Evaluation of the Microbial Quality and Total Phenolic Content of a Local Smoked Cheese

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Abstract
The increasing of the organic foods consumption to prevent the loss of cultural and social traditions that have survived for centuries, requires a proper assessment of the safety and quality of these products. The purpose of this study was the microbiological analysis of smoked cheese and non-smoked cheese, in order to assess the antimicrobial effect of smoke, as well as the determination of water-soluble phenolic compounds. The microbiological analyzes that were performed are: total number of aerobic mesophilic germs, coliform bacteria, coagulase-positive staphylococci, total number of yeasts and molds. The Folin–Ciocalteu test was chosen to measure total phenolic content of smoked cheese extracts. Microbial activity is reduced in smoked cheese types compared to non-smoked. The registered values were below the maximum admissible limits in the case of the total aerobic germs and shown coliform absence and inhibition in the growth of coagulase-positive staphylococci. Yeast and molds have been also inhibited through the smoking process. The total content of phenols varied from one assortment to another, with higher values in traditionally smoked cheese. The study shows that the microbiological quality of the cheese is greatly influenced by the hygienic conditions of processing and storage.

Keywords: cheese, phenolic content, microorganisms

Introduction
Consumers interest in organic and traditional products is increasing and international authorities and organizations are encouraging the research in this area. Traditional cheeses are obtained by technologies that differ according to geographic location, even if they are processed under poorer hygienic conditions. Developing countries, including Romania, have poor hygiene standards in traditional milk production, and that is reflected in the poor hygiene quality of traditionally made cheeses (Georgescu et al., 2014). In general, fresh cheese is characterized by a soft texture, low salt content, acidity and high humidity, which favor the growth of microorganisms, including pathogens capable of surviving even at refrigeration temperatures (Fogarasi et al., 2019). Also, there is a wide range of cheese varieties obtained by different processes and technologies on the Romanian market in order to meet the highly demanding standards of the customers.

The Romanian ‘Cascaval’ is a fine, soft, semi-hard, hard cheese processed of cow’s or sheep’s milk which has many varieties. Smoking is being practiced to improve its preservation degree and also to increase its sensory quality. Generally, smoking affects food which is high in protein with aromatic components and also plays bacteriostatic and antioxidant roles (Amran and Abbas, 2011).
Due to their nutritional value, especially the high protein and lipid content, dairy products are a suitable growth environment for a range of microorganisms (Laslo and György, 2018). The presence of coliform bacteria in cheese are indicators of faecal contamination that can cause undesirable defects in the product. The presence of coliforms in raw milk cheeses is often reported, because coliforms are common in raw milk, but in the case of the cheese obtained through the pasteurization process, they should be destroyed. Any coliforms present in the final products are the result of post-processing contamination that can occur in the processing or maturing plant through contact with contaminated water, air, equipment, humans (Martin et al., 2016).

In the case of dairy products, their shelf life is reduced because cheese is a favorable growth medium for a wide range of microorganisms (Kunová et al., 2015). The diversity of microorganisms present in cheese depend on the microbial characteristics, handling and heat treatment of the milk, manufacturing and curd-handling conditions, temperature and humidity during ripening, amount and manner of salting, and exposure of the cheese to exogenous microorganisms during and after manufacture (Banjara et al., 2015; Torkar and Teger, 2006).

The smoke resulting from the combustion of woody tissue, contains phenolic compounds belonging to the group of natural antioxidants and due to their molecular structure they are considered to be very efficient peroxide radical cleaning agents (Shaiban et al., 2006). The presence of the polyphenolic compounds in milk and later in cheese is the result of their transfer from plants to milk (Levkov et al., 2014), since pasture plants contain a significant amount of bioactive components that can be transferred into the mammary secretion and subsequently to the processed cheese (Hilario et al., 2010). They can influence both the milk and cheese taste and can also affect their antioxidant activity (Cheynier, 2005).

The purpose of this study consists in the microbiological analysis of the smoked cheese varieties existing on the local market, obtained by both traditional and technological procedures, in order to test the antimicrobial effect of the smoke, and also in the determination of the water soluble phenolic compounds from the dairy product, testing their antioxidant activity.

