

## Effect of gluten on the quality of meat products

Jana Výrostková<sup>1</sup>, Ivana Regecová<sup>1\*</sup>, František Zigo<sup>2</sup>, Nikola Dančová<sup>3</sup>

<sup>1</sup>University of Veterinary Medicine and Pharmacy in Košice, Department of Food Hygiene, Technology and Safety, Slovakia

<sup>2</sup>University of Veterinary Medicine and Pharmacy in Košice, Department of Nutrition and Animal Husbandry, Slovakia

<sup>3</sup>University of Veterinary Medicine and Pharmacy in Košice,  
Department of Public Veterinary Medicine and Animal Welfare, Slovakia

Article Details: Received: 2020-10-14 | Accepted: 2020-11-27 | Available online: 2021-01-31

<https://doi.org/10.15414/afz.2021.24.mi-prap.80-84>



Licensed under a Creative Commons Attribution 4.0 International License



The aim of the study was the evaluation of gluten quality of selected meat products. Gluten is a vegetable protein, which, due to its properties, is very often used in various sectors of the food industry and therefore in the production of meat products. Sensory evaluation, microbiological examination and gluten detection were performed in samples of sausages, Poultry frankfurter, Fine salami and Malokarpatská salami. The meat products came from different producers and were purchased from a retail network. Sensory quality was determined by assessing color, aroma, juiciness, fragility and taste using a 5-point rating scale. The microbiological examination included the determination of the total number of microorganisms, the number of psychrotrophic, coliform microorganisms and coagulase-positive staphylococci. The presence of gluten alone was detected using a commercially produced GlutenTox Home test kit as well as a PCR reaction. Individual testing of selected meat products showed minor differences in the overall quality of gluten free and gluten-free meat products.

**Keywords:** meat products, salami, gluten, quality, microbiological examination

### 1 Introduction

Cereals are an essential and very important part of our diet. After consumption and processing in the gastrointestinal tract, they provide the body primarily with energy in the form of carbohydrates, minerals, vitamins and are also a source of fiber. Wheat, rice and corn are the most consumed in the world. Consumption of cereals is often associated with various diseases. Those we can divide into autoimmune, non-autoimmune, allergic and non-allergic. Celiac Disease is one of the most famous autoimmune disease. In particular, the Triticeae (wheat, barley, rye) and Aveneae (oats) cereal families cause health problems for patients suffering from celiac disease and cereal allergies (Abaffyová et al., 2015).

Gluten is part of cereal protein. It is found mainly in the endospeme of cereal grains, where it is bound with starch. Unlike gluten, starch is soluble in water and the two substances can be separated from each other. It is a complicated system consisting of prolamins and glutelins in a ratio of 2 : 3, whose toxicity is different. For celiacs, prolamins are much more harmful than glutelins (Frič and Keil, 2011).

Gluten-containing cereals (wheat, rye and barley) are able to induce allergic reactions mediated by immunoglobulins E when they enter the body upon consumption or inhalation. In contrast, they have gained much more interest in their association with celiac disease (Taylor, 1992).

Methods for the determination of gluten proteins can be divided into several groups. These are immunological methods and this group includes ELISA, Dip Stick test, Biosensor. Immunological methods are based on the reaction of an antigen with an antibody. Another group consists of molecular-biological methods. We include PCR and Real Time-PCR to these group. The essence of these methods is the amplification of a specific section of DNA (van Hengel, 2007).

\***Corresponding Author:** Ivana Regecová, University of Veterinary Medicine and Pharmacy in Košice, Department of Food Hygiene, Technology and Safety, Komenského 73, 041 81 Košice, Slovakia; e-mail: [ivana.regecova@uvlf.sk](mailto:ivana.regecova@uvlf.sk)

Today's market offers celiacs a large number of different gluten-free products. The meat processing industry, which offers a wide range of meat products from different producers, is no exception. As the quality of individual gluten-free products and gluten-containing products may vary based on the amount and type of ingredients added to the meat product, our work deals with sensory and microbiological examination of selected types of meat products placed on the market. At the same time, the presence of gluten in the products is determined using a commercial GlutenTox Home test kit and a PCR reaction.

