

High efficiency of supercritical rosemary extract in long term oxidative stabilization of pork liver pâté

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A b s t r a c t: The study was aimed to investigate potential of supercritical extract of rosemary (RSE) in the long term oxidative stabilization of pork liver pâté (five years). Determination of peroxide value (PV) and thiobarbituric acid (TBA) assay were used to assess and compare lipid deterioration in pâté samples containing the same content of RSE, butylated hydroxyanisole (BHA) and commercial rosemary antioxidant Flavor 'Plus (FP). The first evidence of primary oxidation was reported after five years in all the samples except in those containing low concentrations of RSE (200-500 mg/kg) and FP (500 mg/kg). Increasing concentration of the RSE in pâté (1000 mg/kg) resulted in the lower efficiency in preventing primary oxidation of lipids (PV=0.27 meq/kg). The BHA was reported for the lowest efficiency among tested antioxidants (PV=0.26-0.3 meq/kg). The TBA assay showed no evidence of secondary oxidation products in all the samples due to insufficient amount of formed peroxides. General chemical composition of the pâté samples after five years was not significantly different from the initial one. Since RSE is isolated and deodorized using supercritical fluid technology it doesn't contain traces of organic solvents. For that reason, the RSE would provide an added value to commercial liver pâté due to both natural origin and potential bioactive properties.

Key words: Supercritical rosemary extract; Liver pâté; Compositional analysis; Oxidative stabilization; Peroxide value.

Introduction

Lipid oxidation, as one of the major causes of chemical spoilage of food, leads to the formation of off-flavors and reduces nutritional quality. Process of lipid oxidation adversely affects food safety due to formation of reactive oxygen species (ROS), which have been related to carcinogenesis, inflammation, early aging and cardiovascular diseases (Siddhuraju and Becker 2003). The addition of antioxidants is one of the processes currently used to increase the resistance to lipid deterioration in foods at the industrial level (Silva et al., 2001).

Synthetic antioxidants (i.e., butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tertiary butylhydroquinone (TBHQ), etc.) widely used for prevention of lipid oxidation in foods, have been already prohibited in many countries due to recent suspicion of their undesirable long-term toxicological effects, including carcinogenicity (Shahidi

and Zhong, 2005). Increasing resistance of consumers to usage of synthetic additives and their requirements for high added value foods have additionally stimulated the search and evaluation of natural compounds with antioxidant properties (Yanishlieva et al., 2006; Caldera et al., 2012; Skotti et al., 2014).

Rosemary (*Rosmarinus officinalis* L) represents one of the most effective spices widely used in food processing. The extracts isolated from rosemary leaves are the only commercially available herbal extracts for use as natural alternatives to synthetic food antioxidants in Europe and the United States (Yanishlieva et al., 2006). Antioxidant properties of rosemary extracts are mainly associated with the presence of phenolic diterpenes such as carnosol, rosmanol, carnosic acid, methyl carnosate, and phenolic acids such as rosmarinic acid (Yanishlieva et al., 2006; Babovic and Petrovic, 2011).

Rosemary essential oil was reported to have better efficiency than BHT in inhibition of oxidative

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Table 1. Literature review on the efficiency of the supercritical rosemary extracts in oxidative stabilization of various food systems**Tabela 1.** Pregled literature o efikasnosti superkritičnih ekstrakata ruzmarina u oksidativnoj stabilizaciji različitih sistema hrane

