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Effect of selected plant extracts on the inhibition of enzymatic browning in fresh-cut apple

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Summary

This study describes the evaluation of the anti-browning effect of 36 plant extracts, divided into three groups: (1) fruits, vegetables, and oil seeds, (2) herbs and tea plants, and (3) medicinal plants, on minimally processed fresh apples. The extracts were applied to fresh-cut apple slices as dipping solutions. Development of browning was analyzed by measuring *L**, *a**, and *b** values. The greatest inhibition of browning was caused by extracts from pumpkin seed (group 1), hibiscus flower (group 2), and pelargonium root (group 3). However, the latter caused intense passive staining. The inhibitory potential might be attributable to the antioxidative activity of secondary plant metabolites, especially phenolic compounds. Furthermore, these bioactive substances might influence enzyme activity directly by acting as competitive or non-competitive inhibitors.

Introduction

Fresh-cut fruits and vegetables have become a popular snack in recent years because of an increased awareness by consumers of the health benefits of fresh fruits and vegetables, as well as an increased demand for convenience food (TOIVONEN, 2006). A major concern is enzymatic browning, which can lead to changes in colour, taste and texture of fresh-cut products and is thus associated with quality deterioration. Enzymatic browning is induced by the action of polyphenoloxidase (PPO) on its phenolic substrates in the presence of oxygen. The oxidation products that are formed can undergo further reactions resulting in complex polymers, which are responsible for browning (MCEVILY et al., 1992). Especially potatoes, mushrooms, bananas, peaches, and apples are prone to oxidative browning (MCEVILY et al., 1992).

For browning control of fresh cut fruits and vegetables ascorbic acid and its derivatives can be used due to their reducing properties; however these effects are temporary. Long-term antibrowning effects can be achieved by sulphur dioxide and sulphites. Thus, these food additives are industrially used to inhibit PPO-induced browning reactions for a wide range of products (MCEVILY et al., 1992), including fresh cut apples sold as intermediate products to gastronomic business areas. However, sulphiting can reduce the uptake of thiamine by degradation of the vitamin and lead to asthmatic reactions in sensitive individuals (MCEVILY et al., 1992; RANGAN and BARCELOUX, 2009). Hence, the replacement of such compounds is an important issue for the food industry. Several approaches, such as the use of dipping treatments with reducing or complexing agents, edible coatings and modified atmosphere packaging have been explored to control the browning of fresh-cut apples (OMS-OLIU et al., 2010; ROJAS-GRAÜ et al., 2009). However, owing to concerns about food safety, off-flavours and off-odours, economic feasibility, and the effectiveness of inhibition, only a few substances and methods have shown potential for use in the food industry (MCEVILY et al., 1992; PARK, 1999). Thus, further investigation of alternative methods is required. One approach is the application of plant extracts to inhibit browning in apple products. Such extracts could serve as reducing agents because of the antioxidant capacity of the secondary plant metabolites present. Furthermore, phenolic compounds might influence PPO activity directly by acting as, for example, competitive or non-competitive inhibitors. Promising results have been obtained with rhubarb juice, pineapple juice, and green tea extract, which were all able to prevent discolouration of cut surfaces of apples (SOYSAL, 2009; SON et al., 2000; LOZANO-DE-GONZALEZ et al., 1993). At the same time several phenolic compounds can be PPO substrates depending on the presence and position of substituents (CHANG, 2009).

To compare the anti-browning effects of different plant-derived PPO inhibitors on fresh-cut apples it is essential to conduct assays under identical conditions. In the study described herein, 36 plant extracts were screened and their polyphenol content and antioxidant capacity determined to identify promising anti-browning agents.

Material and methods

Plant material and chemicals

Apple cv. 'Jonagold' was chosen for all investigations in order to obtain comparable results for all extracts. The fruits were delivered by Meyer Gemüseverarbeitung GmbH (Twistringen, Germany). Following harvest at commercial maturity, the fruits were stored at 10 - 12 °C until processed.

Folin-Ciocalteu reagent, 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) were obtained from Fluka (Neu-Ulm, Germany). Caffeic acid was purchased from Sigma Aldrich (Steinheim, Germany). All other chemicals were obtained from Merck (Darmstadt, Germany).

Dipping solutions of plant extracts

Thirty-six different plant extracts that were divided into three major groups (Tab. 1) were tested. Except for the grapefruit extract, which was obtained from Eurochem Feinchemie GmbH (Gröbenzell, Germany), all extracts were provided by Frutarom Switzerland Ltd (Wädenswil, Switzerland). The extracts were used as aqueous dipping solutions at a concentration of 1 % (w/v). After dilution, the extract solutions were autoclaved (121 °C, 15 min) and centrifuged (7025 *g*, 10 min). As a reference, 0.14 % sodium bisulphite solution was prepared by the same method except for the centrifugation step.

Characterization of plant extracts

The plant extracts were characterized by determining the pH value, the total polyphenol content (TPP), and the antioxidative capacity of the 1 % aqueous extract solutions. The TPP of the extracts was determined by the Folin-Ciocalteu method, modified after TANNER and BRUNNER (1987) and RITTER (1994). Folin-Ciocalteu reagent (75 µL) was mixed with 15 µL of extract and 1260 µL of distilled water. After 3 min, 150 µL of a saturated Na_2CO_3 solution were added. After 1 h, the absorbance of the sample was measured at 720 nm. The TPP was calculated from a calibration curve obtained using caffeic acid and thus was expressed as caffeic acid equivalents. The antioxidant capacity of the extract was determined using the

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Tab. 1: Plant extracts investigated for their anti-browning properties (extract sources, botanical names, and commercial names according to supplier: www.frutarom.com, www.eurochem-feinchemie.com).

