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Yield and Aging of Cheddar Cheeses Manufactured from Milks with Different Milk Serum Protein Contents*

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ABSTRACT

Whey proteins in general and specifically β-lactoglobulin, α-lactalbumin, and immunoglobulins have been thought to decrease proteolysis in cheeses manufactured from concentrated retentates from ultrafiltration. The proteins found in whey are called whey proteins and are called milk serum proteins (SP) when they are in milk. The experiment included 3 treatments; low milk SP (0.18%), control (0.52%), and high milk SP (0.63%), and was replicated 3 times. The standardized milk for cheese making of the low milk SP treatment contained more casein as a percentage of true protein and more calcium as a percentage of crude protein, whereas the nonprotein nitrogen and total calcium content was not different from the control and high SP treatments. The nonprotein nitrogen and total calcium content of the milks did not differ because of the process used to remove the milk SP from skim milk. The low milk SP milk contained less free fatty acids (FFA) than the control and high milk SP treatment; however, no differences in FFA content of the cheeses was detected. Approximately 40 to 45% of the FFA found in the milk before cheese making was lost into the whey during cheese making. Decreasing the milk SP content of milk by 65% and increasing the content by 21% did not significantly influence general Cheddar cheese composition. Higher fat recovery and cheese yield were detected in the low milk SP treatment cheeses. There was more proteolysis in the low milk SP cheese and this may be due to the lower concentration of undenatured β-lactoglobulin, α-lactalbumin, and other high molecular weight SP retained in the cheeses made from milk with low milk SP content.
(**Key words:** serum protein, cheese, yield, proteolysis)

Abbreviation key: DF = diafiltration, **MF** = microfiltration, **RMF** = retentate from microfiltration, **SNPTN** = soluble nitrogen as a percentage of total nitrogen, **SP** = serum protein, **SPC** = serum protein concentrate, **TN** = total nitrogen.

INTRODUCTION

Native β-LG inhibits the proteolysis of CN by plasmin more so than denatured β-LG (Bastian et al., 1993). Native and denatured β-LG inhibited chymosin hydrolysis of α_{s1}-CN in simulated milk ultrafiltrate (Lo and Bastian, 1997). Denatured α-LA inhibited chymosin activity on α_{s1}-CN in simulated milk ultrafiltrate (Lo and Bastian, 1997). Whey proteins present in cheese made from milk with moderate or no heat treatment would be in solution in the water phase of the cheese. Nelson et al. (2004b) reported that whey proteins are present in the expressible serum of Cheddar cheese. Slower proteolysis during aging in Cheddar cheese manufactured from concentrated UF retentates has been well documented (Covacevich and Kosikowsi, 1978; Creamer, 1987). Lelievre et al. (1990) reported that other high molecular weight whey proteins (e.g., immunoglobulins) inhibit proteolysis of α_{s1}-CN by rennet. Akaeda et al. (1971) reported that α_2-macroglobulin bound directly to κ-CN and decreased rennin activity. Plasmin is also inhibited by the large molecular weight proteins α_2-macroglobulin (Steiner et al., 1987) and α_2-antiplasmin (Precetti et al., 1997). Higher whey protein retention in cheeses was attractive with respect to increasing cheese yield, but the decreased proteolysis accompanying higher whey protein retention was detrimental to Cheddar cheese flavor development. Cheddar cheese made from unconcentrated milk usually contains very little whey protein (O'Keeffe et al., 1978), generally <1% of the cheese protein, and yet whey proteins comprise most of the protein in the expressible serum of these cheeses (Nelson et al., 2004b). The water phase of cheeses manufactured from concentrated retentates from UF would contain even more whey proteins. Therefore, one could hypothesize that reducing protease inhibitors in the water phase of cheese could increase the rate of CN proteolysis, and potentially in-

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crease the rate of development of typical Cheddar flavors.

