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Effect of β-lactoglobulin phenotype on whey protein nitrogen index and sulphydryl content of skim milk powder

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Abstract — The heat labile nature of whey proteins suggests that compositional differences in these protein fractions may affect physicochemical characteristics and functional properties during the thermal processing of milk products. For this reason, a study was set out to determine the effect of β-lactoglobulin (β-lg) polymorphism on the protein content of skim milk, its denaturation during skim milk powder (SMP) manufacture and the consequential impact on the heat classification of the resultant powders. Furthermore, the effect of β-lg variant on sulphydryl (SH) group content of SMP and its potential to augment the whey protein nitrogen index (WPNI) method as a means of heat classification was explored. The study revealed that the β-lg AA phenotype was associated with higher levels of whey protein and its respective β-lg fraction in raw milk, as well as a higher total SH content in SMP irrespective of preheat temperature used during manufacture. This was reflected in the lower WPNI values for the AA phenotype measured at each processing step (preheated skim: $P < 0.004$, concentrate: $P < 0.023$ and powder: $P < 0.001$). A significantly lower α-lactalbumin (α-la) content was associated with the AA phenotype compared to the AB and BB phenotypes for preheated skim milk ($P < 0.042$), concentrate ($P < 0.001$) and powder ($P < 0.046$). Moreover, it was found that the β-lg AA phenotype SMP contained a higher percentage of slow-reacting SH groups (as a percentage of total), which suggests greater resistance to heat denaturation. A potentially interesting relationship between slow-reacting SH groups and WPNI emerged in the course of data analysis, which may warrant further study. Manufacture of milk powders to meet low-heat classification is favoured by selective segregation of β-lg AA phenotype milks. © Inra/Elsevier, Paris

β-lactoglobulin / skim milk powder / WPNI / sulphydryl

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Résumé — Effet du phénotype de la β-lactoglobuline sur l'azote des protéines de lactosérum non dénaturées (WPNI), et la teneur en sulphydryl de la poudre de lait écrémé. Les protéines de lactosérum étant sensibles au chauffage, des différences de composition des fractions protéiques peuvent affecter les caractéristiques physicochimiques et les propriétés fonctionnelles des produits laitiers selon les traitements thermiques. Des études ont donc été conduites pour déterminer l'effet du polymorphisme de la β-lactoglobuline (β-lg) sur la teneur en protéines du lait écrémé, sur leur dénaturation pendant la fabrication de poudre de lait écrémé et par conséquent sur la classification thermique des poudres résultantes. De plus, l'effet du polymorphisme de la β-lactoglobuline (β-lg) sur la teneur en sulphydryl (groupe SH) de la poudre du lait écrémé et sa capacité à augmenter l'azote des protéines du sérum non dénaturées comme méthode de classification thermique ont été étudiés. Les résultats obtenus montrent que le phénotype β-lg AA est associé à des niveaux plus élevés de protéines du lactosérum, et de β-lg dans le lait cru, ainsi qu'à une teneur plus élevée de sulphydryl dans la poudre du lait écrémé indépendamment de la température de préchauffage au cours de la fabrication. Les valeurs du WPNI pour le phénotype β-lg AA étaient réduites (lait écrémé préchauffé : $p < 0{,}004$; concentré : < 0,023, et poudre : $p < 0{,}001$). Une teneur en lactalbumine significativement plus basse était associée au variant β-lg AA par rapport aux variants AB et BB (lait écrémé préchauffé : $p < 0{,}042$; concentré : $p < 0{,}001$ et poudre : $p < 0{,}046$). De plus la poudre obtenue avec le variant β-lg AA contenait un pourcentage plus élevé de sulphydryl réagissant lentement, ce qui suggère une plus grande résistance à la dénaturation au chauffage. On a découvert un rapport intéressant entre le sulphydryl réagissant lentement et WPNI qui nécessite des études complémentaires. La fabrication des poudres du lait pour atteindre les normes « low heat » est favorisée par le choix de laits contenant de la β-lg AA.

β-lactoglobuline / poudre de lait écrémé / WPNI / sulphydryl

1. INTRODUCTION

β-Lactoglobulin (β-lg) accounts for 50–60 % of total whey protein in bovine milk. The primary structure of bovine β-lg consists of a chain of 162 amino acids with a monomeric molecular weight of ~ 18 000 [20]. Native β-lg has two internal disulphide groups and one free thiol group. Disulphide bridges occur at positions Cys^{66}–Cys^{160} and Cys^{106}–Cys^{119} [9, 10]. The free thiol occurs at residue 121 [10].

The most commonly occurring genetic variants of bovine β-lg in western breeds are A and B, as first discovered by Aschaffenberg and Drewry [6]. Differences between these two variants are due to rather minor changes in the amino acid sequence: β-lg A has aspartic acid and valine at residues 64 and 118, respectively, while β-lg B has glycine and alanine, respectively, at these positions [39]. The worldwide frequency of the occurrence of β-lg AA, AB and BB phenotypes is 11–19, ~ 50 and 30–40 %, respectively.

Genetic polymorphism of β-lg is associated with variations in the composition of milk, especially its protein content. A number of studies reveal that milk protein content is significantly influenced by β-lg phenotype in the following order: AA > AB > BB [18, 40, 41]. However, many other studies indicate that there is no significant difference between the protein content of β-lg AA, AB and BB milks [1, 3, 11, 23, 36, 54]. The effect of β-lg phenotypes on whey protein and casein content appears to be more consistent and reveals that β-lg AA is associated with higher whey protein and lower casein levels than the BB phenotype [3, 17, 23, 30, 32, 38, 44, 46]. In some cases, it is thought that the higher whey protein content in β-lg AA milk is counterbalanced by a lower casein content and, thus, explains why the total protein content of β-lg AA and BB milks may be quite similar [26, 27].

β-lg AA milk contains higher levels of β-lg than AB and BB phenotype milk [1, 11, 23, 39, 42, 46] and lower levels of (α-la), serum albumin and immunoglobulins [1, 23, 24, 31, 42, 46].

Genetic manipulation of milk proteins has far reaching implications for the dairy industry given the potential to alter functional properties [8], and numerous studies have been carried out on the effects of β-lg phenotypes on the processing characteristics of milk, e.g. cheese manufacture and heat stability. Interest is now growing regarding the potential influence of β-lg variants on milk powder production. Recent studies indicate that heating surfaces (e.g. UHT plants and evaporators) are fouled to a lesser degree by β-lg BB milk [25], and therefore, allows longer manufacturing run times than the AA phenotype. It has also been found that β-lg BB milk concentrates are less viscous, and permit a higher total solids feed to the drier [35].

β-lg B denatures at a faster rate than the A variant [4, 13, 21, 47, 49]. In the present study, it was considered that this may be of consequence during the manufacture of skim milk powder where the residual level of undenatured whey protein dictates its heat classification according to the American Dry Milk Institute (ADMI) [2]. Three categories – low-, medium- and high-heat skim milk powder (SMP) – are identifiable according to the ADMI bands of undenatured whey protein nitrogen (WPN), falling within the limits of ≥ 6.0, 1.51–5.99 and ≤ 1.5 mg·WPN·g^{-1} powder, respectively. The degree of denaturation taking place during milk drying [45] highlights that preheat treatment of the milk before evaporation is the single most influential unit operation involved. Thus, there is particular interest in the susceptibility to denaturation during the drying of milks containing higher whey protein levels as a result of dairy cow selection according to β-lg A variant.

