Effect of Milk Protein Standardization Using Different Methods on the Composition and Yields of Cheddar Cheese

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ABSTRACT

Two sets of Cheddar cheese were made in which the milk protein level (% wt/wt) was increased from 3.3 (Control A, CA) to 3.6 (set A) or from 3.3 (control B, CB) to 4.0 (set B) by the addition of phosphocasein (PC), milk protein concentrate (MPC), or freshly prepared ultrafiltered milk retentate (UFR). The cheeses were denoted CA, PCA, MPCA, and UFRA from set A, and CB, PCB, MPCB, and UFRB, from set B, respectively. The level of cheese moisture decreased significantly on increasing milk protein level from 3.3 to 3.6 or 4.0% (wt/wt), but was not affected significantly by the method of protein standardization. The percentage milk fat recovered to cheese increased significantly on increasing the level of milk protein from 3.3 to 3.6% (wt/wt) with PC, and from 3.3 to 4.0% (wt/wt) with PC, MPC, and UFR. Increasing milk protein level from 3.3 to 4.0% (wt/wt) with PC significantly increased the percentage of milk protein recovered to cheese. Actual cheese yield increased significantly with milk protein level. The yield of cheese per 100 kg of milk normalized to reference levels of fat (3.4%, wt/wt) and casein (2.53%, wt/wt) indicated no significant effects of protein content or standardization treatment on yield. However, the moisture-adjusted yield per 100 kg of milk with reference levels of fat and casein increased significantly on increasing the protein content from 3.3 to 3.6% (wt/wt) with MPC and from 3.3 to 4.0% (wt/wt) with PC, MPC, and UFR.

Key words: milk protein standardization, phosphocasein, milk protein concentrate, Cheddar cheese

INTRODUCTION

Cheese yield and manufacturing efficiency (e.g., percentage recovery of milk fat or protein to cheese) are major determinants of the profitability accruing to cheese manufacturing plants. Consequently, these aspects of cheese and the factors affecting them have been

the subject of numerous publications and reviews (IDF, 1991; Fox et al., 2000). Perhaps the single most important factor affecting cheese yield is milk composition, in particular the concentrations of fat and protein, which together constitute ~93% of the DM of Cheddar cheese. The importance of their contribution is highlighted by the generic nature of prediction formulas relating cheese yield (Y) to levels of fat (F) and casein (C) in milk and moisture (M) in cheese: $Y = (aF + bC) / (100 - M)$, where the values of the coefficients a and b are dependent on many factors (IDF, 1991).

However, marked changes occur in the composition of milk throughout the year owing to the combined effects of differences in stage of lactation (which alters udder physiology and metabolism), plane of nutrition (especially for cows fed on pasture grass), somatic cell count, and season. These changes are most marked in countries where much of the milk supply is from spring-calving herds fed on pasture grass, as in Ireland (Mehra et al., 1999; O’Brien et al., 1999), New Zealand (Auldist et al., 1998; Nicholas et al., 2002), and parts of Australia (Auldist et al., 1996; Broome et al., 1998; Walker et al., 2004). However, seasonal changes in milk composition have also been reported for Canada (Kroeker et al., 1985), France (Martin and Coulon, 1995), and the United Kingdom (Grandison 1986; Banks and Tamime, 1987). Seasonal changes in milk composition and quality, especially those that occur in late lactation, have a major impact on curd forming properties and on the yield and composition of the resultant cheeses (O’Keeffe, 1984; Banks and Tamime, 1987; Auldist et al., 1996; O’Brien et al., 1999), and are thus conducive to inconsistencies in cheese quality (Lawrence et al., 2004). The effect of seasonal changes in milk composition and their impact on cheese making can be reduced through a combination of different approaches such as optimization of dairy husbandry practices, calving patterns, diet management, and cheese-making procedures (e.g., the standardization of protein to fat ratio, rennet to casein ratio, pH of the milk at set). However, the seasonal variation in the level of protein (or more precisely, casein) has a major influence on gelation rate and cheese making characteristics, especially in large modern dairy plants where the rennet gel tends to be

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cut on the basis of time rather than on gel firmness or gel firming rate and other steps, such as speed and duration of cut program, are fixed. In these circumstances, standardization of the levels of milk protein, or casein, to a target value across the cheese-making season would provide a very effective means of minimizing the effects of natural seasonal-related variations in milk composition on cheese composition, quality, and manufacturing efficiency. Moreover, standardization of milk protein to higher-than-normal levels offers the advantage of increasing plant output without investment of capital expenditure on extra cheese vats.

Consequently, the use of low concentration UF as a means of standardizing the level of milk protein and cheese-making characteristics of milk has been investigated extensively (IDF, 1994; Guinee et al., 1996). More recently, Broome et al. (1998) found ultrafiltration of milk to 4.0 to 4.5% (wt/wt) protein to be an effective means of reducing the high levels of moisture in nonfat substances (e.g., >56%, wt/wt), which occur in cheese from late lactation milk and have a negative impact on Cheddar quality (Lawrence et al., 2004), to values more typical of those in cheese from midlactation milk. Hence, UF milk retentate is now being used commercially; for example, in the United States (Fassbender, 2004; Mistry and Maubois, 2004), for the standardization of milk protein level in the manufacture of some rennet-curd cheeses such as Cheddar and Mozzarella. However, it is difficult to quantify the extent to which it is practiced commercially as there are few, if any, records available.

In recent years, membrane processes (UF, microfiltration) have led to the development of new ingredients, including milk protein concentrates (MPC) and phosphocasein (PC), which offer potential as means of standardizing milk protein content for cheese manufacture (Novak, 1992; Kelly et al., 2000), pending their permitted use by legislation. Feedback from the marketplace indicates that MPC (Fassbender, 2004) and secondary starter media, which have the proximate composition of MPC, are currently being used, at least in the United States, in the manufacture of nonstandard fermented milk products and cheeses. Investigation of PC and MPC under varying conditions of reconstitution (e.g., composition of diluent, ionic strength, pH, and calcium ion level) has shown that they have rennet coagulation properties very similar to those of milk at similar protein concentrations and ionic strength (Famelart et al., 1996; Kameswaran and Smith, 1999; Kelly et al., 2000). Little, if any, information is available on their performance in cheese manufacture. Shakeel-Ur-Rehman et al. (2003) found that increasing milk protein level from 2.45 in the control milk to ~5.3% (wt/wt) by the addition of MPC (63%, wt/

wt protein) reduced the numbers of nonstarter lactic acid bacteria and levels of primary and secondary proteolysis in reduced-fat Cheddar; the slower rate of maturation may be attributed to the addition of chymosin on a volume basis rather than on a casein basis. In contrast, the addition of MPC gave an increase in the moisture-adjusted cheese yield greater than that expected from the higher levels of added casein and fat in the fortified milk. Because the percentage recovery of milk fat to cheese did not differ significantly between control and MPC-fortified milk cheeses, the increased moisture-adjusted yield suggests a relatively high level of whey protein denaturation in the MPC, and hence, a high recovery of milk protein in the MPC-fortified milk cheese. Kuo and Harper (2003) reported that a model Feta-type cheese made from recombinant milk using a high protein MPC (83%, wt/wt protein) was softer and had a more open, sponge-like microstructure than cheese from recombinant milk with the same protein level but prepared using a lower protein (56%, wt/wt) MPC.

The objective of the current study was to compare the addition of UF milk retentate (UFR), PC, or MPC as means of milk protein standardization on the composition, yield, and manufacturing efficiency of Cheddar cheese. Milk protein was standardized upwards from 3.3 to 3.6 or 4.0% (wt/wt), levels that incorporate the seasonal variation in composition found in Irish manufacturing milks (O'Brien et al., 1999). The effects of the different means of milk protein standardization on age-related changes in proteolysis, texture, and cooking properties are currently being examined.

