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Dairy Sci. & Technol. (2013) 93:303–314
DOI 10.1007/s13594-013-0123-8

ORIGINAL PAPER

# Formation of biogenic amines and vitamin K contents in the Norwegian autochthonous cheese Gamalost during ripening

**Tahir Mahmood Qureshi · Cees Vermeer · Gerd E. Vegarud · Roger K. Abrahamsen · Siv Skeie**

Received: 30 August 2012 / Revised: 28 February 2013 / Accepted: 12 March 2013 /
Published online: 9 April 2013


**Abstract** Gamalost, a Norwegian mould (*Mucor mucedo*) ripened autochthonous cheese, is a potential functional food due to a high content of peptides that might reduce hypertension, however it has a high content of free amino acids which may be precursors for biogenic amines. This study aimed to investigate if Gamalost might have further health benefits or risks by determination of the formation of vitamin K and biogenic amines. The development of biogenic amines and vitamin K was analysed during ripening. Putrescine was the only biogenic amine detected by liquid chromatography in ripened Gamalost, in the range from 11 to 25 $mg.kg^{-1}$. The presence of very low concentrations of biogenic amines may suggest that Gamalost is devoid of hazards posed on health. The menaquinones (vitamin $K_2$) detected in Gamalost by high-performance liquid chromatography were MK-4 to MK-10 and among them, MK-9 was found in the significantly highest concentration (46 $\mu g.100\ g^{-1}$). The menaquinone content of Gamalost was attributed to the activities of the starter lactic acid bacteria used for fermentation during manufacture. Gamalost contained a significantly higher menaquinone content than Norvegia, a Norwegian cheese.

**Keywords** Gamalost · Ripening · Biogenic amines · Vitamin K

## 1 Introduction

Gamalost, an autochthonous mould ripened Norwegian cheese, is made by acid precipitation of pasteurised skimmed milk as described by Qureshi et al. (2012a).

T. M. Qureshi · G. E. Vegarud · R. K. Abrahamsen · S. Skeie (✉)
Department of Chemistry, Biotechnology and Food Sciences, Norwegian University of Life Sciences, Chr. Magnus Falsensvei 1, 1432 Ås, Norway
e-mail: siv.skeie@umb.no

C. Vermeer
VitaK and Cardiovascular Research Institute CARIM, Maastricht University, Maastricht, The Netherlands

The DL starter used is a mesophilic starter consisting of *Lactococcus* (*L.*) *lactis* ssp. *lactis*, *L. lactis* ssp. *cremoris*, *L. lactis* ssp. *lactis* biovar *diacetylactis* and *Leuconostoc cremoris.* As the cheese is made from skimmed milk, it contains negligible amounts of fat. The cheese ripens up to 30 days and during ripening *Mucor* (*M.*) *mucedo* cause extensive proteolysis. Normally, the cheese is sold after 10–12 days of ripening. Gamalost is a potential functional food due to its high angiotensin I-converting enzyme (ACE) inhibitory activity (Qureshi et al. 2012a). Foods with high ACE inhibitory activity might reduce hypertension and therefore have a positive impact on blood pressure. Although the proteolysis of Gamalost now has been well characterised (Qureshi et al. 2012a), we know little of the development of other compounds in Gamalost which may influence health such as the content and development of biogenic amines and vitamin K during ripening.

