Potato and Mushroom Polyphenol Oxidase Activities Are Differently Modulated by Natural Plant Extracts

ARTICLE *in* JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY · DECEMBER 2013

Impact Factor: 2.91 · DOI: 10.1021/jf4043375 · Source: PubMed

7 AUTHORS, INCLUDING:

Teunie van Herk
University of Applied Sciences Leiden
4 PUBLICATIONS **85** CITATIONS
SEE PROFILE

Deborah Louisa Narh Mensah
CSIR-Food Research Institute, Accra, Ghana
11 PUBLICATIONS **11** CITATIONS
SEE PROFILE

Willem J H van Berkel
Wageningen University
415 PUBLICATIONS **7,610** CITATIONS
SEE PROFILE

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Potato and Mushroom Polyphenol Oxidase Activities Are Differently Modulated by Natural Plant Extracts

Tomas F. M. Kuijpers,[†] Teunie van Herk,[‡] Jean-Paul Vincken,[†] Renske H. Janssen,[†] Deborah L. Narh,[‡] Willem J. H. van Berkel,[‡] and Harry Gruppen*,[†]

[†]Laboratory of Food Chemistry, Wageningen University, P.O. Box 17, 6700 AA Wageningen, The Netherlands

[‡]Laboratory of Biochemistry, Wageningen University, Wageningen, The Netherlands

Supporting Information

ABSTRACT: Enzymatic browning is a major quality issue in fruit and vegetable processing and can be counteracted by different natural inhibitors. Often, model systems containing a single polyphenol oxidase (PPO) are used to screen for new inhibitors. To investigate the impact of the source of PPO on the outcome of such screening, this study compared the effect of 60 plant extracts on the activity of PPO from mushroom (*Agaricus bisporus*, AbPPO) and PPO from potato (*Solanum tuberosum*, StPPO). Some plant extracts had different effects on the two PPOs: an extract that inhibited one PPO could be an activator for the other. As an example of this, the mate (*Ilex paraguariensis*) extract was investigated in more detail. In the presence of mate extract, oxygen consumption by AbPPO was found to be reduced >5-fold compared to a control reaction, whereas that of StPPO was increased >9-fold. RP-UHPLC-MS analysis showed that the mate extract contained a mixture of phenolic compounds and saponins. Upon incubation of mate extract with StPPO, phenolic compounds disappeared completely and saponins remained. Flash chromatography was used to separate saponins and phenolic compounds. It was found that the phenolic fraction was mainly responsible for inhibition of AbPPO and activation of StPPO. Activation of StPPO was probably caused by activation of latent StPPO by chlorogenic acid quinones.

KEYWORDS: *enzymatic browning, tyrosinase, LC-MS, plant extracts, Ilex paraguariensis*

INTRODUCTION

Polyphenol oxidases (PPOs) catalyze enzymatic browning by oxidizing phenolic compounds to their respective *o*-quinones, which subsequently undergo nonenzymatic reactions with each other and other compounds present, resulting in the formation of dark-colored pigments, also referred to as melanins.[1] This phenomenon is a major quality problem in fruit and vegetable processing (e.g., apple, potato, mushroom), but it is also associated with discoloration of shrimps[2] and formation of hyperpigmentation in human skin.[3]

To control enzymatic browning, much research is dedicated to finding natural inhibitors of enzymatic browning.[4−7] In such research, it is important to distinguish between (i) actual PPO inhibitors that prevent the formation of *o*-quinones, (ii) compounds that reduce the *o*-quinones to their *o*-diphenolic precursors (e.g., ascorbic acid[8]), and (iii) compounds that form colorless addition products with *o*-quinones (e.g., cysteine[9]).

Inhibition studies have been conducted on PPOs from a range of sources. Because of its commercial availability, mushroom tyrosinase is often used in model browning systems, both for browning of food products and for skin pigmentation.[10] Little is known on whether results of inhibition studies with one PPO can be extrapolated to another PPO, as most such studies were conducted with a single PPO. To address this issue, we compared the effect of a selection of plant extracts on two different PPOs in the same model system of enzymatic browning. A commercially available mushroom (*Agaricus bisporus*) tyrosinase (AbPPO) and a PPO isolated from potato tubers (*Solanum tuberosum*) (StPPO) were used.

MATERIALS AND METHODS

Materials. Potato (*S. tuberosum* cv. Celino) tubers were obtained from Gemüse Meyer (Twistringen, Germany). Mushroom (*A. bisporus*) tyrosinase, L-3,4-dihydroxyphenylalanine (L-DOPA), chlorogenic acid, and theobromine were obtained from Sigma-Aldrich (St. Louis, MO, USA). Ultrahigh-performance liquid chromatography−mass spectrometry (UHPLC-MS) grade acetonitrile (ACN) was obtained from Biosolve BV (Valkenswaard, The Netherlands), and caffeine was from Boom (Meppel, The Netherlands). Water was prepared using a Milli-Q water purification system (Millipore, Billerica, MA, USA).

Plant Extracts. Commercial, food grade plant extracts (Table 1) were obtained from Frutarom (Wädenswil, Switzerland). The solvents used in the production of the extracts are specified in Table S1 in the Supporting Information.

Purification of Mushroom Tyrosinase. The mushroom tyrosinase was purified by a single gel filtration step.[11] A HiLoad 26/60 Superdex 200 column connected to an Akta Explorer system (GE Healthcare, Uppsala, Sweden) was used. Fifty milligrams of the commercial enzyme (dissolved in 50 mM HEPES buffer, pH 6.8) was loaded and eluted with 50 mM HEPES buffer, pH 6.8, at 4 mL/min. Fractions (5 mL) were collected, and activity was assayed by a spectrophotometric assay: 50 μL of each fraction was combined with 100 μL of 0.8 mM tyrosine in a 96-well plate, and absorbance at 520 nm was monitored in time. Active fractions were pooled and stored at −20 °C until use. Tyrosinase activity was expressed in units (U), according to the suppliers definition (1 U increases the A_{280} by 0.001/