**Materials and methods**

**Cheese making and samples collection**

‘Cascaval’ is a type of cheese spread in Romania, and it has many varieties, not only in our country but also around the world. For the implementation of this research, seven cheese samples were selected, two of which were obtained by the traditional method in the household system, purchased from the market of the local producers from Baia Mare, and the other five samples were purchased from the supermarket. One of the two samples obtained in the traditional system was not subjected to the smoking process (non-smoked cheese-NSC), and the second sample was smoked (traditional smoked cheese-TSC). The smoked cheese samples purchased from the trade market were selected from five different brands as follows: smoked cheese B - SCB; smoked cheese D - SCD; smoked cheese H - SCH; smoked cheese P - SCP; smoked cheese L - SCL. The samples submitted to the study were obtained approximately 30 days ago. Traditional cheese is obtained by coagulating the fresh cow’s milk, scalding the pieces of curd in salt water, at a temperature of 90-92˚C and applying of a 24 hours fermentation. The obtained paste is placed in the mold, molded by pressing, so that no air remains in the curd. The cheese is dried for 2-3 days, and in the case of smoking preservation, the cheese is smoked for 4-5 days with cold smoke obtained from beech wood until the cheese gets a dark yellow color over the entire surface of the shell and gets its specific smoke flavor. After smoking under optimum conditions, the cheese enters the maturing stage for 14-18 days, at a temperature of 18-20 °C, being turned from one side to the other, on the first 3 days, 2 times / day, then, once every 2-3 days.

**Microbiological analysis**

Cheese samples were transported to the laboratory for microbiological analysis in accordance with the recomandations of SR EN ISO 6887-5: 2011. Microbiological analysis were performed in order to test the antimicrobial effect on smoked cheese samples, such as the total number of aerobic mesophilic germs, the total number of coliform bacteria, the number of coagulase-positive staphylococci and other species, as well as the total number of yeasts and molds.
Plate Count Agar (Standard Methods Agar) was used for the total aerobic bacteria count. Coliform bacteria detection was performed according to the national standard for the enumeration method of coliforms (SR EN ISO 4831:2009). Enumeration of Staphylococcus sp. was performed according to the SR EN ISO 6888-1:2002. The characteristic black colonies, brilliant, convex, surrounded by a clear area that may be partially opaque (Lazar et al., 2010) were counted. Microbiological analysis regarding the determination of the number of yeasts and molds present in smoked cheese was achieved according to a standardized method (STAS ISO 7954-2001) using Sabouraud Agar with Chloramphenicol as culture medium.

**Total Phenolic Content**

The Folin–Ciocalteu test was chosen to measure Total Phenolic Content (TPC) in the cheese samples. The preparation of the cheese extract was performed from 2 g of the homogenized sample and extracted for 30 minutes with 100 ml of 80% methanol containing 1% HCl at about 80°C on an orbital shaker at 200 rpm. The mixture was cooled, filtered and the residue was washed with the same solvent (Shaiban et al., 2006). Each sample was prepared in triplicate. 1 ml of extract was placed in an 100 ml volumetric flask, 60-70 ml of distilled water was added, shaked and then homogenised together with 5 ml of Folin-Ciocalteu reagent. 15 ml of 7.5% Na₂CO₃ solution was also added. After being placed for 30 minutes at room temperature, the absorbance was measured by a Perkin Elmer UV/VIS Spectrometer Lambda25 at a wavelength of 760 nm. The TPC content was calculated by the standard curve using standard gallic acid solution. The concentrations of the gallic acid in the solution from which the curve was prepared were 5, 25, 50, 100, 200 mg/L. TPC was presented as gallic acid equivalent (GAE) in milligrams per 100 g of extract ± standard deviation of triplicate analysis.

Both microbiological analysis and total phenol content were performed for all samples taken in the study on the first day (t₀) C and also at 21 days (t₂₁) after storage by refrigeration.

