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Inhibition of protein and lipid oxidation by rapeseed, camelina and soy meal in cooked pork meat patties

Abstract In this study protein-containing by-products of deoiling processes rich in phenolics were applied to meat to be used as potential food ingredients in developing meat products with antioxidant effect. The effect of rapeseed meal (*Brassica rapa* L.), camelina meal (*Camelina sativa*), soy meal and soy flour (from soybean, *Glycene max* L.), in inhibiting oxidation of lipids and proteins was tested in cooked pork meat patties. A commercial CO_2 extract from rosemary (*Rosmarinus officinalis*) was used as a reference material alone and in combination with the other plant materials. The cooked pork meat with added plant materials was oxidized for 10 days at 5 °C under light. The oxidation was followed by measuring the formation of hexanal, pentanal and propanal by headspace gas chromatography and the formation of protein carbonyls by converting them to 2,4-dinitrophenylhydrazones (DNPH). Rapeseed meal (0.5 and 0.7 g/100 g meat) and camelina meal (0.7 g/100 g meat) as such and their combination (addition of 0.5 g/100 g) with rosemary extract (0.04 g/100 g) were effective antioxidants toward both protein and lipid oxidation while soy meal and flour were effective only in combination with rosemary extract.

Keywords Protein oxidation · Lipid oxidation · Antioxidants · Phenolics · Meat

Introduction

Oxidative reactions are a major cause of the chemical deterioration of muscle foods during cooking and storage [1].

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Meat is especially prone to oxidation processes due to its complex structure including muscular extracts, myofibrillar systems, polyunsaturated fatty acids, and pro-oxidants [2]. Oxidative reactions in muscle foods are accelerated by heat processing which releases protein-bound iron, activates heme-containing proteins, disrupts cellular membrane systems, and inactivates enzymes [3, 4], and by addition of sodium chloride which increases the pro-oxidant activity of iron [5]. Quality losses in oxidized meat products are generally characterized by flavor deterioration, discolorization, destruction of nutrients, and possible formation of toxic compounds [6].

Protein and lipid interactions contribute to number of functional properties of meat and meat products [7]. In the presence of oxidizing lipids, protein oxidation is manifested by free radical chain reactions similar to those for lipid oxidation [8]. The attack of reactive oxygen species (ROS) on muscle proteins leads to destruction of amino acid residues and the generation of carbonyl compounds. Formation of carbonyls is one of the most salient changes in oxidized proteins. Therefore, the concentration of carbonyl groups is highly indicative of protein oxidation [9]. Although oxidatively modified proteins exhibit alterations in functionality as manifested in the gel-forming ability, water-binding capacity, emulsifying capacity, solubility, and viscosity [7, 10, 11], the exact mechanism underlying in the textural changes has not been elucidated [9].

In order to control the type and extent of protein modification, selection of specific antioxidants, which inhibit both lipid and protein oxidation, can be used [1, 9]. The effectiveness of plant materials such as herbs and essential oils against lipid oxidation is linked to the presence of phenolic compounds and has been proved in different types of meat products [12–15]. Thus, phenolic containing raw materials applied to meat could substantially improve the quality of meat by affecting its oxidative status and improve the nutritional value of the product, and extend the shelf life. As an application of using by-products of deoiling processes of different oilseeds, rapeseed and camelina meals, and soybean, could thus be used as rich sources of phenolic compounds, proteins and essential fatty acids [16]. Soy is

already widely used by food industry as the most abundant and economical source of protein, particularly as a good balance of amino acids, and as source of lysine. The use of less refined soy materials rather than the highly refined isolates and concentrates may be also more beneficial from the antioxidant activity perspective. As shown by Wang and Murphy [17, 18], producing soy protein isolates and concentrates resulted in a significant loss of total isoflavone contents including genistein, daidzein, and glycitein during the processing steps between the raw material and refined product. In general, phenolic compounds present in plant materials are considered to be potential in reducing the risk of diseases [19, 20]. Phenolic extracts and isolated fractions of various plant phenolics have been attributed with positive properties such as antimutagenic, antimicrobial, anticarcinogenic, and anti-inflammatory effects as well as being antioxidants in different model systems including LDL, liposomes and, meat [21–25]. According to Vuorela et al. [26] most of the phenolic compounds remain in the meal when the oil is pressed from the seeds. Therefore, the meat, poultry, and fisheries industries stand to benefit from use of different co-products or by-products of food industry in the development of functional foods.

