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MICROBIOLOGICAL EVALUATION OF POULTRY SAUSAGES STORED AT DIFFERENT TEMPERATURES

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ABSTRACT

The aim of our study was to evaluate the microbiological quality of poultry sausages, which were stored at different temperatures (4 °C, 15 °C). Total bacterial count, coliform bacteria, yeasts and filamentous microscopic fungi were detected in poultry sausages. Microbiological quality was evaluated using the horizontal method for the determination number of microorganisms. Total bacterial count in sausages stored at 4 °C ranged from 1×10^1 CFU.g^{-1} in sample 1 (after opening) to 4.35×10^4 CFU.g^{-1} in sample 1 (7th day of storage). Total bacterial count in sausages stored at 15 °C ranged from 3.25×10^3 CFU.g^{-1} in sample 1 (after opening) to 3.12×10^6 CFU.g^{-1} in sample 1 to 3.12×10^6 CFU.g^{-1} in sample 1 (7th day of storage). Coliform bacteria in sausages stored at 4 °C ranged from 1×10^1 CFU.g^{-1} to 3.15×10^5 CFU.g^{-1}. Coliform bacteria in sausages stored at 15 °C ranged from 1.54×10^3 CFU.g^{-1} to 1.40×10^6 CFU.g^{-1}. Yeasts and microscopic filamentous fungi in sausages stored at 4 °C ranged from 2.75×10^4 CFU.g^{-1} to 1.40×10^6 CFU.g^{-1}. Yeasts and microscopic filamentous fungi in sausages stored at 15 °C ranged from 1.30×10^4 CFU.g^{-1} to 1.44×10^6 CFU.g^{-1}. Total bacterial count, coliform bacteria, yeast and microscopic fungi were not in accordance with Codex Alimentarius of Slovak Republic on 3rd day in samples stored at 15 °C.

Keywords: quality; poultry sausage; meat product; shelf life; microbiological quality

INTRODUCTION

Meat production is one of the major activities in Europe. The main type of meat produced is pork (48.7 %) followed by poultry (23.6 %) and bovine (23.3 %). Meat and meat products present an ideal substrate supporting the growth of several spoilage and pathogenic bacteria **(Mataragas et al., 2006)**.

Kozelová et al. (2011) investigated consumer's opinion about quality of meat and meat products of Slovak and foreign production on the Slovak markets. Quality of foreign products is highly appreciated by 15% of respondents; higher quality was highlighted by 36% of respondents, 30% of respondents highlighted the quality as lower, 19% of respondents labelled the quality of those products as very low.

In the last decade, chicken-based meat products have become increasingly popular worldwide due to their high nutritional quality and low cost and are available as either fresh or precooked (i.e. fried) chicken and/or microbiological products, which after subsequent packaging are usually stored under refrigeration **(Barbut, 2002)**. Additionally, frozen chicken-based meat products also available on the market include specialties such as: nuggets, meatballs, hamburgers, frankfurters, etc.

Pathogenic non-spore-forming/spore-forming bacteria and viruses constitute a large proportion of all foodborne illness **(EFSA, 2007)**. The presence of these microorganisms in raw pork and poultry is the result of their contamination from the live animal, equipment, employees and environment **(Gianfranceschi et al., 2003, Gudbjornsdottir et al., 2004, Reij and Den Aantrekker, 2004 and Gibbons et al., 2006)**.

Poultry meat spoils after 4-5 days under refrigerated conditions **(Morshedy and Sallam, 2009)**, limiting trade in fresh product and causing considerable financial loss to the poultry industry **(Jimenez et al., 1997 and Patsias et al., 2006)**. Shelf-life is the period of time a product may be stored without becoming unfit for human consumption. The sensory shelf life is defined by organoleptic parameters and the product may be considered as spoilt when discolouration, off-odours and/or slime develop **(Nychas et al., 2008)**. The microbial shelf-life of poultry may be defined by the Total bacterial count (TBC) and the product is generally spoilt when bacterial counts reach 10^7-10^8 CFU.g^{-1}. The time to spoilage, and therefore shelf-life, depends on the initial carcasses counts. Psychrotrophic (cold tolerant) total viable count are used as an indicator of shelf-life for poultry **(Nychas et al., 2008)** while mesophilic (organisms that grow between 20 and 45 °C) total viable count, *Enterobacteriaceae*, *Pseudomonas* spp., lactic acid bacteria and yeast/moulds are used in the poultry industry as indicators of processing hygiene and microbiological quality **(Alonso-Calleja et al., 2004 and Álvarez-Astorga et al., 2002)**.

