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Short communication

Stability of α-tocopherol, γ-tocopherol and β-carotene during ripening of pasta-filata cheese made from raw and pasteurised milk with different vitamin contents

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ABSTRACT

Ripening stability of α-tocopherol, γ-tocopherol and β-carotene in cheese was evaluated in relation to different milk vitamin content and to the use of either pasteurised or raw milk. Milk from two farms with different management systems was used to obtain different vitamin content. Milk was divided into two parts, of which only one was pasteurised. Four blocks of cheese were made from each batch and ripened for 0, 15, 30, or 60 days at 14–16 °C. There was a notable variation in cheese vitamin levels, with the differences in milk vitamin content due to farm management having the highest impact. Pasteurisation had no effect on cheese vitamin content. Cheese γ-tocopherol and β-carotene content decreased after 30 and 60 days, respectively, whereas α-tocopherol content remained stable. γ-Tocopherol appeared to be the most efficient antioxidant in cheese, followed by β-carotene. Vitamin stability was not influenced by milk vitamin content or pasteurisation.

1. Introduction

Fat-soluble antioxidants delay initiation reactions in free-radical chain reactions. Even small changes in the fat-soluble vitamin content of milk could have important effects on oxidation processes and could interact with the ripening process to influence cheese quality.

Farm practices may represent the main factor that influences milk vitamin content. Feeding and mastitis are important as feeds contain different vitamin levels (Agabriel et al., 2007), and mastitis influences the oxidative stress state of the animals (Turk et al., 2012). Of the factors associated with dairy technology, the heating of milk for hygienic purposes (Smet et al., 2008) may be the greatest influence on cheese vitamin content.

The objectives of this study were to examine the ripening stability of α-tocopherol, γ-tocopherol and β-carotene in pasta-filata cheese in relation to different contents of the respective vitamins in milk, which may occur under realistic production conditions, and to the use of either raw or pasteurised milk.

2. Material and methods

2.1. Experimental design

Two Sicilian dairy farms with different management systems were chosen to obtain milk with different α-tocopherol and β-carotene content. Little attention has been paid to variation in γ-tocopherol content. Both farms had herds of Friesian cows in mid-late lactation, similar in production, but with different feeding systems. On the first farm, cows grazed for approximately 8 h each day and had integration with concentrates and hay. On the second farm, the cows were fed a total mixed ration (TMR). In addition, there were probably more animals with mastitis in the TMR-fed compared with the pasture-fed herd. Bulk milk from the TMR-fed herd had a high somatic cell count (SCC) of over 432,000 cells mL^{-1} (termed TMR/HSC milk), whereas pasture milk had levels of only 240,000 cells mL^{-1} (termed P/LSC milk). It is assumed that the different mastitis status could have increased the difference in α-tocopherol and β-carotene levels due to the different feeding practices, and led to a wide and at the same time, realistic range of possible milk vitamin levels.

Bulk milk from each farm was collected 3 times within the grazing season, in March 2012. On each test-day, on each farm, bulk

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milk samples from the combined milking of the morning plus the preceding evening were collected and divided into two parts. One was treated with a pasteurisation system at high temperature of 72 °C for a short time of 15 s, without homogenisation, whereas the other was not heated.

Ragusano-type pasta-filata cheeses from each farm and treatment were made according to the method described by Melilli et al. (2003) except for the addition of an artisanal whey starter culture of similar amounts to all samples. The curds were divided into 4 parts to produce blocks of about 4 kg each, for 4 different ripening times. One of these blocks was analysed before brining. The remaining three were placed into saturated salt brine for two days at 14–16 °C and, once removed, sampled after 15, 30 and 60 days. In accordance with the disciplinary norms for PDO Ragusano cheese, the cheeses were not packaged, but exposed to air in a ventilated room at 14–16 °C at a relative humidity of about 80–90%. Cheeses were stored on wooden *shelves* and rotated periodically to allow uniform rind development. Cheeses samples for analysis were stored at −20 °C.

2.2. Chemical analyses

Milk samples were analysed for fat, protein, dry matter, and lactose contents by MilkoScan™ Minor (FOSS, Padova, Italy). Milk pH was determined by a potentiometric method. Somatic cell counts (SCC) were measured with Fossomatic (FOSS). Cheese samples were analysed for dry matter and fat using the APHA (2004) method and the IDF (2008) method (ISO 3433), respectively. The determination of milk and cheese fat-soluble vitamin contents was as described by Marino, La Terra, Licitra, and Carpino (2010).