Biogenic amines (BA) have been identified in non-fermented foods such as fish, fruit, juices, vegetables, meat as well as in fermented foods like fermented vegetables, fermented meat, wine, beer and fermented fish (Silla-Santos 1996). They have also been identified in many cheese varieties such as Cheddar, Ras, Gouda (Ibrahim and Amer 2010), Spanish traditional cheeses (Roig-Sagués et al. 2002), Dutch-type hard cheeses (Komprda et al. 2007), Italian Pecorino cheese (Schirone et al. 2012) and many others (Stratton et al. 1991). In cheese, BA results from decarboxylation of amino acids which are primarily produced from degradation of cheese proteins (Ibrahim and Amer 2010) and microbes with decarboxylase activities (Galgano et al. 2001; Gardini et al. 2001). Formation of BA in cheese depends on various factors; such as ripening time, ripening temperature, pH and the presence of microorganisms having BA-producing capability through their proteolytic and decarboxylase activities (Linares et al. 2012). Generally, BA cause nausea, sweating and headache, but particularly phenylethylamine and tyramine may cause hypertension whereas histamine may be associated with hypotension (Halász et al. 1994). Stratton et al. (1991) reported that consumption of ripened cheese with high contents of BA may pose a risk on public health and histamine poisoning has been reported from Gouda, Swiss, Cheddar, Cheshire, Gruyere (Stratton et al. 1991). The proteolysis of Gamalost is substantial and the content of free amino acids in this cheese is rather high as compared to other cheeses (Qureshi et al. 2012a). The ripening of Gamalost is caused by a *Mucor* mould, which makes the ripening of Gamalost completely different from other cheeses, and the contribution of this mould to the production of menaquinones (MK) is unknown.

Vitamin K has been found in three forms, phylloquinone (vitamin $K_1$), menaquinones (vitamin $K_2$) and menadione (vitamin $K_3$, a synthetic vitamin K form; Booth, 2012). Menaquinone has a variable side chain length of 2–15 isoprene units and it is commonly written as MK-*n* where *n* represents the number of isoprenoid residues (Sato et al. 2001). Phylloquinone ($K_1$) is naturally present in an appreciable concentration in green plants and in certain vegetable oils (Binkley and Suttie 1995; Booth et al. 1995; Booth 2012) whereas the menaquinone, also known as vitamin $K_2$, is formed either from the products of bacterial reactions or from conversion of dietary phylloquinone during digestion. The menaquinones have been observed in many foods such as meat, chicken, eggs, milk and cheeses (Elder et al. 2006). Geleijnse et al. (2004) and Gast et al. (2009) suggested that sufficient dietary intake of menaquinone could be important in preventing coronary heart disease. Increased risk

of hip fracture has for instance been shown to be associated with low intake of dietary vitamin $K_1$ (Booth et al. 2003; Feskanich et al. 1999).

The aim of this present study was to investigate the formation of vitamin K and BA (as anticipated due to presence of high content of amino acids) during ripening of Gamalost. Gamalost was compared with Norvegia, the most consumed cheese in Norway.

## 2 Materials and methods

### 2.1 Collection and ripening of cheeses

Gamalost cheeses were manufactured as described by Qureshi et al. (2012a) and were kindly supplied by the dairy company TINE Meieriet Vik (Vik i Sogn, Norway). A peculiar step during manufacture is the cooking of the moulded cheese in whey at 90–95 °C for 1–2 h. Five cheeses from each of three separate batches were selected randomly at the dairy and one cheese from each batch was frozen fresh (day 0), before the mould *M. mucedo* was added. The other four cheeses from each batch were ripened for 10 days and then frozen. The frozen cheeses were transported to the Department of Chemistry, Biotechnology and Food Science (Ås, Norway). The frozen 10-day-old cheeses were thawed and ripened further for 20, 30 and 60 days at 4 °C. After sampling, the cheeses were refrozen and kept frozen until analysis. For comparison, three commercial Norvegia cheeses (ripened for ~90 days) from three different productions were included in the analytical work. The cheese was sampled according to International Dairy Federation (IDF) standard 50C (IDF 1995) and then grated with a manual grinder. Two different lactic acid bacteria (LAB) starter cultures (CHN 11 and CHN 22, Chr. Hansen, Hørsholm, Denmark) normally used for production of Gamalost and the mould *M. mucedo* were also tested for menaquinones content.