SC-CO ₂ conditions/ SC-CO ₂ uslovi	Substrate/ Supstrat	SFE or CA content/ Sadržaj SFE ili CA (mg/kg) ^a	Time/Temperature Vreme/Temporatura	Antioxidant efficiency/ Antioksidantska efikasnost	Reference/ Literatura
35 MPa; 100°C	Sunflower oil/ <i>Suncokretovo ulje</i>	200	6-12 h / 98°C	PV: SFE > BHA > Flavor' Plus™	(Babović, Žižović, et al., 2010b)
50 MPa; 100°C	Prime steam lard/ <i>mast</i> ; Canola oil/ <i>Ulje repice</i> ; margarine/ <i>margarin</i> ; Beef/ pork sausage/ <i>Govede/svinjske kobasice</i>	300-1000	18 h / 100°C	PV: SFE > BHA:BHT	(Nguyen et al., 1991)
30 MPa; 40°C	“Ultra-high” ω3 concentrates (80%) from fish oils as TAG or EE/ „Ultra-visoke“ ω3 koncentrati (80%) iz ribljih ulja TAG ili EE	500	50 days (<i>dana</i>) / 50–90°C	Rancimat test, p-AnV, PV:SFE+ TOC > SFE ≈ TOC	(Martin et al. 2012)
35 MPa; 100°C; 5% MeOH	Wheat germ oil/ <i>Ulje pšeničnih klica</i>	100	10 days (<i>dana</i>) / 50°C	PV: SFE > CSE p-AnV: SFE > CSE	(Celiktaş et al. 2007)
20-30 MPa; 40-80°C	Coconut oil/ <i>Kokosovo ulje</i>	140	3.5 h / 70°C	SFE > CSE > BHT	(Caldera et al. 2012)
10-20 MPa; 35-60°C	Sunflower oil/ <i>Suncokretovo ulje</i>	200 (CA) 763-802 (SFE)	11 days (<i>dana</i>) / 98°C	PV: SFE > CSE	(Hadolin et al. 2004)
CRE (4-20% CA)	Hazelnuts hacked/ <i>Lešnici</i>	40-80 ppm CA (1000-10 000 mg SFE/kg)	50-800 days (<i>dana</i>) / ambiental-80°C	PV: SFE (80 ppm CA) > RSE (40 ppm CA) Rancidity test: SFE(80 ppm CA) > RSE (60 ppm CA)	(Grüner- Richter et al. 2012)
CRE RAPS GmbH & Co. (Kulmbach, Germany).	Frozen beef burgers/ <i>Zamrznuti govedí burgeri</i>	200	180 days (<i>dana</i>) / -18°C	PV and TBA: RSE+CHI>TOC+CHI > CHI >RSE>TOC	(Georgantelis et al. 2007)

Legend/Legenda: ^aper kg of fat; SFE-supercritical fluid extract; CSE-conventional solvent extract; CRE-commercial SFE. AA-ascorbic acid; BHA:BHT-mixture (1:1); CA-carnosic acid; CHI-chitosan; FP-Flavor' Plus™; TOC- α -tocopherol; TAG-triacylglycerols; EE-ethyl esters; p-AnV-p-anisidine value; PV-peroxide value; TBA-Thiobarbituric Acid Assay/po kg masti; SFE-superkritični ekstrakt fluid; CSE – konvencionalni ekstrakt rastvarač; CRE – komercijalni SFE. AA– askorbinska kiselina; BHA: BHT–mešavina (1: 1); CA – karnozinska kiselina; Chi–Chitosan; FP-Flavor' Plus™; TOC- α -tokoferol; Tag–triacilglicerin; EE – etil estri; p-ANV-P-anizidina vrednost; PV–peroksidna vrednost; TBA–tiobarbiturna kiselina test.

deterioration of fats and proteins in refrigerated stored liver pâté (Estévez et al. 2007). Recent study of (Makri, 2013) indicated high potential of rosemary essential oil in the inhibition of oxidation in minced gilthead sea bream muscle up to three months at concentration of 500 mg/kg at -22°C. Rosemary extracts

obtained by conventional extraction with different organic solvents (including commercial ones), alone or in the combination with synthetic antioxidants, were reported to have high efficiency in oxidative stabilization of walnut oil under different storage conditions (Martínez et al., 2013), vacuum-packed

chicken frankfurters (Rižnar *et al.*, 2006), refrigerated retail packaged beef (McBride *et al.*, 2007), frozen and precooked-frozen pork sausages (Sebranek *et al.*, 2005), retail packed patties (Formanek *et al.*, 2001), frozen beef patties (Thongtan *et al.*, 2005), frozen cooked sea bream (Özyurt *et al.*, 2010), dehydrated chicken meat at room temperature (Nissen *et al.*, 2000), refrigerated lamb meat fillets (Ortuño *et al.*, 2014) and edible vegetable oil (Chen *et al.*, 2014; Cordeiro *et al.*, 2013; Urbančič *et al.*, 2014).

