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Plant Phenolics Affect Oxidation of Tryptophan

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The effect of berry phenolics such as anthocyanins, ellagitannins, and proanthocyanidins from raspberry (*Rubus idaeus*), black currant (*Ribes nigrum*), and cranberry (*Vaccinium oxycoccus*) and byproducts of deoiling processes rich in phenolics such as rapeseed (*Brassica rapa* L.), camelina (*Camelina sativa*), and soy (*Glycine max* L.) as well as scots pine bark (*Pinus sylvestris*) was investigated in an H_2O_2-oxidized tryptophan (Trp) solution. The oxidation of Trp was analyzed with high-performance liquid chromatography using both fluorescence and diode array detection of Trp and its oxidation products. Mechanisms of antioxidative action of the phenolic compounds toward the oxidation of Trp were different as the pattern of Trp oxidation products varied with different phenolic compounds. The antioxidant protection toward oxidation of Trp was best provided with pine bark phenolics, black currant anthocyanins, and camelina meal phenolics as well as cranberry proanthocyanidins.

KEYWORDS: Tryptophan; protein oxidation; HPLC; antioxidants; phenolic compounds; berries

INTRODUCTION

The oxidation of proteins and peptides occurs during processing and storage of food and under physiological conditions leading to altered physicochemical and functional properties and may even result in toxic products (*1*). The oxidation of proteins contributes to changes in food structure, decreases in protein solubility, color changes (browning reactions), and changes in nutritive value (*2, 3*). The nature and extent of reactions involved in food processing depend on the composition of food and food processing conditions, such as temperature, pH, the presence of oxygen, the application of chemicals, fermentation, and irradiation (*4, 5*). Processing-induced changes leading to denaturation of proteins may improve the digestibility, especially of plant proteins containing antinutritional compounds, or impair the digestibility and biological availability by destruction of essential amino acids, conversion of amino acids into nonmetabolizable derivatives, and intra- or intermolecular cross-linking. In addition, the Maillard reaction initiated by reaction between amines and carbonyl compounds at elevated temperatures also has a great impact on the organoleptic and nutritional properties of proteins (*4*). The essential amino acid tryptophan (Trp) is exceptional in its diversity of biological functions such as a precursor for a series of metabolic reactions. Therefore, its stability in processed foods is a major concern as the oxidation products of Trp are biologically active (*5*). In addition to Trp-derived oxidation products, kynurenines (Kyn), Trp-derived nitroso compounds, and the carbolines formed in the presence of carbonyl compounds and/or at high temperatures, for example, during broiling/grilling of meat products, exhibit mutagenic and carcinogenic activities (*5*). Enhanced Trp degradation is observed during pregnancy and in a variety of diseases and disorders, for example, infectious diseases, autoimmune diseases, and neuropsychiatric and neurological disorders such as Alzheimer's and Parkinson's diseases as well as dementia (*6*).

In relation to potential health effects, phenolic bioavailability and phenolic-mediated cell effects may involve the interactions of phenolic compounds with specific biological targets, mainly proteins, and possible participation of phenolic compounds in the regulation of gene expression for specific proteins (*7*). The interaction of tannins (oligomeric procyanidins) with salivary proteins developing sensation of astringency is currently the sole binding process with clear in vivo biological significance (*8*).

The mechanisms of inhibiting oxidation by phenolic compounds, as food components or potential drugs, are related to their reducing ability (antioxidant properties by electron or H-atom donation), their ability to interact with proteins, and the formation of complexes between protein molecules. These flavonoid−protein or protein−protein complexations may provide protection to the protein against oxidative degradation. In addition, flavonoid−protein interactions can modify the redox properties of flavonoids that underline their antioxidant, and eventually their pro-oxidant, activity (*7*).

The molecular interactions responsible for phenolic−protein complexation can be divided into van der Waals (dispersion) interactions and electrostatic interactions. Flavonoid oxidation by autoxidation, radical scavenging, or enzymatic oxidation leads to the formation of flavonoid oxidation products. Being both electrophilic and oxidizing, the flavonoid oxidation products

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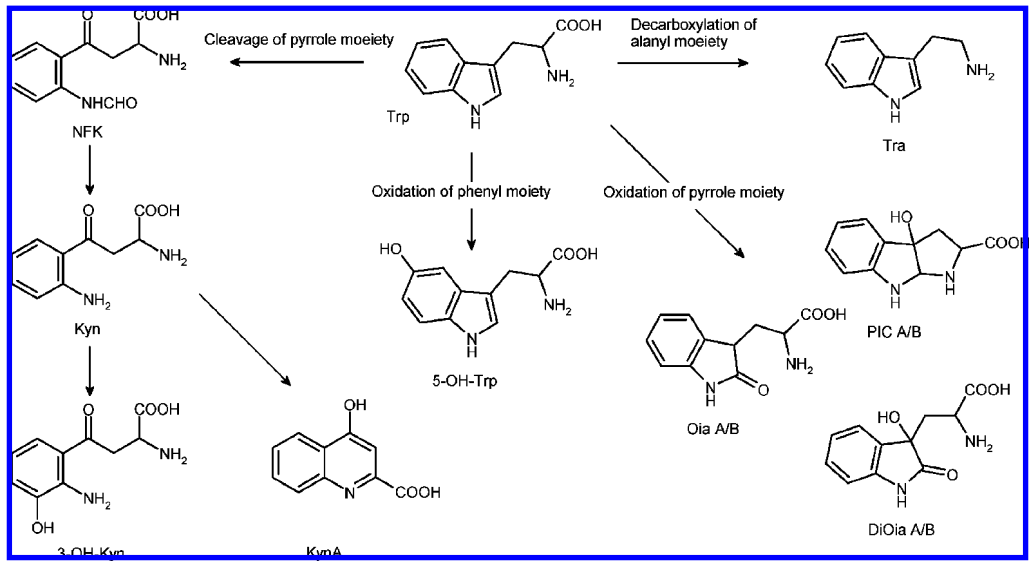

Figure 1. Formation of Trp-derived oxidation products. See panel A for abbreviations.

(aryloxyl radicals, quinones, and quinonoid compounds) may react with nucleophilic or oxidizable amino acid residues, thereby irreversibly modifying the protein by oxidation or covalent bonds (*7*).

The oxidative attack on Trp residues occurs first on the pyrrole ring and second on the phenyl moiety (*9*). Therefore, Trp is oxidized (**Figure 1**) with the formation of primary oxidation products such as diastereomers A and B of 3a-hydroxypyrroloindole-2-carboxylic acid (PIC), β-oxindolylalanine (Oia) (*10*), and dioxindolylalanine (DiOia) (*11*), respectively, as the pyrrole ring remains uncleaved. The phenyl moiety of Trp is susceptible to oxidation forming 5-hydroxy-tryptophan (5-OH-Trp), and the alanyl moiety undergoes changes forming, for example, tryptamine (Tra) and indole derivatives. Cleavage of the pyrrole ring of Trp leads to the formation of *N*-formylkynurenine (NFK), Kyn, and 3-hydroxykynurenine (3-OH-Kyn) (*9*). In mammalian cells, Trp is degraded primarily by a complex enzymatic cascade known as the Kyn pathway (*12*).

To our knowledge, the role of plant phenolics on the oxidation of proteins and amino acids is not well-established. In addition, the mechanisms of phenolic−protein interactions are still incompletely understood in part due to lack of specific methods for measuring oxidation products of proteins. To control and prevent oxidation, the oxidation mechanisms and protein−phenolic interactions must be elucidated. The objective of this study was to investigate the effects of plant phenolics on the oxidation of Trp and the pattern of Trp-derived oxidation products. All plant materials selected were found antioxidatively active in our previous studies (*13–15*), and these materials included byproducts of deoiling processes rich in phenolics, such as rapeseed, camelina and soy meal, soy flour, pine bark, and berry phenolics from raspberry, black currant, and cranberry. The main phenolic compounds found in the selected plant materials are comprised of anthocyanins, hydroxycinnamic acids, hydroxybenzoic acids, catechins, procyanidins, flavonols, and isoflavones (**Figure 2**).

MATERIALS AND METHODS

Materials. The rapeseed (*Brassica rapa* L.) meal used was the residue of a rapeseed deoiling process in which the oil was expelled from the seeds at an elevated temperature by Mildola Ltd. (Finland). Camelina (*Camelina sativa*) meal was obtained from Raisio Ltd. (Finland). Soy flour (from soybean *Glycine max* L.) (Soyolk) was from Cereform Ltd. (Northampton, England), and soy meal (Risetti) was from Risetti Ltd. (Finland). Protein, fatty acid, and tocopherol compositions as well as isoflavone and lignan contents of the oilseed materials have been reported in a previous study by Salminen et al. (*14*). Scots pine (*Pinus sylvestris* L.) bark drink was obtained by extraction with water so that it contained 30% pine bark and phloem (Ravintorengas Ltd., Siikainen, Finland). All berries (raspberry, black currant, and cranberry) were purchased from a market place. Samples were packed immediately into a vacuum and stored in a freezer at −20 °C until use.