One of the more important interactions that β-lg undergoes is that which occurs with α-la and κ-casein (κCN) when heated. Studies have shown that β-lg interacts with α-la and κ-cn via the formation of disulphide bonds and sulphydryl/disulphide interchange. Sawyer et al. [50] proposed that heating causes exposure of sulphydryl (SH) groups in β-lg, leading to intermolecular disulphide bond formation between β-lg and κCN. This was confirmed by Noh et al. [43] and Dalgleish [12]. Heat-induced interaction of α-la/β-lg via sulphydryl/disulphide interchange was demonstrated by Melo and Hansen [37] and Elfagm and Wheelock [14, 15]. Due to its thermal reactivity [22, 29], SH group measurement has been used as an indicator of the extent to which milk has been heated. The commonest method is that of Ellman [16], who used 5'5-dithiobis (2-nitrobenzoic) acid (DNTB) as a colorimetric reagent. Much work has been done to improve the suitability of the method for food samples, e.g. milk [7, 28, 29, 33, 52].

A recent analytical development [51] which distinguishes between SH groups present in native β-lg and those resulting from heat-induced sulphydryl/disulphide interchange provides an opportunity to probe for differences in molecular unfolding and interactions between the β-lg variants. This is achieved by comparing the speed of reaction of SH groups with DNTB in the presence of sodium dodecyl sulphate (SDS). In an unheated milk sample containing native β-lg, the free SH group reacts slowly with DNTB (in the presence of SDS), giving rise to the term 'slow-reacting' SH group. In a heated sample, the position of the free thiol changes and it reacts quickly with DNTB, resulting in the so-called 'fast-reacting' SH group. Thus, of the total SH groups the proportion of slow/fast-reacting SH groups will change, depending on the heat treatment received by the sample.

The aim of this study was to determine the effect of β-lg genetic polymorphism on the whey protein content of skim milk, its denaturation during SMP manufacture and the consequential impact on the heat classi-

fication of the resultant powders. The effect of β-lg phenotype on SH group content of SMP and the potential for estimation of SH group content as an alternative method of heat classification was also investigated.

2. MATERIALS AND METHODS

2.1. Manufacture of SMP containing known variants of β-lg

Approximately 300 Holstein-Friesian cows in the dairy herd at Moorepark were phenotyped for κCN and β-lg variants (AA, AB and BB) by non-denaturing polyacrylamide gel electrophoresis of milk samples, according to the method of O'Hara [44]. Sixty of these lactating animals were selected according to β-lg phenotype for milk collection purposes. Where possible, the κCN phenotype was kept constant while selecting the β-lg phenotypes. Sufficient milk was then collected from individual cows of known β-lg phenotype, and pooled to make up approximately 200 L before processing. In the case of each bulk raw milk, the cream was removed by centrifugal separation (Westfalia Separator MSD 50-01-071, Germany) at 60 °C, and the resulting skim milk was divided into three equal batches (numbered 1, 2 and 3) which were preheated to 75, 85 and 95 °C, respectively, for 30 s in an APV Pasilac SSP Pilot Plant (APV, Silkeborg, Denmark). Each of the preheated skim milks was concentrated to ~ 45 % total solids in a single effect falling film evaporator (Type F1-Lab 3, Anhydro A/S, Copenhagen, Denmark). Concentrates were spray-dried in an Anhydro Lab 3 spray-drier (Anhydro A/S, Copenhagen, Denmark) using disc atomisation with air inlet and outlet temperatures of 190 and 90 °C, respectively. Skim milk powder (for each variant and at each preheat temperature) was manufactured at four different times during the study, identified as Trials 1–4. Trials 1 and 4 took place in October (late lactation), while Trials 2 and 3 took place in May (early lactation) and August (mid-lactation), respectively.

2.2. Determination of whey protein denaturation during manufacture of SMP

Whey protein denaturation was monitored during the SMP manufacturing process by taking samples of raw skim milk, preheated skim milk, concentrate and powder and analysing them for residual undenatured whey protein. Concentrates and powders were reconstituted to the total solids level of the original skim milk with distilled water. The total protein (TP), non-casein nitrogen (NCN) and non-protein nitrogen (NPN) content of samples was determined by the Kjeldahl procedure according to the FIL-IDF Standard (20B:1993). The whey protein nitrogen index (WPNI) was determined by the American Dry Milk Institute method [2].

β-lg and α-la concentrations were quantified using gel permeation fast protein liquid chromatography (GP-FPLC). Standard solutions of β-lg (0–2 $mg \cdot mL^{-1}$) and α-la (0–1.6 $mg \cdot mL^{-1}$) were applied to the FPLC column and standard curves were constructed from the resulting peak areas. The content of β-lg and α-la in test samples was determined using these standard curves. A Superose 12 non-denaturing HR 10/30 FPLC column (Pharmacia LKB Biotechnology, Uppsala, Sweden) was used in this study. The β-lg and α-la standard solutions were made up with running buffer which consisted of 0.1 $mol \cdot L^{-1}$ Tris/HCl, pH 7.0, containing 0.1 $mol \cdot L^{-1}$ NaCl and 10 % (v/v) methanol. Buffer was filtered and degassed through a 0.45 μm filter in an all glass vacuum filtration unit (Millipore Corporation, Bedford, USA). Whey protein samples (previously prepared for NCN analysis) were filtered through 0.45 μm Whatman Puradisc filters (Whatman Int., Maidstone, Kent, UK) into FPLC sample vials and 100 μL was automatically injected into the column. Flow rate through the column was 0.5 $mL \cdot min^{-1}$. Data collected on channel 4 from the 280-nm monitor (single path UV-1 280 nm monitor) was analysed by a Minichrom Chromatography package (VG Data Systems, Altrincham, Cheshire, UK). Total analysis time for a single sample was 71 min, with β-lg and α-la eluting at ~ 29 and 41 min, respectively.

2.3. Determination of the sulphydryl (SH) content of β-lg AA, AB and BB SMP

The reaction of DTNB with protein SH groups at neutral and alkaline pH to yield a thionitrophenylated protein and a thionitrophenylate anion in an equimolar ratio is the basis of the colorimetric method of Shimada and Cheftel [51] which was adapted to suit milk powders. The

following reagents were used: i) standard buffer consisting of 0.086 mol·L^{-1} Tris, 0.09 mol·L^{-1} glycine, 4 mmol·L^{-1} EDTA, pH 8.0; ii) standard buffer plus denaturants - as for (i) above plus 8 mol·L^{-1} urea and 17.3 mmol·L^{-1} SDS; iii) Ellman's reagent solution: 40 mg DTNB per 10 mL standard buffer.

The amount of SMP required to give 50 mL of a 0.1 % protein solution was dissolved readily in standard buffer plus denaturants (ii), made up to the volume in a 50-mL volumetric flask and allowed to stand for 1 h. Three mL of this solution was placed in a test tube, 0.03 mL of Ellman's reagent was added and mixed rapidly. The absorbance of this solution at 412 nm was recorded (Hitachi U-1100 spectrophotometer, Tokyo, Japan) at the following intervals: every 1 min for 10 min, every 2.5 min for a further 10 min and every 5 min for a further 10 min. These absorbance values were incorporated into the formula used by Beveridge et al. [7], so that the SH content of each sample at each time point could be calculated. The amount of slow-reacting and total SH groups was determined graphically according to the method of Shimada and Cheftel [51].

