AUTHENTICITY ANALYSIS OF 100% SHEEP’S BRYNDZA FROM SELECTED ESTABLISHMENTS IN THE SLOVAK REPUBLIC

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ABSTRACT

Cheese was in the past, and still is today, one of the leading commodities. The production of “bryndza” has been preserved over the years, and today it is represented by the Protected Geographical Indication “Slovenská bryndza” (Slovakian bryndza). There are two types of “Slovenská bryndza”, mixed or 100% sheep. However, the name ‘traditional’ belongs only to sheep's bryndza. The high demand for a particular foodstuff also brings risks associated with its quality and fairness. The same is true of the national heritage product, sheep's bryndza. Identifying the originality or authenticity of various animal food products has received considerable attention recently. Our work has focused on the control of adulteration of 100% sheep's bryndza. Purchased samples from different parts of Slovakia were analyzed with a focus on the addition of cow's milk to sheep's bryndza. The reference method for determining the presence of cow's milk in sheep's cheese is based on detecting cow's γ2- and γ3-caseins from cheese after electrophoretic separation on urea-polyacrylamide gels. This method has proven to be reliable and sensitive. We tested sheep's bryndza made from both raw milk and heat-treated milk. The method of checking for adulteration of the cheese proved successful and in one sample we found the presence of cow casein in smaller amounts, probably due to accidental contamination. Isoelectric focusing of γ-caseins after plasmylosis is suitable for qualitatively determining sheep and cow proteins in traditional Slovak bryndza.

Keywords: Slovak bryndza; isoelectric focusing; adulteration

INTRODUCTION

Throughout centuries, Slovakia has cherished its rich dairy heritage, and at the heart of this tradition lies the exquisite sheep's lump cheese and the prized sheep's bryndza, celebrated for their exceptional flavors and cultural significance. A Slovakian man named Ján Vagač was the first to manufacture bryndza commercially in 1787. His process yielded a cheese that was fatter than what the shepherds were making, making it easier to spread while improving the shelf life. In the 20th century, further developments in bryndza's production occurred, creating a texture that was even more spreadable. At this time, around 80 producers were making the cheese in Slovakia. Teodor Wallo was among them and enhanced the creaminess by introducing a saline solution method. This gave the Slovakian version of bryndza its own specific quality, distinct from versions in Romania and neighboring countries (Keresteš, 2008). Among Slovakia's most sought-after dairy products are the traditional sheep lump cheese and the traditional sheep's bryndza made from it, which has been awarded the Protected Geographical Indication (PGI) designation. “Slovenská bryndza” (Slovak bryndza) is the traditional cheese of Slovakia (EC, 2007). Typical Slovak dishes like “bryndzová halušky” (a national dish made with dumplings and bryndza cheese), “bryndzové pagáčiky” (scones made with bryndza cheese), “prohol” (stuffed with bryndza cheese), and many more are created with this cheese. Sheep's bryndza is specific regarding its taste, aroma, and positive properties for the human body. Bryndza cheese includes several predominant lactic acid bacteria (LAB) from the Lactobacillus spp., Enterococcus spp., Lactococcus spp., and Streptococcus spp. (Jurkovič et al., 2006; Berta et al., 2009). It was created by accident and its recipe has been slightly modified to achieve greater health safety and sensory quality or durability (EC, 2008). A lump of matured ewe's cheese, a mixture of this cheese and a lump of cow's cheese, or a combination of a stored lump of matured ewe's cheese and a lump of cow's cheese, is used to make “Slovenská bryndza” a natural cheese. “Slovenská bryndza” must have a minimum of 50% w/w ewe's cheese in its dry matter (MARD SR, 2016). The quality of bryndza cheese depends on many factors, for example milk production period, the quality of ewe milk and the use of starter (Planý et al., 2016). The quality of bryndza cheese including its composition, physico-chemical properties and microbial diversity depends on the quality of the ewe milk (Chebeňová-Turcovská et al., 2011; Pangallo et al., 2014; Šádecká et al., 2014). However, it has retained its essence and effects. The widespread interest in this type of product may mean that these animal products are susceptible to substitution by cheaper milk for economic gain. Milk adulteration is an emerging problem worldwide, affecting consumers, the food industry, and inspection authorities (Zajac et al., 2023). Milk authenticity testing in dairy products is necessary to protect consumers from fraudulent products, mislabelling, and health risks and to prevent unfair competition from the food industry. Thirteen Slovak producers of sheep bryndza from different parts of Slovakia were presented. The experimental research aimed to analyze the authenticity of the sheep's bryndza and check whether it was adulterated by adding milk from another type of dairy animal. The analysis was preceded by defatting the samples in a Soxhlet extractor and obtaining powder for the detection of cow proteins. The procedure used in the analysis of bryndza was presented, and finally, the obtained results were presented and compared with other analyses of sheep bryndza.

MATERIAL AND METHODS

Material

We analyzed 13 different samples of sheep's chard. The samples were purchased from shepherd's huts in different towns in Slovakia. The purchased samples were kept in a refrigerator at -12 °C until the day of testing.