Caffeic acid, chlorogenic acid, cyanidin-3-glucoside, delphinidin-3-glucoside, ellagic acid, ferulic acid, gallic acid, daidzein, genistein, procyanidin B1, rutin, sinapic acid, and taxifolin were from Extrasynthèse (Genay, France). Catechin, quercetin, L-Trp, 3-hydroxy-tryptophan, 5-hydroxy-L-tryptophan, L-Kyn, Tra, and kynurenic acid (KynA) were obtained from Sigma. Vinylsyringol was synthesized as described by Rein et al. (*16*). Oia was synthesized as described by Simat et al. (*17*). Catalase (EC 1.11.1.6) from bovine liver (1824 units/mg) was from Sigma. Sodium tetraborate decahydrate, potassium iodide, and acetic acid were from Riedel-de Haën (Germany). Trifluoroacetic acid (TFA) was from Sigma. All solvents were high-performance liquid chromatography (HPLC) grade and purchased from Rathburn Chemicals Ltd. (Walkerburn, Scotland). A MilliQ water purification system was used (Millipore, Bedford, MA). Chemicals used were of analytical purity.

Isolation of Plant Phenolics. The extractions of camelina meal, soy meal, and soy flour and the extraction of rapeseed meal were performed according to methods of Salminen et al. (*14*) and Vuorela et al. (*15*) with some modifications, respectively. Plant material (0.8 g) and 20 mL of 80% methanol (70% ethanol for rapeseed) was put in a centrifuge tube that was shaken in a water bath (75 °C) for 60 min. The clear phenolic extract was collected after centrifugation (6000 rpm, 15 min).

Berry samples were freeze-dried prior to analysis and stored at −20 °C until use. Extraction and isolation of berry anthocyanins and raspberry ellagitannins were carried out as described by Kähkönen et al. (*18*). The berry anthocyanin fractions were further purified by preparative HPLC as described by Kähkönen et al. (*18*). A method by Määttä-Riihinen et al. (*19*) was followed to isolate cranberry proanthocyanidins. Berry isolates were freeze-dried and stored at −20 °C.

Total Phenolics and Phenolic Profiles. The amount of total polyphenols was measured colorimetrically according to the Folin−Ciocalteau procedure (*20*). Phenolic extract (0.2 mL) was evaporated to dryness. After that, 0.2 mL of methanol/water (1:2), 1 mL of Folin−Ciocalteau reagent (1:10), and 0.8 mL of sodium dicarbonate

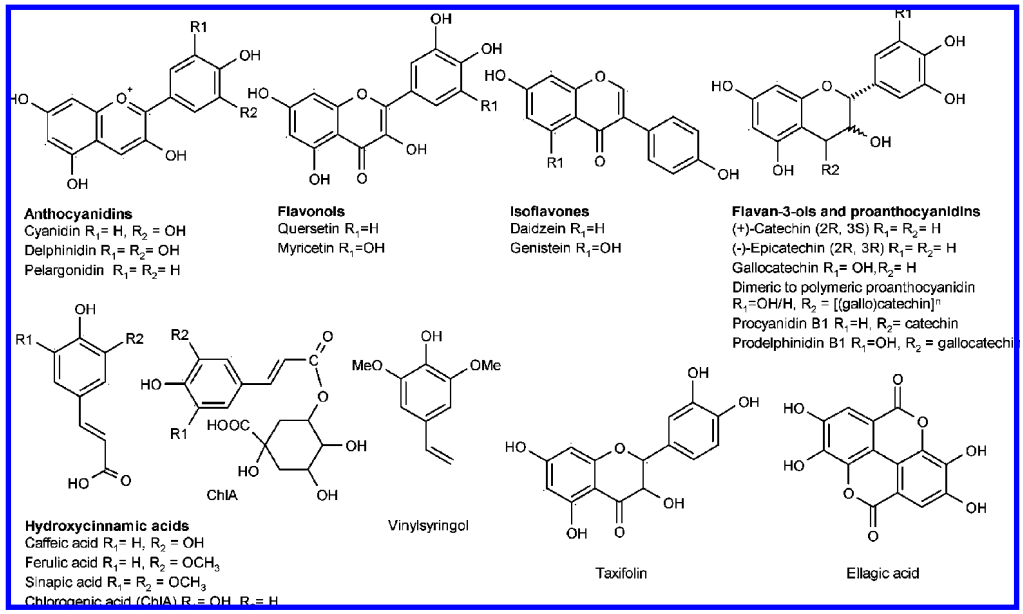

Figure 2. Structures of phenolic compounds.

Table 1. Phenolic Composition of Berry Isolates (mg/g, Mean ± SD)[a]

concentration (mg/g)	raspberry anthocyanins	raspberry ellagitannins	black currant anthocyanins	cranberry proanthocyanidins
anthocyanins[b]	534 ± 25	18 ± 0	314 ± 44	ND
ellagitannins[c]	ND	369 ± 44	ND	ND
proanthocyanidins[d]	ND	ND	ND	554
ellagic acid[c]	ND	16 ± 2	ND	ND
flavanols[e]	ND	ND	12 ± 1	ND
hydroxybenzoic acids[f]	ND	ND	ND	ND
hydroxycinnamic acids and derivatives[g]	ND	ND	ND	ND
flavonols[h]	ND	8 ± 1	ND	1

[a] ND, not detected/concentration under detection limit. [b] Cyanidin 3-glucoside as standard. [c] Ellagic acid as standard. [d] Procyanidin B1 as standard. [e] Catechin as as standard. [f] Gallic acid as standard. [g] Chlorogenic acid as standard. [h] Rutin as standard.

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). Gallic acid was used as a standard compound. The results are expressed as gallic acid equivalents (GAE), μg/g of plant material. The HPLC analysis of phenolics was performed according to the method outlined by Koski et al. (*21*) for phenolic acids and their derivatives, and by Kähkönen et al. (*22*) for other phenolic compounds. Catechin, chlorogenic acid, cyanidin-3-glucoside, ellagic acid, gallic acid, procyanidin B1, rutin, sinapic acid, and vinylsyringol were used as standard compounds.

Oxidation of Trp. A 2 mM Trp model solution using 0.1 M borate (pH 6.3) in the presence of selected extracts of phenolic compounds (at concentrations of 10, 50, and 100 μM) was oxidized with hydrogen peroxide (H_2O_2) added hourly (final concentration of 1.05 M) for 6 h at 37 °C in dark. The addition of H_2O_2 (1.05 M) resulted in a final pH of 4.6. Oxidation was performed by modifying a method outlined by Simat and Steinhart (*9*). H_2O_2 was used as an oxidant since it is an oxidant in many biological molecules, and it is a member of the reactive oxygen species (*23*). Borate/boric acid buffers have been reported to catalyze hydrogen peroxide oxidation reactions leading to the formation of peroxoborates, which may accelerate some reactions. The pH of borate buffer solution decreased as H_2O_2 was added due to the rapid equilibria in the formation of monoperoxoborate (eq 1) and diperoxiborate (eq 2). However, at lower pH, peroxoboric acid was formed (eq 3) (*24*).

$$B(OH)_3 + H_2O_2 \leftrightarrow (HO)_3BOOH^- + H^+ \quad (1)$$

$$(HO)_3BOOH^- + H_2O_2 \leftrightarrow (HOO)_2B(OH)_2^- + H_2O \quad (2)$$

$$B(OH)_3 + H_2O_2 \leftrightarrow (HO)_2BOOH + H_2O \quad (3)$$

It has been observed that at pH ~4.6 only one major boron-containing species, $B(OH)_3$, is present in 0.1 M solution with 1.0 M H_2O_2. In addition, at lower pH levels (acid or neutral), boric acid has no effect on the photodegradation of H_2O_2 (*24*). In acidic solutions, peroxoborates have not been detected due to their low concentrations. Therefore, under the experimental conditions used in this study, the amount of peroxoborates formed may be negligible. It has also been shown that borate and boric acid have insignificant rates of reaction with hydroxyl radicals as compared with hydrogen peroxide and thus cannot act as free radical scavengers in the decomposition of hydrogen peroxide (*25*).

The concentrations of added phenolic compounds (presented as molar equivalent concentrations) were based on the total phenolic content in the cases of soy and pine bark drink and on the total hydroxycinnamic acid derivatives in the cases of rapeseed and camelina meal. The amount of raspberry and black currant anthocyanin isolates, raspberry ellagitannin isolate, and cranberry proanthocyanidin isolate was calculated as cyanidin-3-glucoside, ellagic acid, and procyanidin B1 equivalents, respectively. All tested berry isolates and other plant materials were dissolved in methanol, ethanol (rapeseed), or water (pine bark).

