The effect of essential oil from sage (Salvia officinalis L.) herbal dust (food industry by-product) on the microbiological stability of fresh pork sausages

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Abstract. The effect of essential oil obtained from sage (Salvia officinalis L.) herbal dust (a food industry by-product) (SEO), on the pH value, microbiological stability and sensory properties of fresh pork sausages prepared without chemical additives was evaluated during 8 days of aerobic storage at 3±1°C. The addition of SEO significantly (p<0.05) reduced the microbial growth in fresh pork sausages. Moreover, SEO added at a level of 0.05 μL/g had no negative effect on sensory properties of this meat product. Hence, the results of this study showed significant antimicrobial activity of SEO obtained from sage filter tea processing by-products and the potential for utilising SEO in fresh pork sausages in order to enhance their stability and safety.

1. Introduction

Fresh pork sausages are among the most common and the most popular processed meat products all around the world [1]. Due to their very high water activity, relatively high fat content, comminuted structure of raw materials, high total number of microorganisms and lack of thermal processing, these products are characterized by a short shelf life. Spoilage of these products can be caused by microbial contamination and lipid oxidation, leading to development of unacceptable sensory characteristics and even foodborne diseases. Hence, the inhibition of microbial growth and delay of lipid oxidation are primary goals that can significantly extend shelf life [1,2]. In order to reduce oxidative changes and to prevent bacterial growth, several synthetic food additives are regularly used by meat processors. However, in recent years due to increasing consumer awareness about potentially toxic effects and health issues, the use of nitrites and synthetic antioxidants (butylated hydroxytoluene – BHT; butylated hydroxyanisole – BHA; tertiary butylhydroquinone – TBHQ) has decreased, while demand for natural additives has rapidly increased [1,3,4,5]. Many natural antioxidants also exhibit antimicrobial activity, and thus, have the advantage of being readily accepted by both consumers and meat processors [1,6]. However, natural antioxidants are often more expensive and less effective than synthetic ones. Consequently, increasing attention is recently being paid to the extraction of antioxidants from agro-food industry by-products [7,8]. The aim of this study was to determine the
antimicrobial effect of sage essential oil (SEO), obtained from sage tea processing by-products, when utilised in fresh pork sausage during refrigerated storage.

2. Materials and Methods

2.1. Plant material
Sage (Salvia officinalis L.) originated from Montenegro and was kindly donated by a local filter tea factory producing herbal teas (Fructus DOO, Bačka Palanka, Serbia). Sage herbal dust was obtained as by-product in the filter tea factory while all processing steps and applied unit operations were described elsewhere [9]. Plant material had a mean particle size of <0.315 mm and moisture content of 7.24±0.05%. Conventional (hydrodistillation) and novel (supercritical fluid extraction – SFE) extraction techniques were used for recovery of SEO. The official procedure from the fourth edition of the Yugoslavian Pharmacia was applied for hydrodistillation. Briefly, 20 g of sage herbal dust and 400 mL of water were mixed in a 1 L round flask connected with Clevenger-type apparatus. Hydrodistillation was performed for 2 h and essential oil was separated from the aqueous phase after determination of its yield (%).

2.2. Preparation of fresh pork sausage
Fresh pork sausages (of the type called in Serbian Petrovská klobása) were produced in the meat processing pilot plant within the Institute of Food Technology Novi Sad (FINS). Pork shoulder and back fat were minced using an electric meat grinder with an 8 mm grinding plate. The sausage batter was obtained by mixing minced pork (80%) and back fat (20%) with salt (1.80%), sweet paprika powder (1.00%), red hot paprika powder (0.70%), caraway (0.20%) and garlic paste (0.07%), in a meat mixer. The amount of seasonings was calculated in relation to minced meat and back fat weight. The resulting mixture was divided into four batches. SEO was added separately to three batches, at concentrations of 0.05 µL/g (SEO1), 0.075 µL/g (SEO2) and 0.1 µL/g (SEO3). The remaining batch was used as the control (C). All batches were stuffed into natural casings (pig small intestines; Ø ≈ 32 mm). Sausages were stored at 3±1°C in the dark for 8 days.

2.3. Samples
Samples taken at distinct periods of storage comprised three randomly selected sausages from each batch after 0, 2, 4, 6 and 8 days. Analyses were carried out on the day of sampling, and were conducted in duplicate for each sample.

2.4. pH determination
The pH of samples was measured using the portable pH meter Testo 205 (Testo AG, USA) equipped with a combined penetration tip with temperature probe. The pH meter was calibrated before the readings using two buffer solutions (pH=4.00±0.05 and pH = 7.00±0.01 at 20±2 °C).

2.5. Microbiological analysis
Microbiological analyses were performed on three samples from each group of the fresh pork sausages in duplicate. Twenty grams of samples were homogenized for 10 minutes at 200 rpm (Unimax 1010, Heidolph, Germany) in 180 mL 1 g/L buffered peptone water (Merck, Darmstadt) and then serial decimal dilutions were prepared (up to 10⁻¹). Pour plates were prepared with 1 mL of each dilution in separate sterile Petri plates, and with appropriate media depending on the type of microorganism studied. The following microbiological analyses were performed: total number of aerobic mesophilic bacteria (TBC), Salmonella spp., Escherichia coli, and Listeria monocytogenes [4]. Results were expressed as log cfu/g.

