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Effect of Plant Phenolics on Protein and Lipid Oxidation in Cooked Pork Meat Patties

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Rapeseed and pine bark are rich sources of phenolic compounds that have in previous studies been shown to exhibit antioxidant and anti-inflammatory properties. In this study, the antioxidant effect of rapeseed and pine bark phenolics in inhibiting the oxidation of lipids and proteins in meat was tested as a possible functional food application. The cooked pork meat with added plant material was oxidized for 9 days at 5 °C under light. The suitable level of plant material addition was first screened by following lipid oxidation only. For further investigations plant materials were added at a level preventing lipid oxidation by >80%. The oxidation was followed by measuring the formation of hexanal by headspace gas chromatography and the formation of protein carbonyls by converting them to 2,4-dinitrophenylhydrazones and measured by spectrophotometer. It was shown that rapeseed and pine bark were excellent antioxidants toward protein oxidation (inhibitions between 42 and 64%). These results indicate that rapeseed and pine bark could be potential sources of antioxidants in meat products.

KEYWORDS: Meat; protein oxidation; lipid oxidation; antioxidants; phenolics

INTRODUCTION

Lipid oxidation is the main cause of deterioration of cooked meat in inducing an acceleration of oxidative processes during processing at high temperature. The destruction of cellular structures allows the interaction between polyunsaturated fatty acids and pro-oxidants. In cooked meat, iron is released from heme pigments. This nonheme iron is a known pro-oxidant of lipid hydroperoxides. At cooking temperatures, the denaturation of myoglobin makes it more susceptible toward oxidation due to the catalytic heme group. In meat, the higher the level of polyunsaturated fatty acids, the faster the oxidation process is. The cooking process leads to the development of the "warmed-over" flavor in refrigerated cooked meats (*1*). In food, also proteins undergo oxidation, leading to quality deterioration. Shacter et al. (*2*) define protein oxidation as the covalent modification of a protein induced either directly by reactive oxygen species or indirectly by secondary byproducts of oxidative stress. Meat proteins can be modified by oxidized lipids as well as metal- or enzyme-catalyzed oxidative reactions or other chemical and biological processes (*3*).

The proteins in meat are myofibrillar proteins, sarcoplasma proteins, and stromaproteins. Myofibrillar proteins (such as actin and myosin) make up 60.5% of the total proteins, sarcoplasma proteins (enzymes and myoglobin), 29%, and connective tissue proteins (collagen and elastin), 10.5%. Connective tissue proteins are mostly insoluble in water or salt solutions (*4*).

In meat, amino acids with reactive side chains, for example, amino and sulfhydryl groups, are especially susceptible to oxidation. Formation of carbonyls is one of the most important changes in oxidized proteins. Carbonyl compounds can be generated via direct oxidation of amino acid side chains, fragmentation of the peptide backbone, reaction with reducing sugars, or binding to nonprotein carbonyl compounds (*3*).

Lipid as well as protein oxidation can be inhibited by using antioxidants. In meat, food additives, such as ascorbic acid and phosphates, increase the antioxidant protection. The antioxidant activity of plant extracts containing phenolic compounds against lipid oxidation has been investigated earlier, and thus plant extracts might provide an alternative to food additive use. It has been shown that several plants or their phenolic extracts such as rosemary (*5*, *6*), potato peel (*7*), tea catechins (*6*, *8*−*11*), sage (*6*, *12*), cloudberry, beetroot, and willow herb (*13*) can act as lipid antioxidants in meat. In addition, Rababah et al. (*11*) found that tea catechins also inhibited protein oxidation in meat.

In a previous study, the antioxidant activity of 92 phenolic extracts from edible and nonedible plant materials was examined (*14*), and the plant materials exhibiting the greatest potential were examined further. Among these plant materials was pine

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bark; it was an excellent antioxidant against oxidation of methyl linoleate. In our previous study (*15*), pine bark also showed good antioxidant activity against oxidation of liposomes. According to earlier studies, rapeseed phenolics from rapeseed meal and crude rapeseed oil have shown antioxidant activity in several model systems (*15*−*19*). Rapeseed and pine bark phenolics have also shown anti-inflammatory properties (*15*, *20*). The earlier studies thus indicate that different plant materials including pine bark and rapeseed would be potential sources of natural antioxidants with positive health related effects. In the development of functional foods, the bioactivities of plant phenolics such as antioxidant activity may prove to be beneficial. The aim of this study was to investigate the antioxidant activity of certain plant materials toward protein and lipid oxidation in cooked pork meat patties.

