Biblid: 1450-5029, (2025) 29; 1; p 1 - 5
UDK: 662.7
DOI: 10.5937/jpea29-56582

Original Scientific Paper

SAGE ESSENTIAL OIL EXHIBITS ANTIOXIDATIVE AND ANTIMICROBIAL ACTIVITY IN CHICKEN-COOKED SAUSAGES

ETARSKO ULJE ŽALFIJE POKAZUJE ANTIOKSIDATIVNU I ANTIMIKROBNU AKTIVNOST U BARENIM KOBASICAMA OD PILEĆEG MESA

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ABSTRACT

Sage (Salvia officinalis L.) represents a medicinal plant from the Lamiaceae family, which has been recognized for various biological activities due to its complex chemical profile. Besides a wide application in the pharmaceutical industry, sage has found application as a flavoring agent in food products. The effect of sage essential oil (SEO) obtained from sage (Salvia officinalis L.) on antioxidative and antimicrobial activity in cooked chicken sausages was investigated. pH, instrumental parameters of color (CIE L, CIE a* and CIE b*), thiobarbituric acid-reactive substance (TBARS) value, microbial profile and sensory panel scores were examined. The addition of SEO in concentrations from 0.010 to 0.100 µl/g significantly (p<0.05) affected the color of the product by reducing in lightness (CIE L*) and increasing in redness (CIE a*). The inclusion of SEO in concentrations of 0.075 and 0.100 µL/g significantly (p<0.05) reduced the lipid oxidation defined by the TBARS test. All five tested concentrations of SEO resulted in significant (p<0.05) inhibition of bacterial growth. This study shows that the sage essential oil could be used in the processing of cooked chicken sausages in order to improve oxidative and microbial stability as well as to enhance their color characteristics, without negative implications on the flavor of the final meat products.*

***Keywords**: sage essential oil; cooked sausage; novel antioxidants; antimicrobial activity*

REZIME

Mikrobiološka kontaminacija i oksidacija lipida su jedan od najčešćih uzročnika kvara proizvoda od mesa. Jedan od načina da se uspori oksidacija lipida i produži rok upotrebe ovih proizvoda je dodavanje sintetskih i prirodnih antioksidanasa. S obzirom da sintetski antioksidansi i konzervansi imaju toksična i prokancerogena svojstva i predstavljaju potencijalnu opasnost po zdravlje potrošača, interesovanje istraživača za izučavanje prirodnih antioksidanasa izolovanih iz različitog biljnog materijala (etarska ulja i ekstrakti) u poslednjih nekoliko godina rapidno je poraslo. Žalfija (Salvia officinalis L.) je lekovita biljka iz porodice Lamiaceae, koja je poznata po širokom spektru bioloških aktivnosti, pre svega, zahvaljujući svom složenom hemijskom profilu.

U ovom radu ispitan je uticaj etarskog ulja (SEO) dobijenog iz žalfije (Salvia officinalis L.) na antioksidativnu i antimikrobnu aktivnost u barenim kobasicama od pilećeg mesa. U tu svrhu korišćeni su sledeći parametri: pH vrednost, instrumentalni parametri boje (CIE L, CIE a* i CIE b*), TBARS vrednost, mikrobiološki profil i ocene senzorskog panela. Aplikacija SEO u koncentracijama od 0,010 do 0,100 µl/g značajno (p<0,05) je uticala na smanjenje svetloće (CIE L*) i povećanje uela crvene boje (CIE a*). Dodatak SEO u koncentracijama od 0,075 i 0,100 µL/g značajno (p<0,05) je uticao i na smanjenje oksidacije lipida izražene preko TBARS vrednosti. Svih pet testiranih koncentracija SEO rezultiralo je značajnom (p<0,05) inhibicijom rasta mikroorganizama. Ova studija pokazuje da se etarsko ulje žalfije može koristiti u proizvodnji barenih kobasica od pilećeg mesa u cilju poboljšanja njihove oksidativne i mikrobiološke stabilnosti kao i poboljšanja boje, bez negativnih reperkusija na miris i ukus finalnog proizvoda.*

