Europ.J.Hort.Sci., **75** (5). S. 214–220, 2010, ISSN 1611-4426. © Verlag Eugen Ulmer KG, Stuttgart

Phenolic Acids and Flavonoids in Tea Processed from Flowers of Black Elder (*Sambucus nigra* L.) Stored in Different Packing Materials

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Summary

The aim of the present study was to investigate the effects of cultivar, packing material and storage time on the content of phenolic acids and flavonoids in elderflower tea (ET) processed from flowers of the black elder (*Sambucus nigra*) cultivars 'Sampo', 'Sambu' and 'Samyl'. ET was processed by placing elderflowers in a thin layer on nylon screens in woody frames placed in a cool room and dried using a dehumidified air flow above and below the nylon screens for 96 h at 5 °C. The yield of ET for the cultivars 'Sampo', 'Sambu' and 'Samyl' were 12.8 %, 15.7 % and 8.9 %, respectively. A clear significant difference in the content of polyphenols was found between ET processed from the three cultivars. The total content of phenolic acids was significant higher in 'Samyl' (41.3 mg g^{-1} ET) compared to 'Sampo' (33.8 mg g^{-1} ET) and 'Sambu' (34.0 mg g^{-1} ET). 'Sambu' had the lowest content of flavonoids (25.7 mg g^{-1} ET), whereas the highest content was found in 'Sampo' (33.5 mg g^{-1} ET). ET samples were packed in bags of brown paper at normal pressure and under vacuum in polyethylene plastic bags and in aluminium foil bags comprised of polyamide, aluminium and polyethylene, respectively, and stored at 0 (non-stored), 3, 6, 9, 12, 15, 18 and 21 months. Principal components analysis and multiple linear regression analysis taking into account the correlation and interactions between packing material, storage time and cultivar showed, however, that packing materials and storage time only had a minor effect on the content of phenolic acids and flavonoids in ET. The overall conclusion of the following study is that polyphenols in ET are relatively stable during storage up to 21 months and that the content of phenolic acids and flavonoids in ET is mainly determined by the choice of cultivar/genotype. To maintain a good flavour of ET during storage paper bags are clearly less preferable than aluminium foil and plastic packing materials and therefore the best choice of packing material for ET seem to be aluminium foil based packing materials.

Key words. drying method – packing materials –vacuum – storage – *Sambucus nigra* – elderflower tea – flavonol glycosides – phenolic acids

Introduction

Elderflower extracts are used as a natural flavour ingredient in alcoholic and non-alcoholic beverages, fruit brandies and other spirits, sparkling wine, yoghurt, and ice cream (Kaack et al. 2006). The commercial use of elderflower tea (ET) seem to be limited, although ET processed from fresh flowers are known to have a delicious and attractive flavour (Kaack and Christensen 2008) that are due to aroma compounds, which also are responsible for the flavour of elderflower extracts (Jørgensen et al. 2000; Kaack et al. 2006). Aroma compounds in elderflower products have previously been shown to be useful for the separation of elderflower products with attractive flavours (Jørgensen et al. 2000; Kaack et al. 2006; Kaack and Christensen 2008) that may be preferred in different countries and consumer groups.

The health promoting properties of food products may be improved by optimisation of the content of bioactive compounds such as flavonoids and phenolic acids that have been considered as beneficial agents in a multitude of diseases, such as cancer, cardiovascular disease, and neurodegenerative disorders (Lopez-Lazaro 2002; Youdim et al. 2002; Steinberg et al. 2003), and to be able to inhibit infectious diseases probably due to their antibiotic and immune stimulating properties (Middleton and Kandaswami 1992; Hernandez et al. 2000; Barak et al. 2001). Consequently, flavonoids and phenolic acids are considered to be important bioactive compounds in elderflower products, including ET (Christensen et al. 2008). This is also in accordance with the fact that a high concentration of phenolic acids and flavonoids in Sambuci flos (dried elderflowers) has been considered as a good old fashioned remedy against colds, and it is an almost infallible cure for an attack of influenza (Serkedjieva 1996; Za-

KAY-RONES et al. 2004). The positive effects of flavonoids and phenolic acids in relation to cardiovascular diseases is probably associated with their ability to increase the antioxidative capacity of the blood plasma and prevent oxidation of low density lipoprotein (LDL) and platelet aggregation (OLTHOF et al. 2001; NETZEL et al. 2002; MURKOVIC et al. 2004). The potential cancer preventive effects of flavonoids and phenolic acids in ET and other foodstuff may be due to their ability to modulate enzyme activities resulting in decreased carcinogenicity of xenobiotics and preventing the development of oxidative stress induced cancer (MOON et al. 2006; VALKO et al. 2006). Finally, polyphenols may also provide elderflower products with profound anti-inflammatory properties (LIU et al. 1991; BARAK et al. 2002).

