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Antioxidant Activity of *Juniperus communis* L. Essential Oil in Cooked Pork Sausages

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Abstract

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The antioxidant and antimicrobial activity of *Juniperus communis* L. essential oil (JO) in cooked sausages was examined. Sausages with different concentrations of JO (0.15.0 µl/g) and a control were prepared. Instrumental parameters of colour (CIE *L*a*b**), TBARS values, DPPH scavenging activity assay, microbial profile, and sensory panel scores have been assessed. Batches produced with the JO addition were darker and redder compared to the control. Sausages with the addition of 1.0, 2.0 and 5.0 µl/g JO showed the significantly ($P < 0.05$) lower TBARS values compared to the control. The addition of JO decreased radical formation and reduced the growth of total aerobic mesophilic bacteria. The flavour of sausages produced with the addition of 0.1 and 0.5 µl/g JO was slightly/moderately and significantly ($P < 0.05$) different from the control. This study demonstrates the significant antioxidant and antimicrobial activity of *Juniperus communis* L. essential oil, as well as the potential of its utilisation in the production of cooked pork sausages to enhance quality.

Keywords: natural antioxidant; lipid oxidation; microbiology; meat products

Cooked sausages represent the most popular type of meat products, making up even 50% of the total industrial production of meat in Serbia (Šojić *et al*. 2011, 2015).

Variability of raw materials (meat, spices, and other ingredients), high temperatures in thermal treatment, as well as different storage conditions affect chemical (Kulkarni *et al*. 2011; Hayes *et al*. 2011), microbiological (Sachindra *et al*. 2005), and sensory degradation (Hayes *et al*. 2011) of cooked sausages.

Lipid oxidation is one of the most common causes of chemical degradation (De Almeida *et al*. 2015; Šojić *et al*. 2015). Lipid oxidation leads to negative changes in colour, flavour, taste and texture, as well as to a decrease in the nutritional value of the product (Hayes *et al*. 2011; Qi & Zhou 2013). Antioxidants are used in order to reduce the lipid oxidation. Due to potentially toxic and carcinogenic effects of synthetic antioxidants (BHT, BHA), numerous studies have been focused on research on natural antioxidants (Huang *et al*. 2011; Kulkarni *et al*. 2011; Zhang *et al*. 2013; Šojić *et al*. 2015). Essential plant oils and herbal extracts are the most commonly used natural antioxidants in meat products. Numerous studies have

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shown that essential oils, owing to different volatile, natural and aromatic compounds, besides antioxidants, possess antimicrobial and anti-inflammatory properties (Viuda-Martos *et al*. 2010).

Juniperus communis L., known in Serbia as 'kleka', is a shrub or tree up to 12 m in height with the oval or conical crown. This type of juniper is widespread throughout Europe, North Asia, and North America (Lesjak 2011). For centuries, *Juniperus communis* L. has been used in traditional medicine (for treatment of opportunistic infections), as a spice for meat and meat products, and as a flavour in the preparation of gin and raki (Höferl *et al*. 2014). The major components in the composition of the essential oil of *Juniperus communis* L. are α-pinene, limonene, and myrcene (Wei & Shibamoto 2007). The essential oil of *Juniperus communis* L. also contains other active components which exhibit antiradical activity against the DPPH radical (Wei & Shibamoto 2007) and contribute to the reduction of lipid peroxidation (Lesjak 2011; Höferl *et al*. 2014).

In addition to antioxidant activity, the essential oil of *Juniperus communis* L. exerts antimicrobial activity as well (Tserennadmid *et al*. 2010; Abbassy & Marei 2013).

In recent scientific literature, there is a lack of data regarding the use of *Juniperus communis* L. essential oils (JO) in cooked sausages. Hence, the aim of this study was to assess the effects of different concentrations (0.15.0 µl/g) of *Juniperus communis* L. essential oil addition on antioxidant and antimicrobial activity in cooked pork sausages. Value of pH, colour, and sensory characteristics of colour and flavour were also determined.

MATERIAL AND METHODS

Preparation of cooked sausage. Cooked sausages were produced in a local industrial plant. The main mixture consisted of meat from pork shoulder (14.00 kg), pork back fat (5.00 kg), pork skin emulsion (5.00 kg), ice water (4.50 kg), textured soy protein (0.60 kg), nitrite salt (0.54 kg), and spice mix (Lay Gewurze OHG, Germany) (0.25 kg). The minced meat was mixed with all other ingredients in a bowl chopper (Taifun 200, Nowicki, Poland) to obtain sausage batter. *Juniperus communis* L. essential oil was purchased from Herba d.o.o. (Serbia).

JO was added to the sausage batter at concentrations of 0.1 µl/g (JO1), 0.5 µl/g (JO2), 1.0 µl/g (JO3), 2.0 µl/g (JO4), and 5.0 µl/g (JO5). The sausage batter without *Juniperus communis* L. essential oil addition was used as control. All batches were stuffed into artificial cellulose casings (diameter of 40 mm) and pasteurised (in steam at 75°C) until an internal temperature of 72°C was reached. Immediately after the heating process sausages were cooled and stored in the cooling chamber (to 4°C) until an analysis. Processing was repeated three times for each batch (control, JO1, JO2, JO3, JO4, and JO5).

pH and colour determination. The pH of samples was measured using a Testo 205 portable pH meter (Testo AG, USA) equipped with a combined penetration tip with a temperature probe. The value of pH was measured in three samples from each batch in duplicate.

