EFFECT OF SPICES COMMERCIAL MIXTURE WITH GLUCONO-DELTA-LACTONE ON THE QUALITY OF FERMENTED DRY-CURED SAUSAGES

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ABSTRACT
The main fermented meat products are fermented sausages in which lactic acid bacteria (LAB) are the essential agents of the ripening process. Their application as starter organisms ensures the dominance of the starter during the whole ripening process. However, when no starter cultures are used, direct addition of acids like a glucono-delta-lactone (GdL) is preferred. The goal of this study was to determine the influence of commercial spices mixture (containing GdL) on selected technological parameters of fermented dry-cured sausages – Danube sausage in comparison with currently available conventional spices. Comparison was evaluated also with addition of starter cultures. Determinations of technological (value of pH, water activity, color) and microbiological properties (count of Lactobacillus spp., Enterobacteriaceae family, yeasts and moulds) were realized after 24 hours, 5 and 30 days. The sensory analysis of sausages was carried out after 30 days of ripening process. In sausages with the addition of commercial spice mixture in combination with starter culture were determined the lowest values of pH and aw at the end of ripening process (30 days). Bacteria of Enterobacteriaceae family were occurred in the samples with the addition of currently available conventional spices at the beginning of ripening, but after 5 days of ripening were bacteria of this family not detected. The counts of yeasts in analyzed samples were not detected. Counts of LAB at the end of ripening process (30 days) were lower in comparison with result obtained after 5 days; however their count was comparable with count determined at the beginig of the ripening. Our results show, that the combination of starter culture and commercial spice mixture containing GdL may cause excessive sour taste and sensory defect of dry fermented meat products.

Keywords: GdL; dry-fermented sausages; starter culture; colour; sensory evaluation

INTRODUCTION
Nowadays, fermented sausage production can be considered more than a method of preservation – as, instead, a process of transformation, diversification which is strongly linked to culture and tradition of individual countries (Fernández et al., 2000, Liu et al., 2011).

Fermented meat is produced with the addition of microbes when different condiments are mixed together with meat. The microbiota involved in the fermenting process is diverse and complex, and closely related to the ripening technique. Lactic acid bacteria (LAB) are usually present in high hygienic quality raw meat at low amounts and dominate the fermentation later (Tu, et al., 2010). Their presence effectively prevents harmful bacteria growth and controls the fermentation processes. During the fermentation, acids and alcohols are produced, leading to a decrease of pH (Xu, et al., 2008). Lactobacillus species are the most prevalent microorganisms in dry fermented sausages, and their use as starter cultures is widespread (Hammes et al., 1990). Even though lactic acid bacteria are known as weak lipolytic and proteolytic organisms (Johansson et al., 1994).

In many cases, particularly when no starter cultures are used, direct addition of acids is preferred in order to assure pH lowering within a very short time. Common organic acids are used for this purpose, mainly lactic and citric acids, as well as their sodium and potassium salts, which show much less ability to lower pH values. Besides these, an acid-related molecule may also be used: glucono-delta-lactone (GdL) (Toldrá, 2007).

GdL is a Generally Recognized as Safe (GRAS) substance and is a weak acid, which converts to gluconic acid in water and slowly dissociates into hydrogen ions with time (Chang et al., 2009). After all, GdL slowly hydrolyzes to gluconic acid with a resulting reduction in pH, which finally causes the residual nitrite reduction (Juncher et al., 2000). However, GdL does not control the indigenous flora, and consequently, using only GdL might result in post-acidification giving fault fermentation and sensory drawbacks (Andersen and Cislaghi, 2007). Using a combination of starter culture and spices has resulted in changes in certain microbial properties, change in free fatty acids and effectively in product lowers the pH and aw (Zhao et al., 2011). Therefore, the aim of this study was to evaluate the effect of commercial spice mixture containing GdL in combination with starter culture on physical, microbiological and sensory characteristics of fermented sausages during ripening.
Pork and beef lean meat in ratio 2 : 1 in combination with back pork fat (30%) were trimmed and cured (2.0% salt and 0.01% nitrite). Then the cured trimmed meat mix was divided into four equal parts. Currently available conventional spices paprika (100 g kg⁻¹), pepper (4 g kg⁻¹), caraway (4 g kg⁻¹), garlic (10 g kg⁻¹), spicy paprika (4 g kg⁻¹) were added to the first (C) and second parts (CC) of sausage mixture. Commercial spice mixture designed for meat processing plants contain GdL, was added to the third (M) and fourth part (MC) of sausage mixture. Added conventional spices and commercial spice mixture came from the same manufacture company and were sold under the same brand. Furthermore, second part (CC) and fourth part (MC) of sausage mixture contains starter culture Lyocarni SHI-59 (Clerici Sacco, Italy) in amount 0.2 g kg⁻¹. Each part of sausage mixture was separately minced (4 mm blade) and filled into 34 mm natural sausage casings, smoked and ripened in climatic chamber for 30 days.