Group I: Fruits, vegetables & oil fruits/ seeds				
Extract source	**Botanical name**	**Plant part**	**Extraction solvent**	**Commercial name**
Acai	*Euterpe oleracea* Mart.	Fruit	Water	Acai 4:1
Grapefruit	*Citrus paradisi* Macf.	Fruit	Water	Grapefruitextrakt WS
"Superberry"	*Vitis vinifera* L.,	Fruit	Water	Superberry 4000
	Punica granatum L.,			
	Vaccinium macrocarpon Ait.,			
	Vaccinium corymbosum L.,			
	Vaccinium myrtillus L.,			
	Fragaria vesca L.,			
	Rubus idaeus L.			
Grape	*Vitis vinifera* L.	Seed	Water	OPC Grape seed
Baobab	*Adansonia digitata* L.	Fruit	Ethanol	u. d.
Broccoli	*Brassica oleracea* L. var. *italica*	Seed	CO_2 (supercritical)	BroccoRaphanin
Horseradish	*Armoracia rusticana*	Root	Ethanol	u. d.
Artichoke	*Cynara scolymus* L.	Leaf	Water	Artichoke leaf powder extract
Garlic	*Allium sativum* L.	Bulb	Ethanol	White garlic powder extract
Pumpkin	*Curcubita pepo* L.	Seed	Ethanol	Pumpkin seed powder extract
Soy	*Glycine max*. L.	Hypocotyl	Ethanol	SoyLife 40%
Flax	*Linum usitatissimum* L.	Hull	Ethanol	LinumLife EXTRA
Cranberry	*Vaccinium macrocarpon* Ait.	Fruit	Water	Cranberry high PAC
Purslane	*Portulaca oleracea* L.	Herb	Ethanol	Purslane herb powder extract
Group II: Herbs & tea plants				
Extract source	Botanical name	Plant part	Extraction solvent	Commercial name
Oregano	*Origanum vulgare* L.	Herb	Water	Origanox WS
Rosemary	*Rosmarinus officinalis* L.	Leaf	Water	Rosemary leaf powder extract
Lemon balm	*Melissa officinalis* L.	Herb	Water	Balm leaf powder extract
Green tea	*Camellia sinensis* L.	Leaf	Ethanol	Green tea leaf powder extract
Chamomile	*Chamomilla recutita* L.	Flower	Ethanol	Chamomile flower powder extract
Hibiscus	*Hibiscus* L.	Flower	Water	u. d.
Pink rockrose	*Cistus incanus* L.	Herb	Ethanol	Pink rockrose herb powder extract
Green mate	*Ilex paraguariensis* A. St.-Hil.	Leaf	Ethanol	Finomate
Nettle	*Urtica dioica* L.	Leaf	Water	Nettle leaf powder extract
Sage	*Salvia officinalis* L.	Leaf	Water	Sage leaf powder extract
Olive	*Olea europaea* L.	Leaf	Ethanol	Benolea
Group III: Medicinal plants				
Extract source	Botanical name	Plant part	Extraction solvent	Commercial name
Red wine	*Vitis vinifera* L.	Leaf	Water	Red vine leaf powder extract
Pelargonium	*Pelargonium sidoides* DC.	Root	Ethanol	Pelargonium root powder extract
Goldenrod	*Solidago* sp.	Herb	Ethanol	Goldenrod herb powder extract
Eyebright	*Euphrasia* sp.	Herb	Water	Eyebright herb powder extract
Java tea	*Orthosiphon stamineus* Benth.	Leaf	Ethanol	Java tea powder extract
Bearberry	*Arctostaphylos uva-ursi* (L.) Spreng.	Leaf	Water	Bearberry leaf powder extract
Chasteberry	*Vitex agnus-castus* L.	Fruit	Ethanol	Chaste berry powder extract
Oat	*Avena sativa* L.	Herb	Water	Oats herb powder extract
Willow	*Salix* sp.	Bark	Water	Willow bark powder extract
Passion Flower	*Passiflora incarnata* L.	Herb	Ethanol	Passion flower herb powder extract
Ivy	*Hedera helix* L.	Leaf	Ethanol	Ivy leaf powder extract

u. d. = under development

method of KAO and CHEN (2006) with modifications. An aqueous solution of 7 mM ABTS was prepared, to which 2.45 mM potassium persulphate were added to generate the ABTS radical ($ABTS^{\bullet+}$) by incubation in the dark at ambient temperature for 12 h. The $ABTS^{\bullet+}$-solution obtained was diluted with 1 mM PBS buffer (pH 7.4) to an absorbance at 734 nm of 1.00 (± 0.02). Trolox dissolved in 1 mM PBS buffer was used as a reference. Ten microliters of the standard or sample were mixed with 1 mL of $ABTS^{\bullet+}$; as a control, 10 µL of PBS buffer were mixed with 1 mL of $ABTS^{\bullet+}$. After 60 min, absorbance at 734 nm was recorded in triplicate against PBS as a blank. The degree of quenching of the $ABTS^{\bullet+}$ radical was calculated as follows (FOGLIANO et al., 1999):

$$\text{Inhibition (\%)} = 1 - A_S/A_C \times 100$$

where A_C was the absorbance of the control and A_s the absorbance of the sample at 734 nm. The antioxidant property of the extracts was calculated from a calibration curve of $ABTS^{\bullet+}$ inhibition plotted against Trolox concentration and was expressed as Trolox equivalent antioxidant capacity (TEAC).