### 2.2 Chemical analysis of cheese

The fat content was determined by the Gerber–van Gulik method using a butyrometer (Ardö and Polychroniadou 1999) and the dry matter (DM) content was determined according to IDF standard 4/ISO 5534 (IDF 2004). The ash content was determined according to IDF standard 27 (IDF 1965) and the pH was monitored using a PHM 92 Lab pH-meter (Radiometer, Copenhagen, Denmark; Ardö and Polychroniadou 1999). The total nitrogen (TN) and soluble nitrogen (SN) content were determined by the Kjeldahl method according to the IDF standard 20B (IDF 1993) whereas the trichloroacetic acid soluble nitrogen (TCA-SN) was determined by the Kjeldahl method according to the procedure described by Christensen et al. (1991). Gamalost was not fully soluble in any of the solvents (water or citrate water) used and therefore produced some precipitates during solubilisation. In addition, foaming was a problem during preparation and Kjeldahl digestion. Due to these shortcomings, the analysis of the TN of Gamalost could not be reliable. As Gamalost does not contain measurable amounts of fat, the DM of the cheese may be considered to be approximately the same as the content of protein after subtracting the ash content from the DM. Therefore, the SN/DM of Gamalost was calculated, but SN/protein was calculated for Norvegia.

### 2.3 Determination of biogenic amines

The preparation of the samples and the determination of biogenic amines was done by liquid chromatography according to Smělá et al. (2003). Briefly, a homogenised aliquot of 10±2 g of grated cheese was extracted with 0.5 mL of 1,7-diaminoheptane (Sigma, St. Louis, USA) as internal standard and 15 mL of 5% TCA. After centrifugation (~2,550×*g*, 10 min, 4 °C), filtration through a disposable nylon membrane filter (13 mm, 0.45 μm, Chromatography Research Supplies, Addison, USA) was carried out after two time extraction of the sample. The separation of the biogenic amines was performed using a liquid chromatograph HP 1100 (Agilent Technologies, Waldbronn, Germany) consisting of a quaternary pump (G1311A), a vacuum degasser (G1322A), an auto sampler (G1313A), a UV/VIS detector with variable wavelength (G1314A), and a fluorescence detector (G1321A). The separation of biogenic amines after dansyl chloride (DnsCl) (Fluka, St. Louis, USA) derivatisation, was carried out by gradient elution with $H_2O$/ACN on a ZorbaxSB-C18 column (3.5 μm 3.0×150 mm) (Agilent Technologies) with a guard column Meta Guard ODS-2 (30×4.6 mm, particle size 5 μm) with a flow rate of 0.8 mL.min$^{-1}$ using a photometric UV/VIS detector at 254 nm. The detection limit for the BA in this study was 1 mg.kg$^{-1}$ of cheese.

### 2.4 Measurement of vitamin K

The vitamin K contents were measured according to Schurgers and Vermeer (2000) with some modifications. Briefly, aliquots of 1 g of cheese were extracted with 4 mL of 2-propanol, 20 ng internal standard (vitamin K1(25), i.e. phylloquinone equipped with an aliphatic side chain containing five instead of four isoprenoid residues) (GLSynthesis, Worcester, USA) and 2 mL of distilled water. Authentic K vitamins used as reference were: Phylloquinone and menaquinone-4 (Sigma, St Louis, MO, USA) whereas menaquinones MK-5 through MK-10 were kind gifts from Hoffmann-La Roche (Basel, Switzerland). The mixture was homogenised with a blender (Ultra Turrax, Janke and Kunkel, Staufen, Germany), warmed to 60 °C and extracted with 8 mL of *n*-hexane and pre-purified on Silica Sep-Pak cartridges (Millipore. Milford, MA, USA). The cartridges were washed with 100% hexane and vitamin K was eluted with 3% diethyl ether in *n*-hexane and evaporated until dryness under a stream of nitrogen at 50 °C. The residue was taken up in 80 μL of isopropanol and separation of vitamin K (both phylloquinone and MK) was performed by high-performance liquid chromatography (HPLC) on a C-18 reversed phase column (Thermoscientific BDS Hypersil; length, 100 mm; internal diameter, 3 mm; particle size, 3 μm; pore size, 120 Å) using fluorescence detection after post-column reduction on a zinc column (Riedel-DeHaën, Seelze, Germany) at 40 °C. The mobile phase for the HPLC system consisted of MeOH/acetonitrile/acetic acid/distilled water at a ratio (*v*/*v*) of 88:10:1:1 to which zinc acetate was added at a concentration of 1.1 g.L$^{-1}$. All reagents were of HPLC grade. Each sample was run in duplicate.

