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OXIDATIVE STABILITY OF CHICKEN'S BREAST AFTER VACUUM PACKAGING, EDTA, SAGE AND ROSEMARY ESSENTIAL OILS TREATMENT

Adriana Pavelková, Marek Bobko, Peter Haščík, Miroslava Kačániová, Jana Tkáčová

ABSTRACT

In the present work, the effect of the sage and rosemary essential oils on oxidative stability of chicken breast muscles during chilled storage was investigated. In the experiment were chickens of hybrid combination Cobb 500 after 42 days of the fattening period slaughtered. All the broiler chickens were fed with the same feed mixtures and were kept under the same conditions. The feed mixtures were produced without any antibiotic preparations and coccidiostats. After slaughtering was dissection obtained fresh chicken breast with skin from left half-carcass, which were divided into five groups (n = 5): C - control air-packaged group; A1 - vacuum-packaged experimental group; A2 - vacuum-packaged experimental group with EDTA solution 1.50% w/w; A3 - vacuum-packaged experimental group with *Salvia officinalis* L. oil 2.0% v/w and A4 - vacuum-packaged experimental group with *Rosmarinus officinalis* L. essential oil 2.0% v/w. The sage and rosemary essential oils were applicate on surface chicken breasts and immediately after dipping, each sample was packaged using a vacuum packaging machine and storage in refrigerate at 4 ±0.5 °C. The value of thiobarbituric acid (TBA) expressed as amount of malondialdehyde (MDA) in 1 kg sample was measured during storage in 1st, 4th, 8th, 12th and 16th day. The treatments of chicken breasts with sage and rosemary essential oils show statistically significant differences between all testing groups and control group, where higher average value of MDA measured in breast muscle of broiler chickens was in samples of control group (0.396 mg.kg^{-1}) compared to experimental groups A1 (0.060 mg.kg^{-1}), A2 (0.052 mg.kg^{-1}), A3 (0.042 mg.kg^{-1}) and A4 (0.041 mg.kg^{-1}) after 16-day of chilled storage. The results of experiment showed that the treatment of chicken breast with sage and rosemary essential oils had positive effect on the decrease of oxidative processes in breast muscles during chilling storage and use of plant essential oils is one of the possibilities increase shelf life of fresh chicken meat.

Keywords: oxidative stability; chicken breast; essential oil; sage, rosemary

INTRODUCTION

Meat and meat products are essential components in the human diets and their consumption is affected by various factors, e.g. product characteristics, consumer and environment related **(Jiménez-Colmenero et al., 2001)**. Chicken meat has many desirable nutritional characteristics such as a low lipid content and relatively high concentration of polyunsaturated fatty acids (PUFAs) which can be further increased by specific dietary strategies **(Bourre, 2005)**. However, a high degree of polyunsaturation accelerates oxidative processes leading to deterioration in meat flavour, colour, texture and nutritional value **(Mielnick et al., 2006)**.

Lipid oxidation causes degradation of polyunsaturated fatty acids (PUFA) and generation of residual products, such as malondialdehyde (MDA) and lipid-derived volatiles leading to sensory and nutritional deterioration of meat **(Kanner et al., 1991)**. Oxidative reactions in foodstuffs are enhanced after cooking and refrigerated storage through the increase of their oxidative instability due to the degradation of natural antioxidants and the release of free fatty acids and iron from the haem molecule **(Estévez and Cava, 2004; Kingston et al., 1998; Kristensen and Purslow, 2001)**.

The higher level of PUFAs in muscle membranes increases the susceptibility of oxidative deterioration of lipid **(Engberg et al., 1996)**, which impairs the organoleptic characteristics and shortens the shelf-life of meat and meat products.

The major strategies for preventing lipid oxidation are the use of antioxidants and restricting the access to oxygen during storage vacuum-packaging **(Tang et al., 2001)**. The antioxidant additives are added to fresh and further processed meats to prevent oxidative rancidity, retard development of off-flavours, and improve colour stability **(Nam and Ahn, 2003)**.

For chicken meat products, freshness, as one of the most important quality attributes, has attracted attention from producers and consumers and has a strong relationship with product sales and consumption **(Rzepka et al., 2013)**. One option for reducing lipid oxidation is the use of various natural plant antioxidants presented in essential oils.