2.4. Statistical analysis of whey protein data

Data was analysed by analysis of variance (ANOVA) using the statistical package GENSTAT. Differences stated in the Results and Discussion section are significant (at least) at $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Effect of β-lg genetic variants on the protein content of skim milk

No trend emerged to link differences in the TP and NPN contents of the milks with the three herds segregated according to the different β-lg phenotypes (AA; AB; BB). For example, in Trial 1, the order for total protein was β-lg BB > AA > AB, while in Trial 4 the order was AA > AB > BB (*table I*). Previous authors [3, 11, 23, 36] also failed to establish a trend between β-lg genetic variants and total protein content of milk.

β-lg AA milk contained higher levels of whey protein than the AB and BB phenotypes and is in agreement with the findings of previous authors [3, 23, 27, 38, 44, 46]. Furthermore, GP-FPLC revealed that β-lg AA raw skim milk generally contained higher levels of β-lg, but lower levels of α-la than the BB variant, e.g., in Trial 1, β-lg AA milk contained 4.81 and 1.15 mg·mL^{-1} of β-lg and α-la, respectively, while β-lg BB milk contained 4.07 mg·mL^{-1} of β-lg and 1.32 mg·mL^{-1} of α-la (*table II*). This finding has also been reported by previous authors [1, 23, 42]. β-lg levels in the AB phenotype milk were generally intermediate between those of the AA and BB phenotypes.

Table I. Total protein (TP) and non-protein nitrogen (NPN) content of β-lactoglobulin (β-lg) AA, AB and BB raw skim milk used subsequently for skim milk powder manufacture.

Tableau I. Teneur en protéines totales (TP) et en azote non protéique (NPN) dans les β-lactoglobuline (β-lg) AA, AB and BB de lait cru écrémé utilisé par la suite pour la préparation de poudre de lait écrémé.

	β-lg AA milk		β-lg AB milk		β-lg BB milk	
Trial no.	% TP	% NPN	% TP	% NPN	% TP	% NPN
1 (Oct. 95)	3.74	0.035	3.68	0.028	3.85	0.039
2 (May 96)	3.34	0.031	3.22	0.031	3.09	0.038
3 (Aug. 96)	2.93	0.036	3.48	0.040	3.49	0.043
4 (Oct. 96)	4.19	0.046	4.15	0.044	3.79	0.021

Table II. Whey protein content of β-lg AA, AB and BB raw skim milk in Trials 1–4, represented by % non-casein nitrogen (NCN), whey protein nitrogen index (WPNI) and β-lactoglobulin [β-lg] and α-lactalbumin [α-la] concentrations.

Tableau II. Teneur en protéines du lactosérum dans les β-lg AA, AB et BB de lait cru écrémé pour les essais 1-4, représentée par le pourcentage d'azote non caséique (NCN), l'azote des protéines du lactosérum non dénaturées (WPNI) et les concentrations de β-lactoglobuline [β-lg] et d'α-lactalbumine [α-la].

Trial 1					Trial 2				
β-lg phenotype	NCN (%)	WPNI*	[β-lg]**	[α-la]**	β-lg phenotype	NCN (%)	WPNI*	[β-lg]**	[α-la]**
AA	0.193	6.50	4.81	1.15	AA	0.126	8.99	4.66	1.51
AB	0.132	7.50	4.82	1.20	AB	0.135	8.55	4.17	1.42
BB	0.148	6.15	4.07	1.32	BB	0.098	7.67	2.95	1.49
Trial 3					Trial 4				
AA	0.139	8.00	3.91	0.99	AA	0.192	9.50	5.64	1.20
AB	0.142	8.62	4.38	1.25	AB	0.169	8.95	5.20	1.35
BB	0.147	8.06	3.21	1.11	BB	0.140	9.03	3.22	1.28

* WPNI levels are in $mg \cdot g^{-1}$ eq. ** [β-lg] and [α-la] are given in $mg \cdot mL^{-1}$.
* Niveaux de WPNI exprimés en équivalent $mg \cdot g^{-1}$. ** β-lg et α-la sont donnés en $mg \cdot mL^{-1}$.

3.2. Effect of β-lg genetic variants on whey protein denaturation during SMP manufacture and heat classification of resultant powders

The initial whey protein levels of the raw milks throughout all trials were reflected by the range of values for % NCN (AA: 0.126–0.193 %); (AB: 0.132–0.169); (BB: 0.098–0.148) and WPNI (AA: 6.50–9.50); (AB: 7.50–8.95) and (BB: 6.15–9.03) for the different phenotypes (*table II*). In three of the four trials, the β-lg AA raw skim milk had higher % NCN and WPNI values. For example, in Trial 1, the AA phenotype skim milk had % NCN and WPNI values of 0.193 % and 6.5 $mg \cdot WPN \cdot g^{-1}$ eq., respectively, while the BB phenotype milk had corresponding values of 0.148 % and 6.15 $mg \cdot WPN \cdot g^{-1}$ eq.

NCN data measured during processing indicated that whey protein denaturation was influenced greatly by β-lg genetic phenotype (*table III*), i.e. there was a significant difference in % NCN values between phenotypes in the preheated skim milks ($P < 0.002$), concentrates ($P < 0.001$) and powders ($P < 0.01$). However, the extent of the influence was dependent on the stage of lactation (*table IV*). For example, in Trial 1, β-lg AA and BB reconstituted powders had mean % NCN values of 0.176 and 0.131 %, respectively. However, there was no significant difference between the corresponding values in Trial 3 (AA = 0.090 % and BB = 0.097 %) (*table IV*). Trial 4 data (*figure 1*) illustrate that differences in % NCN between powder samples of the different phenotypes are clearly seen at the lower preheat temperatures of 75 and 85 °C than at 95 °C.

In line with % NCN, WPNI, measured at individual processing stages during SMP manufacture, was also influenced significantly by β-lg genetic phenotype, with the BB phenotype samples having lower WPNI values than the AA (preheated skim: $P < 0.004$, concentrate: $P < 0.023$ and powder: $P < 0.001$) (*table III*). While the β-lg

Table III. Whey protein denaturation – reflected by % non-casein nitrogen (NCN), whey protein nitrogen index (WPNI), β-lactoglobulin [β-lg] and α-lactalbumin [α-la] content – recorded for different β-lg phenotypes at various stages of milk powder manufacture.

Tableau III. Dénaturation des protéines du lactosérum représentée par le pourcentage d'azote non caséique (NCN), l'azote des protéines du lactosérum non dénaturées (WPNI), la teneur en β-lactoglobuline (β-lg) et en α-lactalbumine (α-la) - enregistrée pour différents phénotypes de β-lg à différentes étapes de la fabrication de poudre de lait.