Aliquots of plant phenolics were pipetted into 4 mL screw-top vials, and the solvent was evaporated with nitrogen. In control sample, no antioxidant was added. Small magnets were placed into vials. The Trp solution (2 mL) and 40 μL (0.19 M) of H_2O_2 (30%) were then added into the vials resulting in final Trp concentration of 317 μg/mL. Every hour during the incubation, an aliquot of 40 μL of H_2O_2 was added to samples to keep the catalyst abundant. Samples were agitated on a vortex mixer after additions of H_2O_2, and the mixtures in sealed vials were stirred during incubation. After incubation, the reaction was stopped by 30 min of treatment with 100 μL of catalase (about 300 units). The absence of H_2O_2 was subsequently checked by potassium iodide test. After acidification with 164 μL of 2 M acetic acid, the sample was filtered (0.45 μm GPH) prior to HPLC analysis.

Analysis of Trp Oxidation Products with HPLC. Trp and its oxidation products were determined using an analytical HPLC method combined with diode array and fluorescence detection outlined by Simat and Steinhart (*9*) with some modifications. The HPLC system (Waters, Milford, MA) consisted of 717 plus autosampler, 515 and 510 pumps with a pump control module, a column oven with a temperature control module, a PDA 996 diode array detector, and a HP 1046 scanning fluorescence detector (Hewlett-Packard, Palo Alto, CA). The separation

Table 2. Phenolic Composition of Plant Extracts (μg/g, Mean ± SD)[a]

concentration (μg/g)	rapeseed meal	camelina meal	soy meal	soy flour	pine bark drink (μg/mL)
total phenolics[b]	6730 ± 290	3940 ± 110	2770 ± 65	2650 ± 50	3400 ± 250
flavanols[c]	ND	2110 ± 410	760 ± 10	790 ± 70	80 ± 10
hydroxybenzoic acids[b]	ND	ND	ND	ND	3 ± 0
hydroxycinnamic acids and derivatives[d]	8030 ± 140	3020 ± 160	ND	ND	ND
sinapine[e]	1800 ± 180	650 ± 40			
sinapic acid[e]	140 ± 20	30 ± 3			
vinylsyringol[f]	64 ± 7	ND			
flavonols[g]	ND	2150 ± 70	ND	ND	ND

[a] ND, not detected/concentration under detection limit. [b] Gallic acid as standard. [c] Catechin as standard. [d] Chlorogenic acid as standard. [e] Sinapic acid as standard. [f] Vinylsyringol as standard. [g] Rutin as standard.

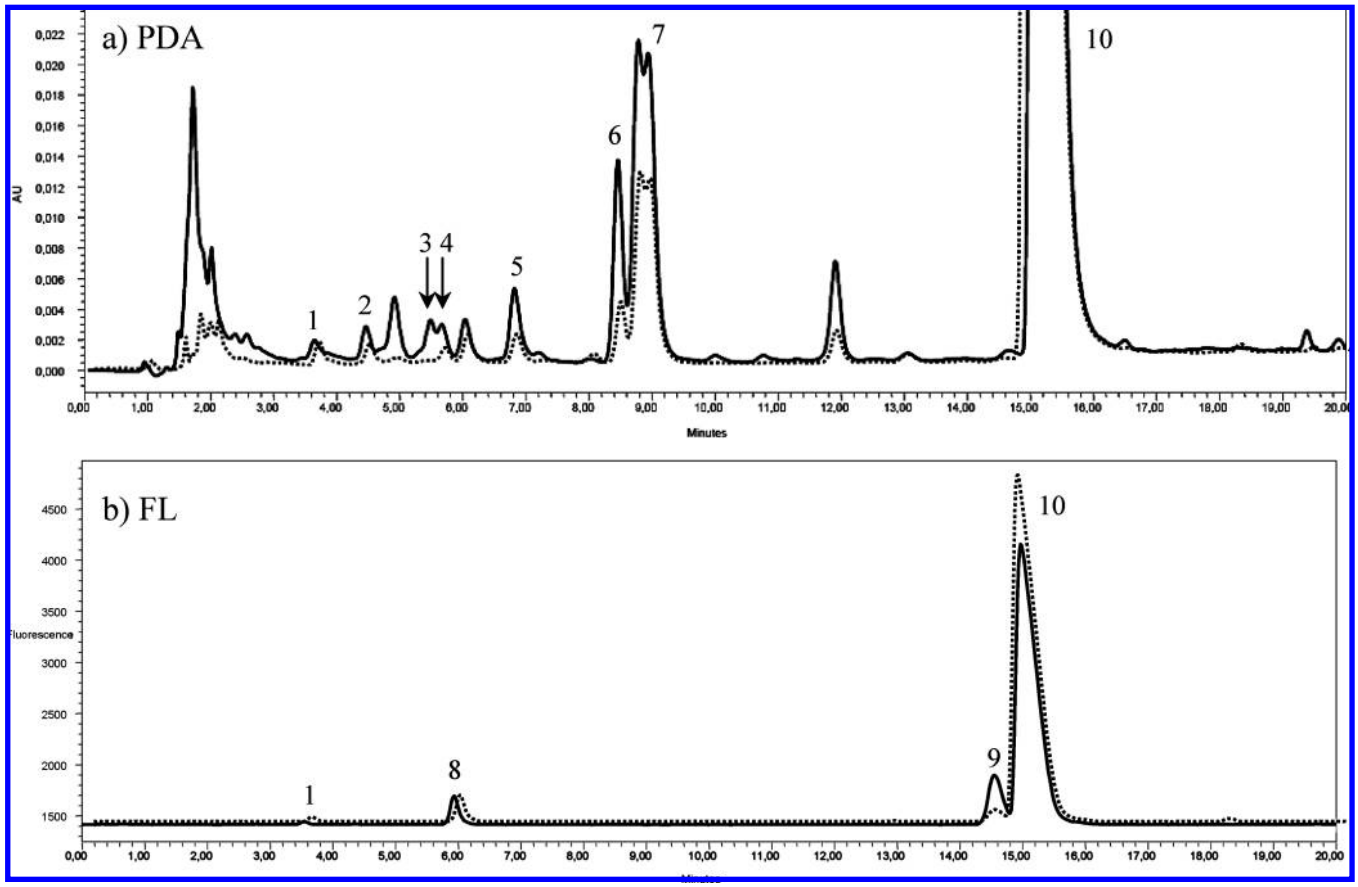

Figure 3. Separation by RP-HPLC of Trp-derived oxidation products of H_2O_2-oxidized Trp (solid line) and with added camelina meal phenolics at 10 μM (dotted line) after 6 h of oxidation. (**a**) PDA 260 nm. (**b**) Fluorescence detection, 0−13 min: Ex, 230 nm; Em, 342 nm; 13−46 min: Ex, 280 nm; Em, 335 nm. The Trp concentration of the control sample (without added phenolics) before oxidation was 317 μg/mL and after oxidation was 158 μg/mL. Peaks: 1, PIC A; 2, DiOia A; 3, DiOia B; 4, PIC B; 5, Kyn; 6, NFK; 7, Oia; 8, 5-OH-Trp; 9, Tra; and 10, Trp. See panel A for abbreviations.

of oxidation products was carried out on a Nova-Pak C18 column (3.9 mm × 150 mm, 4 μm) equipped with a Spherisorb S5 ODS2 precolumn (20 mm × 2 mm, 5 μm) (Waters, Milford, MA). The mobile phase consisted of 0.1% (v/v) TFA in water (solvent A), methanol (solvent B), and acetonitrile (solvent C). The elution conditions were as follows: linear gradient from 95% A and 5% B to 86% A and 14% B, 0−10 min; to 46% A, 14% B and 40% C, 10−40 min; to 26% A, 14% B, and 60% C, 40−42 min; to 95% A and 5% B, 42−46 min. The flow rate was 1.0 mL/min, and the column oven temperature was 35 °C. The injection volume was 20 μL. The Trp oxidation products were confirmed by spectral identification and retention times. The Trp oxidation product standards were dissolved in 0.1 M borate (pH 6.3) to a concentration of approximately 0.5 mg/25 mL except for Trp to 6 mg/25 mL.

A photodiode array (PDA) detector wavelength of 260 nm was used to monitor oxidation products. The fluorescence program was modified as follows: from Ex 230 nm and Em 342 nm, 0−13 min; to Ex 280 and Em 335, 13−46 min. The quantitative determination of Trp and Trp oxidation products by HPLC was made using Trp, Tra, Kyn, Oia, and 5-OH-Trp as external standards. The quantifications for NFK, PIC (A/B), and DiOia (A/B) were calculated as equivalents to 5-OH-Trp as external standard since these standards were not commercially available. The results are given as the mean values of triplicate analyses.

Statistical Analysis. Statistical analysis of multivariance (ANOVA) was performed using Statgraphics Plus (STCC Inc., Rockville, MD). Differences at $p < 0.05$ were considered to be significant.

