Evaluation of Natural Food Preservatives in Domestic and Imported Cheese

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Abstract

In milk and milk products, a number of organic acids naturally occur. We investigated the contents of some naturally occurred food preservatives (sorbic acid, benzoic acid, propionic acid, nitrite, and nitrate) contained in domestic and imported cheeses to establish the standard for the allowable range of food preservatives content in cheese. 8 kinds of domestic precheeses (n=104), 16 kinds of domestic cured cheeses (n=204) and 40 kinds of imported cheeses (n=74) were collected. Each domestic cheese was aged for a suitable number of months and stored for 2 mon at 5°C and 10°C. No preservatives were detected in domestic soft and fresh cheeses, except cream cheese. In case of semi-hard cheeses, 2-5 mg/kg of benzoic acid was detected after 1-2 mon of aging. In imported cheeses, only benzoic acid and propionic acid were detected. The average benzoic acid and propionic acid contents in semi-hard cheese were 8.73 mg/kg and 18.78 mg/kg, respectively. Specifically, 1.16 mg/kg and 6.80 mg/kg of benzoic acid and propionic acid, respectively, were contained in soft cheese, 3.27 mg/kg and 2.84 mg/kg, respectively, in fresh cheese, 1.87 mg/kg and not detected, respectively, in hard cheese, and 2.07 mg/kg and 182.26 mg/kg, respectively, in blended processed cheese.

Keywords: domestic cheese, imported cheese, benzoic acid, propionic acid

Introduction

In milk and milk products, a number of organic acids naturally occur, such as lactic, citric, sialic, benzoic, sorbic, propionic, and others (Urbiene and Leskauskaite, 2006). Benzoic, sorbic, and propionic acids are present in milk in less amounts. Note, however, that they are important because of their preservative properties (Kato et al., 1992; Lee et al., 1995). They are generally effective in controlling mold and inhibiting yeast growth and against a wide range of bacterial attack (Paster, 1979; Saad et al., 2005).

During fermentation, benzoic acid could be produced in fermented products (European Commission, 1995). It could be derived from benzaldehyde, which may be present at high concentrations in many cultured dairy products (Ihmof et al., 1995). According to the US Food and Drug Administration, benzoic acid and their potassium and sodium salts are “generally recognized as safe” (GRAS) (Boer and Nielsen, 1995). Although it is GRAS, adverse effects such as asthma, urticarial, metabolic acidosis, and convulsions were observed at low doses in sensitive persons (Mota et al., 2003; Qi et al., 2009; Saad et al., 2005). Sorbic acid and its salts (sorbates) are also considered GRAS additives (Boer and Nielsen, 1995). Note, however, that it has low toxicity because it is rapidly metabolized by pathways similar to those of other fatty acids. A few cases have been reported on the idiosyncratic intolerance to sorbic acid in human (Deuel et al., 1954; Hannuk-sela and Haahela, 1987). Increased propionic acid levels may interfere with the overall cellular metabolism. In propionic studies, genetic disorder could occur (Macfabe et al., 2007). Potassium or sodium nitrate prevents late blowing and gassy defects in cheese (Gray et al., 1979). It could be found in raw milk and loading, depending mainly on the quality of feed given to livestock (Baranova et al., 1993). Nitrate in cheese is reduced to nitrite by the xanthine oxidase present in milk or by the nitrate reductase produced by microorganisms (Munksgaard and Wermer, 1987). If the nitrate and nitrite level is exceeded, it can cause severe gastroenteritis with abdominal pain, blood in stool and urine, weakness, and fainting (Magee, 2015).

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1983). Nitrite is also involved in the formation of nitrosamines, compounds that are known to be carcinogenic (Gray et al., 1979).

Due to such adverse effects of natural food preservatives, a general standard is necessary. Because the standards of food preservatives in cheese in Korea are not clearly defined, or it does not reflect natural occurrence, we investigated the production of natural food preservatives in cheese during the ripening and storage period to establish the standard for the allowable range of food preservatives content in cheese.