2.3. Statistical analyses

The statistical software (version 8.0.1, SAS Institute Inc., Cary, NC, USA) was used. The data of milk samples was processed with management (M), heating (H) and the interactions M × H as fixed effects, and replicate within M as random effect. The data of cheese samples was processed with M, H, ripening (R) and the interactions M × H, M × R, H × R and M × H × R as fixed effects, and replicate within M as random effect. Differences were considered significant at $P < 0.05$.

3. Results and discussion

3.1. Milk composition

Neither management nor pasteurisation had impact on crude protein or fat levels or pH of milk, with average values of 3.4 ± 0.02 g 100 mL^{-1}, 3.6 ± 0.15 g 100 mL^{-1} and 6.7 ± 0.02, respectively. Lactose level was slightly higher in TMR/HSC milk than in P/LSC milk (4.8 ± 0.02 and 4.7 ± 0.02 g 100 mL^{-1}, respectively).

As expected, different farm management regimes provided large differences in milk vitamin content. Milk α-tocopherol, γ-tocopherol and β-carotene levels were in the ranges 10.8–21.1, 0.7–2.3, and 1.2–8.1 μg g^{-1} fat, respectively. Alpha-tocopherol and β-carotene, but not γ-tocopherol content, were higher in P/LSC milk than in TMR/HSC milk.

Fat-soluble vitamins are sensitive to light, oxygen and temperature. However, the fat-soluble vitamin levels in milk fat were not affected by pasteurisation in this study, as reported by Marino et al. (2010).

3.2. Cheese chemical composition

3.2.1. Management and heat treatment effect on cheese chemical composition

There were no significant differences in mean moisture, fat content or cheese yield between cheeses in relationship to management or pasteurisation (Table 1). The fat-soluble vitamin contents of milk and cheese were highly correlated, which is in accordance with the observations made by Lucas et al. (2006). α-Tocopherol and β-carotene contents were higher in cheese made from P/LSC milk compared with that made from TMR/HSC milk, whereas management had no effect on γ-tocopherol levels. α-Tocopherol and β-carotene levels in cheese fat derived from P/LSC milk were 2.7 and 6.0 times, respectively, higher than those derived from TMR/HSC milk (Table 1). Pasteurisation had no effect on cheese vitamin contents.

3.2.2. Ripening effect on cheese chemical composition

The contents of dry matter and fat increased during the cheese ripening period (Table 2). In the present study, ripening time had no effect on cheese α-tocopherol content. Furthermore, there were no interactive effects between ripening time and original milk vitamin content on any of the measured vitamin contents in cheese. However, cheese γ-tocopherol content decreased by about 25% at 30 days of ripening or longer, and cheese β-carotene content by about 22% at 60 days of ripening, compared with the levels in unripened cheese (Table 2).

Ripened cheese is supposed to be a basically anaerobic and reducing environment with small redox-potential, and oxidation is not expected to be significant. Perna, Intaglietta, Simonetti, and Gambacorta (2015) reported an increasing antioxidant activity of pasta-filata cheeses during ripening, which was attributed to proteolysis and the formation of peptides with antioxidant activity. However, the decrease in γ-tocopherol and β-carotene contents in the present study suggest oxidation processes during ripening.

Table 1
Effects of management and heating on cheese composition.[a]

Item	Management			Heating			Effects (*P* value)						
	P/LSC	TMR/HSC	SEM	Raw	HTST	SEM	M	H	R	M × H	H × R	M × R	M × H × R
Chemical composition (g 100 g^{-1})													
Moisture	41.45	40.71	0.37	41.10	41.06	0.30	NS	NS	***	NS	NS	**	NS
Fat	28.35	29.90	0.01	29.34	28.91	0.01	NS	NS	***	NS	NS	NS	NS
Fat-soluble vitamins (μg g^{-1}fat)													
α-Tocopherol	15.49^{a}	5.82^{b}	0.82	11.00	10.30	0.74	***	NS	NS	NS	NS	NS	NS
γ-Tocopherol	0.47	0.86	0.08	0.70	0.61	0.06	NS	NS	***	NS	NS	NS	NS
β-Carotene	7.75^{a}	1.29^{b}	0.41	4.56	4.49	0.30	***	NS	***	NS	NS	NS	NS

[a] Abbreviations are: P/LSC, pasture feeding, low somatic cell count (240,000 cells mL^{-1}); TMR/HSC, total mixed ration feeding, high somatic cell count (432,000 cells mL^{-1}); Raw, raw milk; HTST, heat treatment short time (72 °C, 15 s); M, management; H, heating; R, ripening time. Under management, least squares means within a row with different superscripts differ significantly ($P < 0.05$). Significance of effects indicated by:**$P < 0.01$; ***$P < 0.001$; NS, not significant ($P > 0.05$).