EFFECT OF THYME AND OREGANO AQUEOUS TEA INFUSIONS ON THE MICROBIOLOGICAL CHARACTERISTICS OF SAUSAGES

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ABSTRACT
In this work the antimicrobial effect of Thymus vulgare and Origanum vulgare aqueous tea infusion on the total mesophilic bacterial count (TVC), psychrotrophic bacteria count (PBC) and enterococci count in the heat treated meat product – sausages were evaluated. To prepare 1 kg of sausage in experimental groups were used 10 cm$^3$ of Thymus vulgare resp. Origanum vulgare aqueous tea infusions. It was found that value of TVC and PBC in the experimental groups of sausages with Thymus vulgare addition after 7 days of storage (4 °C) were 2.78 resp. 2.14 log cfu.g$^{-1}$ and with the Origanum vulgare addition were 2.49 resp. 1.90 log cfu.g$^{-1}$. The value of TVC and PBC in the control group of sausage were 3.13 resp. 2.72 log cfu.g$^{-1}$. During 10 days of storage (4 °C) the TVC and PBC in the sausages with Thymus vulgare addition increase and reached the value 4.81 resp. 3.52 log cfu.g$^{-1}$. In the sausages with the Origanum vulgare addition TVC and PBC after 10 days of storage reached the value 3.67 resp. 1.60 log cfu.g$^{-1}$. The value of TVC and PBC in the control group of sausage after 10 days of storage were 6.47 resp. 5.47 log cfu.g$^{-1}$. Counts of enterococci in control and experimental groups of sausages during 10 days of storage were not detected. Thyme and oregano aqueous tea infusions suppressed the development of TVC and PBC and reached the value 4.81 resp. 3.52 log cfu.g$^{-1}$.

Keywords: thyme; oregano; sausages; bacterial count; enterococci; psychrotrophic bacteria

INTRODUCTION
The possibility of compensation of synthetic antimicrobial and antioxidant compounds in animal husbandry as well as food production, with substances of plant or animal origin naturally occurring in the environment with a high content of bioactive substances has a world interest. Their impact is examined primarily in food to increase oxidative stability, inhibition of pathogenic and spoilage bacteria and improve their quality and safety with prolonged period of consumption.

Raw meat can be easily contaminated by microorganisms and support the growth of pathogens, leading to serious food-borne illnesses. Refrigeration is the most common preservation method of raw meat and meat products. In order to extend the shelf life time of refrigerated storage, synthetic additives may be added to meat foods (Solomakos et al., 2008). Progress in a new data relating chemical additives with toxicological problems (Kaur and Kapoor, 2001) resulting in a tendency by the informed consumer to use natural food products. Many research groups are examining the chemical nature and activity of natural antimicrobials and antioxidants in fruits, vegetables, grains, herbs and other foods (Atoui et al., 2005; Gill and Holley, 2006). Thyme (Thymus vulgaris) and oregano (Origanum vulgare) were often used in meat industry mainly as spices. Chemical analysis of thyme and oregano essential oils revealed the presence of more than 60 components which mainly thymol and carvacol are responsible for antibacterial effect against a board spectrum of gram-positive and gram-negative bacterial species (Gill and Holley, 2006).

The various thyme and oregano extracts were tested for the antioxidant and antimicrobial activity in the foods. In the in vitro studies were analyzed mainly ethanol extracts, acetone extracts, methanol extracts and hexane extracts (Ozen et al., 2011). Also were analyzed the edible coating or gelatin films from polysaccharides, protei...
system is generally reduced when compared to in vitro work, as the presence of fats, carbohydrates, proteins, salts and pH strongly influence the effectiveness of these agents (Burt, 2004).

The objective of this study was to examine antimicrobial activity of thyme and origanum aqueous tea infusions in pork sausages, to estimate their efficacy against to enterococci, mesophilic and psychrotrophic bacteria growth.

MATERIAL AND METHODOLOGY
Preparation of aqueous tea infusions
Dried origanum (Origanum vulgare) and thyme (Thymus vulgaris) were purchased from a local store and kept in dark until the use. Aqueous extracts were prepared by one-step extraction with 3 g of each pulverized plants, placed in a flask with added 100 cm³ distilled water. The suspensions were incubated at 70 °C in water bath for 2 hours. After filtration through Whatman No. 4 the 10 cm³ of each aqueous extracts were used per 1 kg of meat (Kulišić et al., 2007; Matsuura et al., 2003).

Preparation of meat products
The sausages were prepared from freshly boneless pork. Meat was from local abattoir approximately 24 h after slaughter. The pH value of pork was measured 45 min and 24 hours post mortem by pH apparatus Gryf 209L so as to avoid the purchase of PSE meat. The muscle was aseptically sliced and sausages were prepared using the following ingredients per 1 kg of meat: 18 g of mixture sodium nitrite and sodium chloride, 1.5 g powdered black pepper (Piper nigrum), 1 g sweet pulverized paprika (Capsicum annuum), 0.2 g powdered nutmeg (Myristica fragrans), 0.2 g powdered allspice (Pimenta officinalis), 10 g cutter mix and 200 cm³ water. Following mincing, raw materials were assigned to one of six treatments. Control sausages (no added extracts); sausages with added 10 mL/kg thyme; sausage with added 10 and mL/kg origanum. The sliced meat with ingredients was fine chopped by bowl vertical cutter PSP 500 for 5 minutes. Mixing, filling and striking of sausage mixtures were carried out in aseptically conditions with minimal possibility of secondary contamination. Emulsified sausages were stuffed into polyamide casings (Ø 22 mm), cold smoked for 4 hours and heat treated in water bath until the temperature in the center of sausages reached the value 70 °C for 10 min. The sausages were stored in air conditions at 4 ±1 °C and relative humidity 95% and evaluated for microbial growth on 1st, 7th, and 10th days.