**Materials and Methods**

**Cheese sample**
8 kinds of domestic precheeses (n=104), 16 kinds of domestic cured cheeses (n=204) and 40 kinds of imported cheeses (n=74) were collected from the market. Each domestic precheese was aged for a suitable number of months. And, each domestic cured cheese was stored for 2 mon at 5°C and 10°C.

**HPLC-PDA analysis of benzoic acid and sorbic acid contents**

**Standard and chemicals**
Commercial-standard (Sigma-aldrich, USA) benzoic acid and sorbic acid were used. HPLC-grade solvents were purchased from J.T. Baker (Phillipsburg, USA). Other reagents (analytical grade) were purchased from Wako (Japan).

**Sample preparation**
Sample preparation was conducted in accordance with the Korean Food Additives Codex (MFDS, 2014). Five gram of cheese sample was accurately weighed, with distilled water added until the total volume was 50 mL. It was vortexed for 1 min and sonicated for 20 min, and then filtered. 5 mL of filtrate was mixed with 1.5 mL of 0.1 N HCl and 0.5 mL of 0.005 M cetyltrimethylammonium chloride (CTA) solution.

Sep-Pak C18® cartridges (Waters Associates, USA) were prepared before use by successively washing each cartridge with 10 mL of methanol and 10 mL of 0.005 M CTA solution; the mixed solution was then applied to the Sep-Pak cartridge at flow rate of 2 mL/min. After washing with 10 mL of water, the solution was eluted with 10 mL of methanol. The solution was filtered with a 0.45-μm filter paper.

**HPLC-PDA analysis**
HPLC analysis of benzoic acid and sorbic acid was also conducted in accordance with the Korean Food Additives Codex (MFDS, 2014). An analysis of benzoic acid and sorbic acid was performed using high-performance liquid chromatography (HPLC) with photodiode array (PDA) (Shiseido Co., Ltd., Japan). The operating conditions were as follows: column temperature of 35°C; flow rate of 1.0 mL/min; injection volume of 10 μL; and PDA detection at 217 nm. The maximum extinction wavelength of benzoic acid and sorbic acid was 230 nm and 259 nm, respectively. Chromatographic separations were performed on SP^Column MF C8 (5.0 μm particle size, 150×4.6 mm i.d.; Shiseido), with the mobile phase consisting of 0.1% Tetrabutylammonium hydroxide (TBA-OH) (Phase A) and 100% acetonitrile (Phase B). The gradient elution conditions are given in Table 1.

**GC-FID analysis of propionic acid contents**

**Standard and chemicals**
Commercial-standard (Sigma) propionic acid was used.

**Sample preparation**
Sample preparation was conducted in accordance with the Korean Food Additives Codex (MFDS, 2014). 30 g of cheese sample was mixed with 100 mL of distilled water and neutralized with 10% NaOH or 10% HCl. Then, 10 mL of 15% tartaric acid solution, 80 g of NaCl, 1 drop of silicone resin, and 100 mL of distilled water were mixed in a 500 mL round-bottomed flask. 100 mL of distillate was obtained using steam distiller. 1 mL of trans-crotonic acid and 1 mL of 85% phosphoric acid were added and extracted with 50 mL of ether. After repeating the process twice, the ether was obtained and concentrated.

**Table 1. Gradient elution conditions of HPLC-PDA analysis of benzoic acid and sorbic acid contents**

| min | Phase A (%) | Phase B (%) |
|-----|-------------|-------------|
| 0   | 75          | 25          |
| 2.5 | 75          | 25          |
| 7.0 | 65          | 35          |
| 12.0| 60          | 40          |
| 15.0| 70          | 30          |

\(^{1}0.1\%\) TBA-OH. \(^{2}100\%\) Acetonitrile.
performed using gas chromatography (GC) with flame ionization detection (FID) (Hewlett-Packard Co., USA). The operating temperature conditions were as follows: temperature of 100°C (1 min) - 5 min - 170°C (4 min); injection temperature of 230°C; oven temperature of 100°C; and detector temperature of 250°C. HP-FFAP column (25 mm × 0.32 mm, 0.5 µm) (Hewlett-Packard Co.) was used in the analysis.