2.6. Sensory analysis
Sensory evaluation of the investigated sausages was performed by ranking tests [10]. Sausages were taken from refrigerated storage (3°C) and baked in an oven at 165°C for 1h. The sausages were served warm (50-60°C) and analysed by a panel of 70 consumers. Consumers were asked the following: “Please rank the samples in numerical order according to your preference” (from like extremely – 1 to dislike extremely – 7). Consumers between 19 and 65 years old were students and staff members of the Faculty of Technology, Novi Sad.

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

3. Results and Discussion
The effect of SEO on the pH value of fresh pork sausages is shown in Table 1. At the beginning of storage, pH values ranged from 5.60 to 5.66 in the sausages.

| Storage day | C       | SEO1     | SEO2     | SEO3     |
|-------------|---------|----------|----------|----------|
| 0           | 5.62±0.04<sup>A/a</sup> | 5.60±0.05<sup>H/a</sup> | 5.60±0.01<sup>H/a</sup> | 5.66±0.02<sup>H/a</sup> |
| 2           | 5.54±0.03<sup>C/b</sup> | 5.58±0.03<sup>B/a</sup> | 5.62±0.01<sup>A/b</sup> | 5.61±0.02<sup>B/a</sup> |
| 4           | 5.47±0.02<sup>C/C</sup> | 5.52±0.01<sup>B/b</sup> | 5.55±0.03<sup>B/c</sup> | 5.57±0.04<sup>C/b</sup> |
| 6           | 5.46±0.01<sup>C/C</sup> | 5.45±0.01<sup>B/C</sup> | 5.53±0.03<sup>A/c</sup> | 5.47±0.01<sup>B/c</sup> |
| 8           | 5.40±0.02<sup>H/d</sup> | 5.40±0.01<sup>B/d</sup> | 5.46±0.06<sup>A/d</sup> | 5.44±0.03<sup>A/d</sup> |

Values with different letters (A–C) in the same row are significantly different (p<0.05); Values with different letters (a–c) in the same column are significantly different (p<0.05).

The results obtained corresponded very well with literature data for this type of sausage [11]. A significant drop of pH was registered in each sausage group during 8 days of refrigerated storage. Most probably, this was result of growth and metabolic activity of lactic acid bacteria, as was previously reported by a number of authors [6, 11]. The microbiological profile of fresh pork sausage during 8 days of storage under refrigeration is shown in Table 2.

| Storage day | C       | SEO1     | SEO2     | SEO3     |
|-------------|---------|----------|----------|----------|
| 0           | 6.90±0.02<sup>A/c</sup> | 5.74±0.01<sup>C/c</sup> | 5.59±0.01<sup>D/c</sup> | 6.03±0.05<sup>B/c</sup> |
| 2           | 6.10±0.01<sup>A/e</sup> | 6.00±0.02<sup>B/d</sup> | 5.88±0.01<sup>C/d</sup> | 5.80±0.02<sup>B/d</sup> |
| 4           | 6.63±0.03<sup>A/d</sup> | 6.38±0.02<sup>B/c</sup> | 6.01±0.01<sup>D/c</sup> | 6.23±0.01<sup>BC/b</sup> |
| 6           | 7.15±0.03<sup>A/b</sup> | 6.84±0.02<sup>C/b</sup> | 6.73±0.02<sup>Db</sup> | 6.98±0.02<sup>H/a</sup> |
| 8           | 7.66±0.04<sup>A/a</sup> | 7.29±0.01<sup>H/a</sup> | 7.15±0.01<sup>Ca</sup> | 7.10±0.02<sup>A/a</sup> |

Values with different letters (A–E) in the same row are significantly different (p<0.05); Values with different letters (a–c) in the same column are significantly different (p<0.05).

The addition of SEO significantly (p<0.05) reduced the TBC. Probably, it was the consequence of sage antimicrobial properties [11]. The initial TBC ranged from 5.59 log cfu/g (SEO2) to 6.90 log cfu/g (C). As expected, for all sausages, the TBC significantly (p<0.05) increased during 8 days of storage.
storage. At the end of storage, TBC was significantly (p<0.05) different between the sausages, being in the order as follows: C>SEO1>SEO2>SEO3. None of the three analysed foodborne pathogenic bacteria (Salmonella spp., E. coli, or L. monocytogenes) were detected, neither in control nor in sausages with added SEO.

The sensory attribute of preference of C and SEO fresh pork sausages are shown in Figure 1.

![Figure 1. Summary of rank sums for sensory attribute of consumer preference for control sausages and sausages with added SEO](image)

Values with different letters (A-C) are significantly different (p<0.05)
SEO levels in sausages: 0.05 µL/g (SEO1), 0.075 µL/g (SEO2) and 0.1 µL/g (SEO3)

SEO addition to fresh pork sausage at the concentration of 0.05 µL/g had no negative effect on the sensory attribute of preference. This was in accordance with literature data [12].

4. Conclusion
The results of this study showed that the utilisation of SEO retarded microbial growth in fresh pork sausages. Use of SEO (at 0.05 µL/g) did not have a negative effect on the sensory attribute of preference by consumers, compared with control sausages. The overall results show that essential oil obtained from sage herbal dust (a food industry by-product) could be successfully applied in the formulation of fresh pork sausages as an antimicrobial agent.

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