When it comes to the production and utilization of alternative antioxidants from natural sources, the food industry faces strict regulations and complies with measures for safety, reliability, and standardization of natural product (Shahidi & Zhong, 2005). Firstly, plant extracts for potential use as food antioxidants should have sufficient antioxidant activity to allow usage at levels equivalent to recommended concentrations of synthetic antioxidants (for meat products: 100–400 mg/kg of fat) without influencing sensory characteristics and food safety. Use of both, natural or synthetic antioxidants, is limited not just due to economic and technological reasons but also because of their physicochemical characteristics (e.g., solubility) and toxicological profiles (Silva *et al.*, 2001).

In order to achieve similar or stronger antioxidant activity compared to synthetic food antioxidants (i.e., Ascorbylpalmitate, BHA, BHT, TBHQ, Octylgallate, α -tocopherol) the amount of added rosemary extracts (isolated with organic solvents) or essential oil in aforementioned studies (Formanek *et al.*, 2001; Georgantelis *et al.*, 2007; McBride *et al.*, 2007; Estévez *et al.*, 2007; Cordeiro *et al.*, 2013; Martínez *et al.*, 2013; Chen *et al.*, 2014) was 3.3–25 times higher in comparison to synthetic antioxidants. Recent studies also indicate that the addition of rosemary essential oil despite its antioxidant activity greatly influences the aromatic profile of the products since some volatile components of these essential oils were terpenes which contribute to specific aromatic notes (Estévez *et al.*, 2007; Estévez *et al.*, 2004; Olmedo *et al.*, 2013). Solvent extraction which is generally used for the extraction of antioxidants from plant material has many drawbacks, including antioxidant transformation, low selectivity and extraction of solvent residues which are often prohibited by food regulations.

Extraction with supercritical CO₂ is environmentally friendly technology which enables isolation of solvent free extracts suitable for applications in food. Since the CO₂ selectivity can be adjusted by tuning pressure/temperature conditions, this property can be used to concentrate antioxidant compounds (antioxidant activity improvement) and/or to remove

volatiles (dearomatization) of extracts for potential use as food antioxidants (Babovic and Petrovic, 2011; Babovic *et al.*, 2010a; Ivanovic *et al.*, 2009).

Supercritical CO₂ extract of rosemary isolated at 34.5 MPa and 80°C has been reported for stronger scavenger activity against free DPPH in comparison to BHA, Trolox and ascorbic acid of same dosages (Peng *et al.*, 2007). Our recent study also proved superior scavenging activity of rosemary, sage and hyssop supercritical extracts isolated at 35 MPa and 100°C against free hydroxyl radicals compared to BHA at same concentrations (Babovic *et al.*, 2010a). Several other studies have also indicated superiority of the supercritical rosemary extracts over synthetic antioxidants (BHA and BHT), commercial rosemary antioxidant and conventional rosemary extract in oxidative stabilization of various types of foods alone or in combination with commercial antioxidants (Table 1).

As can be seen from Table 1, there is scant data on efficiency of rosemary supercritical extract in long term oxidative stabilization (several months to more than one year) of foods (Grüner-Richter *et al.*, 2012; Martín *et al.*, 2012). Apparently, no data on efficiency of supercritical rosemary extract on the long term oxidative stability of meat products is available.

Pork liver pâté is a very popular and cheap cooked meat product manufactured and consumed all around the world. It is made of minced by-products from meat industry (back fat, liver and, sometimes, low category meat) mixed with water and several seasonings added (i.e., spices, curing salt, brandy, etc.) according to the recipe of a manufacturer. Due to high amounts of fat and non-heme iron as well as manufacturing process itself, liver pâté is highly susceptible to lipid oxidation (Lorenzo and Pateiro, 2013).

The main goal of the present work was to investigate the efficiency of supercritical rosemary extract in oxidative stabilization of liver pâté over five years and to compare it with commercially available rosemary antioxidant Flavor'Plus (FP) and BHA (E 320).

Materials and Methods

Materials and chemicals

Rosemary extract (RSE) was isolated and deodorized using supercritical carbon dioxide fractional extraction (Babovic *et al.*, 2010), making it sensory suitable for use in food (Babović and Petrović, 2011). Essential oil fraction rich in volatiles was extracted first at pressure of 11.5 MPa and temperature of 40°C. Extraction of antioxidant fraction followed at pressure of 35 MPa and temperature of 100°C.