Sample	AA	AB	BB	SED	Significance*
% NCN - PS	0.126[a]	0.101[b]	0.103[b]	0.005	0.002
% NCN - RC	0.142[a]	0.099[b]	0.102[b]	0.003	< 0.001
% NCN - RP	0.114[a]	0.111[a]	0.103[b]	0.003	0.01
WPNI - PS	5.36[a]	5.25[a]	4.01[b]	0.306	0.004
WPNI - RC	5.66[a]	5.51[a]	4.61[b]	0.323	0.023
WPNI - RP	5.53[a]	5.60[a]	4.39[b]	0.218	< 0.001
[β-lg] - PS	2.627[a]	2.262[b]	1.533[c]	0.180	< 0.001
[β-lg] - RC	2.331[a]	2.112[a]	1.388[b]	0.142	< 0.001
[β-lg] - RP	2.410[a]	2.363[a]	1.464[b]	0.140	< 0.001
[α-la] - PS	0.976[a]	1.026[ab]	1.089[b]	0.037	0.042
[α-la] - RC	0.853[a]	0.974[b]	0.942[b]	0.020	< 0.001
[α-la] - RP	0.998[a]	1.082[b]	1.073[b]	0.032	0.046

* Significant at $P < 0.05$. PS: preheated skim; RC: rediluted concentrate; RP: reconstituted (10 % w/v) powder. Significant differences exist between values across rows which have different superscript letters.
* Significatif à $p < 0,05$. PS = lait préchauffé, RC = concentré redilué, RP = poudre reconstituée (10 % p/v). Des différences significatives existent entre les valeurs suivies de lettres différentes.

Table IV. Whey protein denaturation (reflected by % non-casein nitrogen), recorded for β-lactoglobulin (β-lg) AA, AB and BB at various stages of skim milk powder manufacture in Trials 1–4.

Tableau IV. Dénaturation des protéines du lactosérum (représentée par le pourcentage d'azote non caséique (NCN), enregistrée pour les β-lactoglobulines (β-lg) AA, AB et BB à différentes étapes de la fabrication de poudre de lait écrémé dans les essais 1–4.

Sample	β-lg phenotype	Trial 1 (Oct. 95)	Trial 2 (May 96)	Trial 3 (Aug. 96)	Trial 4 (Oct. 96)	SED	Significance*
PS	AA	0.155[a]	na	0.102[a]	0.121[a]	0.008	0.009
	AB	0.092[b]	na	0.102[a]	0.109[ab]		
	BB	0.115[c]	na	0.093[a]	0.102[b]		
RC	AA	0.203[a]	na	0.094[a]	0.131[a]	0.005	< 0.001
	AB	0.090[b]	na	0.103[a]	0.104[b]		
	BB	0.112[c]	na	0.094[a]	0.098[b]		
RP	AA	0.176[a]	0.064[a]	0.090[a]	0.125[a]	0.006	< 0.001
	AB	0.114[b]	0.095[b]	0.102[a]	0.103[b]		
	BB	0.131[c]	0.086[b]	0.097[a]	0.098[b]		

* Significant at $P < 0.05$. PS: preheated skim; RC: rediluted concentrate; RP: reconstituted (10 % w/v) powder; na: data not available. Significant differences exist between values of variants which have different superscript letters (read vertically).
* Significatif à $p < 0,05$. PS = lait écrémé, RC = concentré redilué, RP = poudre reconstituée (10 % p/v). na = donnée non disponible. Des différences significatives existent entre les valeurs des variants suivies de lettres différentes (verticalement).

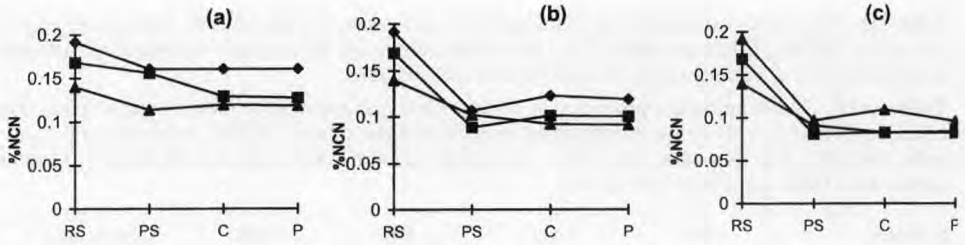

Figure 1. The effect of processing steps on % non-casein nitrogen (% NCN) during manufacture of β-lactoglobulin AA (◆), AB (■) and BB (▲) skim milk powders (Trial 4, Oct. 96), employing preheat temperatures of (a) 75 °C, (b) 85 °C and (c) 95 °C for 30 s. RS: raw skim; PS: preheated skim; C: concentrate; P = powder.

Figure 1. Effet des étapes de traitement sur le pourcentage d'azote non caséique (NCN) pendant la fabrication des β-lactoglobulines AA (◆), AB (■) et BB (▲) des poudres de lait écrémé (essai 4 oct. 96), utilisant des températures de préchauffage de (a) 75 °C, (b) 85 °C et (c) 95 °C pendant 30 s. RS = écrémé cru, PS = écrémé préchauffé, C = concentré, P = poudre.

AA SMP displayed higher WPNI values than the BB phenotype, consistent with a higher initial whey protein content, the AB phenotype was either higher or intermediate in value relative to the other two (*figure 2*). For example, at a preheat temperature of 85 °C (Trial 4), β-lg AA, AB and BB SMP had WPNI values of 5.69, 4.96 and 3.6 mg·WPN·g^{-1}, respectively. WPNI as an analytical method which measures the residual undenatured whey protein nitrogen in powder is a useful guide of the extent to which milk is preheat treated during processing. Although the technique in practice does not reflect the initial concentration of undenatured whey protein in raw milk, increasing preheat temperature is the single most influential factor that is used to increase whey protein denaturation in bulk milks during drying. Approximately two-

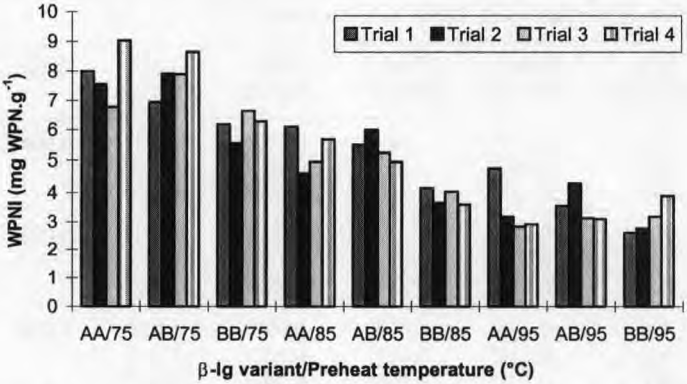

Figure 2. Effect of β-lactoglobulin (β-lg) genetic variants (AA, AB and BB) on whey protein nitrogen index (WPNI) of skim milk powder manufactured in Trials 1–4 at preheat temperatures of 75, 85 and 95 °C.

Figure 2. Effet des variants génétiques β-lg (AA, AB and BB) sur l'azote des protéines du lactosérum non dénaturées (WPNI) de poudre de lait écrémé fabriquée dans les essais 1-4 aux températures de préchauffage de 75, 85 et 95 °C.

Table V. Heat classification of β-lactoglobulin (β-lg) AA, AB and BB skim milk powder (SMP) according to the American Dry Milk Institute method [2].

Tableau V. Classification thermique des poudres de lait écrémé (SMP) contenant β-lactoglobulines (β-lg) AA, AB et BB selon la méthode de l'Institut américain du lait.