**HPLC-UV analysis of nitrate contents**

**Standard and chemicals**
Commercial-standard (Sigma) nitrate was used.

**Sample preparation**
Sample preparation was conducted in accordance with Jo et al. (2010). 10 g of sample was mixed with distilled water and homogenized. Mass of 100 mL was derived by adding deionized water. After 20 min in the water bath at 80°C, it was filtered with a membrane filter (0.25 µm).

**HPLC-UV analysis**
A modified version of a method of HPLC analysis of nitrate by Jo et al. (2010) was used. An analysis of nitrate was performed using HPLC with ultraviolet rays (UV) (Shiseido). The operating conditions were as follows: flow rate of 1.2 mL/min; injection volume of 20 µL; and UV detection at 230 nm. Chromatographic separations were performed on IonPac®AS14 (5.0 µm particle size, 250×4.0 mm i.d.; Thermo, USA), with the mobile phase consisting of 1.7 mM NaCO₃ (Phase A) and 1.8 mM NaHCO₃ (Phase B).

**Spectrophotometric analysis of nitrite contents**

**Standard and chemicals**
Commercial-standard (Sigma) nitrite was used. All chemicals used in the analysis were also obtained from Sigma.

**Nitrite analysis**
Nitrite production was measured according to the spectrophotometric analysis method of the Food Additives Codex (MFDS, 2014). 10 g of sample was mixed with 90 mL of 80°C water. 10 mL of mixed solution (0.5 N NaOH: 12% zinc sulfate [1:1]) was added and reacted in the water bath at 80°C. After cooling at room temperature for 3 h, 20 mL of 10% ammonium acetate (adjusted to pH 9.1 with ammonia solution) was added until the mass was 200 mL. It was left for 10 min and filtered. 20 mL of the filtrate, 1 mL of sulfanilamide, 1 mL of n-(1-naphthyl) ethylenediamine, and 3 mL of D.W were mixed. After 20 min, the absorbance was measured at 540 nm using an Optizen UV/visible spectrophotometer (Mecasys, Korea) (A). The concentrations of nitrite were determined as:

$$\text{Nitrite (g/kg)} = \frac{A}{\text{Sample (g)}} \times \frac{1}{100}$$

**Validation procedures**
For the method validation of the analysis of benzoic acid, sorbic acid, propionic acid, and nitrate, validation of the in-house laboratory method determining the limit of detection (LOD), limit of quantification (LOQ), linearity, repeatability, and accuracy was done. LOD and LOQ were calculated according to the following equations (Miller and Miller, 1993):

$$\text{LOD} = 3.3 \frac{S_a}{b} \quad \text{and} \quad \text{LOQ} = 10 \frac{S_a}{b},$$

where $S_a$ is the standard deviation of the intercept and $b$ is the slope of regression line obtained from the calibration.

Standard solutions were prepared at three concentrations of benzoic acid (1.0, 2.0, and 4.0 µg/mL), sorbic acid (1.25, 2.5, and 5.0 µg/mL), and nitrite (2.5, 5.0, and 10.0 µg/mL). After the preconditioning process, recovery was determined by HPLC analysis. In the case of propionic acid, standard solutions were prepared at concentrations of 10.0, 20.0, and 40.0 µg/kg, and recovery was determined by GC analysis after the preconditioning process.

**Statistical analysis**
Statistics were analyzed using a statistical analysis system (SAS, SAS Institute, Inc., USA). The results were showed as the mean and standard deviation (SD). The significance of the differences was assessed using one-way analysis of variance (ANOVA) together with Duncan’s multiple range tests. Values of $p<0.05$ were considered statistically significant.

**Results and Discussion**

**Method validation**
The linearity and sensitivity data of natural food preservatives are presented in Table 2. The linearity range was 0.2-8.0 µg/mL for benzoic acid, 0.25-10.0 µg/mL for sorbic acid, 1.0-80.00 µg/mL for propionic acid, and 0.3-20.0 µg/mL for nitrate. For each analysis, the linear regression curve showed a correlation coefficient ($R^2$) of over 0.99. The LOD values for benzoic acid, sorbic acid, propionic acid, and nitrate were 0.08, 0.38, 0.71, and 0.52 µg/mL, respectively. The LOQ values for benzoic acid, sorbic acid,
propionic acid, and nitrate were 0.23, 1.14, 2.14, and 1.58 μg/mL, respectively.