Methodology

We needed to defeat the bryndza samples before the analysis as it is a high-fat product and we wanted to get the most accurate results. Before isoelectric focusing we separated the fat from the samples on a Soxhlet extractor, the water was evaporated during this process, so we obtained a powder composed of protein and carbohydrate. We performed the extraction three times to separate the fat from the samples as much as possible.

Isoelectric focusing

First, we turned on the heat sink (Serva, Germany) to cool the ceramic plate (Serva, Germany) to 10 °C. The ceramic plate was cleaned with distilled water and wiped dry. We then pipetted 3 ml of the H-shaped cooling mixture. We placed the 0.65 mm GEL-FIXTM polyacrylamide gel fixation film on the prepared plate without air bubbles in a precise position, which was important to record the results in the correct boxes. Prefocusing was started with the Method PrefMIIl program and was
run at 1000 V, 50 mA, and 10 W for 20 min. We then applied thirteen samples, each of 5 μl, and two markers (Table 1). We placed electrodes on the gel. The isoelectric focusing program was carried out in three phases at 500 - 2000 V, 20 mA, and 10 - 30 W at different time lengths for a total of 3.5 hours.

Figure 1 Map with locations of sheep’s bryndza sample production

Table 1 Location of the sample on the gel

<table>
<thead>
<tr>
<th>Position on the gel</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>16</th>
<th>17</th>
<th>18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample number</td>
<td>M</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9</td>
<td>10</td>
<td>11</td>
<td>12</td>
<td>13</td>
<td>M</td>
</tr>
</tbody>
</table>

M - marker

After isoelectric focusing was completed, we carefully removed the gel from the ceramic plate with tweezers and placed it in a tray, which we fixed.

Fixing, coloring, bleaching, and drying

Using fixative solutions, we stabilized the gel. We placed the gel in a plastic container with tweezers, covered it with 20% TCA solution, and covered it. We placed the tray on a shaker (GFL, The Netherlands) where the gel was washed. After 45 minutes, we removed the gel with tweezers and immersed it in 5% TCA solution, in which it was washed for 30 minutes, and covered. We removed the sample and immersed it in the regenerative decolorizing solution for half a minute. This immersion was performed twice. We immersed the gel in a tray of copper sulfate pentahydrate staining solution for 45 minutes and rewashed it at 100 - 200 rpm on a shaker (GFL, The Netherlands). The stained gel was immersed for 20 min in a tub of decolorizing solution. Then we rinsed the gel with distilled water. Washing is important to obtain the correct result. Once the gel was decolored and the desired color was obtained, we placed it on a paper handkerchief and blotted the underside of the foil. The top part with the gel dried in the air.

Photography and analysis of the gel photo

This was followed by photo documentation with Azure biosystems (Dublin) photo equipment designed for photographing gels. The obtained photographs were analyzed using the computer program Gel-Pro Analyzer 6.0. We labeled the protein fractions (bands) captured on the gel in lanes according to their size and density. A report was generated from our recordings in which the migration distances of the proteins on the gel were displayed, and the sizes of the bands (bands) were plotted on graphs according to the height of the peaks. A high concentration of a particular protein in a given band (strip) represents its darker coloration and higher peak height in the graph than other bands.

Canning

Finally, gel preservation was performed by immersion in glycerol solution for 30 min and final drying was done free air drying. We archived the gel obtained.

RESULTS AND DISCUSSION

The aim of the experimental part was to the detection of adulteration of Traditional Slovak Sheep’s Bread by the addition of cow’s milk. Thirteen samples of sheep’s bryndza were purchased during the winter months from the end of September to October, i.e. winter bryndza samples. The samples were kept in a freezer at -12 °C, which was then convenient for us when preparing the samples for extraction, as they were easier to grate into smaller pieces. Defatting the samples before isoelectric focusing was the more time-consuming part of the work as we did all the samples in two repetitions making 26 samples. To obtain perfectly fat-free solids, we repeated the extraction three times until the metal container where the fat flowed was empty and dry. Thanks to the triple extraction of the samples, we obtained a representative result recorded on the gel after isoelectric focusing. After isolation and hydrolysis of casein by plasmin, we obtained separated sheep γ2- and γ3-caseins in each sample in the photographs. Other protein fractions (bands) are also captured on the resulting gel without a scattered fat layer. We obtained the following results:

Samples analyzed
M - MARKER (numbers 1 and 15 on the gel)
1. Salaš Pastierska (number 2 on the gel)
2. Syrmix Žázrivá (number 3 on the gel)
3. Agronova Liptov (number 4 on the gel)
4. Bryndziareň Turčianske Teplice (number 5 on the gel)
5. Syrex Žázrivá (number 6 on the gel)
6. Salaš Pružina (number 7 on the gel)
7. Bryndziareň Slatina (number 8 on the gel)
8. Gemerské ovečky (number 9 on the gel)
9. Jožko Farmárik (number 10 on gel)
10. GreenSheep Zvolenská Slatina (number 11 on the gel)
11. Farm Strelnica (number 12 on the gel)
12. Ecofarm Važec (number 13 on the gel)
13. Farm Dlhé nad Cirochou (number 14 on the gel)

The results indicate that no cow’s milk was added to samples 2 - 13 (numbers 3 - 14 on the gel) and this means that the samples are fine, have not been adulterated, and are 100% sheep's bryndza.