β-lg variant SMP (and manufacture date)	Medium heat (1.51–5.99 mg WPN g^{-1} SMP)			Low heat (≥ 6.0 mg·WPN·g^{-1})
	1.51–2.99	3.0–4.49	4.5–5.99	
AA (Oct. 95)			▲	●, ■
AB		▲	■	●
BB	▲	■		●
AA (May 96)		▲	■	●
AB		▲		●, ■
BB	▲	■	●	
AA (Aug. 96)	▲		■	●
AB		▲	■	●
BB		■, ▲		●
AA (Oct. 96)	▲		■	●
AB		▲	■	●
BB		■, ▲		●

Preheat temperature: ● 75 °C, ■ 85 °C, ▲ 95 °C.
Température de préchauffage : ● 75 °C, ■ 85 °C, ▲ 95 °C.

thirds of experimentally produced powders were classified as medium-heat powders *(table V)*, although a relatively high preheat temperature of 95 °C was used in the course of one treatment. None of the samples satisfied the high-heat classification criterion, i.e. WPNI ≤ 1.5 mg·WPN·g^{-1}. Virtually all powders produced at a preheat temperature of 75 °C were classified as low heat. The % decrease in WPNI during preheat treatment of the milks of all phenotypes indicates that the β-lg BB phenotype milk displayed a greater degree of whey protein denaturation than either of the AA or AB phenotype milks (*table VI*). Previous authors have found that β-lg B denatures at a faster rate than the A variant [4, 21, 47, 49].

Changes in the concentrations of individual whey protein fractions of the β-lg phenotypes provide some insights into how powder heat classification may be influenced (*table III*). Significantly higher β-lg contents were associated with the AA phenotype ($P < 0.001$). In the SMP samples, the mean β-lg content for the BB phenotype (1.46 mg·mL^{-1}) was much lower than either the AA (2.41 mg·mL^{-1}) or AB phenotypes (2.36 mg·mL^{-1}) (*table III*). A different trend was recorded for α-la content, i.e., significantly lower α-la content was associated with the AA phenotype compared to the AB and BB phenotypes for preheated skim milk ($P < 0.042$), concentrate ($P < 0.001$) and powder ($P < 0.046$).

3.3. Effect of β-lg genetic polymorphism on the sulphydryl content of SMP

It was considered worthwhile to examine the effects of β-lg phenotypes on the SH content of milk powder, given that β-lg accounts for a significant proportion of the SH content in milk. The method of Shimada and Cheftel [51] was adapted to determine total (SH_T) and slow-reacting (SH_S) SH content of SMP in order to provide some insight

Table VI. Percentage decrease (% dec.) in whey protein nitrogen index (WPNI) of skim milk during preheat treatment (at 75, 85 and 95 °C) for β-lactoglobulin (β-lg) AA, AB and BB variants in Trials 1, 3 and 4.

Tableau VI. Pourcentage de diminution l'azote des protéines du lactosérum non dénaturées dans le lait écrémé durant le préchauffage (à 75, 85 et 95 °C) pour les β-lactoglobulines (β-lg) AA, AB et BB dans les essais 1, 3 et 4.

		← % dec. in WPNI* →		
Trial	β-lg phenotype	75 °C	85 °C	95 °C
1 (Oct. 95)	AA	1.54	16.92	26.15
	AB	6.67	28.67	53.33
	BB	7.32	38.21	71.54
3 (Aug. 96)	AA	10.5	35.00	65.25
	AB	7.08	44.90	66.01
	BB	23.33	56.82	66.75
4 (Oct. 96)	AA	9.79	47.37	68.84
	AB	9.27	48.60	67.04
	BB	32.00	62.35	67.55

* % decrease = $\frac{\text{WPNI (RS)} - \text{WPNI (PS)}}{\text{WPNI (RS)}} \times 100$, where RS and PS denote raw and preheated skim, respectively.

* % décroissance = $\frac{\text{WPNI (RS)} - \text{WPNI (PS)}}{\text{WPNI (RS)}} \times 100$ avec RS = lait cru et PS = lait préchauffé.

into the thermal denaturation behaviour of whey proteins in milks originated from different β-lg phenotypes. β-lg AA was associated with higher levels of SH_T than the BB phenotype, e.g. in Trial 2 at a preheat temperature of 85 °C, the SH_T values recorded for the AA, AB and BB variant SMP were 4.76, 3.86 and 2.52 μmol $SH \cdot g^{-1}$ protein, respectively (*figure 3*, *table VII*). The AB phenotype SMP gave SH_T levels that were intermediate between the other phenotypes in two of the four trials. Overall, the occurrence of higher levels of SH_T in SMP was consistent with higher concentrations of β-lg in the first instance. An attempt at correlating SH_T (*table VII*) with undenatured β-lg (*table VII*) according to each phenotype using scatter plots (not shown) shows that a linear relationship emerges as increasing preheat treatment reduces the concentration of both constituents. The correlation is higher in the case of the AB phenotype ($r^2 = 0.86$) than either AA ($r^2 = 0.43$) or BB ($r^2 = 0.33$) although AA appeared to have a good concentration of points about the trend line, but had a lower slope (mean = 0.32) compared to the other two (AB = 0.54 and BB = 0.61).

SH_S represents those SH groups that are present at position 121 of native β-lg and its presence would be expected to reflect the residual amount of native (i.e. undenatured) β-lg remaining after processing. All β-lg AA SMPs had higher SH_S contents than the BB phenotypes, and in most cases, the AB phenotype powders were intermediate in value (*figure 3, table VII*). For example, in Trial 4 and at a preheat temperature of 75 °C, the SH_S content of the powders was 4.32, 3.38 and 2.4 μmol $SH \cdot g^{-1}$ protein for β-lg AA, AB and BB SMP, respectively. Against a background of where the skim milks of all β-lg phenotypes (AA, AB and BB) received

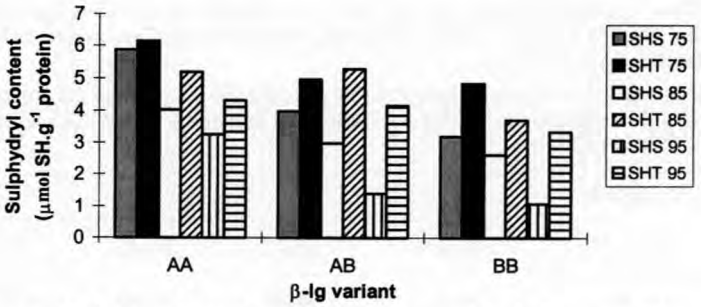

Figure 3. Effect of (β-lg) variants AA, AB and BB on sulphydryl content (slow-reacting and total) of Trial 1 SMP (preheat temperatures of 75, 85 and 95 °C).

Figure 3. Effet des variants β-lg AA, AB et BB sur la teneur en groupes *slow-reacting* sulphydryl (SH_S) de l'essai 1 SMP (températures de préchauffage 75, 85 et 95 °C).

Table VII. The effect of β-lactoglobulin (β-lg) genetic variant on slow-reacting (SH_S) and total (SH_T) sulphydryl content of skim milk powder (SMP) (preheat temperatures at 75, 85 and 95 °C).

Tableau VII. Effet des variants génétiques de β-lg sur la teneur en slow-reacting sulphydryl (SH_S) et les sulphydryl totaux (SH_T) de SMP (préchauffés à 75, 85 et 95 °C).