Determination of antimicrobial activity
The samples of sausages (5 g) were taken after specified storage periods and homogenized in saline for 30 second by apparatus Heidolph DIAX 900. Mesophilic bacterial count, resp. total viable count (TVC) and count of psychrotrophic bacteria (PBC) were determined on diagnostic Plate count agar (HiMedia, India). Samples were incubated at temperature 30 ±1 °C for 72 ±2 h (TVC) and at the temperature 6.5 ±1 °C for 10 days (PBC).

The samples for enumeration of enterococci count were cultured on selective diagnostic Slanetz – Bartley agar at temperature 37 ±1 °C for 48 ±2 h (Biokar Diagnostic, France). Microbial counts were transformed to log 10 cfu.g⁻¹.

Statistical analysis
The significance of differences among treatments at 1st, 7th, and 10th day of storage was determined by analysis of variance (ANOVA). Differences were considered significant at the p <0.05 level. The geometric mean and standard deviation of the difference was also calculated. The entire experiment was replicated three times.

RESULTS AND DISCUSSION
The TVC in sausages without added spices reached the value 3.00 ±0.25 log cfu.g⁻¹ after 24 hours of storage. The values of TVC in sausages treatment with spice aqueous tea infusions after 24 hours of storage were lower about 0.58 (thyme) resp. 0.96 (origanum) log cfu.g⁻¹ compared with control. Also it was found lower values of PBC in sausages with added thyme and origanum aqueous tea infusions compared with control (Figure 1). The differences between counts of bacteria in control and tested samples were after 24 hours and 7 days of chilling storage not significant (p >0.05). Slovak requirements not include the obligation to evaluate the count of mesophilic and bacteria in meat products. Nevertheless, in our opinion the enumeration of psychrotrophic bacteria is important especially in animal products stored at refrigeration temperatures. In matter of fact the group of psychrotrophic bacteria including mainly spoilage bacteria with proteolytic and lipolytic properties but also the pathogenic bacteria. Considering to their mentioned properties the psychrotrophic bacteria occurred in meat products represent one of the ultimate factors affected their shelf life during the chilling storage.

The TVC and PBC increased steadily during 10 days of storage in sausages treated with spice aqueous tea infusions and also in control. The highest increase of TVC and PBC were observed in sausages without spice treatment after 10 days of storage and reached the value 6.47 ±0.80 resp. 5.47 ±0.15 log cfu.g⁻¹. It was found statistically significant differences (p <0.05) in values of TVC and PBC between control and sausages treated with spice aqueous tea infusions. The TVC and PBC in sausages treated with thyme after 10 days of storage were lower about 1.66 respectively 1.95 log cfu.g⁻¹ (p <0.05) and in sausages treated with origanum were lower about 2.8 (for TVC) resp. 3.87 (for PBC) log cfu.g⁻¹ relative to control samples of sausages.

Occurrence of enterococci in tested and control sausages was not detected during of storage.

Discussed authors also found comparable results, however they tested the essential oils. Busatta et al. (2008) found that addition of Origanum majorana essential oil to fresh sausages exerted bactericidal effect but higher concentration of essential oil also caused alterations in the taste of product.

The Fratianne et al. (2010) found comparable results of TVC after chicken breast meat treatment by thyme essential oil. They reported that TVC of chicken breast meat with thyme treatment reached the value 2.9 x 10³ cfu.cm⁻³ and 4.4 x 10⁹ cfu.cm⁻³ after 7 resp. 21 days of storage. These results were significantly lower than those
of the control sample after both 7 days (3.6 x 10^4 cfu.cm^-2) and 21 days (3.6 x 10^4 cfu.cm^-2) of storage at 4 °C. Also, Nessrien-Yasin and Abou-Taleb (2007) treated the fish fillets with 2.5% solution of thyme and origanum and found the decrease of TVC about more than 4 log cfu.g^-1 and PBC about more than 2 log cfu.g^-1 after 6 days of storage at 4 °C.

Tsigarida et al. (2000) reported a reduction of beef meat fillets microflora by 2 to 3 log cfu.g^-1 with the addition of 0.8% origanum essential oil after 7 days of storage. Skandamis and Nychas (2001) and Chouliara et al. (2007) also reported a suppression of TVC in poultry and minced meat when origanum oil was added.

CONCLUSION

Results obtained in this work indicated the technical viability of using the origanum and thyme aqueous tea infusions in relative low concentration, which is possible to enlarge the shelf-life of fresh sausages with the desired slight alteration of the original taste parameters. Results indicate that thyme and origanum aqueous tea infusions compare to essential oils can be cheaper alternative incorporate into pork sausages as natural antimicrobials.

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