Acta Agraria Kaposváriensis (2014) Vol 18 Supplement 1, 103-108.
Kaposvári Egyetem, Agrár- és Környezettudományi Kar, Kaposvár
Kaposvár University, Faculty of Agricultural and Environmental Sciences, Kaposvár

Effect of cheesemaking with microparticulated whey proteins on the concentration of low molecular thiols in cheese

G. Niero, A. Sturaro, A.R. Trentin, A. Masi, M. De Marchi, M. Cassandro

Department of Agronomy, Food, Natural resources, Animals and Environment
University of Padova, Agripolis, Viale dell'Università 16, 35020 Legnaro (PD), Italy

ABSTRACT

Aim of this work was to investigate different concentrations of microparticulated whey proteins (MWP) added during cheesemaking process on the recovery of low molecular weight thiols (LMT) in cheese. Historically, milk whey has been considered an industrial waste, because of its high cost of disposal and its polluting potential. In recent years the re - utilization of this waste represents an interesting perspective. Nowadays, several procedures are available to whey constituents recovery: some of the main are whey proteins (WP) and LMT. Whey ultrafiltration is the most common treatment to WP recovery. Thereafter, the WP undergo processes that lead to the production of protein aggregates (microparticulated-MWP), useful in different sectors of the food industry for their high gelling power and potential in LMT linkage. Mini-cheesemaking trial using milk standardized at 3.5% of protein with 3.0% or 4.0% MWP were carried out. Cheesemaking were performed in 6 days, 3 days for each treatment. Within a day, 3 replicates of the same treatment were carried out (n=18). The LMT of milk and whey were determined using RP - HPLC, while LMT in cheese were calculated by difference. Data were analyzed through a generalized linear model as fixed effects of the MWP concentration, replicate, and day of cheesemaking nested within MWP. Results showed that the quantified concentration of LMT in cheese were quite stable in both the percentages of MWP. The soluble properties of LMT represent a problem in their recovery in cheese; indeed beyond a certain concentration they are not retained in the curd, but are released in the whey.

(Keywords: microparticulated whey proteins, RP - HPLC, thiols, whey protein)

INTRODUCTION

Animal cells produce energy by reducing molecular oxygen in water. This process generates few amounts of free radicals called reactive oxygen species (ROS), that can damage the chemical structure and the biological function of cells molecules (*Droge*, 2002; *Siliprandi et al.*, 2008). The negative effects of ROS are on a large scale: they mainly act on lipid peroxidation of plasma membrane, oxidative alterations of proteins, up to the DNA cleavage. Cells have developed several mechanism to remove and inactivate ROS, by producing antioxidants. The molecules that act as antioxidants are enzymatic (catalase, superoxide dismutase and glutathione peroxidase) or non - enzymatic. Among the aforementioned, the main are fat - soluble vitamins A and E,

ascorbic acid and glutathione (GSH) in the cytosol (*Robbins et al.*, 2008). In particular, GSH, tripeptide composed of glycine, glutamate and cysteine, belonging to the class of low molecular thiols (LMT), plays a central role in deactivation of ROS (*Fang et al*., 2002).

Milk whey, a by - product of cheesemaking, has a great antioxidant activity, mainly due to its content in whey proteins (WP) rich in cysteine, key element in the GSH biosynthesis. The whey protein concentrates (WPC) and aggregates (MWP) produced by ultrafiltration and microparticulation of whey, have attracted the scientific community attention because of their higher content in WP (*Hakkak et al.*, 2000). Nowadays, several studies have been carried out to increase GSH production by WP and WPC administration in immunodeficient patients with HIV infection. Products based on WP have been used as a cystein source to increase intracellular levels of GSH production (*Micke et al.*, 2002).

This study aimed to investigate the variation of LMT, cysteine (Cys), cystein – glycine (Cys - Gly), γ–glutamylcysteine (γ – GC), and GSH, after MWP addition during cheesemaking process.