Commercial rosemary antioxidant Flavor'Plus™ (FP) purchased from Naturex (Avignon, France) and butylated hydroxyanisole (BHA) purchased from Sigma Chemical Co., St. Louis, USA were used as positive controls in comparative analysis of efficiency in oxidative stabilization of liver pâtés. All the reagents used for determination of chemical composition of pâtés and oxidative stability were analytical grade (p.a.) and purchased from Merck (Darmstadt, Germany).

Preparation of liver pâté

Experimental conditions for preparation of liver pâté were similar to those used for preparation of the commercial products. Liver pâté (10 kg) was prepared using pork head meat and trimmings (25.02%), solid fat (32.72), soup (28.87%), pork liver (9.62%), soybean flour (1.92%), sodium chloride (1.45%), sodium nitrite (0.009%), flavoring mixture (0.38%). Meat and fat were boiled in water for 1.5 h at 80°C, and then milled in a blender, with the addition of other ingredients to obtain a homogenous emulsion.

After cooking, the resulting slurry was divided into experimental groups, which were supplemented with 200 mg, 500 mg and 1000 mg of tested antioxidants per kg of fat. Antioxidants were dissolved in sunflower oil prior to their supplementation to pâté in order to provide homogeneous mixing. Concentrations of the antioxidants have been chosen in accordance with Codex General Standard for Food Additives (GSFA) provisions for BHA (E 320) in meat and meat products and EFSA (European Food Safety Authority) opinion on use of rosemary extracts as a food additive (EFSA, 2008).

Samples of pâté were filled into hermetically aluminum cans (á 150g) and sterilized in the autoclave at a temperature of 120°C for 15 min. Canning represents the oldest and most important means of preparing ambient stable, long shelf-life foods. In practice, the long storage meat products filled in sealed containers are exposed to temperatures above 100°C in order to inactivate/kill the most heat resistant bacterial microorganisms, such as the spores of *Bacillus* and *Clostridium*. After cooling, the pâté samples were stored at room temperature ($20 \pm 2^\circ\text{C}$) and analyzed at regular intervals of storage (once per year).

General composition analysis

General composition analysis of liver pâté was determined using standard ISO methods. The protein content in liver pâté samples ($N \times 6.25$) was determined by the Kjeldahl method using the Kjeltec 8400 Analyzer Unit (Foss, Sweden). Moisture

content was determined by drying samples at $103 \pm 2^\circ\text{C}$ to the constant mass (ISO 1442:1997). Total fat content was determined by extraction of weighted amount of liver pâté samples with petroleum ether (30–50°C b.p.) in a Soxhlet apparatus, after acid hydrolysis of the sample (ISO 1443:1973).

Standardized procedure (AOAC, 1984) was used to determine sodium chloride content in the tested pâté samples. Samples were acidified with concentrated nitric acid and 0.1 M silver nitrate is added in excess. This excess of silver nitrate was titrated with 0.1 M ammonium thiocyanate. Standardized procedure for determination of total ash in meat and meat products was used (ISO 936:1998). Testing samples were dried, carbonized and torrefied at $550 \pm 25^\circ\text{C}$ and subsequently weighted after cooling down.

Oxidative stability evaluation

Peroxide value (PV). Standardized procedure for determination of peroxide value (PV) for animal and vegetable fats and oils was applied (EN ISO 3960:2010). Lipids were extracted from the tested samples with chloroform. An aliquot of the extract was poured in solution containing acetic acid and isooctane and treated with potassium iodide solution. Released iodine was titrated with standard solution of sodium thiosulfate.

Thiobarbituric acid (TBA) assay. Lipid oxidation potential was assessed by determining of 2-thiobarbituric acid-reactive substances (TBARS) according to modified procedures described elsewhere (Tarladgis et al., 1960; Holland, 1971). It included extraction of malonaldehyde (MDA) with aqueous acidic solution from the samples (20 g) and addition of TBA in equivalent quantity of distillate. The resulting red color was measured using spectrophotometer JENWAY 6405 (Keison International Ltd., United Kingdom) at 530 nm. Absorbance readings *versus* concentration of MDA were plotted.