The aim of the study was to investigate the effect of protein containing by-products of deoiling processes rich in phenolics, such as rapeseed meal, camelina meal, soy meal, and soy flour, in inhibiting oxidation of lipids and proteins in cooked pork meat patties. Rosemary extract, commercially used as an antioxidant in meat, was used as a reference material both alone and in combination with the other plant materials.

Materials and methods

Materials

The rapeseed (*Brassica rapa* L.) meal used was the residue of rapeseed deoiling process in which the oil was expelled from the seeds at elevated temperature by Mildola Ltd (Finland). Camelina (*Camelina sativa*) meal was obtained from Raisio Ltd (Finland). Soy flour (from soybean *Glycene max* L.) (Soyolk) was from Cereform Ltd (Northampton, England), and soy meal (SoyUp) was from Eco Food Factory Ltd (Finland). Rosemary extract (Stabiloton WS®) used was the supercritical extract from *Rosmarinus officinalis* L. by RAPS Ltd (Germany). Meat and fat from pork were obtained from local butcher shop. Quersetin was from Sigma, chlorogenic acid and genistein were from Extrasynthèse (Genay, France). Hexanal was from Aldrich (Germany). All solvents were HPLC grade and purchased from Rathburn Chemicals Ltd (Walkerburn, Scotland). MilliQ water purification system was used (Millipore, Bedford, Mass.). The sodiumphosphate buffer with 6-M guanidine hydrochloride (Aldrich) was made of disodiumhydrogenphosphate (NaHPO$_4$·12H$_2$O) (J.T. Baker, Deventer, Holland) and sodiumdihydrogenphosphate (NaH$_2$PO$_4$·H$_2$O) (Merck, Darmstadt, Germany) adjusted to pH 6.5. Trichloroacetic acid and potassiumchlo-

ride were obtained from Merck (Darmstadt, Germany). 2,4-Dinitrophenylhydrazine was purchased from Fluka. Chemicals used were of analytical purity.

Characterization of plant materials

Extraction of plant phenolics

The extraction of rapeseed, camelina and soy meal, soy flour and rosemary extract phenolics was performed according to method of Vuorela et al. [23] with some modifications. One to two grams of plant material and 20 ml 80% methanol was put in a centrifuge tube and was shaken in a water bath (75°C) for 60 min. The clear phenolic extract was collected after centrifugation (6000 rpm, 15 min).

Phenolic content

The amount of total polyphenols was measured colorimetrically according to the Folin-Ciocalteu procedure [27]. Phenolic extract (0.2 ml) was evaporated to dryness. After that 0.2 ml of methanol/water (1:2) 1 ml Folin-Ciocalteu reagent (1:10) and 0.8 ml sodium dicarbonate solution (7.5%) were added. After 30 min the total phenolic content was measured at 765 nm by Perkin-Elmer λ25 UV-Vis spectrophotometer, Norwalk, CT [26]. Gallic acid was used as standard compound. The results were expressed as gallic acid equivalents (GAE), in milligram of plant material. The HPLC analysis of phenolics was performed according to the method outlined by Koski et al. [28] for phenolic acids and their derivatives, and by Kähkönen et al. [29] for other phenolic compounds. Catechin, gallic acid, chlorogenic acid, sinapic acid and rutin were used as standard compounds. Isoflavones and lignans were analyzed in soy meal and flour according to the methods by Nurmi et al. [30] and Nurmi et al. [31], respectively. Also the content of tocopherols in the pressed cakes of camelina and rapeseed, soy meal, soy flour, and rosemary extract were analyzed [32].

Protein concentration of plant phenolics

The protein concentration was measured by determination of nitrogen according to the Kjeldahl procedure [33], and calculated with a 6.25 nitrogen conversion factor.