MATERIALS AND METHODS

Preparation of PC, MPC, and UFR

Raw milk (3.25%, wt/wt protein) was obtained from a local dairy in April 2002, and separated at 55°C; the resultant skim milk (2,500 kg) was cooled to 4°C and used for the preparation of PC and MPC powders on the same day.

Phosphocasein powder was prepared by dia-microfiltration of skim milk at 50°C, evaporation of the microfiltered retentate, and spray drying of the concentrate, according to the method of Kelly et al. (2000). A total volume of diafiltration water, equivalent to 140% of initial milk volume, was incorporated into the microfiltration process in 2 stages: 77% of diafiltration water was added before initial microfiltration separation, and the remaining 23% added to the resulting retentate before further microfiltration processing using a Tetra Pak Alcross, Type 2 × 19 Special, crossflow microfiltration plant fitted with a 0.1-μm pore size Membralox ceramic membrane with a filtration area of 13.3 m²

(Tetra Pak Filtration Systems, Arhus, Denmark). The resultant retentate (~6% wt/wt total solids) was evaporated to 20% (wt/wt) total solids on a falling film evaporator (Niro A/S, Copenhagen, Denmark) and spray dried on a pilot scale tall-form drier (Niro A/S); the powder was stored in polythene-lined paper sacks at room temperature. The moisture, protein, and ash contents of the PC powder were 4, 87, and 8% (wt/wt), respectively.

Milk protein concentrate was prepared by UF and diafiltration of skim milk. Following initial concentration by UF to ~15% (wt/wt) total solids, diafiltration water was continuously fed into the process until such time that lactose levels in the UF permeate were not detectable by refractometer reading. The resultant retentate was spray dried and stored as for PC. The moisture, protein, and ash contents of the MPC powder were 3.8, 84, and 8% (wt/wt), respectively.

For each cheese-making trial, UFR was prepared on the day before cheese manufacture by concentration of fresh skim milk (3.3% wt/wt protein) at 50°C to ~14% (wt/wt) protein using a Memtech (Lion Bay, Morriston, Swansea, UK) ultrafiltration plant fitted with Koch (Wilmington, MA) spiral-wound polysulfone membranes with a 10-kDa molecular weight cut-off and a filtration area of 144 m². The UFR was cooled to 10°C and used for milk standardization on the same day.

Preparation of Milks for Cheese Manufacture

Two series of experiments, A and B, were undertaken in April and May 2002, respectively, to study the effect of increasing milk protein level from 3.3 to 3.6% (wt/wt) in series A, and from 3.3 to 4.0% (wt/wt) in series B. In each experimental series, cheese-making trials were undertaken on 4 separate occasions over a 2-wk period to compare 4 treatment milks. For series A, the treatment milks were the control milk with 3.3% (wt/wt) protein (**CA**), and 3 protein-standardized (fortified) milks in which the protein content was increased from 3.3 to 3.6% (wt/wt) protein using PC (**PCA**), MPC (**MPCA**), or UFR (**UFRA**). Similarly for experimental series B, the protein was increased from 3.3 in the control (**CB**) to 4.0% (wt/wt) using PC, MPC, or UFR to give **PCB**, **MPCB**, and **UFRB** treatment milks, respectively. For all cheese-making experiments, standardized milks were prepared on d 1, stored at 10°C overnight, and pasteurized and made into cheese on d 2.

Control Milks. Raw milk was obtained from a local dairy on d 1. Part of the milk was separated at 55°C to give skim and cream (48%, wt/wt, fat). The control cheese milks (**CA**, **CB**) were prepared by adding the resultant skim milk to whole milk to get the desired protein to fat ratio of ~0.97. The protein content of the

raw milk varied from 3.2 to 3.4% (wt/wt) between replicate cheese-making trials. To ensure a similar protein content of ~3.3% (wt/wt) for the control milks (**CA** and **CB**) in all trials, part of the skim milk was ultrafiltered to 14% (wt/wt) protein, and the protein content of the control was standardized, where necessary, using either the UF retentate or permeate.

Protein-Standardized Milks. On d 1, PC or MPC powders were dispersed in freshly skimmed milk at 50°C using a high-speed Silverson mixer (model GX10, Silverson Machines Limited, Chesham, UK) at 5,000 rpm for 10 min to give concentrates with a total protein content of ~13.3% (wt/wt), with 10% (wt/wt) protein contributed by the PC or MPC. The concentrates were allowed stand for 20 min with intermittent agitation, as described above, for 1 min every 5 min. The solubility index of the concentrates was checked (IDF, 1988) and found to be <0.1 mL in all cases.

The PC-, MPC-, and UFR-standardized milks were prepared on d 1 by blending the appropriate quantities of raw milk and cream, and PC concentrate, MPC concentrate, or UFR, respectively. Standardized milks were stored in jacketed stainless steel silos overnight and maintained at 10°C. On the following day (d 2), the milks were pasteurized at 72°C × 15 s, cooled to 31°C, pumped to 4 identical cheese vats, and converted to Cheddar cheese.

Cheese Manufacture

Cheese making was performed in cylindrical, jacketed, stainless steel 500-L vats, with variable speed cutting and stirring (APV Schweiz AG, CH-3076, Worb 1, Switzerland). Pasteurized milks were inoculated with a starter culture, comprising *Lactococcus lactis* ssp. *lactis* strains 303 and 227 (Chr. Hansen Ireland Ltd., Little Island, Co. Cork, Ireland), at a weight ratio of 1:1. The starters were grown separately overnight at 23°C in 10% (wt/vol) reconstituted, low heat skim milk powder heat-treated at 90°C for 30 min. The weight (% wt/wt) of starter culture added to milk was based on milk protein level: i.e., ~1.35, 1.48, and 1.64 for milk protein levels of 3.3, 3.6, and 4% (wt/wt), respectively.

After a 30-min ripening period at 31°C, rennet (Chymax Plus, Pfizer Inc., Milwaukee, WI), diluted to 1:10 with deionized water, was added to milk at a fixed rennet to protein ratio, equivalent to 0.18 mL of undiluted rennet per liter of milk with 3.3% (wt/wt) protein or 5.5 mL of undiluted enzyme per kilogram of milk protein. All gels were cut at a similar strength of 54 Pa, and cheese making was then performed as described by Fenelon and Guinee (1999). Following overnight

Figure 1. Effect of standardization of milk protein from 3.3 (●) to 4.0% (wt/wt) using phosphocasein (○), milk protein concentrate (▲), or UF milk retentate (△) on the elastic shear modulus, G' , of the rennet-treated milk; the broken line indicates the value of G' at cut.

pressing, cheeses were vacuum-packaged and placed at 4°C.

Rennet Coagulation Properties of Cheese Milks

Two minutes after rennet addition and stirring, a sample of cheese milk was taken from the cheese vat, and placed in an insulated glass container. Within 2 min, a 13-g subsample was placed in the cell of a controlled stress rheometer (CSL²₅₀₀ Carri-Med; TA Instruments, Inc., New Castle, DE). The elastic shear modulus, G' , which was used as an index of curd firmness, was measured as a function of time at 31°C using low-amplitude strain oscillation. For each milk, cutting of the gel in the cheese vat was initiated when the firmness in the rheometer sample reached 54 Pa; depending on the treatment, this required ≤55 min (Figure 1).

Sampling and Mass Balance

The pasteurized cheese milks were weighed to the nearest 0.1 kg into 500-L cheese vats, as described by Fenelon and Guinee (1999). Representative samples of pasteurized milks were taken immediately before addition of starter culture.

Wheys were collected, filtered (through a 1-mm sieve to exclude curd particles), and weighed as described by Fenelon and Guinee (1999). The bulk whey comprised the wheys collected during whey drainage (pump out) and curd cheddaring; the white (salty) whey refers to the whey expressed from the curd during salting/mellowing and pressing. Before sampling, wheys were stirred and heated to 40°C.

The weight of the pressed cheeses was recorded and the actual yield determined (Fenelon and Guinee, 1999).