β-lg phenotype & SMP preheat temperature (°C)	Trial 1		Trial 2		Trial 3		Trial 4	
	SH_S	SH_T	SH_S	SH_T	SH_S	SH_T	SH_S	SH_T
AA - 75	5.87	6.15	4.71	4.97	4.17	6.46	4.32	6.00
AB - 75	3.98	4.96	3.73	4.45	3.30	4.89	3.38	5.57
BB - 75	3.19	4.83	1.73	2.83	2.65	5.11	2.40	6.14
AA - 85	4.04	5.18	2.12	4.76	2.52	5.42	2.76	5.32
AB - 85	2.98	5.29	1.86	3.86	1.82	3.86	1.62	4.08
BB - 85	2.62	3.72	1.02	2.52	0.76	4.23	0.99	6.21
AA - 95	3.27	4.32	1.36	4.08	1.14	4.82	1.43	5.27
AB - 95	1.39	4.16	1.42	3.41	0.85	3.27	1.12	3.71
BB - 95	1.08	3.34	0.77	3.11	0.59	4.07	1.12	4.52

the same heat treatment, the higher SH_S levels associated with β-lg AA SMP suggest that it is less susceptible to heat denaturation. Thresher [53] found that purified β-lg variants react with Ellman's reagent in the order A > B > C and that above 55 °C the SH group becomes exposed and available for reaction.

When expressed as a percentage of SH_T (*table VIII*), the proportion of slow-reacting sulphydryls was substantially higher in the case of the AA phenotype compared to the BB. For example, in Trial 2 (preheat temperature = 75 °C), 94.88 % of the total SH groups was slow-reacting in the case of the AA phenotype, while the value for the corresponding BB phenotype powder was much lower at 61.05 %. In both absolute and relative terms, the AA phenotype had a higher SH_S content, which indicates that this phenotype was less susceptible to heat denaturation. This observation is consistent with

Table VIII. Slow-reacting sulphydryl groups (SH_S) as a percentage of total sulphydryls (SH_T) of β-lactoglobulin (β-lg) AA, AB and BB skim milk powders (preheat temperatures at 75, 85 and 95 °C) manufactured in Trials 1–4.

Tableau VIII. Groupes *slow-reacting* sulphydryl (SH_S) en tant que pourcentage des groupes sulphydryl totaux (SH_T) des β-lactoglobulines (β-lg) AA, AB et BB de poudre de lait écrémé des essais 1-4, (préchauffés à 75, 85 et 95 °C).

β-lg phenotype	Preheat temp. (°C)	% SH_S Trial 1	Trial 2	Trial 3	Trial 4
AA	75	95.5	94.88	64.53	72.02
AB	75	80.32	83.96	67.48	60.73
BB	75	66.21	61.05	51.8	39.13
AA	85	77.96	44.64	46.42	51.84
AB	85	56.40	48.16	46.98	39.67
BB	85	70.48	40.33	18.81	15.4
AA	95	75.65	33.26	23.61	27.21
AB	95	33.53	41.7	26.03	30.15
BB	95	32.3	24.74	14.56	24.81

% SH_S was calculated as follows: $\frac{SH_S}{SH_T} \times 100$, where SH_T = total sulphydryl content.

% SH_S calculé par : $\frac{SH_S}{SH_T} \times 100$, avec SH_T = total sulphydryl total.

the percentage decrease in WPNI (*table VI*) of each phenotype when subjected to different preheat temperatures during evaporation and drying (which also suggests that the A phenotype is less susceptible to heat denaturation). Furthermore, it is in agreement with the findings of previous authors who concluded that β-lg A was less heat susceptible than the B phenotype [4, 5, 21, 47, 49].

3.4. Determination of SH content as an alternative method of heat classification of milk powders

The residual undenatured whey protein nitrogen level of reconstituted skim milks has been traditionally used to classify powders [2] into three heat treatment categories: low (> 6.0 mg·WPN·g^{-1} powder) medium 1.51–5.99 mg·WPN·g^{-1} powder) and high (< 1.5 mg·WPN·g^{-1} powder) where it is evident that the greater the heat treatment applied usually at the preheat treatment stage, the lower the residual undenatured whey protein. Some limitations have been identified with the use of the WPNI method, e.g. it has been suggested that this method has poor sensitivity and is prone to errors due to its reliance on turbidity development [34, 45, 48]. Thus, there is interest in the use of additional or complementary methods which would give better qualitative information on the state of whey protein denaturation. Many of the powders manufactured in the present study are clustered within the rather broad band that is represented by the 'medium-heat' classification category (*table V*), with the result that there is poor differentiation of the effects of the three different preheat temperature treatments in particular. Increasing preheat temperature results in a lower SH_T content of SMP (*table VII, figure 3*). For example, the SH_T content of

β-lg AA SMP prepared with preheat temperatures of 75, 85 and 95 °C were 6.15, 5.18 and 4.32 μmol·L^{-1} SH·g^{-1} protein, respectively (*table VII*). It was also found that the SH_S levels of SMP decreased when powder preheat temperature increased. Furthermore, the proportion of slow-reacting SH groups (relative to the total SH content) was decreased with increasing preheat temperature, e.g. in Trial 3, the proportion of SH_S groups (as a % of SH_T) of β-lg AA SMP were 64.53, 46.42 and 23.61 % for preheat temperatures of 75, 85 and 95 °C, respectively (*table VIII*). This trend was noted for each β-lg variant. If SH_S groups represent those SH groups present in undenatured β-lg, then it would be expected that increasing preheat temperature would result in their decrease.

A scatter-plot diagram (*figure 4*) of SH_S concentration at each WPNI value of the powders is used to explore the extent of the relationship between the two parameters. Both WPNI and SH_S values decreased with increasing preheat treatment. While approximately two-thirds of the powders manufactured in the present study were classified as medium heat powders according to the traditional WPNI method, it is evident that these powders have widely differing SH_S contents (ranging from ~ 0.5–3.5 μmol·L^{-1} SH·g^{-1} protein) (*figure 4*). At lower preheat temperatures, there was a noticeable difference in the SH_S content of powders which had very similar WPNI values. For example, two powders having similar WPNI values (~ 6.7 mg·WPN·g^{-1} SMP) had SH_S contents of 4.17 and 2.65 μmol·L^{-1} SH·g^{-1} protein, respectively. These results suggest that monitoring changes in SH groups may be beneficial when manipulating the heat classification of milk powders during processing. Low-heat SMP is usually demanded for recombination applications where it is intended to prepare pasteurised milk products with good flavour profiles. In addition, low-heat powders with minimal whey protein denaturation are desirable for the manufacture of cheeses from recombined milks with good rennet clotting and synergic properties. Medium-heat powders are frequently used to fortify the protein content of yoghurt milks, where precise control of the rheological and water-binding characteristics is afforded by the degree of whey protein denaturation. Virtually complete whey protein denaturation, arising from high preheat temperatures of skim milk, is needed in the course of attaining high-heat classification. High-heat SMP is used for bakery applications or recombined concentrated milk products where a high degree of heat stability is required. Hence, further work to explain the

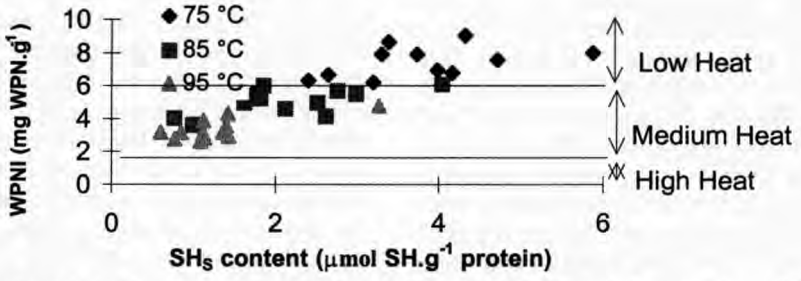

Figure 4. Association between whey protein nitrogen index (WPNI) and slow-reacting sulphydryl group (SH_S) content of skim milk powders, which were preheated to 75, 85 and 95 °C. Low, medium and high heat refer to classification categories of the WPNI method.