MATERIALS AND METHODS

Bulk milk used for cheese making was collected in the Soligo dairy cooperative (Soligo, Italy) added using 3.0% or 4.0% of MWP and standardized at 3.5% of proteins using proper quantity of milk protein concentrate (MPC). Samples of MWP and MPC were obtained in the same dairy, after ultrafiltration by polyethersulphone membrane (10,000 Da; TetraPak Food Engineering, Lund, Sweden) at 10°C, and than treated at 95°C for 10 min, at 40 bar of pressure. The milk used in each day was analysed with MilkScan FT2 (Foss Electric, Hillerod, Denmark).

Ten litres of standardized milk has been used for mini - cheesemaking. Coagulation was monitored with sensor CoAguLite (Reflectronics Inc., Lexington, KY; *Fagan et al.*, 2007). Milk is added to a freeze–dried starter culture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (TB, MicroMilk, Crema, Italy) and to a solution of commercial liquid rennet (chymosin 75:25 bovine pepsine, rennet De Longhi Michele & CSas, Treviso, Italy) diluted in water (1:3). Finally the crud is placed in a mold and is incubated at 37°C for about 3 hours, until pH is under 5.5. The form is placed in a saline solution for one hour (1.14 kg of NaCl per L) and then in chilled room for curing (10 days at 4°C and 85% of relative humidity). Cheesemaking were performed in 6 days, 3 days for each treatment. Within a day, 3 replicates of the same treatment were carried out (n=18).

Soluble LMT quantification in milk and whey was carried out by RP–HPLC method, using C18 column, after the application of a derivatization protocol as proposed by *Masi et al.* (2002). LMT concentration in cheese was calculated by difference between milk and whey LMT. The normal distributions of LMT in milk, whey and cheese were checked using Shapiro-Wilk's Test. Data regarding concentrations of LMT in milk, whey and cheese were analysed with a linear model, using the GLM procedure of SAS (SAS 9.2, 2008). The model included the fixed effect of the MWP concentration (2 levels), replicate (3 levels) and day of cheesemaking nested within MWP. The effect of MWP concentration has been tested on error line within day variance. Bonferroni's test was used in order to determine differences between LMT means concentration. Significance was established at $P \leq 0.05$.

RESULTS AND DISCUSSION

The effect of cheesemaking with MWP was tested to assess any changes of the LMT concentrations in milk, whey and cheese. In *Table 1* are summarized the descriptive statistics of thiols in milk added with 3 and 4% of MWP, whey and Caciotta cheese obtained after cheesemakings. The results of the Shapiro-Wilk's test showed for all traits a normal distribution of data. The cysteine was the most abundant thiol in milk as well in whey and cheese. The high concentration of cysteine in milk and whey has been reported by several authors (*Parodi*, 1998; *Bounous*, 2000). While, in the same matrices γ – GC and GSH showed lower concentrations. Finally, the Cys – Gly, was more concentrated in milk, but at the end of the cheesemaking process it is found in the whey. For this reason it has fairly low levels in cheese.

Table 2 shows the results of the variance analysis conducted for the LMT present in milk, whey and cheese. The effect of the date is highly significant in explaining the variability of the γ – GC in whey, and Cys – Gly in milk and cheese. Such daily variability could be due to different LMT concentration in the starting milk. For all the studied traits, no statistically significant effects are observed for replicate and MWP.

In *Table 3* are reported the least squares means of milk, whey and Caciotta cheese thiols across different concentrations of MWP (3.0% and 4.0%). LMT concentration in milk was not affected by the two MWP treatments, except for the γ – GC. While, in whey the concentration of thiols, γ – GC and GSH, was affected by MWP. By increasing concentration of MWP an increment in both thiols losses was observed. In cheese, MWP treatment did not affect thiol concentration. All LMT in all matrices analyzed, evidenced an increasing trend by increasing percentage of MWP used. The soluble properties of LMT (*Guttemberger et al.*, 1992) had reduced their potential recovery on cheese. Adjustments in MWP production, to retain major LMT, and during cheesemaking, should be made to improve the LMT concentration in cheese produced with MWP.