***Ključne reči:** etarsko ulje žalfije; barena kobasica; novi antioksidansi; antimikrobna aktivnost*

INTRODUCTION

Chicken meat is a very popular animal-based food due to its high biological value, relatively low cost and short production cycle. Fresh chicken meat is recognized as a reservoir of essential amino acids, different types of vitamins, and other vital nutrients (*Sharma et al., 2017*). Cooked sausages, made from various types of meat such as pork and chicken, as well as poultry mechanically separated meat, fat tissue, plant and animal proteins, water, various salts, and different ingredients (spices, flavorings, additives, etc.), are among the most popular cured-cooked meat products in Serbia. After the sausage batter is stuffed into casings, the sausages undergo additional thermal processing (e.g., sterilization, cooking, or pasteurization) (*Šojić et al., 2015, 2020*). Microbiological contamination and lipid oxidation are the primary factors contributing to the spoilage of cooked sausages and leading to a relatively short shelf-life for these meat products *(Sharma et al., 2017*). The primary (e.g., peroxides) and secondary products of lipid oxidation (e.g., aldehydes, alcohols) outcome in rancidity, discoloration, and the gathering of potentially toxic compounds, which have a significant impact on human health *(Ozaki et al., 2020*).

The use of synthetic antioxidants and preservatives is one of the most effective approaches to enhancing the microbiological and chemical quality, as well as the shelf-life of cooked meat products *(Riel et al., 2017*). Sodium and potassium nitrite have been effectively used as preservatives to inhibit the proliferation of a wide spectrum of pathogenic bacteria, such as *Listeria monocytogenes*, *Clostridium* sp., *Salmonella* spp. and *Staphylococcus* spp., which are common pathogens linked to foodborne illnesses (*Ozaki et al., 2021*). Additionally, these preservatives possess strong antioxidant properties and help promote the formation of the typical color and flavor in cooked meat products (*Honikel, 2008*).

However, many synthetic antioxidants (e.g., BHT, BHA, and TBHQ) and preservatives (e.g., sodium nitrite and nitrate) can cause both acute and chronic adverse reactions, such as toxic metabolic responses that may lead to allergies and long-term pro-carcinogenic implications. Owing to these concerns, the customer market has gradually shifted away from synthetic additives, displaying a rising interest in natural and functional foods that processed with the use of natural antioxidants and antimicrobials (*Munekata et al., 2020; Ozaki et al., 2020; Sucu & Turp, 2018*).

Among the most frequently used natural antioxidants in meat processing are essential oils derived from aromatic and medicinal plants, as well as herbal extracts (*Sharma et al., 2017*). Essential oils are promising alternatives for synthetic antioxidants and artificial preservatives, given their antimicrobial (e.g., antibacterial, antifungal) and antioxidant activity which are connected to key bioactive compounds such as carvacrol and thymol (*Pavlić et al., 2021*). Particularly, essential oils and plant extracts isolated from different plants (e.g., spices, vegetables, fruits, aromatic herbs, by-products from the food sector) are gaining importance as potential emerging food antioxidants and/or preservatives, subsequently, they are generally marked as safe (GRAS) and have varying levels of acceptance among consumers (*Burt, 2004*).

Sage (*Salvia officinalis*) was initially established in parts of the Mediterranean and Middle East, but today it is harvested all over the world (Šojić et al., 2021). It is well known that sage has been used in traditional medicine and food processing throughout history. Monoterpenes (α- and β-thujone, camphor, etc.), diterpenes and sesquiterpenoids are dominant compounds of sage essential oil (*Šojić et al., 2018*).

In the past decade, sage herbal dust, a by-product of the filter tea industry, has been recognized as a potential reservoir of a wide spectrum of bioactive compounds, i.e., essential oils (*Pavlić et al., 2018*). In order to obtain bioactive compounds with strong bioactivity, different extraction procedures (e.g., hydrodistillation, supercritical fluid extraction) were applied *(Danilović et al., 2021*).

