Evaluation of microbiological quality of cooked meat products during their shelf life

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Abstract

The aim of this work was to analyze the microbiological quality of cooked meat products (ham salami and Spiš sausages) over their storage period. The analyses were focused on the determination of total viable counts of microorganisms, *Enterobacteriaceae* and coliforms. The results showed that the maximum growth of microorganisms occurred on storage day 21 (ham salami) and day 5 (Spiš sausages) at temperatures from 2 to 6 °C after the packages have been open. Storing meat products at refrigerated temperatures is safe as long as all the requirements of the manufacturer are observed.

Microbiological quality, cooked meat products, shelf life, storage

Introduction

Meat is defined as the flesh of animals used as food. The term 'fresh meat' includes meat from recently processed animals as well as meat that is vacuum-packed or meat in controlled atmosphere packaging, which has undergone no treatment other than chilling to ensure preservation (Storia et al. 2008). The diverse nutrient composition of meat makes it an ideal environment for the growth and propagation of meat spoilage microorganisms and common foodborne pathogens. It is therefore essential that adequate preservation technologies are applied to maintain its safety and quality (Aymerich et al. 2008). Meat products are defined as products containing at least 50% of meat. These products are among the most frequently consumed food products. The shelf life of cooked meat products depends on a number of factors. The most important is the microbiological quality of the raw materials (Afshin et al. 2011). The detection of microbial contamination, particularly of the total viable counts (TVC), sterility testing and selective determination of microorganisms are common microbiological tests used on a large scale on food, environmental, medical and biological samples (Baylis 2003). The increasing importance of food and microbial safety dictates the need for adequate methods that can provide simple and rapid TVC assays to screen an ever increasing number of samples. Conventional culture methods remain the most popular and are regarded as gold standard benchmarks by many laboratories. Major pathogens that need to be controlled in fresh meat include *Salmonella, Campylobacter,* and enterohaemorrhagic *E. coli* O157:H7. Though progress is being made in their control, some of these pathogens will continue to be of concern well into the future (Bacon and Sofos 2003). Additional pathogens that may emerge as a serious risk factor in meat products in the future include non-O157 shigatoxin producing *E. coli* serotypes, *Mycobacterium avium* subsp. *paratuberculosis*, *Escherichia albertii*, *Clostridium difficile*, etc. The emergence of pathogens should not be a surprise as approximately 60–70% of outbreaks and 40–50% of reported cases of food-borne illness are caused by unknown etiologic agents (Sofos 2008). Sliced cooked and dry cured ham, ready-to-eat products, and marinated beef loin, products intended to be eaten

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cooked, are convenience meat products whose safe shelf life can be compromised due to cross-contamination during slicing and packaging operations (Garriga et al. 2004). The microbiological and biochemical events taking place in meat and meat products depend on their history, with both being mostly affected by temperature and water activity. Temperature seems to be the most important factor influencing the spoilage of meat. Breaks in the cold chain may result in overgrowth of specific spoilage organisms (SSO) and overproduction of biogenic amines (Nychas et al. 2008). There is a general need in the food supply chain for rapid methods to monitor microbial quality, and to identify hygienic and safety conditions in order to enable necessary corrective actions at the appropriate time. Safety inspections of pork products are also needed because the industry and regulatory bodies need to ensure that pork is safe for the market and meets the highest standards (El Barbri et al. 2008). Packaged meat products are stored at the temperature specified by the manufacturer (Table 1) (Staruch 2009).

Table 1. The recommended shelf life of meat and meat products at different temperatures in households (Staruch 2009)

Meat	Product storage temperature		
Meat products	up to 5 °C	up to 15 °C	over 15 °C
Ground meat	8-hour-preparation is only for direct consumption on the same day		
Meat in small pieces, pieces	8 hours	2 hours	-
Cooked meatballs	12 hours	4 hours	-
Soft and boiled products	26 hours	6 hours	2 hours
Lard	3 months	14 days	3 days

Materials and Methods

The aim of this work was to analyze the microbiological quality of cooked meat products in relation to their storage periods. To met the objective, we focused on sensory and microbiological testing of the quality of Spiš sausages and ham salami, which are part of a group of cooked meat products made from pork that have been subjected to heat treatment and salting.

