Effect of Different Somatic Cell Levels on Nitrogen Components of Yoghurt Milk and Probiotic Set Yoghurt During Storage Life

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ABSTRACT

Nitrogen components of yoghurt milk samples and resulting probiotic set yoghurt were determined. Analyses included total nitrogen (TN), non-casein nitrogen (NCN), non-protein nitrogen (NPN), casein nitrogen (CN) and proteolysis index (PI). Yoghurt samples were analyzed 1, 7, 14 and 21days after production and storage at 5±1°C. TN, CN and PI contents of yoghurt milk samples with high Somatic Cell SC level were lower than yoghurt milk with low SC ($p<0.05$). NCN and NPN concentrations of yoghurt milk increased by the elevation of SC level, however differences between low and medium milk samples were not significant. During storage life, TN and CN contents of all yoghurt samples decreased significantly. NCN and NPN concentrations of probiotic yoghurt samples increased ($p<0.05$) after 21 days. It was concluded that SC level elevation in milk decreased casein as a percentage of true protein in the resulting probiotic yoghurt throughout storage for 21 days.

Keywords: Nitrogen Components; Probiotic Set Yoghurt; Proteolysis; Somatic Cell (SC) Level; Storage Life; Yoghurt Milk.

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INTRODUCTION

Somatic cells (SCs) in milk are a pivotal part of mammary gland immunity and used as an indicator of the severity of mammary infection and milk quality (Bradley, 2002). In healthy mammary gland of cows, the predominant cell type is macrophages (37-79 percent) followed by lymphocytes (16-28 percent), polymorphonuclear neutrophils (PMNs) (3-26 percent) and epithelial cells (2-15 percent) (Lindmark-Mansson *et al*., 2006). When there is a bacterial infection, tissue damage, or other inflammation processes affecting the mammary tissue, the recruitment of huge number of defensive PMNs results in somatic cell count (SCC) elevation (Considine *et al*., 1999; Gargoury *et al*., 2007).

SCs are associated with a range of proteolytic enzymes; including elastase, collegenase and cathepcins B, C, D and G, which contribute to hydrolysis of casein (Le Roux *et al*., 2003; O'Brien *et al*., 2004). In addition, plasmin is the principle proteolytic enzyme in milk from both healthy udders and udders with elevated SCC (Leitner *et al*., 2006). Plasmin is a heat-stable alkaline serine proteinase which exists in milk as a component of a complex system, including its zymogen (plasminogen), plasminogen activators (PAs) and inhibitors of both plasmin and PAs (Kelly *et al*., 2006). Level of active plasmin in milk depends on the activator- inhibitor balance, with the balance in mastitic milk being in favor of activation (Chen *et al*., 2003).

Proteolysis of casein leads to decrease in the relative proportion of caseins (α_{s1} and β-casein) with simultaneous clear increased levels of γ-caseins and proteose peptones (Leitner *et al*., 2006). Proteolysis in processed milk has been measured by monitoring changes in nitrogen levels such as decreases in casein nitrogen (CN) or increases in non-protein nitrogen (NPN). These changes have been linked to changes in functionality, such as microstructural changes (e.g. casein flocculation) and increases in viscosity in UHT milk (Chen *et al*., 2003). The contribution of the activities of certain enzymes to the quality of certain dairy products has been the subject of considerable researches, for example, increased plasmin activity is accompanied by increasing clotting time, loss of moisture in cheese, reduced curd stability and yield (Kelly & Fox, 2006).

Probiotic fermented dairy products, mainly yoghurt, has been consumed with a long history of safe use (Maragkoudakis *et al*., 2006). The term probiotic is defined as live microorganisms that when administered in adequate amounts confer a health benefits on the host (Vasiljevic & Shah, 2008). Probiotic bacteria as well as yoghurt bacteria have proteolytic activity. Proteinases and peptidases constitute the primary enzymes in lactic acid bacteria for proteolysis in milk protein as a source of amino acids and nitrogen (Donkor *et al*., 2006). Previous studies have shown that the proteolytic activity of *S. thermophilus, L. delbrueckii spp. bulgaricus*, and *L.acidophilus* was much higher than that of *Bifidobacterium spp*. (Shihata & Shah, 2000).

Present study was determined to investigate the possible effect of different SC levels in milk on the nitrogen components and proteolysis index of yoghurt milk and resulting probiotic set yoghurt.