Sage essential oil and lipid extract obtained by supercritical fluid extraction, were effectively applied as natural antioxidants and antimicrobials in fresh pork sausages (*Šojić et al., 2018*). Considering the importance of the development of emerging ingredients in meat processing, the main goal of this research was to investigate the antioxidative and antimicrobial potential of sage essential oil (SEO), obtained from sage tea processing by-products in the processing of cooked chicken sausage. This approach will improve the utilization of by-products from the food industry.

MATERIALS AND METHODS

Plant material

Sage (*Salvia officinalis* L.) herbal dust was processed as a by-product in the filter tea industry (Fructus DOO, Bačka Palanka, Serbia). Herbal dust was discarded after sieve fractionation as a fraction with particle size <0.315 mm, which could not be used for filter tea production (*Šojić et al., 2018*).

Chemicals

2-Thiobarbituric acid was purchased from Sigma-Aldrich GmbH (Germany). All other chemicals used were of analytical reagent grade.

Essential oil extraction

Hydrodistillation was used for the isolation of SEO according to standard procedure from *Ph. Jug*. IV at Clevenger-type apparatus. Hydrodistillation was completed after 2 h and SEO was separated from the aqueous phase after the determination of its yield (%; v/w).

Preparation of cooked chicken sausage

Cooked chicken sausages were made in a local meat processing plant. The sausage batter, in the form of a meat emulsion, consists of meat from chicken shoulder (45%), mechanically separated meat (20%), chicken skin emulsion (15%), ice water (15%), soy protein (2%), nitrite salt (2%) and spice mix (1%). The procedure was described in detail by *Šojić et al.* (*2015*). SEO was applied to the shaped batter at the following levels: 0.010 μL/g (SEO1), 0.025 μL/g (SEO2), 0.050 μL/g (SEO3), 0.075 μL/g (SEO4) and 0.100 μL/g (SEO5). The sausage batter without SEO addition was marked as control. All batches were stuffed into artificial casings (diameter of 36 mm) and pasteurized until an internal temperature of 70 °C was reached. Immediately after the heating process sausages were cooled and stored in the cooling chamber (at 4 °C) until an analysis.

pH and color determination

The pH value of samples was measured using the portable pH meter Testo 205 (Testo AG, USA). pH was determined on three sausages from each batch in duplicate. Color features were expressed in the CIE $L^*a^*b^*$ system (*CIE, 1976*). Twelve replicate measures of surface color were completed on three sausages from each batch.

TBARS determination

TBARS (2-thiobarbituric acid reactive substances) test was done on three sausages from each batch in duplicate according to the method described in *Šojić et al.* (*2015*). TBARS values were expressed as milligrams of malondialdehyde (MDA) per kilogram of sample (mg MDA/kg).

Microbiological analysis

Microbiological evaluations were done on three sausages from each group in duplicate. The succeeding microbiological analyses were completed: total number of aerobic mesophilic bacteria -TBC (ISO 4833:2003), *Salmonella* spp. (ISO 6579:2008), *Escherichia coli* (ISO 16649-2:2005) and *Listeria monocytogenes* (ISO 11290-2:2014). Results were expressed as a cfu/g.

Sensory analysis

The Difference-Control-Test was carried out by 7 trained assessors, who were able to discriminate samples in relation to the investigated attributes (i.e. color and flavor). Panelists 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, 1993*).

Statistical analysis

Statistical analysis was carried out using STATISTICA 12.0 (StatSoft, Inc., Tulsa, OK, USA). All data were presented as mean values with their standard deviation indicated (mean ± SD). Variance analysis (ANOVA) was performed, with a confidence interval of 95% ($p<0.05$). Means were compared by Fisher's LSD test.

RESULTS AND DISCUSSION

pH and color determination

The effect of SEO obtained from filter tea industry by-products on the pH value of cooked chicken sausages is displayed in Table 1. The inclusion of SEO had no significant effect (p>0.05) on the pH values of these cured meat products. pH values fluctuated within a very narrow interval from 6.30 to 6.39. The obtained values are consistent with literature data for similar chicken meat products (*Guerra et al., 2023; Schmidt et al., 2017*).

*Table 1. Effect of different concentrations of sage essential oil (SEO) on pH, color (CIE L*a*b*) and TBARS values of cooked chicken sausages*

a–d Means ± SD with different superscript letters in the same column differ significantly (p<0.05).