The content and composition of phenolic acids and flavonoids in elderflowers and elderflower extracts resulted recently in the identification and quantification of 9 phenolic acids (3-*O*-, 4-*O*- and 5-*O*-caffeoylquinic acid and 3-*O*- and 5-*O*-*p*-coumaroylquinic acid, 1,5-di-*O*-, 3,4-di-*O*-, 3,5-di-*O*- and 4,5-di-*O*-caffeoylquinic acid) and 6 flavonol glycosides (quercetin-3-*O*-rutinoside, quercetin-3-*O*-glucoside, quercetin-3-*O*-glucoside-6"-acetate, kaempferol-3-*O*-rutinoside, isorhamnetin-3-*O*-rutinoside and isorhamnetin-3-*O*-glucoside) using liquid chromatography–mass spectrometry (LC–MS) and high-performance liquid chromatography–diode array detection (HPLC–DAD), respectively (CHRISTENSEN et al. 2008). Furthermore, we have recently shown that volatile compounds with flowery, fruity and/or sweet notes are important contributors to the flavour of ET and that tea processed from the flowers of the cultivars 'Sampo' and 'Sambu', packed in plastic and aluminium foil bags had a satisfactory flavour and content of volatile compounds several months after processing in contrast to ET samples stored in paper bags. All tea samples processed from the cultivar 'Samyl' had for example an unpleasant grassy off-flavour (KAACK and CHRISTENSEN 2008). Hence, packing material, storage time and cultivar has a significant effect on the flavour of ET.

Flavonoids and other phenolics are known to undergo numerous enzymatic and chemical reactions during postharvest food storage and processing (ES-SAFI et al. 2007), but whether packing material, storage time and cultivar has an effect on the content of potential bioactive phenolic acids and flavonoids in ET is, however, not known. Therefore, the aim of the present study was to investigate the effects of cultivar (genotype), packing material and storage time on the content of phenolic acids and flavonoids in ET.

Materials and Methods

Raw materials and processing

In 2003, elderflower umbels were sampled from the cultivars 'Sampo', 'Sambu' and 'Samyl' in the morning between 9 and 12 a.m. from 18-year-old bushes grown at Pometet in Taastrup, Denmark. The flower umbels were kept at 3 °C during transport to the laboratory, where the flowers were removed from the stalks using sharp scissors and leaving 5 mm stalk only. Dry matter in the raw flowers was determined in two replicates by drying 500 g fresh whole elderflowers corresponding to 42 umbels with 5 mm stalks, at 80 °C for 16 h in a drying cabinet.

Processing of tea was carried out in a cool room at 5 °C by placing 5 kg prepared elderflowers in a thin layer on nylon screens in woody frames and dried using dehumidified air flow above and below the nylon screens for 96 h at the same temperature. Packaging was carried out in another room at 20 °C. The yield of elderflower tea (ET) was determined by weighing the fresh flowers and the tea at 20 °C before and after drying, respectively.

Fifteen gram ET processed from the cultivars 'Samyl', 'Sampo', and 'Sambu' were weighed into aluminium foil bags comprised of 15 μm oriented polyamide, 9 μm aluminium, 15 μm oriented polyamide and 70 μm low linear density polyethylene glued together, width 130 mm, height 200 mm (Amcor Flexibles, Horsens, Denmark), polyethylene plastic bags with thickness of 0.08 mm, width 130 mm, height 200 mm (Danisco Flexible, Højbjerg, Denmark) and brown paper bags with thickness of 0.15 mm, width 65 mm, depth 35 mm, height 190 mm (Rhedar, Odense, Denmark). The aluminium foil bags and plastic bags were sealed with approximately 99 % vacuum, which means that approximately 1 %, atmosphere was left in the sealed bags (WEBO, Webo Matic, Bochum, Germany) and that the pressure in the bags was practically zero. The brown paper bags were closed using paper clips. The bags were stored at 20 °C in a thermostated cabinet without light (Termaks series, Lytzen Lab, Denmark) for 0 (non-stored), 3, 6, 9, 12, 15, 18, and 21 months until analysis for phenolic acids and flavonoids.

