

## EFFECT OF ROSEMARY IN COMBINATION WITH YEAST EXTRACT ON MICROBIOLOGY QUALITY, OXIDATIVE STABILITY AND COLOR OF NON-FERMENTED COOKED SALAMI “INOVEC”

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### ABSTRACT

The aim of this study was to determine antimicrobial and antioxidant effect of dried rosemary (1 g/kg) in combination with yeast extract 1 g/kg (1<sup>st</sup> experimental group) and 2 g/kg (2<sup>nd</sup> experimental group) in compare to control sample (without rosemary extract and treated with E316 and E621) in non-fermented heat-treated product Inovec salami. Inovec salami is a product produced all over the Slovakia. The similar product can be found in Polish (Polish salami) or in the Czech Republic (Vysocina salami). The samples were vacuum packaged and were kept at 4 °C for 30 days. Determination of psychrotrophic bacteria count (PBC), count of *Enterobacteriaceae* family (ETB), yeast and moulds, *Lactobacillus* spp. bacteria count (LAB) was done by cultivation methods. Oxidative stability was determined by TBARS value. Color spaces L\*, a\*, b\* of Inovec salami was determined by CM 2600D spectrophotometer (Konica Minolta, Germany). It was found non-significant ( $p > 0.05$ ) color loss (decrease in redness) over time, and the treatments had a non-significant impact ( $p > 0.05$ ) on redness of the salami in compare to control sample. TBARS values of the rosemary treated cooked salami with different amount of yeasts extracts were significantly lower compare to control sample at the end of the shelf life. Also, lower value of TBARS was determined in salami with rosemary in combination with higher amount of yeast extract. The lowest count of PBC was determined in the second experimental group after 15 days. The rosemary extract in combination of yeast extract treatments were not able to influence the growth of the yeasts and moulds. It was found that rosemary extract in combination with yeast extract in the salami of the second experimental group after 15 days of storage significantly ( $p \leq 0.05$ ) decrease count of *Lactobacillus* bacteria.

**Keywords:** rosemary; salami; oxidative stability; color; antimicrobials.

### INTRODUCTION

Nowadays people are showing greater interest in foods that contain bioactive or functional components, which will give additional benefits to their health status (Cofrades et al., 2008). Many consumers believe meat and meat product consumption is unhealthy, because of their high animal fat, cholesterol, synthetic antioxidants and antimicrobials contents which may be associated with the several degenerative diseases (Serrano et al., 2007).

The extracts and essential oils of herbs and spices are widely known for their strong antioxidant, antimicrobial and antifungal activities in foods. These properties of herb and spice extracts are due to the presence of many bioactive components, including flavanoids, terpenoids, vitamins, minerals, carotenoids and phytoestrogens (Rodríguez Vaquero et al., 2010).

Addition of natural antioxidants to meat and meat products is one of the important strategies in development of healthier and novel meat products. In this regard several studies utilizing herbs, spices, fruits and vegetable extracts, and have shown that addition of these extracts to raw and cooked meat products decreased lipid oxidation, improved color stability and total antioxidant capacities which are

important characteristics for shelf stable meat products (Hygreeva et al., 2014).

Meat and meat products are good substrates for spoilage and pathogenic microorganisms because of their nutrient contents (Zhang et al., 2009). Antimicrobials are in meat products used for mainly two reasons: to preserve the food for long time (control natural spoilage process) and to increase food safety (control growth of pathogenic microorganisms). The term natural antimicrobials implies, antimicrobials derived from natural sources like plants, animals and microbes (Hygreeva et al., 2014).

Many species and herbs exert antimicrobial activity due to their essential oil fractions. Some scientists reported the antimicrobial activity of essential oils from oregano, thyme, sage, rosemary, clove, coriander, garlic, and onion against both bacteria and moulds. The composition, structure, as well as functional groups of the oils play an important role in determining their antimicrobial activity (Omidbeygi et al., 2007; Yesil Celiktas et al., 2007).

Because of concerns for the synthetic antioxidants and antimicrobials by some human health professionals and consumers, many meat processors have been seeking alternative “natural” antioxidants and antimicrobials (Karre et al., 2013). Rosemary (*Rosmarinus officinalis*)

extracts have been found to be effective in meat systems (Formanek et al., 2003; Lawrence et al., 2004).