## 2 Material and methods

### 2.1 Sensory evaluation of meat products

Total of 16 samples of meat products were subjected to sensory examination. Specifically, these were 4 samples of Poultry Sausages, 4 samples of "Špekáčky", 4 samples of Fine Salami and 4 samples of "Malokarpatska Salami". Two samples from each type of meat product tested were declared gluten-free by the manufacturer. All meat products were purchased in the sales network from various manufacturers. Samples of Poultry Sausages and samples of "Špekáčky" were evaluated hot. Samples of meat products were evaluated by a 5-member sensory evaluation committee (three were trained evaluators and two were amateurs). A maximum of 5 points were determined for each quality mark. The lower the number, the worse the view of the sample. The sum of the points of all the characters indicates the final quality of the sample.

The quality characteristics that were assessed were: colour, aroma, taste, juiciness and fragility.

### 2.2 Microbiological examination of meat products

The determination of the total number of microorganisms (TVC) was performed according to STN EN ISO 4833-1 (2014), using the arbitrary agar medium Plate Count Agar (PCA). Incubation was at 30 °C/72 hours. To determine the number of psychrotrophic microorganisms, also prepared Petri dishes were incubated at 6 °C/10 days.

To determine the number of coliform bacteria, STN ISO 4832 (1997) was used, bacterial strains were tested on VRBL agar medium.

To determine the number of coagulase-positive staphylococci STN EN ISO 6888-1/A1 (2001), BairdParker agar was used as a solid arbitration selective-diagnostic medium.

### 2.3 Gluten Detection in Food "Commercial GlutenDetectionKit in food"

The GlutenTox Home (Figure 1) is a fast and easy-to-use test for the detection of gluten in solid foods and beverages that may be present as part of additives and preservatives.



Figure 1 GlutenTox Home test kit

## 2.4 Detection of gluten by PCR reaction

DNA from the examined samples was isolated using a NucleoSpin® Tissue column kit (MACHEREY-NAGEL GmbH & Co. KG, Germany). Subsequent detection of gluten by the PCR method was performed according to Olexová et. al. (2006) using HOT Firepol® Blend Master Mix (Solis BioDyne, Tartu, Estonia). The resulting PCR product of 200 bp was sequenced at GATC Biotech AG (Cologne, Germany). The obtained sequences were searched homologously to the sequences available in the GenBank-EMBL database using the BLAST program (software package NCBI). Isolated DNA from coarse wheat flour was used as a positive control in PCR reaction.

## 3 Results and discussion

### 3.1 Results of sensory evaluation of meat products

Based on an overall evaluation of the results of sensory evaluation of meat products, the sample of “Malokarpatská salami” not declared as gluten-free obtained the largest number of points in the summary as well as in all evaluated features, namely 21.3 points out of a possible 25 points. Overall, the lowest number of points in the sensory evaluation was obtained by all samples of Poultry Sausages and Fine Salami.

If we compare the results of sensory evaluation of the sample, samples not declared by the manufacturer as gluten-free were evaluated better overall, which may be due to the presence of several additives in the product.

### 3.2 Microbiological examination of meat products

The total number of microorganisms (TVC), the number of psychrotrophic microorganisms (NPM), the number of coliform bacteria (NCB) and the number of coagulase-positive staphylococci (CPS) were determined by culture microbiological examination of samples.

The culture examination of the samples showed a higher TVC in the samples Malokarpatská salami ( $2.4 \pm 0.08$  log CFU/ml) and Malokarpatská salami BLP ( $3.4 \pm 0.10$  log CFU/ml).

As shown in Table 1, the higher TVC was detected in samples of meat products that were not labeled as gluten-free by the manufacturer.

**Table 1** Microbiological examination of meat products/BLP-gluten-free

Meat product	TVC	NPM	NCB	CPS
	log CFU/ml			
Špekáčky	$2.4 \pm 0.07$	$<1.0 \pm 0.00$	$<1.0 \pm 0.00$	$<2.0 \pm 0.00$
Špekáčky BLP	$2.0 \pm 0.10$	$<1.0 \pm 0.00$	$<1.0 \pm 0.00$	$<2.0 \pm 0.00$
Poultry Sausages	$2.9 \pm 0.08$	$2.04 \pm 0.02$	$<1.0 \pm 0.00$	$<2.0 \pm 0.00$
Poultry Sausages BLP	$2.7 \pm 0.08$	$<1.0 \pm 0.00$	$<1.0 \pm 0.00$	$<2.0 \pm 0.00$
Fine Salami	$2.9 \pm 0.11$	$<1.0 \pm 0.00$	$<1.0 \pm 0.00$	$1.0 \pm 0.06$
Fine Salami BLP	$2.8 \pm 0.11$	$<1.0 \pm 0.0$	$<1.0 \pm 0.00$	$<2.0 \pm 0.00$
Malokarpatská Salami	$2.4 \pm 0.08$	$2.7 \pm 0.08$	$<1.0 \pm 0.00$	$1.5 \pm 0.12$
Malokarpatská Salami BLP	$3.4 \pm 0.10$	$2.3 \pm 0.10$	$<1.0 \pm 0.00$	$<2.0 \pm 0.00$

Psychrotrophic microorganisms are characterized mainly by the ability to grow and reproduce even at colder temperatures. The recorded numbers of these microorganisms in the examined samples were relatively low. The higher NPM was recorded mainly in the samples of Malokarpatska salami, namely  $2.7 \pm 0.08$  log CFU/ml (Table 1).