Received: June 27, 2013
Revised: December 17, 2013
Accepted: December 17, 2013

dx.doi.org/10.1021/jf4043375 | J. Agric. Food Chem. XXXX, XXX, XXX−XXX

Table 1. Effect of Plant Extracts on the Rate of Dopachrome Formation by AbPPO and StPPO

	extract	plant species	relative activity[a] (%) AbPPO	StPPO
1	green mate leaf	*Ilex paraguariensis*	27 ± 1.0	354 ± 41.5
2	oats herb	*Avena sativa*	73 ± 1.8	88 ± 10.6
3	olive leaf	*Olea europaea*	85 ± 3.2	224 ± 2.3
4	echninacea dry pressed juice	*Echinaceae purpurea*	104 ± 3.7	105 ± 6.7
5	echninacea purpurea root	*Echinaceae purpurea*	91 ± 2.2	102 ± 7.3
6	pumpkin seed	*Curcubita pepo*	85 ± 1.6	101 ± 3.7
7	green tea leaf	*Camillia sinensis*	22 ± 1.1	61 ± 1.9
8	nettle leaf	*Urtica dioica, Urtica urens*	84 ± 2.7	106 ± 3.8
9	balm herb	*Melissa officinalis*	30 ± 2.9	216 ± 9.9
10	sage leaf	*Salvia officinalis*	31 ± 2.6	181 ± 4.1
11	rhubarb	*Rheum rhabarbarum*	50 ± 1.1	85 ± 3.4
12	red vine leaf	*Vitis vinifera*	63 ± 7.2	89 ± 0.8
13	peppermint leaf	*Mentha piperita*	44 ± 3.3	124 ± 13.8
14	dandelion herb and root	*Taraxum officinale*	79 ± 8.6	100 ± 8.4
15	thyme herb	*Thymus vulgaris*	37 ± 1.8	156 ± 0.2
16	pink rockrose herb	*Cistus incanus*	37 ± 2.1	70 ± 1.5
17	passion flower herb	*Passiflora incarnata*	67 ± 3.5	95 ± 4.3
18	damiana leaf	*Turnera diffusa*	75 ± 3.3	93 ± 10.7
19	goldenrod herb	*Solidago* sp.	43 ± 0.2	189 ± 11.2
20	artichoke leaf	*Cynara scolymus*	63 ± 2.2	102 ± 25.4
21	java tea	*Orthosiphon stamineus*	34 ± 0.2	107 ± 0.13
22	eyebright herb	*Euphrasia* sp.	42 ± 2.7	146 ± 3.9
23	ivy leaf	*Hedera helix*	53 ± 3.8	123 ± 1.4
24	marshmallow root	*Althea officinalis*	99 ± 2.7	96 ± 1.4
25	bearberry leaf	*Arctostaphylos uva-ursi*	36 ± 6.6	61 ± 0.9
26	schisandra fruit	*Schisandra chinensis*	88 ± 5.7	92 ± 2.2
27	licorice root	*Glycyrrhiza glabra*	4 ± 1.8	15 ± 1.1
28	chasteberry	*Vitex agnus-castus*	51 ± 12.8	115 ± 0.4
29	juniper fruit	*Juniperus* sp.	84 ± 3.2	96 ± 1.3
30	rosemary leaf	*Rosmarinus officinalis*	29 ± 3.4	160 ± 0.0
31	devil's claw root	*Harpagophytum procumbens* and/or *H. zeyheri*	41 ± 1.5	117 ± 1.6
32	pelargonium root	*Pelargonium sidoides*	25 ± 1.0	44 ± 9.6
33	chamomile flower	*Chamomilla recutita*	33 ± 0.2	102 ± 1.9
34	caraway seed	*Carum carvu*	56 ± 1.0	97 ± 2.4
35	puslane herb	*Potulaca oleracea*	62 ± 3.3	86 ± 8.7
36	rosehip	*Rosa canina*	98 ± 0.5	84 ± 13.0
37	SoyLife 40	*Glycine max*	48 ± 0.5	103 ± 17.0
38	LinumLife EXTRA	*Linum usitatissimum*	82 ± 2.9	103 ± 9.4
39	brocoraphanin 10% glucoraphanin	*Brassica oleracea* var. *italica*	118 ± 11.5	80 ± 12.7
40	biocurcumin	*Curcuma longa*	–[b]	–[b]
41	Pomactiv AGE	*Malus* sp.	44 ± 2.7	90 ± 5.8
42	SuperBerry 6000	blend of seven berries	37 ± 8.0	78 ± 3.7
43	acai	*Euterpe oleracea*	94 ± 5.5	82 ± 10.2
44	black currant 25% anthocyanins	*Ribes nigrum*	11 ± 10.3	90 ± 18.1
45	Origanox WS-LB	*Origanum vulgare* and/or *Melissa officinalis*	31 ± 2.4	213 ± 3.0
46	Origanox WS	*Origanum vulgare*	44 ± 2.1	125 ± 3.0
47	Cranberry High PAC 25:1	*Vaccinum macrocarpon*	176 ± 4.5	90 ± 1.6
48	OPC Grape Seed ActiVin	*Vitis vinifera*	18 ± 0.4	67 ± 0.7
49	neohesperidine dihydrochalcone (NHDC)	extracted from citrus and chemically modified	19 ± 0.8	73 ± 2.3
50	neohesperidine	extracted from citrus	40 ± 4.9	97 ± 4.0
51	hesperidine	extracted from citrus	45 ± 5.1	99 ± 1.3
52	black garlic	*Allium sativum*	109 ± 3.0	95 ± 0.6
53	horseradish	*Armoracia rusticana*	110 ± 1.2	86 ± 12.9
54	hibiscus	*Hibiscus* sp.	114 ± 12.3	54 ± 15.0
55	baobab	*Adansonia digitata*	107 ± 0.1	94 ± 1.5
56	coriander	*Coriandrum sativum*	79 ± 4.5	105 ± 2.4
57	cinnamon	*Cinnamomum* sp.	69 ± 5.6	91 ± 4.3
58	cinnamon 2	*Cinnamomum* sp.	104 ± 40.4	95 ± 4.0
69	Pomactiv HFV	*Malus* sp.	48 ± 1.8	107 ± 13.7

dx.doi.org/10.1021/jf4043375 | J. Agric. Food Chem. XXXX, XXX, XXX–XXX

Table 1. continued

	extract	plant species	relative activity[a] (%) AbPPO	StPPO
60	Pomactiv Shape	*Malus* sp.	40 ± 2.4	183 ± 4.6

[a]The rate of dopachrome formation relative to the appropriate control (L-DOPA with AbPPO or StPPO and the solvent used to dissolve the plant extract). [b]Biocurcumin was not included in the spectrophotometric assay due to interfering color of the extract.