Determination of technological and microbiological properties were realized after 24 hours, 5 and 30 days.

**Determination of pH value:**

The pH value of Danube sausages was measured using a Gryf 209 (Gryf HB, Czech Republic) apparatus during whole period of ripening.

**Determination of color:** Color spaces L*, a*, b* was determined by apparatus Heidolph DIAX 900 (Heidolph, Germany). After homogenization of samples. Color on the surface of homogenized sausages was measured with SCE (Specular Component Excluded).

**Determination of a_w:** was determined by Testo 645 (Germany).

**Microbiological examination**

The samples of sausages (5 g) were taken after specified storage periods and homogenized in saline for 30 second by apparatus Heidolph DIAX 900 (Heidolph, Germany). The samples for enumeration of mesophilic bacteria (MBC) were cultured on selective diagnostic plate count agar (Biokar Diagnostic, France) at temperature 30 ± 1 °C for 72 hours. The samples without addition of starter cultures (M and C) for enumeration of indigenous lactic acid bacteria count (LAB) were cultured on MRS agar (Himedia, India) at temperature 30 ± 1 °C for 5 days. The samples with addition of starter cultures (CC and MC) for enumeration of lactic acid bacteria count of genus Lactobacillus (LAB) were cultured on MRS agar (Himedia, India) at temperature 37 ± 1 °C for 5 days in anaerobic conditions (Anaerogen, Oxoid UK). Count of Enterobacteriaceae family (ETB) was determined on VRBG agar (Himedia, India) at temperature 37 °C after 24 hours of cultivation. Count of yeasts and moulds were determined on DRBC and DG18 agar (Merck, Germany) at temperature 25 °C after 5 days.

**Sensory evaluation**

The sensory analysis of sausages was carried out after 30 days of ripening process. Samples of sausages before and after heat treatment (heating in 80°C water, while in core of sausages reached the temperature 70 °C for 10 minutes) were evaluated by a 6-member semi-trained panel of laboratory co-workers. Panelists evaluate, color, aroma, taste on 5-point hedonic scale where 1 (the worst) and 5 (the best) were the extremes of each characteristic.

**RESULTS AND DISCUSSION**

The following 4 types of sausages were evaluated:

- sample C – sausages with addition of conventional spices,
- sample M – sausages with addition of commercial spice mixture (with GdL),
- sample CC – sausages with addition of conventional spices and starter culture,
- sample MC – sausages with addition of commercial spice mixture (with GdL) and starter culture.

The lowest values of pH in samples of fermented sausages were determined after 5 days of ripening. The lowest pH values (5.25) after 30 days of ripening were determined in the samples of M and MC fermented sausages (Figure 1). According to Slovak decree no. 1895/2004-100, the both samples containing commercial spices mixture (M and MC) may be included into subgroups with pH value below 5.5, and mark them as fermented products. The products C and MC were classified according to pH and a_w value to subgroups dried (pH 5.5 to 6.2). The higher pH value of the sample C may be due to indigenous lactobacilli with the low acidifying ability (Casaburi et al., 2007).

Continuous decline of the water activity was observed during whole ripening process. The most significant decrease of water activity was found in products M. The water activity after 24 hours in all samples was...
characterized by values close to the level which is an average value of 0.945 (Figure 2).