Figure 4. Association entre l'azote des protéines du lactosérum non dénaturées(WPNI) et la teneur en groupes slow-reacting sulphydryl (SH_S) des poudres de lait écrémé, qui sont préchauffées à 75, 85 and 95 °C. Basse, moyenne et haute température selon la classification de la méthode WPNI.

spread of SH_S values for a given WPNI figure may lead to a better understanding of structural changes that take place in whey proteins during processing. Such studies should address the role of other milk constituents such as minerals on WPNI and β-lg denaturation.

4. CONCLUSION

It was not possible to establish a relationship between the protein content of milk and β-lg phenotypes. While total protein content was higher in late lactation for all three phenotypes, a comparison of results within one trial revealed that no phenotype could be associated with a particularly high milk protein content. These results mirror the inconsistencies found in published data to date, in which widely differing trends are reported. It would appear that variations in the ratios of whey protein/casein for different phenotypes counterbalance each other, resulting in similar total protein levels. The present study shows that higher whey protein levels in milk are associated with the occurrence of the β-lg AA phenotype. FPLC analyses confirmed that this milk contains higher levels of β-lg and lower levels of α-la than the BB phenotypes.

Analyses of samples taken during manufacture of SMP revealed that the higher level of whey protein associated with β-lg AA was maintained in the undenatured form throughout the manufacturing process. Thus, if SMP is manufactured from milks containing different β-lg phenotypes under identical processing conditions, the powder containing the AA phenotype would be expected to contain higher levels of whey protein. This has potentially important implications for the heat classification of the resulting powders, as a higher level of residual undenatured whey protein content in powder will assist in achieving a lower heat classification. Furthermore, when the percentage decrease in WPNI (as a result of the preheating step) was considered, it was noted that there was a much greater loss of undenatured whey protein for the BB phenotype. This implies that β-lg B denatures at a faster rate than the A variant and will create a bias in the heat classification of powders towards the high-heat side because of the lower residual whey protein remaining after heat treatment. Therefore, β-lg genetic polymorphism may play a role in powder manufacture particularly where the residual amount and state of denaturation of whey proteins have a bearing on the functionality of end products.

The AA phenotype of β-lg was associated with a higher total SH content of SMP at all preheat temperatures. This is not unexpected since β-lg AA SMP contained higher levels of β-lg. β-lg AA SMP also contained higher absolute levels of slow-reacting SH groups, which suggests that it was less susceptible to heat denaturation. Moreover, it was found that the AA phenotype powders contained a higher percentage of slow-reacting SH groups (as a percentage of total), which also suggests greater heat resistance for this variant. These findings are in agreement with the conclusion drawn from the WPNI data.

SMP samples produced from milks exposed to higher heat treatments during processing were shown to have lower slow-reacting and total SH contents. This suggests that SH analysis could be used to distinguish between powders that have received different heat treatments. A basis for establishing a correlation between slow-reacting SH content and WPNI values was attempted (*figure 4*). As demonstrated in *table V*, WPNI classification does not always differentiate clearly between powders. Further work is required to investigate certain aspects (such as the spread of SH values for a given WPNI) before the method could be considered sufficiently reliable before putting into general use to augment existing techniques employed to determine the heat classification of milk powders.

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REFERENCES

[1] Aaltonen M.-L., Antila V., Milk renneting properties and the genetic variants of proteins, Milchwissenschaft 42 (1987) 490–492.

[2] ADMI (American Dry Milk Institute), Standards for grades of dry milk including methods of analysis, Bull. Am. Dry Milk Institute 916 (1971) 7–12.

[3] Aleandri R., Buttazzoni L.G., Schneider J.C., Caroli A., Davoli R., The effects of milk protein polymorphisms on milk components and cheese producing ability, J. Dairy Sci. 73 (1990) 241–255.

[4] Alexander S.S., Pace C.N., A comparison of the denaturation of bovine β-lactoglobulins A and B and goat β-lactoglobulin, Biochemistry 10 (1971) 2738–2743.

[5] Anema S.A., Hill J.P., Boland M.J., McKenna A.B., Kinetic and thermodynamic study of the denaturation of α-lactalbumin, β-lactoglobulin A and β-lactoglobulin B in heated reconstituted milk, in: Brief Communications of the XXIV International Dairy Congress, Melbourne, Australia, 1994, p. 181.

[6] Aschaffenberg R., Drewry J., Occurrence of different β-lactoglobulins in cows milk, Nature 176 (1955) 218–219.

[7] Beveridge T., Toma S.J., Nakai S., Determination of SH- and SS-groups in some food proteins using Ellman's reagent, J. Dairy Sci. 39 (1974) 49–51.

[8] Boland M.J., Hill J.P., Creamer L.K., Genetic manipulation of milk proteins and its consequences for the dairy industry, Australas. Biotechnol. 2 (1992) 355–360.

[9] Bewley M.C., Qin B.Y., Jameson G.B., Sawyer L., Baker E.N., Bovine β-lactoglobulin and its variants: a three-dimensional structural perspective, in: Milk Protein Polymorphism, FIL-IDF, special issue 9702, 1997, pp. 100–109.

[10] Brittan H., Mudford J.C., Norris G.E., Kitson T.M., Hill J.P., Labelling the free sulphydryl group in β-lactoglobulin A, B and C, in: Milk Protein Polymorphism, FIL-IDF, special issue 9702, 1997, pp. 200–203.

[11] Cerbulis J., Farrell Jr., H.M., Composition of milks of dairy cattle. 1. Protein, lactose, and fat contents and distribution of protein fraction, J. Dairy Sci. 58 (1975) 817–827.

[12] Dalgleish D.G., Denaturation and aggregation of serum proteins and caseins in heated milk, J. Agric. Food Chem. 38 (1990) 1995–1999.

[13] Donovan M., Mulvihill D.M., Thermal denaturation and aggregation of whey proteins, Irish J. Food Sci. Technol. 11 (1987) 87–100.

[14] Elfagm A.A., Wheelock J.V., Interaction of bovine α-lactalbumin and β-lactoglobulin during heating, J. Dairy Sci. 61 (1978) 28–32.

[15] Elfagm A.A., Wheelock J.V., Heat interaction between α-lactalbumin, β-lactoglobulin and casein in bovine milk, J. Dairy Sci. 61 (1978) 159–163.

[16] Ellman G.L., Tissue sulfhydryl groups, Arch. Biochem. Biophys. 82 (1959) 70–77.

[17] Feagan J.T., Factors affecting protein composition of milk and their significance to dairy processing, Aust. J. Dairy Technol. 34 (1979) 77–81.

[18] Feagan J.T., Bailey L.F., Hehir A.F., McLean D.M., Ellis N.J.S., Coagulation of milk proteins. I. Effect of genetic variants of milk proteins on rennet coagulation and heat stability of normal milk, Aust. J. Dairy Technol. 27 (1972) 129–134.

[19] FIL-IDF, Milk – determination of nitrogen content, Standard 20B, 1993.

[20] Fox P.F., The milk protein system, in: Fox P.F. (Ed.), Developments in Dairy Chemistry. 4. Functional Milk Proteins, Elsevier Applied Science, London, New York, 1982, pp. 1–53.

[21] Gough P., Jenness R., Heat denaturation of β-lactoglobulins A and B, J. Dairy Sci. 45 (1962) 1033–1039.