Rosemary (*Rosmarinus officinalis*) is a plant species of the *Labiatae* family, and its major and most active extract components (e.g. carnosol, carnosic acid, carnosol, rosmarinic acid etc.) have been proved to be against cancer and inflammation diseases in experimental animals and humans (Johnson, 2011; Ngo et al., 2011). Rosemary extract also possesses antioxidant and antimicrobial properties. The diterpenes, carnosol and carnosic acid, have been shown to account for the antioxidant properties of rosemary (Offord, 2004; Rižnar et al., 2006). The phenolic compounds in rosemary extract are believed to enhance antimicrobial properties by affecting the function of the bacterial cellular membrane, the synthesis of DNA, RNA, protein and lipids, and the function of the mitochondrion (Belantine et al., 2006).

Thus, it was found that rosemary extract showed a synergistic effect with different antimicrobials in reducing the total bacterial counts fresh sausages but when it was used on its own, it was not effective (Mathenjwa et al., 2012). Seydim et al. (2006) found a similar effect when they used rosemary extract for reduction of total bacterial counts in vacuum packaged ground ostrich meat. Rosemary extract was more effective when used in a mixture or in combination with sodium lactate.

In this context the aim of this study was to determine antimicrobial and antioxidant effect of dried rosemary in non-fermented heat-treated product Inovec salami.

## MATERIAL AND METHODOLOGY

### Preparation of salami:

Pork (70%) and beef (30%) meat were trimmed, cured (2.0% salt and 0.01% nitrite) and together with spices extracts cooled 24 hours at 4 °C. The next day was meat minced (4 mm blade) and divided into three equal parts: meat mixture without rosemary extract and treated with E316 and E621 (C-positive control), treated with dried rosemary in amount 1 g/kg and yeast extract in amount 1 g/kg (1<sup>st</sup> experimental group), and treated with the same amount of dried rosemary, but yeast extract was in amount 2 g/kg (2<sup>nd</sup> experimental group). Each part was separately filled into 90 mm fibrous casings, dried, smoked and heat treated until the temperature in the core reached the value 70 °C for 10 min. Samples from each of the group were sliced and vacuum packaged. Samples were kept at 4 °C for 7, 15 and 30 days.

### Determination of microbiological contamination:

The samples of salami (5 g) were taken after specified storage periods and homogenized in saline for 30 s by apparatus Heidolph DIAX 900 (Heidolph, Germany). The samples for enumeration of psychrotrophic bacteria count (PBC) were cultured on plate count agar at temperature 4 ±1 °C for 10 days (Himedia, India). The samples for enumeration of *Lactobacillus* spp. bacteria count (LAB) were cultured on MRS agar (Himedia, India) at temperature 37 ±1 °C for 5 days. Count of *Enterobacteriaceae* family (ETB) was determined on VRBG agar (Himedia, India) at temperature 37 ±1 °C after 24 hours of cultivation. Count of yeast and moulds were

determined on DG18 agar (Merck, Germany) at temperature 25 ±1 °C after 5 days.

### Determination of antioxidant activity:

Lipid oxidation was assessed in triplicate by the 2-thiobarbituric acid test (TBARS) following the recommendations of Grau et al. (2000) and measured by spectrophotometric method at 532 nm (Jenway UV/VIS – 7305, UK). TBARS values were calculated from a standard curve of malondialdehyde (MDA) and expressed as mg MDA/kg sample.

### Determination of pH value:

The pH value of Inovec salami was measured using a Gryf 209 (Gryf HB, Czech Republic) apparatus during whole period of storage.

### Determination of color:

Color spaces L\*, a\*, b\* of Inovec salami was determined by CM 2600D spectrophotometer (Konica Minolta, Germany) after homogenization. Color on the surface of homogenized salami was measured with SCE (Specular Component Excluded).