Storage experiment

A full factorial storage experiment using elderflowers from 3 cultivars, 3 packing materials (aluminium foil, plastic, paper) and 7 sampling dates in two replicates resulted in 126 samples that was supplied with 6 samples of non-stored tea (control) processed from 3 cultivars so that the total number of samples was 132.

Identification and quantification of flavonoids and phenolic acids

Elderflower tea (ET) samples (1 g) was mixed with 20 ml 50 % acetonitrile (acetonitrile, 99.9 % HPLC grade, Sigma-Aldrich) using an Ultra Turrax blender (IKA-Labortechnik, T25, Staufen, Germany) and the sample left at 22 °C for 1 h under stirring until filtration into a HPLC vial through a 0.45-μm Minisart SRP 25 HPLC filter (Bie & Berntsen, Denmark). This extraction method ensured almost complete extraction of flavonoids and phenolic acids as shown by sequential extraction experiments on the same plant material. Phenolic acids and flavonoids in ET extracts were identified and quantified by LC-MS and analytical HPLC-DAD, respectively, according to the methods described previously by CHRISTENSEN et al. (2008). The analysis of ET extracts resulted in the identification of the flavonol glycosides quercetin-3-*O*-rutinoside, quercetin-3-*O*-glucoside, quercetin-3-*O*-glucoside-6"-acetate, kaempferol-3-*O*-rutinoside, isorhamnetin-3-*O*-rutinoside and isorhamnetin-3-*O*-glucoside and the phenolic acids 3-*O*- and 5-*O*-caffeoylquinic acid, 3-*O*- and 5-*O*-*p*-coumaroylquinic acid, and 1,5-di-*O*-, 3,4-di-*O*-, 3,5-di-*O*- and 4,5-di-*O*-caffeoylquinic acid

(Christensen et al. 2008). Quantification of phenolic acids and flavonol glycosides in the analysed samples were based on calibration curves of authentic standards of 5-*O*-caffeoylquinic acid and quercetin-3-*O*-rutinoside, respectively. Molecular weight correction factors were taken into account in the quantification of the individual polyphenols.

Statistical analysis

Multiple factorial analysis of variance and factor analysis were carried out using Version 4 of Statgraphic Statistical Package (Statistical Graphics Corporation, Rockville, USA) with separation of averages using different letters ($P < 0.05$). Factor analysis was carried out according to Überla (1971) and Sharma (1996).

Results and Discussion

Effect of cultivar on the content of flavonol glycosides and phenolic acids in non-stored elderflower tea

Eight phenolic acids and six flavonoids were repeatedly detected in elderflower tea (ET) of which the flavonol glycosides quercetin-3-*O*-rutinoside, kaempferol-3-*O*-rutinoside and isorhamnetin-3-*O*-rutinoside was the major flavonoids in ET (Table 1). These flavonoids comprised 92.7, 88.9 and 84.6 % of the total flavonoid content in the non-stored ET samples (control) of the cultivars 'Sampo', 'Sambu' and 'Samyl', respectively (Table 1). The major phenolic acids in the tea were 5-*O*-caffeoylquinic acid and 1,5-di-*O*-caffeoylquinic acid, which comprised 79.0, 80.0 and 84.3 % of the total phenolic acid content in non-stored ET samples (control) of the cultivars 'Sampo', 'Sambu' and 'Samyl', respectively. These results are in accordance with previous investigations on the content of polyphenols in fresh elderflowers (Seitz 1991; Pietta et al. 1992; Hawryl et al. 2002; Dawidowicz et al. 2003, 2006; Christensen et al. 2008).

The total content of phenolic acids in ET of 'Samyl' (41.3 mg g^{-1} ET) was significantly higher compared to the total content of phenolic acids in ET of 'Sambu' (34.0 mg g^{-1} ET) and 'Sampo' (33.8 mg g^{-1} ET) (Table 1). In particular the major phenolic acids 5-*O*-caffeoylquinic acid and 1,5-di-*O*-caffeoylquinic acid was present in significant higher amounts in ET of 'Samyl' compared to ET of 'Sampo' and 'Sambu' (Table 1). 3,4-Di-*O*-caffeoylquinic acid also appeared in higher concentrations in 'Samyl', whereas the content of the minor phenolic acids such as 3-*O*-*p*-coumaroylquinic acid, 3-*O*-caffeoylquinic acid, 3,5-di-*O*- and 4,5-di-*O*-caffeoylquinic acid was significantly higher in ET processed from the flowers of 'Sambu' compared to the content found in ET samples of 'Sampo' and 'Samyl' (Table 1).

