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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 origanum aqueous tea infusions suppressed the development of TVC and PBC compare to control samples.

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 prologated 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 origanum (*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 origanum 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, proteins and lipids with thyme and origanum adding which can extend the shelf-life of meats, poultry and seafood **(Gennadios et al., 1997; Gómez-Estaca et al., 2009; Min and Oh, 2009)**.

The main advantage of essential oils with antibacterial and antioxidant activity is that they can be used in any food and are generally recognized as safe (GRAS) **(U.S Food and Drug Administration, 2006)**, as long as their maximum effects are attained with minimal change in the organoleptic properties of the food **(Viuda-Martos, 2009)**. It is well known that potency of essential oil in food

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 vulgare*) 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 cm^3 distilled water. The suspensions were incubated at 70 °C in water bath for 2 hours. After filtration through Whatman No. 4 the 10 cm^3 of each aqueous extracts were used per 1kg 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 1kg 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 cm^3 water. Following mincing, raw materials were assigned to one of six treatments. Control sausages (no added extracts); sausages with added 10 $mL.kg^{-1}$ thyme; sausage with added 10 and $mL.kg^{-1}$ 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 22mm), 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 geomean 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 psychrotorophic 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 **Fratianni 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 10^3 $cfu.cm^{-3}$ and 4.4 x 10^4 $cfu.cm^{-3}$ after 7 resp. 21 days of storage. These results were significantly lower than those

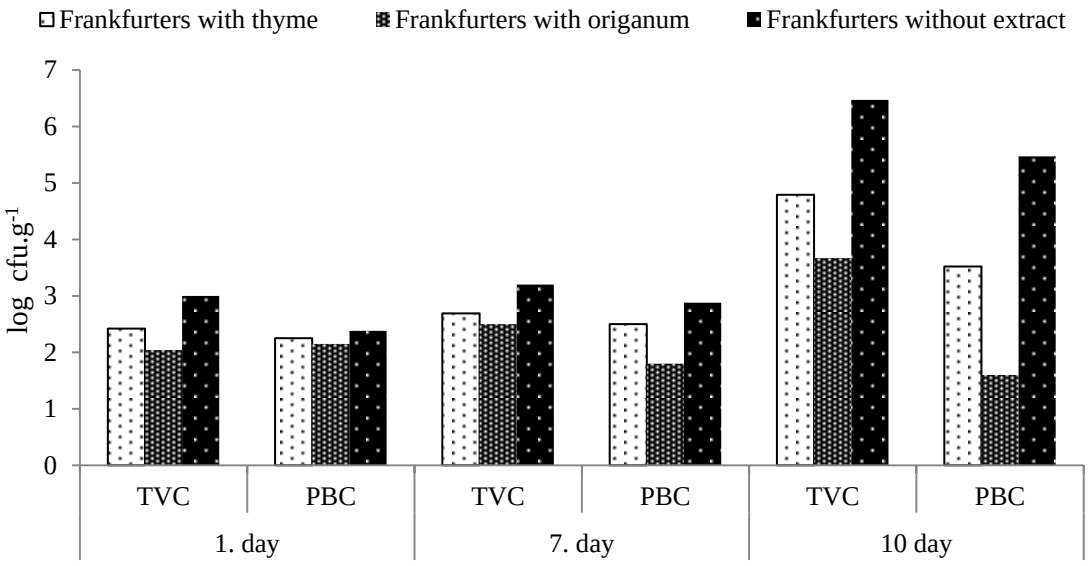

Figure 1 Total aerobic mesophilic bacterial count (TVC) and count of psychrotrophic bacteria (PBC) in tested sausages during 10 days of storage.

of the control sample after both 7 days (3.6 x 10^4 cfu.cm^{-3}) and 21 days (3.6 x 10^4 cfu.cm^{-3}) of storage at 4 °C. Also **Nessrien-Yasin and Abou-Taleb (2007)** treated the fish filets 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.

REFERENCES

Atoui, A. K., Mansouri, A., Boskou, G., Kefalas, P. 2005. Tea and herbal infusions: their antioxidant activity and phenolic profile. *Food Chemistry*, vol. 89, no. 1, p. 27-36. https://doi.org/10.1016/j.foodchem.2004.01.075

Burt, S. 2004. Essential oils: their antibacterial properties and potential applications in foods. *International Journal of Food Microbiology*, vol. 94, no. 3, p. 223-253. https://doi.org/10.1016/j.ijfoodmicro.2004.03.022 PMid:15246235

Busattaa, C., Vidala, R. S., Popiolskia, A. S., Mossia, A. J., Darivab, C., Rodriguesc M. R. A., Corazzaa, F. C., Corazzaa, M. L., Oliveiraa, J. V., Cansian, R. L. 2008. Application of *Origanum majorana L.* essential oil as an antimicrobial agent in sausage. *Food Microbiology*, vol. 25, no. 1, p. 207-211. https://doi.org/10.1016/j.fm.2007.07.003 PMid:17993397

Fratianni, F., De Martino, L., Melone, A. 2010. Preservation of chicken breast meat treated with thyme and balm essential oils. *Journal of Food Science*, vol. 75, no. 8, p. 528-535. https://doi.org/10.1111/j.1750-3841.2010.01791.x PMid:21535509