Compositional Analysis of Milks

For clarity of presentation, cheese milk refers to the pasteurized milk with added starter culture; all other milks, where mentioned, are supplied with the appropriate description (e.g., raw, standardized, or pasteurized). Samples of raw, standardized, and pasteurized milks were analyzed for fat, total N, casein, and NPN using IDF standard methods (Fenelon and Guinee, 1999). The levels of protein, fat, and casein in the cheese milk were calculated as described by Fenelon and Guinee (1999) taking account of the weight fractions of pasteurized milk and starter culture, and their respective levels of protein, fat, and casein. Casein calculated for the starter inoculum (Fenelon and Guinee, 1999) consists of true casein and denatured whey protein complexed with the casein. However, it was not possible to calculate true casein for the starter culture, as the casein content of the milk used to prepare the skim milk powder, from which the starter medium was prepared, was not available. Hence, for the purpose of this study, it was assumed that the casein content of the starter inoculum as calculated in the above formula is true casein.

The levels of undenatured whey protein and whey protein denaturation were calculated as described by Fenelon and Guinee (1999).

Compositional Analysis of Wheys

Wheys were analyzed for fat and protein using IDF standard methods, and for fines as described by Fenelon and Guinee (1999).

Analysis of Cheese

Grated cheese samples were analyzed in triplicate at 14 d for moisture, protein, fat, salt ash, Ca, and P using IDF standard methods (Fenelon and Guinee, 1999). The pH was measured on a cheese slurry prepared from 20 g of cheese and 12 g of H₂O.

Measuring Component Losses/Recoveries and Cheese Yields

The percentage of milk fat lost (%FL) in whey streams (e.g., bulk whey) was calculated using the formula:

$$\%FL = \frac{100 \times (\text{weight of bulk whey} \times \% \text{ wt / wt, fat in bulk whey})}{(\text{weight of cheese milk} \times \% \text{ wt / wt, fat in cheese milk})}$$

The percentage fat recovery to cheese (%FRC) was calculated using the formula:

$$\%FRC = \frac{100 \times (\text{weight of cheese} \times \% \text{ wt / wt, fat in cheese})}{(\text{weight of cheese milk} \times \% \text{ wt / wt, fat in cheese milk})}$$

The percentages of milk protein lost to whey and recovered to cheese were calculated using similar formulas.

Cheese yield was expressed in a number of formats, which have been described previously (Fox et al., 2000; Guinee et al., 2005):

(i) **Ya**, actual yield per 100 kg of cheese milk (kg/100 kg of cheese milk);

(ii) **Yafpam**, actual yield per 100 kg of cheese milk normalized to reference levels of fat (3.4%, wt/wt) and protein (3.3%, wt/wt) at the protein to fat ratio of the standardized milks (~0.97); Yafpam eliminates the effects of differences in milk composition to yield and, hence, allows yields of cheese from treatment milks of different composition to be compared. This yield, referred to as actual yield (Ya) per 100 kg of fat (f) and protein (p) adjusted milk (am), was calculated using the formula:

$$Yafpam = Ya \times \left(\frac{Frm + Prm}{Fcm + Pcm} \right)$$

where Fcm and Pcm correspond to the actual fat and protein contents of the cheese milk, and Frm and Prm to the percentages fat and protein in the reference cheese milk (i.e., 3.4 and 3.3%, wt/wt), respectively.

(iii) **Yma**, moisture-adjusted (to 38.5%, wt/wt) cheese yield (kg/100 kg of cheese milk); Yma eliminates the direct effect of differences in cheese moisture to yield and, hence, allows the yield of treatment cheeses with different moisture contents to be compared. It was calculated using the formula:

$$Yma = Ya \times \left(\frac{100 - Ma}{100 - Mr} \right)$$

where Ma and Mr correspond to the actual moisture and reference moisture (38.5%, wt/wt), respectively.

(iv) **Ymafpm**, moisture-adjusted cheese yield (Yma) per 100 kg of cheese milk adjusted to reference levels of fat (3.4%, wt/wt) and protein (3.3%, wt/wt) (kg/100 kg of normalized cheese milk). The use of this expression allows the direct effect of treatment on cheese yield to be determined, without the interfering effects of inter-treatment differences in milk composition and cheese moisture. It was calculated using the formula:

$$Ymafpm = Yafpam \times \left(\frac{100 - Ma}{100 - Mr} \right)$$

where Yafpam, Ma, and Mr are as described above.

(v) **Yafcam**, actual yield of cheese (Ya) per 100 kg of fat- (f) and casein- (c) adjusted milk (am); permits the direct comparison of cheese yields from treatment milks with different levels of fat and casein. It was calculated using the formula:

$$Yafcam = Ya \times \left(\frac{Frm + Crm}{Fcm + Ccm} \right)$$

where Fcm and Ccm correspond to the actual fat and casein contents of the cheese milk, and Frm and Crm to the percentages of fat and casein in the reference milk (i.e., 3.4 and 2.53% wt/wt, respectively).

(vi) **Ymafcam**, moisture-adjusted cheese yield (Yma) per 100 kg of casein- and fat-adjusted milk, was calculated from Yafcam using a formula similar to (iv) above.

Statistical Analyses

For both series of experiments (A and B), a randomized complete block design incorporating the 4 treatment milks and cheeses, namely control, and PC-, MPC-, or UFR-standardized milks, was used. There were 4 replicate trials (blocks) for each treatment. The ANOVA was carried out using a SAS procedure (SAS Institute, 1995), where the effect of treatment and replicates were estimated. Duncan's multiple-comparison test was used as a guide for pair comparisons of the treatment means.

When the relationship between Ya and milk protein level was investigated, linear regression of data from all cheeses, with intercept, was performed. The significance of the regression was determined by applying Students *t*-test to r^2 with $n - 2$ df, in which n is the actual number of data points, and df is the degrees of freedom.

RESULTS AND DISCUSSION

Milk Composition

The mean compositions of the raw milks in experimental series A and B did not differ significantly (Table 1). The values for the different compositional parameters were typical of those for commercial Irish milks in April and May (Mehra et al., 1999; O'Brien et al., 1999).

The compositions of the standardized milks are presented in Table 2. For both experimental series, increasing the milk protein content (% wt/wt) from ~3.3 to

Table 1. Compositions of raw milks used in Cheddar cheese manufacture¹

Experimental series	Fat (%, wt/wt)	Protein (%, wt/wt)	Lactose (%, wt/wt)	Casein number ²	NPN (% total N)	Whey protein (% total)
A: Increasing milk protein from 3.3 to 3.6 % (wt/wt)	3.84 ^a	3.33 ^a	4.79 ^a	78.09 ^a	5.76 ^a	16.15 ^a
B: Increasing milk protein from 3.3 to 4.0 % (wt/wt)	3.76 ^a	3.33 ^a	4.77 ^a	76.80 ^a	5.80 ^a	17.41 ^a
SED ³	0.10	0.21	0.05	1.24	0.62	0.78

^aValues in a row with a common superscript letter do not differ significantly ($P > 0.05$).

¹Presented values are means of 4 replicate milks.

²Casein number = casein expressed as percentage of total protein.

³SED = Standard error of difference.

3.6% (series A) or 4.0 (series B) resulted in a significant increase in fat content, due to the standardization of protein to fat ratio by the addition of cream, and a reduction in the level of NPN, due to the dilution effect of added protein. The addition of the protein ingredients also significantly increased the casein number of most milks. Comparison of the different methods of protein standardization showed that PCA and PCB milks had significantly higher casein number and lower whey protein, as percentage total protein, than the corresponding MPC and UFR milks. The higher casein number of the PCA and PCB milks is because the protein in the added PC is composed of casein only, compared with casein and whey protein in the MPC and UFR. The

higher casein number in the MPCA, UFRA, and UFRB milks, relative to the control CA and CB milks suggests some denaturation of whey proteins and their complexing with the casein micelles (Anema and Li, 2003) during the preparation of the MPC and UFR. However, the absence of significant differences in the whey protein levels (as % total protein) between the C, MPC, and UFR milks indicates that the levels of whey protein denaturation were small.