Batch	pH	CIE L^* value	CIE a* value	CIE b^* value	TBARS (mg MDA/kg)
Control	6.30±0.03^a	57.4±0.6^a	14.5±0.3^b	12.8±0.1^a	0.48±0.05^a
SEO1	6.39±0.04^a	54.8±0.5^c	15.1±0.1^a	13.1±0.3^a	0.47±0.06^a
SEO2	6.35±0.03^a	53.4±1.0^d	15.2±0.3^a	13.0±0.7^a	0.47±0.03^a
SEO3	6.36±0.02^a	56.2±0.2^b	15.0±0.1^a	12.8±0.4^a	0.44±0.01^a
SEO4	6.33±0.04^a	55.0±0.4^c	15.1±0.3^a	12.8±0.4^a	0.38±0.03^b
SEO5	6.34±0.05^a	53.2±0.6^d	16.5±0.5^a	13.0±0.2^a	0.25±0.01^b

The main instrumental parameters of color in the *CIELab* system: CIE L^* value - lightness, CIE a^* - value redness and CIE b^* value - yellowness are presented in Table 1. The typical reddish-pink color of cooked sausages is one of the most essential features to customers and its development is based on the reactions between nitric oxide and pigment myoglobin *(Ozaki et al., 2021; Sharma et al., 2017*). SEO addition significantly (p<0.05) affected changes in lightness and redness. All five concentrations of SEO resulted in a lower (p<0.05) CIE L^* value and a higher (p<0.05) CIE a^* value compared to control. The reduction in lightness and the increase in redness was undoubtedly the results of the interaction between SEO bioactive compounds (α-thujone, eucalyptol and camphor) and meat pigment myoglobin. Similar results were detected in our previous research (*Šojić et al., 2019*). *Zhang et al.* (*2013*) also suggested that SEO addition influenced the lower lightness and higher redness on the surface of Chinese-style sausage. It should be noticed that terpenes, phenolics and other bioactive compounds supported the reaction between the nitrite and myoglobin in order to form the pink nitrosyl-hemochrome pigment (Armenteros et al., 2013). Moreover, *Liu et al.* (*2009*) stated, that the oxidation of phenolic compounds in the presence of polyphenol oxidases and oxygen led to the formation of dark compounds. In the case of CIE b^* value, the inclusion of SEO had no significant (p>0.05) impact on this instrumental parameter of the color.

Lipid oxidation

Essential oils isolated from different plant materials (e.g., aromatic herbs, spices and medicinal plants) are characterized by radical scavenging properties, suggesting that they could be used as natural food ingredients, predominantly in animal-origin products (*Hassoun & Emir Çoban, 2017*). Lipid oxidation was assessed by TBARS values (Table 1). TBARS values diverse in the interval from 0.25 mg malondialdehyde/kg (SEO5) to 0.48 mg malondialdehyde/kg (SEO1). These values are consistent with literature data with literature data (Da Silveira et al., 2014). SEO in concentrations above 0.050 µL/g affected (p<0.05) the reduction in TBARS values. These results undoubtedly demonstrate the strong antioxidative potential of oxygenated monoterpenes (α-thujone, eucalyptol and camphor) and diterpene polyphenols (viridiflorol) present in SEO (*Šojić et al., 2018*). The obtained results correlate well with our previous study, which established a strong antioxidant potential of SEO in fresh pork sausages obtained by conventional and "green" extraction technologies (*Šojić et al., 2018*).

Microbiological analysis

Sage has been earlier described to have a powerful bactericidal potential (*Danilović et al., 2021*). The microbiological profile (TBC, *Salmonella* spp., *Escherichia coli* and *Listeria monocytogenes*) of cooked chicken sausages is presented in Table 2. The inclusion of SEO significantly (p<0.05) reduced the TBC. As expected, the lowest TBC (40 cfu/g) was identified in the batch with the highest level of SEO5 (0.100 µL/g). Generally, the obtained results advocated that SEO possesses important potential to be used as a natural preservative in cooked chicken sausage.