Slicing and dipping

Selected apples of uniform size and colour were manually washed, cored, and sliced longitudinally using a manual corer-slicer (Tchibo GmbH Hamburg, Germany). For each extract, three apple slices (thickness of inner cutting edge 9 mm, width 25 – 35 mm) were soaked in dipping solution at ambient temperature for 2 min. The excess liquid was removed and the samples were stored at room temperature for 4 h.

Colour measurement

The development of browning in the treated apple slices and the reference slices was measured with a colorimeter (CR 400, Konica Minolta, Tokyo, Japan) with the standard illuminant D65. The instrument was calibrated with a white tile before the first measurement. Colour (L^*, a^* and b^* values) was measured in untreated apple slices (initial colour directly after cutting; L_C, a_C, b_C), at zero time (directly after dipping; L_{0h}, a_{0h}, b_{0h}), and after 4 h (L_{4h}, a_{4h}, b_{4h}). The results are presented as the means of three replicates of three measured samples. The difference in lightness (ΔL_{4h}), which is associated with the degree of browning (MASKAN, 2006), was calculated as: $L_{0h} - L_{4h}$. The passive staining by the plant extracts was determined separately as the total colour change given by the parameter ΔE, which was calculated using the following equation:

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$$

with $\Delta L = L_{0h} - L_C$, $\Delta a = a_{0h} - a_C$, and $\Delta b = b_{0h} - b_C$.

Statistical analysis

To determine significant differences between treatments, the experimental data for ΔL_{4h} were analyzed with Duncan's multiple range tests ($p = 0.05$) as implemented in SPSS 18.0 (IBM Deutschland GmbH, Ehningen, Germany). Prior to this post-hoc test, normal distribution and homogeneity of the variance were tested by Kolmogorov-Smirnov test and Levene test.

Results

Many secondary plant compounds, such as polyphenols and carotenoids, are known to have antioxidative properties (MOURE et al., 2001). Thus, they might serve as reducing agents to prevent food products from enzymatic browning by, for example, reducing pigment intermediates such as quinones. Some polyphenols are also known to inhibit PPO competitively, non-competitively, or by acting as a chelating agent (CHANG, 2009). Fruits, vegetables, and oil fruits/seeds are rich in polyphenols, carotenoids, and other antioxidative secondary plant compounds. Fourteen extracts from such sources were tested as anti-browning agents (group I). Also many herbs and tea plants contain significant amounts of secondary plant metabolites, such as flavonoids, which possess strong antioxidant properties (IVANOVA et al., 2005). Hence, 11 extracts from herbs and tea plants were tested (group II). Medicinal plants are known to contain considerable amounts of phenolic acids and flavonoids, especially hydrolyzed and condensed tannins, respectively. As described above, tannins are able to inactivate PPO by binding copper or proteins. In the present study, 11 extracts from medicinal plants were tested for their inhibitory effect on browning in apple slices (group III). Fig. 1 shows the ΔL_{4h} values of the extract-treated apple slices, which represent the difference in browning. The total change in colour of the apple slices that had occurred after dipping is an indicator of the passive staining by the extracts and data are shown in Tab. 2. As pH influences PPO activity, the pH values of the used extract solutions were measured. The results are shown in Tab. 3.