Microfiltration (**MF**) enables β-LG and α-LA to be separated from CN in skim milk. Cheddar cheeses manufactured from low concentration factor retentate from microfiltration (**RMF**; 1.2 to 1.8×) had lower soluble nitrogen as a percentage of total nitrogen (**SNPTN**) and less CN degradation over 180 d of aging (Neocleous et al., 2002b). By adjusting the moisture-in-the-nonfat substance of cheese from the 1.8× treatment to the same level as the control and adding more chymosin (Neocleous et al., 2002b), the cheese SNPTN and CN degradation increased to a level similar to that of the control cheese. Neocleous et al. (2002b) indicated, as have others, that large molecular weight proteins retained in cheese might reduce chymosin and plasmin activity (Akaeda et al., 1971; Steiner et al., 1987; Lelievre et al., 1990; Precetti et al., 1997). Milk minus milkfat is called milk plasma, whereas milk plasma minus CN micelles is called milk serum. Milk serum proteins (**SP**) are largely present in milk serum in molecular form or as very small aggregates (Walstra et al., 1999). Because the milk SP concentration in the standardized milks of Neocleous et al. (2002a) increased (0.52 to 0.59%) with increasing concentration factor (1.2 to 1.8×), they concluded that large molecular weight milk SP may be retained by the MF membrane. The work of Jost et al. (1999) supports their conclusion that immunoglobulins are retained by a 0.1-μm MF membrane.

Recently, a diafiltration (**DF**) process was reported to remove 95% of the milk SP, mostly β-LG and α-LA, from skim milk (Nelson and Barbano, 2005). Milk SP are whey proteins that have been removed from milk before the cheese making process and are therefore not part of the whey. We are unaware of any studies that measured the yield and proteolysis of Cheddar cheese from milk with 95% of the milk SP removed before cheese making. In this experiment, we removed about 95% of the milk SP from the skim milk before standardizing with cream so that the direct impact of variation in milk SP on cheese proteolysis could be determined. We hypothesize that removing milk SP from milk before cheese making should increase proteolysis but have little or no detectable influence on cheese yield in cheeses with the lower milk SP content.

MATERIALS AND METHODS

Milk Processing

One batch of whole milk was pasteurized (72°C for 15 s) then separated into cream and skim milk using a centrifugal separator. The skim milk was divided into 3 portions: 1 to be used for a low milk SP treatment, 1 for an high milk SP treatment, and 1 to be used for a control with typical milk SP content. A 3-stage process with 1 MF stage (0.1 μm pore size, 3× concentration factor) and 2 DF stages was used to remove SP from skim milk for the low milk SP treatment and produce the serum protein concentrate (**SPC**) for the high milk SP treatment, as described by Nelson and Barbano (2005). Permeate from the UF of the permeate from MF was used as the diafiltrant instead of water so that the lactose, NPN, and calcium concentrations in the milk with decreased SP content would remain the same as the original milk (Nelson and Barbano, 2005). The 3× MF skim retentate from the third stage of the process was diluted with permeate from UF to reduce the casein concentration back to the level in the original skim milk. To determine the amount of dilution needed, the true protein content of the diluted retentate and original skim milk was measured using a MilkoScan 605 infrared milk analyzer (A/S Foss Electric, Hillerød, Denmark). The CN content of the RMF was estimated (0.82 × true protein) from the infrared results. The diluted diafiltered RMF was stored overnight at 4°C before cheese making.

The low milk SP standardized milk was made by combining diluted diafiltered RMF with cream. The control standardized milk was made by combining skim milk and cream. The high milk SP standardized milk was made by combining skim milk with cream and then adding milk SPC (about 3.2 kg) containing about 10% milk SP. The milk SPC was made by concentrating permeate from the MF of skim milk using UF (Nelson and Barbano, 2005).

Cheese Manufacture

An 18-kg block of Cheddar cheese was manufactured from each of the 3 treatment milks on the day after MF processing. All weights were measured to the nearest gram (model SB32000, Mettler Toledo Instrument Co., Highstone, NJ) for mass balance calculations. Cheeses were manufactured according to the procedure described by Nelson et al. (2004a) with the following exceptions: starter usage rate = 0.2 g/kg of milk; whey draining pH = 6.40; and the salting rate was 3% of the curd weight. The MF processing and cheese making was replicated 3 times in a 3-wk period using 3 different batches of milk.

Milk, Whey, Salt Whey, and Cheese Sampling

Raw whole milk was mixed, sampled at 4°C before pasteurization, and tested for SCC. Milks for cheese making were sampled at 31°C immediately before starter addition. All of the whey drained from each cheese vat up to the time of salting was collected sepa-