The casein number increased in all milks during pasteurization (Table 3), an effect that reflects heat-induced denaturation of whey proteins and their complexing with the casein micelles. The degree of whey protein denaturation varied from ~1.3 to 5.9%; it did not differ

Table 2. Effect of increasing milk protein from 3.3 to 3.6 or 4.0% (wt/wt) using different standardization treatments on the composition of standardized cheese milks prepasteurization¹

	Treatment ² and milk protein level (%, wt/wt); series A				
	CA, 3.3	PCA, 3.6	MPCA, 3.6	UFRA, 3.6	SED ³
Fat (%, wt/wt)	3.44 ^b	3.81 ^a	3.80 ^a	3.83 ^a	0.032
Protein (%, wt/wt)	3.31 ^b	3.62 ^a	3.63 ^a	3.61 ^a	0.010
Lactose (%, wt/wt)	4.78 ^a	4.79 ^a	4.78 ^a	4.75 ^a	0.025
Casein number ⁴	77.99 ^c	79.53 ^a	78.44 ^b	78.57 ^b	0.172
NPN (% total N)	5.81 ^a	5.33 ^b	5.33 ^b	5.26 ^b	0.055
Whey protein (% of total protein)	16.20 ^a	15.15 ^b	16.23 ^a	16.17 ^a	0.179
	Treatment ² and milk protein level (%, wt/wt); series B				
	CB, 3.3	PCB, 4.0	MPCB, 4.0	UFRB, 4.0	SED
Fat (%, wt/wt)	3.42 ^b	4.12 ^a	4.18 ^a	4.16 ^a	0.014
Protein (%, wt/wt)	3.30 ^b	3.98 ^a	4.01 ^a	3.99 ^a	0.009
Lactose (%, wt/wt)	4.77 ^a	4.78 ^a	3.99 ^a	4.72 ^b	0.018
Casein number ⁴	76.75 ^c	78.92 ^a	77.05 ^{cb}	77.81 ^b	0.040
NPN (% total N)	6.00 ^a	5.08 ^b	5.06 ^b	4.86 ^b	0.122
Whey protein (% of total protein)	17.25 ^a	16.00 ^b	17.90 ^a	17.33 ^a	0.322

^{a,b,c}Values within a row not sharing a common superscript differ significantly at $P < 0.05$.

¹Presented values for both A and B treatments are means of 4 replicate milks.

²Milk protein was increased from 3.3 (CA) to 3.6% (wt/wt) in experimental series A by the addition of phosphocasein (PCA), milk protein concentrate (MPCA), or UF milk retentate (UFRA), and from 3.3 (CB) to 4.0% (wt/wt) in experimental series B by the addition of phosphocasein (PCB), milk protein concentrate (MPCB), or UF retentate (UFRB).

³SED = Standard error of difference.

⁴Casein number = casein expressed as percentage of total protein.

Table 3. Effect of increasing milk protein from 3.3 to 3.6 or 4.0% (wt/wt) using different standardization treatments on the composition of pasteurized cheese milks¹

	Treatment ² and milk protein level (% wt/wt); series A				SED ³
	CA, 3.3	PCA, 3.6	MPCA, 3.6	UFRA, 3.6	
Fat (% wt/wt)	3.42 ^b	3.79 ^a	3.82 ^a	3.83 ^a	0.028
Protein (% wt/wt)	3.30 ^b	3.62 ^a	3.61 ^a	3.59 ^a	0.014
Lactose (% wt/wt)	4.77 ^a	4.78 ^a	4.76 ^a	4.72 ^a	0.027
Casein number ⁴	78.63 ^c	80.02 ^a	79.01 ^{bc}	79.55 ^{ab}	0.244
NPN (% total N)	5.62 ^a	5.19 ^b	5.25 ^b	5.26 ^b	0.091
Whey protein (% of total protein)	15.76 ^a	14.78 ^b	15.74 ^a	15.19 ^b	0.210
Denatured whey protein (% of total whey protein)	2.67 ^a	2.36 ^a	3.54 ^a	5.90 ^a	1.66
	Treatment ² and milk protein level (% wt/wt); series B				SED
	CB, 3.3	PCB, 4.0	MPCB, 4.0	UFRB, 4.0	
Fat (% wt/wt)	3.36 ^b	4.14 ^a	4.18 ^a	4.18 ^a	0.038
Protein (% wt/wt)	3.29 ^b	3.97 ^a	3.97 ^a	3.97 ^a	0.009
Lactose (% wt/wt)	4.76 ^a	4.77 ^a	4.74 ^a	4.70 ^a	0.013
Casein number ⁴	77.66 ^c	79.41 ^a	77.99 ^b	78.18 ^b	0.301
NPN (% total N)	5.99 ^a	5.02 ^b	5.02 ^b	4.92 ^b	0.082
Whey protein (% of total protein)	16.43 ^a	15.57 ^b	16.99 ^a	16.91 ^a	0.294
Denatured whey protein (% of total whey protein)	1.32 ^b	3.89 ^{ab}	5.17 ^a	2.45 ^{ab}	1.46

^{a,b,c}Values within a row not sharing a common superscript differ significantly at $P < 0.05$.

¹Presented values for both A and B treatments are means of 4 replicate milks.

²Milk protein was increased from 3.3 (CA) to 3.6% (wt/wt) in experimental series A by the addition of phosphocasein (PCA), milk protein concentrate (MPCA), or UF milk retentate (UFRA), and from 3.3 (CB) to 4.0% (wt/wt) in experimental series B by the addition of phosphocasein (PCB), milk protein concentrate (MPCB), or UF retentate (UFRB).

³SED = Standard error of difference.

⁴Casein number = casein expressed as percentage of total protein.

significantly with treatment in the A milks but did in the B milks, with the value of MPCB being significantly higher than that of CB.

The estimated compositions of the cheese milks are shown in Table 4. These are similar to those of the pasteurized apart from the slightly lower level of fat, because of the addition of starter culture, which was made from reconstituted low heat skim milk powder.

Rennet Coagulation and Cheese-Making Characteristics

In agreement with previous results (Guinee et al., 1997), increasing the milk protein level enhanced its rennet coagulability (Figure 1), as reflected by reductions in gelation time (i.e., time at which G' begins to increase), increases in curd-firming rate (slope of the G' /time curve in the linear region), and curd firmness (value of G' at any time after the onset of gelation).

Consistent with the changes in rennet coagulability, increasing the protein content resulted in a significant reduction in set to cut time (time to reach 54 Pa) during cheese making; that is, ~12 and 23 min when the protein was standardized to 3.6 and 4.0%, respectively (Table 5). For both A and B milks, the method of standardizing milk protein content did not affect the set to cut time significantly, even though that for PCA and PCB milks

was numerically lower than the other A and B milks. Despite the relatively large differences in set to cut time between the control and protein-standardized milks, differences in step times (e.g., starter to drain time) diminished with progression of cheese making, and there were no significant differences between treatments in the time from starter addition to curd milling at pH 5.25. Such a trend was expected because starter culture was added to the cheese milk based on protein content rather than on volume and all milks were set at a similar pH (6.55).

Cheese Composition

The values for moisture, fat, protein, moisture in non-fat substances (MNFS), salt, and pH for all cheeses (Table 6) were typical of those found in commercial Cheddar cheeses and within the range prescribed for good quality Cheddar (Lawrence et al., 2004).