RESULTS AND DISCUSSION

Phenolic Profiles of Berry and Plant Extracts. The phenolic composition of berry isolates is shown in **Table 1**. Raspberry anthocyanin isolate consisted of purely anthocyanins. The main phenolics in black currant isolate were anthocyanins with a modest amount of flavanols present. Raspberry ellagitannin

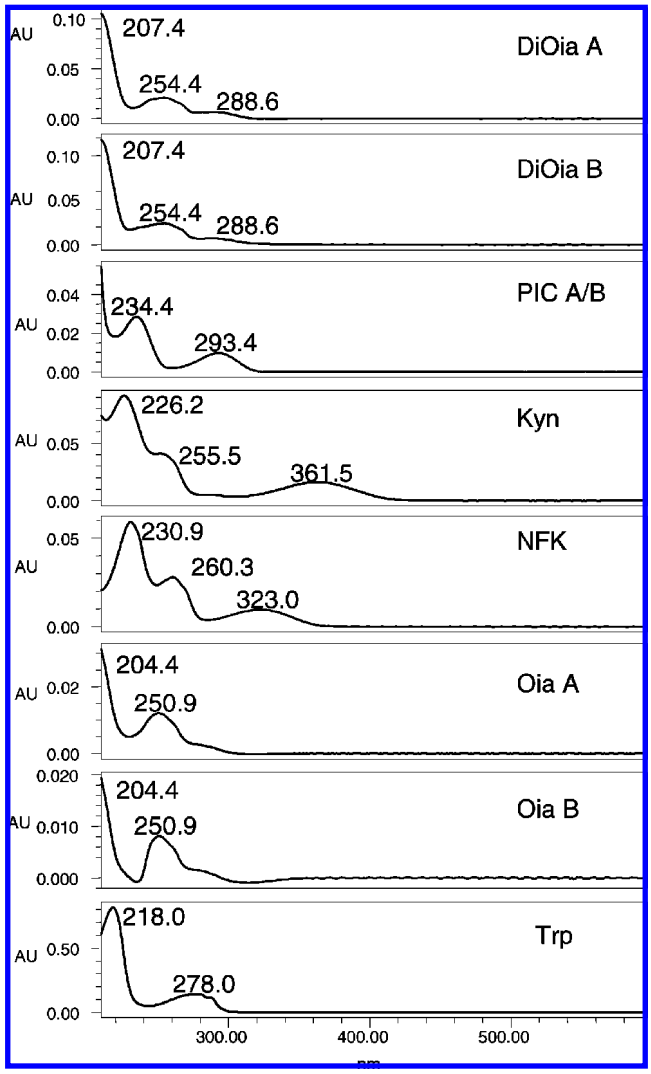

Figure 4. UV-vis spectra of Trp and its oxidation products from PDA detector of the HPLC. See panel A for abbreviations.

isolate consisted mainly of ellagitannins and ellagic acid with minor amounts of anthocyanins and flavonols. Cranberry proanthocyanidin isolate contained mainly proanthocyanidins with minor amounts of anthocyanins and flavonols. The phenolic composition of plant extracts is presented in **Table 2**. Camelina meal extract was predominated by hydroxycinnamic acids, flavanols, and flavonols, whereas rapeseed meal extract was predominated by hydroxycinnamic acids. Soy meal and flour consisted mainly of flavanols. The main phenolics in pine bark drink were flavanols with hydroxybenzoic acids.

Oxidation of Trp. Nine Trp-derived oxidation products detectable with the modified HPLC method were identified by PDA and fluorescence (FL) detection (**Figure 3**). Separation of Trp and its oxidation products was achieved within 16 min by using the selected column. DiOia and PIC each gave two peaks, and Oia gave a double peak, referring to their diastereomers. Some peaks remained unidentified. The identification of Trp and its oxidation products was confirmed by their spectral properties (**Figure 4**). The loss of Trp in the model system was approximately 50% after 6 h of oxidation with H_2O_2. Only ~20% of the determined Trp loss in free Trp could be elucidated by the determined Trp oxidation compounds (**Table 3**). Oia and NFK were shown to be the main oxidation products of Trp, followed by Tra, DiOia B, PIC B, Kyn, DiOia A, 5-OH-Trp, and PIC A. A corresponding oxidation susceptibility of formation of Trp-derived products has been observed (*9*) reporting a higher Trp loss (68%). However, it was reported that the amount of degradation products related to Trp content was approximately 11 and 13% with Trp loss of 50 and 68%, respectively (*9*). Thus, under strong H_2O_2 treatment, Trp is oxidized to such an extent (50%) that secondary oxidation products (~20%) are efficiently formed. The major differences were the temperature and pH used; instead of pH 8.3 and a temperature of 40 °C, pH 6.3 and a temperature of 37 °C were used. Degradation patterns of Trp and rate of oxidation depend on the oxidative conditions such as incubation time, the amount of oxidant, and temperature (*5, 9*). Oxidation of Trp did not reach its end point since Trp loss could have been more substantial by extending the incubation time. It has been also observed that under strong oxidative conditions (1.05 M H_2O_2) PIC A is decreased, and the amount of Kyn, NFK, and DiOia is increased (*9*). This is due to the fact that Trp oxidation products (PIC A, Oia, DiOia, Kyn, and 5-OH-Trp) are even more susceptible to oxidation than Trp is itself. In addition, the oxidation rate for Trp oxidation products is more rapid (*9*).

Table 3. Concentrations of Trp-Derived Oxidation Products (after 6 h of Oxidation with H_2O_2) in a Trp Model Solution (μg/mL, Mean ± SD, $n = 36$)[a]

Trp-derived oxidation product	concentration (μg/mL)
Trp	157.7 ± 13.5
Oia	12.6 ± 0.9
NFK	4.9 ± 0.6
Tra	3.2 ± 0.3
DiOia B	2.1 ± 0.2
PIC B	1.2 ± 0.1
Kyn	1.3 ± 0.2
DiOia A	1.1 ± 0.1
5-OH-Trp	0.7 ± 0.2
PIC A	0.6 ± 0.0

[a] See panel A for abbreviations.

It still remains unclear as to what other compounds Trp is degraded into as 80−90% of the degradation products related to Trp loss have not been explicated. However, the formation of Trp oxidation products, NFK and Kyn, has been shown to correlate with a loss of Trp due to oxidation (*26*). Oxidative modification of Trp residues to Kyn type products may also induce covalent aggregation in proteins (*27*), and oxidized milk proteins have been observed to polymerize (*26*). In addition, oxidation of myofibrillar proteins at high H_2O_2 and $FeCl_3$ concentrations has been reported to cross-link mainly from disulfide bonds, but also, dityrosine and ditryptophan bonds may be involved (*28*).

Effect of Plant Phenolics toward Oxidation of Trp. The loss of Trp affected by different phenolic compounds was 30−50% after 6 h of oxidation (**Table 4**). However, as only 5−20% of the determined oxidation products depending on the Trp loss could be elucidated, the appearance of less polar UV and fluorescent compounds was detected (**Figure 3**) after Trp was eluted. However, the formation of the putative Trp−dimers, Trp−phenolic, or phenolic−phenolic adducts was not substantiated. Labieniec et al. (*29*) showed that phenols can react with proteins and modify the formation of CO groups.

After 6 h of oxidation, PIC A and B, the primary oxidation products of Trp, exhibited higher concentrations levels with most of the phenolic compounds than with Trp control regardless of the amount of Trp loss (**Table 5**). However, at this point of oxidation, the formation of PIC has been observed to be decreasing (*9*). Therefore, this may be due to the fact that Trp oxidation is slowed down by the phenolic compounds, but further oxidation of PIC itself proceeds. Thus, the further