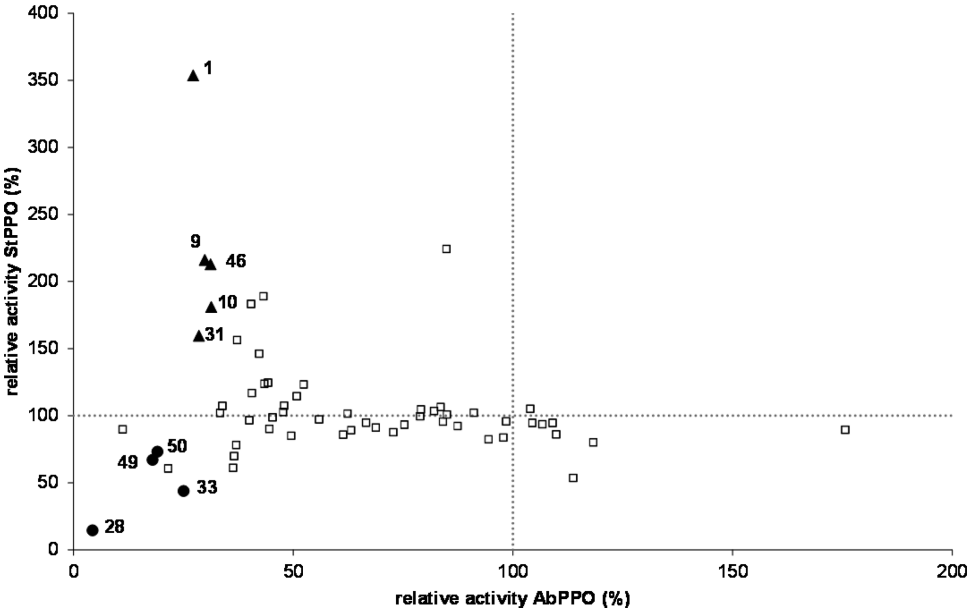

Figure 1. Comparison of the influence of different plant extracts on the conversion of L-DOPA to dopachrome by AbPPO and StPPO. Solid circles indicate extracts that were relatively good inhibitors for both reactions, solid triangles indicate extracts that showed a large difference in inhibition, and open squares indicate all other plant extracts. Numbers refer to Table 1.

min with L-tyrosine as substrate, at pH 6.5 and 25 °C). Purified mushroom tyrosinase is further referred to as AbPPO.

Purification of Potato PPO. One kilogram of potatoes was cooled to 4 °C, washed, and homogenized in 1 L of ice-cold 50 mM HEPES buffer, pH 6.9, containing 1% ascorbic acid and two tablets of protease inhibitor (Complete - EDTA free, Roche Diagnostics GmbH) using a commercial blender. The homogenate was filtered through four layers of cheesecloth, and the filtrate was centrifuged at 13600*g* at 4 °C for 1 h (crude extract, CE). Ammonium sulfate was added to 40% saturation, and the resulting solution was stirred overnight at 4 °C. The precipitate was collected by centrifugation at 13600*g* at 4 °C for 1.5 h and dissolved in a minimal amount of 50 mM HEPES buffer, pH 6.9, containing 1% ascorbic acid. The obtained protein solution was three times dialyzed against 2 L of 50 mM HEPES buffer, pH 6.9, containing 0.1% ascorbic acid and two tablets of the protease inhibitor, after which it was centrifuged at 13600*g* at 4 °C for 30 min.

All subsequent chromatographic steps were performed using an Akta Explorer system (GE Healthcare) at room temperature. The dialyzed protein solution (268 mL) was applied onto a Fast Flow Q-sepharose column (2.6 × 10 cm), pre-equilibrated with 50 mM HEPES buffer, pH 6.9. After a washing with 400 mL of starting buffer, bound protein was eluted using an 800 mL linear gradient of 0−1 M NaCl in the same buffer. The flow rate was 4 mL/min, and 15 mL fractions were collected. Fractions showing maximal PPO activity were pooled and concentrated using a 10 kDa Amicon membrane filter (Millipore) under air pressure.

The concentrated enzyme solution (5 mL) was applied onto a Superdex S-200 column (2.6 × 100 cm) and eluted with 50 mM HEPES buffern pH 6.9n at a flow rate of 1.5 mL/min. Fractions (5 mL) showing maximal PPO activity were concentrated as described above.

This purification procedure gave an activity yield of 10.6% and resulted in an 11-fold purified enzyme preparation with a specific activity of 5.9 U/mg (Supporting Information Table S2).

Fractionation of Mate Extract. Mate extract was fractionated using a 12 g Reveleris C18 column on a Reveleris flash chromatography system (Grace, Deerfield, IL, USA) operated at 30 mL/min. Twenty milliliters of a 30 g/L solution of mate extract in MQ was applied onto the column. Water acidified with 0.1% (v/v) acetic acid, eluent A, and ACN acidified with 0.1% (v/v) acetic acid, eluent B, were used as eluents. The following elution profile was used: 0−2 min, isocratic on 0% (v/v) B; 2−3 min, linear gradient from 0 to 30% (v/v) B; 3−8 min, isocratic at 30% (v/v) B; 8−13 min, linear gradient from 30 to 100% (v/v) B; 13−15 min, isocratic at 100% (v/v) B. Fractions of 10 mL were collected and pooled on the basis of RP-UHPLC-MS analysis.

Screening of Plant Extracts. Inhibitory activity of plant extracts was assayed using a spectrophotometric assay. Plant extracts (5 g/L) dissolved in either water or dimethyl sulfoxide were diluted (0.16 g/L) into 0.2 mM L-DOPA and 6.5 U/mL AbPPO or 0.4 mM L-DOPA and 0.19 U/mL StPPO in 50 mM HEPES buffer, pH 6.9, in a total volume of 155 μL in a 96-well plate. The absorbance at 520 nm was measured every 20 s for 20 min at 25 °C. The initial rate of color formation (100−240 s) was compared to the appropriate water or dimethyl sulfoxide controls and expressed as relative activity. To ensure that potential competitive inhibition could be observed, substrate concentrations were chosen below the K_m of the two PPOs for L-DOPA.

Oxygen Consumption Measurements. Oxygen consumption of AbPPO or StPPO with selected extracts was measured using an Oxytherm System (Hansatech, Kings Lynn, UK). Incubations with plant extracts or fractionated mate extracts (0.16 g/L) were done with 0.4 mM L-DOPA and 0.19 U/mL StPPO or 0.2 mM L-DOPA and 65 U/mL AbPPO in a total volume of 1 mL of 50 mM HEPES buffer, pH

dx.doi.org/10.1021/jf4043375 | J. Agric. Food Chem. XXXX, XXX, XXX−XXX

6.9, at 25 °C. Data acquisition and analysis were performed using Oxygraph Plus software (Hansatech).

RP-UHPLC Analysis. Samples were analyzed on an Accela UHPLC system (Thermo Scientific, San Jose, CA, USA) equipped with a pump, autosampler, and PDA detector. Samples (1 μL) were injected onto a Hypersil Gold column (2.1 × 150 mm, particle size = 1.9 μm; Thermo Scientific). Water acidified with 0.1% (v/v) acetic acid, eluent A, and ACN acidified with 0.1% (v/v) acetic acid, eluent B, were used as eluents. The flow rate was 400 μL/min, and the column oven temperature was controlled at 30 °C. The PDA detector was set to measure the range 200−600 nm. The following elution profile was used: 0−1 min, isocratic on 5% (v/v) B; 1−21 min, linear gradient from 5 to 75% (v/v) B; 21−21.1 min, linear gradient from 75 to 100% (v/v) B; 21.1−24 min, isocratic on 100% (v/v) B; 24−24.1 min, linear gradient from 100 to 5% (v/v) B; 24.1−27 min, isocratic on 5% (v/v) B.