Gennadios, A., Hanna, M. A., Kurth, L. B. 1997. Application of Edible Coatings on Meats Poultry and Seafoods. *LWT – Food Science and Technology*, vol. 30, no. 4, p. 337-350. https://doi.org/10.1006/fstl.1996.0202

Gill, A. O., Holley, R. A. 2006. Disruption of *E. coli*, *Listeria monocytogenes* and *Lactobacillus sakei* cellular membranes by plant oil aromatics. *International Journal of Food Microbiology*, vol. 108, no. 1, p. 1-9. https://doi.org/10.1016/j.ijfoodmicro.2005.10.009 PMid:16417936

Gómez-Estaca, J., Bravo, L., Gómez-Guillén, M. C., Alemán, A., Montero, P. 2009. Antioxidant properties of tuna-skin and bovine-hide gelatin films induced by the addition of oregano and rosemary extracts. *Food Chemistry*, vol. 112, no. 1, p. 18-25. https://doi.org/10.1016/j.foodchem.2008.05.034

Chouliara, E., Karatapanis, A., Savvaidis, I. N., Kontominas, M. G. 2007. Combined effect of oregano essential oil and modified atmosphere packaging on shelf life extension of fresh chicken breast meat, stored at 4 °C. *Food Microbiology*, vol. 24, no. 6, p. 607-617. https://doi.org/10.1016/j.fm.2006.12.005 PMid:17418312

Kaur, C., Kapoor, H. C. 2001. Antioxidants in fruits and vegetables-the millennium's health. *International Journal of Food Science and Technology*, vol. 36, no. 7, p. 703-725. https://doi.org/10.1046/j.1365-2621.2001.00513.x

Kulišić, T., Dragović-Uzelac, V., Miloš, M. 2007. Antioxidant activity of aqueous tea infusions prepared from oregano, thyme and wild thyme. *Food Technology and Biotechnology*, vol. 44, no. 2, p. 485-492.

Matsuura, H., Chiji, H., Asakawa, C., Amano, M., Yoshihara, T., Mizutani, J. 2003. DPPH radical scavengers from dried leaves of oregano (*Origanum vulgare*). *Bioscience Biotechnology Biochemistry*, vol. 67, no. 11, p. 2311-2316. https://doi.org/10.1271/bbb.67.2311 PMid:14646188

Min, B. J., Oh, J. H. 2009. Antimicrobial activity of catfish gelatin coating containing origanum (*Thymus capitatus*) oil against gram-negative pathogenic bacteria. *Journal of Food Science*, vol. 74, no. 4, p. 143-148. https://doi.org/10.1111/j.1750-3841.2009.01115.x PMid:19490330

Nessrien-Yasin, M. N., Abou-Taleb, M. 2007. Antioxidant and antimicrobial effects of marjoram and thyme in coated refrigerated semi fried mullet fish fillets. *World Journal of Dairy & Food Sciences*, vol. 2, no. 1, p. 1-9.

Ozen, T., Demirtas, I., Aksit, H. 2011. Determination of antioxidant activities of various extracts and essential oil compositions of *Thymus praecox subsp. skorpilii var. Skorpilii*. *Food Chemistry*, vol. 124, no. 1, p. 58-64. https://doi.org/10.1016/j.foodchem.2010.05.103

Skandamis, P., Nychas, G. E. J. 2001. Effect of oregano essential oil on microbiological and physico-chemical attributes of minced meat stored in air and modified atmospheres. *Journal of Applied Microbiology*, vol. 91, no. 6, p. 1011-1022. https://doi.org/10.1046/j.1365-2672.2001.01467.x PMid:11851808

Solomakos, N., Govaris, A., Koidis, P., Botsoglou, N. 2008. The antimicrobial effect of thyme essential oil, nisin, and their combination against *Listeria monocytogenes* in minced beef during refrigerated storage. *Food Microbiology*, vol. 25, no. 1, p. 120-127. https://doi.org/10.1016/j.fm.2007.07.002 PMid:17993385

Tsigarida, E., Skandamis, P., Nychas, G. J. E. 2000. Behaviour of *Listeria monocytogenes* and autochthonous flora on meat stored under aerobic, vacuum and modified atmosphere packaging conditions with or without the presence of oregano essential oil at 5°C. *Journal of Applied Microbiology*, vol. 89, no. 6, p. 901-909. https://doi.org/10.1046/j.1365-2672.2000.01170.x PMid:11123463

U.S Food and Drug Administration, 2014. Food additive status list [online] 2014-12-16 [cit. 2017-01-17] Available at: https://www.fda.gov/food/ingredientspackaginglabeling/foodadditivesingredients/ucm091048.htm

Viuda-Martos, M., Ruiz-Navajas, Y., Fernández-López, J., Pérez-Álvarez, J. A. 2009. Effect of adding citrus waste water, thyme and oregano essential oil on the chemical, physical and sensory characteristics of a bologna sausage. *Innovative Food Science and Emerging Technologies*, vol. 10, no. 4, p. 655-660. https://doi.org/10.1016/j.ifset.2009.06.001

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