Table 4. Inhibition of Trp Loss (%) with Phenolics after 6 h of Oxidation with H_2O_2 Determined by HPLC[a]

	inhibition of Trp loss (%) after oxidation		
	10 μM	50 μM	100 μM
	plant phenolics		
black currant as	4.1 ± 8.5 c	41.9 ± 3.7 a	3.0 ± 9.1 f
raspberry as	−11.1 ± 10.0 d	6.0 ± 1.2 d	2.7 ± 9.9 f
cranberry pro	20.7 ± 5.9 ab	33.3 ± 4.3 ab	27.0 ± 2.1 b
raspberry et[b]		16.6 ± 6.7 cd	11.5 ± 2.9 de
pine bark	20.0 ± 9.3 b	29.8 ± 6.4 b	35.8 ± 0.7 a
camelina meal	31.8 ± 1.5 a	10.4 ± 14.8 cd	5.8 ± 1.4 ef
rapeseed meal	10.1 ± 8.9 bc	8.4 ± 5.8 cd	22.9 ± 0.7 bc
soy meal	12.5 ± 3.3 bc	17.8 ± 2.5 c	6.9 ± 2.7 ef
soy flour	7.9 ± 0.9 c	13.8 ± 7.0 cd	15.2 ± 2.6 cd
	phenolic compounds		
cyanidin-3-glucoside	15.2 ± 1.4 cde	24.4 ± 1.4 c	30.1 ± 3.3 d
delphinidin-3-glucoside	32.2 ± 3.9 a	30.6 ± 5.5 b	37.9 ± 3.5 bc
caffeic acid	23.8 ± 0.9 b	35.0 ± 1.8 b	38.2 ± 0.5 b
catechin	8.8 ± 5.8 fg	18.7 ± 3.1 de	25.3 ± 2.2 de
chlorogenic acid	8.6 ± 3.0 fg	23.3 ± 1.6 cd	30.2 ± 5.5 cd
ellagic acid	17.9 ± 3.4 cd	46.3 ± 1.6 a	50.6 ± 1.9 a
ferulic acid	17.0 ± 3.6 cd	22.0 ± 3.8 cd	23.0 ± 3.7 de
procyanidin B1	−11.1 ± 7.1 h	−3.5 ± 4.8 g	−4.8 ± 6.9 g
quercetin	6.4 ± 3.1 g	21.9 ± 1.9 cd	26.1 ± 5.2 de
sinapic acid	19.9 ± 1.3 bc	24.5 ± 0.8 c	27.1 ± 0.7 d
taxifolin	−5.4 ± 3.9 efg	3.9 ± 2.8 f	8.2 ± 0.2 ef
vinylsyringol	−19.0 ± 3.5 i	−9.0 ± 4.2 h	−18.5 ± 11.0 h
daidzein	13.2 ± 1.9 def	16.5 ± 0.5 ef	14.7 ± 2.2 f
genistein	13.3 ± 1.7 def	15.5 ± 1.2 ef	11.7 ± 1.5 f

[a] Trp loss (%) of the control after oxidation was 50.3 ± 4.2 (n = 36). Values in the same column at the same concentration within plant phenolics and within phenolic compounds followed by different letters are significantly different (p < 0.05) (percent inhibition, mean ± SD, n = 3). [b] Raspberry ellagitannin isolate concentrations at 50 and 100 μM correspond to 58 and 115 μM, respectively. Abbreviations: As, anthocyanins; pro, proanthocyanidins; and et, ellagitannins.

oxidation of primary oxidation products PIC A, Oia, and DiOia yielding Kyn and NFK, DiOia and Kyn, and Kyn, respectively (*9*), results in higher concentrations of Kyn, NFK, and DiOia. This phenomenon is observed with results of raspberry and black currant anthocyanins (**Table 5**). However, the formation of DiOia A and B with following oxidation susceptibility to PIC A/B and Oia is retarded with most of the plant phenolics and phenolic reference compounds (**Tables 5** and **6**). Therefore, the faster oxidation of primary oxidation products may thus lead to an increased formation of secondary oxidation products. That explains why some phenolic compounds inhibited the formation of primary oxidation products poorly or at a moderate level while they were excellent antioxidants toward the formation of secondary oxidation products.

Berry Phenolics. Black currant anthocyanins (50 μM) exhibited the best inhibition (42%) among plant phenolics against Trp loss as compared to the inhibition levels of pine bark phenolics (100 μM) of 36%, cranberry procyanidins of (50 μM) of 33%, and camelina meal phenolics (10 μM) of 32% (**Table 4**). However, black currant anthocyanins at other concentrations (10 and 100 μM) were poor in inhibiting Trp loss. Even though the amount of Trp loss in the presence of black currant anthocyanins at 100 μM did not differ from the control sample, the pattern of the Trp-derived oxidation products was similar to that at black currant anthocyanin concentration level of 50 μM (**Table 5**). Black currant anthocyanins were able to inhibit almost totally Tra, indicating that the oxidation of alanyl moiety of Trp is highly affected by black currant anthocyanins. In addition, black currant anthocyanin isolate (100 μM) was an excellent inhibitor of DiOia B. NFK as the second most important oxidation product formed as well as Kyn and DiOia A were inhibited approximately 70% by black currant isolate. The inhibition of Oia was about 40% by black currant. Raspberry anthocyanins in all concentrations showed no difference in Trp loss as compared to oxidized Trp (**Table 4**). The antioxidant effect of different berry anthocyanins reflects their anthocyanin composition (**Table 1**). Raspberry consists mainly of the 3-sophorisides (59%), 3-glucosides (16%), and 3-glucosylrutinosides (16%) of cyanidin with minor amounts of pelargonidin (4%) with different 3-glucosyl substituents, while black currant contains four major anthocyanins: the 3-glucosides and 3-rutinosides of cyanidin (7 and 38%) and delphinidin (16 and 39%) (*30*). Cyanidin-3-glucoside and delphinidin-3-glucoside were able to inhibit the Trp loss by 30% (**Table 4**). Effects of cyanidin-3-glucoside and delphinidin-3-glucoside (although with less effect) (**Table 6**) showed a consistency in the pattern of oxidation products formed with black currant and raspberry anthocyanins. Therefore, according to these results, it seems that cyanidin- and delphinidin-3-glucosides present are the main compounds responsible for the antioxidant activities in black currant isolates. It has been reported (*31*) that black currant consists also of procyanidins (43%), and prodelphinidins (57%) with low molecular weight (LMW) (1−10 μg/g) and insoluble high molecular weight (HMW) (100 μg/g) proanthocyanidins, which may also contribute to the antioxidant activity. Prodelphinidins in the form of trimeric gallocatechins (*31*) and flavan-3-ols such as catechin (8 μg/g) and epicatechin (11 μg/g) have been identified from black currant (*32*). This suggests that the amount of 3.7% of flavanols in black currant isolate (**Table 1**) may also affect the oxidation of Trp, which is in accordance with the results that catechin as a reference compound inhibited the Trp oxidation (**Tables 4** and **6**).

Cranberry proanthocyanidins (50 and 100 μM) exhibited approximately 30% inhibition toward Trp loss (**Table 4**). However, Trp with cranberry proanthocyanidins showed a higher susceptibility to oxidation as compared to pine bark (**Table 5**) although the inhibition of Trp oxidation was similar (30%) (**Table 4**). Cranberry procyanidins were less efficient in retarding oxidation of Oia and DiOia A as they still yielded DiOia and Kyn, respectively (**Table 5**). Although procyanidin

Table 5. Inhibitions of Trp Oxidation Products (after 6 h of Oxidation) by Plant Phenolics in a Trp Model Solution (Percent Inhibition, Mean ± SD)[a]