Electrospray Ionization Mass Spectrometry (ESI-MS). Mass spectrometric data were obtained by analyzing samples on an LTQ-Velos (Thermo Scientific) equipped with a heated ESI probe coupled to the RP-UHPLC system. Nitrogen was used as sheath gas and auxiliary gas. Data were collected over the m/z range 150−1500. Data-dependent MS^n analysis was performed with a normalized collision energy of 35%. The MS^n fragmentation was performed on the most intense product ion in the MS^{n-1} spectrum. Most settings were optimized via automatic tuning using Tune Plus (Xcalibur 2.1, Thermo Scientific). The system was tuned with a mate extract in negative ionization (NI) mode. The source heater temperature was 45 °C, the transfer tube temperature was 350 °C, and the source voltage was 3.5 kV. Data acquisition and analysis were done with Xcalibur 2.1 (Thermo Scientific).

RESULTS

Screening of Plant Extracts for PPO Inhibitory Activity. The effect of 60 different plant extracts on dopachrome formation by AbPPO and StPPO was compared by expressing the rate of dopachrome formation as activity relative to the appropriate control (L-DOPA with AbPPO or StPPO and the solvent used to dissolve the plant extract) (Table 1). To facilitate this comparison, the relative activities were plotted against each other (Figure 1). From this plot it can be observed that, in general, the StPPO-mediated color formation seemed to be less inhibited than color formation caused by AbPPO. Interestingly, some extracts seemed to inhibit AbPPO, whereas they stimulated StPPO. It should be taken into account that the observed effect on color formation is not necessarily caused by influencing the enzymatic activity. An alternative explanation for an observed inhibition of color formation could be the presence of reducing compounds in the plant extracts, which can either reduce the enzymatically formed *o*-quinone back to the corresponding *o*-diphenol (e.g., ascorbic acid[12]) or combine with the *o*-quinone in an addition product (e.g., cysteine[9] or sulfite[13]). An explanation for enhanced color formation could be the presence of substrates for enzymatic browning in the plant extract itself. To investigate whether enzymatic activity was truly affected, five extracts that showed a large difference in effect on AbPPO and StPPO and four extracts that appeared to be relatively good inhibitors for both the AbPPO and StPPO-mediated color formation were selected for oxygen consumption measurements.

Oxygen Consumption Measurements Discriminate between Inhibition of Color Formation and Enzyme Activity. The four extracts indicated with a circle in Figure 1 (licorice root, pelargonium root, OPC grape seed ActiVin, and NHDC) were used in oxygen consumption measurements to determine whether the observed inhibitory effect on dopachrome formation for both enzymes was caused by inhibition of PPO activity. Biocurcumin was also used in this assay, as the color of this extract was found to interfere with the spectrophotometric assay. The trend of color formation with StPPO being less inhibited than that with AbPPO (Figure 1) was confirmed by oxygen consumption of the two enzymes (Figure 2). Remarkably, the relative oxygen consumption of

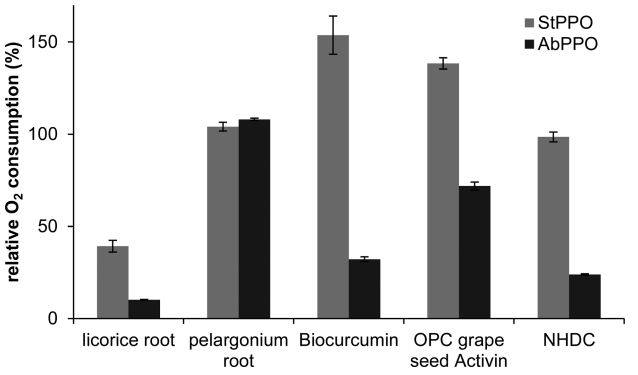

Figure 2. Relative oxygen consumption rates of StPPO and AbPPO with combinations of plant extracts and L-DOPA. Bars represent averages of duplicate determinations; error bars indicate standard deviation.

StPPO in the presence of biocurcumin, grape seed, pelargonium root, and NHDC was 100% or more, whereas the relative color formation with StPPO and these extracts was below 100%. Possibly, the extract contained substrates for StPPO, the oxidation of which did not result in products with an absorption at 520 nm, the wavelength used for the screening assay. If these substrates are preferentially used by StPPO, no color development at 520 nm would be observed, while oxygen consumption occurs. It is unlikely that this effect could be attributed to the presence of reducing compounds in the extracts, as no differences between StPPO and AbPPO would then be expected.

The influence on oxygen consumption of StPPO and AbPPO of five extracts that showed a large difference in their effect on StPPO- and AbPPO-mediated color formation (mate, balm herb, sage leaf, rosemary, and oregano, indicated with triangles in Figure 1) was investigated. Because compounds present in the extracts could potentially be substrates for either of the PPOs, in this way possibly enhancing enzyme activity, incubations of PPO with only plant extract were compared to incubations of PPO, plant extracts, and L-DOPA (Figure 3). The oxygen consumption of StPPO with all of the plant extracts alone was higher than that of the control reaction (StPPO with L-DOPA) used for standardization, indicating that these plant extracts contained either substrates that had a higher affinity for StPPO than L-DOPA or substrates in a considerably higher concentration than the L-DOPA used (0.4 mM). Assuming a molecular weight of 150−300 g/mol for possible substrates in the plant extracts and assuming that these extracts consisted only of substrate, it can be calculated that the maximum theoretical substrate concentration is approximately 0.5−1 mM, with the concentration of plant extracts used in the assay. Balm herb, sage, rosemary, and oregano are all members of the Lamiaceae plant family, which are known to contain a variety of phenolics, including phenolic acids, flavonoids, and phenolic terpenes.[14,15] LC-MS analysis of the extracts used confirmed the presence of a range of compounds, with the most abundant compounds being rosmarinic acid and derivatives of

dx.doi.org/10.1021/jf4043375 | J. Agric. Food Chem. XXXX, XXX, XXX−XXX

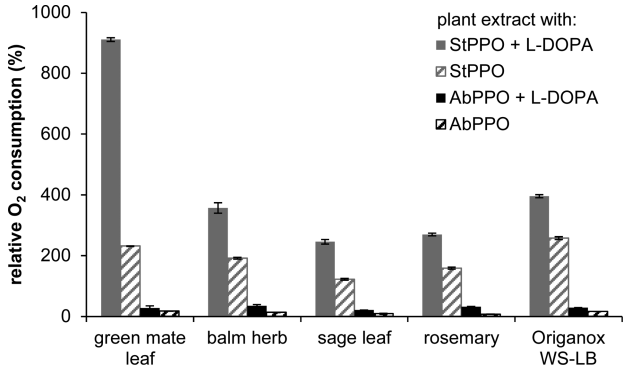

Figure 3. Relative oxygen consumption rates of StPPO and AbPPO in the presence of plant extracts alone or combinations of plant extracts and L-DOPA. Oxygen consumption was expressed relative to the control reaction of either StPPO or AbPPO with L-DOPA. Bars represent verages of duplicate determinations; error bars indicate standard deviation.

