The effect of winter savory (*Satureja montana* L.) extract on the quality of cooked pork sausages

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Abstract. The effect of winter savory (*Satureja montana* L.) supercritical critical extract on pH, Thiobarbituric acid reactive substance value, microbiological profile and sensory attribute of flavour was examined. All four tested concentrations of supercritical fluid extract (0.025, 0.050, 0.075 and 0.100 µL/g) resulted in significant (p<0.05) reduction of thiobarbituric acid reactive substances and inhibition of microbial growth. The addition of supercritical fluid extract had no negative impact on flavour. Therefore, winter savory extract could be successfully applied as natural antioxidant and antimicrobial agent in order to improve quality of cooked pork sausages.

1. Introduction

Cooked sausages are broadly consumed meat products in Serbia [1]. During different stages of processing, distribution and storage, cooked sausages undergo chemical (lipid and protein oxidation), microbiological and sensory deterioration [2,3]. One of the main strategies to slow down deterioration of cooked sausages is the use of synthetic antioxidants and antimicrobial agents. On the other hand, these compounds are recognized as potentially unhealthy owing to their carcinogenic properties [1-3].

Therefore, the replacement of synthetic antioxidants by natural antioxidants/antimicrobials, including plant essential oils and extracts has been proposed in different type of meat products [1, 2, 4, 5].

Winter savory (*Satureja montana* L.) has been designated as an important aromatic plant broadly spread in the Balkans. Due to the high content of monoterpen polyphenols, thymol and carvacrol, winter savory and its extracts can be used as aroma additives with functional potential in food and pharmaceutical industry products [6]. Conventional extraction techniques (Soxhlet extraction with organic solvents and hydrodistillation) were predominantly used for obtaining *Satureja montana* L. essential oil and volatile extracts. On the other hand, these methods retain various drawbacks: low extraction efficiency, longer period of extraction, solvent residues in the extracts which decrease their quality and have negative influence on the environment due to huge consumption of organic solvents [7,8]. In order to overcome these disadvantages and achieve high quality and high bioactivity of extracts, novel extraction methods, including supercritical fluid extraction, have been developed [8]. There are no studies in the available literature concerning the application of supercritical fluid extracts of *Satureja montana* L. in meat products. Therefore, the aim of this work was to assess the effect of supercritical fluid extract (SFE) obtained from winter savory (*Satureja montana* L.) on pH, oxidative and microbiological stability, and sensory properties of cooked pork sausages during refrigerated storage.
2. Materials and Methods

2.1. Plant material
Winter savory (Satureja montana L.) was produced at the Institute of Field and Vegetable Crops, Novi Sad, Serbia. The collected plant material (aerial parts) was air dried and stored at room temperature. Plant material was grounded in a domestic blender and the mean particle size of raw material (0.377 mm) was determined using sieve sets. Moisture content of plant material (9.71%) was analysed using the standard procedure, i.e., by drying the plant material at 105 °C until constant weight.

2.2. Supercritical fluid extraction
The supercritical fluid extract was obtained using a laboratory-scale high pressure extraction plant (HPEP, NOVA, Swiss, Efferikon, Switzerland) described in Šojić et al. [8]. Plant material (50.0 g) was placed in an extractor vessel and extraction process was carried out at 100 bar, 40 °C, for 4 h using 0.2 kg/h CO₂ flow rate. Solvent-free SFE was recovered in a separator under following conditions: 15 bar and 25 °C. Total extraction yield was measured and result was expressed as grams of total extractable compounds per 100 grams of plant material (g/100 g), i.e. percentage (%).