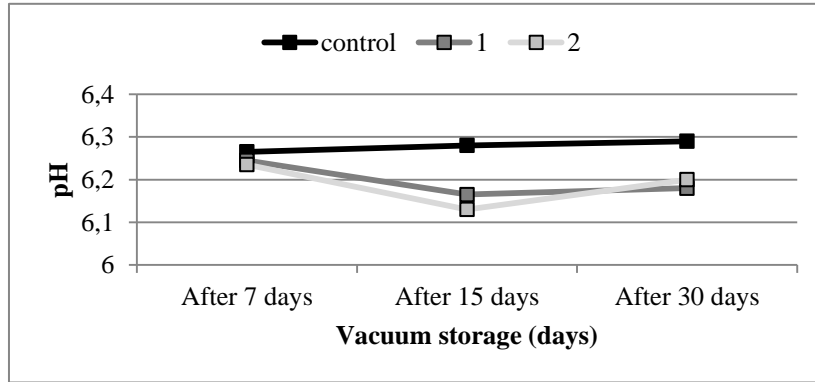
## RESULTS AND DISCUSSION

The pH values of salami were similar up to the seventh day of storage (6.23 – 6.26). The significant ( $p \leq 0.05$ ) decline of pH values were recorded in experimental groups compare to control after fifteen days of storage (figure 1). Significantly ( $p \leq 0.05$ ) lower values of pH in experimental groups were determined also at the end of storage.

The color values for lightness and redness are shown in figures 2 and 3. All samples were characterized without any significant colour discrepancies. There was non-significant ( $p > 0.05$ ) color loss (decrease in redness) over time, and the treatments had a non-significant impact ( $p > 0.05$ ) on redness of the salami in compare to positive control. Our results are in agreement with McCarthy et al. (2001b) and Mirshekar et al. (2009) who found that rosemary extract has been shown to reduce redness in broiler meat, fresh pork and frozen pork patties. A synergistic effect of rosemary with ascorbic acid has been observed in modified packaged fresh pork sausage, whereby the redness of the product was maintained for 12 days (Martinez et al., 2007). In contrary to our results Sebranek et al. (2005) found that rosemary extract significantly improve redness and was very effective in preserving the color of frozen pork sausage.

Yu et al. (2002) reported that rosemary extracts improved the color stability of cooked turkey rolls. Lawrence et al. (2004) also observed improved color stability as a result of rosemary extracts injected into beef loins.

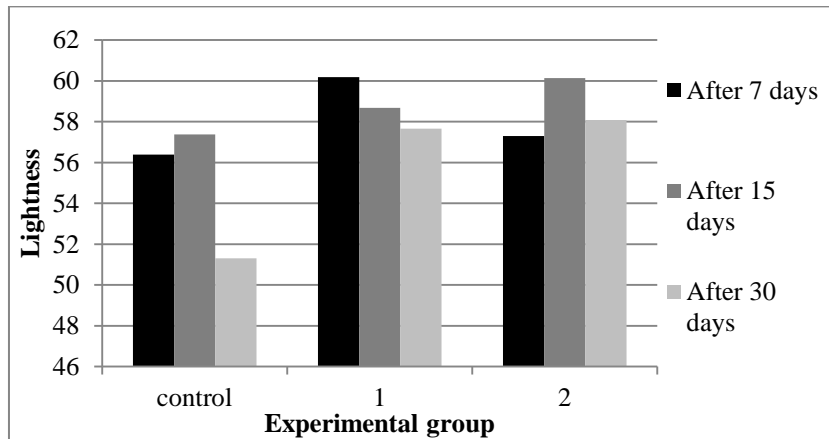
However treatment of rosemary extract in our study was more effective in maintaining lightness than the control. Results of lightness are in accordance with McCarthy et al. (2001a), who also found no significant differences over the storage period in either raw or cooked patties. Fernandez-Lopez et al. (2003) apply rosemary extracts to cooked pork meat and stored for 8 days at 4 °C. They found that rosemary extract inhibited the lipid oxidation and degradation of heme pigments caused by cooking and storage.