rosmarinic acid (data not shown). The fact that the extracts consisted of multiple components indicated that substrates present in the extracts had higher affinities for StPPO than L-DOPA. The relative activity of AbPPO on the plant extracts alone was lower than the activity on combinations of plant extract and L-DOPA, and activity of both reactions was lower than that of the control reaction (AbPPO with L-DOPA). This indicated that the selected plant extracts effectively reduced the rate of AbPPO-mediated color formation by inhibiting the enzyme. The observation that AbPPO showed some activity on the plant extracts alone indicated that besides inhibitors for AbPPO, the extracts also contained substrates for AbPPO. Furthermore, comparing the activity of StPPO and AbPPO on the plant extracts alone, it can be concluded that the substrates present in the extracts are probably better substrates for StPPO than for AbPPO.

Comparing the effect of the plant extracts in combination with L-DOPA on StPPO to the activity of StPPO on the plant extracts alone, the effect of the mate extract stands out. Whereas the activity of StPPO on the mate extract alone was approximately double that of StPPO on L-DOPA, the activity of StPPO on a combination of L-DOPA and mate extract was >9 times that of StPPO on L-DOPA alone. It seems unlikely that this effect can be attributed only to the presence of substrates in the mate extract. As an example of the contrasting effects a plant extract can have on the activity of different PPOs, the effect of mate extract on StPPO and AbPPO was further investigated.

Mate Extract Accelerates Oxygen Consumption of StPPO, whereas It Inhibits That of AbPPO. The effect of sequential addition of mate extract and L-DOPA to StPPO was compared to a control incubation of StPPO with L-DOPA alone and an incubation of StPPO with L-DOPA and mate extract added simultaneously (Figure 4A). The initial activity of StPPO on mate extract is comparable to that on the combination of mate extract and L-DOPA, both of which are higher than the activity on L-DOPA alone. The activity of StPPO on mate extract alone started to decrease shortly after the beginning of the reaction and eventually leveled off, indicating that the substrates present in mate extract were all converted. When L-DOPA was added after complete conversion of the substrates present in the mate extract, enhanced StPPO activity was still observed. This is an indication that the enhanced oxygen consumption of StPPO with combinations of L-DOPA and mate extract not only is due to supplementation of L-DOPA with additional substrates present in the extract but rather points toward activation of StPPO.

The activity of AbPPO on mate extract was much lower than that of StPPO, and the L-DOPA oxidation rate of AbPPO was lower in the presence of mate extract than without mate extract (Figure 4B). AbPPO was also inhibited upon sequential addition of mate extract and L-DOPA.

These results seemed to indicate that the mate extract used contained one or more activators for StPPO and one or more inhibitors for AbPPO. It is not known whether the same compounds are responsible for the activation of StPPO and the inhibition of AbPPO or whether activators for StPPO are present alongside inhibitors for AbPPO.

Characterization of Mate Extract. To identify the compound(s) responsible for activation of StPPO and inhibition of AbPPO, the composition of the mate extract was characterized on the basis of RP-UHPLC-MS analysis (Figure 5A). Peaks were annotated on the basis of comparison of MS/MS fragmentation with published data (Table 2).[16−18] Two distinct groups of compounds were found, hydroxycinnamic acid conjugates and saponins. Furthermore, quercetin-3-*O*-rutinoside, a glycosylated flavonol, caffeine, and theobromine were found. The hydroxycinnamic acid conjugates were, besides a small amount of feruloylquinic acid, all chlorogenic acid-like compounds, that is, different caffeoyl- and dicaffeoylquinic acid isomers. Chlorogenic acid is a well-known substrate

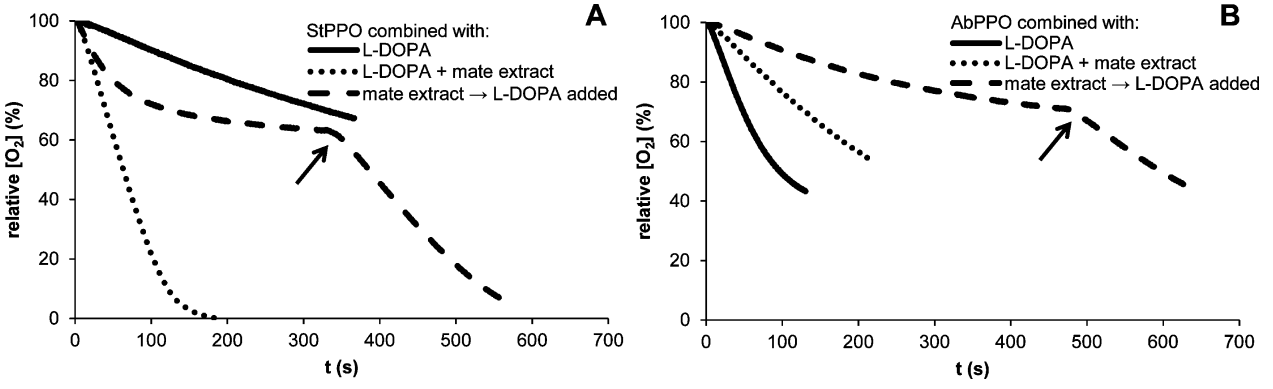

Figure 4. Oxygen consumption in time of incubations of StPPO (A) and AbPPO (B) with L-DOPA and mate extract. Arrows indicate the time point at which L-DOPA was added.