Statistical analysis

Each measurement was performed three times in duplicate for a total of six repetitions per sample. The mean value and standard deviation were calculated using the OriginPro 8 software (OriginLab Corporation, Northampton, MA, USA). A one-way ANOVA (analysis of variance) method followed by post hoc Tukey's test was used to evaluate the significant difference among various treatments with the criterion of $p < 0.05$. Pearson product moment correlation is used to assess linear correlation coefficients (r) among means.

Results and Discussion

Compositional analysis of liver pâtés

Initial chemical composition of liver pâté samples containing RSE, FP and BHA was investigated and compared to their composition after five years. Moisture, fat, protein, ash and sodium chloride contents in the tested samples of liver pâtés are given in Table 2.

The mean content of solid fat in all the samples was 28.97 % (initial) and 29.35% (after 5 years). Recent study of *Lorenzo and Patiero* (2013) demonstrate higher potential of foal pâtés with 30% of pork back fat for consumer appeal, being healthier low-fat meat product with better physicochemical properties and higher oxidative stability in comparison to pâté with 40% of fat. Higher fat content (> 30%) has been recently correlated to lower protein content due to their oxidative deterioration in stored porcine liver pâté, chilled foal liver pâté and frozen beef patties (*Estévez et al.*, 2005; *Lorenzo and Pateiro*, 2013; *Utrera et al.*, 2014).

The relative change of the protein and fat content after five years has been presented in Fig. 1. Relative change of fat and protein in samples supplemented with RSE was lower than for samples containing BHA (Fig. 1). Minor relative change of the fat content in the samples with FP might be due to its hydrophilic nature and consequently to its lower solubility in fats in comparison to BHA and RSE. There was a strong correlation of the RSE concentration with the relative change of fat content ($r = 0.831$) and moderate correlation with the relative change of protein content ($r = 0.603$).

In accordance to the specification of the manufacturer, commercial water soluble rosemary antioxidant (FP) contained 45–55% of dry water-soluble rosemary extract with 4–5% of rosmarinic acid (active component), 20–35% polyethylene glycol and citric acid (pH of 10% solution is 4.1–4.5%). Detailed chemical composition of the RSE used in this study has been reported in our previous work (*Babovic et al.*, 2010a). The main compounds in the lyophilic RSE considered to be responsible for the antioxidant activity of RSE used in this study were

Table 2. General chemical composition of the liver pâtés containing RSE, FP and BHA*

Tabela 2. Opšti hemijski sastav jetrene paštete koji sadrže RSE, FP i BHA*

Sample/ Uzorak	Water/ Voda (%)		Proteins/ Protein (%)		Fat/ Mast (%)		Ash/ Pepeo (%)		NaCl (%)	
	Initial/ Početni	After 5 years/ 5 godina	Initial/ Početni	After 5 years/ 5 godina	Initial/ Početni	After 5 years/ 5 godina	Initial/ Početni	After 5 years/ 5 godina	Initial/ Početni	After 5 years/ 5 godina
RSE-I	56.71±0.11	56.62±0.01	6.98±0.11	7.07±0.03	28.98±0.10	29.23±0.06	2.10±0.01	2.08±0.01	1.51±0.04	1.38±0.03
RSE-II	56.48±0.11	56.20±0.32	7.05±0.04	7.22±0.04	28.84±0.07	29.43±0.04	2.10±0.03	2.08±0.04	1.41±0.01	1.31±0.01
RSE-III	56.44±0.25	56.125±0.29	7.20±0.07	7.22±0.05	29.21±0.06	29.84±0.07	2.11±0.03	1.85±0.03	1.42±0.03	1.32±0.03
FP-I	56.40±0.04	56.73±0.08	6.94±0.07	6.98±0.09	28.96±0.07	29.18±0.14	2.11±0.00	2.00±0.00	1.45±0.00	1.39±0.03
FP-II	56.39±0.11	55.96±0.30	6.99±0.11	7.22±0.04	29.30±0.07	29.10±0.04	2.10±0.01	1.96±0.01	1.41±0.03	1.36±0.04
FP-III	56.40±0.25	56.12±0.27	7.01±0.10	7.06±0.08	29.05±0.06	29.01±0.01	2.09±0.03	2.10±0.03	1.42±0.01	1.34±0.01
BHA-I	56.66±0.04	56.46±0.06	7.03±0.01	7.16±0.06	28.96±0.07	29.52±0.07	2.08±0.01	1.85±0.03	1.31±0.03	1.41±0.03
BHA-II	56.70±0.06	56.00±0.05	6.99±0.00	7.20±0.04	28.59±0.13	29.33±0.04	2.10±0.07	1.94±0.04	1.30±0.01	1.35±0.03
BHA-III	56.54±0.14	56.28±0.33	6.99±0.10	7.08±0.09	28.82±0.10	29.55±0.03	1.92±0.03	1.94±0.01	1.36±0.03	1.36±0.00