### 2.5 Statistical analysis

Statistical analysis was performed by Minitab statistical software version 15 (Minitab Inc., State College, PA, USA), using the general linear model and

Tukey's test for pair-wise comparison of means in analysis of variance (ANOVA). The Shapiro–Wilk test was performed to test the normal distribution of all variables and normality assumptions were found to be satisfied. Two-way ANOVA was used to compare the different ripening steps of Gamalost. The age (fixed variable) and batches (random variable) were used as classification factors in the statistical model, with the assumption that the individual cheeses from the same batch were independent. One-way ANOVA was used to compare the content of MK of 20 days Gamalost with Norvegia. The level of significance for all comparisons was set to $P<0.05$.

## 3 Results

### 3.1 Gross composition

Table 1 shows the pH, DM, SN/DM and TCA-SN/DM of the cheeses analysed. The pH of Gamalost increased up around 7 during the first 10 days of ripening whereas Norvegia had a pH around 5.4 after approximately 90 days of ripening compared to Gamalost. The ash content of ripened Gamalost was lower than Norvegia. The DM content of Gamalost increased during ripening, whilst Norvegia had 1% higher content of DM. The fat content in Gamalost was not measurable as the cheese was made from skimmed milk, while the fat content and fat in DM (in percent) of Norvegia was 26.4% and 46.2%, respectively. The protein (in percent) content of Norvegia was 26.5%. The SN/DM of Gamalost increased significantly to >10% during the first 10 days of ripening, thereafter the content remained statistically unchanged until 30 days of ripening. However during further ripening, the SN/DM increased further and after 60 days, it was significantly higher than in cheese ripened

**Table 1** The pH, ash, dry matter (DM), soluble nitrogen (SN), trichloroacetic acid (TCA)-SN (mean±SD, $n=3$) in Gamalost during ripening and in ripened Norvegia

| Cheese type | Age (days) | pH | Ash (%) | DM (%) | SN/DM[a] or SN/protein[b] (%) | TCA-SN/DM[c] or TCA-SN/protein[d] (%) |
|---|---|---|---|---|---|---|
| Gamalost | 0 | $4.64^{a}$±0.04 | $1.13^{a}$±0.01 | $45.11^{a}$±0.56 | $0.34^{a}$±0.14 | $0.26^{a}$±0.01 |
| Gamalost | 10 | $6.93^{b}$±0.04 | $1.45^{b}$±0.03 | $55.54^{b}$±1.17 | $10.47^{b}$±0.07 | $9.10^{c}$±0.16 |
| Gamalost | 20 | $7.04^{bc}$±0.02 | $1.49^{b}$±0.02 | $55.70^{c}$±1.16 | $10.41^{b}$±0.22 | $8.70^{b}$±0.20 |
| Gamalost | 30 | $7.08^{cd}$±0.02 | $1.50^{b}$±0.02 | $56.90^{c}$±0.63 | $10.64^{bc}$±0.13 | $9.20^{c}$±0.05 |
| Gamalost | 60 | $7.19^{d}$±0.08 | $1.48^{b}$±0.01 | $56.86^{c}$±0.58 | $11.06^{c}$±0.08 | $9.98^{d}$±0.09 |
| Norvegia | 90 | 5.41±0.01 | 3.14±0.11 | 57.25±0.28 | 2.86±0.07 | 1.93±0.09 |