MATERIALS AND METHODS

The rapeseed (*Brassica rapa* L.) meal (fat 9%, not removed) used was the residue of the rapeseed deoiling process, whereby the oil expelled from the seeds at elevated temperature and the crude postexpelled rapeseed oil were obtained by Mildola Ltd., Tuusula, Finland. Scotch pine (*Pinus sylvestris* L.) bark drink (Ravintorengas Ltd., Siikainen, Finland) was obtained by extraction with water so that it contained 30% of pine bark and phloem. Meat and fat from pork were obtained from a local butcher shop.

Sinapic acid, ferulic acid, and taxifolin were from Extrasynthèse (Genay, France). Vinylsyringol, the main phenolic compound existing in postexpelled crude rapeseed oil, was synthesized as described in Rein et al. (*21*). Hexanal was from Aldrich (Germany), and sodium pyrophosphate was from Merck (Darmstadt, Germany). All solvents were of HPLC grade and purchased from Rathburn Chemicals Ltd. (Walkerburn, Scotland). A Milli-Q water purification system was used (Millipore, Bedford, MA). Ultraflo L, the enzyme preparation with β-glucanase as main activity and ferulic acid esterase as side activity, was provided by Novozymes (Bagsvaerd, Denmark).

The sodium phosphate buffer with 6 M guanidine hydrochloride (Aldrich) was made of disodium hydrogen phosphate ($NaHPO_4 \cdot 12\ H_2O$) (J. T. Baker, Deventer, The Netherlands) and sodium dihydrogen phosphate ($NaH_2PO_4 \cdot H_2O$) (Merck) adjusted to pH 6.5 (with NaOH). Trichloroacetic acid and potassium chloride were obtained from Merck. 2,4-Dinitrophenylhydrazine was purchased from Fluka. Chemicals used were of analytical purity.

Extraction of Rapeseed Phenolics. The phenolics of rapeseed meal were extracted with aqueous ethanol (70%), enzymatically with Ultraflo L enzyme preparation, and those of crude rapeseed oil with aqueous methanol (80%).

Extraction with Aqueous Ethanol. Extraction of rapeseed phenolics was performed according to the method outlined by Cai and Arntfield with some modifications (*22*). Rapeseed meal (0.8 g) and 20 mL of 70% aqueous ethanol or hot water were put in a centrifuge tube that was then shaken in a water bath (75 °C) for 60 min. The clear phenolic extract was collected after centrifugation (3500 rpm, 15 min).

Enzymatic Treatment. The enzymatic treatment was carried out according to the method of Vuorela et al. (*16*) with some modifications. Rapeseed meal (0.8 g) and 20 mL of 0.02 M ammonium dihydrogen phosphate buffer solution (pH 5.5) containing 0.1% Ultraflo L enzyme preparation (calculated toward total phenolic content) were put in a shaking water bath (37 °C) for 2 h. The enzymatic reaction was stopped by boiling for 10 min. After centrifugation (3500 rpm, 20 min), the phenolic extract was collected and stored at −20 °C.

Extraction of Phenolics from Crude Postexpelled Rapeseed Oil. The extraction was performed according to the method of Koski et al. (*19*). Oil (3 g) was dissolved in heptane. The phenolics were extracted with 3 × 20 mL of aqueous methanol (80%) in a separation funnel. The methanolic fractions were combined and washed with heptane (20 mL), filtered, and evaporated to dryness. The residue was dissolved in methanol (5 mL) and stored at −20 °C.

Total Phenolic Content. An aliquot of the phenolic extract (0.2 mL) was evaporated to dryness. After that, 0.2 mL of methanol/water (1:2) for rapeseed and water for pine bark, 1 mL of Folin−Ciocalteau reagent (1:10), and 0.8 mL of 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) (*16*). Sinapic acid for rapeseed and gallic acid for pine bark were used as standard compounds. The total phenolic contents of rapeseed and pine bark extracts are given in **Table 1**.