The use of natural preservatives to increase the shelf life of meat products is a promising technology since many

vegetal substances have antioxidant and antimicrobial properties. Functional ingredients in meat products may improve the nutritional and health qualities and prolonging their self-life **(Fernández-Ginés et al., 2005)**. Plants' extracts rich in polyphenols are good candidates, since they are easily obtained from natural sources and they efficiently prevent lipid oxidation in food products.

Studies have shown wide effective in spices to retard lipid oxidation in meat products **(Juntachote et al., 2006, 2007; Chouliara et al., 2007; Mariutti et al., 2008; Sasse et al., 2009; Lee et al., 2010; Marcinčák et al., 2010; Viuda-Martos et al., 2011; Tkáčová et al., 2015)**.

Essential oils (EOs) are aromatic oily liquids obtained from plant material (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots). They can be obtained by expression, fermentation, enfleurage or extraction but the method of steam distillation is most commonly used for commercial production of EOs **(Van de Braak and Leijten, 1999)**. EOs obtained from various herbs are widely used in cosmetics and food manufacturing and can be used for prolonging the shelf-life of food for their antimicrobial **(Skandamis et al., 2002; Mihajilov-Krstev et al., 2009)**, and antioxidant activities **(Burt, 2004; Bobko et al., 2015a, b)**.

In the last years, many researchers have evaluated the antioxidant properties of extracts from different plants and vegetables **(Chen et al., 2002; Ibanez et al., 2003; Ichikawa et al., 2003)**.

Essential oils represent a small fraction of the plant composition; the main compounds are terpenes and sesquiterpenes, and several oxygenated derivatives compounds (alcohols, aldehydes, ketones, acids, phenols, ethers, esters, etc.) all of them responsible for the characteristic plant odour and flavour **(Yanishlieva et al., 2006)**. These compounds include natural flavourings such as sage, oregano, rosemary and others **(Mariutti et al., 2008)**.

Sage (*Salvia officinalis*) and rosemary (*Rosmarinus officianalis*) are popular *Labiatae* herbs with a verified potent antioxidant activity **(Dorman et al., 2003)**. The antioxidant activity of sage and rosemary essential oils is mainly related to two phenolic diterpenes: carnosic acid and carnosol which are considered two effective free-radical scavengers **(Dorman et al., 2003; Ibanez et al., 2003)**.

Sage (*Salvia officinalis*) is a variety of aromatic herb which has been planted widely throughout much of the world. It is not only used as raw material in the pharmaceutical and cosmetic industries but also used to improve flavours of foods **(Tepe et al., 2006)**. Sage has been reported to have excellent activities in scavenging radicals, reducing metal ions and inhibiting lipid peroxidation **(Dorman et al., 2003; Grzegorczyk et al., 2007)**. The phenolic compounds, such as carnosol, carnosic acid and rosmarinic acid, in the plant may account for the antioxidant activity of sage. Some researchers have reported that sage, or sage extracts, can effectively retard lipid oxidation of muscle foods **(Fasseas et al., 2007; McCarthy et al. 2001a; Tanabe et al., 2002)**.

Among natural antioxidant sources, rosemary (*Rosmarinus officinalis* L.), a woody aromatic herb that is native to the Mediterranean countries, has recently been authorized by the European Union under Directive 95/2/EC and assigned E-392 as its E number (European Union Directives 2010/67/EU and 2010/69/EU) for use in meat product preservation. The addition of rosemary extract to poultry products has been shown to be effective in retarding lipid oxidation, and previous studies in chicken sausages **(Liu et al., 2009)** and patties **(Naveena et al., 2013)** have pointed to the protective effect of rosemary extract (500–1500 ppm) and leaves (22.5–130 ppm) in inhibiting lipid oxidation.

Rosemary antioxidant activity is related to components such as phenolic diterpenes, carnosol (CAS No. 5957-80-2) and carnosic acid (CAS No. 3650-09-7) **(Rodriguez-Rojo et al., 2012)**. The antioxidant capacity of phenolic compounds is due to their ability to scavenge free radicals, donate hydrogen atoms and chelate metal cations **(Shan et al., 2005)**. Previous studies **(Azmir et al., 2013; Wang et al., 2013)** have reported that the yield of bioactive compounds can be changed or modified by using different extraction procedures, solvents, temperatures, pressures and times.