**Results and discussions**

Cheese processors try to bring on the market a wide range of products that meet the needs of consumers and at the same time they try to preserve the authenticity and the local tradition. However, the hygienic conditions for obtaining and fulfilling the legal requirements regarding the quality of the final product must be taken into account.

Thus, the microbiological analysis were performed on the smoked cheese assortments existing on the traditional market (TSC) and in supermarkets (SCB, SCD, SCH, SCP, SCL), as well as on a non-smoked cheese sample (NSC).

The results regarding the total number of germs indicated a higher number of germs in the non-smoked cheese sample, between 5.76 - 5.85 log CFU/g compared to the smoked samples in which the number varies between 5.04-5.03 log CFU/g in the initial test and 4.26-5.52 log CFU/g after 21 days of refrigeration storage (Figure 1). The lowest number of germs was registered in the commercial smoked cheese, SCB with 4.04-4.26 log CFU/g.
log CFU/g, followed by SCL 4.2-4.83 log CFU/g, respectively TSC 4.3-4.96 log CFU/g. In SCH, the number of germs is similar to the non-smoked sample, being registered a value of 5.03-4.52 log CFU/g.

Total aerobics increased during storage period by refrigeration. However, the values obtained in this study are lower compared to the results obtained by Amran and Abbas, (2011), on the local Yemen smoked cheese. In the cheese obtained under traditional conditions, the degree of contamination can be higher, because the manual manipulation is performed at different stages of processing.

Smoking has an inhibitory effect on coliforms, and their presence is not detected in smoked cheese samples obtained in both traditional and commercial systems. This was also demonstrated by the result recorded in the case of non-smoked cheese, in which the presence of fecal contaminants was reported in a number of 45 CFU/g in the initial phase (t₀) and of 92 CFU/g after 21 days of storage. This result indicates the degree of contamination of the non-smoked cheese, which occurred during the handling, maturation and storage of the product. Previous studies of Brooks et al., (2012) and Trmčić et al., (2016), showed that in raw and pasteurized milk cheeses there were samples that were positive for coliforms in concentrations greater than 10 cfu/g.

Contamination with pathogenic microorganisms, such as bacteria from the group Staphylococcus sp., is commonly found in cheese, given its tolerance to salt. The presence of coagulase-positive staphylococci is a parameter that is frequently determined in cheese to avoid the formation of staphylococcal enterotoxins that can trigger foodborne toxicity to consumers. In the analyzed cheese samples, the incidence of coagulase-positive staphylococci contamination is reduced, being inhibited by the smoking process. According to Regulation no. 2073/2005 in smoked and non-smoked matured cheeses, coagulase-positive staphylococci should not exceed 1000 CFU/g, regarding microbiological criteria for foodstuffs.

In the non-smoked cheese, the number of coagulase-positive staphylococci exceeds the maximum allowed value, being registered a number of 4.14 log CFU/g initially, and after 21 days it reaches 4.23 log CFU/g. In smoked cheese samples the number of Staphylococcus sp. does not exceed 3 log CFU/g; in the initial phase of determination being even absent in the samples from the SCB trade, respectively SCL, while a slow growth being recorded after 21 days (Fig. 2).

In all of the cheese variants, there is a tendency of increasing the number of pathogenic bacteria when the samples are kept, a fact also reported by Ramsaran et al. (1998), who have showed that pathogens that have survived in the product can grow in large numbers of cells during cheese maturation and storage.

It is known that yeasts and molds significantly affect the cheese; the sources of contamination come either from the environment or from the

![Figure 2. Variation of CFU coagulase-positive staphylococci](image-url)
brine, the processing equipment, the workers, etc. (Borelli et al., 2006). Sometimes they can have beneficial effects, but most of the time they can cause the food alteration.

In the non-smoked cheese, the number of yeasts and molds is higher than in the smoked samples, which indicates the benefit of preserving the cheeses by smoking. If in the case of non-smoked sample NSC the number is between 3.62-3.75 log CFU/g, in the smoked samples the value of 3 log CFU/g is not exceeded, being observed a tendency to increase in the TSC sample obtained in the household system (2.74-2.86 log CFU/g) according to fig.3.