In agreement with previous studies (Bush et al., 1983; Guinee et al., 1994; Broome et al., 1998), increasing milk protein from 3.3 to 3.6 or 4.0% (wt/wt) resulted in significant decreases in the levels of moisture and MNFS, and a significant increase in fat content. Although the method of standardizing milk protein level did not significantly affect the moisture content, the lowest moisture and MNFS contents were observed in

Table 4. Effect of increasing milk protein from 3.3 to 3.6 or 4.0% (wt/wt) using different standardization treatments on the estimated composition of cheese milks with added starter culture¹

	Treatment ² and milk protein level (% wt/wt); series A				SED ³
	CA, 3.3	PCA, 3.6	MPCA, 3.6	UFRA, 3.6	
Fat (% wt/wt)	3.38 ^b	3.74 ^a	3.77 ^a	3.78 ^a	0.028
Protein (% wt/wt)	3.30 ^b	3.63 ^a	3.59 ^a	3.62 ^a	0.038
Casein (% wt/wt)	2.58 ^c	2.90 ^a	2.82 ^b	2.85 ^{ab}	0.028
Protein to fat ratio	0.97 ^a	0.97 ^a	0.96 ^a	0.96 ^a	0.007

	Treatment ² and milk protein level (% wt/wt); series B				SED
	CB, 3.3	PCB, 4.0	MPCB, 4.0	UFRB, 4.0	
Fat (% wt/wt)	3.31 ^b	4.07 ^a	4.12 ^a	4.11 ^a	0.014
Protein (% wt/wt)	3.29 ^c	3.98 ^a	3.99 ^a	3.97 ^a	0.009
Casein (% wt/wt)	2.53 ^b	3.15 ^a	3.08 ^b	3.08 ^b	0.018
Protein to fat ratio	0.98 ^a	0.98 ^a	0.97 ^{ab}	0.96 ^b	0.006

^{a,b,c}Values within a row not sharing a common superscript differ significantly at $P < 0.05$.

¹Presented values for both A and B treatments are means of 4 replicate milks. Compositions were estimated by consideration of weights of pasteurized standardized cheese milk and starter culture, using equation in text.

²Milk protein was increased from 3.3 (CA) to 3.6% (wt/wt) in experimental series A by the addition of phosphocasein (PCA), milk protein concentrate (MPCA), or UF milk retentate (UFRA), and from 3.3 (CB) to 4.0% (wt/wt) in experimental series B by the addition of phosphocasein (PCB), milk protein concentrate (MPCB), or UF retentate (UFRB).

³SED = Standard error of difference.

the UFRA and UFRB cheeses. The reduction in moisture level on using the UFR standardized milks (~0.36 and 0.29%, wt/wt, per 0.1%, wt/wt, increase in milk protein in the A and B milks, respectively) is similar in magnitude to that (0.29%, wt/wt, per 0.1%, wt/wt, increase in milk protein) reported previously for Cheddar cheese on increasing milk over the same range using UF (Guinee et al., 1996). A tentative explanation for inverse relationship between milk protein level and

cheese moisture may be the concomitant increase in the ratio of casein to soluble salts. This would favor a more rapid aggregation of the *para*-casein micelles and a coarser gel network (Auty et al., 2005), which would be more porous and prone to syneresis on cutting and stirring (Green et al., 1981). The increases in the number and volume fraction of curd particles in the curd/whey mixture, and consequently in the number of collisions between curd particles and between curd particles

Table 5. Effect of increasing milk protein from 3.3 to 3.6 or 4.0% (wt/wt) using different standardization treatments on Cheddar cheese making characteristics¹

Cheese making characteristic	Treatment ² and milk protein level (% wt/wt); series A				SED ³
	CA, 3.3	PCA, 3.6	MPCA, 3.6	UFRA, 3.6	
Set-to-cut time at 54 Pa ⁴ (min)	51.5 ^a	38.1 ^b	38.4 ^b	40.6 ^b	1.4
Starter-to-drain time (min)	195 ^a	195 ^a	192 ^a	196 ^a	2.6
Starter-to-curd milling time (min)	290 ^a	298 ^a	292 ^a	297 ^a	5.7

	Treatment ² and milk protein level (% wt/wt); series B				SED
	CB, 3.3	PCB, 4.0	MPCB, 4.0	UFRB, 4.0	
Set-to-cut time at 54 Pa ⁴ (min)	56.1 ^a	31.1 ^b	32.5 ^b	34.2 ^b	1.8
Starter-to-drain time (min)	199 ^a	193 ^b	187 ^c	193 ^b	1.8
Starter-to-curd milling time (min)	297 ^a	304 ^a	308 ^a	304 ^a	9.8

^{a,b,c}Values within a row not sharing a common superscript differ significantly at $P < 0.05$.

¹Presented values for both A and B treatments are means of 4 replicate cheese trials.

²Milk protein was increased from 3.3 (CA) to 3.6% (wt/wt) in experimental series A by the addition of phosphocasein (PCA), milk protein concentrate (MPCA), or UF milk retentate (UFRA), and from 3.3 (CB) to 4.0% (wt/wt) in experimental series B by the addition of phosphocasein (PCB), milk protein concentrate (MPCB), or UF retentate (UFRB).

³SED = Standard error of difference.

⁴As determined using low-amplitude strain oscillation rheometry.

Table 6. Effect of increasing milk protein from 3.3 to 3.6 or 4.0% (wt/wt) using different standardization treatments on the composition of Cheddar cheese at 14 d¹

Cheese composition	Treatment ² and milk protein level (% wt/wt); series A				SED ³
	CA, 3.3	PCA, 3.6	MPCA, 3.6	UFRA, 3.6	
Moisture (% wt/wt)	37.16 ^a	36.39 ^b	36.31 ^b	36.05 ^b	0.26
Fat (% wt/wt)	31.37 ^b	31.9 ^a	32.24 ^a	32.41 ^a	0.24
Protein (% wt/wt)	26.0 ^a	26.06 ^a	25.96 ^a	26.15 ^a	0.22
MNFS ⁴ (% wt/wt)	54.14 ^a	53.44 ^b	53.58 ^{ab}	53.35 ^b	0.27
NaCl (% wt/wt)	1.68 ^a	1.75 ^a	1.80 ^a	1.74 ^a	0.27
Ash (% wt/wt)	3.85 ^a	3.95 ^a	3.97 ^a	3.90 ^a	0.07
Ca (mg/g of protein)	29.3 ^a	29.7 ^a	29.3 ^a	29.4 ^a	0.53
P (mg/g of protein)	20.2 ^a	20.6 ^a	20.5 ^a	20.2 ^a	0.34
pH	5.19 ^a	5.21 ^a	5.22 ^a	5.22 ^a	0.03

Cheese composition	Treatment ² and milk protein level (% wt/wt); series B				SED
	CB, 3.3	PCB, 4.0	MPCB, 4.0	UFRB, 4.0	
Moisture (% wt/wt)	37.52 ^a	36.27 ^b	36.15 ^b	35.82 ^b	0.41
Fat (% wt/wt)	31.48 ^c	31.80 ^{bc}	32.12 ^{ab}	32.36 ^a	0.17
Protein (% wt/wt)	26.37 ^a	26.45 ^a	26.12 ^a	26.13 ^a	0.18
MNFS ⁴ (% wt/wt)	54.08 ^a	53.18 ^b	53.26 ^b	52.96 ^b	0.24
NaCl (% wt/wt)	1.69 ^a	1.75 ^a	1.83 ^a	1.82 ^a	0.23
Ash (% wt/wt)	3.88 ^b	3.94 ^{ab}	4.00 ^{ab}	4.06 ^a	0.08
Ca (mg/g of protein)	28.93 ^b	29.47 ^a	29.48 ^a	30.20 ^a	0.25
P (mg/g of protein)	20.95 ^a	20.97 ^a	21.35 ^a	21.66 ^a	0.41
pH	5.07 ^b	5.13 ^{ab}	5.17 ^a	5.19 ^a	0.03

^{a,b,c}Values within a row not sharing a common superscript differ significantly at $P < 0.05$.

¹Presented values for both A and B treatments are means of 4 replicate cheese trials.