In this study we aimed to investigate the combined effect of ethylenediaminetetraacetate (EDTA) and plant essential oils (*Salvia officinalis* L. and *Rosmarinus officinalis* L.) on the oxidative stability of fresh chicken breasts stored under vacuum packaging (VP), at 4 ±0.5 °C for a period of 16 days.

MATERIAL AND METHODOLOGY

The experiment was implemented in the local poultry station (Hydinaren a.s., Zamostie). The tested were broiler chickens of hybrid combination Cobb 500 both sexes. All the broiler chickens were fed with the same feed mixtures and were kept under the same conditions. The feed mixtures were produced without any antibiotic preparations and coccidiostats. At the end of the fattening period (42. day) were chickens slaughtered for analysis in laboratory of Slovak University of Agriculture in Nitra. After slaughtering was dissection obtained fresh chicken breast with skin from left half-carcass, which were divided into five groups (n = 5):

- Air-packaged (C, control group): chicken breast fresh meat was packaging to polyethylene backs and stored aerobically in refrigerator;
- Vacuum-packaged (A1, experimental group): chicken breast fresh meat was packaging to polyethylene backs and stored anaerobically in vacuum and in refrigerator;
- VP with EDTA solution 1.50% w/w (A2, experimental group): chicken breast fresh meat was treated with EDTA for 1 min and packaging to polyethylene backs and stored anaerobically in vacuum and in refrigerator;
- VP with *Salvia officinalis* L. 2.0% v/w (A3, experimental group): chicken breast fresh meat was treated with *Salvia officinalis* L. oil for 1 min and packaging to polyethylene backs and stored anaerobically in vacuum and in refrigerator;
- VP with *Rosmarinus officinalis* L. 2.0% v/w, (A4, experimental group): chicken breast fresh meat was treated with *Rosmarinus officinalis* L. oil for 1 min and packaging to polyethylene backs and stored anaerobically in vacuum and in refrigerator.

Immediately after dipping, each sample was packaged using a vacuum packaging machine type VB-6 (RM Gastro, Czech Republic).

Ethylenediaminetetraacetic acid (EDTA) (C10H14N2O8.Na2.2H2O) was 99.5% purity, analytical grade, (Invitrogen, USA). A stock solution of 500 mM concentration was prepared by diluting 186.15 $g.L^{-1}$ distilled water. A final concentration of 50 mM EDTA solution was prepared from the stock solution. The pH of the solution was adjusted to 8.0 with the addition of the appropriate quantity of NaOH solution. The amount of EDTA added to the treat chicken breasts was 0.28 $g.kg^{-1}$. Essential oil (Calendula, Nova Lubovna, Slovakia) were added to the coated chicken beast surface (both sides) of each sample using a micropipette so as to achieve a 0.2% v/w final concentration of essential oils.

TBA value expressed in number of malondialdehyde (MDA) was measured in the process of first storage day of 1^{st}, 4^{th}, 8^{th}, 12^{th} and 16^{th} day. TBA number was determined by **Marcinčák et al. (2004)**. Absorbance of samples was measured on UV-VIS spectrophotometer T80 (PG Limeted Instruments, UK) at a wavelength of 532 nm, the translation results on the amount of MDA in 1 kg samples.

Results of the experiment were evaluated by statistical program SAS 9.3 with using application Enterprise Guide 4.2. The variation-statistical values (mean, standard deviation) were calculated and to determine the significant difference between groups was used variance analyse.

RESULTS AND DISCUSSION

Jo et al. (2006) stated that oxidation of lipids can have significant impact to meat industry. Meat containing unsaturated fatty acids is very sensitive to lipid oxidation especially during storage, because polyunsaturated fatty acid esters are easily oxidized by molecular oxygen. This kind of oxidation is called autoxidation and proceeds by a free radical chain mechanism **(Brewer, 2011)**.

The results of the oxidation stability of fresh chicken breast muscles of chicken Cobb 500 after application EDTA and plant essential oils (*Salvia officinalis* L. and *Rosmarinus officinalis* L.) during 16 days storage at 4 °C are shown in Table 1 and Figure 1.