2.3. Preparation of cooked pork sausage
Cooked pork sausages were created in a local industrial meat company (Strand, Novi Sad, Serbia). The sausage batter consisted of meat from pork shoulder (50%), pork back fat (15%), pork skin emulsion (15%), ice water (15%), soy protein (2%), nitrite salt (2%) and spice mix (Lay Gewurze OHG, Germany) (1%). Procedure was described in details by Šojić et al. [1]. SFE was added to the sausage batters at concentrations of 0.025 µL/g (SFE1), 0.050 µL/g (SFE2), 0.075 µL/g (SFE3) and 0.100 µL/g (SFE4). SFE was mixed with salt and added to sausage batters prior to stuffing. The remaining batch (without SFE) was assigned as the control sausage type (C). All sausages were stuffed into artificial casings (Ø ≈ 36 mm) and pasteurized until an internal temperature of 70°C was reached. Immediately after the heating process, sausages were cooled and stored in a cooling chamber (to 4°C) until analysis.

2.4. Sausage sampling
Samples taken at distinct periods of storage were three randomly selected sausages from each sausage group after 0, 15 and 30 days. Analyses were carried out on the day of sampling, and were completed in duplicate for each day of sampling.

2.5. pH determination
The pH of sausages 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).

2.6. TBARS determination
2-Thiobarbituric acid reactive substances (TBARS) were determined as described by Šojić et al. [1]. TBARS were expressed as milligrams of malondialdehyde (MDA) per kilogram of sample.

2.7. Microbiological analysis
Microbiological analyses were performed on three samples from each group of the cooked pork sausages in duplicate. Twenty grams of sausage sample were homogenized for 10 minutes at 200 rpm (Unimax 1010, Heiolph, Germany) in 180 mL 1 g/L buffered peptone water (Merk, Darmstadt) and then serial decimal dilutions were prepared (up to 10⁻³). One millilitre of each dilution was placed in a sterile Petri plate and overlaid with appropriate media depending on the type of tested microorganism. The following microbial analyses were performed: total number of aerobic mesophilic bacteria (TBC), Salmonella spp., Escherichia coli, Listeria monocytogenes [8]. Results were expressed as a cfu/g.
2.8. Sensory analysis
The Difference-Control-Test was carried out by 10 trained sensory assessors, who were able to discriminate sausages in relation to the investigated attributes (flavour). Panellists were asked to evaluate the control sausage first and then to determine how different the other coded sausages were from the control 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 [1].

2.9. 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 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 SFE on the pH of cooked pork sausages is shown in Table 1. At the beginning of storage, pH ranged from 6.33 to 6.36. Storage period significantly (p<0.05) affected the decrease of pH. Significant drops of pH were registered in each sausage group during 30 days of refrigerated storage. Most probably, this was the result of growth and metabolic activity of lactic acid bacteria [3,9]. Lipid oxidation was estimated by determining the ranks of TBARS (mg malondialdehyde/kg) (Table 1). The initial TBARS values varied from 0.09 mg malondialdehyde/kg (SFE1; SFE3) to 0.12 mg malondialdehyde/kg (control). During the storage period, TBARS values significantly (p<0.05) increased for all treatments. Most probably, this was the result of lipid oxidation [10]. After 15 days of storage, all four concentrations of SFE significantly (p<0.05) affected the reductions of TBARS values.

Additionally, at the end of storage (day 30), the TBARS levels varied between the treatments in the following order: C>SFE1≥SFE2≥SFE3≥SE4. These results indicate the strong antioxidative effect of SFE. Antioxidant activity of SFE could be attributed to the presence of its major monoterpene phenolics, particularly carvacrol (67.58%) [6]. A similar result was observed by de Oliveira et al. [2].