The precision and accuracy data for the determination of natural food preservatives are shown in Table 3. Experiments of repeatability and recovery were performed on three different days with the same instrument but different operators. Repeatability values were calculated as result dispersion in terms of standard deviation (RSD%). Recovery data were calculated by comparing the concentration of spiked cheese samples and determined by interpolation on the calibration curve with nominal fortification level. The intra-day RSDs for benzoic acid, sorbic acid, propionic acid, and nitrate were 1.82-4.02%, 0.78-4.44%, 2.28-5.31%, and 1.12-5.29%, respectively. In the case of inter-day RSDs for benzoic acid, sorbic acid, propionic acid, and nitrate, RSDs were 1.97-3.19%, 3.46-7.78%, 3.60-6.39, and 3.60-9.53, respectively. For method validation, intra- and inter-day RSDs must be less than or equal to 15% at all quality control and dilution control concentrations (Pereira et al., 2000). In our experiment, all of RSDs were less than 15%. Recovery values for benzoic acid, sorbic acid, propionic acid, and nitrate were 95.35-99.83%, 97.84-101.96%, 92.80-99.56%, and 94.27-95.22%, respectively. Compared to the international-level recovery value of 80-120 %, the recovery values in the test were good.

Natural food preservatives in domestic and imported cheeses

The amounts of five kinds of natural food preservatives (sorbic acid, benzoic acid, propionic acid, nitrite, and nitrate) in domestic cheeses during the ripening and storage period were investigated. Except benzoic acid, four kinds of natural food preservatives were not detected in domestic cheeses. The benzoic acid concentrations in domestic cheeses during the ripening and storage period are listed in Tables 4 and 5. In case of domestic soft and fresh cheeses except cream cheese, benzoic acid was not detected in all periods. The benzoic acid was detected in cream cheese at 3.44-3.83 mg/kg after 1-2 mon of aging in all types of semi-hard cheeses, and the concentrations were 2-5 mg/kg. The benzoic acid content was highest in Appenzeller cheese and one of Gouda cheese. The benzoic acid concentration was showed the tendency to increase during aging period and it did not increase highly during the storage period.

In imported cheeses, only benzoic acid and propionic acid were detected (Table 6). The average benzoic acid.

| Table 2. Linearity and sensitivity data of natural food preservatives |
|-----------------|-----------------|-----------------|-----------------|
| Compounds       | Linearity range | Regression equation<sup>1)</sup> | LOD (µg/mL) |
|                 | (µg/mL)         | y=ax+b          | (µg/mL) |
| Benzoic acid    | 0.20-8.00       | 8.63x10<sup>4</sup>-2.34x10<sup>3</sup> | 0.08 |
| Sorbic acid     | 0.25-10.00      | 5.35x10<sup>4</sup>-2.63x10<sup>3</sup> | 0.38 |
| Propionic acid  | 1.00-80.00      | 1.05x10<sup>2</sup>-1.09x10<sup>2</sup> | 0.71 |
| Nitrate         | 0.30-20.00      | 1.33x10<sup>3</sup>3.89x10<sup>3</sup> | 0.52 |

<sup>1)</sup>The regression equation is y=ax+b, where y is the peak area, x is the concentration of compounds (µg/mL), a is the slope, and b is the intercept. 2) r is the correlation coefficient.