dx.doi.org/10.1021/jf4043375 | J. Agric. Food Chem. XXXX, XXX, XXX−XXX

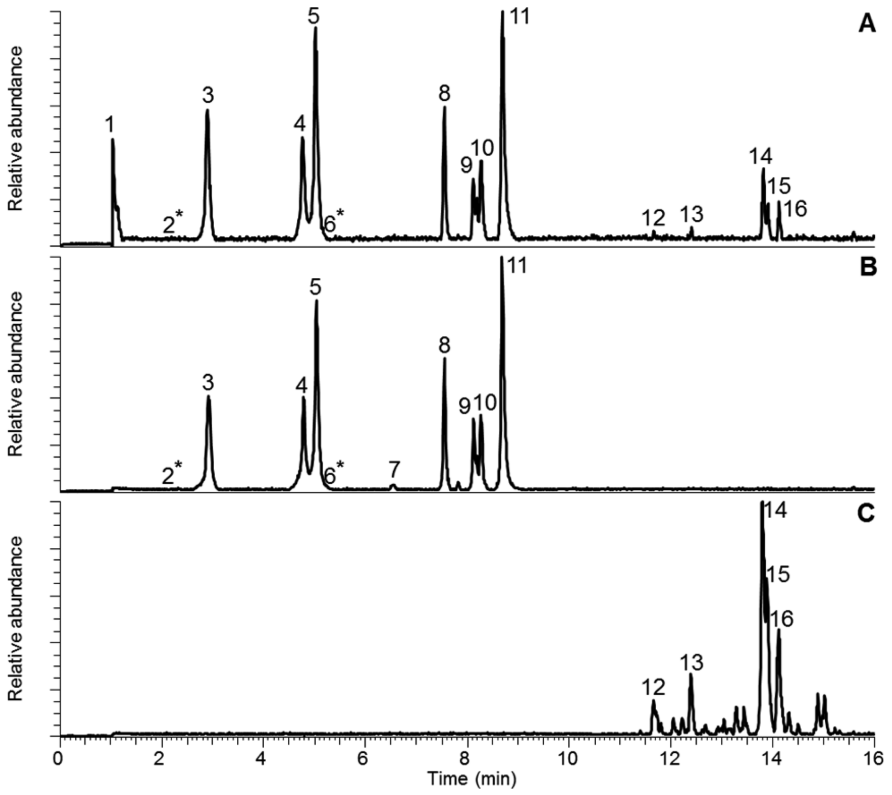

Figure 5. RP-UHPLC-MS traces of mate extract (A), polar mate fraction (B), and apolar mate fraction (C). Peak numbers refer to annotation in Table 2. ∗ indicates the retention time of theobromine and caffeine, which were observed only in the UV trace, not in negative ionization mode MS.

Table 2. RP-UHPLC-MS Characterization of Mate Extract[a]

peak	retention time	$[M-H]^-$	MS^2 fragments (relative abundance)	tentative identification
1	1.04	191	191 (100), 111 (42), 127 (30), 85 (30), 93 (17), 87 (7), 109 (6), 155 (4), 71 (3), 153 (3)	quinic acid
2	2.15			theobromine
3	2.90	353	191 (100), 179 (39), 353 (8), 135 (3), 173 (1)	3-*O*-caffeoylquinic acid
4	4.76	353	191 (100), 179 (1)	5-*O*-caffeoylquinic acid
5	5.02	353	173 (100), 179 (72), 191 (28), 353 (9), 135 (4), 155 (1)	4-*O*-caffeoylquinic acid
6	5.18			caffeine
7	6.57	367	191 (100), 173 (73), 193 (15), 285 (7), 307 (3), 367 (2), 203 (2), 325 (1), 155 (1)	feruloylquinic acid
8	7.56	609	301 (100), 300 (48), 609 (9), 343 (8), 271 (8), 255 (5), 179 (2), 273 (1)	quercetin-3-rutinoside
9	8.12	515	353 (100), 335 (11), 173 (1), 515 (8), 179 (8), 191 (6), 203 (3), 255 (2), 299 (2)	dicaffeoylquinic acid
10	8.28	515	353 (100), 191 (3), 179 (1)	dicaffeoylquinic acid
11	8.70	515	353 (100), 203 (6), 173 (5), 299 (5), 255 (4), 179 (3), 335 (2), 317 (2), 191 (1)	dicaffeoylquinic acid
12	11.67	1073	911 (100), 749 (76), 893 (12), 603 (10), 983 (9), 927 (6), 765 (5), 901 (5), 1043 (5)	matesaponin 3
13	12.42	1219	895 (100), 733 (13), 937 (2)	matesaponin 4
14	13.82	1057	895 (100), 733 (14), 937 (8), 587 (5)	matesaponin 2
15	13.91	1057	895 (100), 733 (64), 893 (8), 587 (7)	matesaponin 2 isomer
16	14.13	911	749 (100), 791 (19)	matesaponin 1

[a]Peaks were annotated on the basis of MS^2 fragmentation.

for PPO,[19,20] so the presence of these compounds explains the observed activity of StPPO and AbPPO when incubated with mate extract as substrate. Chlorogenic acids are the most abundant phenolic compounds in potato,[21] which might explain the much higher activity of StPPO than AbPPO on the mate extract, considering that chlorogenic acid is likely to be the natural substrate for StPPO. To investigate whether indeed the different chlorogenic acid isomers were used as substrate by PPO, RP-UHPLC-MS analysis of incubations of mate extract with StPPO and AbPPO was done. The MS traces of these samples revealed that the different chlorogenic acids and quercetin-3-*O*-rutinoside were converted, whereas the saponins remained (data not shown). No reaction products of the oxidation of the different substrates were found, whereas the mate extract after incubation with PPO visibly turned brown. This might be explained by the formation of a wide variety of reaction products from the *o*-quinones resulting from PPO oxidation, which individually fall below the detection limit of the RP-UHPLC analysis.

Polar Compounds in Mate Extract Are Responsible for Activation of StPPO. The fact that the saponins remained after PPO activity led to the hypothesis that they might be responsible for the observed activation of StPPO and inhibition of AbPPO. Saponins extracted from *Paris polyphylla* have been

dx.doi.org/10.1021/jf4043375 | J. Agric. Food Chem. XXXX, XXX, XXX−XXX

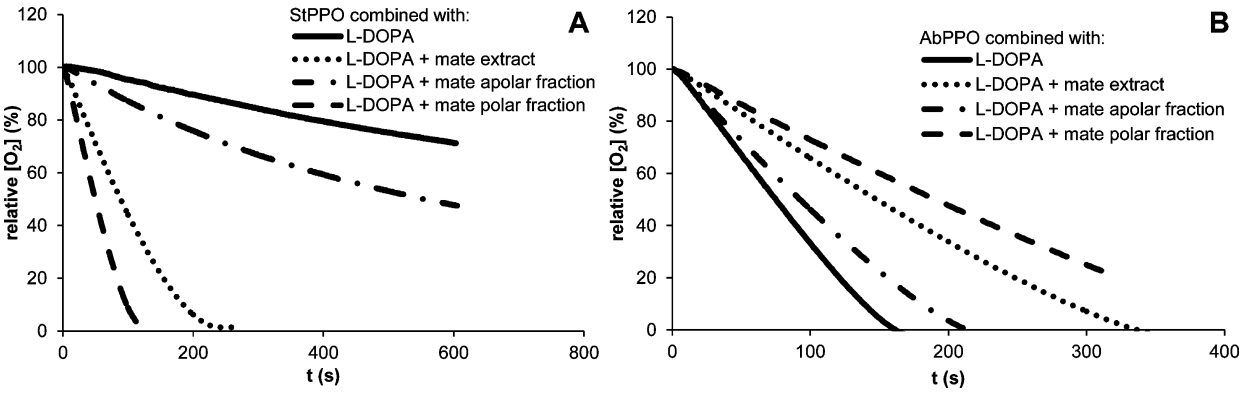

Figure 6. Oxygen consumption in time of incubations of StPPO (A) and AbPPO (B) with L-DOPA (blank) and L-DOPA combined with mate extract, the polar mate fraction, and the apolar mate fraction.

found to inhibit AbPPO.[22] To investigate this, reversed-phase flash chromatography was used to fractionate the mate extract to investigate the effect of the saponins separately from the chlorogenic acid-like compounds. Fractions from flash chromatography were pooled such that a fraction containing mainly chlorogenic acid-like compounds (polar fraction, Figure 5B) and a fraction containing mainly saponins (apolar fraction, Figure 5C) were obtained.