Minimising of microbial contamination on meat, including poultry, is dependent on the strict application of good farming practices (GFP) and hygienic processing. The latter is documented in the prerequisite (GMP/GHP) programme and hazard analysis and critical control point (HACCP) plans. HACCP includes critical control points (CCP), where an intervention may be used to prevent,

reduce or eliminate microbial contamination **(Loretz et al., 2010)**.

The aim of this study was to evaluate the microbiological quality of poultry sausages on the first day of storage, after three days of storage, and after seven days of storage of the products at different temperatures (4 °C and 15 °C). In poultry sausages microbiological parameters: total bacterial count, coliform bacteria, yeasts and filamentous microscopic fungi were observed.

MATERIAL AND METHODOLOGY

Microbiological quality of poultry sausages was evaluated. These products are categorized of soft meat products.

Microbiological evaluation consisted of three parts:
- determination of total bacterial count,
- determination of coliform bacteria,
- determination of yeasts and filamentous microscopic fungi.

There were evaluated 10 samples of poultry sausage, two analyzes were performed. Evaluation of poultry sausages were performed as follows:
- two samples were evaluated immediately after opening (first day of storage),
- two samples were evaluated after three days of storage at temperature 4 °C,
- two samples were evaluated after three days of storage at temperature 15 °C,
- two samples were evaluated after seven days of storage at temperature 4 °C,
- two samples were evaluated after three days of storage at temperature 15 °C.

Characteristic of coliform bacteria

Coliforms are commonly used bacterial indicators of sanitary quality of water and foods. They are rod-shaped Gram-negative non-spore forming bacteria which ferment lactose into acid and gas at 35-37 °C. Coliforms are common inhabitants of the gut of the warm-blooded animals, but they can be found in the environment, on vegetation and in soil. Their presence indicates the potential presence of pathogenic organisms. *Escherichia coli* is a facultative mixed-acid fermenting member of the coliform group being capable of fermenting lactose at 44 °C. Presence of *E. coli* is considered as an almost sure sign of fecal contamination **(Harwood et al., 2002)**.

Characteristic of total bacteria count

Detection of microbial contamination, particularly total bacterial count, sterility testing and selective determination of microorganisms, are common microbiological tests used on a large scale on food, environmental, medical and biological samples. Total bacterial count includes determination of mesophilic aerobic and facultative anaerobic microorganisms **(Baylis, 2003)**.

Characteristic of microscopic fungi

Microscopic fungi include yeasts and microscopic filamentous fungi (moulds) are very important organisms. They are employed in the production of pharmaceuticals, enzymes, organic acids and food, and some of them are associated with several diseases affecting humans and other animals **(Domingues et al., 2005)**.

Determination of total bacterial count, coliform bacteria, yeasts and filamentous microscopic fungi

The total bacterial count (TBC), coliform bacteria (CB), yeasts (Y) and microscopic filamentous fungi (MF) were determined. Plate diluting method was applied for quantitative CFU (Colony Forming Units) counts determination of respective groups of microorganisms in 1 g of meat products. Homogenized samples of meat components were prepared in advance by sequential diluting based on decimal dilution system application. Basic dilution (10^{-1}) was prepared as follows: 5 g of meat product was added to the bank containing 45 mL of distilled water. The cells were separated from substrate in shaking machine (30 minutes). Petri dishes of gelatinous nutritive substrate were inoculated with 1 mL of meat samples (TBC, CB, Y, MF) in three replications. For microorganism cultivation three types of cultivating mediums were used, to segregate individual microorganism groups. Plate count agar (E. coli) was used for CFU segregation of TBC (incubation 48-72 h at 30 °C, aerobic cultivation method). Dilutions of 10^{-3} and 10^{-4} were used to determine of TBC. Violet red bile agar (E. coli) was used for CFU segregation of CB (incubation 24 h at 37 °C, aerobic cultivation method). Dilutions of 10^{-1} and 10^{-2} were used to determine of CB. Chloramfenicol yeast glucose agar (E. coli) was used for CFU segregation of Y and MF (incubation 5-7 days at 25 °C, aerobic cultivation method). Dilutions of 10^{-1} and 10^{-2} were used to determine of Y and MF.