Legend/Legenda: RSE-rosemary supercritical extract; FP-Flavor'Plus™; BHA- Butylatedhydroxyanisole.; Concentration of antioxidant: I-200 mg/kg, II-500 mg/kg, and III-1000 mg/kg (calculated on the basis of the fat weight in liver pâté); For all analyses, mean differences in the same column are not significant at $p = 0.05$ level; *The results are expressed as the mean value ± standard deviation of six replicates per test (n=6)/ RSE- superkritični ruzmarina ekstrakt; FP-Flavor 'Plus™; BHA-Butilizovani hidroksianizol; Koncentracija antioksidanata: I-200 mg/kg, II-500 mg/kg, i III-1000 mg/kg (izračunat na osnovu težine masti u jetrenoj pašteti); Za sve analize, razlike srednjih vrednosti u istoj koloni nisu značajni na nivou u $p = 0,05$; * Rezultati su izraženi kao srednja vrednost ± standardna devijacija od šest ponavljanja po testu (n = 6)

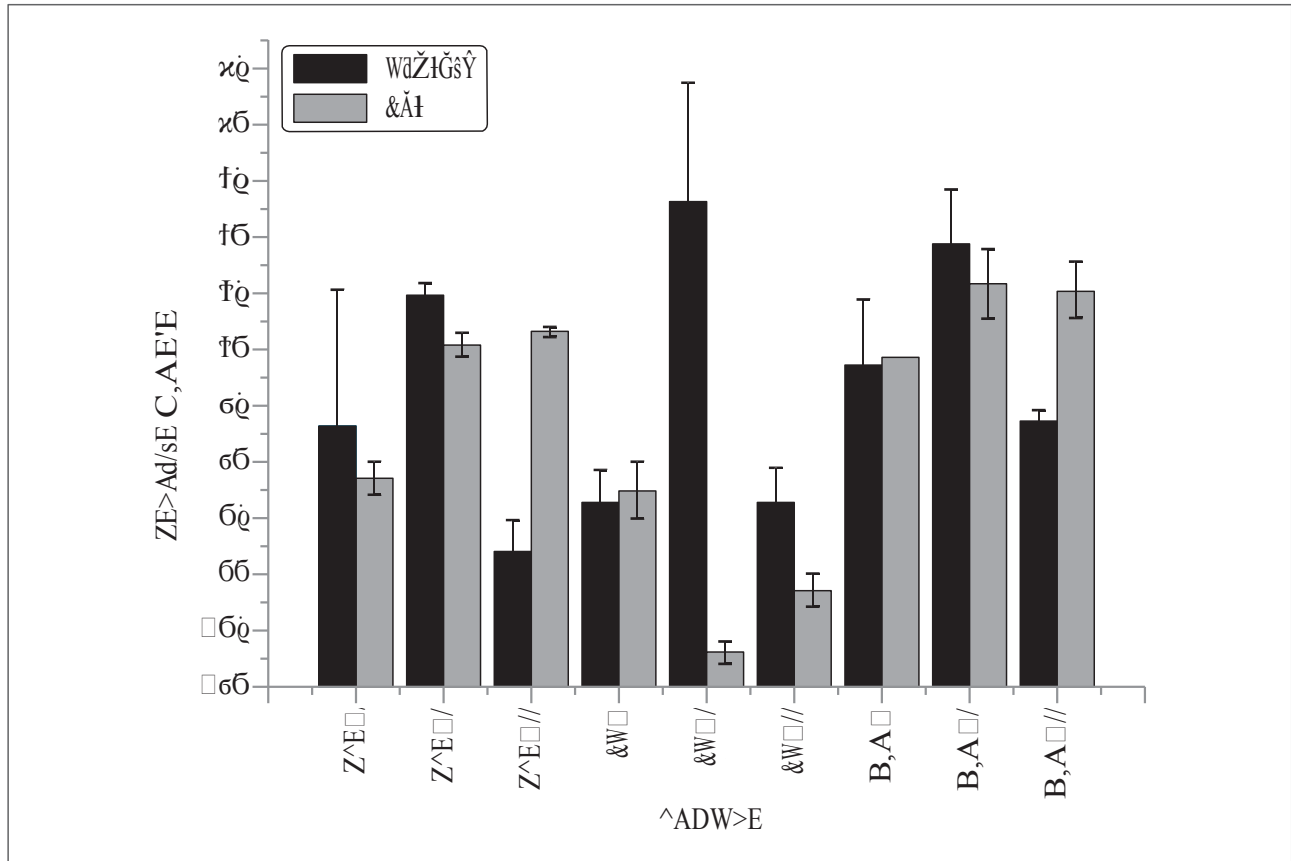


Figure 1. Relative change of protein and fat content during five years (with respect to initial content)

Figura 1. Relativna promena proteina i masti tokom pet godina (u odnosu na početni sadržaj)

carnosol (3.9368 g/100g extract) and carnosic acid (4.7596 g/100g extract) (Babovic et al., 2010a). Rosmarinic acid was not identified in the RSE.

Oxidative stability evaluation

The peroxide value (PV) and thiobarbituric acid (TBA) assays were used to assess lipid deterioration in the liver pâté samples. Determination of PV has been one of the most important and implemented quality control measurements for assessment of food quality and safety. The PV indicates the concentration of peroxides and hydroperoxides that are produced during the early stages of lipid oxidation (primary oxidation products). Sharp increase of PV should indicate the end of the shelf-life of a sample. In this study, PV analysis was used to indicate the primary oxidation status of the pork liver pâté by measuring the concentration of hydroperoxides. The quantity of the peroxides was reported as milliequivalents of active oxygen in 1 kg of fat in the sample.

