids in whey varied from 6.85% for bulk tank mixed phenotype milk to 7.20 for AB/AA, BB/AA, BB/AA and BB/AB milk. Heating of milk at 80°C decreased total solids in whey which ranged from 6.51% for bulk tank mixed phenotype milk to 6.76% for AA/AA milk. The decrease in whey total solids with increasing preheating temperatures is due to more milk fat and protein being incorporated into the cheese and less of those two components being lost in the whey as shown in figure 3. In general there was more fat in whey (0.26-0.43%) for milk preheated at 30 and 70°C than for milk preheated at 75 and 80°C (0.10-0.36%). The range of values for fat in whey was from 0.10% for BB/AA milk heated at 80°C to 0.43% for AB/AA milk heated at 70°C. Overall mean for protein in whey was 0.87% and in all cases, protein in whey decreased as the preheating temperature was increased for 30°C to 80°C. This decrease in protein in the whey was most prevalent in BB/AA milk with values of 1.19, 1.06, 0.91 and 0.60% at 30, 70, 75, 80°C, respectively.

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