Table 1. Phenolic Composition of Rapeseed and Pine Bark Samples

sample[a]	phenolic composition (μg/g ± SD)	total phenolics (μg/g ± SD)
rapeseed meal ex I	sinapine,[b] 2861 ± 7 sinapic acid,[c] 275 ± 2	4751 ± 114
rapeseed meal ex II	sinapic acid, 2381 ± 76 sinapine, 275 ± 2	5885 ± 109
rapeseed oil ex	vinylsyringol,[d] 463 ± 5 sinapic acid, 22 ± 1 sinapine, 3 ± 0	785 ± 28
pine bark ex	phenolic acid derivatives,[e] 70 ± 4 flavonoids,[f] 336 ± 8 lignan glycosides,[e] 83 ± 2	762 ± 01

[a] Aqueous ethanolic (70%) extract of rapeseed meal phenolics (rapeseed meal ex I), extract obtained by enzymatic treatment with Ultraflo L (rapeseed meal ex II), aqueous methanolic (80%) extract of crude rapeseed oil (rapeseed oil ex), and commercial pine bark extract (pine bark ex). [b−d] Amount based on sinapic acid as a standard compound. [e] Amount based on *p*-hydroxybenzoic acid. [f] Amount based on catechin and taxifolin.

HPLC Analysis. The HPLC analysis of phenolics was performed according to the method outlined by Koski et al. (*19*) for rapeseed and that outlined by Karonen et al. (*20*) for pine bark drink. The phenolic composition of rapeseed and pine bark extracts is shown in **Table 1**.

Preparation of Pork Meat Patties. The meat model system was performed according to the method of Kivikari et al. (*23*). Red meat from the longissimus dorsi muscle of pork and fat from pork back (2:1) were homogenized in a Tecator 1094 cutter for 3−4 min. After that, the mass was divided in portions, and to each portion were added water (10%), salt (1.4%), and the tested antioxidants by homogenizing and blending by hand. 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 overwrap, and kept at 5 °C under light. Oxidation was followed by measuring the lipid and protein oxidation at days 1, 6, and 9. The oxidation tests were performed in triplicate.

Lipid Oxidation. The suitable level of plant material addition was first screened by following lipid oxidation only. For further investigations plant materials were added at a level preventing lipid oxidation by >80%. The oxidation was followed by measuring the formation of hexanal by headspace gas chromatography. A sample (2.0 g) was put in a headspace vial, and the formation of hexanal 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 an automatic sampler (Perkin-Elmer HS 40XL). Vials were thermostated for 20 min at 80 °C. The oven temperature was held constant at 60 °C. The inhibition of rapeseed or pine bark against the formation of hexanal was calculated as

$$(A_0 - A_1)/A_0 \times 100$$

where A_0 is the area of hexanal in the control sample and A_1 is the area of hexanal in the tested sample. The inhibitions were expressed as percentages.

Protein Oxidation. Protein oxidation was measured according to the method outlined by Oliver et al. (*24*). Two different measurements were made for protein oxidation: (a) carbonyl quantification and (b) protein quantification (**Figure 1**). Meat samples of ∼1 g (amount of protein = 0.7−1 mg of a sample) were homogenized with 10 mL of 0.15 M KCl with an UltraTurrax homogenizor for 60 s. One hundred microliters of homogenate was transferred into a 2 mL Eppendorf vial, where 1 mL of 10% trichloroacetic acid was added. The sample was

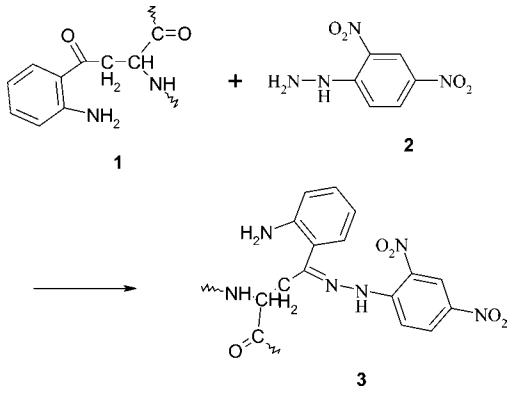

Figure 1. Formation of dinitrophenylhydrazone (**3**) from dinitrophenylhydrazine (DNPH) (**2**) and the oxidized form of tryptophan, *N*-formylkynurenine (**1**).