With respect to the set objectives, we took samples from food business operators over a period of 5 months. The samples were of the same type of ham salami (completely packaged and weighing about 1 kg) and sausage (5 pieces), which we subsequently analyzed at the Department of Hygiene and Food Safety. Samples of the ham salami were subjected to microbiological and sensory analysis on their day of production (A), then after 7 (B), 14 (C) and 21 days (D) while refrigerated at 2 – 6 ° C. These analyses were focused on determining both the total viable counts of microorganisms and the presence of coliform bacteria and *Enterobacteriaceae*. The first sausage sample (A) was analyzed on its day of purchase, the second (B) on day 5, which was also the expiration date set by the manufacturer.

During the observation period, we took 25 g samples from individual packages of ham salami and sausage under aseptic conditions. We inserted the samples into 225 ml of peptone solution and homogenized them. In this way we obtained a basic dilution of 10^{-1}, from which a set of further dilutions was prepared. For the microbiological analysis of the samples, we used PETRIFILM™ and 3M™ PETRIFILM™ plates, which are among the certified and commercially available analytical methods. After incubating the samples (30 °C ± 1 °C over a period of 48 ± 2 hours for TVC and 37 °C over a period of 24 hours for coliform bacteria and *Enterobacteriaceae*), we used a magnifier and a light source to count the colonies grown, which we recalculated on 1 g of the product. We proceeded according to STN ISO 8261 (1998).

We established our methodology based on Commission Regulation (EC) No. 1441/2007, which specifies microbiological criteria for meat and meat products. According to this regulation, it is the responsibility of the food business operator to follow safety criteria (*Salmonella*), as well as hygiene criteria (total viable counts of microorganisms and *E. coli)*. It should be noted that although the determination of the total viable counts of microorganisms is not strictly intended for soft meat products, we wondered what effect their storage in refrigeration after a period of 21 and 5 days will have on microbiological safety. Our in-house standard that we availed ourselves of specifies TVC of 5.69 log cfu·g^{-1} for the duration of the shelf life of the meat products provided the integrity of the packaging is maintained.

Results and Discussion

In light of the set objective, we analyzed the samples of ham salami and Spiš sausage for the presence of the total number of microorganisms, *Enterobacteriaceae* and coliform bacteria in relation to storage period in refrigeration at 2 – 6 °C. Table 2 gives the results of the determination of total viable counts in the analyzed samples of ham salami. The table shows that the samples taken on the day of production, as well as those after 7 days, complied with the microbiological criteria laid down by the in-house regulations in all months. It should also be noted that samples were not analyzed in the place of production, but only after transfer to the laboratory under aseptic conditions and while adhering to cold chain shipment principles. At the same time, the growth of microorganisms during the course of the storage period was the most dramatic in the summer months, as well as in the month of October, when the total viable counts were elevated as early as the day of ham salami production.

Table 2. Determining the total viable counts of microorganisms in relation to the storage period of ham salami

Season	Total viable counts (log cfu·g^{-1})			
	Sample A	Sample B	Sample C	Sample D
	(n = 25)	(n = 25)	(n = 25)	(n = 25)
June	4.96	5.33	5.97	6.97
July	5.01	5.44	6.00	7.03
August	5.01	5.54	6.04	7.05
September	4.99	5.38	6.01	7.02
October	5.03	5.47	6.01	7.04
$\bar{x}$	5.00	5.43	6.00	7.02
x_{min}	4.92	5.29	5.91	6.92
x_{max}	5.08	5.61	6.10	7.12
s	4.04	4.77	5.05	6.17
$v_{k,\%}$	11.02	21.8	11.00	14.19

The average value of the total viable counts (TVC) in 1 g of product reached a value of 5.00 log cfu·g^{-1} on the day of production. After 7 days of storage in refrigeration the TVC had increased to 5.43 log cfu·g^{-1}, after 14 days to 6.00 and on day 21 to 7.02 log cfu·g^{-1}. The coefficient of variation ranged from 11% (after 14 days) to 21.8% (after 7 days).