Figure 2 Gel showing protein fractions

Figure 3 Gel image showing the thickness, size, and number of fractions present

In the test field of sample number 1, Salaš Pastierska (number 2 on the gel), a small amount of protein not belonging to sheep protein was detected between two bands in the field. There are several reasons or causes for the occurrence of this protein fraction. Due to the weaker intensity of the band, it is difficult to discern the correct reason. It is, however, a band from the cow fraction. The cause of this 'non-sheep' fraction may be an attempt to adulterate the product by adding cow’s milk before making the lump cheese, or improper, inconsistent sanitation of the equipment in the production area of the plant/shed, or a sales error where the salesperson did not use different tools when serving the customer for each range.
The adulteration problem has also been detected and controlled in Romania, where cow’s milk is also added to buffalo, goat, and sheep milk. To avoid unfair competition between producers, methods have been devised to identify the authenticity of dairy products. Most of them are based on milk protein analysis. However, the European methodology for identifying the authenticity of IEF cheeses by analysis of gamma casein fractions has the disadvantage in this case that goat’s and sheep’s milk cannot be distinguished. Therefore, the study by Balteanu et al. (2011) aimed to test the possibility of using all alleles described so far to distinguish between the milk of these species. Interspecies milk mixtures were prepared from reference samples containing all described genetic variants of the four farmer species, combinatorially with each other. The IEF behavior of each major milk protein and their species-specific genetic variants were used to distinguish each milk type, including differentiation between goat and sheep milk, and cow and Romanian buffalo milk. The preliminary results of their work indicate the applicability of this proposed method for the authentication of dairy products.

They also indicate the possibility of using \(\beta\)-casein CBT as a marker to identify the authenticity/origin of Romanian buffalo cheeses. According to the analysis by Illou et al. (2020) of sheep milk from the Ouled-Djellal and Rembi sheep species, reared in North Africa, we can agree and confirm the presence of \(\alpha\)-caseins in our samples, despite the different breed from the Slovak breed. The analysis results were obtained in the same way using isoelectric focusing.

Figure 6 Negative gel image with different types of caseins

Figure 7 Negative gel image confirming \(\alpha\)-caseins

Figure 8 is a photograph of the gel after isoelectric separation of \(\gamma\)-casein from cheese samples along with one sample of 100% cow and one sample of 100% sheep protein. The results of the research by Špoljaric et al. (2013) confirm the adulteration of sheep’s cheese by the addition of cow’s milk.

Table 2 Identified protein fractions and their isoelectric points

<table>
<thead>
<tr>
<th>Protein fraction</th>
<th>Isoelectric point</th>
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<tbody>
<tr>
<td>(\gamma)-2-casein</td>
<td>7.48 – 7.56</td>
</tr>
<tr>
<td>(\gamma)-3-casein</td>
<td>6.53 – 6.74</td>
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</table>

Zajác et al. (2021) state that the IEF laboratory method is suitable for determining the proportion of sheep and cow lump cheeses in Slovak bryndza if the producer has followed the production process given by legislation without incorrect technological steps.
We have been checking the adulteration of 100% sheep's bryndza in Slovakia. Samples purchased from different parts of Slovakia were analyzed with a focus on adding cow's milk to sheep's bryndza. The reference method for determining the presence of cow's milk in sheep's cheese is based on detecting cow's y2- and y3-caseins from cheese after electrophoretic separation on urea-polyacrylamide gels. This method has proven to be reliable and sensitive. We tested sheep's bryndza made from both raw milk and heat-treated milk. The method for checking adulteration of cheese proved successful and we found the presence of cow casein in one sample in a minor amount, probably due to unintentional contamination. Isoelectric focusing of γ-caseins after plasmolysis is suitable for qualitatively determining sheep and cow proteins in traditional Slovak bryndza. The method was more time-consuming with our number of samples. We defatted the samples by triple extraction on a Soxhlet extractor, thus obtaining representative samples recorded after isoelectric focusing on the gel without fat spots around the protein fractions. The method can be shortened by omitting the staining procedure after isoelectric focusing and photographing or scanning the unstained fixed gel. From the results of our analysis, we can confirm the honesty of Slovak producers and their focus on preserving tradition and producing quality traditional products. Although we evaluate the obtained results of the work positively, our positive result should not represent an omission of controls in the following years. It is important to check the adulteration of traditional Slovak bryndza on a targeted basis in regular and random monitoring so that any attempts at adulteration can be traced and reported publicly if illegal practices are detected. Announced checks on random samples by the official inspection bodies would provide a degree of respect and order in the area of adulteration of sheep's bryndza. They would also create some certainty for honest producers and their competitiveness, as the conditions and order in the area of adulteration of sheep's bryndza. They would also create some certainty for honest producers and their competitiveness, as the conditions

Concluding remarks could lead to economic losses and loss of traditions.

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REFERENCES