centrifuged for 5 min at 5000 rpm, and the supernatant was removed. For sample a 1 mL of 2 M HCl with 0.2% 2,4-dinitrophenyl hydrazine (DNPH) was added, and for sample b 1 mL of 2 M HCl was added. After an incubation of 1 h (shaken every 20 min), 1 mL of 10% trichloroacetic acid was added. The sample was vortexed and centrifuged for 5 min at 5000 rpm. Supernatant was removed carefully without damaging the pellet with the Pasteur pipet. The pellet was washed with 1 mL of ethanol/ethyl acetate (1:1), shaken, and centrifuged for 5 min at 10000 rpm; this procedure was repeated two to three times. After this, the pellet was completely dried with nitrogen. The pellet was dissolved in 1.5 mL of 20 mM sodium phosphate buffer with 6 M guanidine hydrochloride, final pH 6.5, shaken, and centrifuged for 2 min at 5000 rpm. Carbonyls (sample a) and protein concentration (sample b) were measured spectrophotometrically at 370 and 280 nm, respectively.

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. Concentration (nanomolar) of carbonyls was calculated as [Abs_{370nm}/21.0 mM^{-1} cm^{-1}) × 1000], where 21.0 mM^{-1} cm^{-1} is the molar extinction coefficient of carbonyls. The inhibition of rapeseed and pine bark against formation of protein carbonyls was calculated as

$$(C_0 - C_1)/C_0 \times 100$$

where C_0 is the concentration (nanomolar) of protein carbonyls per mg of protein in a control sample and C_1 is the concentration of protein carbonyls per mg of protein in a tested sample. The inhibitions were expressed as percentages.

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

RESULTS

Lipid Oxidation. The levels of plant materials exhibiting an inhibitory effect of >80% toward lipid oxidation after 9 days of oxidation were selected for further analyses (**Figure 2**). Rapeseed meal was tested at four different addition levels (141−424 mg/100 g of meat, which contained 0.67−2.0 mg of phenolic compounds). Rapeseed phenolic extracts were tested at levels of 15 and 29 mL (in rapeseed ethanolic extract, the amounts of phenolic compounds were 2.9 and 5.6 mg, and in rapeseed enzyme-assisted extract, the amounts were 3.5 and 6.9 mg), phenolic extract of crude rapeseed oil at 2 and 5 mL (the amounts of phenolic compounds were 0.9 and 2.2 mL), and pine bark extract at 7 and 11 mL/100 g of meat (the amounts of phenolic compounds were 5.3 and 8.4 mg), respectively. Sinapic acid was tested at levels of 7−24 mg/100 g of meat, and 24 mg was chosen as the general standard concentration to be tested.