We also refer to coliform microorganisms as indicator microorganisms, because by determining them we can detect hygienic deficiencies during the food production process. Examination of the samples did not confirm the presence of NCB.

Subsequently, the presence of coagulase-positive staphylococci was confirmed in the tested samples of meat products in two cases (Table 1). Higher numbers were determined in the sample Malokarpatská salami ( $1.5 \pm 0.12$  log CFU/ml) and Fine salami ( $1.0 \pm 0.06$  log CFU/ml).

The results of determining the number of TVCs in this work were relatively low, so they did not pose any risk to the consumer. The highest numbers were recorded in the samples of both Malokarpatská salamis, due to the addition of a starter culture, which increases the level of quality and safety of the product. At the same time, it positively influences sensory properties, prolongs shelf life and reduces the risk of the presence of undesirable microorganisms (Toldrá, 2002).

### 3.3 Results of the commercial kit *GlutenTox Home*

The test can be used to detect the presence of prolamins of wheat, barley, rye, but also oats in small quantities in food samples, specifically in samples of meat products. These can be harmful and dangerous for people suffering from celiac disease.

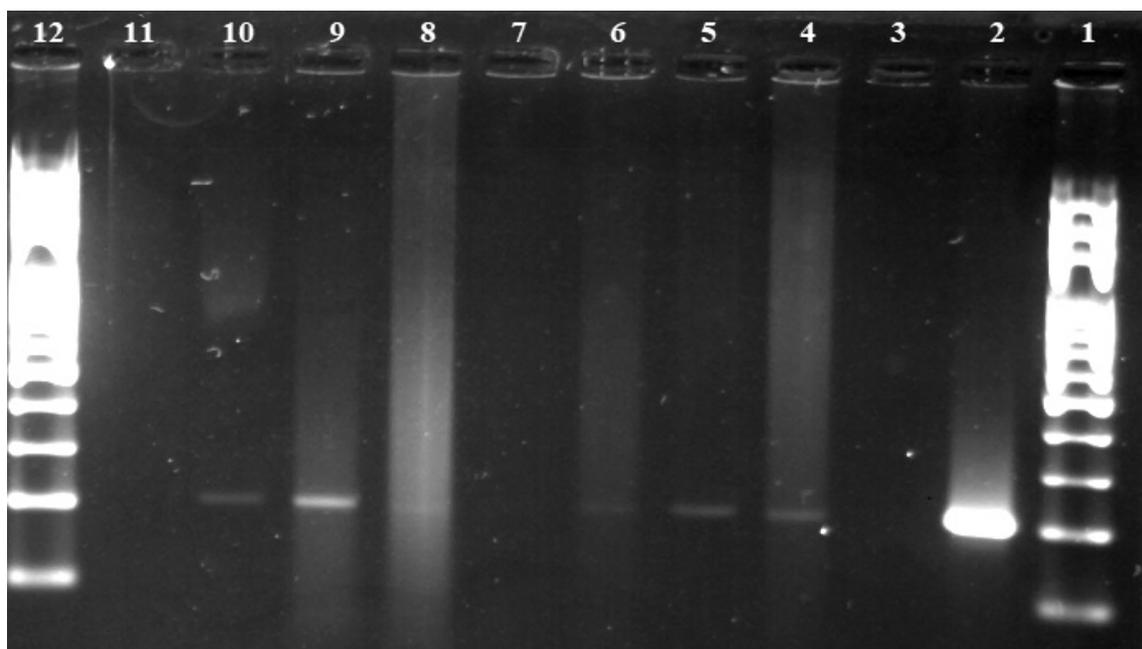


**Figure 2** Negative result *GlutenToxHome* test for Poultry sausages and Fine salami not declared as a “gluten-free” product

All sixteen samples were tested this way. The presence of gluten was not confirmed in any of the samples examined, whether the products were or were not declared gluten-free by the manufacturer.