Table 2. Effect of different concentrations of sage essential oil (SEO) on microbiological profile (cfu/g) of cooked chicken sausages

Batch	TBC	*Salmonella* spp.	*E.coli*	*L. monocytogenes*
Control	600±10^a	ND	ND	ND
SEO1	340±10^b	ND	ND	ND
SEO2	220±10^c	ND	ND	ND
SEO3	180±10^c	ND	ND	ND
SEO4	117±6^d	ND	ND	ND
SEO5	40±10^e	ND	ND	ND

a–e Means ± SD with different superscript letters in the same column differ significantly (p<0.05).

The decline of TBC, undoubtedly, was the result of antimicrobial activity of the main antimicrobial terpenoids compounds of SEO (e.g., camphor, α-thujone and eucalyptol). The antimicrobial activity of SEO could be linked with their chemical shape and the percentage of terpenoid compounds with different antimicrobial activity. The chemical shape of SEO was shown in our previous study (*Šojić et al., 2018*). It is well known that essential oils are recognized for their hydrophobicity, which is liable for the destruction of bacterial macromolecules *(Mujović et al., 2023*). Some studies have exhibited the strong antimicrobial impact of SEO on different pathogenic bacteria (*Danilović et al., 2021; Šojić et al., 2021; Yazgan, 2020*). The most abundant pathogenic bacteria in cooked meat products: *Salmonella* spp., *E. coli* and *L. monocytogenes* were not identified both in control and the batches produced with SEO addition. Undoubtedly, it was the result of good hygiene procedures during the cooked chicken sausages processing.

Sensory analysis

Sensory analysis of cooked chicken sausages is shown in Table 3. The inclusion of SEO resulted in only slight differences in the sensory attribute of the color. This outcome is in accordance with the instrumental parameters of color.

Table 3. Effect of different concentrations of sage essential oil (SEO) on sensory properties of cooked chicken sausages

Batch	Color	Flavor
Control	0.11±0.22^b	0.11±0.20^d
SEO1	1.59±0.22^a	0.22±0.51^d
SEO2	1.57±0.44^a	0.22±0.55^d
SEO3	1.22±0.33^a	0.55±0.41^c
SEO4	1.35±0.60^a	1.59±0.58^b
SEO5	1.12±0.67^a	3.58±0.78^a

a–c Means ± SD with different superscript letters in the same column differ significantly (p<0.05).

The addition of SEO in concentrations 0.010-0.075 µL/g resulted in slight to moderate, but significant ($p<0.05$) differences in the sensory attribute of flavor compared to the control. Also, it should be noticed that the highest concentration of SEO (0.100 µL/g) caused significant ($p<0.05$) and moderate to large differences in the same attribute compared to the control. The strong and atypical flavor of this type of meat products detected by a sensory panel is undoubtedly related to a high concentration of monoterpenes. It has been noted that conventional hydrodistillation provides "pure" essential oil and monoterpene compounds with strong odors and flavors. Hence, in order to process emerging ingredients with better sensory characteristics, the utilization of novel extraction technologies is essential.

CONCLUSIONS

It can be concluded that SEO, at a relatively low concentration (≤0.075 µL/g), effectively retarded lipid oxidative reactions and reduced the growth of TBC, without negatively affecting the typical color and flavor of cooked chicken sausages. Therefore, SEO obtained from sage herbal dust could be effectively used as an emerging ingredient in cooked chicken sausages. Valorizing by-products from the food industry has significant ecological and economic importance and aligns with the goals of the latest European Green Deal. Encapsulation is one of the best ways to increase the solubility and stability of lipophilic extracts. Therefore, further investigations should focus on encapsulating SEO to enhance its stability and bioactive potential in different types of meat products.

Acknowledgment of funding: This research was supported by the Ministry of Science, Technological Development and Innovations, Serbia, program (451-03-65/2024-03/200134). Corresponding author would like to acknowledge CEEPUS grants to the Slovak University of Nitra and the Universities of Banja Luka and Lodz (RS-1512-05-2425 - Improving Food Quality with Novel Food Processing Technologies).

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Received: 06. 02. 2025. Accepted: 25. 02. 2025.