Intensity of red color ($a^*$) during the whole period of ripening was determined in fermented sausages with the addition of commercial spice mixture, regardless of the addition of starter culture (CM and MC). These results are in contrary with previous studies (Hong et al., 2008; Hong and Chin 2010) noted increasing the GdL level significantly decreased the $a^*$ value (redness) of the fermented meat products.

The cause is probably due to the addition of colorant cochineal also called carmine (E 120) in a commercial spice mixture.

The addition of starter culture to the products with the addition of currently available conventional spices (CC) also increased the intensity of the red color (Figure 3). In products with only the addition of currently available conventional spice (C) was found after 24 hours the number of microorganisms of the family Enterobacteriaceae 1.94 log CFU.g$^{-1}$ (Figure 4). During the next period of maturation were not bacteria of this family detected. The reason for the occurrence of the family Enterobacteriaceae could be contamination of traditional spices used in manufacture.

In the all samples of fermented sausages count of LAB up to 5 days of ripening increased. Counts of LAB at the end of ripening process (30 days) were lower in comparision with result obtained after 5 days; however their count was comparable with count determined at the beginig of the ripening. Addition of commercial spice mixture which contains GdL had no effect on the count of LAB in the samples of fermented sausages during whole period of ripening process.

The rapid growth of LAB in the initial stage of fermentation is beneficial in reducing the pH of the fermented product and inhibits undesirable bacteria such as microorganisms of the family Enterobacteriaceae (Essid and Hassouna, 2013).

Occurrences of yeasts in fermented meat products were not detected. According to Fernández-López et al. (2008) the number of yeast in fermented meat products stored 30 days is usual. Yeasts are characterized by lipolitic activity of secondary importance and can also contribute to the formation of an organoleptic profile of the final products. The sensory evaluation of fermented sausages was in raw state (uncooked), which was supposed to evoke the same possibilities for evaluating as consumers in stores. The highest values for appearance were after 30 days of ripening assigned to both samples of fermented sausages with addition of starter cultures (CC, MC). The products with added conventional spices (C) and commercial spice mixtures (M) without addition of starter cultures were characterized by strongly wrinkled surface and yellowish fat grains. The characterized intensity of red color in sliced samples was determined in sausages with added commercial spice mixtures (M, MC). Fermented sausages produced with currently available conventional spices (C), were characterized by a weaker shade color after red paprika. In the final assessment of raw sausages without heat treatment were the highest values for overall

![Figure 3](image3.png)

**Figure 3** Intensity of red color ($a^*$) during ripening of fermented sausages.

![Figure 4](image4.png)

**Figure 4** Occurrence of microorganisms in fermented sausages after 24 hours of ripening.
acceptability assigned to the samples with the addition of commercial spice mixture with combination of starter culture (MC).

The highest intensity of characteristic odor evaluated after heat treatment was found in products using currently available conventional spices (C) and commercial spice mixtures (M) without addition of starter cultures. On the other hand sausages with commercial spice mixture and starter culture (MC) due to sour taste reached the lowest values for overall taste acceptability. Increased sour taste of these products was probably caused by combining of commercial spice mixture containing GdL and starter culture. Also Schillinger and Luecke, (1989) and Feiner (2006) reported that application of GdL might negatively influence the taste (metallic off-flavour and bitterness), texture (grittiness), and color (paleness) of the salami relative to the amount of GdL added. Furthermore, high levels of GdL might promote growth of peroxide-forming lactobacilli resulting in rancidity and further color problems.
CONCLUSION
Composition of commercial spice mixtures designated for the manufacture of meat products is currently considered as know-how of manufacturing companies. Therefore, the packaging of these spice products does not provide the exact composition. It is necessary to label on the package of commercial spice mixtures the presence and quantity of Gdl, especially in production where is probably possibility to use starter cultures. Our results show, that the combination of starter culture and commercial spice mixture containing Gdl may cause excessive sour taste and sensory defect of dry fermented meat products.

REFERENCES

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