| Table 3. Precision and accuracy data for the determination of natural food preservatives in spiked cheese |
|-------------------------------------------------|-------------------------------------------------|-----------------|-----------------|-----------------|-----------------|
| Compound            | Spiked concentration (µg/mL) | Detected concentration (µg/mL) | Recovery (%) | Intra-day test (n=3, RSD%) | Inter-day test (n=9, RSD%) |
|--------------------|-----------------------------|-------------------------|---------------|-----------------------------|-----------------------------|
| Benzoic acid       | 4.00                        | 3.81±0.08               | 95.35±1.99   | 2.09                        | 3.19                        |
|                    | 2.00                        | 1.97±0.04               | 98.60±1.79   | 1.82                        | 1.97                        |
|                    | 1.00                        | 1.00±0.04               | 99.83±3.99   | 4.02                        | 2.66                        |
| Sorbic acid        | 5.00                        | 5.06±0.04               | 101.14±0.30  | 0.78                        | 3.46                        |
|                    | 2.50                        | 2.55±0.15               | 101.96±4.64  | 4.44                        | 6.26                        |
|                    | 1.25                        | 1.27±0.07               | 97.84±5.03   | 3.77                        | 7.78                        |
| Propionic acid     | 40.00                       | 37.12±0.82              | 92.80±2.04   | 2.28                        | 3.60                        |
|                    | 20.00                       | 19.24±0.79              | 96.22±3.97   | 4.43                        | 6.39                        |
|                    | 10.00                       | 9.96±0.46               | 99.56±3.75   | 5.31                        | 5.78                        |
| Nitrate            | 10.00                       | 9.43±1.63               | 94.27±1.04   | 1.12                        | 9.53                        |
|                    | 5.00                        | 4.78±0.86               | 95.22±1.89   | 2.00                        | 8.89                        |
|                    | 2.50                        | 2.51±0.45               | 95.07±4.58   | 5.29                        | 3.60                        |

Values are shown as the mean±standard deviation of triplicate.
The benzoic acid content was highest in Emmental cheese made from Germany with 35.12 mg/kg. In the case of propionic acid, the concentration was highest in smoked cheese made in the Netherlands at 182.28 mg/kg. Moreover, it was generally high in Emmental cheeses. That is because Propionibacterium, the strain used in the production of emmental cheese, produces propionic acid. In the Korean Food Additives Codex (MFDS, 2014), the propionic acid content in cheese is limited to 3.0 g/kg. All of the values detected in imported cheese did not exceed the limit. This implies that lower limits may be set for propionic acid.

The benzoic acid content was not more than 35.12 mg/kg in all the cheeses we investigated. According to the report of Iammarino et al. (2011), they analyzed the benzoic acid content in 100 samples of cheese, and it was found to be 11.8-28.7 mg/kg. They estimated the maximum admissible limit of benzoic acid in cheese to be 40.0 mg/kg. The result corresponds to our study; we could also estimate that 40.0 mg/kg is a permissible amount of benzoic acid in cheese.

In a previous report of Kyriakidis et al. (1997), nitrate and nitrite were determined in 140 samples of Greek

### Table 4. Content of benzoic acid in domestic cheese during the ripening period (mg/kg)

| Co. | Cheese   | Number of sample | Aging period (mon) | 0 | 1 | 2 | 3 |
|-----|----------|------------------|--------------------|---|---|---|---|
| A   | Camembert| 8                | N.D.               | N.D. | N.D. | N.D. | - |
|     | Brie     | 8                | N.D.               | N.D. | 3.54±0.31" | 4.51±0.08" | - |
| B   | Appenzeller| 8                | N.D.               | N.D. | 2.60±0.40" | 2.98±0.22" | 2.91±0.20" |
|     | Caciocavallo| 21              | N.D.               | N.D. | 0.74±0.50" | 1.66±0.10" | - |
| C   | Gouda     | 16               | N.D.               | N.D. | 2.60±0.26" (3 mon) | 3.13±0.22" (4 mon) | 4.46±0.08" (6 mon) |
| D   | Raclette  | 21               | N.D.               | 1.93±0.38" | 2.13±0.20" | 2.12±0.24" | - |

"Not detected. Values are shown as the mean±standard deviation in triplicate. Mean values with different superscript in each row are significantly different (p<0.05)