RESULTS AND DISCUSSION

The total bacterial count (TBC) in poultry sausages after opening of the products was 5.70×10^3 CFU.g^{-1} (sample 1) and 1×10^1 CFU.g^{-1} (sample 2). TBC on 3rd day of storage at 4 °C was 3.65×10^3 CFU.g^{-1} (sample 1) and 2.10×10^3 CFU.g^{-1} (sample 2).

TBC in poultry sausages on 7th day of storage at 4 °C was 4.35×10^4 CFU.g^{-1} (sample 1) and $2.56.10^4$ CFU.g^{-1} (sample 2) (Tab. 1). The Codex Alimentarius of Slovak republic (CA SR) indicates TBC (10^5), number of coliform bacteria (5.10^2) and microscopic fungi ($<10^1$). TBC in poultry sausages stored at 4 °C were in accordance with **CA SR (2006)**.

TBC in sausages on 3rd day of storage at 15 °C was 3.25×10^3 CFU.g^{-1} in sample 1 and 4.45×10^3 in sample 2. TBC in poultry sausages on 7th day of storage at 15 °C was 3.12×10^6 CFU.g^{-1} in sample 1 and 4.05×10^5 CFU.g^{-1} in sample 2 (Tab. 2). TBC in sausages on 3rd day of storage at 15 °C were in accordance with CA SR. Samples examined after seven days of storage at 15 °C were not in accordance with **CA SR (2006)**.

Al-Dughaym and Altabari (2010) found that the total bacterial count (TBC) in chicken nuggets were 2.7×10^4 and 3.0×10^6 CFU.g^{-1}. The *Staphylococcus aureus* counts were less than 10^2 CFU.g^{-1} and *Escherichia coli* was isolated from chicken nuggets in incidence of 60%, while *Salmonella* sp. was not detected.

Coliform bacteria (CB) in sausages after opening of product was lower than 1×10^1 CFU.g^{-1} in sample 1 and 1.0×10^1 CFU.g^{-1} in sample 2. CB on 3rd day of storage at 4 °C was lower than 1×10^1 CFU.g^{-1} in sample 1 and 2.20×10^2 CFU.g^{-1} in sample 2. CB in poultry sausages on 7th day of storage at 4 °C was lower than 1×10^2 CFU.g^{-1} in sample 1 and 3.15×10^5 CFU.g^{-1} in sample 2 (Tab. 3). The number of coliform bacteria in meat products on 7th day of storage at 4 °C were not in accordance with **CA SR (2006)**.

Olsen et al. (2000) found that the most common pathogens per meat category were (% mean values): a) bovine, *E. coli* (40.4); *Salmonella* spp. (26.9); and *Clostridium perfringens* (21.2), b) pork, *Salmonella* spp. (40); *Yersinia enterocolitica* (20); *Clostridium perfringens* (10); and *Staphylococcus aureus* (10), c) chicken, *Salmonella* spp. (60); *Staphylococcus aureus* (10); *Shigella* spp. (10); *Bacillus cereus* (10); and virus (10), and d) turkey, *Salmonella* spp. (50); *Staphylococcus aureus* (33.3); and *Clostridium perfringens* (16.7). Most common places of exposure were home and restaurants (malpractices/mishandling during food preparation).

CB in poultry sausages on 3rd day of storage at 15 °C was 1.95×10^3 CFU.g^{-1} in sample 1 and 1.54×10^3 in sample 2. CB in meat products on 7th day of storage at 15 °C was 3.05×10^4 CFU.g^{-1}in sample 1 and 1.40×10^6 CFU.g^{-1} in sample 2 (Tab. 4). The number of coliform bacteria in meat products at 15 °C were not in accordance with **CA SR (2006)**.

Enterobacteriaceae, a hygiene indicator **(Zeitoun et al., 1994)**, were also part of the microbiota of ground chicken meat. The population of *Enterobacteriaceae* (3.4 log CFU.g^{-1}) is indicative of adequate hygiene conditions of production in the poultry plant.

According to **Adams and Moss (1997)** *Enterobacteriaceae* can grow under vacuum packaging and high-pH values in meat and produce high levels of H_2S giving meat objectionable odours.