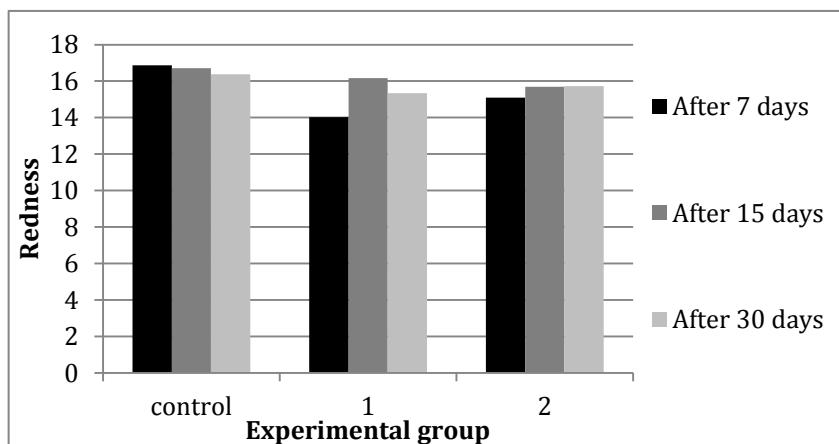
**Figure 1** Effect of rosemary with combination of yeast extract on pH value of Inovec salami during vacuum storage at 4 °C.

Also, delayed metmyoglobin formation and stabilized the red meat color of the cooked meat during storage. TBARS values of the rosemary treated cooked salami with different amount of yeasts extracts were significantly lower ( $p \leq 0.05$ ) compare to control at the end of the shelf life. Also, lower value of TBARS was determined in salami with rosemary in combination with higher amount of yeast extract.

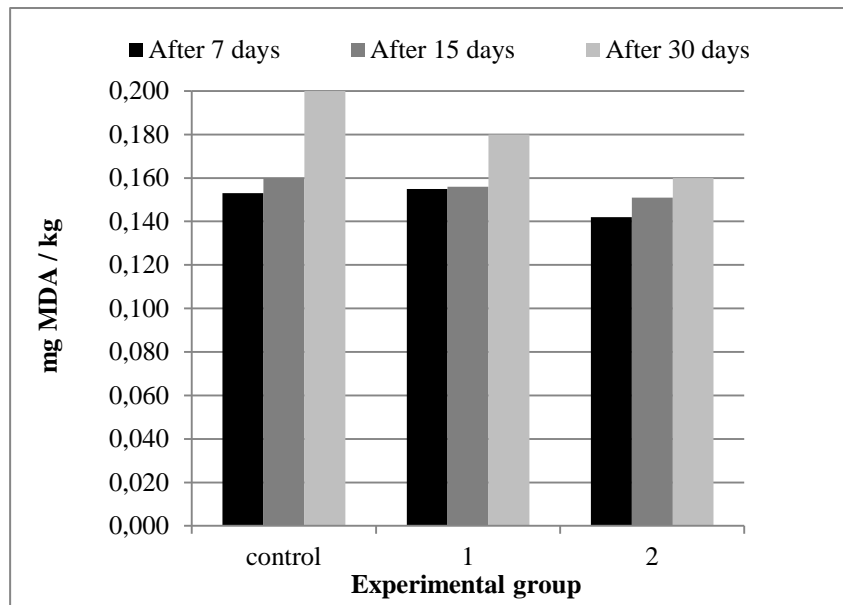
Lipid oxidation (apart from microbial spoilage) in meat and meat products is the main cause of their quality loss. A large number of compounds are generated during the oxidation processes which adversely affect texture, color, flavor, nutritive value and safety of meat products (Lahucky et al., 2010) and this limits the shelf-life of meat and meat products (Karakaya et al., 2011).



**Figure 2** Effect of rosemary with combination of yeast extract on lightness of Inovec salami during vacuum storage at 4 °C.



**Figure 3** Effect of rosemary with combination of yeast extract on redness of Inovec salami during vacuum storage at 4 °C.



Oil-soluble carnosic acid is one of the major antioxidant compounds of rosemary extract that stabilize unsaturated fatty acids and thus retard their deterioration (Estévez et al., 2005). Martínez et al. (2006) found that rosemary extract plus ascorbic acid strongly inhibited lipid oxidation and therefore off-odour formation, delayed sausage discolouration and inhibited microbial growth, extending the shelf-life of salted fresh pork sausages by at least 4 days.