Oxygen consumption measurements with L-DOPA and StPPO in the presence of the apolar or polar mate fraction (Figure 6A) showed that the polar mate fraction seemed to be mainly responsible for activation of StPPO: whereas StPPO was slightly more active in the presence of the apolar mate fraction than with only L-DOPA, activity in the presence of the polar mate fraction was much higher than the blank incubation.

Besides chlorogenic acid-like compounds, caffeine and theobromine were also present in the polar fraction. Whereas the chlorogenic acid-like compounds were converted after incubation with PPO, caffeine and theobromine remained. Experiments with pure caffeine and theobromine showed that they were not responsible for activation of StPPO or inhibition of AbPPO (data not shown). Apparently, the substrates present in the mate extract activated StPPO, also after they had reacted (Figure 4A). To investigate whether the activation of StPPO was indeed due to the chlorogenic acid-like substrates present in the mate extract, StPPO was incubated with pure chlorogenic acid. When oxygen consumption had leveled off, L-DOPA was added. The results obtained were similar to those obtained with mate: oxygen consumption with sequential chlorogenic acid and L-DOPA addition was accelerated compared to a control of StPPO with only L-DOPA (data not shown).

Because of the inhibition of AbPPO by mate, fractionation of the extract indicated that the polar fraction was mainly responsible for inhibition (Figure 6B). In the presence of the apolar mate fraction, oxygen consumption of AbPPO with L-DOPA was comparable to that in the blank reaction, whereas in the presence of the polar fraction the oxygen consumption was decreased compared to that with the blank.

DISCUSSION

Our results of screening 60 plant extracts for StPPO and AbPPO inhibitory activity demonstrated that the effect that an extract can have on PPO-catalyzed browning is dependent on the source of PPO. Some extracts showed inhibitory activity toward both PPOs, whereas other extracts inhibited only one PPO or even inhibited one and activated the other PPO. As an example, a mate extract was investigated in more detail, and it was found to be an activator for StPPO and an inhibitor for AbPPO.

Inhibition of PPOs by Plant Extracts. Of 60 plant extracts screened for inhibitory effects on StPPO and AbPPO, 4 were further investigated by measuring their influence on oxygen consumption. Only the extract of licorice root was found to inhibit the activity of both StPPO and AbPPO (Figure 2), indicating that the observed inhibitory effect on color formation of the other extracts was most likely caused by reducing compounds in the extracts. These compounds only prevent formation of color, but do not inhibit enzyme activity.

The inhibitory effect of licorice root on AbPPO has been described before, and several inhibitory (iso)flavonoids and chalcones have been identified.[23−25] Licorice extracts were found to inhibit both mushroom tyrosinase in vitro and melanin formation in cultured human melanocytes.[24] In addition to this, our results demonstrated that licorice also inhibited StPPO. This might indicate that the inhibitors present in licorice are inhibitors with a wide application range.

Activation of StPPO by Mate Extract. When StPPO was incubated with mate extract, oxygen consumption and conversion of substrates present in the extract was observed. Incubation of StPPO with mate extract had an activating effect on L-DOPA oxidation by the enzyme (Figure 4A). Fractionation of the mate extract demonstrated that chlorogenic acid-like compounds were responsible for this activation, which was confirmed by experiments with pure chlorogenic acid. An explanation for the increased StPPO activity observed might be activation of StPPO, which was extracted in a latent state, by *o*-quinones resulting from chlorogenic acid oxidation. Similarly, activation of latent PPO from red clover (*Trifolium pretense*) by *o*-quinones resulting from oxidation of endogenous substrates has been demonstrated.[26] It was proposed that a conformational change of clover PPO occurred through interaction of *o*-quinones with the protein. Conformational changes have been implicated before in the activation of latent plant PPOs. Treatment with surfactants such as SDS, addition of fatty acids, and pH-induced conformational change have been reported to activate plant PPOs, by making the active site more accessible.[27−30] Possibly, interaction of chlorogenic acid quinones with StPPO induced a conformational change, in this way making its active site more accessible.

In conclusion, our results showed that care should be taken when results of inhibitor studies obtained with one specific PPO are generalized to broader food or cosmetic applications.

dx.doi.org/10.1021/jf4043375 | J. Agric. Food Chem. XXXX, XXX, XXX−XXX

Although screening with a commercially available PPO might be a convenient way to find potential inhibitors, experiments with the target PPO should be done before an inhibitor can be applied in a specific product. Moreover, when a different PPO is used for inhibitor screening than for the final application, potentially useful inhibitors might be overlooked.

■ ASSOCIATED CONTENT

Supporting Information

Solvents used in the production of plant extracts and purification table of potato polyphenol oxidase. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*(H.G.) Phone: +31-317-483211. Fax: +31-317-484893. E-mail: harry.gruppen@wur.nl.

Funding

This study has been carried out with financial support from the Commission of the European Communities within the Seventh Framework Programme for research and technological development (FP7), Grant Agreement 226930, title "Replacement of sulphur dioxide (SO_2) in food keeping the SAme qualitY and shelf-life of the products", acronym SO2SAY.

Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Friedman, M. Food browning and its prevention: an overview. *J. Agric. Food Chem.* **1996**, *44*, 631−653.

(2) Nirmal, N. P.; Benjakul, S. Effect of ferulic acid on inhibition of polyphenoloxidase and quality changes of Pacific white shrimp (*Litopenaeus vannamei*) during iced storage. *Food Chem.* **2009**, *116*, 323−331.

(3) Smit, N.; Vicanova, J.; Pavel, S. The hunt for natural skin whitening agents. *Int. J. Mol. Sci.* **2009**, *10*, 5326−5349.

(4) Chang, T.-S. An updated review of tyrosinase inhibitors. *Int. J. Mol. Sci.* **2009**, *10*, 2440−2475.

(5) Loizzo, M. R.; Tundis, R.; Menichini, F. Natural and synthetic tyrosinase inhibitors as antibrowning agents: an update. *Compr. Rev. Food Sci. Food Saf.* **2012**, *11*, 378−398.