Total fat and fatty acid composition

The total fat of plant materials was determined by using a Soxtec Avanti 2050 automatic extraction system. The fatty acid composition was measured after hydrolyzing and methylating the fat extracts by gas chromatography. Nonadecanoic acid (C 19:0 fatty acid) was used as an internal standard. A commercial methyl ester mixture (Nu Chek

Prep, GLC-63A) was used as a standard. The results were expressed as methyl ester equivalents of fatty acids.

Preparation of pork meat patties

The meat model system was performed according to the method of Vuorela et al. [23]. Red meat from *longissimus dorsi* muscle of pork, and fat from pork back (2:1), water (10%) and salt (1.4%) were homogenized in a Tecator 1094 cutter for 3–4 min. The mass was then divided in portions, and to each portion the tested antioxidants were added by blending by hand. Rapeseed meal, camelina meal, soy meal, and soy flour were tested at levels of 0.3, 0.5, and 0.7 g/100 g meat. In addition, these plant materials were tested at level of 0.5 g/100 g meat in combination with rosemary extract of addition level of 0.04 g/100 g meat. Rosemary extract was tested at levels 0.04 g and 0.08 g/100 g meat, which were the recommended usage levels (0.4–0.8 g/kg) by manufacturer. Standard compounds, quersetin, chlorogenic acid, and genistein, were tested at levels of 0.01 g/100 g meat. Samples were then vacuum-packed and heated in a water bath (80 °C, 10 min). After heating, the bags were opened, and the samples were transferred to decanter flasks, covered with plastic wrapper, and kept at 5 °C under light. Oxidation was followed by measuring the lipid and protein oxidation at the day 0, at the day 3, at the day 6, and at the day 10. The oxidation experiments were performed in triplicate.

Analysis of oxidation products

Lipid oxidation

Lipid oxidation was followed by measuring the formation of volatile aldehydes, hexanal, pentanal and propanal, by headspace gas chromatography. A sample (2 g) was put in a headspace vial, and the formation of hexanal, pentanal and propanal was measured by headspace gas chromatography equipped with a capillary column (Nordibond NB-54, 25 m, 0.32 mm), and a flame ionization detector. Samples were injected with automatic sampler (Perkin Elmer HS 40XL). Vials were thermostated for 20 min at 80°C. Oven temperature was held constant at 60°C. The inhibition of plant phenolics against formation of different aldehydes was calculated at day 6: $(A_0-A_1)/A_0 \times 100$, where A_0 is the area of aldehyde in control sample and A_1 is the area of aldehyde in tested sample. The inhibitions were expressed as percentages.

Protein oxidation

Protein oxidation was followed by measuring the formation of protein carbonyls by converting them to 2, 4-dinitrophenylhydrazones (DNPH) and measured by spectrophotometer according to method outlined in Oliver et al. [34]. Concentration (nM) of carbonyls was calculated as $[Abs_{370nm}/21.0_{mM-1cm-1}) \times 1000]$, where $21.0_{mM-1\ cm-1}$ is the molar extinction coefficient of carbonyls. For protein quantification a standard solution of bovine serum albumin (BSA) in 20 mM sodiumphosphate buffer with 6 M guanidine hydrochloride (pH 6.5) was prepared and the protein concentrations were determined according to a standard curve. The inhibition of plant materials against formation of protein carbonyls was calculated: $(C_0-C_1)/C_0 \times 100$, where C_0 is the concentration (nM) of protein carbonyls per mg of protein in a control sample and C_1 is the concentration (nM) of protein carbonyls per mg of protein in a tested sample. The inhibitions were expressed as percentages.

Statistical analysis

Statistical analysis was performed using Statgraphics Plus (STCC Inc., Rockville, ML, USA) one-way ANOVA.