MATERIAL AND METHODS

Milk collection

Thirty lactating Holstein cows selected among animals (A herd of a commercial dairy farm, Tehran province, Iran) in the intermediate stages of lactation, which were not treated with antibiotics 7 days prior to milk collection. Milk from individual cows was analysed for somatic cell counts and distributed in three groups upon their SCC status: Low SCC (<200,000 cells ml^{-1}), medium SCC (200,000-800,000 cells ml^{-1}) and high SCC >800,000 cells ml^{-1}. Milk samples from selected cows were kept in sterile bottle and transported immediately to The Research and Development Laboratory of Iran Dairy Industries Company (Pegah).

Probiotic set yoghurt preparation

For probiotic set yoghurt production, raw milk was heat treating at 85°C for 30 min and cooled to the fermentation temperature. After inoculation with the starter culture (ABY-1; Containing *Lactobacillus acidophilus* (LA-5), *Bifidobacterium* (BB-12) and yoghurt bacteria, Chr. Hansen, Denmark), yoghurt milk was distributed to 100 ml plastic retail container, sealed and incubated (37°C) until pH reached 4.6, then cooled and stored at 4-6°C for 21 days.

Milk and probiotic set yoghurt analyses

Total protein (TN), non casein nitrogen (NCN), non protein nitrogen (NPN), were determined by Kjeldahl method according to the Association of Official Analytical Chemists (AOAC, 2002). Casein nitrogen (CN) was calculated by CN=TN-NCN and proteolysis index (PI) by dividing the CN by true protein (expressed as [TN – NPN] ×6.38) (Fernandes *et al*., 2007). Analyses of probiotic set yoghurt samples were carried out one day after preparation and after 7, 14 and 21 days of storage by kjeldahl method (AOAC, 2002).

Statistical analyses

A split-plot design was used to monitor the effects of treatment, storage time and the interaction of the measured response variables at intervals during 21days of storage. Statistically significant differences (p<0.05) between different treatment levels were determined by Duncan's significant difference test.

RESULTS AND DISCUSSION

Nitrogen component of yoghurt milk

TN and CN contents of yoghurt milk significantly decreased (p<0.05) by the elevation of SC levels (Table 1). There was a highly significant linear relationship (R^2=0.803) between SC level and TN content of yoghurt milk. It was found that by increase of one unit of SCC, the TN content decreased 0.03 percent in each category (p<0.05) (Table 2). Reduced amount of TN in yoghurt milk samples by elevation of SC levels was due to the reduction in the synthesis and secretion ability of mammary tissue as a result of mastitis (Lee *et al*., 1991; Petrovski & Emanuel, 2006). A significant reduction was observed in the CN concentration of yoghurt milk as the SC level increased. Also, the relationship (R^2= 0.78) between SCC and CN concentration was strongly significant (p<0.0001). Decreasing trend in CN concentration is likely related to activation of plasmin system resulting in partial CN degradation (Leitner *et al*., 2006). Most previous studies have concerned cathepcin C and G, collagenase and elastase activities in blood during the inflammatory response, which exhibit proteolytic activity towards bovine casein (Haddadi *et al*., 2006).

Table 1: Nitrogen components of yoghurt milk at different levels of somatic cell count (Mean ± Standard deviation)

Somatic cell count (1000 cells/mL)	Somatic cells (log/ml)	TN (g/100 g)	NCN (g/100 g)	NPN (g/100 g)	CN (g/100 g)	PI (%)
<200	$4.87^a \pm 0.36$	$0.53^a \pm 0.01$	$0.11^a \pm 0.01$	$0.015^a \pm 0.005$	$0.43^a \pm 0.02$	$82.25^a \pm 1.67$
200-800	$5.67^b \pm 0.14$	$0.5^b \pm 0.01$	$0.12^a \pm 0.02$	$0.03^b \pm 0.014$	$0.38^b \pm 0.02$	$82.04^a \pm 5.03$
>800	$6.12^c \pm 0.13$	$0.47^c \pm 0.01$	$0.14^b \pm 0.02$	$0.055^c \pm 0.015$	$0.33^c \pm 0.03$	$78.22^b \pm 2.95$

TN, Total nitrogen; NCN, Non-casein nitrogen; NPN, Non-protein nitrogen; CN, Casein nitrogen; PI, Proteolysis index.
[a,b,c] Within a row, means with different letters differ statistically ($p<0.05$).