Data in columns with different superscripts are significantly different ($P<0.05$) using Tukey's pair-wise comparison test

[a] Percentage of SN on dry matter (DM) basis in case of Gamalost

[b] Percentage of SN on protein basis in case of Norvegia

[c] Percentage of TCA-SN on dry matter (DM) basis in case of Gamalost

[d] Percentage of TCA-SN on protein basis in case of Norvegia

for 20 days or less. The SN/protein of Norvegia was 2.86%. The TCA-SN/DM (in percent) of Gamalost increased significantly during the first 10 days of ripening, but from 30 to 60 days of ripening, a significant further increase of TCA-SN/DM was established. The TCA-SN/protein content of Norvegia was 1.93%. No significant variation between the different batches of Gamalost was observed for the pH, DM, ash, SN/DM and TCA-SN/DM.

### 3.2 Biogenic amines in Gamalost

The BA detected in Gamalost and Norvegia are presented in Table 2. Putrescine was the only amine present in Gamalost in an identifiable concentration (>1 $mg.kg^{-1}$). Putrescine was not detected in unripened cheese, but appeared after 10 days of ripening. The content of putrescine increased significantly from ~12 $mg.kg^{-1}$ in cheese after 10 days of ripening to ~25 $mg.kg^{-1}$ in cheese after 20 and 30 days of ripening. During the remaining ripening period until 60 days, the content decreased, but a large standard deviation shows a varying decrease between the batches. There was however no significant difference in the content of putrescine between the batches. Putrescine was not detected in Norvegia; however, Norvegia had a low content of both tyramine, histamine and spermine. No other amines were present in Gamalost and Norvegia, at levels above the detection limits (1 $mg.kg^{-1}$).

### 3.3 Vitamin K content of Gamalost and Norvegia

The content of phylloquinone and menaquinones (MK-4 to MK-10) in Gamalost (development from 0 to 60 days) and Norvegia (at 90 days) is presented in Table 3. Phylloquinonein was present in very small concentrations compared to menaquinones (MK-4 to MK-10) in Gamalost. The concentrations of MK-4, MK-5, MK-6, MK-7 and MK-8 were not significantly influenced by the ripening of Gamalost. The MK-9 and MK-10 content increased significantly from 0 to 10 days of ripening, but did not change significantly during further ripening. Compared to

**Table 2** The formation of biogenic amines (milligram per kilogram; mean±SD, $n=3$) in Gamalost during ripening and the content in ripened Norvegia cheese

| Cheese type | Age (days) | Putrescine | Tyramine | Histamine | Spermine |
|---|---|---|---|---|---|
| Gamalost | 0 | <1 | <1 | <1 | <1 |
| Gamalost | 10 | $11.87^{a}$±0.76 | <1 | <1 | <1 |
| Gamalost | 20 | $25.17^{b}$±1.57 | <1 | <1 | <1 |
| Gamalost | 30 | $24.57^{b}$±2.28 | <1 | <1 | <1 |
| Gamalost | 60 | $16.15^{ab}$±7.00 | <1 | <1 | <1 |
| Norvegia | 90 | <1 | 5.56±0.06 | 1.43±0.29 | 1.09±0.10 |

Data in columns with different superscripts are significantly different ($P<0.05$) using Tukey's pair-wise comparison test