[22] Guingamp M.-F., Humbert G., Linden G., Determination of sulfhydryl groups in milk using Ellman's procedure and Clarifying Reagent®, J. Dairy Sci. 76 (1993) 2152–2155.

[23] Hill J.P., The relationship between β-lactoglobulin phenotypes and milk composition in New Zealand dairy cattle, J. Dairy Sci. 76 (1993) 281–286.

[24] Hill J.P., Boland M.J., Creamer L.K., The alteration of bulk milk composition through the selection of β-lactoglobulin phenotypes, Livest. Prod. Sci. 35 (1992) 193.

[25] Hill J.P., Boland M.J., Smith A.F., The effect of β-lactoglobulin variants on milk powder manufacture and properties, in: Milk Protein Polymorphism, FIL-IDF, special issue 9702 (1997) 372–379.

[26] Hill J.P., Thresher W.C., Boland M.J., Creamer L.K., Anema S.G., Manderson G., Otter D.E., Paterson G.R., Lowe R., Burr R.G., Motion R.L., Winkelman A., Wickham B., The polymorphism of the milk protein β-lactoglobulin. A review. Biotechnology for Dairy Production, in: Proceedings OECD Conference, Wellington, New Zealand, 7–9 February 1996, CAB International, 1997, pp. 173–202.

[27] Jakob E., Effect of genetic polymorphism on milk protein composition, in Implications of Genetic Polymorphism of Milk Proteins on Production and Processing of Milk, Bull. FIL-IDF 304 (1995) 7–8.

[28] Kalab M., Factors affecting the Ellman determination of sulfhydryl groups in skim milk powder and gels, J. Dairy Sci. 53 (1970) 711–718.

[29] Koka M., Mikolajcik E.M., Gould I.A., Spectrophotometric method for determination of heat-activated sulfhydryl groups of skim milk, J. Dairy Sci. 51 (1968) 217–219.

[30] Kristiansen K.R., Influence of genetic variants on milk composition and technological properties, in: XXIII International Dairy Congress, Montreal, Posters and Brief Communication, Vol. 1, 1990, p. 59.

[31] Kroeker E.M., Ng-Kwai-Hang K.F., Hayes J.F., Moxley J.E., Effect of β-lactoglobulin variant and environmental factors on variation in the detailed composition of bovine milk serum proteins, J. Dairy Sci. 68 (1985) 1637–1641.

[32] Lunden A., Nilsson M., Janson L., The relevance of genetic milk protein variants for yield and composition of milk, in: Implications of Genetic Polymorphism of Milk Proteins on Production and Processing of Milk, Bull FIL-IDF 304 (1995) 8–9.

[33] Lyster R.L.J., The free and masked sulphydryl groups of heated milk and milk powder and a new method for their determination, J. Dairy Res. 31 (1964) 41–51.

[34] Manji B., Kakuda Y., Determination of whey protein denaturation in heat processed milks: comparison of three methods, J. Dairy Sci. 70 (1987) 1355–1361.

[35] Marshall K., $77m R&D investment boost profitability, Food Technol. (New Zeal.) 31 (1996) 19–22.

[36] McLean D.M., Graham E.R.B., Ponzoni R.W., McKenzie H.A., Effects of milk protein genetic variants on milk yield and composition, J. Dairy Res. 51 (1984) 531–546.

[37] Melo T.S., Hansen A.P., Effect of ultra-high-temperature steam injection on model systems of α-lactalbumin and β-lactoglobulin, J. Dairy Sci. 61 (1978) 710–713.

[38] Ng-Kwai-Hang K.F., Genetic polymorphs of milk proteins; influence on milk yield and composition, in: Implications of Genetic Polymorphism of Milk Proteins on Production and Processing of Milk, Bull. FIL-IDF 304 (1995) 6–7.

[39] Ng-Kwai-Hang K.F., Grosclaude F., Genetic polymorphism of milk proteins, in: Fox P.F. (Ed.), Advanced Dairy Chemistry, Vol. 1, Proteins, Elsevier Science Publishers, London, 1992, pp. 405–455.

[40] Ng-Kwai-Hang K.F., Hayes J.F., Moxley J.E., Monardes H.G., Association of genetic variants of casein and milk serum proteins with milk, fat and protein production by dairy cattle. J. Dairy Sci. 67 (1984) 835-840.

[41] Ng-Kwai-Hang K.F., Hayes J.F., Moxley J.E., Monardes H.G., Relationships between milk protein polymorphisms and major milk constituents in Holstein-Friesian cows, J. Dairy Sci. 69 (1986) 22–26.

[42] Ng-Kwai-Hang K.F., Hayes J.F., Moxley J.E., Monardes H.G., Variation in milk protein concentrations associated with genetic polymorphism and environmental factors, J. Dairy Sci. 70 (1987) 563–570.

[43] Noh B., Richardson T., Creamer L.K., Radiolabelling study of the heat-induced interactions between α-lactalbumin, β-lactoglobulin and κ-casein in milk and in buffer solutions, J. Food Sci. 54 (1989) 889–893.

[44] O'Hara H.M., Genetic variants of milk proteins and their effect on the composition and processing properties of bovine milk, thesis, University College, Cork, Ireland, 1995.

[45] O'Sullivan A.C., O'Connor F., Aspects of heat treatment classified skim milk powder manufacture, Dairy Ind. Int. 36 (1971) 340–344.

[46] O'Sullivan A., Kelly P.M., FitzGerald R., Murphy J., Murphy M.F., Characterisation of skim milk powders prepared from milks selected according to β-lactoglobulin phenotype, in: Proceedings of the Dairy Ingredient Dehydration 2000 Symposium, 27–28 February 1997, Teagasc, DPRC-Moorepark, Fermoy Co., Cork, Ireland, 1997.

[47] Parris N., Anema S.G., Singh H., Creamer L.K., Aggregation of whey proteins in heated sweet whey, J. Agric. Food Chem. 41 (1993) 460–464.

[48] Sanderson W.B., Seasonal variations affecting the determination of the whey protein nitrogen index of skim milk powder, N.Z. J. Dairy Sci. Technol. 5 (1970) 48.

[49] Sawyer W.H., Heat denaturation of bovine β-lactoglobulin and relevance of disulphide aggregation, J. Dairy Sci. 51 (1968) 323–329.

[50] Sawyer W.H., Coulter S.T., Jenness R., Role of sulphydryl groups in the interaction of k-casein and β-lactoglobulin, J. Dairy Sci. 46 (1963) 564–565.

[51] Shimada K., Cheftel J.C., Sulfhydryl group/disulfide bond interchange reactions during heat-induced gelation of whey protein isolate. J. Agric. Food Chem. 37 (1989) 161–168.

[52] Stapelfeldt H., Bjerrum K., Skibsted L.H., Ellman's reagent for determination of the heat treatment of milk powder. Improved analytical procedure based on a stopped-flow kinetic study. Milchwissenschaft 52 (1997) 146–149.

[53] Thresher W.C., Interactions of β-lactoglobulin variants with κ-casein and DTNB: formation of a covalent complex, in: Milk Protein Polymorphism, FIL-IDF, special issue 9702 (1997) 138–145.

[54] Van den Berg G., Escher J.T.M., Bovenhuis H., de Koning P.J., Genetic variants of milk proteins. I. Composition of bulk milk and whey, in: XXIII International Dairy Congress, Montreal, Posters and Brief Communications, Vol. 1, 1990, p. 79.