The higher average value of MDA measured in breast muscle in 0 day of experiment was in samples of vacuum-packaged chicken breasts group with *Rosmarinus officinalis* L. oil 2.0% v/w group A4 (0.026 $mg.kg^{-1}$) compared to experimental groups A1 (0.022 $mg.kg^{-1}$), A2 (0.023 $mg.kg^{-1}$), A3 (0.024 $mg.kg^{-1}$) and air-packaged control group (0.024 $mg.kg^{-1}$). We have not found statistically significant differences between testing groups chicken breasts. During chilled storage of the breast muscles were noticed increased content of malondialdehyde in comparison to the first day of storage.

On the fourth day of storage were measured below the values of malondialdehyde in all experimental groups (0.028 $mg.kg^{-1}$ in group A2, 0.030 $mg.kg^{-1}$ in group A3, 0.034 $mg.kg^{-1}$ in group A4, and 0.036 $mg.kg^{-1}$ – group A1) opposite control group C (0.182 $mg.kg^{-1}$). We have found statistically significant differences ($p \leq 0.05$) between control group C and all tested groups.

A similar tendency of improving the oxidation stability after eight days of refrigerate storage in the breast muscle of hybrid combination Cobb 500 we found in the experimental groups (0.031 $mg.kg^{-1}$ – A3, A4 to 0.048 $mg.kg^{-1}$ - A1) compared with control group C (0.191 $mg.kg^{-1}$).

After 12 days of breast muscle storage was statistic significantly ($p \leq 0.05$) improved the oxidative stability of all test groups chicken breasts (0.033 $mg.kg^{-1}$ – A4 to 0.055 $mg.kg^{-1}$ – A1) compared to the control group C (0.229 $mg.kg^{-1}$). We have found statistically significant differences ($p \leq 0.05$) between control group C and tested groups, between group A1 and A2, A4 and between tested group A2 and groups A3, A4.

During testing period of chilled storage were higher values of malondialdehyde measured in control group C

Table 1 Effect of sage and rosemary essential oils on the concentration of MDA ($mg.kg^{-1}$) in breast muscle (mean ±*SD*) (n = 5).

Day	C	A1	A2	A3	A4
0	0.024 ±0.006	0.022 ±0.007	0.023 ±0.006	0.024 ±0.005	0.026 ±0.007
4	0.182 ±0.007^{a}	0.036 ±0.004^{b}	0.028 ±0.007^{b}	0.030 ±0.004^{b}	0.034 ±0.004^{b}
8	0.191 ±0.006^{a}	0.048 ±0.005^{b}	0.044 ±0.007^{b}	0.031 ±0.008^{c}	0.031 ±0.007^{c}
12	0.229 ±0.019^{a}	0.055±0.006^{b}	0.043 ±0.005^{c}	0.037 ±0.009^{cd}	0.033 ±0.005^{d}
16	0.396 ±0.027^{a}	0.060 ±0.005^{b}	0.052 ±0.004^{c}	0.042 ±0.004^{d}	0.041 ±0.005^{d}

Note: C - air-packaged control group; A1 - vacuum-packaged control group; A2 - vacuum-packaged control samples with EDTA solution 1.50% w/w; A3 - vacuum-packaged experimental group with *Salvia officinalis* L. oil 2.0% v/w; A4 - vacuum-packaged experimental group with *Rosmarinus officinalis* L. oil 2.0% v/w. Mean values in the same lines with different superscripts (a, b, c) are significantly different at $p \leq 0.05$ level.

compare to experimental groups. The higher average value of MDA measured in breast muscle of broiler chickens Cobb 500 was in samples of control group C (0.396 mg.kg^{-1}) compared to experimental groups A1 (0.060 mg.kg^{-1}), A2 (0.052 mg.kg^{-1}), A3 (0.042 mg.kg^{-1}) and A4 (0.041 mg.kg^{-1}) after 16-day of chilled storage. At the end of the test period we have found statistically significant differences ($p \leq 0.05$) between all testing groups and control group of chicken breasts.

Botsoglou et al. (2007) reported that a higher concentration of antioxidants in poultry meat has the effect of reducing lipid oxidation, i.e. there is a reduction in malondialdehyde values during chilling storage. **Gong et al. (2010)** used TBARs values as an indicator of secondary lipid oxidation products, which were determined in minced breast and thigh muscles from chicken, turkey and duck during -4 °C storage. TBARs formation was slowest in minced chicken thigh, intermediate in duck thigh and fastest in turkey thigh ($p < 0.01$).

The plant essential oils such as oregano, thyme, sage etc. **(Economou et al., 1991; Yanishlieva and Marinova, 1995; Man and Jaswir, 2000)**, show positive effect on oxidation stability of lipids in meat.