### Table 5. Benzoic acid content of domestic cheese depending on the temperature and storage period (mg/kg)

| Co. | Cheese   | Number of sample | Storage period (mon) | 0 | 1 | 2 | 3 |
|-----|----------|------------------|----------------------|---|---|---|---|
| A   | Camembert| 20               | 5°C                  | N.D. | N.D. | N.D. | N.D. |
|     | Brie     | 20               | 10°C                 | N.D. | N.D. | N.D. | N.D. |
|     | Mozzarella| 5               | 5°C                  | N.D. | N.D. | N.D. | N.D. |
|     | String   | 5                | 10°C                 | N.D. | N.D. | N.D. | N.D. |
| B   | Appenzeller| 15              | 5°C                  | 4.51±0.08" | 4.33±0.17" | 4.35±0.27" | 4.54±0.11" |
|     | Caciocavallo| 25              | 10°C                 | 2.91±0.20" | 2.74±0.10" | 2.82±0.13" | 2.64±0.08" |
| C   | Gouda     | 25               | 5°C                  | 3.61±0.18" | 3.95±0.25" | 4.06±0.20" | 4.23±0.06" |
|     | Raclette  | 25               | 10°C                 | 2.12±0.24" | 2.19±0.11" | 2.16±0.13" | 2.14±0.07" |
| D   | Gouda     | 25               | 5°C                  | N.D. | N.D. | N.D. | N.D. |
|     | Raclette  | 25               | 10°C                 | N.D. | N.D. | N.D. | N.D. |
| F   | Camembert| 5                | 5°C                  | N.D. | N.D. | N.D. | N.D. |
|     | Brie     | 5                | 10°C                 | N.D. | N.D. | N.D. | N.D. |
|     | Mozzarella| 5               | 5°C                  | N.D. | N.D. | N.D. | N.D. |
| G   | Cream     | 9                | 5°C                  | 3.62±0.43" | 3.66±0.18" | 3.44±0.20" | 3.83±0.17" |
|     | Queso blanco| 5               | 10°C                 | 3.62±0.43" | 3.66±0.18" | 3.44±0.20" | 3.46±0.41" |

"Not detected. Values are shown as the mean±standard deviation in triplicate. Mean values with different superscript in each row are significantly different (p<0.05)

and propionic acid contents in semi-hard cheeses were 8.73 mg/kg and 18.78 mg/kg, respectively. Moreover, the average amounts of benzoic acid and propionic acid were 1.16 mg/kg and 6.80 mg/kg, respectively, in soft cheese, 3.27 mg/kg and 2.84 mg/kg, respectively, in fresh cheese, 1.87 mg/kg and N.D., respectively, in hard cheese, and 2.07 mg/kg and 182.26 mg/kg, respectively, in blended processed cheese. The benzoic acid content was highest in Emmental cheese made from Germany with 35.12 mg/kg. In the case of propionic acid, the concentration was highest in smoked cheese made in the Netherlands at 182.28 mg/kg. Moreover, it was generally high in Emmental cheeses. That is because Propionibacterium, the strain used in the production of emmental cheese, produces propionic acid. In the Korean Food Additives Codex (MFDS, 2014), the propionic acid content in cheese is limited to 3.0 g/kg. All of the values detected in imported cheese did not exceed the limit. This implies that lower limits may be set for propionic acid.

The benzoic acid content was not more than 35.12 mg/kg in all the cheeses we investigated. According to the report of Iammarino et al. (2011), they analyzed the benzoic acid content in 100 samples of cheese, and it was found to be 11.8-28.7 mg/kg. They estimated the maximum admissible limit of benzoic acid in cheese to be 40.0 mg/kg. The result corresponds to our study; we could also estimate that 40.0 mg/kg is a permissible amount of benzoic acid in cheese.

In a previous report of Kyriakidis et al. (1997), nitrate and nitrite were determined in 140 samples of Greek cheese.
The nitrate contents of cheeses varied from 0.7 up to 13.1 ppm. Only sample with value above 10 ppm was found. Nitrites had generally low values (below 1 ppm), with only 6 samples having values above 1 ppm. In the Greek Food Law (Greek Food Codex, 1994), only the presence of naturally existing nitrates and nitrites is accepted; the maximum permitted values are 10 mg/kg for nitrates and 2 mg/kg for nitrite. Because nitrate and nitrite were not detected in the cheeses we investigated, it could be permitted as per the Greek Food Law.