### 3.4 Results of the PCR reaction

After the initial detection of gluten in the meat product samples using the *GlutenTox Home* diagnostic kit, gluten detection was performed by a more sensitive molecular PCR method. The isolated DNA from meat product samples was used as a template in PCR reaction, which consisted of the following three steps: denaturation, hybridization and extension. The resulting reaction products, PCR amplicons of approximately 200 bp in size, were visualized on a 1.5% agarose gel using an ultraviolet transilluminator (Figure 3) and sequenced using the Sanger method. Subsequently, the sequences were compared with the sequences of the genes encoding gluten in the GenBank-EMBL database.



**Figure 3** Detection of gluten by PCR (PCR product 200 bp)  
lane 1 – 100 bp standard; lane 2 – positive control (wheat flour); lane 3 – negative control 1; lane 4 – BLP salami; lane 5 – Fine salami; lane 6 – Poultry sausages; lane 7 – Poultry sausages BLP; lane 8 – Malokarpatská salami BLP; lane 9 – Malokarpatská salami; lane 10 – Špekáčky; lane 11 – BLP Špekáčky; 12 – 100 bp standard path.

In the tested products, the presence of gluten was confirmed by this method in all eight products that were not labeled gluten-free by the manufacturer. At the same time, the gluten content was also demonstrated in the samples of Fine BLP Salami and Malokarpatská BLP Salami. In špekáčky and poultry sausages labeled as gluten-free products, the presence of gluten was not detected (Figure 3).

Currently, the most commonly used methods for detection of microorganisms in food as well as allergenic proteins are polymerase chain reaction (PCR) and Real Time PCR. The ELISA method is also used to detect allergens in food (van Hengel, 2007). The PCR reaction has a sensitivity of approximately 10 times higher compared to the ELISA method (Hulín et al., 2008). These statements are also confirmed by our results, where in the case of the detection of gluten in our selected meat products, the high sensitivity and accuracy of the PCR reaction was confirmed.

#### 4 Conclusions

The obtained results showed small differences in quality between meat products with and without gluten content, either from a microbiological point of view or sensory evaluation of products. The commercial GlutenTox Home test kit showed lower sensitivity compared to the PCR method. The kit did not detect the presence of gluten, regardless of whether the meat product was tested with or without gluten. Thus, we can state that this method of gluten detection is not accurate and the obtained results should be only indicative. Examination using a commercial kit should be combined with a more accurate and sensitive method such as PCR.

#### Acknowledgments

This work was supported by Slovak grants No. APVV-18-0039 and KEGA no. 006UVLF-4-2020.

#### References

- Abaffyová, Z. et al. (2015). Cereal grain a little different. *Pediatrica pre prax*, 4, 140–146.
- Frič, P. and Keil, R. (2011). Celiac disease for practice. Olomouc. *Medicina pro praxi*, 8, 354–359.
- Hulín, P., Dostálek, P. and Hochel, I. (2008). Methods for determination of gluten proteins in food. *Metódy stanovení lepkových bílkovin v potravinách. Chemické listy*, 102(5), 327–337. [https://www.academia.edu/18759054/Methods\\_for\\_determination\\_of\\_gluten\\_proteins\\_in\\_food](https://www.academia.edu/18759054/Methods_for_determination_of_gluten_proteins_in_food)
- Olexová, L. et al. (2006). Detection of gluten-containing cereals in flours and “gluten-free” bakery products by polymerase chain reaction. *Food Control*, 17(3), 234–237. <https://doi.org/10.1016/j.foodcont.2004.10.009>
- STN EN ISO 4833-1. Microbiology of food chain. Horizontal method for the enumeration of microorganisms. Part 1: Colony count at 30 degrees C by the pour plate technique. (ISO 4833-1:2013).
- STN EN ISO 6888-1. Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species). Part 1: Technique using Baird-Parker agar medium (ISO 6888-1:1999).
- STN EN ISO 4832. Microbiology. General guidance for the enumeration of coliforms. Colony count technique.
- Taylor, S. (1992). Chemistry and detection of food allergens. *Food Technology*, 46(5), 148–152. <https://doi.org/10.1080/10408399609527761>
- Toldrá, F. (2002). Fermentation and starter cultures. *Dry-cured meat products*, Trumbull : Food & Nutrition Press Inc., 89–112. <https://doi.org/10.1002/9780470385111>
- van Hengel, A. J. (2007). Food allergen detection methods and the challenge to protect food-allergic consumers. *Analytical and Bioanalytical Chemistry*, 389(1), 111–118. <https://doi.org/10.1007/s00216-007-1353-5>