concentration (μM) of extracts	PIC A	DiOia A	DiOia B	PIC B	5-OH-Trp	Kyn	NFK	Oia	Tra
				raspberry anthocyanins					
10	−25.1 ± 17.1 bc	−37.7 ± 23.4 e	−0.8 ± 2.7 e	−4.3 ± 4.7 d		−46.6 ± 17.0 d	15.0 ± 3.9 e	10.4 ± 6.0 f	8.3 ± 6.9 e
50	−56.7 ± 2.6 ab	3.5 ± 7.8 e	50.5 ± 0.3 d	−162.9 ± 0.0 f		−4.2 ± 27.1 e	65.5 ± 13.2 c	26.0 ± 4.5 c	85.0 ± 2.4 c
100	−76.6 ± 6.7 c	41.1 ± 1.1 e	98.0 ± 0.3 b	31.0 ± 9.8 a		63.5 ± 0.9 c	100.0 ± 0.0 a	25.6 ± 0.6 f	93.3 ± 0.2 e
				black currant anthocyanins					
10	−6.8 ± 1.4 a	16.0 ± 4.6 d	31.2 ± 2.8 c	−21.1 ± 1.0 e		−13.4 ± 14.9 c	37.8 ± 2.4 d	22.9 ± 9.0 e	48.1 ± 7.4 d
50	−23.0 ± 2.0 ab	63.4 ± 5.2 bc	78.0 ± 5.4 b	50.5 ± 3.3 a		71.0 ± 6.3 bc	71.9 ± 1.4 c	40.1 ± 4.2 b	100.0 ± 0.0 a
100	−81.9 ± 1.4 c	68.5 ± 0.7 b	95.2 ± 0.3 c	−6.1 ± 10.2 b		71.5 ± 1.1 b	73.2 ± 4.6 c	41.1 ± 0.9 d	97.8 ± 0.5 b
				cranberry proanthocyanidins					
10	−14.5 ± 2.6 ab	19.5 ± 0.5 cd	18.6 ± 13.0 d	38.6 ± 10.2 b		22.4 ± 0.6 b	12.7 ± 11.7 e	8.4 ± 4.6 f	
50	−29.5 ± 13.6 ab	56.3 ± 10.5 cd	70.8 ± 8.7 c	44.9 ± 2.9 a		56.7 ± 14.0 c	24.5 ± 2.6 d	36.2 ± 7.3 b	74.7 ± 2.8 e
100	−21.3 ± 6.2 b	47.6 ± 1.8 d	58.8 ± 3.4 f	31.9 ± 3.5 a		41.7 ± 3.1 d	39.0 ± 5.4 f	34.1 ± 2.3 e	100.0 ± 0.0 a
				raspberry ellagitannins					
57.5	−5.4 ± 4.0 a	14.7 ± 16.6 e	97.6 ± 1.5 a	−21.2 ± 16.8 c		93.0 ± 2.0 a	100.0 ± 0.0 a	51.1 ± 8.2 a	99.0 ± 0.2 ab
115	−341.3 ± 2.3 f	36.4 ± 2.2 f	89.3 ± 0.3 d	−6.4 ± 0.6 b		87.0 ± 0.4 a	100.0 ± 0.0 a	22.7 ± 0.4 g	
				pine bark					
10	−36.4 ± 3.5 c	68.3 ± 0.2 ab	93.2 ± 0.3 a	50.1 ± 1.6 a		80.0 ± 3.0 a	81.2 ± 0.7 ab	50.4 ± 2.0 ab	100.0 ± 0.0 a
50	−30.2 ± 1.5 ab	73.5 ± 0.5 b	97.9 ± 0.1 a	39.7 ± 1.9 a		80.3 ± 1.1 ab	85.8 ± 0.5 b	55.4 ± 3.8 a	100.0 ± 0.0 a
100	1.5 ± 0.4 a	61.6 ± 0.1 c	100.0 ± 0.0 a	35.5 ± 1.4 a		64.6 ± 1.8 c	79.9 ± 0.3 b	51.6 ± 0.8 b	100.0 ± 0.0 a
				camelina meal					
10	−78.6 ± 1.8 d	71.7 ± 0.2 a	95.2 ± 0.3 a	24.1 ± 0.6 c	−767.1 ± 71.0 e	86.1 ± 1.0 a	87.7 ± 0.5 a	53.4 ± 0.4 a	98.8 ± 0.3 ab
50	−79.4 ± 4.6 c	41.9 ± 0.2 d	96.1 ± 0.5 a	−86.9 ± 20.6 d		92.1 ± 0.5 a	83.7 ± 0.5 b	52.5 ± 7.0 a	100.0 ± 0.0 a
100	−170.2 ± 4.1 d	29.8 ± 1.1 g	95.5 ± 0.1 c	−143.8 ± 0.6 d		90.9 ± 0.0 a	73.8 ± 0.9 c	55.1 ± 1.3 a	100.0 ± 0.0 a
				rapeseed meal					
10	−36.4 ± 9.3 c	54.9 ± 1.2 b	79.9 ± 1.2 b	36.2 ± 1.8 b	−224.5 ± 17.8 c	74.0 ± 1.0 a	72.0 ± 0.9 c	45.5 ± 2.8 bc	89.7 ± 0.6 c
50	−93.0 ± 1.1 bc	47.7 ± 1.4 d	84.2 ± 0.1 b	11.1 ± 1.1 b	−577.8 ± 123.5 d	79.1 ± 1.5 ab	73.0 ± 0.8 c	51.4 ± 2.6 a	97.2 ± 0.4 bc
100	−210.6 ± 11.4 e	15.8 ± 1.5 h	72.5 ± 0.2 e	−44.8 ± 1.5 c	−1077.1 ± 131.2 f	71.9 ± 2.1 b	62.9 ± 0.7 d	44.1 ± 0.5 c	97.3 ± 0.2 b
				soy meal					
10	−251.1 ± 10.9 e	35.3 ± 0.7 c	95.2 ± 0.2 a	−2.7 ± 5.8 d		74.1 ± 0.3 a	80.7 ± 1.1 ab	41.5 ± 2.0 cd	94.2 ± 1.2 abc
50	−825.8 ± 15.2 d	−101.3 ± 2.6 f	100.0 ± 0.0 a	−98.7 ± 1.6 d		35.1 ± 5.2 d	100.0 ± 0.0 a	26.0 ± 1.7 c	98.2 ± 1.8 ab
100	−1817.5 ± 20.2 h	−110.3 ± 4.1 i	100.0 ± 0.0 a	−188.8 ± 3.0 e		36.6 ± 5.4 e	59.1 ± 1.7 de	10.3 ± 1.7 h	96.6 ± 0.4 c
				soy flour					
10	−298.9 ± 3.9 f	32.9 ± 0.4 c	90.9 ± 0.1 a	−8.4 ± 2.1 d	−99.0 ± 12.2 b	72.4 ± 4.0 a	75.9 ± 0.6 bc	37.1 ± 0.6 d	92.5 ± 0.3 bc
50	−1150.2 ± 2.0 e	100.0 ± 0.0 a	100.0 ± 0.0 a	−141.7 ± 1.2 e	−81.5 ± 12.2 ab	59.4 ± 3.6 c	65.4 ± 2.7 c	16.2 ± 3.1 d	95.7 ± 0.7 c
100	−1418.8 ± 11.6 g	100.0 ± 0.0 a	100.0 ± 0.0 a	−262.3 ± 1.6 f	31.9 ± 117.9 a	24.9 ± 1.9 f	57.8 ± 2.6 e	−6.5 ± 2.6 i	95.4 ± 0.3 d

[a] Values in the same column at the same concentration followed by different letters are significantly different ($p < 0.05$). See panel A for abbreviations.

B1 used as a reference compound was effective in inhibiting the formation of Trp oxidation products (**Table 6**), it showed no effect in inhibiting Trp loss as compared to control (**Table 4**). Cranberry procyanidin fractions have been found to be effective antioxidants in DPPH test and toward lipid oxidation such as inhibiting the oxidation of methyl linoleate emulsion and LDL (*33*). Cranberry, blueberry, and grape seed extracts alone and in combinations showed antioxidant activity assayed by using a DPPH radical inhibition test (*34*). In addition, cranberry juice powder and its synergies with ellagic and rosmarinic acids have been shown to reduce oxidative stress and mediate antioxidant enzyme responses in porcine muscle tissue induced by H_2O_2 (*35*).

Raspberry ellagitannin isolate provided only a weak protection against the oxidation of Trp (**Table 4**). Among plant phenolics such as pine bark drink and soy meal (10 and 50 μM), cranberry proanthocyanidins (10 and 100 μM), and rapeseed (100 μM) with the same Trp loss (40%), raspberry ellagitannins had a more pronounced effect on oxidation products such as DiOia A and B, Kyn, NFK, and Tra. In contrast, rapeseed meal, pine bark, and soy meal affected the oxidation of Oia about the same as raspberry ellagitannins. According to Kähkönen et al. (*36*), the main compounds in ellagitannin fraction consist of mixture of monomers ($M_W \sim 936$ g/mol), dimers (sanguiin H6), trimers (lambertianin C), and polymers. Raspberry is reported to contain minor amounts of flavonols such as 3-glucosides and 3-glucuronides of quercetin (*19*), which is accordance with our results (**Table 1**). Ellagic acid and raspberry ellagitannins have been attributed with antioxidative properties (*37*). Ellagic acid with increasing concentration exhibited the best activity against oxidation of Trp (**Table 4**) by decreasing the Trp loss by 50%.

Oilseed Processing Byproducts. Camelina meal phenolics inhibited the oxidation of Trp by 32% already at a concentration of 10 μM in comparison to rapeseed meal phenolics with 23% inhibition at 100 μM (**Table 4** and **Figure 3**). Camelina meal phenolics at 50 and 100 μM exhibited a similar rate of Trp oxidation with oxidized Trp (control), whereas the amount of Trp-derived oxidation products was clearly decreased as compared to less oxidized Trp with camelina meal phenolics at 10 μM (**Table 5** and **Figure 3**). In general, camelina meal phenolics showed more pronounced effects in retarding the oxidation of Oia and Kyn as compared to other plant phenolics. The inhibition of Kyn may be due to the fact that oxidation of Oia was slowed down, thus yielding less Kyn. The phenolic composition of camelina meal extract was predominated by flavanols, hydroxycinnamic acids, and flavonols, while rapeseed meal phenolics were mainly hydroxycinnamic acids (**Table 2**), which is accordance with results reported earlier (*14, 15*). Consequently, flavanols and flavonols, such as quercetin glucosides, contribute to the antioxidant activity of camelina meal, which was also concluded in an earlier study (*14*). With regard to the reference compounds, catechin and quercetin provided a protection toward Trp and Trp oxidation compounds (**Table 4**).

