Wild thyme (*Thymus serpyllum* L.) supercritical extract as antioxidant in precooked pork chops during chilled storage

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Abstract. The effect of thyme supercritical extract on oxidative stability of precooked and cold-stored (at 4°C for 6 days) pork chops was analysed. Thyme extract was applied with a marinated process (SF1), or was introduced on the surface of the chops after cooking (SF2). Thyme extract in SF1 samples showed a significant protective effect towards oxidation of lipids during the cooking process. Both processes of thyme application showed potential for lipid oxidation inhibition throughout the refrigerate storage period of precooked pork chops, thus protecting colour and sensory characteristics of the samples. Finally, at the end of storage period, the lowest oxidative changes were determined for SF1 chops.

1. Introduction

The importance of ready meals in the food markets has been increasing constantly. The increase is especially for chilled ready meals, because most consumers (75%) rather choose this type of product over its frozen counterpart [1]. Consumers choose products which do not require much time, skill or energy for complete home meal preparation. That is why food convenience become one of the main trends in nowadays lifestyles. Convenience foods are produced by high industrial processing degrees [2, 3]. Considering meat products, precooked or ready-to-eat meat products could be the representatives of convenience foods [4]. During industrial processing and storage, meat products are subjected to several changes that could have negative effects to appearance, colour and overall acceptability of the products. In addition, products could undergo formation of off-odours and off-flavours [5]. The main cause of processed meat quality deterioration is lipids oxidation, facilitated by high temperatures during cooking. Lipids oxidation leads to the development of rancid flavour, known as warmed-over flavour (WOF). WOF is especially noticeable in meat which is reheated after being cooked and refrigerate stored. That is why the modern trend of controlling lipid oxidation is increasingly important [6]. Rancidity and WOF development in meat products could be slow down with both synthetic and natural antioxidants. Since there is an increasing preference for natural products, antioxidants of plant origin are gaining an increasing advantage over synthetic ones [5, 7].
For isolation of plants’ bioactive components supercritical fluid extraction, as an excellent process, could be used. This extraction could reduce solvent, time and energy consumption. It could gain better extraction yield and prevent degradation of sensitive compounds [8].

Regarding the problem of precooked and chilled stored meat quality deterioration caused by WOF formation, the objective of this paper was to evaluate the effect of wild thyme supercritical extract on oxidative stability and sensory characteristics of cooked and refrigerated pork chops.

2. Materials and Methods

2.1. Supercritical fluid extraction
Supercritical fluid extraction, performed on a laboratory-scale high pressure extraction plant (HPEP, NOVA, Swiss, Effretikon, Switzerland) was used for isolation of thyme extracts and it was described in detail by Pavlić et al. [8]. Extract was obtained at: pressure = 100 bar, temperature = 40°C and CO₂ flow rate = 0.3 kg/h, while separator conditions were 15 bar and 25°C.

2.2. Preparation of marinated pork chops
Three fresh (24 h post-mortem) pork loins were purchased from a slaughterhouse. All separable connective tissue, fascia and external fat were removed, and loins were cut perpendicular to the muscle fibres into 2-inch thick chops. Chops were divided to three following treatments: C (meat marinated in water/salt – control), SF1 (meat marinated with thyme supercritical extract – 0.2 μl/g), SF2 (meat marinated in water/salt, cooked, introduced into plastic bags with supercritical extract – 0.2 μl/g, and massaged for 10 min). Chops were cooked in a convection oven (175°C) until 72°C was achieved in the centre of the chop. In order to simulate the common household conditions after cooling chops were transferred to plastic boxes and covered with aluminium foil, to be exposed to air, and stored in refrigerator (4°C) for 6 days. Analyses were performed after cooking (day 0) and during 6 days of storage.

2.3. Colour measurement
Colour measurements were performed on the surface of cooked pork chops using a MINOLTA Chroma Meter (Model CR-400), with aperture of 8 mm in the measuring head and standard additions to measure CR-A33b (Konica Minolta Inc., Osaka, Japan). Colour characteristics were expressed in CIE L* a* b* system (L*– lightness, a*– redness; b*– yellowness), and total colour changes (ΔE) were calculated using obtained CIE L* a* b* values [9].