Results and discussion

The lipid and protein oxidation curves during 10 days of oxidation in the presence of standard compound quersetin, and camelina meal are shown in Fig. 1. The effect of the plant materials toward oxidation are presented as inhibition values at day 6 of oxidation (Table 1) since there were no changes in the inhibition of lipid oxidation between
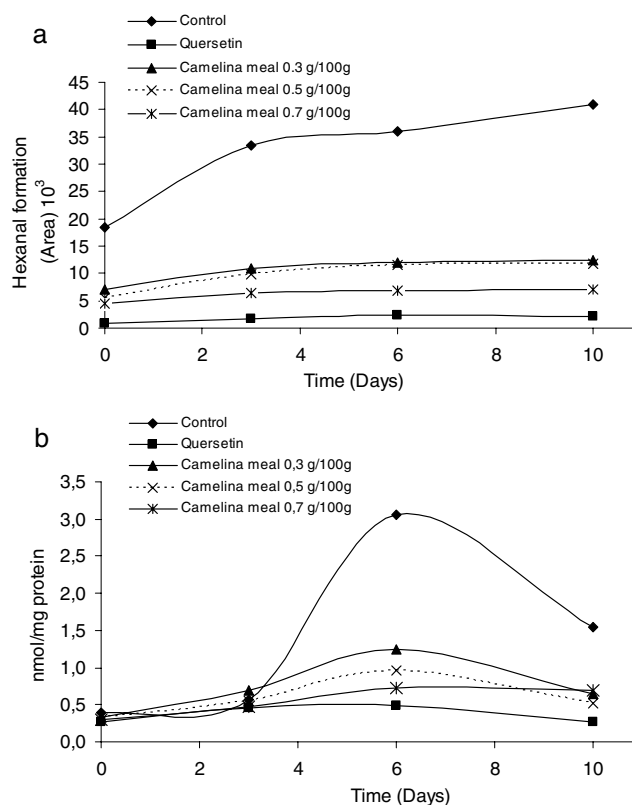

Fig. 1 Formation of **a** hexanal (area 10^3) and **b** protein carbonyl compounds (nmol/mg protein) in cooked meat during storage at +5 °C

Table 1 Inhibition of Protein and lipid oxidation (after 6 days of oxidation) with plant phenolics (percent inhibition, mean ($n=3$) ± SD)

Materials	Addition level g/100 g meat	Hexanal	Propanal	Pentanal	Protein carbonyls
Camelina meal	0.3	66.4±6.7 fg	58.0±8.5 d	61.1±6.0 ef	59.1±7.1 bcdef
	0.5	67.5±2.1 fg	58.7±7.3 d	61.3±4.2 ef	68.6±5.3 abc
	0.7	80.6±2.6 d	68.5±3.7ab	74.4±5.2 cd	76.2±6.4 a
Rapeseed meal	0.3	72.2±0.9 ef	58.6±6.0 d	65.6±1.6 de	63.9±13.6 abcd
	0.5	88.7±0.7 bc	70.7±2.2 ab	80.3±2.3 bc	62.1±10.7 abcde
	0.7	95.5±1.0 ab	74.0±3.0 a	85.0±0.5 b	52.8±1.7 cdefg
Soy flour	0.3	28.3±2.4 kl	35.6±2.6 ef	46.5±10.6 ghi	28.5±17.3 hi
	0.5	39.8±2.7 hi	36.7±2.0 ef	53.1±9.1 fgh	43.9±12.3 fgh
	0.7	43.6±6.9 hi	26.8±2.9 g	37.3±4.8 ij	28.5±4.1 hi
Soy meal	0.3	31.5±0.4 jk	34.2±2.0 f	35.0±1.0 j	23.4±4.0 i
	0.5	39.3±2.8 ij	35.4±1.1 ef	44.5±2.2 hij	39.6±9.3 ghi
	0.7[a]	48.2±7.3 h	38.5±3.3 ef	51.0±4.9 fgh	49.0±0.5 defg
Rosemary	0.04	21.7±2.3 l	36.1±2.1 ef	22.3±3.6k	37.5±12.3 ghi
	0.08	62.5±14.1 g	60.9±9.2 cd	56.4±17.5 efg	46.2±6.4 efg
Rapeseed meal + Rosemary	0.5+0.04	97.4±0.8 a	42.5±3.2 e	96.7±0.4 a	70.2±11.1 ab
Camelina meal + Rosemary	0.5+0.04	94.6±1.3 ab	39.8±4.4 ef	93.8±0.4 a	70.0±11.6 ab
Soy meal + Rosemary	0.5+0.04	84.9±4.0 cd	55.8±1.6 d	84.2±3.9 bc	66.2±8.0 abcd
Soy flour + Rosemary	0.5+0.04	78.3±4.3 de	66.4±3.2 bc	83.5±5.1 bc	53.5±8.7 cdefg
Standards					
Quersetin	0.01	93.6±0.4	81.6±0.9	93.3±1.4	76.8±1.7
Chlorogenic acid	0.01	36.9±5.2	44.3±23.5	43.2±7.0	45.0±6.5
Genistein	0.01	−12.7±2.6	−9.7±5.8	−14.3±2.9	−3.8±17.0