*<1* the levels were beyond the detection limit

**Table 3** The content of vitamin $K_1$ (phylloquinone) and vitamin $K_2$ (menaquinones) (MK-4 to MK-10) (μg/100 g; mean±SD, $n$=3) in Gamalost (0–60 days) and Norvegia (90 days)

| Cheese type | Rip days | MK-4 | MK-5 | MK-6 | MK-7 | MK-8 | MK-9 | MK-10 | Σ $K_2$[a] | $K_1$ | Σ ($K_1$+$K_2$) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Gamalost | 0 | 1.09[a]±0.09 | 0.58[a]±0.03 | 0.21[a]±0.01 | 0.68[a]±0.04 | 3.63[a]±0.18 | 30.57[a]±2.11 | 1.44[a]±0.23 | 38.20[a] | 0.16[a]±0.01 | 38.36[a] |
| Gamalost | 10 | 1.08[a]±0.13 | 0.67[a]±0.21 | 0.31[b]±0.10 | 0.96[b]±0.25 | 5.40[b]±1.62 | 45.59[b]±11.20 | 2.19[b]±0.44 | 56.20[c] | 0.17[ab]±0.01 | 56.37[c] |
| Gamalost | 20 | 1.02[a]±0.04 | 0.60[a]±0.04 | 0.27[ab]±0.01 | 0.91[b]±0.05 | 4.83[b]±0.44 | 41.42[b]±5.17 | 2.03[b]±0.28 | 51.06[b] | 0.18[b]±0.01 | 51.24[b] |
| Gamalost | 30 | 1.02[a]±0.18 | 0.63[a]±0.22 | 0.31[b]±0.10 | 1.06[b]±0.23 | 5.41[b]±1.39 | 46.21[b]±7.91 | 2.31[b]±0.5 | 56.93[c] | 0.18[b]±0.02 | 57.11[c] |
| Gamalost | 60 | 1.01[a]±0.14 | 0.58[a]±0.11 | 0.27[ab]±0.03 | 0.93[b]±0.04 | 4.84[b]±0.44 | 42.72[b]±4.37 | 2.28[b]±0.33 | 52.64[b] | 0.18[b]±0.01 | 52.82[b] |
| Norvegia | 90 | 5.10±0.90* | – | 0.30±0.04 | 1.33±0.15* | 5.25±0.52 | 29.55±3.64* | – | 41.53* | 4.38±0.38* | 45.91* |

Data in columns with different superscripts are significantly different ($P$<0.05) using Tukey's pair-wise comparison test

[a] Sum of vitamin $K_2$ (MK-4 to MK-10)

*$P$<0.05, significant difference between Norvegia and Gamalost ripened for 20 days

Norvegia, Gamalost contained very low amounts of vitamin $K_1$ and MK-4 and high concentrations of MK-9. The MK-6, MK-7 and MK-8 contents were almost similar in both cheese varieties. The MK-5 and MK-10 were not detected in Norvegia but in certain amounts in Gamalost. In total, the menaquinone content ($\sum K_2$) of Gamalost ripened for 20 days was significantly ($P<0.05$) higher than that of Norvegia.

Table 4 shows the content of MK-4 to MK-10 (μg.100 $g^{-1}$ cells) in the DL starter (CHN) and *M. mucedo* used for the manufacturing of Gamalost. The starter cultures contained a high content of menaquinone whereas *M. mucedo* was not found to contain even traces of menaquinone. The content of menaquinones seemed to be dependent of the dry matter content of Gamalost, where the content of vitamin K is calculated in percent of DM. Most of the menaquinone residues (except MK-4) showed no significant difference during ripening, whilst phylloquinone, although present in only small amounts, was significantly reduced from 0 to 10 days of ripening.

The content of MK-4 to MK-10 varied between the different batches of Gamalost and an interaction (batches and age) effect ($P<0.05$) was also observed (results not shown). The inter batch variance increased with increased ripening and for MK-4 it was 0.08, 0.15, 0.2 and 3.15 in 0, 10, 20 and 30 days ripened Gamalost, respectively, whereas for MK-10 it was 0.55, 1.96, 0.75, 2.49 in 0, 10, 20 and 30 days ripened Gamalost, respectively. The content of vitamin $K_1$ did not vary between the different production batches.