2.4. TBARS determination
Lipid oxidation was estimated as 2-thiobarbituric acid reactive substances test (TBARS), and was expressed as milligrams of malondialdehyde per kilogram of the sample (mg MDA/kg). The TBARS test was performed according to Botsoglou et al. [10].

2.5. Sensory analyses
Nine panellists, with experience in meat products sensory evaluation, performed sensory analysis using a 15-point descriptive scale (where 1 = not noted; 15 = extremely present). Prior to the sensory analyses, chops were reheated for 10 minutes in a convection oven (at 175°C), and were served warm to the panellists. Panellists evaluated odour (cardboard-like, linseed oil-like) and flavour (rancid, vegetable oil-like, marinated oil-like) characteristics of the samples, regarding the descriptors as described by Byrne et al. [11]. Before starting the sensory evaluation, panellists were introduced with referent materials for descriptors.
2.6. Statistical analysis
STATISTICA 12.0 (StatSoft, Inc., Tulsa, OK, USA) was used for statistical analysis. The obtained results were analysed using ANOVA (One-way), and differences among treatment means were compared according to Duncan’s multiple range test (p ≤ 0.05).

3. Results and Discussion
Formation of TBARs was used to determine the extent of lipid oxidation in the samples of precooked pork chops, and the results, expressed in mg malondialdehyde (MDA)/kg of sample, are shown in Fig. 1. For the samples of raw meat TBARs value was 0 mg MDA/kg indicating the absence of lipid oxidation. The TBARs values slightly increased after marinating, what could probably be due to salt addition and meat massaging [12]. Heat is recognised as the one of the main initiators for the lipid oxidation [13] and that is why first increase in TBARs value was determined after cooking. Significantly higher TBARs value increase was determined for control sample, comparing to experimental ones. Marinating meat with thyme extract slow down lipid oxidation caused by high temperatures during cooking, and consequently, SF1 samples had almost no change in TBARs values after cooking, comparing to samples after marinating. During the storage period oxidative rancidity increased in all samples, and significant differences (p<0.05) in TBARs values among samples were noted throughout the entire storage period. The highest increase was for control samples, and after 4 days of storage TBARs value for this sample reached 2.08 mg MDA/kg. Thyme extract also showed a significant protective effect towards lipid oxidation during chilled storage, but to the different extents. The lowest TBARs values were determined for marinated chops during the whole storage period. Samples treated with thyme extract after cooking had, during the whole storage period, TBARs values that were higher than in marinated chops, but significantly lower (p < 0.05) than in the control ones. Thyme extract’s antioxidant activity could be attributed to the monoterpene phenolics, especially carvacrol and thymol, what was in accordance with the results of Šojić et al. [14].

Figure 1. Effect of thyme on TBARS values in precooked pork chops during refrigerate storage

The results of instrumental colour characteristics measurements during 6 days of storage (+4°C) are shown in Table 1. Lipid oxidation influence colour changes. Along with lipid oxidation in a coupled lipid-pigment reaction haem pigments also oxidize, resulting in colour change [15]. Differences in \( L^* \) values were noted among samples during the storage period, but no clear pattern was observed. In the experiment for model raw pork batters Hernandez-Hernandez et al. [16] correlated higher TBARs values, as indicators of lipid oxidation, to lower \( L^* \) values (darker samples). On the other hand, Oliveira et al. [15] did not find any correlation between lipid oxidation and lightness in mortadella. Redness (\( a^* \) values) of all chops was within the range 0.87-7.25, and majority of the samples were in the range perceived as of grey colour [17]. The redness of the chops decreased during storage, what
was in accordance with results of Fernandez-Lopez et al. [18], who reported that decrease in $a^*$ value for meat products could be correlated with oxidation processes. At the end of storage period, the lowest $a^*$ value was determined for control samples.

In general, the yellowness ($b^*$ values) did not change significantly during 6 days of storage. Changes of colour characteristics during storage could be shown by the value of total colour change, $\Delta E$. When $\Delta E$ is higher than 2, modifications in colour are considered to be occurred [19]. For control chops, $\Delta E$ was higher than 2 for the whole storage period, while SF1 and SF2 chops reached this value after 6 days.