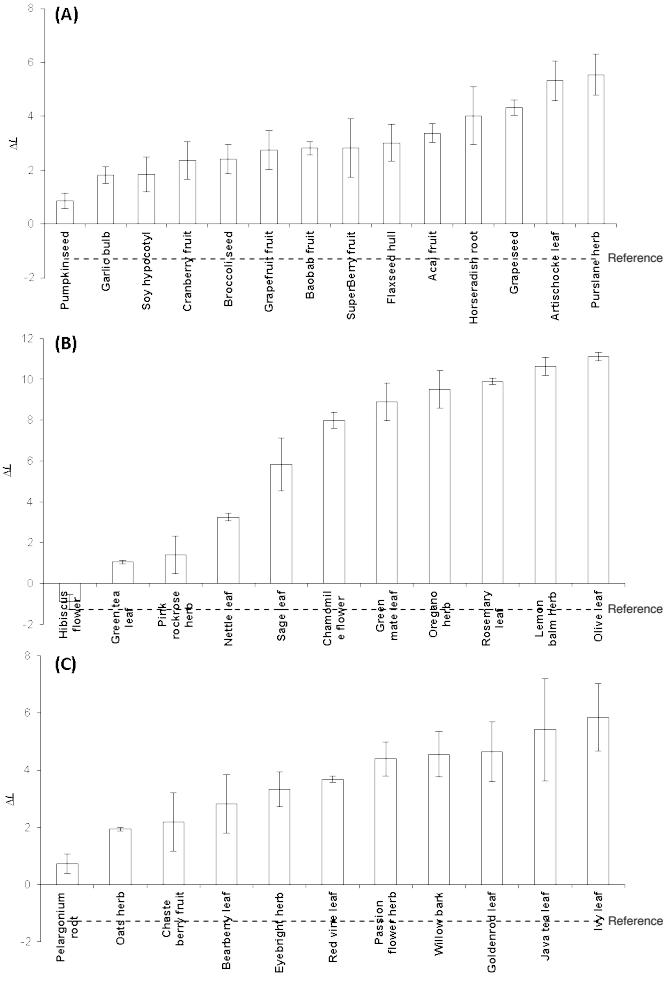

Fig. 1: Browning of fresh-cut apples treated with 1 % plant extract solutions 4 h after treatment compared to the sodium bisulfite solution (reference). Apple samples were treated with extracts from (A) fruits, vegetables, and oil fruits/seeds, (B) herbs and tea plants, and (C) medicinal plants. Vertical bars represent the standard deviation of the mean (n = 3).

Tab. 2: Passive staining of apples by the 36 chosen plant extracts (expressed as ΔE); for comparison: the sodium bisulfite solution (reference) had a ΔE of 0.76.

Fruits, vegetables and oil fruits / seeds	Passive staining (*ΔE*)	Herbs and tea plants	Passive staining (*ΔE*)	Medicinal plants	Passive staining (*ΔE*)
Pumpkin seed	2.04	Hibiscus flower	3.94	Pelargonium root	26.25
Garlic bulb	2.54	Green tea leaf	6.94	Oats herb	1.35
Soy hypocotyl	2.49	Pink rockrose herb	4.85	Chasteberry fruit	3.60
Cranberry fruit	2.01	Nettle leaf	7.01	Bearberry leaf	2.53
Broccoli seed	1.88	Sage leaf	3.51	Eyebright herb	3.02
Grapefruit fruit	2.83	Chamomile flower	0.99	Red wine leaf	6.49
Baobab fruit	0.43	Green mate leaf	1.40	Passion flower herb	4.13
"Superberry" fruit	14.11	Oregano herb	2.60	Willow bark	6.60
Flaxseed hull	2.85	Rosemary leaf	4.51	Goldenrod herb	1.27
Acai fruit	1.85	Lemon balm herb	3.55	Java tea leaf	6.37
Horseradish root	1.72	Olive leaf	2.04	Ivy leaf	1.54
Grape seed	23.03				
Artichoke leaf	0.96				
Purslane herb	7.53				

Tab. 3: pH values, TPP (mg/100 mL) and TEAC (mM) of plant extract solutions (1 %) at room temperature (18 – 22 °C); for comparison: the sodium bisulfite solution (reference) had a pH of 3.7.

Group I				Group II				Group III			
Fruits, vegetables and oil fruits/seeds	pH	TPP (mg/ 100 mL)	TEAC (mM)	Herbs and tea plants	pH	TPP (mg/ 100 mL)	TEAC (mM)	Medicinal plants	pH	TPP (mg/ 100 mL)	TEAC (mM)
Pumpkin seed	5.7	12.38	2.43	Hibiscus flower	2.8	18.79	2.83	Pelargonium root	5.0	484.00	131.80
Garlic bulb	6.0	16.46	4.25	Green tea leaf	4.4	445.52	162.96	Oats herb	5.6	26.93	3.81
Soy hypocotyl	5.3	41.82	22.96	Pink rockrose herb	4.9	196.14	36.83	Chasteberry fruit	4.7	41.21	7.10
Cranberry fruit	3.6	2.73	17.02	Nettle leaf	7.0	52.29	7.99	Bearberry leaf	4.4	408.36	83.84
Broccoli seed	6.0	13.25	15.34	Sage leaf	5.3	174.78	31.01	Eyebright herb	5.6	55.64	8.90
Grapefruit fruit	4.4	141.62	45.28	Chamomile flower	4.8	61.65	13.54	Red wine leaf	4.7	131.96	34.48
Baobab fruit	3.2	14.31	2.87	Green mate leaf	5.0	309.97	40.70	Passion flower herb	5.1	61.82	14.65
Superberry fruit	3.8	311.43	49.89	Oregano herb	4.5	275.43	62.95	Willow bark	5.4	214.54	42.43
Flaxseed hull	6.4	88.58	22.98	Rosemary leaf	5.3	204.02	34.44	Goldenrod herb	5.1	145.86	36.03
Acai fruit	3.6	4.92	7.44	Lemon balm herb	5.6	249.66	33.09	Java tea leaf	5.4	187.78	32.59
Horseradish root	5.2	8.62	1.66	Olive leaf	4.4	132.83	22.33	Ivy leaf	4.6	61.00	10.98
Grape seed	4.0	623.78	106.13								
Artichoke leaf	5.4	37.10	2.50								
Purslane herb	5.3	74.65	15.23								

Discussion

Extracts of group I

The greatest inhibition of browning was shown by pumpkin seed extract; apple slices treated with this extract showed only a slight darkening over the 4 h period of measurement. Analysis with Duncan's multiple range test ($p = 0.05$) showed that pumpkin seed, garlic bulb, and soy hypocotyl extracts formed a homogeneous subgroup of inhibitors (subgroup 1). In this subgroup, pH did not influence the inhibitory effect of the extracts, which had pH values between 5.3 and 6.0. These values were similar to the optimal pH (5.5) for PPO from the apple cv. 'Golden Delicious' that was reported by SOYSAL (2009). Approximately half of the other extracts had pH values lower than 5 but were identified to be less potent inhibitors of browning.