## 4 Discussion

### 4.1 Biogenic amines

The present study showed that the BA's were present in very low concentrations in Gamalost and Norvegia. According to Kebary et al. (1999) and Ordóñez et al. (1997) the development of BA depends on the starter cultures used and the bacterial strains present in the cheese. Many strains of LAB such as Enterococci, Carnobacteria, *Lactobacillus curvatus*, *Lactobacillus brevis*, *Lactobacillus buchneri* may be producers of tyramine, whereas formation of putrescine mainly has been caused by Enterobacteriaceae (Bover-Cid and Holzapfel 1999). Due to cooking of Gamalost at 90–95 °C for 1–2 h in whey, the bacteria and its enzymes present in the cheese were most probably dead and inactivated and BA expected from the activity of LAB in the cheese during ripening

**Table 4** The content of vitamin $K_2$ (menaquinones) (MK-4 to MK-10) (μg/100 g cells) (mean±SD, $n=3$) in the starter CHN 11 and CHN 22 (the species found in the starters: *L. lactis* ssp. *lactis*, *L. lactis* ssp. *cremoris*, *L. lactis* ssp. *lactis* biovar *diacetylactis* and *L. cremoris*) and in the mould (*M. mucedo*) used for the manufacturing of Gamalost

| Culture | MK-4 | MK-5 | MK-6 | MK-7 | MK-8 | MK-9 | MK-10 |
|---|---|---|---|---|---|---|---|
| CHN 11 | 1.29±0.11 | 6.23±0.62 | 4.57±0.18 | 14.10±0.17 | 94.80±4.24 | 472.40±22.63 | 12.09±0.11 |
| CHN 22 | 2.11±0.04 | 6.92±0.16 | 4.86±0.06 | 13.81±0.30 | 92.60±2.26 | 390.30±10.38 | 13.14±0.59 |
| *M. mucedo* | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

was therefore not observed. Only the mould (*M. mucedo*) which was added after cooking of the cheese was active in Gamalost throughout the ripening. Usually, the formation of BA in cheese increase during ripening (Kebary et al. 1999; Ordóñez et al. 1997) but in Gamalost the development of putrescine did not increase further during ripening beyond 10 days until 30 days even though the content of the precursors of putrescine, i.e. arginine and glutamine increased during ripening in our previous study (Qureshi et al. 2012a). After 30 days, the content of putrescine seemed to be decreased in the Gamalost, but as the standard deviation at 60 days was very high, that decrease seemed to be batch dependent. It may also be possible that putrescine was converted into spermine and spermidine but their concentration might be below the detection limit. The optimum pH range for the formation of BA through the decarboxylation of amino acids by the action of bacteria is 2.5–6.5 (Halász et al. 1994). Gamalost at day 0 had an acidic pH (4.6) but due to the inactivation of LAB, no decarboxylation activity was expected as manifested by the absence of BA in the cheese at this stage. The presence of a very low content of BA, with the exception of putrescine, in ripened Gamalost might indicate a very low decarboxylase activity of *M. mucedo*. Moreover, the strain of *Mucor* mould was not tested for BA and mycotoxin production. A positive correlation between free amino acids and the content of BA was found in low fat Ras, Semicotto Caprino and raw milk Hispánico cheese (Kebary et al. 1999; Fernández-García et al. 2000; Galgano et al. 2001). Norvegia was found to have low levels of tyrosine (precursor of tyramine), histidine (precursor of histamine) and glutamine (precursor of spermine) as determined previously (Qureshi et al. 2012b); therefore, it was reasonable that tyramine, histamine and spermine could only be formed in very low concentrations in Norvegia.

As the BA were not detected, or detected at very low concentrations in Gamalost and Norvegia, health hazards should not be expected from consumption of these cheeses. Moreover, healthy individuals have di-amino oxidase (EC 1.4.3.6), mono-amino oxidase (EC, 1.4.3.4) and polyamine oxidase (EC, 1.5.3.11) present in their tissues (especially present in the intestinal tract), and these enzymes play an important role in the detoxification of BA through acetylation and oxidation reactions during and after absorption of BA (Karovičová and Kohajdová 2005). But on the other hand, people having gastrointestinal disorders may be at risk due to the lower activity of the oxidases (Karovičová and Kohajdová 2005).