Increased proteolytic activity in infected quarters resulted in increase amount of NPN and NCN in yoghurt milk when compared to those from uninfected quarters; however the trend for NCN content was not significant ($p\geq0.05$) between low and medium yoghurt milk samples (Table 1). There were positive linear regression coefficient (Table 2) between SCC level and NCN, NPN contents of yoghurt milk ($p<0.0001$).

Proteolysis index (PI) or casein as a percentage of true protein decreased by the elevation of SC level in yoghurt milk samples (Table 1) while the difference between low and medium milk samples was not significant ($p\geq0.05$). A weak relationship ($R^2= 0.16$) obtained between elevation of SCC and PI (Table 2). This result was predictable due to the non-significant differences in NCN content resulted between low and medium milk samples.

Table 2: Regression coefficient of milk nitrogen components versus SCC

	Intercept	RC	R^2
TN	0.563	-0.031*	0.803
NCN	0.084	0.019*	0.473
NPN	0.007	0.02*	0.66
CN	0.48	-0.05*	0.78
PI	0.849	-0.02*	0.16

TN, Total nitrogen; NCN, Non-casein nitrogen; NPN, Non-protein nitrogen; CN, Casein nitrogen; PI, Proteolysis index.
Regression coefficient, RC.
* $p\leq0.0001$

Nitrogen component of probiotic set yoghurt

During storage life, significant decrease was observed in TN content of probiotic yoghurt samples prepared from milk with higher level of SCC (Table 4). The SC level and the storage life were found to have significant effect on TN content of probiotic set yoghurt. However, the linear relationship ($R^2= 0.46$) between SC level and TN content was greater than storage life ($p<0.0001$). Results showed that probiotic yoghurts from high SC level milk had lower CN content in comparison with samples from low SCC (Table 3). CN content of yoghurt samples had a negative significant relationship with SC level and storage life (Table 5). Furthermore, 58.73 percent of the variability in CN content during storage life could be explained by SC level elevation ($p<0.0001$). Based on previous works (Maragkoudakis *et al.*, 2000; Santos *et al.*, 2003) milk with high SCC has native milk protease activity which could survive pasteurization.

Table 3: Non-casein nitrogen, non-protein nitrogen and casein nitrogen contents of probiotic set yoghurt prepared from milk with different levels of somatic cell count (Mean ± Standard deviation)

Storage life	NCN			NPN			CN		
	L	M	H	L	M	H	L	M	H
1	$0.105^{aA} \pm 0.004$	$0.114^{aB} \pm 0.005$	$0.124^{aC} \pm 0.006$	$0.032^{aA} \pm 0.002$	$0.031^{aB} \pm 0.01$	$0.038^{aC} \pm 0.011$	$0.43^{aA} \pm 0.01$	$0.40^{aB} \pm 0.01$	$0.37^{aC} \pm 0.02$
7	$0.107^{abA} \pm 0.004$	$0.119^{abB} \pm 0.005$	$0.126^{abC} \pm 0.005$	$0.031^{aA} \pm 0.01$	$0.04^{aB} \pm 0.004$	$0.043^{aC} \pm 0.01$	$0.42^{aA} \pm 0.01$	$0.38^{aB} \pm 0.01$	$0.36^{aC} \pm 0.01$
14	$0.111^{bcA} \pm 0.006$	$0.122^{bcB} \pm 0.005$	$0.128^{bcC} \pm 0.004$	$0.037^{bA} \pm 0.01$	$0.048^{bB} \pm 0.006$	$0.050^{bC} \pm 0.01$	$0.40^{bA} \pm 0.01$	$0.37^{bB} \pm 0.01$	$0.34^{bC} \pm 0.01$
21	$0.113^{cA} \pm 0.005$	$0.125^{cB} \pm 0.007$	$0.134^{cC} \pm 0.005$	$0.042^{bA} \pm 0.01$	$0.050^{bB} \pm 0.004$	$0.054^{bC} \pm 0.005$	$0.38^{bA} \pm 0.01$	$0.35^{bB} \pm 0.01$	$0.32^{bC} \pm 0.02$

L, low somatic cell; M, Medium somatic cell; H, High somatic cell.
NCN, non-casein nitrogen; NPN, non-protein nitrogen; CN, casein nitrogen.
^{abc} within a column, means with different letters differ statistically (p<0.05)
^{ABC} within a row, means with different letters differ statistically (p<0.05)

Table 4: Total nitrogen and proteolysis index of probiotic set yoghurt prepared from milk with different levels of somatic cell count (Mean ± Standard deviation)