One class of secondary plant compounds that is characteristic of the three extracts in subgroup 1 and present in considerable amounts are phytoestrogens. The major class that is present in pumpkin seed and garlic are lignans, such as secoisolariciresinol, whereas in soy the main phytoestrogens are isoflavones, such as genistein, daidzein, glycitein, and their glycosides, as well as lignans (MURPHY and

Hendrich, 2002). Although phytoestrogens are known to possess antioxidant activity (Moure et al., 2001) in subgroup 1 only soy extract showed a strong antioxidant activity, with a TEAC value of 22.96 mM (Tab. 3), which was correlated with its polyphenol content (Fig. 2A).

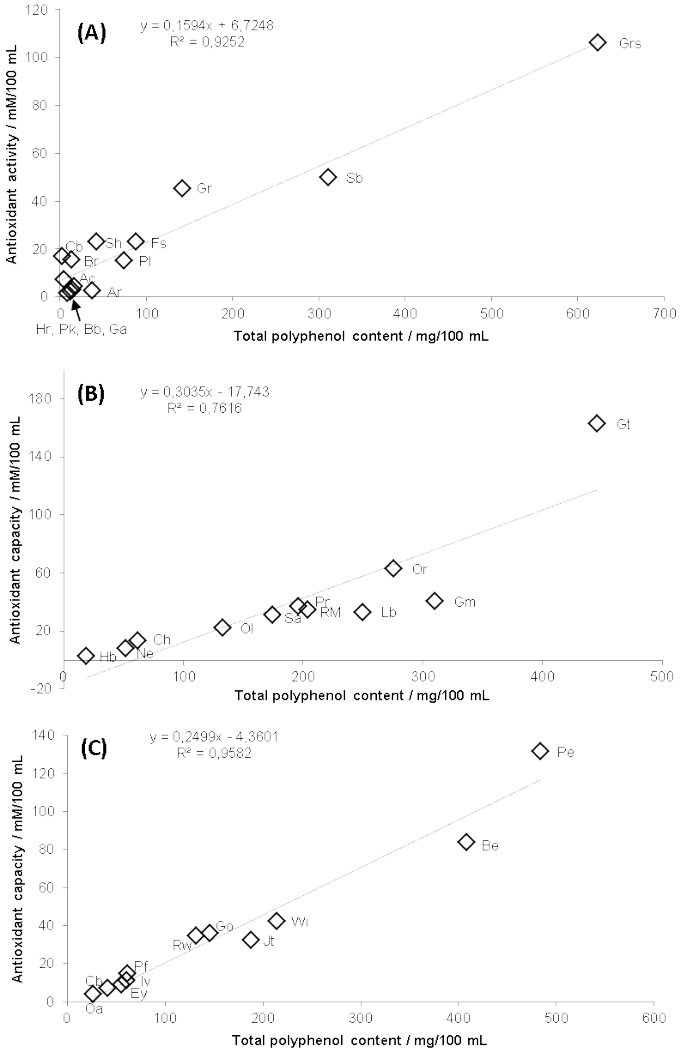

Fig. 2: Linear correlation of total polyphenol content (mg/100 mL) and antioxidant capacity (mM/100 mL). Fresh-cut apple samples were treated with 1 % solutions of extract from (A) fruits, vegetables, and oil fruits/seeds (Ac: Acai fruit, Ar: Artichoke leaf, Bb: Baobab fruit, Br: Broccoli seed, Cr: Cranberry fruit, Fs: Flaxseed hull, Ga: Garlic bulb, Gr: Grapefruit fruit, Grs: Grape seed, Hr: Horseradish root, Pk: Pumpkin seed, Pl: Purslane herb , Sb: Superberry fruit, Sh: Soy hypocotyl), (B) herbs and tea plants (Ch: Chamomile flower, Gm: Green mate leaf, Gt: Green tea leaf, Hb: Hibiscsus flower, Lb: Lemon balm herb, Ne: Nettle leaf, Ol: Olive leaf, Or: Oregano, Pr: Pink rockrose herb, Rm: Rosemary leaf, Sa: Sage leaf), and (C) medicinal plants (Be: Bearberry leaf, Cb: Chasteberry fruit, Ey: Eyebright herb, Go: Goldenrod herb, Iv: Ivy leaf, Jt: Java tea leaf, Oa: Oats herb, Pe: Pelargonium root, Pf: Passion flower herb, Rw: Red wine leaf, Wi: Willow bark).

Hence, this extract might act as reducing agent to retard enzymatic browning. In addition to their reducing properties, the phytoestrogens that are present in the extracts could influence PPO activity directly as enzyme inhibitors. In the present study, subgroup 1 did not cover all the extracts from plants that contain high amounts of lignans. The highest concentrations of lignans are found in flaxseed (3710 µg/g dry weight) followed by pumpkin seeds (214 µg/g dry weight) (Adlercreutz and Mazur, 1997). However, in the present study, pumpkin seed extract was a more potent inhibitor of browning than flaxseed hull extract. A reason for flaxseed lignans being weak PPO inhibitors might be their complex structure. Lignans are present mainly in bound form and usually occur as a lignan complex of esterified secoisolariciresinol diglucosides (Struijs et al., 2009). Karioti et al. (2007) investigated the inhibition of tyrosinase by lariciresinol glycosides from species of Lamiaceae and found that diglycosides are less potent inhibitors than glycosides, probably because of a lack of free hydroxyl groups.