As shown in Figure 1, the most significant increase in the total viable counts was recorded on day 21, while at the same time we can also state that the microbiological changes were accompanied by sensory changes (a slimy surface on day 21 and an off-odor). In addition to determining the TVC, analyses were also aimed at the determination of coliform bacteria and *Enterobacteriaceae*, and concluded that none of the studied samples exhibited any of these types of microorganisms.

While analyzing the samples of Spiš sausages (Table 3), the average value of TVC on the day of production was 4.86 log cfu·g^{-1}, and on the expiration date the number of studied microorganisms investigated had increased to 5.38 log cfu·g^{-1}. The standard deviation of the total number of microorganisms was 4.43 log cfu·g^{-1} on the day of production and in the analysis of the samples of sausages on the expiration date the standard deviation was 4.59 log cfu·g^{-1}. The sausage samples also met the requirements set by the Codex Alimentarius of the Slovak Republic for *Enterobacteriaceae* and coliform bacteria. No colonies arose on the Petrifilm plates during analysis and the average value of *Enterobacteriaceae* and coliforms were no detectable.

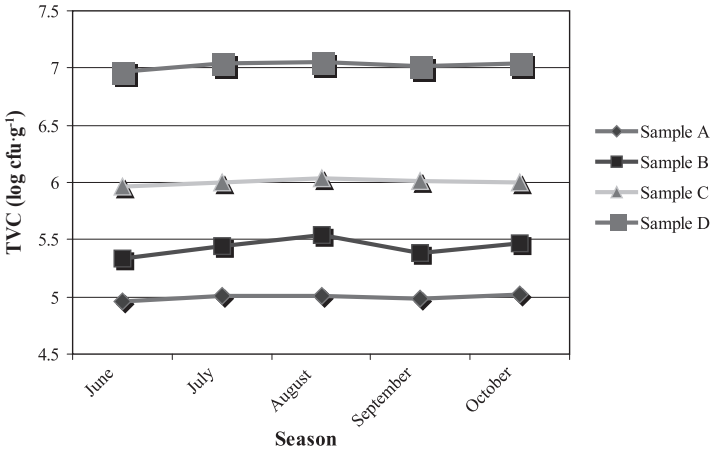

Fig. 1. Changes in total viable counts of microorganisms in ham salami during the storage

Table 3. Determining the total viable counts of microorganisms in relation to the storage period of Spiš sausage

Season	Total viable counts (log cfu·g^{-1})	
	Sample A (n = 25)	Sample B (n = 25)
June	4.64	5.29
July	4.98	5.44
August	4.98	5.43
September	4.86	5.39
October	4.83	5.33
$\bar{x}$	4.86	5.38
x_{min}	4.50	5.26
x_{max}	5.11	5.50
s	4.43	4.59
$v_{k,\%}$	37.54	16.41

Sachindra et al. (2005) carried out a microbiological survey of Buffalo sausage and reported the presence of coliform bacteria. They isolated coliforms from both raw and cooked sausage, but less in cooked sausage than in raw sausage. Their results demonstrate that cooking substantially reduces the microbial counts in the sausage.

It needs to be emphasized that the manufacturer determined the expiration date of the ham salami to be 21 days and the Spiš sausages 5 days from the date of production if the integrity of the packaging is maintained. Despite the fact that we did not apply this condition during our research, it can be stated that storing meat products at refrigeration temperatures is safe provided that all of the manufacturer's requirements have been met. It follows from this that the purchase of larger packages that are then consumed by the end user over several days is not appropriate from the point of view of preserving the sensory and microbiological characteristics of the meat product. After opening the package, it is necessary to consume the products within 24 and 48 hours respectively.

Korkeala et al. (1985) studied vacuum packed cooked ring sausage during storage and found a shelf life of 20 – 28 days and 43 days. Shelf life of 49 days for Hot smoked sausage packed in CO was recorded.