Table 1

Descriptive statistics of milk, whey and Caciotta cheese thiols for both treatments (n=18)

Thiols[1], µM	Mean	SD	Minimum	Maximum
Milk				
Cys	33.24	3.86	27.93	43.41
Cys – Gly	1.35	0.23	1.08	1.93
γ – GC	0.54	0.22	0.23	0.84
GSH	0.81	0.28	0.43	1.58
Whey				
Cys	26.73	3.53	19.22	32.15
Cys – Gly	1.15	0.16	0.90	1.60
γ – GC	0.43	0.24	0.14	0.80
GSH	0.54	0.22	0.16	1.05
Cheese				
Cys	6.51	4.17	0.33	15.43
Cys – Gly	0.19	0.15	0.00	0.52
γ – GC	0.11	0.08	0.00	0.25
GSH	0.26	0.20	0.03	0.65

[1]Cys=Cysteine; Cys–Gly=Cysteine–Glycine; γ–GC=γ–Glutamylcysteine; GSH=Glutathione

Table 2

Results from ANOVA (*F* - value and significance) for milk, whey and Caciotta cheese thiols

Thiols[1], µM	Effect			RMSE[4]	R^2
	Date (MWP)[2]	Replicate	MWP[3]		
Milk					
Cys	0.86	1.15	1.28	3.88	0.41
Cys – Gly	6.47**	0.47	0.01	0.15	0.73
γ – GC	16.49**	1.33	0.48	0.10	0.88
GSH	2.08	1.12	1.32	0.26	0.51
Whey					
Cys	0.93	0.27	0.12	3.84	0.30
Cys – Gly	1.20	1.71	0.02	0.15	0.45
γ – GC	31.09***	1.81	0.26	0.08	0.93
GSH	2.87	0.88	2.29	0.16	0.66
Cheese					
Cys	0.23	0.94	1.53	4.74	0.24
Cys – Gly	9.45**	1.62	0.01	0.09	0.80
γ – GC	1.41	0.20	0.18	0.08	0.39
GSH	1.35	0.74	0.06	0.20	0.41

[1]Cys=Cysteine; Cys–Gly=Cysteine–Glycine; γ–GC=γ –Glutamylcysteine; GSH=Glutathione
[2]Data(MWP) = Data of analysis nested on MWP
[3]MWP = microparticulated whey proteins 3.0% and 4.0%
[4]RMSE = root mean square error
***P* < 0.01; ****P* <0.001

Table 3

Least squares means of milk, whey and Caciotta cheese thiols across different concentrations of MWP[1]

Thiols[2], µM	MWP 3%	MWP 4%
Milk		
Cys	32.28[a]	34.20[a]
Cys – Gly	1.35[a]	1.35[a]
γ – GC	0.48[a]	0.61[b]
GSH	0.72[a]	0.90[a]
Whey		
Cys	26.43[a]	27.03[a]
Cys – Gly	1.15[a]	1.16[a]
γ – GC	0.38[a]	0.49[b]
GSH	0.44[a]	0.64[b]
Cheese		
Cys	5.85[a]	7.17[a]
Cys – Gly	0.20[a]	0.19[a]
γ – GC	0.10[a]	0.12[a]
GSH	0.28[a]	0.25[a]

[1]MWP = concentration of microparticulated whey proteins
[2]Cys=Cysteine; Cys–Gly=Cysteine–Glycine; γ – GC=γ –Glutamylcysteine; GSH = Glutathione

CONCLUSIONS

Cheesemaking trials with different MWP concentrations has not led to significant changes in the concentration of LMT as cysteine, γ - glutamylcysteine, cysteine - glycine and glutathione in cheese. Moreover the cheesemaking leads to the loss of such molecules, most of which are found in the whey. Other studies should be performed to confirm that LMT are lost in the whey after cheesemaking; in particular need to be developed further methods for the quantification of non-soluble thiols, that may be bound to whey proteins, due to glutathionylation reactions that occur in the microparticulation process.

ACKNOWLEDGEMENTS

The authors express their gratitude to the dairy industry Latteria di Soligo for equipment and samples provided. The research was funded by the Project Acquadolce (PSR 2008 – 2013, Mis. 124).

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Corresponding author:

Giovanni Niero
Department of Agronomy, Food, Natural resources, Animals and Environment
University of Padova, Agripolis
Viale dell'Università 16, 35020 Legnaro (PD), Italy
Phone: +39 - 3155405
E-mail: giovanni.niero@studenti.unipd.it