(6) Kim, Y. J.; Uyama, H. Tyrosinase inhibitors from natural and synthetic sources: structure, inhibition mechanism and perspective for the future. *Cell. Mol. Life Sci.* **2005**, *62*, 1707−1723.

(7) Parvez, S.; Kang, M.; Chung, H.-S.; Bae, H. Naturally occurring tyrosinase inhibitors: mechanism and applications in skin health, cosmetics and agriculture industries. *Phytother. Res.* **2007**, *21*, 805−816.

(8) Ros, J. R.; Rodriguez-Lopez, J. N.; Garcia-Canovas, F. Effect of L-ascorbic acid on the monophenolase activity of tyrosinase. *Biochem. J.* **1993**, *295*, 309−312.

(9) Richard, F. C.; Goupy, P. M.; Nicolas, J. J.; Lacombe, J. M.; Pavia, A. A. Cysteine as an inhibitor of enzymic browning. 1. Isolation and characterization of addition compounds formed during oxidation of phenolics by apple polyphenol oxidase. *J. Agric. Food Chem.* **1991**, *39*, 841−847.

(10) Adhikari, A.; Devkota, H. P.; Takano, A.; Masuda, K.; Nakane, T.; Basnet, P.; Skalko-Basnet, N. Screening of Nepalese crude drugs traditionally used to treat hyperpigmentation: *in vitro* tyrosinase inhibition. *Int. J. Cosmetic Sci.* **2008**, *30*, 353−360.

(11) Schurink, M.; van Berkel, W. J. H.; Wichers, H. J.; Boeriu, C. G. Novel peptides with tyrosinase inhibitory activity. *Peptides* **2007**, *28*, 485−495.

(12) Iyengar, R.; McEvily, A. J. Anti-browning agents: alternatives to the use of sulfites in foods. *Trends Food Sci. Technol.* **1992**, *3*, 60−64.

(13) Kuijpers, T. F. M.; Narváez-Cuenca, C.-E.; Vincken, J.-P.; Verloop, A. J. W.; van Berkel, W. J. H.; Gruppen, H. Inhibition of enzymatic browning of chlorogenic acid by sulfur-containing compounds. *J. Agric. Food. Chem.* **2012**, *60*, 3507−3514.

(14) Hossain, M. B.; Rai, D. K.; Brunton, N. P.; Martin-Diana, A. B.; Barry-Ryan, A. C. Characterization of phenolic composition in lamiaceae spices by LC-ESI-MS/MS. *J. Agric. Food Chem.* **2010**, *58*, 10576−10581.

(15) Koukoulitsa, C.; Karioti, A.; Bergonzi, M. C.; Pescitelli, G.; Di Bari, L.; Skaltsa, H. Polar constituents from the aerial parts of *Origanum vulgare* L. ssp. *hirtum* growing wild in Greece. *J. Agric. Food Chem.* **2006**, *54*, 5388−5392.

(16) Bravo, L.; Goya, L.; Lecumberri, E. LC/MS characterization of phenolic constituents of mate (*Ilex paraguariensis*, St. Hil.) and its antioxidant activity compared to commonly consumed beverages. *Food Res. Int.* **2007**, *40*, 393−405.

(17) Puangpraphant, S.; Berhow, M. A.; de Mejia, E. G. Mate (*Ilex paraguariensis* St. Hilaire) saponins induce caspase-3-dependent apoptosis in human colon cancer cells *in vitro*. *Food Chem.* **2011**, *125*, 1171−1178.

(18) Martinet, A.; Ndjoko, K.; Terreaux, C.; Marston, A.; Hostettmann, K.; Schutz, Y. NMR and LC-MS^n characterisation of two minor saponins from *Ilex paraguariensis*. *Phytochem. Anal.* **2001**, *12*, 48−52.

(19) Kermasha, S.; Goetghebeur, M.; Monfette, A.; Metche, M.; Bovel, B. Studies on inhibition of mushroom polyphenol oxidase using chlorogenic acid as substrate. *J. Agric. Food Chem.* **1993**, *41*, 526−531.

(20) Oszmianski, J.; Lee, C. Y. Enzymic oxidative reaction of catechin and chlorogenic acid in a model system. *J. Agric. Food Chem.* **1990**, *38*, 1202−1204.

(21) Narváez-Cuenca, C.-E.; Vincken, J.-P.; Gruppen, H. Identification and quantification of (dihydro) hydroxycinnamic acids and their conjugates in potato by UHPLC−DAD−ESI-MS^n. *Food Chem.* **2012**, *130*, 730−738.

(22) Devkota, K. P.; Khan, M. T. H.; Ranjit, R.; Meli Lannang, A.; Samreen; Iqbal Choudhary, M. Tyrosinase inhibitory and antileishmanial constituents from the rhizomes of *Paris polyphylla*. *Nat. Prod. Res.* **2007**, *21*, 321−327.

(23) Fu, B.; Li, H.; Wang, X.; Lee, F. S. C.; Cui, S. Isolation and identification of flavonoids in licorice and a study of their Inhibitory effects on tyrosinase. *J. Agric. Food Chem.* **2005**, *53*, 7408−7414.

(24) Nerya, O.; Vaya, J.; Musa, R.; Izrael, S.; Ben-Arie, R.; Tamir, S. Glabrene and isoliquiritigenin as tyrosinase inhibitors from licorice roots. *J. Agric. Food Chem.* **2003**, *51*, 1201−1207.

(25) Yokota, T.; Nishio, H.; Kubota, Y.; Mizoguchi, M. The inhibitory effect of glabridin from licorice extracts on melanogenesis and inflammation. *Pigment Cell Res.* **1998**, *11*, 355−361.

(26) Winters, A. L.; Minchin, F. R.; Michaelson-Yeates, T. P. T.; Lee, M. R. F.; Morris, P. Latent and active polyphenol oxidase (PPO) in red clover (*Trifolium pratense*) and use of a low PPO mutant to study the role of PPO in proteolysis reduction. *J. Agric. Food Chem.* **2008**, *56*, 2817−2824.

(27) Kanade, S. R.; Paul, B.; Rao, A. G. A.; Gowda, L. R. The conformational state of polyphenol oxidase from field bean (*Dolichos lablab*) upon SDS and acid-pH activation. *Biochem. J.* **2006**, *395*, 551−562.

(28) Mayer, A. M. Polyphenol oxidases in plants and fungi: going places? A review. *Phytochemistry* **2006**, *67*, 2318−2331.

(29) Pérez-Gilabert, M.; Morte, A.; García-Carmona, F. Histochemical and biochemical evidences of the reversibility of tyrosinase activation by SDS. *Plant Sci.* **2004**, *166*, 365−370.

(30) Golbeck, J. H.; Cammarata, K. V. Spinach thylakoid polyphenol oxidase: isolation, activation, and properties of the native chloroplast enzyme. *Plant Physiol.* **1981**, *67*, 977−984.