Negative values indicate pro-oxidant activity. Values in the same column followed by different letters are significantly different ($p<0.05$)

SD standard deviation

[a]$n=2$

the 6th and 10th day (Fig. 1). The initial level of protein oxidation was approximately 0.4 nmol/mg of protein. The content of protein carbonyls increased during the first 6 days of oxidation to reach a maximum level which was approximately sixfold the initial level in the control sample. In other studies, basal level of carbonyl groups has been reported in fresh beef meat to be approximately 3 nmol/mg protein [35, 36]. Also the decrease of carbonyl groups after a long incubation has already been observed with different oxidation systems [36, 37], which is in accordance with our results (Fig. 1). Thus, for comparison results for protein and lipid oxidation, the inhibition values at day 6 of oxidation are presented (Table 1) as the formation of protein carbonyls is not decreasing. Aroma has an important influence on the final consumer acceptance of the commodity. Although sensory evaluation was not performed during the oxidation, odour of the meat patties with added plant phenolics was mild and acceptable, and no visible changes in texture was observed.

Rapeseed meal at a concentration of 0.7 g/100 g meat as well as both rapeseed meal and camelina meal in combination with rosemary extract were excellent antioxidants toward lipid oxidation in inhibiting hexanal formation (≥95% inhibition). Rapeseed meal at a lower concentration (0.5 g/100 g meat) and camelina meal (0.7 g/100 g meat) as such showed good antioxidant activity toward hexanal formation (≥80% inhibition). These phenolic rich plant materials were also effective in inhibiting formation of pentanal. Methanolic extracts of both camelina and rapeseed meals have been reported to show remarkable radical scavenging activity (DPPH test, electron spin resonance (ESR) test), and antioxidant activity in β-carotene/linoleic acid system [38]. The amount of total phenolics of rapeseed is 5,100 µg/g with the hydroxycinnamic acids dominating (3,100 µg/g, Table 2). According to Vuorela et al. [23, 26] the main hydroxycinnamic acid derivatives in rapeseed meal contributing to antioxidant effect are sinapine (2,400—2,900 µg/g) and sinapic acid (280 µg/g). The effect of sinapic acid (23.5 mg/100 g meat) in inhibiting the formation of hexanal in meat was 89.0±10.3% [23]. However, also much smaller amounts of rapeseed phenolics are effective toward oxidation of meat. The antioxidant effect of rapeseed meal (0.3 g of meal containing 1.5 mg phenolics/100 g meat) in inhibiting the formation of hexanal in meat was 66.4±6.7% (Table 1), which is accordance with our earlier findings [23]. The combination of rapeseed meal (0.5 g/100 g meat) with the commercial CO_2 extract of rosemary (0.04 g/100 g meat) was excellent toward oxidation of meat lipids. Although the amount of phenolic compounds (3.6 mg) present in 0.7 g of rapeseed meal was lower compared to 0.7 g camelina meal (4.3 mg phenolics), rapeseed meal showed higher antioxidant activity toward lipid oxidation. This difference in the efficacy of rapeseed and camelina phenolics toward oxidation of