In contrast to synthetic antioxidants, the use of natural antioxidants from spices is increasing since their application is less stringently regulated in most countries around the world. Active essential oil compounds in rosemary, oregano, borage and sage are for example phenolic diterpenes, derivates of hydroxycinnamic acid, flavonoides and triterpenes **(Oberdieck, 2004; Ryan et al., 2009; Sanchez-Escalante et al., 2003).** For rosemary, sage and oregano, the most active substances with a high antioxidant potential are carnosic acid, carnosol, and rosmarinic acid **(Oberdieck, 2004).**

Estévez et al. (2007) evaluated the antioxidant effect of two plant essential oils (sage and rosemary essential oils) and one synthetic antioxidant (BHT) on refrigerated stored liver pâté (4 °C/90 days). The addition of antioxidants significantly ($p \leq 0.05$) reduced the total amount of lipid-derived volatiles isolated from liver pâtés HS. Plant essential oils inhibited oxidative deterioration of liver pâtés to a higher extent than BHT did.

Fasseas et al. (2007) showed that porcine and bovine ground meat treated with the essential oils of oregano and sage (3%w/w) had increased oxidative stability and the antioxidant capacity of the raw and cooked meat (85 °C for 30 min) was high during storage at 4 °C for 12 days. They also suggested that addition of antioxidants is much more important for cooked meat products than the raw products.

Mohamed et al. (2011) reported that addition of herbal extracts of marjoram, rosemary and sage at concentration of 0.04% (v/w) to ground beef prior to irradiation (2 and 4.5 kGy) significantly lowered the TBARS values, off odour scores and increased colour and acceptability scores.

Sampaio et al. (2012) examined the effect of combinations of sage, oregano and honey on lipid oxidation in cooked chicken meat (thigh and breast) during refrigeration at 4 ±0.5 °C for 96 h as measured by TBARs numbers. The analysis of variance on the TBARs data indicated that the TBARs values were significantly affected by natural antioxidants throughout refrigeration ($p < 0.05$). Analysis their data showed that all of the three combinations of natural antioxidants tested would be beneficial for reducing the velocity of lipid oxidation in