²Milk protein was increased from 3.3 (CA) to 3.6% (wt/wt) in experimental series A by the addition of phosphocasein (PCA), milk protein concentrate (MPCA), or UF milk retentate (UFRA), and from 3.3 (CB) to 4.0% (wt/wt) in experimental series B by the addition of phosphocasein (PCB), milk protein concentrate (MPCB), or UF retentate (UFRB).

³SED = Standard error of difference.

⁴MNFS = Moisture in nonfat substance.

and the vat knives and walls, may also contribute to the reduction in moisture content as milk protein was increased. Such collisions are expected to increase the number/magnitude of the pressure gradients across the surfaces of the curd particles and thereby provide greater opportunity for local deformation of the matrix within each curd particle, rearrangement of the protein matrix into a more compact structure (Dejmek and Walstra, 2004), and hence, more syneresis, especially during cutting and the early stages of stirring. At first approximation, the above results appear to be at variance with those reporting that syneresis of rennet-induced milk gels, as measured using laboratory-scale methods, decreases markedly as milk is concentrated by UF (Dejmek and Walstra, 2004). However, these methods measure the volume of whey expressed only and not the moisture of the residual curd; naturally, the level of whey expressed from a rennet gel prepared from UF milk decreases relative to a control because much of the whey is removed from the former as UF permeate before measuring syneresis.

In contrast to moisture, the level of fat in cheese increased significantly as the milk protein was in-

creased for both the A and B cheeses (Table 6). The inverse relationship between fat and moisture levels in the cheese, which was shown by linear regression to be statistically significant for the A and B cheeses ($r = -0.80$, $df = 14$), is consistent with results of previous studies (Fenelon and Guinee, 1999). Fat globules may act as "stoppers" occluded within the *para*-casein matrix, which reduce the loss of whey exuding from the pores of the matrix. Consequently, the decrease in the percentage MNFS, which reflects the moisture associated with the protein matrix, was lower in magnitude than the percentage reduction in moisture on increasing the milk protein level.

The method of increasing milk protein content generally did not significantly affect composition of cheeses from the protein-standardized A or B milks, an exception being the significantly higher levels of ash and calcium in the UFRB cheese compared with the PCB and MPCB cheeses.

Whey Composition

Bulk Whey. The mean compositions of the CA and CB bulk wheys (Table 7) are similar to those reported

Table 7. Effect of increasing milk protein from 3.3 to 3.6 or 4.0% (wt/wt) using different standardization treatments on the composition of, and losses of fat and protein in, the bulk cheese whey¹

	Treatment ² and milk protein level (% wt/wt); series A				SED ³
	CA, 3.3	PCA, 3.6	MPCA, 3.6	UFRA, 3.6	
Weight (kg/100 kg of cheese milk)	90.0 ^a	88.8 ^b	88.8 ^b	89.0 ^b	0.10
Fat (% wt/wt)	0.35 ^a	0.32 ^a	0.34 ^a	0.33 ^a	0.009
Protein (% wt/wt)	0.87 ^c	0.91 ^b	0.95 ^a	0.94 ^a	0.001
Curd fines (mg/kg)	159 ^b	273 ^a	225 ^{ab}	210 ^{ab}	43
Fat (% of total milk fat)	9.33 ^a	7.58 ^b	8.06 ^{ab}	7.80 ^{ab}	0.701
Protein (% of milk protein)	23.7 ^a	22.33 ^c	23.46 ^{ab}	23.2 ^b	0.201
	Treatment ² and milk protein level (% wt/wt); series B				SED
	CB, 3.3	PCB, 4.0	MPCB, 4.0	UFRB, 4.0	
Weight (kg/100 kg of cheese milk)	90.1 ^a	88.3 ^b	88.2 ^b	88.2 ^b	0.23
Fat (% wt/wt)	0.36 ^a	0.35 ^a	0.38 ^a	0.38 ^a	0.01
Protein (% wt/wt)	0.86 ^c	0.95 ^b	1.02 ^a	1.03 ^a	0.008
Curd fines (mg/kg)	176 ^b	254 ^a	235 ^{ab}	267 ^a	27.6
Fat (% of total milk fat)	9.61 ^a	7.64 ^c	8.02 ^{cb}	8.17 ^b	0.204
Protein (% of milk protein)	23.7 ^a	21.2 ^c	22.6 ^b	22.9 ^b	0.231

^{a,b,c}Values within a row not sharing a common superscript differ significantly at $P < 0.05$.

¹Presented values for both A and B treatments are means of 4 replicate cheese trials.

²Milk protein was increased from 3.3 (CA) to 3.6% (wt/wt) in experimental series A by the addition of phosphocasein (PCA), milk protein concentrate (MPCA), or UF milk retentate (UFRA), and from 3.3 (CB) to 4.0% (wt/wt) in experimental series B by the addition of phosphocasein (PCB), milk protein concentrate (MPCB), or UF retentate (UFRB).

³SED = Standard error of difference.

previously for milk of a similar composition (Fenelon and Guinee, 1999) but somewhat higher (~10 to 15%) than those of bulk wheys from Irish dairy plants (Guinee et al., 2005).

The weight of bulk whey decreased significantly as the protein level was increased from 3.3 to 3.6 or 4.0% (wt/wt) but was not affected by the means of milk protein standardization. The weight of white whey, which accounted for only ~1.5% of total whey weight, was not affected significantly by milk protein level.

In agreement with previous results (Guinee et al., 1994), increasing the milk protein content from 3.3 to 3.6 or 4.0% (wt/wt) did not significantly influence the fat level in the bulk cheese whey. Such a trend was expected because of the constancy of the protein to fat ratio, which avoids dilution, or concentration, of the casein matrix relative to fat, and, hence, its ability to occlude or to compress/deform embedded fat globules. Moreover, cutting the gels of all treatment milks at similar firmness values, together with the short cut cycle (170 s), reduces the potential for differences in curd particle shattering during cutting and the early stages of stirring. Excessive firmness at cutting, which could occur if gels from high-protein milks were cut at times similar to those of control milks, leads to tearing of the curd and high fat losses (Guinee et al., 1994).

The percentage of total milk fat lost in the bulk whey decreased significantly on increasing the level of protein milk from 3.3 to 3.6% (wt/wt) with PC and from 3.3 to

4.0% (wt/wt) with PC, MPC, or UFR. The above trends were expected because of the constancy of actual fat levels (% wt/wt) in the bulk whey and the significant decrease in volume of bulk whey as the level of milk protein increased (Table 7). The reduction in the percentage of milk fat lost in the bulk whey of the protein-standardized B milks relative to the control CB milk was highest with PC (e.g., ~2.0%) and similar for MPC and UFR (~1.5%). The low loss of fat from the PC-standardized milk gels may be due to their slightly higher casein to fat ratio at cutting and during the early stages of cutting, when the rate of syneresis of (Dejmek and Walstra, 2004), and presumably the loss of fat globules from, the freshly-cut surfaces of curd particles is highest.