Colour of each sample was measured immediately after slicing, according to the procedure described by Tomović *et al*. (2013). Colour characteristics were expressed in the CIE $L^*a^*b^*$ system (CIE 1976). Ten replicate measures of surface colour were performed on three samples from each batch.

TBARS determination. The TBARS (2-thiobarbituric acid reactive substances) test was performed according to the method of Botsoglou *et al*. (1994), with modifications described by Šojić *et al*. (2015). TBARS values were expressed as milligrams of malondialdehyde per kilogram of the sample (mg MDA/kg). The TBARS test was performed on three samples from each batch in duplicate.

DPPH scavenging activity assay. The free radical scavenging activity was evaluated using the DPPH scavenging activity assay as was described by Vaštag *et al*. (2010). The DPPH scavenging activity was calculated as follows (Eq. 1):

$$\text{Antioxidant activity (\%)} = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100 \quad (1)$$

where: $A_{control}$ – concentration of DPPH in a blank (in the presence of ethanol instead of extract); A_{sample} – concentration of DPPH in the sample (in the presence of sample extract), after 30 min of reaction

The apparent IC_{50} value of samples was estimated as the concentration of sausage, in µg/ml required to produce 50% antioxidant activity under described conditions.

Microbiological analysis. Microbiological analyses were performed on three samples from each batch in duplicate. Total aerobic mesophilic bacteria count, total yeast, and mould counts, *E. coli*, *Clostridium* spp., and total Enterobacteriaceae count were determined according to procedure described by Šojić *et*

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al. (2015). Results were expressed as the number of colony forming units per gram (CFU/g).

Difference from control test. The difference from control test was carried out by 7 trained assessors who were able to discriminate samples in relation to the investigated attributes (i.e. colour and flavour). Panellists were asked to evaluate the control sample first and then to determine how different the other coded samples were from the control one by rating this difference on a scale from 0 to 6, where 0 = no difference; 1 = very slight difference; 2 = slight/moderate difference; 3 = moderate difference; 4 = moderate/large difference; 5 = large difference; and 6 = very large difference (Meilgaard *et al*. 1999).

Statistical analysis. Statistical analysis was carried out using the STATISTICA 12.0 version (StatSoft, Inc., USA). All data were presented as mean values with their standard deviations (mean ± SD). The analysis of variance (ANOVA) was performed, with a confidence interval of 95% ($P < 0.05$). Means were compared by Duncan multiple range test.

RESULTS AND DISCUSSION

The effect of *Juniperus communis* L. essential oil (JO) on the pH values of cooked sausages is shown in Table 1. The addition of JO had no significant ($P > 0.05$) effect on the pH values of cooked sausages. Values of pH ranged from 6.35 (control) to 6.38 (JO3). Obtained results are in agreement with literature data for this type of sausages (Viuda-Martos *et al*. 2010; De Oliveira *et al*. 2012).

Colour is one of the most important parameters by which consumers evaluate meat and meat product quality (Cachaldora *et al*. 2013). The colour parameters, lightness (CIE L^* value), redness (CIE a^* value), and yellowness (CIE b^* value) are shown in Table 1. All batches produced with JO addition had significantly ($P < 0.05$) lower lightness (CIE L^* value) and yellowness (CIE b^* value) than the control. Regarding redness (CIE a^* value), the JO addition in concentrations from 0.1 to 1.0 µl/g significantly ($P < 0.05$) increased this colour parameter. On the contrary, higher concentrations of JO (2.0 and 5.0 µl/g) had no significant ($P > 0.05$) effect on CIE a^* values. It was in accordance with literature data. De Oliveira *et al*. (2012) reported that the addition of a high concentration of essential oil can lead to the prooxidant effect, which is associated with a reduction in redness. However, since no relationship was found between TBARS values and instrumental colour parameters, the improvement in the colour of cooked sausages was probably a consequence of the present active compounds of JO (De Oliveira *et al*. 2012).

Lipid oxidation was evaluated by determining the levels of TBARS (mg malondialdehyde/kg) and the DPPH assay (Table 1).

In this study, the addition of JO in concentrations lower than 1.0 µl/g did not affect ($P > 0.05$) TBARS values, while concentrations of JO from 1.0 to 5.0 µl/g significantly ($P < 0.05$) decreased TBARS values compared to control samples. TBARS values were in the range of 0.14 (JO5) up to 0.29 mg MDA/kg (JO1). The obtained results very well corresponded with the literature data for similar products in the type of cooked sausages (Kulkarni *et al*. 2011; Šojić *et al*. 2015). In the case of DPPH, expressed by the IC_{50} value, all five concentrations (0.1–5.0 µl/g) significantly ($P < 0.05$) reduced radical formation compared to the control.