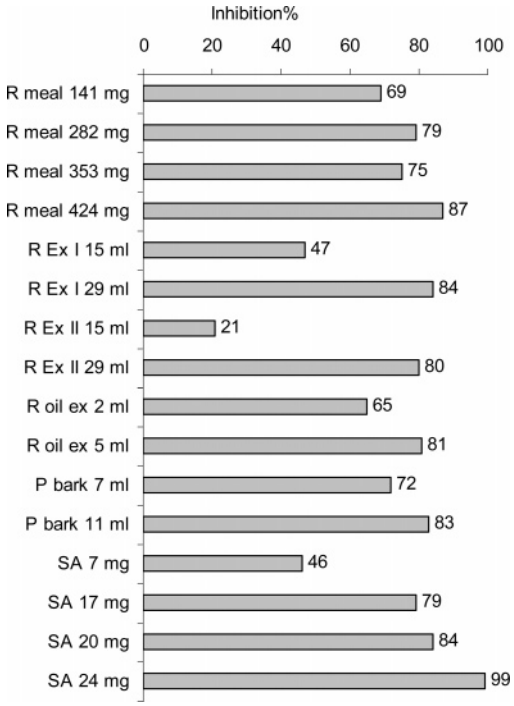

Figure 2. Plant phenolics as antioxidants toward lipid oxidation (inhibition % of hexanal formation): rapeseed meal after pressing the oil at elevated temperature and pressure (R meal); aqueous ethanolic (70%) extract of rapeseed meal (R Ex I); enzyme-assisted extract with Ultraflo L of rapeseed meal (R Ex II); phenolic extract of crude postexpelled rapeseed oil (R oil ex); commercial pine bark extract (P bark); sinapic acid (SA).

Table 2. Effect of Rapeseed and Pine Bark on the Formation of Hexanal and Carbonyl Compounds (Inhibition Percent ± SD)

material[a]	addition level/ 100 g of meat	amount of phenolics in sample (mg)	hexanal	protein carbonyls
rapeseed meal	282.4 mg	1.3	79.5 ± 12.0b[b]	41.7 ± 17.7b[b]
rapeseed meal ex I	29.4 mL	5.6	93.6 ± 6.2ab	61.1 ± 6.4ab
rapeseed meal ex II	29.4 mL	6.9	90.6 ± 7.8ab	71.5 ± 9.1a
rapeseed oil ex	4.7 mL	2.2	84.1 ± 2.9ab	58.0 ± 8.2ab
pine bark ex	10.6 mL	8.1	98.2 ± 0.5a	63.5 ± 7.6ab
standards				
sinapic acid	23.5 mg		89.0 ± 10.3	59.7 ± 19.3
vinylsyringol	23.5 mg		100 ± 0	77.5 ± 6.0
taxifolin	23.5 mg		85.2 ± 1.2	66.6 ± 5.6

[a] Rapeseed meal after pressing the oil at elevated temperature and pressure (rapeseed meal), aqueous ethanolic (70%) extract of rapeseed meal (rapeseed meal ex I), enzyme-assisted extract with Ultraflo L of rapeseed meal (rapeseed meal ex II), phenolic extract of crude postexpelled rapeseed oil (rapeseed oil ex), and commercial pine bark extract (pine bark ex). [b] Values in the same column with different letters are significantly different ($p < 0.05$).

In dry rapeseed meal, 1.3 mg of phenols was the sufficient amount for 80% of inhibition against hexanal formation. Of tested plant extracts, rapeseed ethanolic extract with 5.6 mg of phenolic compounds, rapeseed enzyme-assisted extract with 6.9 mg, phenolic extract of crude rapeseed oil with 2.2 mg, and pine bark extract with 8.1 mg were the sufficient amounts of phenolic compounds for an effective antioxidant activity against lipid oxidation in meat.

Lipid oxidation in the presence of standard compounds is shown in **Figure 3**. Vinylsyringol was the most powerful antioxidant, followed by sinapic acid and taxifolin. The materials selected for further investigation inhibited the hexanal formation between 79.5 and 98.2% (**Table 2**). Pine bark extract was the most effective antioxidant against lipid oxidation in meat