The protein level (% wt/wt) of the bulk whey increased significantly as the milk protein content was increased from 3.3 to 3.6 or 4.0% (wt/wt). This trend is consistent with the concomitant increases in the levels of native whey proteins (for the MPC and UFR milks) and glycomacropeptide (which accounts for 4 to 5% of total casein), both of which are lost in the cheese whey. The level of protein in PC wheys was significantly lower than that in the corresponding MPC and UFR wheys, a trend reflecting the relatively lower ratio of whey protein to casein in the PC milks (Table 3). In contrast to the above, the percentage of total milk protein lost in the bulk whey decreased significantly on increasing the milk protein from 3.3 to 3.6% (wt/wt) using PC and

Table 8. Effect of increasing milk protein from 3.3 to 3.6 or 4.0% (wt/wt) using different standardization treatments on the composition of, and losses of fat and protein in, the white cheese whey¹

	Treatment ² and milk protein level (% wt/wt); series A				SED ³
	CA, 3.3	PCA, 3.6	MPCA, 3.6	UFRA, 3.6	
Weight (kg/100 kg of cheese milk)	1.29 ^a	1.32 ^a	1.36 ^a	1.33 ^a	0.05
Fat (% wt/wt)	3.08 ^a	2.91 ^a	2.90 ^a	2.88 ^a	0.206
Protein (% wt/wt)	1.02 ^a	1.03 ^a	1.06 ^a	1.04 ^a	0.018
Curd fines (mg/kg)	1,229 ^a	1,144 ^a	1,291 ^a	1,173 ^a	117
Fat (% of total milk fat)	1.14 ^a	1.05 ^a	1.04 ^a	1.01 ^a	0.061
Protein (% of milk protein)	0.40 ^a	0.40 ^a	0.38 ^a	0.38 ^a	0.020
	Treatment ² and milk protein level (% wt/wt); series B				SED
	CB, 3.3	PCB, 4.0	MPCB, 4.0	UFRB, 4.0	
Weight (kg/100 kg of cheese milk)	1.30 ^a	1.32 ^a	1.36 ^a	1.33 ^a	0.12
Fat (% wt/wt)	3.09 ^a	3.32 ^a	3.36 ^a	2.98 ^a	0.258
Protein (% wt/wt)	1.06 ^a	1.11 ^a	1.12 ^a	1.13 ^a	0.048
Curd fines (mg/kg)	1,156 ^{ab}	1,083 ^{ab}	900 ^b	1,412 ^a	152
Fat (% of total milk fat)	1.24 ^a	1.03 ^a	1.07 ^a	1.06 ^a	0.111
Protein (% of milk protein)	0.40 ^a	0.35 ^a	0.39 ^a	0.39 ^a	0.036

^{a,b}Values within a row not sharing a common superscript differ significantly at $P < 0.05$.

¹Presented values for both A and B treatments are means of 4 replicate cheese trials.

²Milk protein was increased from 3.3 (CA) to 3.6% (wt/wt) in experimental series A by the addition of phosphocasein (PCA), milk protein concentrate (MPCA), or UF milk retentate (UFRA), and from 3.3 (CB) to 4.0% (wt/wt) in experimental series B by the addition of phosphocasein (PCB), milk protein concentrate (MPCB), or UF retentate (UFRB).

³SED = Standard error of difference.

from 3.3 to 4.0% (wt/wt) with PC, MPC, or UFR. This decrease, which was expected for the PCA and PCB wheys because of the relatively low whey protein to casein ratio in the PC-standardized milks, suggests that some of the whey proteins in the MPC and UFR milks were denatured and recovered with the curd during cheese manufacture. Thus, the casein number of the MPCA, MPCB, UFRA-, and UFRB milks were higher than those of the corresponding CA and CB milks (Table 3).

White Whey. The weights and compositions of white wheys from the CA and CB cheeses (Table 8) are similar to those reported previously (Fenelon and Guinee, 1999) for Cheddar cheese making under similar conditions. The composition and percentage losses of fat and protein in white whey were not affected significantly by increasing protein content or by the method of milk protein standardization.

Recoveries of Milk Fat and Protein

The percentage of total milk fat recovered to cheese increased significantly when the milk protein level was increased from 3.3 to 3.6% (wt/wt) using PC and from 3.3 to 4.0% (wt/wt) with PC, MPC, or UFR (Figure 2a; Table 9). This trend is consistent with the reduction in percentage of total milk fat lost in the bulk whey, which contributed ~89% of the total fat lost to cheese whey (Table 7).

The percentage of milk protein recovered to cheese increased significantly as the milk protein was standardized from 3.3 to 4% (wt/wt) using PC (Figure 2b). Otherwise, protein recovery to cheese was not significantly influenced by milk protein level or the method of milk protein standardization (Table 9). This result concurred with the opposite effect noted for the percentage of milk protein lost in the bulk whey.

Cheese Yields

The yields of cheeses are given in Table 9. In an attempt to assign potential differences in cheese yield to the direct effect of treatment per se rather than to intertreatment differences associated with milk composition (levels of fat, protein, or casein) or cheese composition (moisture), cheese yield was expressed in a number of formats as defined earlier and discussed separately below.

Ya and Yafpam. Actual yield, Ya, increased significantly as milk protein increased from 3.3 to 3.6 or 4.0% (wt/wt), due to pro rata increases in the content of added protein and milk fat. Linear regression analysis indicated a significant ($P < 0.01$) linear relationship ($r = 0.99$, $df = 30$) between milk protein level and Ya, with the latter increasing by ~0.32 kg per 100 kg of milk for every 0.1% (wt/wt) increase in milk protein (Figure 3); the rate of increase concurs with that reported for skim milk cheese (0.33 kg per 0.1% wt/wt milk protein; Gilles

Figure 2. Effect of standardizing milk protein from 3.3 (control, C) to 3.6 (white bars) or 4.0 (gray bars) %, wt/wt, with phosphocasein (PC), milk protein concentrate (MPC), or UF milk retentate (UFR) on the recoveries of milk fat (a) and protein (b) to cheese. Presented values are the means from 4 replicate cheese trials; error bars show standard deviations of the means.

and Lawrence, 1985) and for full-fat Cheddar cheese from milk standardized to a protein to fat ratio of 0.97 (0.33 kg per 0.1% wt/wt milk protein; Fox et al., 2000).

The normalized yield, Yafpam, per 100 kg of milk with reference levels of fat (3.4, % wt/wt) and protein (3.3%, wt/wt) increased significantly on increasing milk protein from 3.3 to 4% (wt/wt) with PC (Table 9), with the mean increase over the control cheese (CB) amounting to ~0.18 kg of cheese/100 kg of milk (Figure 4a). The higher Yafpam for PCB is in agreement with its significantly higher recoveries of milk fat and protein to cheese. Although the percentage fat recovery for the

PCA, MPCA, UFRA, MPCB, and UFRB cheeses was also significantly higher than those the CA or CB cheeses, the corresponding protein recovery values were not.

Yma and Ymafpam. In the current study, the moisture content of the cheeses decreased significantly on standardization of milk protein level to 3.6 or 4.0% (wt/wt). No attempts were made to intervene in the cheese-making process to offset the moisture decrease, as the objective of the study was to consider the effect of one treatment variable—method of protein standardization—at 2 different milk protein levels. However, process intervention would, of course, be very feasible at the commercial level to counteract the moisture reduction and, thereby, enhance the yield cheeses from the protein-standardized milks. The moisture content of cheeses from the latter milks could be increased by a number of process alterations, such as increasing milk pasteurization temperature, gel firmness at cutting, curd particle size, cooking rate of the curd/whey mixture or pH at curd milling, and reducing temperature of maximum scald (Mayes and Sutherland, 1989; IDF, 1991, 1994; Fox et al., 2000; Dejmeck and Walstra, 2004; Guinee et al., 2005).

The moisture-adjusted yield, Yma, normalizes cheese moisture to a reference level, and thereby facilitates the comparison of the yields of treatment cheeses with different moisture contents. The Yma increased significantly on increasing the milk protein from 3.3 to 3.6 or 4.0% (wt/wt) with all standardization methods, because of reasons similar to that discussed above for Ya. However, the differences between Yma for the control cheeses (CA or CB) and the corresponding cheeses from the protein-standardized milks (PCA, MPCA, and UFRA, or PCB, MPCB, and UFRB) were larger than those for Ya (Table 9), because of the inverse relationship between the levels of milk protein and cheese moisture (Table 6).

The normalized moisture-adjusted (38.5% wt/wt) yield, Ymafpam, per 100 kg of fat- (f) and protein- (p) adjusted milk (am), increased significantly as milk protein level was increased from 3.3 to 3.6% (wt/wt) with PC and MPC, and from 3.3 to 4.0% (wt/wt) with PC, MPC, and UFR. This contrasts with the Yafpam, which increased significantly only when milk protein was standardized from 3.3 to 4.0% (wt/wt) using PC. However, as for Yafpam, the numerically highest values of Ymafpam at milk protein levels of 3.6 and 4.0% (wt/wt) were obtained with PC (Figure 4a), a trend that is consistent with its highest recoveries of milk fat and protein to cheese (Figure 3).