The effect of the rosemary extract, yeast extract and storage time on the total bacterial psychrotrophic count of chosen groups is indicated in figure 5. The count of PBC in control, the first and the second experimental group after seven days of storage was 3.53; 2.78 respectively 3.05 log CFU.g<sup>-1</sup>. The numbers of other groups of bacteria after 7 days of storage were not detected. Also, the occurrence of bacteria *Enterobacteriaceae* family in salami during the whole period of storage was not determined. The lowest count of PBC was determined in the second experimental group

after 15 days. Jiang et al. (2011) identified that rosemary extract had strong antibacterial activity against Gram-positive and Gram-negative bacteria. Although rosemary extract in work of Mathenjwa et al. (2012) was not identified as a good antimicrobial, its antimicrobial and antioxidant properties were improved when used in combination with the SO<sub>2</sub>. The rosemary extract in combination of yeast extract treatments were not able to influence the growth of yeasts and moulds. Also, Ibrahim and Al-Ebady (2014) found that rosemary treatments exhibited no effect in preventing fungal infection. Šošo et al. (2013) found a strong antifungal activity of rosemary oleoresin against *Aspergillus clavatus*. It was found that rosemary extract in combination with yeast extract in the salami of the second experimental group after 15 days of storage significantly ( $p \leq 0.05$ ) decrease count of *Lactobacillus* bacteria. Especially susceptibility to the activity of rosemary extracts of the *Lactobacillus* and *Brochothrix* genus also found Fernandez-Lopez et al. (2005).

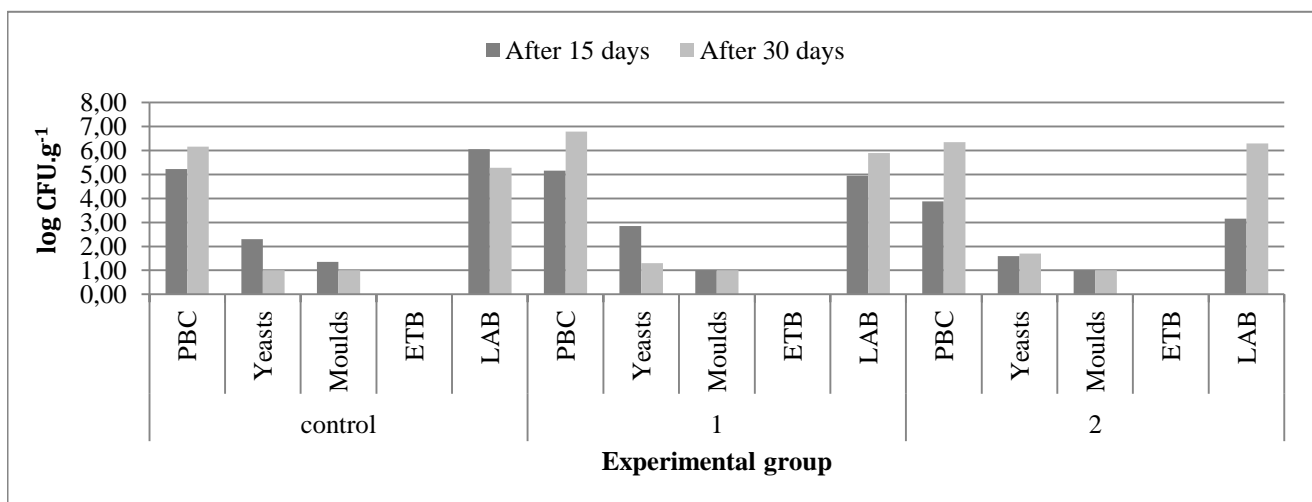


Figure 5 Effect of rosemary with combination of yeast extract on count of selected bacteria.

## CONCLUSION

The negative consumer perception towards chemical antimicrobials/preservatives led to extensive search for natural antimicrobials. Numerous studies have compared plant derivatives with synthetic antimicrobials and antioxidant and reported that they are more potent and safer than synthetic compounds. While rosemary extracts have been found in this study to be not effective in preserving the color the further research has to be carried out for its natural antioxidant and antimicrobial potential in application in shelf stable non-fermented cooked salami. Also further research are required to find the appreciate doses and combinations of rosemary extract.

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