Pérez-Rodrígues et al. (2010) determined number of coliforms in cooked meat products from different establishments. Coliforms were found in 65% of analyzed samples, and counts were significantly lower than the other groups of microorganisms. The average value was 1.88 log CFU.g^{-1}, though it was obtained a maximum value of 4.90 log CFU.g^{-1}. *E. coli* was detected in 8 samples (<10 CFU.g^{-1}).

Yeasts and microscopic filamentous fungi (YaMF) in sausages after opening of product was lower than 1×10^1 CFU.g^{-1} in sample 1 and 2. YaMF on 3rd day of storage at 4 °C was lower than 1×10^1 CFU.g^{-1} in sample 1 and also in sample 2. YaMF in poultry sausages on 7th day of storage at 4 °C was 2.75×10^4 CFU.g^{-1} in sample 1 and 2.31×10^5 CFU.g^{-1} in sample 2 (Tab. 5). Number of yeasts and microscopic fungi in both samples on 7th day of storage at 4 °C were not in accordance with **CA SR (2006)**.

YaMF after three days of storage at 15 °C was 1.44×10^4 CFU.g^{-1} in sample 1 and 1.30×10^4 CFU.g^{-1} in sample 2. YaMF in poultry sausages on 7th day of storage at 15 °C was 3.24×10^4 CFU.g^{-1} in sample 1 and 1.44×10^6 CFU.g^{-1} in sample 2 (Tab. 6). Samples investigated on 3rd and 7th day of storage at tepmerature 15 °C were not in accordance with **CA SR (2006)** in number of microscopic filamentous fungi.

A total of 52 samples of ethnic meat products were collected and analyzed by **Rai et al. (2010)**. In all traditionally prepared meat products, lactic acid bacteria (LAB) were found at 10^6-10^8 CFU.g^{-1}. Yeasts were also recovered in all samples at 10^4-10^6 CFU.g^{-1}. The counts of bacilli were $<10^3$ CFU.g^{-1}. Filamentous fungi were also detected in a few samples at less than 10^3 CFU.g^{-1}. The occurrence of *Micrococcaceae* was found at 10^4-10^7 CFU.g^{-1}. The total viable count in the samples, collected from different places of the Himalayas, varied between 10^5 and 10^9 CFU.g^{-1}.

Table 1 Total bacterial count in sausages stored at 4 °C

Sample	TBC (CFU.g^{-1})		
	After opening	3rd day	7th day
1	5.70 x 10^3	3.65 x 10^3	4.35 x 10^4
2	1 x 10^1	2.10 x 10^3	2.56 x 10^4

Table 2 Total bacterial count in sausages stored at 15 °C

Sample	TBC (CFU.g^{-1})	
	3 rd day	7 th day
1	3.25 x 10^3	3.12 x 10^6
2	4.45 x 10^3	4.05 x 10^5

Table 3 Coliform bacteria in sausages stored at 4 °C

Sample	CB (CFU.g^{-1})		
	After opening	3 rd day	7 th day
1	<1 x 10^1	< 1 x 10^1	< 1 x 10^2
2	1.0 x 10^1	2.20 x 10^2	3.15 x 10^5

Table 4 Coliform bacteria in sausages stored at 15 °C

Sample	CB (CFU.g^{-1})	
	3 rd day	7 th day
1	1.95 x 10^3	3.05 x 10^4
2	1.54 x 10^3	1.40 x 10^6

Table 5 Yeasts and microscopic filamentous fungi in sausages stored at 4 °C

Sample	YaMF (CFU.g^{-1})		
	After opening	3rd day	7th day
1	<10	<10	2.75 x 10^4
2	<10	<10	2.31 x 10^5

Table 6 Yeasts and microscopic filamentous fungi in sausages stored at 15 °C

Sample	CB (CFU.g^{-1})	
	3 rd day	7 th day
1	1.44 x 10^4	3.24 x 10^4
2	1.30 x 10^4	1.44 x 10^6

CONCLUSION

The microbiological quality of poultry sausages stored under various temperature conditions was evaluated in this study. Quality of meat products is affected by the quality of raw meat, storage temperature and handling conditions. Current challenges and concerns related to consumption of meat products may be divided into those associated with microbial pathogens and into other meat safety issues. Major challenges related to microbial pathogens include foodborne illness outbreaks, associated product recalls, regulatory compliance, and issues related to microbiological control.

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