Given that the secoisolariciresinol content of broccoli (4.14 µg/g dry weight) is much lower than that of flaxseed (Adlercreutz and Mazur, 1997), a different class of substances must be responsible for the higher anti-browning effects of broccoli seed extract. These compounds might be glucosinolates, which are sulphur- and nitrogen-containing substances derived from amino acids and glucose and are found in considerable amounts in *Brassica* vegetables (Verkerk and Dekker, 2008). Recently, Narváez-Cuenca et al. (2011) found out that sodium hydrogen sulphite reacts with *o*-quinones formed during PPO reactions, particularly with caffeic acid-containing compounds. Following a nucleophilic attack sulpho-adducts are formed, which are not involved in further browning reactions. It needs to be investigated, whether chlorogenoquinones formed of chlorogenic acid, which is a main phenolic compound of apples, is attacked by sulphur groups of glucosinolates in a similar way.

The secoisolariciresinol content in broccoli is similar to that in garlic (3.79 µg/g) (Adlercreutz and Mazur, 1997), but the degree of browning indicated by ΔL_{4h} is higher for apples treated with broccoli seed extract than those treated with garlic bulb extract. The inhibition of apple PPO by the latter might be the result of sulphuric compounds that are found in *Allium* species. Garlic is rich in alliin, which is transformed enzymatically into water-soluble, lipid-soluble, and volatile compounds by alliinase following mechanical rupture of cells (Crozier et al., 2006). Given that aqueous extract solutions were used in the study, the main active agents in the garlic bulb dipping solution would be (partially) water-soluble substances such as *N*-acetylcysteine. It is known to inhibit apple PPO (Son et al., 2001) and loquat PPO (Ding et al., 2002), although the exact inhibitory mechanism is unclear. Comparably, Kim, Kim and Park (2005) have shown that onion extract can inhibit browning of pears and attributed it to the thiol-containing compounds.

Given that soy hypocotyl extract belonged to subgroup 1, the results suggest that isoflavones also act as an inhibitor of apple PPO. Tyrosinase inhibition by isoflavones that are contained in soy was demonstrated by Chang et al. (2007). These authors found that daidzein, glycitein, daidzin, and genistin act as competitive inhibitors that reversibly inhibit the monophenolase activity of mushroom tyrosinase. In addition, there is evidence that the position and number of the hydroxyl group in the A-ring of the isoflavone structure could affect both the strength and mode of inhibition of isoflavones on mushroom tyrosinase (Chang et al., 2007). However, details on the mechanism of inhibition remain unclear.

Within the extracts of fruits, vegetables, and oil fruits/seeds the correlation coefficients between TPP and ΔL_{4h} (0.07) and TEAC and ΔL_{4h} (0.03) respectively underline the lack of correlation between antibrowning and reducing properties. Grape seed and "superberry" fruit extract showed significantly higher values for TEAC and TPP but did not have strong PPO inhibitory potential. The high TEAC values of grape seed extract can mainly be attributed to the procyanidins. The antioxidant potential of proanthocyanidins is 20 times higher than that of vitamin E and 50 times higher than that of vitamin C (Shi et al., 2003). Hence, the inhibition of browning by

procyanidins acting as reducing agents may be expected. The low level of inhibition of PPO by grape seed extract suggests that an additional factor in the extract counteracts the positive effects of the procyanidins with respect to browning. This factor might be the high content of (+)-catechin and gallic acid, which act as PPO substrates (SELINHEIMO et al., 2007; KUBO et al., 2000).

"Superberry" fruit extract, which is obtained from seven different berries [grape and pomegranate (main ingredients), cranberry, blueberry, bilberry, strawberry, and raspberry], also showed high values for TEAC and TPP. The predominant polyphenols in dark blue-, red-, and purple-colored fruits, such as blueberries, cranberries, and strawberries, are anthocyanins. Almost all of these fruits also contain hydroxycinnamic acids, hydroxybenzoic acid derivatives, flavonols, and flavanols, as well as proanthocyanidins (NACZK and SHAHIDI, 2006). The antioxidant properties of these classes of polyphenols indicate that they act mainly as reducing agents, and thus may inhibit browning temporarily. However, given that compounds from grape and pomegranate are the main ingredients, the extract will also contain considerable amounts of PPO substrates, such as (+)-catechin, gallic acid, chlorogenic acid, and ellagic acid.