### Conclusion

In order to investigate the contents of naturally occurred food preservatives in cheese, we were collected domestic and imported cheese and investigated the production of sorbic acid, benzoic acid, propionic acid, nitrite and nitrate. In domestic cheese, benzoic acid is only detected.

### Table 6. Amount of benzoic acid and propionic acid in imported cheese (mg/kg)

| Type                  | Cheese          | Country     | Benzoic acid | Propionic acid |
|-----------------------|-----------------|-------------|--------------|----------------|
| Semi-hard cheeses     | Blue            | Denmark     | N.D.³        | N.D.           |
|                       | Gouda           | Netherlands | 8.25±0.41    | N.D.           |
|                       | Apple smoke gouda | USA         | 4.62±0.34    | N.D.           |
|                       | Cheddar         | UK          | 11.56±0.41   | N.D.           |
|                       | Edam            | Austria     | 2.59±0.24    | N.D.           |
|                       | Edam            | Netherlands | 1.06±0.26    | N.D.           |
|                       | Edam            | Switzerland | 1.58±0.63    | 27.15±7.89     |
|                       | Edam            | France      | 15.9±0.29    | 139.79±3.07    |
|                       | Edam            | Germany     | 6.21±0.82    | Under LOQ      |
|                       | Edam            | France      | 28.39±2.11   | 167.06±3.25    |
|                       | Edam            | Germany     | 35.12±5.27   | 2.22±0.16      |
|                       | Parmigiano reggiano | Italy    | 1.12±0.26    | N.D.           |
|                       | Raclette        | Switzerland | 2.58±0.48    | N.D.           |
|                       | Maasdam         | Netherlands | 4.31±1.20    | N.D.           |
|                       | Colby jack      | USA         | N.D.         | N.D.           |
|                       | Monterey jack   | USA         | 11.96±1.54   | N.D.           |
|                       | Chevrette       | Netherlands | 5.96±0.24    | 20.69±0.75     |
|                       | Rembrandt       | Netherlands | 3.13±0.14    | N.D.           |
|                       | Camembert       | Germany     | N.D.         | N.D.           |
|                       | Camembert       | France      | N.D.         | N.D.           |
|                       | Brie            | Germany     | N.D.         | N.D.           |
|                       | Brie            | France      | 2.42±0.27    | N.D.           |
|                       | Feta            | Greece      | N.D.         | N.D.           |
|                       | Gorgonzola      | Italy        | N.D.         | N.D.           |
|                       | Gorgonzola      | Italy        | N.D.         | N.D.           |
|                       | Saint André     | France       | 13.39±0.34   | N.D.           |
|                       | Rouy            | France       | 5.10±0.36    | 24.05±1.18     |
|                       | Snack           | France       | 5.74±0.33    | 27.88±1.21     |
| Fresh cheese          | Mozzarella      | Italy        | 1.91±0.41    | N.D.           |
|                       | Mozzarella      | USA          | 0.73±0.24    | N.D.           |
|                       | String          | USA          | 1.53±0.46    | N.D.           |
|                       | Cream           | USA          | 20.60±0.84   | N.D.           |
|                       | Mascarpone      | Italy        | 1.38±0.21    | N.D.           |
|                       | Mascarpone      | Italy        | N.D.         | N.D.           |
|                       | Ricotta         | USA          | N.D.         | N.D.           |
|                       | Ricotta         | Italy        | N.D.         | 22.68±3.94     |
| Hard cheese           | Gruyere         | Switzerland | 1.87±0.32    | N.D.           |
| Processed cheese      | Smoked          | Netherlands | 2.07±0.17    | 182.26±8.10    |

³Not detected. Values are shown as the mean±standard deviation in triplicate.
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The content was showed the tendency to increase during aging period and it was maintained during storage period. In case of imported cheese, benzoic acid and propionic acid were detected. These preservatives were generally highly detected in Emmental cheeses. The highest amount of benzoic acid was 167.06 mg/kg and the Emmental cheese was produced in Germany. The highest amount of propionic acid was 35.12 mg/kg and the Emmental cheese was produced in France.

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