### 4.2 Vitamins K

The major menaquinones detected in unripened as well as ripened Gamalost were the long chain MK-8 and MK-9. It has previously been reported that the long chain menaquinones are not present in whole and skimmed milk whereas they have been found in appreciable concentrations in hard (Dutch) cheeses and soft (French) cheeses, probably due to fermentation caused by the bacterial starter (Schurgers and Vermeer 2000). Moreover, Morishita et al. (1999) observed considerable production of menaquinones (MK-8 to 10) by some LAB such as *L. lactis* ssp. *cremoris*, *L. lactis* ssp. *lactis*, *Leuconostoc lactis* when grown on reconstituted non-fat dry milk or in a soymilk medium. The *L. lactis* ssp. were responsible for MK-8 and MK-9 production whereas the *L. lactis* produced MK-9 and MK-10 (Morishita et al. 1999). The DL starter culture used for the manufacturing of Gamalost in the present experiment contained high amounts of menaquinones; therefore, the presence of a high content of MK-8 and MK-9

in Gamalost at day 0 was attributed to the fermentation of skimmed milk by LAB (DL starter) during the initial fermentation of the milk. The remarkable increase in MK-8 and MK-9 from 0 to 10 days of ripening was most probably due to a significant loss of moisture during the initial ripening of Gamalost. In this way, conversely marked increase in DM occurred and vitamin K was then concentrated per unit weight of cheese. As shown when vitamin K was calculated in percent of DM, the concentrations did not change during ripening, showing that the mould did not contribute to the production of vitamin K in Gamalost. This was evidenced also by the fact that the mould in Gamalost did not contain even trace amounts of vitamin K. Our findings of the presence of MK-8 and MK-9 in Gamalost as well as in Norvegia seem to be in agreement with others (Schurgers and Vermeer 2000; Morishita et al. 1999) as regards LAB as vitamin $K_2$ producers. Absence of MK-5 and MK-10 in Norvegia showed inability of the starter LAB to produce them during production of Norvegia, while their presence in Gamalost (even in low quantity) showed that the LAB most probably could produce these menaquinones during the initial fermentation of Gamalost. As a whole, 20-day Gamalost cheeses contained higher ($P<0.05$) contents of vitamin K compared to Norvegia. The most apparent difference between the initial fermentation of Gamalost and Norvegia is the lower pH in the first one. The lowest pH normally obtained in Norvegia is around 5.3 (Skeie 2001) in contrast to 4.6 in Gamalost, a higher fermentation rate in the latter may explain the differences in the content of menaquinones.

Since all K vitamins are lipophilic in nature (Shearer and Newman 2008), an efficient intestinal absorption of vitamin K may require the simultaneous consumption of fat such as butter or margarine (Schurgers and Vermeer 2000). Traditionally, Gamalost is consumed on bread with butter over and under the cheese layer.

## 5 Conclusions

This study showed that Gamalost might have interesting properties as a functional food by its high content of menaquinones in addition to its ACE inhibiting activity as shown previously. It may also be assumed that Gamalost is free from health hazards posed by biogenic amines.

**Acknowledgments** Tahir Mahmood Qureshi was funded by the higher education commission Pakistan. The experimental work was funded by a grant from the Norwegian Research Council, the Norwegian Foundation for Research Levy on Agricultural Products, the Norwegian Agricultural Agreement Research Fund and TINE SA. The authors are obliged to TINE Meieriet Vik through their kind supply of Gamalost cheeses. The authors are thankful to Eurofins Food & Agro Testing Norway AS, for the analysis of biogenic amines. Stefan Pantus (VitaK BV, BioPartner Center Maastricht, Maastricht University, The Netherlands) is also acknowledged for the analysis of vitamin K.

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