Table 2 Phenolic and tocopherol composition of plant materials (µg/g, mean ± SD)

Amount (µg/g)	Rapeseed meal	Camelina meal	Soy meal	Soy flour	Rosemary extract[h]
Total phenolics	5100±155	6200±490	2600±195	1800±150	15600±520
Flavonoids and phenolic acids					
Flavanols[a]	35±4	236±37	92±22	99±14	nd
Hydroxybenzoic acids[b]	14±3	ND	ND	ND	ND
Hydroxycinnamic acids and derivatives[c]	3096±330	747±54	ND	ND	ND
Sinapine[d]	2400–2900[g]	1437±47			
Sinapic acid[d]	280[g]	427±10			
Flavonols[e]	ND	1325±196	ND	ND	ND
Isoflavones[f]					
Total			1310±6	1400±51	
Daidzein			500	520	
Genistein			720	770	
Glycitein			110	110	
Lignans					
Total			15.2±1.38	22.8±0.51	
Secoisolariciresinol			1.0	2.7	
Syringaresinol			3.0	3.3	
Pinoresinol			2.6	3.5	
Lariciresinol			8.6	13.3	
Matairesinol			ND	ND	
Tocopherols and tocotrienols					
Total	128±7	248±12	10±3	287±14	880±100
α-Tocopherol	52±5	24±7	3±3	17±1	832±95
β-Tocopherol	ND	ND	0±0	4±0	4±0
γ-Tocopherol	54±2	177±8	5±0	171±9	7±1
δ-Tocopherol	1±0	3±0	2±0	92±4	4±1
α-Tocotrienol	ND	ND	ND	ND	6±1
γ-Tocotrienol	20±1	43±2	ND	2±0	27±2

ND not detected/concentration under detection limit

[a]Gallic acid as standard

[b]Catechin as standard

[c]Chlorogenic acid as standard

[d]Sinapic acid as a standard

[e]Rutin as standard

[f]As aglycone calculated from total 7-*O*-glucoside and aglycone

[g]Vuorela et al. [23, 26]

[h]According to manufacturer, extract contains 9 ± 1% phenolic diterpenes: carnosic acid, carnosol and rosmanol

meat lipids is in part explained by the more than two times higher content of sinapine in rapeseed meal. The oilseed by-products contained tocopherols with the highest amount of α-tocopherol (52 µg/g) found in rapeseed meal compared to that (24 µg/g) in camelina meal (Table 2). However, camelina meal was rich in γ-tocopherol (177 µg/g). Tocopherols are well known to be important natural antioxidants for the oxidative stability of vegetable oils [39, 40].

In camelina meal there are several phenolic compounds present, which may be responsible for the antioxidant activity. The phenolic composition in camelina meal was predominated by flavonols, hydroxycinnamic acids, flavanols and, tocopherols (Table 2). Our results are comparable to the levels of sinapine (1.7–4.2 mg/g), sinapic acid (56.5 mg/g), and flavanoids (142.79 mg/g) reported earlier [38, 41]. According to Matthäus and Zubr [41] *Camelina sativa* oilseed cakes contain also other bioactive substances such as condensed tannins (1.0–2.4 mg/g) and glucosinolates (14.5–23.4 µmol/g). In the present study, camelina meal had the highest total fat level (24%) whereas rapeseed meal contained 11% of fat. The fatty acid composition of rapeseed meal and camelina meal were in accordance with the profiles of rapeseed and camelina oils [42, 43]. As camelina meal contains a high amount of α-linolenic acid (Table 3), it is the most susceptible to oxidation compared to the other by-products of oilseed processing. Most likely sinapine, the choline ester of sinapic acid, and sinapic acid are responsible for the antioxidant effect of camelina meal (Table 2). In addition to tocopherols, partly flavonols, such as quercetin glycosides, also contribute to the antioxidant activity of camelina meal as the amount of flavonols predominated that of hydroxycinnamic acids.