Table 6. Inhibitions of Trp Oxidation Products (after 6 h of Oxidation) by Phenolic Compounds in a Trp Model Solution (Percent Inhibition, Mean ± SD)[a]

concentration (μM) of extracts	PIC A	DiOia A	DiOia B	PIC B	5-OH-Trp	Kyn	NFK	Oia	Tra
				cyanidin-3-glucoside					
10	−2.6 ± 1.2 a	7.3 ± 1.2 ab	5.5 ± 1.1 g			13.0 ± 1.7 a	12.8 ± 5.5 bc	4.8 ± 1.9 cd	34.8 ± 0.2 ab
50	−15.9 ± 1.7 b	43.4 ± 0.8 c	58.0 ± 0.8 e			22.2 ± 0.5 bc	53.4 ± 2.1 c	31.3 ± 1.1 cd	79.6 ± 0.9 c
100	−69.0 ± 36.8 c	49.8 ± 4.5 c	78.2 ± 0.6 e			42.3 ± 0.1 cd	70.1 ± 4.8 c	40.7 ± 2.2 d	92.9 ± 1.6 bc
				delphinidin-3-glucoside					
10	−39.8 ± 0.9 i	−53.3 ± 25.6 g	−10.7 ± 0.9 i		−50.6 ± 25.2 cd	−4.5 ± 5.6 b	8.9 ± 0.8 bc	1.7 ± 0.8 d	15.5 ± 0.4 cdef
50	−20.9 ± 1.5 c	−6.2 ± 5.1 h	16.8 ± 2.7 j		−60.9 ± 2.2 c	−30.9 ± 3.3 f	26.7 ± 3.9 ef	11.7 ± 1.1 fg	39.0 ± 1.4 f
100	−134.7 ± 10.4 f	23.5 ± 11.2 e	37.9 ± 4.4 i		−84.0 ± 1.5 de	7.5 ± 6.5 i	47.5 ± 2.1 d	23.5 ± 0.5 f	74.5 ± 1.4 e
				caffeic acid					
10	−21.4 ± 0.7 g	10.0 ± 0.2 a	22.8 ± 1.0 cd	9.0 ± 1.9 d		−12.3 ± 20.3 bc	16.4 ± 7.4 bc	8.3 ± 1.6 bc	36.0 ± 0.2 ab
50	−136.3 ± 3.4 l	23.1 ± 0.8 e	61.7 ± 0.1 de	33.7 ± 0.5 c		33.0 ± 13.9 b	51.8 ± 2.4 c	43.6 ± 1.4 ab	92.0 ± 0.3 a
100	−175.6 ± 5.7 g	23.1 ± 0.3 e	75.2 ± 0.2 f	35.4 ± 0.4 ab		44.9 ± 3.0 bc	64.8 ± 2.1 c	55.6 ± 1.8 bc	96.4 ± 0.1 ab
				catechin					
10	−0.8 ± 0.3 a	−12.7 ± 13.6 de	21.2 ± 0.2 cd	−0.9 ± 0.1 e		−20.9 ± 11.7 bcd	17.4 ± 0.1 abc	8.6 ± 3.4 bc	25.8 ± 0.5 bc
50	−60.5 ± 1.9 g	33.7 ± 0.7 d	64.4 ± 0.6 d	27.0 ± 0.6 d		26.2 ± 0.5 bc	51.6 ± 5.4 c	32.7 ± 1.8 cd	81.2 ± 0.2 c
100	−120.0 ± 3.3 ef	54.8 ± 1.5 c	81.3 ± 0.8 d	36.9 ± 0.1 ab		23.3 ± 8.3 efgh	65.1 ± 3.8 c	50.0 ± 4.5 c	93.2 ± 0.0 bc
				chlorogenic acid					
10	−11.8 ± 0.5 de	7.8 ± 3.5 ab	23.9 ± 0.4 c	25.6 ± 1.2 b	−26.5 ± 30.1 bc	−6.1 ± 10.5 bc	16.1 ± 1.6 bc	9.1 ± 4.5 bc	20.7 ± 8.4 cd
50	−49.7 ± 2.4 f	47.2 ± 0.6 b	63.7 ± 0.3 d	33.4 ± 0.0 c	−64.9 ± 8.6 c	9.0 ± 6.5 cde	49.4 ± 2.2 c	37.1 ± 2.6 bc	83.8 ± 0.2 bc
100	−91.1 ± 2.8 cd	67.9 ± 7.5 b	86.4 ± 1.7 c	41.5 ± 0.9 ab	−83.3 ± 24.3 de	14.9 ± 3.0 hi	69.6 ± 5.7 c	61.9 ± 4.7 ab	94.4 ± 3.3 bc
				ferulic acid					
10	−40.4 ± 1.6 i	−28.4 ± 1.7 f	13.5 ± 0.2 ef	8.9 ± 0.6 d	−51.7 ± 0.4 cd	−11.3 ± 10.9 bc	−2.7 ± 37.2 c	5.0 ± 5.7 cd	38.8 ± 2.5 a
50	−91.1 ± 0.9 i	−18.8 ± 2.8 j	26.6 ± 7.9 gh	23.2 ± 1.7 e	−59.6 ± 11.8 c	11.6 ± 11.6 cde	38.7 ± 11.0 d	17.5 ± 6.2 ef	82.4 ± 3.6 c
100	−101.3 ± 1.5 de	−27.7 ± 1.3 fg	30.4 ± 1.1 j	23.4 ± 3.0 bc	−53.8 ± 27.4 cd	13.9 ± 18.7 fghi	47.1 ± 6.7 d	21.5 ± 0.3 f	90.4 ± 0.6 c
				ellagic acid					
10	−7.9 ± 1.3 c	−5.7 ± 3.9 bcd	−2.6 ± 3.6 h	−2.9 ± 1.1 efg	−40.9 ± 5.1 cd	−21.2 ± 11.5 bcd	27.6 ± 2.2 ab	5.0 ± 2.4 cd	7.5 ± 2.5 f
50	−110.7 ± 3.0 j	53.9 ± 1.0 a	82.7 ± 0.3 a	11.6 ± 1.4 f	−81.2 ± 4.3 c	61.1 ± 4.9 a	100.0 ± 0.0 a	51.2 ± 3.0 a	93.2 ± 1.2 a
100	−183.3 ± 3.5 g	82.6 ± 0.3 a	99.0 ± 0.0 a	13.8 ± 1.3 bc	−94.1 ± 2.0 e	94.5 ± 1.0 a	100.0 ± 0.0 a	63.3 ± 1.2 a	100.0 ± 0.0 a
				procyanidin B1					
10	−33.2 ± 1.0 h	−6.7 ± 2.5 cde	61.0 ± 4.7 a	49.6 ± 0.5 a	90.0 ± 10.5 a	−49.8 ± 14.1 e	37.8 ± 10.5 a	26.4 ± 6.2 a	41.8 ± 5.0 a
50	−132.0 ± 0.0 k	25.6 ± 1.1 e	76.7 ± 0.2 b	50.2 ± 1.1 a	27.7 ± 11.1 a	−1.7 ± 21.6 e	63.3 ± 5.9 b	44.3 ± 4.7 ab	87.5 ± 0.7 b
100	−120.2 ± 1.7 ef	72.7 ± 0.1 b	89.4 ± 0.0 b	59.7 ± 0.7 a	31.1 ± 1.3 a	58.0 ± 3.1 b	81.5 ± 1.6 b	60.7 ± 2.4 ab	96.6 ± 0.2 ab
				sinapic acid					
10	−9.7 ± 0.5 cd	−19.4 ± 1.3 ef	−2.4 ± 0.4 h	−2.3 ± 1.3 ef	−3.1 ± 11.8 b	−22.8 ± 3.6 cd	10.1 ± 2.1 bc	4.2 ± 4.2 cd	12.4 ± 2.6 def
50	−30.7 ± 1.8 d	−0.5 ± 0.7 g	29.6 ± 1.7 g	35.9 ± 0.8 b	4.0 ± 12.1 ab	15.5 ± 2.0 cde	25.9 ± 2.1 f	15.3 ± 2.1 efg	61.6 ± 1.9 d
100	−40.5 ± 1.8 b	25.5 ± 0.5 e	44.5 ± 0.5 h	38.6 ± 0.4 ab	−14.4 ± 35.6 b	29.1 ± 4.4 de	45.1 ± 3.1 d	21.7 ± 1.4 f	83.0 ± 0.3 d
				taxifolin					
10	−12.0 ± 1.8 e	−11.4 ± 1.0 de	30.0 ± 1.2 b	−4.7 ± 0.2 g	−24.5 ± 28.4 bc	−36.1 ± 2.8 de	12.5 ± 1.8 bc	6.0 ± 0.6 bcd	6.6 ± 0.3 f
50	−14.9 ± 3.2 ab	−7.4 ± 1.6 h	37.4 ± 5.1 f	12.4 ± 0.2 f	−29.3 ± 9.5 bc	20.3 ± 10.2 bcd	28.1 ± 4.1 ef	11.1 ± 0.4 fg	46.4 ± 1.3 e
100	−85.9 ± 1.8 cd	19.8 ± 0.7 e	64.8 ± 0.3 g	−0.8 ± 0.5 cd	−27.7 ± 5.6 bc	9.6 ± 0.5 ghi	42.4 ± 2.2 d	23.2 ± 0.7 f	82.1 ± 2.7 d
				vinylsyringol					
10	−16.9 ± 2.5 f	9.7 ± 1.0 a	33.7 ± 9.0 b	19.7 ± 1.1 c		−11.5 ± 9.8 bc	23.3 ± 8.1 ab	12.3 ± 7.8 b	14.6 ± 4.8 def
50	−35.8 ± 2.1 e	18.1 ± 0.4 f	60.9 ± 4.8 de	5.0 ± 1.1 g		4.6 ± 13.0 de	34.9 ± 1.5 de	17.5 ± 5.7 ef	45.8 ± 5.2 e
100	−13.4 ± 2.3 a	27.1 ± 11.7 e	76.6 ± 3.1 ef	18.2 ± 6.1 bc		22.6 ± 14.0 efg	50.1 ± 10.5 d	30.8 ± 11.7 e	74.4 ± 6.1 e
				quercetin					
10	−5.5 ± 2.4 b	4.6 ± 0.1 abc	17.7 ± 2.9 de	−3.3 ± 0.9 fg	−69.2 ± 27.4 d	−6.2 ± 12.4 bc	12.1 ± 20.9 bc	3.2 ± 4.2 cd	18.6 ± 21.3 cde
50	−65.3 ± 1.3 h	41.4 ± 2.4 c	71.1 ± 0.3 c	−331.9 ± 1.1 j	−155.9 ± 92.4 d	35.4 ± 16.6 b	58.0 ± 2.7 bc	24.8 ± 18.8 de	83.2 ± 2.7 bc
100	−97.8 ± 32.3 de	40.0 ± 1.7 d	79.2 ± 0.2 de	−1131.7 ± 57.0 e	−306.0 ± 47.2 f	30.1 ± 5.0 de	67.7 ± 5.3 c	42.9 ± 2.9 d	93.2 ± 0.4 bc
				daidzein					
10	−13.9 ± 0.5 e	−13.1 ± 0.8 de	3.2 ± 2.6 g	−8.2 ± 2.2 h	−60.4 ± 5.5 d	−23.1 ± 10.0 cd	−1.0 ± 0.8 c	−0.1 ± 0.8 d	8.0 ± 4.0 ef
50	−23.3 ± 0.7 c	−14.8 ± 1.9 i	18.9 ± 0.4 ij	−7.4 ± 0.6 h	−49.8 ± 4.7 c	−24.9 ± 6.0 f	20.4 ± 3.4 f	6.1 ± 2.1 g	18.6 ± 1.2 h
100	−14.3 ± 0.7 a	−32.4 ± 0.8 g	14.6 ± 0.4 l	−2.7 ± 1.3 cd	−89.1 ± 12.4 de	−51.4 ± 5.8 j	12.7 ± 7.0 f	−0.1 ± 2.3 h	15.8 ± 6.8 g
				genistein					
10	−8.8 ± 1.6 c	−20.0 ± 1.6 ef	7.9 ± 1.8 fg	−2.0 ± 0.9 ef	−5.8 ± 1.1 b	−21.9 ± 2.0 bcd	−33.6 ± 1.5 d	5.1 ± 1.5 cd	14.6 ± 3.4 def
50	−12.1 ± 0.7 a	−26.2 ± 0.4 k	22.5 ± 0.6 hi	−28.5 ± 1.9 i	17.8 ± 9.0 ab	−30.9 ± 7.6 f	22.9 ± 12.3 f	7.0 ± 3.1 g	34.4 ± 5.8 g
100	−19.0 ± 1.0 ab	−20.8 ± 0.5 f	27.0 ± 1.5 k	−20.3 ± 0.5 d	−106.9 ± 6.4 e	27.2 ± 10.4 def	25.4 ± 13.3 e	8.9 ± 4.4 g	43.8 ± 3.2 f