### Table 1. Effect of thyme on colour characteristics of precooked pork chops during refrigerate storage

| Day | Sample | L*  | $a^*$  | $b^*$  | $\Delta E$ | Day | Sample | L*  | $a^*$  | $b^*$  | $\Delta E$ |
|-----|--------|-----|-------|-------|------------|-----|--------|-----|-------|-------|------------|
| 0   | C      | 73.04 | 3.69a | 9.69b |             | SF1 | 72.89 | 3.58a | 10.77a | 9.69a | 2.62       |
|     | SF2    | 74.28 | 2.92b | 11.00b|             | SF2 | 74.49 | 3.07b | 11.23b | 9.84a | 2.62       |
|     |        | 77.26a| 7.25a | 10.38b| 5.56        |        | 75.13 | 2.11b | 11.30b | 12.08a | 1.90       |
| 1   | SF1    | 74.76 | 2.09c | 10.79c |             | SF1 | 74.01 | 2.80a | 11.30b | 10.79 | 3.42       |
|     | SF2    | 74.76 | 2.09c | 10.79c |             | SF2 | 74.01 | 2.80a | 11.30b | 10.79 | 3.42       |
|     |        | 75.48a| 3.07b | 11.23b | 1.09        |        | 76.10a| 2.41a | 10.90 | 3.42       |
| 2   | SF1    | 74.12 | 3.20b | 10.81b | 2.89        | SF2 | 74.76 | 2.62b | 10.79c | 10.28b | 3.67       |
|     | SF2    | 74.12 | 3.20b | 10.81b | 2.89        |        | 75.48 | 3.07b | 11.23b | 10.79c | 3.67       |
|     |        | 72.87c| 2.62b | 10.79c | 1.46        |        | 74.69 | 1.97a | 11.29b | 1.09  | 2.48       |
| 3   | SF1    | 74.76 | 3.07b | 10.79c | 1.46        | SF2 | 74.76 | 2.62b | 10.79c | 1.09  | 2.48       |
|     | SF2    | 74.76 | 3.07b | 10.79c | 1.46        |        | 75.77 | 3.16a | 10.35a | 1.64  | 2.21       |

$a,b,c$ values in the same column, same day, between different treatments – with different superscript letters – are significantly different ($p < 0.05$)

The results of sensory analyses for precooked and chill stored pork chops are presented in Fig. 2. Differences between freshly cooked and chill stored samples in several odour and flavour attributes were determined. Analysed sensory attributes changed differently during the storage period.

### Figure 2. Effect of thyme on sensory characteristics of precooked pork chops during refrigerate storage

The attributes, cardboard like, vegetable and linseed oil odour, and rancid flavour, which are directly associated with WOF development [20], had higher scores during storage period. For control
samples was the highest increase in cardboard odour, followed by SF2 samples. For SF1 samples cardboard odour had the lowest value. Byrne et al. [21] correlated intensity of cardboard flavour with WOF formation, but Campo et al. [22] pointed out that cardboard-like flavour disappeared when the flavour was dominated with rancidity notes.

Rancid flavour increased with storage, and again, the highest values were for control chops. Campo et al. [22] reported positive correlations between rancid flavour and WOF formation with TBARS values. According to Oliveira et al. [15] sensory recognized oxidation, that is WOF formation, for pork and beef products is associated with TBARs values in the range from 0.3 to 1.0 mg MDA/kg. Obtained positive effect of thyme extract on WOF formation was in accordance with the results of Lara et al. [16] which showed efficacy of natural antioxidants, originated from rosemary and lemon balm, in controlling lipid oxidation in processed meat product. In our study, the marinating process resulted in more pronounced thyme aroma, compared with samples treated with thyme extract after cooking.

4. Conclusion

The addition of thyme supercritical extract, as natural antioxidants, in the processing of precooked pork chops, resulted in protective effects against oxidation of lipids during 6 days of storage under refrigeration conditions, consequently preserving colour and sensory characteristics. Usage of extract in the marinating process gain better results, than when applying thyme extract after cooking. Nonetheless, samples with application of extract after cooking were better than the control ones, and the thyme aroma was less pronounced than in extract marinated samples, thus had lower impact in changing the dominant aroma of cooked meat.

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