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Table 3 Protein and fatty acid composition of plant materials (%)

	Rapeseed meal	Camelina meal	Soy meal	Soy flour	Rosemary extract
Protein	31.5±0.3	32.3±0.2	53.1±0.2	40.9±0.3	0.1±0.1
Total fat	10.5±2.9	23.5±9.7	1.1±0.0	23.0±0.1	19.9±0.2
Fatty acid composition					
C 14:1	–	–	–	–	3.0
C 16:0	5.6	7.2	20.7	12.0	22.4
C 18:0	1.6	2.6	4.4	4.3	44.9
C 18:1	55.6	13.8	15.3	19.9	29.6
C 18:2	21.5	19.9	53.4	54.1	–
C 18:3	8.6	35.4	6.2	9.7	–
C 20:0	–	1.7	–	–	–
C 20:1	7.1	9.8	–	–	–
C 20:2	–	2.3	–	–	–
C 20:4	–	1.7	–	–	–
C 22:0	–	5.6	–	–	–
SFA	7.1	17.1	25.1	16.3	67.3
MUFA	62.7	23.6	15.3	19.9	32.7
PUFA	30.1	59.3	59.6	63.8	0.0

SFA saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids

Inhibition of protein oxidation compared to lipid oxidation is only moderate. This may be due to the reason that inhibition of lipid oxidation is the primary target for antioxidants, and protein oxidation the secondary. Rapeseed and camelina meal contained 32% protein (Table 3) contributing to ca. 0.7–1.6% of the meat proteins. Camelina meal phenolics were more potent in inhibiting the oxidation of proteins than rapeseed phenolics. Camelina meal as such (0.7 g/100 g meat) as well as rapeseed meal and camelina meal in combination with rosemary extract were the best antioxidants against protein oxidation with inhibition levels between 76.2 and 70.0%. Lower concentrations were also effective as camelina meal (0.5 g/100 g meat), soy meal in combination with rosemary extract, and rapeseed meal (0.3 g and 0.5 g/100 g meat) were also potent in inhibiting the formation of protein carbonyls. In addition to sinapic acid and its derivative, sinapine, also other phenolic compounds, such as quercetin glycosides are likely to contribute to the antioxidant effect toward protein oxidation. Compared to the effect of sinapic acid with 59.7% inhibition for addition of 23.5 mg/100 g meat [23], quercetin was superior with 76.8% inhibition (Table 1) toward formation of protein carbonyls. Camelina meal exhibited higher antioxidant activity toward protein oxidation than rapeseed meal. This difference in the antioxidant activity of camelina and rapeseed toward oxidation of proteins may partly be explained by the difference in the tocopherol content. In contrast, the amount of tocopherols (201 µg) present in 0.7 g of soy flour was higher than in camelina meal (174 µg) but the antioxidant effect was much weaker. Dietary vitamin E is known to reduce lipid oxidation in the precooked meat products made from pork, beef and poultry [44, 21]. The addition of α-tocopherol to meat products, however, is not considered to be effective toward lipid oxidation [45], although dietary vitamin E reduces lipid oxidation in meat products.

In comparison to the antioxidant activity of rapeseed and camelina meal, soy meal and soy flour were potent in inhibiting lipid oxidation only in combination with rosemary extract (Table 1). Rosemary is known to exhibit the most effective activity among herbs as spice and flavoring agent. Rosemary extract has been shown to act as an effective antioxidant in combination with a chelator such as phosphates in meat [20], in combination with α-tocopherol in frozen-crushed fish meat models [46], in combination of BHA or BHT with citric acid in turkey breakfast sausages [47], in combination with sodium tripolyphosphate in restructured chicken nuggets [48], in pre-cooked roast beef slices [49], and in addition of tertiary butylhydroxyquinone (TBHQ) in restructed beef steaks [50]. In addition, Nassu et al. [51] demonstrated that fermented goat meat containing 0.05% rosemary showed the best characteristics in relation to oxidative stability and to sensory acceptance, when compared with a sample containing 0.025% rosemary. The addition of rosemary essential oil (0.6 g/kg) in refrigerated frankfurters inhibited protein oxidation by 22.8% [52]. The phenolic diterpenes, carnosic acid and carnosol, account for over 90% of antioxidant activity of rosemary [20]. However, in the present study rosemary extract added to the meat in amounts relevant to food use (0.04%), showed poor antioxidant activity (Table 1). It may be assumed that the antioxidant activity of rosemary extract in combination with soy meal or soy flour is due to synergism, since addition of rosemary significantly improved the antioxidant protection of the by-products of soy processing. However, there were no synergistic effects of antioxidant activity with rapeseed and camelina meal in combination with rosemary extract.