According to Turkoglu et al., (2003), the progressive growth of molds and yeasts during storage is due to the fact that these microorganisms can metabolize lactic acid. Abdalla and Mohammed, (2010), also showed tendency to progressive yeasts and molds multiplication in the product.

At the same time, together with the determination of the microbiological parameters, the Total Phenolic Content (TPC) was determined. The total content of phenolic compounds in different types of cheese has been determined in order to test the antioxidant capacity of the smoke used in the product preservation process.

The results expressed as milligrams of gallic acid/100 grams ± the standard deviation of the triplicate analysis, both in the initial stage (t0) and after 21 days (t21) of storage by refrigeration are presented in Tab.1.

In general, milk and milk products have a lower content in compounds with antioxidant action compared to fruits and vegetables. However, in the current research it has been shown that certain categories of dairy products have a content of phenolic compounds accumulated with the ingestion of fodder. Smoke has a beneficial effect on the product, a fact proven by the results obtained on the assortments of smoked cheese both in the

![Figure 3. The Total Molds and Yeasts](image-url)

*NSC- non-smoked cheese; TSC- traditional smoked cheese; SCB- smoked cheese B; SCD- smoked cheese D, SCH - smoked cheese H, SCP - smoked cheese P, SCL - smoked cheese L.

**Table 1. The phenolic content of the smoked cheese (mean ± S.D.)**

<table>
<thead>
<tr>
<th>Samples</th>
<th>Total phenolic content (mg GAE/100 g extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$t_0$</td>
</tr>
<tr>
<td>NSC</td>
<td>114.1 ± 1.97</td>
</tr>
<tr>
<td>TSC</td>
<td>585 ± 2.45</td>
</tr>
<tr>
<td>SCB</td>
<td>697 ± 2.82</td>
</tr>
<tr>
<td>SCD</td>
<td>255.4 ± 3.11</td>
</tr>
<tr>
<td>SCH</td>
<td>333.3 ± 3.23</td>
</tr>
<tr>
<td>SCP</td>
<td>359 ± 2.12</td>
</tr>
<tr>
<td>SCL</td>
<td>497 ± 1.97</td>
</tr>
</tbody>
</table>

Note: NSC- non-smoked cheese; TSC – traditional smoked cheese; SCB, SCD, SCH, SCP, SCL - commercial smoked cheese.
initial stage, at their purchase, and after storage by refrigeration for 21 days.

Following the storage of the product by refrigeration, it was observed an increase of the phenols content in all the cheese variants, the most obvious antioxidant effect being in the case of commercial smoked cheese SCB of 952.3 ± 0.82 mg GAE/100 g extract, followed by the traditional smoked cheese of 859.1 ± 2.23 mg GAE/100 g extract.

Previous research (Levkov et al., 2014; Shaiban et al., 2006) also shows that the microorganisms activity of milk and cheese, as well as the activity of enzymes, may influence the TPC content in the product. During the refrigeration process, the TPC concentration probably varies due to the number of chemical and enzymatic reactions that these unstable compounds undergo.

Conclusions

Following this study, the microbiological analysis show a reduction in the number of microorganisms in the smoked cheese samples, compared to the non-smoked cheese. The absence of coliform bacteria, the presence of coagulase-positive staphylococci in a small number, similar to the yeasts and molds number, highlights the antimicrobial character of the smoke absorbed on the cheese surface.

Also, it has been observed that the cheese obtained in the traditional system has a higher degree of contamination compared to the one purchased from the trade market. The storage time influences the microbiological quality of the cheese, an aspect observed during the determinations made in the first and in the 21 days.

The content of the total phenolic compounds in the two types of cheeses is different, being observed an increase of TPC content in smoked cheese samples, compared to the non-smoked sample. Analysing the evolution of the phenolic content during the determination, a more obvious increase was observed in the case of the traditional smoked cheese sample, compared to the commercial samples. The effect of the progressive increase of the total polyphenol content during storage was probably due to their decomposition into smaller molecules that influence the final results.

References