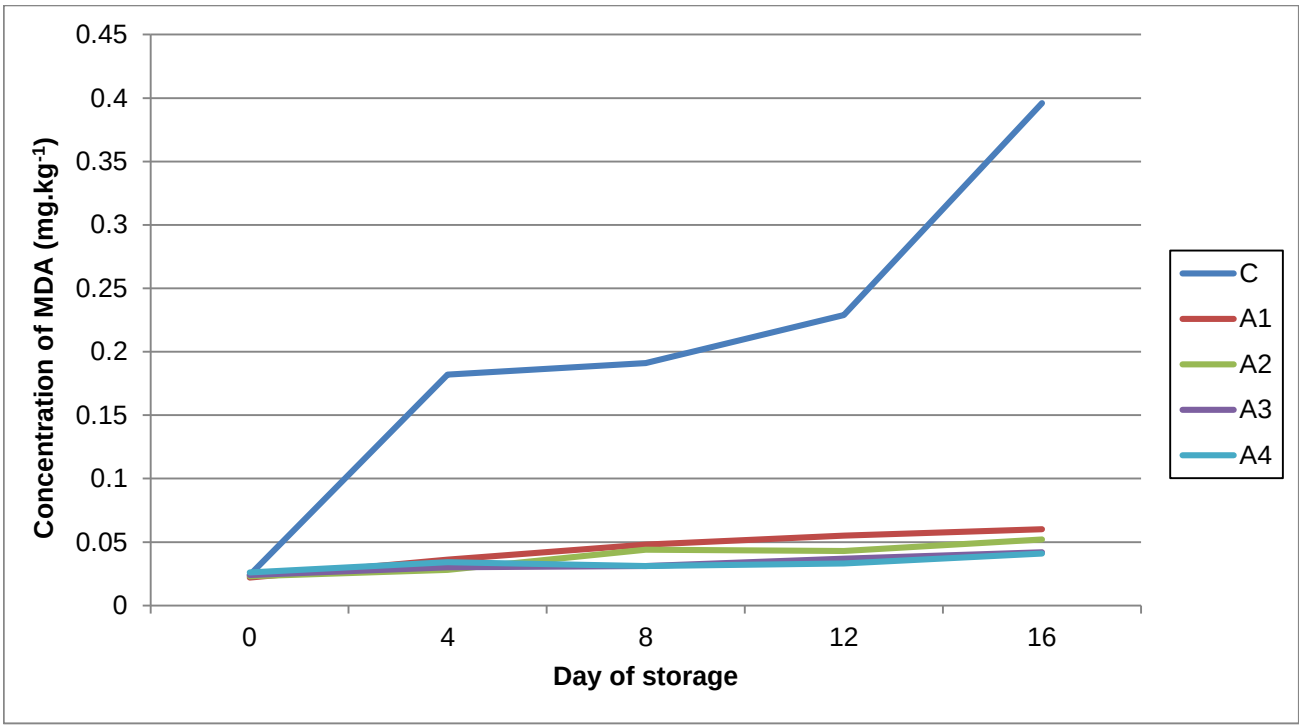

Figure 1 Concentration of MDA (mg.kg^{-1}) in breast muscle.

Note: C - air-packaged control group; A1 - vacuum-packaged control group; A2 - vacuum-packaged control samples with EDTA solution 1.50% w/w; A3 - vacuum-packaged experimental group with *Salvia officinalis* L. oil 2.0% v/w; A4 - vacuum-packaged experimental group with *Rosmarinus officinalis* L. oil 2.0% v/w.

both chicken meats during storage, what are corroborated by other authors who have added honey and herbs and thereby inhibited the development of lipid oxidation in cooked meats during refrigeration time **(McKibben and Engeseth, 2002; Juntachote et al., 2007)**.

Mohamed et al. (2011) reported that addition of herbal extracts of marjoram, rosemary and sage at concentration of 0.04% (v/w) to ground beef prior to irradiation (2 and 4.5 kGy) significantly lowered the TBARS values, off odour scores and increased colour and acceptability scores.

The effectiveness of rosemary essential oil as an inhibitor of lipid oxidation in meat products has been documented **(Esteévez and Cava, 2006; McCarthy et al., 2001; Sebranek et al., 2005)**.

Plant essential oils have been successfully introduced to inhibit oxidative deterioration of meat and fat products, this deterioration being generally referred to the accumulation of lipid-oxidation-derived products and to the generation of lipid-derived volatiles in meat products **(Ahn et al., 2002; Yu et al., 2002)**. **Formanek et al. (2001)** and **McCarthy et al. (2001)** reported the high effectiveness of antioxidants from natural resources against oxidative reactions that showed similar activity to those from synthetic origin such as BHT. **Sebranek et al. (2005)** reported similar antioxidant activities of rosemary essential oils and synthetic ones (BHT/BHA) regarding MDA generation in refrigerated sausages.

Ramos Avila et al. (2013) stated that the degradation pathways of fatty substances play one of the main causes of foods deterioration and unpleasant odours. This factor is also responsible for the loss of sensory properties.

Rhee et al. (1996) observed that raw poultry meat is less prone to lipid oxidation than beef or pork meat because of its lower iron content.

CONCLUSION

The essential oil as well essential oils from *Labiatae* herbs can be used as substitutes to chemical food additives which could prolong of shelf life of the meat and meat products. Results achieved in the experiment show that the treatment of chicken breast muscles with *Salvia officinalis* L. and *Rosmarinus officinalis* L. essential oils in concentration 0.20% v/w with combination vacuum packaging had positive effect on the decrease of oxidative processes in chicken breast muscles during chilling storage at 4 ±0.5 °C in comparative with tested groups - control air-packaged group, vacuum-packaged experimental group and vacuum-packaged experimental group with EDTA solution 1.50% w/w.

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Contact address:

Adriana Pavelková, Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Evaluation and Processing of Animal Products, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, E-mail: adriana.pavelkova@uniag.sk

Marek Bobko, Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Evaluation and Processing of Animal Products, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, E-mail: marek.bobko@uniag.sk.

Peter Haščík, Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Evaluation and Processing of Animal Products, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, E-mail: peter.hascik@uniag.sk.

Miroslava Kačániová, Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Microbiology, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, E-mail: miroslava.kacaniova@uniag.sk.

Jana Tkáčová, Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Evaluation and Processing of Animal Products, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, E-mail: jana.tkacova@uniag.sk.