Extracts of herbs and tea plants

Among the extracts tested, only hibiscus flower extract showed strong potential as an inhibitor of browning and represented a separate homogeneous subgroup, as indicated by Duncan's multiple range test. The extract even seemed to bleach the apple slices, as indicated by the negative value for ΔL_{4h}. The antioxidative properties of hibiscus (*Hibiscus sabdariffa*) flavonoids (e. g. hibiscitrin, gossytrin, quercetrin, sabdaretin, and gossypetin-8-, -7- and -3-glucosides (HEGNAUER, 1990)) and anthocyanins (e. g. cyanidin-3-diglycosides and cyanidin-3,5 bismonoglucoside (HEGNAUER, 1990)) might explain the PPO inhibition. However, given that the TEAC and TPP values of hibiscus flower extract were the lowest among the extracts from herbs and tea plants, the anti-browning effect cannot be attributed solely to polyphenols. The pH of the extract seems to play an important role because it was lower than 3, and thus is within the range of potential to influence PPO activity. MCEVILY et al. (1992) mentioned that PPO is inactivated completely at pH levels below 3. The low pH of the extract derived from flowers of *Hibiscus sabdariffa* might reflect the high content of a number of organic acids, such as citric acid, malic acid, tartaric acid, oxalic acid, and hibiscus acid (HILLER and LOEW, 2009). Besides lowering the pH organic acids are able to form complexes with copper, the cofactor of PPO and thus to influence the enzyme activity. For instance oxalic acid was determined to be a noncompetitive inhibitor due to the binding with copper to form an inactive complex (YORUK and MARSHALL, 2003). Among the tested extracts derived from herbs and tea plants, TPP and TEAC did not correlate with the anti-browning properties of the extracts, approved by the correlation coefficients 0.02 and 0.04. As already mentioned, hibiscus flower extract showed the lowest antioxidant capacity and polyphenol content. The highest values were observed for green tea leaf extract. This finding is consistent with previous studies which indicated that green tea flavonoids possess strong antioxidative potential that is correlated with the total phenolic content of tea (SOYSAL, 2009).

Green tea leaf extract ranked second with respect to the inhibition of browning. This finding is consistent with the results reported by SOYSAL (2009), who found that flavonoid-rich green tea extract has an inhibitory effect on apple PPO activity.

It is reasonable to assume that the inhibitory effect of green tea leaf extract on browning depends on flavonoids, in particular catechins, which can be divided into free catechins and esterified catechins (HARA, 2001). Besides acting as reducing agents, flavonoids with a free 3-hydroxy group inhibit tyrosinase by chelating copper (PARVEZ et al., 2007). Due to their structure, free catechins in green tea extract belong to the group of copper chelating agents. But, as mentioned above, (+)-catechin can also act as PPO substrate. The esterified catechins (–)-epicatechin gallate (ECG) and (–)-epigallocatechin gallate (EGCG) have been identified as competitive tyrosinase and melanogenesis inhibitors, respectively (LIN et al., 2008; SELINHEIMO et al., 2007; PARVEZ et al., 2007). It is assumed that gallate moieties bound to the 3-hydroxyl position of flavon-3-ols are responsible for the strong inhibitory effect of ECG and EGCG (PARVEZ et al., 2007). Furthermore, PPO might be deactivated by reaction with tannins, such as esterified green tea catechins and condensed tannins based on green tea catechins. Tannins are known to form complexes with heavy metal ions such as copper (KRAAL et al., 2006). Given that PPO contains copper in its active site (MCEVILY, 1992), the enzyme might be complexed by the tannins in the extract. Furthermore, tannins can bind proteins resulting in protein precipitation (CROZIER et al., 2006). Relating to enzymes it was shown that *ß*-glucosidase and esterases can be precipitated by tannins from the bark of *Betula*, *Salix*, and *Pinus* species (JUNTHEIKKI and JULKUNEN-TIITTO, 2000). However, the exact mechanisms of PPO inhibition by green tea flavonoids remain to be determined.

Duncan's multiple range test indicated that pink rockrose herb extract and green tea leaf extract formed a homogeneous subgroup. Their comparable anti-browning effects might be attributable to the identical profile of catechins in green tea and pink rockrose (POMPONIO et al., 2003).

Other extracts within the group of herbs and tea plants also showed high TTP contents that were correlated with TEAC (Fig. 2B). However, given that these extracts did not show promising anti-browning effects, they are not discussed further.

Extracts of medicinal plants

Among the group of medicinal plants, pH had no influence on the inhibitory effect of the extracts. The pH values of the extracts ranged from 4.4 to 5.6, which was close to the pH optimum of 5.5 for apple PPO (SOYSAL, 2009). Thus, the effects on browning can be attributed to the effects of substances present in the extracts.

The greatest inhibition of browning was achieved with pelargonium root extract, for which only a slight colour change was observed over the period of measurement. Duncan's multiple range test ($p = 0.05$) indicated that the extracts from pelargonium root, oats herb, and chasteberry fruits formed a homogeneous subgroup of inhibitors. Pelargonium root extract showed the strongest inhibition of browning and the highest levels of TEAC and TPP. The polyphenols in pelargonium root extract are mainly flavonoids, tannins, and coumarins, especially umckalin (WIESENAUER, 2011). The anti-browning potential might be attributed to the reducing properties of the polyphenols, but this effect is only temporary. On the other hand, particular flavonoids and tannins can inactivate PPO by complexing copper or reacting with proteins. Similar mechanisms might occur in apples treated with chasteberry fruit extract which also contains tannins. In addition, chasteberry fruits contain neolignans, such as ficusal, vladirol, and balanophonin (CHEN et al., 2011), which might be responsible for the anti-browning potential of chasteberry extract. TIAN, KANG, KIM and KIM (2005) found out that honokiol, a neolignan found in the cortex of *Magnolia* species, inhibits mushroom tyrosinase significantly.