Friedhoff et al. (2005) described the use of the monitoring of simple microbiological criteria, including aerobic mesophilic colony counts, *Enterobacteriaceae* counts and in some instances, enumeration of yeast, in samples taken during processing in small businesses for the verification of good manufacturing practices. This verification through monitoring was found to be an attractive alternative to the examination of end products, and also coliform bacteria are one of most important indicator organisms that are most commonly used to ensure food safety. Similar analyses were performed by Jay (2005)

and Mantis et al. (2005). Brashears et al. (2012) determined the microbiological risks associated with condensation in harvest, fabrication and ready-to-eat meat processing environments. Significant interactions between season and plant type were observed for nearly all microorganisms, resulting in counts that were generally higher in the summer compared to other seasons. Aerobic plate counts ranged from non-detectable to 3.7 log cfu $100 \cdot mL^{-1}$ of condensation. Total viable counts were so low that data had to be converted to $100 \cdot mL^{-1}$. Coliforms and Enterococci were no detectable in most condensation samples.

Afshin et al. (2011) carried out a study which revealed the pattern of microbial profile associated with the preparation of Hot Smoked Sausage. The results were analyzed by a paired T-test and a comparison of mean microbial counts in different weeks was made. The results showed a significant increase ($P < 0.05$) in microbial counts in different weeks. All of the samples failed in weeks 1 and 2 as they showed optimum microbial counts. No coliforms were detected during the 5-week study period. This indicates that the products had low quality for marketing.

Microorganisms can be considered the most serious factor affecting the quality and safety of pork and the number of microorganisms will directly affect the quality of pork. It has been recommended that the critical value in relation to spoilage, i.e. total viable counts (TVC), has been set at 10^6 cfu$\cdot/g^{-1}$ (Panigrahi et al. 2006).

Conclusions

The objective of the study was to understand the microbial properties of select meat products (ham salami and Spiš sausage) in relation to shelf life. The analyses focused on the determination of total viable counts, *Enterobacteriaceae* and coliforms. The results showed that the highest growth of microorganisms was detected on day 21 of ham salami and on day 5 of sausages at 2 to 6 °C. It is essential to maintain the cold chain and to comply with the requirements set by the manufacturer.

Microbial ecology of meat products mainly depends on the environment, kind of meat and raw materials, equipment handling practices, processing, packaging and storage temperature.

The best strategy for improving the safety of meat and meat products is to apply proper hygiene and antimicrobial intervention technologies that:

- reduce contamination on live animals;
- minimize access and transfer of microorganisms to carcasses and meat;
- reduce, through decontamination, microbial levels on carcasses or meat;
- reduce or eliminate, by killing, microbial contamination on products;
- avoid or minimize cross-contamination; and
- inhibit the growth of surviving. Thus, foodborne pathogen control requires application of interventions at pre-harvest, post-harvest, processing, storage, distribution, merchandizing, preparation, foodservice and consumption.

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Plate I

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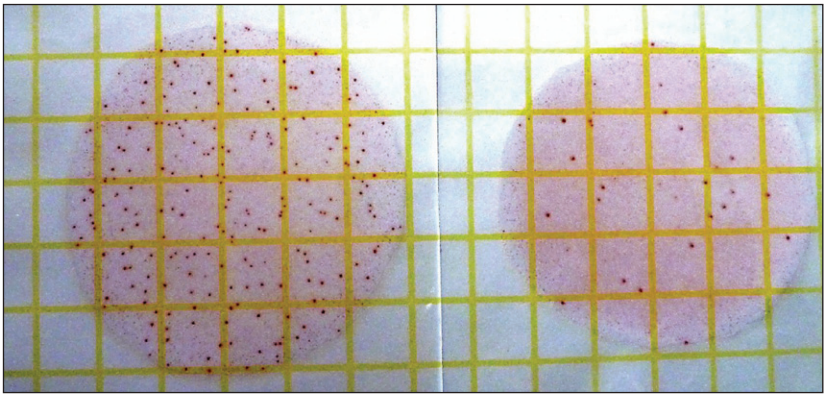

Fig. 2. An increase in the total number of microorganisms in the Spiš sausages on the expiration date (dilution of 10^{-4} and 10^{-5})