The reduced lipid oxidation in cooked sausages could be attributed to the compounds which exhibit antioxidative activity in *Juniperus communis* L. es-

Table 1. Effect of different concentrations of *Juniperus communis* L. essential oil on pH, colour (CIE $L^*a^*b^*$), TBARS values, and DPPH radical scavenging activity of cooked pork sausages

Batch	pH	CIE L^* value	CIE a^* value	CIE b^* value	TBARS (mg malondialdehyde/kg)	DPPH (IC_{50})
Control	6.35 ± 0.01^{d}	67.6 ± 0.8^{a}	13.1 ± 0.3^{b}	14.6 ± 0.4^{a}	0.28 ± 0.02^{a}	95.3 ± 1.5^{a}
JO1	6.34 ± 0.01^{d}	65.7 ± 0.6^{c}	13.6 ± 0.4^{a}	13.4 ± 0.5^{b}	0.29 ± 0.01^{a}	87.8 ± 2.2^{b}
JO2	6.35 ± 0.01^{cd}	66.8 ± 0.7^{b}	13.6 ± 0.3^{a}	13.5 ± 0.2^{b}	0.28 ± 0.01^{a}	81.3 ± 6.3^{c}
JO3	6.38 ± 0.01^{a}	66.6 ± 0.5^{b}	13.7 ± 0.2^{a}	13.5 ± 0.1^{b}	0.18 ± 0.01^{b}	73.9 ± 5.0^{d}
JO4	6.36 ± 0.01^{bc}	66.8 ± 0.5^{b}	13.4 ± 0.2^{ab}	13.5 ± 0.3^{b}	0.16 ± 0.02^{bc}	60.7 ± 1.3^{e}
JO5	6.37 ± 0.03^{ab}	66.7 ± 0.7^{b}	13.3 ± 0.2^{ab}	13.6 ± 0.1^{b}	0.14 ± 0.01^{c}	49.2 ± 0.4^{f}

$^{a–f}$means ± SD with different superscript letters in the same column differ significantly ($P < 0.05$)

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Table 2. Effect of different concentrations of *Juniperus communis* L. essential oil on the microbiological profile (CFU/g) of cooked pork sausages

Batch	Total aerobic mesophilic bacteria count	Total yeasts and moulds count	*E. coli*	*Clostridium* spp.	Enterobacteriaceae
Control	137 ± 15[a]	ND	ND	ND	ND
JO1	36.7 ± 5.8[b]	ND	ND	ND	ND
JO2	26.7 ± 11.5[bc]	ND	ND	ND	ND
JO3	20.0 ± 10.0[bc]	ND	ND	ND	ND
JO4	13.3 ± 5.8[c]	ND	ND	ND	ND
JO5	10.0 ± 0.0[c]	ND	ND	ND	ND

[a–c]means ± SD with different superscript letters in the same column differ significantly ($P < 0.05$); ND – not detected

sential oil (Wei & Shibamoto 2007; Lesjak 2011; Höferl *et al*. 2014).

The microbiological profile of cooked pork sausages is shown in Table 2. The addition of JO significantly reduced ($P < 0.05$) the total number of aerobic mesophilic bacteria compared with the control. It was probably a consequence of antimicrobial properties of *Juniperus communis* L. essential oil (Tserennadmid *et al*. 2010; Abbassy & Marei 2013).

Total yeast and mould counts, *E. coli*, *Clostridium* spp., Enterobacteriaceae in the control, and batches produced with the addition of JO were not detected.

Sensory panel results are shown in Table 3. Sausages produced with the addition of JO were darker and redder compared to the control. It was caused in accordance with the results of instrumental colour values. In this study, all five concentrations of JO significantly ($P < 0.05$) influenced the intensity of flavour. The addition of JO in concentrations of 0.1 and 0.5 µl/g affected slight/moderate differences in flavour. However, moderate/large to very large differences in flavour were observed for JO3, JO4, and JO5 compared with the control.

Table 3. Effect of different concentrations of *Juniperus communis L.* essential oil on the sensory properties of cooked pork sausages

Batch	Colour	Flavour
Control	0.14 ± 0.38[c]	0.43 ± 0.53[d]
JO1	2.29 ± 0.49[b]	2.00 ± 1.00[c]
JO2	2.86 ± 0.90[ab]	2.43 ± 0.53[c]
JO3	3.43 ± 0.79[a]	3.86 ± 1.07[b]
JO4	3.29 ± 0.95[a]	5.14 ± 0.38[a]
JO5	2.71 ± 0.95[ab]	5.86 ± 0.39[a]

[a–d]means ± SD with different superscript letters in the same column differ significantly ($P < 0.05$)

CONCLUSION

Juniperus communis L. essential oil, used in a concentration of 0.01 µl/g (JO1), retarded radical formation and reduced the growth of total aerobic mesophilic bacteria and improved the colour of cooked sausages, with slight to moderate alteration of the original flavour of cooked sausages. Hence, *Juniperus communis* L. essential oil, as a natural plant material, could be successfully used as quality enhancer in cooked pork sausages.

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