The first evidence of primary oxidation was reported after five years in all the samples except in those containing low concentrations

(200–500 mg/kg) of the RSE and 500 mg/kg of the FP (PV = 0 meq/kg) (Table 3). Higher concentration of the RSE in pâté (1000 mg/kg) had lower antioxidant efficiency (PV = 0.27 meq/kg). In this regard, there was a strong correlation between the applied RSE concentration in the pâté and the peroxide value ($r = 0.929$). After five years, the highest PVs were recorded for the pâté samples with BHA, indicating the lowest efficiency in long term oxidative stability. The FP showed low efficiency at lower concentration (200 mg/kg) as well as at high concentrations (1000 mg/kg). Latter might be due to pro-oxidant effect of FP when it is added in the higher concentrations (1000 mg/kg).

According to the comparative analysis, the following order of antioxidant efficiency in oxidative stabilization following order was established for each applied concentration:

- 200 mg/kg: RSE > BHA > FP
- 500 mg/kg: RSE = FP > BHA
- 1000 mg/kg: FP > RSE > BHA.

The relative order of antioxidant efficiency (according to the average PV values for concen-

Table 3. Peroxide value (PV) in the tested samples of liver pâté after five years
Tabela 3. Peroksidna vrednost (PV) u testiranim uzorcima jetrene paštete posle pet godina

Sample/ Uzorak	PV (meq/kg)	Average PV at 200-1000 mg/kg/ Prosečna PV kod 200–1000 mg/kg
RSE-I	0.00	
RSE-II	0.00	0.09
RSE-III	0.27±0.01	
FP-I	0.28±0.04	
FP-II	0.00	0.16
FP-III	0.20±0.04	
BHA – I	0.26±0.08	
BHA – II	0.30±0.04	0.28
BHA – III	0.28±0.02	

tration range 200–1000 mg/kg) follow the order RSE>FP>BHA (Table 3).