Table 1. pH and 2-thiobarbituric acid reactive substance values of cooked pork sausages

| Day of storage | Control | SFE1 | SFE2 | SFE3 | SFE4 |
|----------------|---------|------|------|------|------|
| pH             |         |      |      |      |      |
| 0              | 6.33±0.02<sup>Ac</sup> | 6.34±0.02<sup>Ac</sup> | 6.35±0.01<sup>Ac</sup> | 6.36±0.02<sup>Ac</sup> | 6.35±0.02<sup>Ac</sup> |
| 15             | 6.32±0.04<sup>Bc</sup> | 6.33±0.03<sup>Bc</sup> | 6.33±0.04<sup>Bc</sup> | 6.32±0.03<sup>Bc</sup> | 6.36±0.02<sup>Bc</sup> |
| 30             | 6.07±0.05<sup>Cb</sup> | 6.18±0.03<sup>Cb</sup> | 6.20±0.05<sup>Cb</sup> | 6.12±0.04<sup>Cb</sup> | 6.15±0.03<sup>Cb</sup> |

2-Thiobarbituric acid reactive substances (mg malondialdehyde/kg)

| Day of storage | Control | SFE1 | SFE2 | SFE3 | SFE4 |
|----------------|---------|------|------|------|------|
| 0              | 0.12±0.02<sup>Ac</sup> | 0.09±0.04<sup>Ac</sup> | 0.10±0.01<sup>Ac</sup> | 0.09±0.03<sup>Ac</sup> | 0.10±0.02<sup>Ac</sup> |
| 15             | 0.35±0.01<sup>Bc</sup> | 0.32±0.01<sup>Bc</sup> | 0.29±0.01<sup>Bc</sup> | 0.30±0.02<sup>Bc</sup> | 0.25±0.02<sup>Bc</sup> |
| 30             | 0.54±0.04<sup>Bc</sup> | 0.43±0.02<sup>Bc</sup> | 0.42±0.03<sup>Bc</sup> | 0.39±0.02<sup>Bc</sup> | 0.39±0.02<sup>Bc</sup> |

Values with different letters<sup>**</sup> in the same row are significantly different (p<0.05); Values with different letters<sup>***</sup> in the same column are significantly different (p<0.05); SFE – supercritical fluid extract.

The microbiological profile of cooked pork sausages during 30 days of storage under refrigeration is shown in Table 2. The addition of SFE significantly (p<0.05) reduced the total number of aerobic mesophilic bacteria (TBC). At the end of storage, TBC was significantly (p<0.05) different between the sausage groups, in the order: C>SFE1≥SFE2>SFE3>SFE4. It should be underlined that the antimicrobial potential of SFE can be mainly attributed to the presence of carvacrol, thymol and eugenol [7].
Table 2. Total number of aerobic mesophilic bacteria (cfu/g) of cooked pork sausages

| Day of storage | Control   | SFE1       | SFE2       | SFE3       | SFE4       |
|----------------|-----------|------------|------------|------------|------------|
| 0              | 66.7±15.3 | 46.7±5.8   | 43.3±5.8   | 43.3±5.8   | 16.7±11.6  |
| 15             | 157±12    | 116±6      | 100±10     | 93.3±5.8   | 83.3±5.8   |
| 30             | 710±36    | 520±10     | 500±20     | 426±31     | 323±25     |

Values with different letters (A-D) 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); SFE – supercritical fluid extract.

The initial TBC ranged from 16.7 cfu/g (SFE4) to 66.7 cfu/g (C). As expected, for all treatments the TBC significantly (p<0.05) increased during 30 days of storage. Three analysed foodborne pathogenic bacteria (Salmonella spp., E. coli, L. monocytogenes) were not detected both in control and treated sausages.

Sensory panel results for flavour assessment are shown in Figure 1. All four concentrations of SFE significantly (p<0.05) affected sausage flavour. The intensity of flavour was very slight (SFE1; SFE2) and slight/moderate (SFE3; SFE4), i.e. different to control sausages.

Hence, the results obtained in this study indicate the use of SFE (0.025-0.100 μL/g) had a relatively mild sensory effects on sausage flavour.

4. Conclusion
In conclusion, application of winter savory supercritical fluid extract (SFE) retarded lipid oxidation and reduced microbial growth, with only a very slight to slight/moderate alteration of the original flavour of cooked pork sausages. Hence, these results indicate that winter savory supercritical extract (SFE) could be successfully applied as a natural plant antioxidant and antimicrobial agent in cooked pork sausages.

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