In contrast to pelargonium root and chasteberry fruit extracts, oats herb extract does not contain tannins. Oats herb contains mainly flavonoids; nevertheless, the overall content of phenolic compounds in oats herb extract was low, as indicated by the results of the Folin and TEAC assays. Hence, the main anti-browning activity cannot be attributed to reducing compounds. Additional secondary plant compounds that are contained in oats herb are the phytoalexins

avenalinum I – III. No information is available on the potential effects of these substances on apple PPO. Oats herb also contains minerals such as calcium. The extract was recovered with water (Tab. 1), so it is expected to be rich in minerals compared with extracts obtained by ethanolic extraction. Thus, minerals might contribute to the inhibitory effect of oats herb extract. AYAZ et al. (2008) reported that several minerals, including calcium, reduced the activity of PPO extracted from medlar (*Mespilus germanica* L.) fruit.

The results of the TEAC and Folin assays were not correlated with the anti-browning activity (ΔL_{4h}) of the extracts of medicinal plants, underlined by the correlation coefficients 0.001 and 0.01. The extracts that showed the highest antioxidant activity and polyphenol content were scattered among all the homogeneous subgroups resulting from Duncan's multiple range test, namely pelargonium root extract (subgroup 1), bearberry leaf extract (subgroup 2), willow bark extract (subgroup 3), and java tea leaf extract (subgroup 4). In bearberry leaf extract, arbutin, which is a hydroquinone glycoside, might inhibit PPO competitively because of its structural similarities with the substrate tyrosine. This knowledge is useful owing to the use of arbutin in depigmentation products in the cosmetic industry in recent years (ZHU and GAO, 2008). Willow bark and java tea leaf extracts did not show promising anti-browning effects and are not discussed further.

Comparison of anti-browning activity among the three extract groups according to Tab. 1 showed that extracts from groups 1 and 3 were stronger inhibitors than extracts from group 2, as indicated by the maximum ΔL_{4h} values.

Browning and total colour change of extract-treated apple slices in comparison with the reference

The passive staining was negligible for most of the extracts that were identified as potential inhibitors of browning (Tab. 2). Only treatment with pelargonium root extract, which was the strongest inhibitor within the extract group of the medicinal plants, resulted in orange staining. The ΔE value for pelargonium root extract was at least six times higher than ΔE for apples treated with hibiscus flower or pumpkin seed extract (Fig. 3). The latter two extracts showed ΔE values that were similar to those of the reference samples, which were treated with sodium bisulphite. Regarding the development of browning, only treatment with hibiscus flower extract resulted in a negative ΔL_{4h} value which is comparable to the reference and represents bleaching of the apples. The ΔL_{4h} of apples treated with pumpkin seed extract was close to zero and little passive staining of the apple slices was observed. Overall, hibiscus flower and pumpkin seed extracts showed the highest potential among the extracts investigated to replace sodium bisulphite as an anti-browning agent.

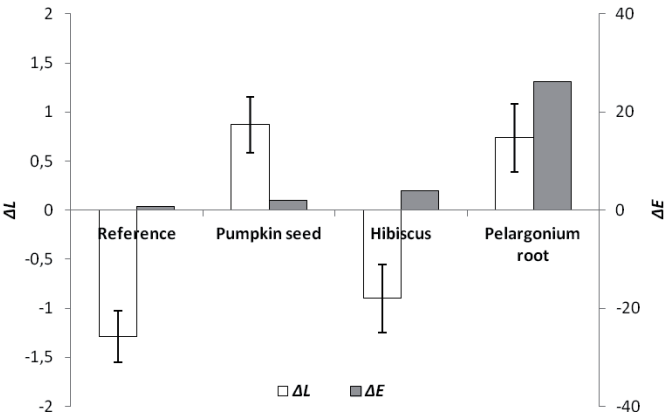

Fig. 3: Browning after 4 h measurement and total colour change at zero time (directly after dipping) of apple-fresh-cuts treated with sodium bisulfite (reference) compared to pumpkin seed, hibiscus and pelargonium root extract. Vertical bars represent standard deviation of the means (n = 3).

Conclusion

The aim of this study was to identify the most effective anti-browning agent(s) for fresh-cut apple from among different plant-derived extracts. Thirty-six extracts that were categorized into three main groups were tested for their inhibitory effects on enzymatic browning. The extracts that showed the highest potential in each group were: pumpkin seed extract (group 1), hibiscus flower extract (group 2), and pelargonium root extract (group 3). Pumpkin seed and hibiscus flower extract can be considered as substitutes for sulfites, whereas pelargonium root extract is not suitable because of the strong passive staining. In general, it was shown that the content of reducing compounds, determined as TPP and TEAC, does not correlate with the anti-browning activity of the investigated plant extracts. Thus, browning control by plant extracts is not only based on reduction of *o*-quinones, but might also be attributed to competitive and non-competitive PPO inhibition. Further investigations are needed to clarify, whether the anti-browning effects might be attributed to particular substances or in case of hibiscus flower extract resulted from the low pH. Likewise, the effectiveness, the economic feasibility, the effects on sensorial quality parameters, and the nutritional hazards of the practical application of extracts like pumpkin seed and hibiscus flower extract have to be investigated. In addition, research on the microbiological potential of these extracts should be conducted because sulfurization is also used to preserve food.

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