The maximum level for edible animal fat (lard, tallow, rendered pork fat) or oil of 10 meq/kg is recommended to ensure its safety for consumption (CODEX-STAN 211. 1999). In this study, reported PVs in the liver pâté samples (0.0–0.30 meq/kg) (Table 3) were much lower than recommended maximum value for fat containing food (10 meq/kg). It means that after five years primary lipid oxidation in pâté samples has just started.

Hydroperoxides (the primary oxidation compounds) are not stable and can easily decompose into variety of volatile and nonvolatile secondary oxidation products (alkanes, ketones, aldehydes, acids, etc.) which are responsible for the off-flavor in oxidized fats and oils (Shahidi and Zhong 2005). Secondary lipid oxidation in pork liver pâté was studied by the TBA assay. Malondialdehyde (MDA) is one of the most abundant aldehydes generated during secondary lipid oxidation and also probably the most commonly used as an oxidation marker. According to the TBA analysis, presence of MDA was not recorded in any of the tested pâté samples. Since the previously determined PVs were very low (0.0–0.3 meq/kg) it was evident that even after five years lipid oxidation

was just at the beginning. In other words, the amount of peroxide in the system was not sufficient to form secondary oxidation products.

Conclusions

The study is the first report on the great potential of the supercritical rosemary extract (RSE) in five year oxidative stabilization of the liver pâté. The RSE showed higher efficiency in preventing primary lipid oxidation than Flavor'Plus and BHA at low concentration (200 mg/kg). During tested period, the RSE prevented secondary oxidation of lipids equally efficiently as Flavor'Plus and BHA. General chemical composition of all the samples was not significantly changed over five years.

The RSE might be promising alternative to existing synthetic food antioxidants for several reasons. First of all, the RSE is isolated and deodorized using the supercritical fluid technology so it contains no organic solvents. Secondly, good solubility in fat and oils, due to its lyophilic nature, makes it convenient for use as antioxidant in fat and oil containing food. Finally, the RSE would give an added value to commercial liver pâté, due to both its natural origin and potential bioactive properties.

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Visoka efikasnost superkritičnog ekstrakta ruzmarina u dugoročnoj oksidativnoj stabilizaciji svinjske jetrene paštete

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Rezime: Studija je imala za cilj da istraži potencijal superkritičnog ekstrakta ruzmarina (RSE) u dugoročnoj oksidativnoj stabilizaciji jetrene svinjske paštete (pet godina). Određivanje peroksidne vrednosti (PV) i tiobarbiturne kiseline (TBA test) korišćeni su za procenu i poređenje lipidnog pogoršanja u uzorcima paštete koji imaju isti sadržaj RSE, butilovanog hidroksianizola (BHA) i komercijalnog antioksidansa ruzmarina Flavor 'Plus (FP). Prvi dokaz primarne oksidacije je zabeležen nakon pet godina u svim uzorcima osim u onim koji sadrže niske koncentracije RSE (200–500 mg/kg) i FP (500 mg/kg). Povećanje koncentracije RSE u pašteti (1000 mg/kg) je rezultovalo u slabijoj efikasnosti u prevenciji primarne oksidacije lipida (PV = 0,27 meq/kg). BHA vrednost utvrđena za najnižu efikasnost testiranih antioksidanasa (PV = 0,26–0,3 meq/kg). TBA test je pokazao da nema dokaza sekundarne oksidacije proizvoda u svim uzorcima zbog nedovoljne količine formiranih peroksida. Opšti (osnovni) hemijski sastav uzoraka paštete posle pet godina nije se bitno razlikovao od prvobitnog sastava. Budući da je RSE izolovana i dezodorisana korišćenjem tehnologije superkritičnog fluida, ne sadrži tragove organskih rastvarača. Iz tog razloga, RSE bi obezbedio dodatnu vrednost komercijalnoj jetrenoj pašteti, kako zbog prirodnog porekla tako i zbog potencijalnih bioaktivnih svojstava.

Ključne reči: Superkritični ekstrakt ruzmarina; jetrena pašteta; analize hemijskog sastava; oksidativna stabilizacija; peroksidna vrednost.

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