[a] Values in the same column at the same concentration followed by different letters are significantly different ($p < 0.05$). See panel A for abbreviations.

Camelina meal has been shown to inhibit protein carbonyls in cooked pork meat patties with similar antioxidant activity as rapeseed meal (*14*). The pattern of Trp oxidation products formed and inhibited with rapeseed meal phenolics was similar to camelina meal phenolics; however, the antioxidant effect was more pronounced with camelina meal (**Table 5**). Rapeseed phenolics have been reported to show moderate radical scavenging activity (DPPH test) (*37*) and antioxidant activity in cooked pork meat patties (protein carbonyls) (*14, 15*) as well as in liposomes (*37*). Among hydroxycinnamic acids, caffeic and chlorogenic acids showed a more profound antioxidant effect than ferulic and sinapic acids toward the oxidation of Trp (**Table 4**). In this regard, the differences in activities may be due to the different structures of hydroxycinnamic acids. Caffeic and chlorogenic acids with only hydroxyl groups are more prone to interaction with Trp than ferulic and sinapic acids or sinapine with one or more methoxy groups. However, as camelina, rapeseed, and soy meal as well as pine bark used in this study

were rich in diverse phenolics, the pure phenolic compounds used as reference compounds may not explicate the exact activity of a certain compound. In addition, the antioxidant activity of certain compound(s) contributing to activity is difficult to demonstrate since there may also be synergistic effects between the different phenolics present in the extracts.

Soy meal and soy flour consist mainly of flavanols (**Table 2**) with isoflavones and lignans dominating (*16*). Soy meal and soy flour phenolics (10 μM) provided more protection toward the formation of Trp oxidation compounds than at higher concentrations (**Table 5**), even though there were only minor differences in Trp loss (**Table 4**). However, soy meal phenolics at 50 and 100 μM were able to inhibit DiOia B but not DiOia A. This may be due to stereoselective reaction. Soy flour phenolics, however, was able to inhibit both diastereomers of DiOia. Isoflavones, daidzein and genistein, as such acted mostly as weak antioxidants toward Trp oxidation. In addition, genistein and daidzein were incapable of affecting oxidation of Oia, thereby yielding more DiOia A/B and Kyn (**Table 6**). In previous study (*14*), soy meal and soy flour were effective in inhibiting protein carbonyls only in combination with rosemary extract due to their synergistic interactions. It has been reported that in foods where phenolic compounds are present together in interactions with other (bioactive) ingredients, one phenolic could synergistically improve the chances of the other phenolics to function effectively (*37*).

Pine Bark. Pine bark was one of the best sources of plant phenolics to exhibit antioxidant activity toward Trp and Trp oxidation products (**Tables 4** and **5**). Pine bark phenolics were able to inhibit the formation of secondary Trp oxidation products (Kyn, NFK, and Tra) and/or inhibiting the further oxidation of primary oxidation products of Trp. Because Trp with these phenolics was less oxidized than the Trp control, the formation of secondary oxidation products is consequently inhibited. However, regardless of the extent of Trp loss (30−40%) within different concentrations of pine bark, there is no difference in the determined oxidation products formed. Therefore, oxidation of Trp may also be stabilized with pine bark. The main phenolics in the pine bark extract were comprised of flavanols (**Table 2**) with catechin dominating (*15*). Catechin (**Table 4**) was more effective in inhibiting Trp oxidation than taxifolin, another phenolic compound reported in pine bark (*15*). As reported by Vuorela et al. (*15*), pine bark phenolics inhibited protein oxidation in cooked pork meat patties.

This paper reports for the first time the antioxidant effects of plant phenolics from berries and byproducts of oilseeds toward Trp oxidation. Pine bark phenolics, black currant anthocyanins, camelina meal phenolics, and cranberry procyanidins provided the best protection toward the oxidation of Trp. The pattern of Trp oxidation compounds derived by oxidation of free Trp by H_2O_2 was different with different phenolic compounds. Therefore, inhibition of Trp oxidation is strongly dependent on the type of phenolics present in the plant material. Each phenolic compound has its own mode of action against a particular target. Pine bark and camelina meal phenolics provided the best protection toward the formation of both primary oxidation products (DiOia A/B and Oia) and secondary oxidation products (Kyn, NFK, and Tra). Soy flour phenolics exhibited antioxidative effects on the formation of Dioia A and B. These modes of actions may be due to their ability to form Trp−phenolic complexes by forming noncovalent interactions or even covalent attachments and due to their ability to participate in different oxidation routes of Trp depending on their structure.

ABBREVIATIONS USED

Trp, tryptophan; NFK, *N*-formylkynurenine; Kyn, kynurenine; 3-OH-Kyn, 3-hydroxykynurenine; KynA, kynurenic acid; 5-OH-Trp, 5-hydroxy-tryptophan; Tra, tryptamine; PIC A/B, 3a-hydroxypyrroloindole-2-carboxylic acid; Oia A/B, oxindolylalanine; DiOia A/B, dioxindolylalanine.

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