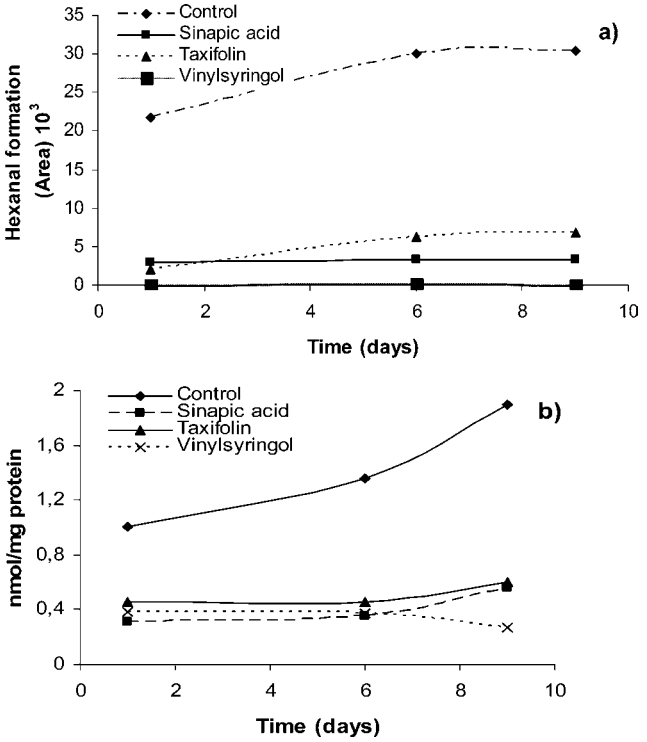

Figure 3. Formation of (**a**) hexanal (area 10^3) and (**b**) protein carbonyl compounds (nmol/mg of protein) in cooked meat during storage at 5 °C.

followed by rapeseed meal extracts. However, rapeseed oil extract as well as rapeseed meal could inhibit at least 80% of hexanal formation.

Protein Oxidation. Protein oxidation was followed for 9 days. The oxidation in the presence of standard compounds is shown in **Figure 3**. In a control sample the concentration of protein carbonyls is at a higher level compared to the standards from the start until the end of oxidation, which is due to cooking process as a catalyst of the oxidation. The inhibitions against protein carbonyl formation were between 41.7 and 71.5%. Enzyme-assisted extract of rapeseed meal (rapeseed meal ex II) was the best antioxidant against protein oxidation followed by pine bark extract (pine bark ex) and aqueous ethanolic extract of rapeseed meal (rapeseed meal ex I). Vinylsyringol was the best standard compound against protein oxidation (**Table 2**).

DISCUSSION

In this study, the effect of selected plant materials rich in phenolics on the antioxidant activity of meat proteins and lipids was tested. Rapeseed meal as well as its phenolic isolates obtained by aqueous ethanolic extraction, and enzyme-assisted extraction, as well as the aqueous methanolic extraction from crude postexpelled rapeseed oil, were investigated for possible functional food development. A natural product already on the market, pine bark drink, a water extraction of pine bark and phloem, was chosen as the source of pine bark phenolics. Rapeseed and pine bark phenolics have been shown to exhibit excellent antioxidant as well as anti-inflammatory activity (*15*, *16*, *20*). In earlier studies, Kivikari et al. (*23*) and Rey et al. (*13*) found that rapeseed meal, camelina seed meal, raspberry, cloudberry, pine bark extract, birch bark extract, and willow herb were very effective antioxidants against meat lipid oxidation. Compared to some phenolic compounds (ellagic acid, myricetin, quercetin, or the commonly used food additive mixture ascorbic acid−phosphate), these plant materials were as good or even better antioxidants in meat. Young et al. (*25*) tested the impact of two diets on the oxidative status of chickens: a conventional diet rich in antioxidants compared to a semisynthetic diet low in antioxidants. They found that the conventional diet including wheat, soybean, and rapeseed press cakes as well as edible plant oils protected the chicken proteins from oxidation better than the semisynthetic diet low in antioxidants containing mostly cellulose and isolated soybean proteins.

In this study, the potential plant materials were first screened according to their capability to prevent lipid oxidation by at least 80% as measured by the inhibition of hexanal formation. Of the tested plant materials selected, pine bark drink (pine bark ex), a water extract of pine bark and phloem, was the best hexanal inhibitor followed by rapeseed meal extracts I (ethanolic extract) and II (enzyme-assisted extract). However, according to statistical analysis, there was no statistically significant difference between pine bark and rapeseed extracts. The selected plant materials were further investigated to test their antioxidant activity against protein oxidation. Both rapeseed and pine materials inhibited the formation of protein carbonyls. Rapeseed meal ex II was the best antioxidant against the formation of protein carbonyls followed by rapeseed meal ex I, rapeseed oil ex, and pine bark ex. However, there was no statistically significant difference between rapeseed and pine bark extracts. The effect of dry rapeseed meal was lower compared to that of the phenolic extracts, but statistically the effect did not differ from rapeseed ex I, rapeseed oil ex, and pine bark drink. In dry material, the phenolic compounds may be bound to cellular structures and there may be some nonphenolic compounds that may act as pro-oxidants. In addition, the amount of phenolic compounds present in the amount of rapeseed meal added (1.3 mg) was considerably lower than in the extracts (5.6 and 6.9 mg).