In soy meal and soy flour isoflavones and lignans were the predominant phenolic compounds present. Soy meal contained higher amount of total phenolics (2,600 µg/g) than soy flour (1,800 µg/g). However, soy flour contained more isoflavones and lignans than soy meal (Table 2). There were also differences in the content of macromolecules, as

soy meal contained 53% protein and only 1% fat compared to 41% protein and 23% fat in soy flour (Table 3). The total isoflavone content of soy flour is reported to range from 1000 to 1650 µg/g, and also the amounts of genistein (460–940 µg/g), daidzein (440–710 µg/g), and glycitein (70–140 µg/g) [53, 54] are in accordance with our findings. The secoisolariciresinol content in soy flour is reported to be 1.3 µg/g [52]. While the amount of total phenolics (1.8 mg) in 0.7 g of soy meal was approximately the same as in 0.3 g of camelina meal (1.9 mg), soy meal contained significantly less tocopherols thus partly explaining why the inhibition of both lipid and protein oxidation was weaker in soy meal. In addition, 0.5 g of soy meal, 0.7 g of soy flour, and 0.08 g of rosemary extract, with total phenolics (1.3 mg) showed poor antioxidant effect compared to 0.3 g of rapeseed meal with approximately the same amount of total phenolics (1.5 mg). Similar findings of antioxidant effects on lipid oxidation were presented with soy protein hydrolysates (2%), which inhibited the formation of conjugated dienes by 46% and the amount of thiobarbituric acid-reactive substances (TBARS) by 32% in cooked pork patties [55]. In frozen pork patties, rosemary (0.1%) and soy protein (0.01%) inhibited the TBARS by 50 and 38%, respectively [56], indicating a less than moderate antioxidant effect. Conflicting results have been reported regarding the antioxidant activity of genistein, daidzein, and glycitein. As genistein exerted antioxidant activity in lipid/aqueous solution, in contrast it possessed very little antioxidant effect on soybean oil [57]. Genistein also lacked antioxidant activity toward oxidation of lipids in emulsions [58], which relates to our findings as genistein showed pro-oxidant activity (Table 1). Osborn-Barnes and Akoh also [58] reported the ability of daidzein to exhibit pro-oxidant activity in structured lipid-based emulsions. Lignans have been suggested to exert antioxidant activity, which is supported by the radical scavenging property of secoisolariciresinol diglucoside [59]. On the other hand, according to Rawel et al. [60] interactions of different phenolic acids such as gallic acid, chlorogenic acid, quersetin, myricetin, caffeic acid and kaempferol, cause a reduction in lysine, cysteine and tryptophan residues in soy proteins. Thus, it is clear that the mode of antioxidant action of isofavones and lignans, as well as that of other phenolic compounds, toward oxidation is affected by the environment. The structure of food and interactions with the other food constituents may alter the antioxidant efficacy.

In conclusion, rapeseed meal was an excellent antioxidant toward oxidation of meat lipids. Both rapeseed and camelina meals as well as their combination with rosemary extract were effective in protecting cooked pork meat patties toward lipid and protein oxidation. Sinapic acid and its derivative, sinapine, were partly responsible for the antioxidant activity of rapeseed and camelina meal. Also tocopherols present in the oilseed by-products may contribute to the antioxidant effect. The antioxidant properties of camelina meal were enhanced by flavonols. In contrast to rapeseed and camelina meal, soy meal and soy flour were effective only in combination with rosemary extract due to their synergistic interactions. The results indicate that residues of deoiling processes containing various bioactive substances could be exploited for example as potential food ingredients in developing functional meat products.

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