Storage life	TN			PI		
	L	M	H	L	M	H
1	$0.53^{aA} \pm 0.01$	$0.51^{aB} \pm 0.01$	$0.49^{aC} \pm 0.02$	$85.53^{aA} \pm 0.7$	$82.8^{aB} \pm 1.56$	$81.33^{aC} \pm 1.13$
7	$0.52^{bA} \pm 0.01$	$0.50^{bB} \pm 0.01$	$0.48^{bC} \pm 0.01$	$84.52^{abA} \pm 1.6$	$82.74^{abB} \pm 1.01$	$81.20^{abC} \pm 1.15$
14	$0.51^{cA} \pm 0.01$	$0.49^{cB} \pm 0.01$	$0.47^{cC} \pm 0.01$	$84.35^{abA} \pm 1.7$	$83.14^{abB} \pm 1.14$	$81.36^{abC} \pm 1.14$
21	$0.50^{dA} \pm 0.01$	$0.48^{dB} \pm 0.01$	$045^{dC} \pm 0.02$	$84.44^{bA} \pm 1.3$	$82.67^{bB} \pm 1.31$	$80.10^{bC} \pm 1.23$

L, low somatic cell; M, Medium somatic cell; H, High somatic cell.
TN, Total nitrogen; PI, proteolysis index.
^{abc} within a column, means with different letters differ statistically (p<0.05)
^{ABC} within a row, means with different letters differ statistically (p<0.05)

Table 5: Regression coefficient of milk nitrogen components versus Somatic Cell (SC) level Storage time

	SC level			**Storage Time**		
	Intercept	RC	R^2	Intercept	RC	R^2
TN	0.54	- 0.02*	0.4614	0.51	- 0.002*	0.263
NCN	0.1	0.009*	0.6081	0.114	0.0005*	0.1268
NPN	0.030	0.005*	0.1835	0.033	0.0008*	0.3151
CN	0.44	- 0.031*	0.5873	0.40	- 0.002*	0.2587
PI	0.866	- 0.186*	0.5808	0.832	- 0.034^{NS}	0.0084

TN, Total nitrogen; NCN, Non-casein nitrogen; NPN, Non-protein nitrogen; CN, Casein nitrogen; PI, Proteolysis index.
Regression coefficient, RC.
NS = Not Significant
* $p \leq 0.0001$

Results showed that the concentration of NCN in probiotic set yoghurt (Table 3) increased at the day 21 when compared to that of at first day, whereas the differences between day 7 and 14 were not statistically significant ($p \geq 0.05$). Strong positive relationship (R^2= 0.61) between SCC and NCN concentration of probiotic yoghurt revealed that the effect of elevation of SC level was more than the effect of cold storage (Table 5).

NPN concentration of probiotic set yoghurt showed significant increase after 14 days of storage. The increasing trend was greater for the yoghurt samples prepared from high SCC milk (Table 3). The linear relationship between NPN and the variable factors (SCC and storage time) showed a greater relationship with the storage time (R^2= 0.315, $p<0.0001$) (Table 5). ABY starter cultures contain yoghurt bacteria, in particular *L.delbrueckii subsp. bulgaricus* which is more proteolytic than *S.thermophilus*. Symbiotic growth also occurs due to the presence of both bacterial species where they can produce amino acids and peptides, which are required for growth and survival of probiotic bacteria. Yoghurt bacteria also appeared to be highly proteolytic as compared to the probiotic bacteria such as *L.acidophilus* and *Bifidobacterium spp*. (Shihata & Shah, 2002).

The PI results showed that the probiotic yoghurt produced from high SCC milk had lower casein content as a percentage of true protein. PI of probiotic yoghurt in each level of SC decreased significantly at day 21 in comparison to first day. However, it was found that there is a weak relationship (R^2= 0.0084) between PI during cold storage ($p \geq 0.05$).

CONCLUSIONS

It can be concluded that increased SC level in milk led to a reduction in casein content as a percentage of true protein. It was suggested that for monitoring the quality of yoghurt milk with different levels of SC, concentrations of NCN and NPN may used as suitable quality factors. During storage life, NCN concentration can be applied as an indicator of deterioration in probiotic set yoghurt produced from raw milk with different SC levels. In order to avoid deterioration or even spoilage in final product during storage life, it is suggested that raw milk used to produce probiotic yoghurt should not contain more than 200,000 cell ml^{-1} somatic cells.

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