The main phenolic compound in rapeseed ex II was sinapic acid, whereas in rapeseed ex I, sinapine was the principal phenolic compound. Sinapic acid and its derivative, choline ester of sinapic acid, that is, sinapine, were responsible for the antioxidant activity of rapeseed meal extracts. This finding is in accordance with our previous studies of rapeseed phenolics as lipid antioxidants (*15*, *16*). Sinapic acid exhibited good antioxidant activity against protein oxidation, but the effect of the rapeseed meal extracts was higher, presumably due to synergy between the different phenolics present in the extracts. In crude rapeseed oil, vinylsyringol was the predominant phenolic compound. Compared to the other standards tested, vinylsyringol showed the best antioxidant properties against the formation of protein carbonyls. This corroborates that the effect of rapeseed oil extract containing 463 μg/g of vinylsyringol was more pronounced even though the amount of phenolics was much lower than in the other rapeseed extracts. This suggests that vinylsyringol is responsible for the antioxidant activity of rapeseed oil extract. In pine bark, there are several phenolic compounds present, which may be responsible for the antioxidant activity. Taxifolin was as effective an antioxidant as sinapic acid against protein oxidation, but it is not the only compound responsible for the antioxidant activity of pine bark as several other compounds, such as lignans and catechins, are present. According to Rababah et al. (*11*), tea catechins are known to be effective inhibitors of protein oxidation in irradiated raw chicken meat. Tea catechins have also shown excellent antioxidant properties toward lipid oxidation in several studies (*6*, *8*−*11*). In our earlier study (*15*), we found pine bark and its phenolic isolates to exhibit excellent antioxidant properties against oxidation of liposomes. The antioxidant effect of the lignans matairesinol and pinoresinol, which are among the major

bioactive phenolic compounds in pine bark, was also excellent. This indicates that lignans may in part explain the antioxidant activity of pine bark in the present study.

Rapeseed and pine bark phenolics were effective antioxidants toward protein oxidation, although the level of inhibition was lower compared to that of lipid oxidation. One possible mechanism is the inhibition of the formation of hexanal, a secondary oxidation product of lipids, in turn capable of inducing protein oxidation. However, whereas vinylsyringol completely inhibited lipid oxidation in meat patties, there were some protein carbonyls present. Thus, it can be assumed that the formation of protein carbonyls in meat with added vinylsyringol may be in part originated from direct oxidation of proteins to form protein carbonyls. In meat, there may also be some hydroperoxides or other oxidative compounds present capable of inducing protein oxidation. The amount of iron released from myoglobin during the cooking process can also react with proteins and act as an oxidizing compound. In turn, phenolic compounds can also protect proteins by chelating metals. In our previous studies (*15*, *16*), we found that rapeseed and pine bark phenolics were excellent antioxidants against liposome oxidation, whereas their radical scavenging activity was only moderate. Because a metal, copper, is used to catalyze liposome oxidation, it can be assumed that the main antioxidant protection of rapeseed and pine bark phenolics includes metal chelation. By chelating metals, for example, the iron(II) released from myoglobin, rapeseed and pine bark phenolics can directly protect meat proteins from undergoing oxidative changes.

Lipid oxidation is a well-known and important problem, which results in a rancid taste of meat, but it does not explain the textural changes such as loss of juiciness and increased toughness of meat, which is due to protein oxidation (*26*). Rapeseed and pine bark phenolics are effective antioxidants toward both oxidation of meat lipids and proteins. The antioxidant function of rapeseed and pine bark phenolics during storage of cooked pork meat patties include inhibition of the formation of lipid oxidation products by scavenging free radicals and also by metal chelating. This finding is in accordance with our previous studies (*15*, *16*). By inhibiting lipid oxidation in meat, rapeseed and pine bark phenolics subsequently also inhibit the oxidation of meat proteins. In conclusion, rapeseed and pine bark may be alternative sources of phenolic compounds as natural antioxidants in inhibiting the protein and lipid oxidation in cooked meat products.

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