Yafcam and Ymafcam. The normalized yield per 100 kg of milk with reference levels of fat (3.4% wt/wt) and casein (2.53%, wt/wt), Yafcam, was not significantly

Table 9. Effect of increasing milk protein from 3.3 to 3.6 or 4.0% (wt/wt) using different standardization treatments on the recoveries of milk fat and protein and on the yields of Cheddar cheese¹

	Treatment ² and milk protein level (% wt/wt); series A				SED ³
	CA, 3.3	PCA, 3.6	MPCA, 3.6	UFRA, 3.6	
Recovery to cheese					
Fat (% total milk fat)	88.4 ^b	90.79 ^a	90.47 ^{ab}	90.21 ^{ab}	0.91
Protein (% total milk protein)	75.24 ^a	76.66 ^a	76.1 ^a	76.64 ^a	0.85
Cheese yield ⁴					
Ya (kg/100 kg)	9.514 ^b	10.63 ^a	10.57 ^a	10.51 ^a	0.07
Yafpam	9.55 ^a	9.67 ^a	9.64 ^a	9.52 ^a	0.08
Yma	9.72 ^b	11.00 ^a	10.94 ^a	10.93 ^a	0.08
Ymafpm	9.76 ^b	10.00 ^a	9.98 ^a	9.90 ^{ab}	0.07
Yafcam	9.48 ^a	9.51 ^a	9.52 ^a	9.40 ^a	0.09
Ymafcam	9.68 ^b	9.83 ^{ab}	9.86 ^a	9.78 ^{ab}	0.07
	Treatment ² and milk protein level (% wt/wt); series B				SED
	CB, 3.3	PCB, 4.0	MPCB, 4.0	UFRB, 4.0	
Recovery to cheese					
Fat (% total milk fat)	88.72 ^b	91.25 ^a	90.56 ^a	90.95 ^a	0.41
Protein (% total milk protein)	75.73 ^b	77.99 ^a	76.41 ^b	76.11 ^b	0.56
Cheese yield ⁴					
Ya (kg/100 kg)	9.46 ^b	11.69 ^a	11.62 ^a	11.56 ^a	0.06
Yafpam	9.49 ^b	9.68 ^a	9.56 ^{ab}	9.56 ^{ab}	0.05
Yma	9.71 ^b	12.11 ^a	12.06 ^a	12.06 ^a	0.07
Ymafpm	9.74 ^b	10.03 ^a	9.93 ^a	9.98 ^a	0.05
Yafcam	9.49 ^a	9.60 ^a	9.57 ^a	9.53 ^a	0.06
Ymafcam	9.73 ^b	9.94 ^a	9.93 ^a	9.94 ^a	0.05

^{a,b}Values within a row not sharing a common superscript differ significantly at $P < 0.05$.

¹Presented values for both A and B treatments are means of 4 replicate cheese trials.

²Milk protein was increased from 3.3 (CA) to 3.6% (wt/wt) in experimental series A by the addition of phosphocasein (PCA), milk protein concentrate (MPCA), or UF milk retentate (UFRA), and from 3.3 (CB) to 4.0% (wt/wt) in experimental series B by the addition of phosphocasein (PCB), milk protein concentrate (MPCB), or UF retentate (UFRB).

³SED = Standard error of difference.

⁴Yield expressions: Ya = actual yield (kg/100 kg of milk); Yafpam = yield per 100 kg of milk normalized to reference fat (3.4%, wt/wt) and protein (3.3%, wt/wt) levels; Yma = moisture-adjusted yield; Ymafpm = moisture-adjusted (38.5%, wt/wt) yield per 100 kg of milk normalized to reference fat and protein levels; Yafcam = yield per 100 kg of milk normalized to reference fat (3.4%, wt/wt) and casein (2.53%, wt/wt) levels; Ymafcam = moisture-adjusted yield per 100 kg of milk normalized to reference fat and casein levels.

affected by increasing milk protein content or by the method of milk protein standardization (Table 9, Figure 4b). This indicates that the significantly higher Yafpam for the PCB cheese was due largely to its higher content of milk casein and significantly higher protein recovery to cheese (Table 7); casein is essentially the only protein fraction (apart from denatured whey protein complexed with the casein) recovered in the protein matrix of the Cheddar cheese.

In contrast to Yafcam, the moisture-adjusted yield per 100 kg of fat and casein-adjusted milk, Ymafcam, increased significantly as milk protein content was increased from 3.3 to 3.6% (wt/wt) using MPC and from 3.3 to 4.0% (wt/wt) with PC, MPC, and UFR (Figure 4a, b). This indicates that the moisture-adjusted yield of cheese from the MPCA, PCB, MPCB, and UFRB milks is greater than that expected as a result of their higher levels of casein and fat. The higher Ymafcam values from the latter milks compared with the control

milks (CA, CB) is due mainly to their higher percentage fat recovery to cheese, which increased with milk protein level and was highest for the PCB-standardized milk.

CONCLUSIONS

With a highly standardized cheese-making procedure, the use of PC, MPC, or UFR to standardize (increase) milk protein content from 3.3 to 4.0% (wt/wt) significantly increased fat recovery to cheese (by ~1.8 to 2.5% of total milk fat). Nevertheless, the improved fat recovery did not give an increase in the yield of cheeses per unit weight of milk normalized to reference levels of casein and fat because of the inverse relationship between the levels of milk protein and cheese moisture. However, the normalized yield of moisture-adjusted cheese per unit weight of milk with reference levels of casein and fat (Ymafcam) increased signifi-

Figure 3. Relationship between level of milk protein content on the actual yield (Y_a , ●) of Cheddar cheeses made from different milks, as described in Table 4; the regression line (—) drawn between experimental data points was significant ($P < 0.05$).

cantly on increasing milk protein level from 3.3 to 4.0% (wt/wt) with all standardization treatments. This result suggests that increasing the moisture content of cheeses from protein-standardized milks through process intervention would have led to increases in cheese yield over the control milk, which would be higher than that expected as a result of the higher levels of fat and protein in the standardized milks. In commercial practice, the moisture content of cheese made from protein-standardized milk could be increased very easily by slight alterations of make procedure. Indeed, some feedback from preliminary evaluations at the commercial level indicates an increase in the level of cheese moisture when using protein-standardized milks, a trend that suggests that gels are cut based on time rather than firmness in such evaluations. An integrated approach to cheese manufacture, involving milk protein standardization/fortification, make procedure standardization (e.g., ratios of rennet activity and starter quantity to casein), and effective process intervention where necessary, enables manufacturers to optimize standard operating procedures and, thereby, to obtain more consistent target values for key process indicators of efficiency and cheese composition. Such an approach is becoming increasingly necessary to remain competitive in the production of commodity-type cheeses such as Cheddar, Gouda, and Mozzarella, in which scale of operation, maximization of cheese-making efficiency, and the delivery of product with consistent composition (e.g., sodium) and quality are key factors in lowering product costs and securing market share. For cheese makers unable to produce fresh UFR before cheese

Figure 4. Effect of standardizing milk protein from 3.3 (control cheese, CB) to 4.0% (wt/wt) with phosphocasein (PCB), milk protein concentrate (MPCB), or UF milk retentate (UFRB) on the actual (white bars) and moisture-adjusted (38.5% wt/wt; gray bars) cheese yields from: (a) 100 kg of milk normalized to reference fat (3.4%, wt/wt) and protein (3.3%, wt/wt) levels, or (b) 100 kg of milk normalized to reference fat (3.4%, wt/wt) and casein (2.53%, wt/wt) levels. Presented values are the means from 4 replicate cheese trials; error bars show standard deviation of the means.

manufacture, equal performance may be achieved by means of MPC addition. Because PC is a relatively new protein ingredient, special commercial arrangements may need to be put in place to secure its availability.

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