The total content of flavonoids was significantly higher in ET processed from flowers of the cultivar 'Sampo' (33.5 mg g^{-1} ET) compared to the total content of flavonoids in 'Samyl' (32.6 mg g^{-1} ET) and 'Sambu' (25.7 mg g^{-1} ET) (Table 1). The highest concentrations of the major flavonol rutinosides, quercetin-3-*O*-rutinoside and isorhamnetin-3-*O*-rutinoside, was found in ET processed from the flowers of 'Sampo', whereas the concentration of quercetin-3-*O*-glycoside-6"-acetate and

Table 1. Content of phenolic acids and flavonoids in non-stored tea samples processed from elderflowers of the cultivars 'Sampo', 'Sambu' and 'Samyl' (average of two replicates; n = 6).

Compound	'Sampo'	'Sambu'	'Samyl'	CV
	(mg g^{-1} ET)[a]			
3-*O*-Caffeoylquinic acid	1.49 b [b]	1.60 a	1.06 c	20.6
3-*O*-*p*-Coumaroylquinic acid	0.91 b	1.18 a	0.73 c	24.1
5-*O*-Caffeoylquinic acid	12.40 c	13.00 b	14.00 a	6.2
5-*O*-*p*-Coumaroylquinic acid	1.55 a	0.89 c	1.46 b	27.5
1,5-Di-*O*-caffeoylquinic acid	14.30 b	14.20 b	20.80 a	23.1
3,4-Di-*O*-caffeoylquinic acid	1.38 b	0.84 c	1.86 a	37.9
3,5-Di-*O*-caffeoylquinic acid	1.09 b	1.38 a	0.78 c	27.8
4,5-Di-*O*-caffeoylquinic aicd	0.67 b	0.84 a	0.63 c	16.2
Total content of phenolic acids	33.80 b	34.00 b	41.30 a	11.7
Quercetin-3-*O*-rutinoside	23.90 a	15.70 c	17.60 b	22.5
Quercetin-3-*O*-glucoside	1.17 b	0.73 c	3.05 a	74.7
Quercetin-3-*O*-glucoside-6"-acetate	0.63 c	1.58 a	0.97 b	64.2
Kaempferol-3-*O*-rutinoside	1.84 c	2.88 b	5.07 a	50.3
Isorhamnetin-3-*O*-rutinoside	5.33 a	4.26 c	4.91 b	13.5
Isorhamnetin-3-*O*-glucoside	0.58 b	0.52 c	1.07 a	41.0
Total content of flavonoids	33.50 a	25.70 c	32.60 b	14.0

[a]ET, elderflower tea. The relative standard deviation was less than 5 % between replicates. CV, Coefficient of variation;
[b]Significant different averages are separated using different letters, $P < 0.05$.

kaempferol-3-*O*-rutinoside was significantly lower in ET samples from this cultivar compared to 'Sambu' and 'Samyl' (Table 1). The content of the flavonol glycosides quercetin-3-*O*-glucoside, kaempferol-3-*O*-rutinoside and isorhamnetin-3-*O*-glucoside was significantly higher in ET processed from flowers of 'Samyl' (Table 1). The present investigation clearly demonstrates that tea processed from elderflowers of the cultivars 'Sampo', 'Sambu' and 'Samyl' contains the same phenolic acids and flavonoids but differ significantly in their content of these polyphenols. This is also in accordance with previous investigations of fresh elderflowers and elderflower extracts of various elderberry genotypes (Kaack and Christensen 2008) and thus shows that the genotype/cultivar has a large impact on the composition of polyphenols in ET and other elderflower products.

Effect of packing material and storage time on the content of flavonoids and phenolic acids in elderflower tea

As shown in Table 2 the differences between ET samples packed in paper, plastic or aluminium foil bags are to a large extent significant different, although the differences are small. The total content of phenolic acids, including the content of 3-*O*-caffeoylquinic acid, 3-*O*-*p*-coumaroylquinic acid, 5-*O*-caffeoylquinic acid, and 1,5-di-*O*-caffeoylquinic acid decreased and for some compounds significantly when packed in plastic and aluminium foil bags compared to packaging in paper bags. The content of 3,4-di-*O*-caffeoylquinic acid on the other hand was found to be significant higher in plastic and aluminium foil bags compared to paper bags. No significant differences in the concentration of 3,5-di-*O*-caffeoylquinic acid and 4,5-di-*O*-caffeoylquinic acid was observed (Table 2). The total content of flavonoids as well as the content of the flavonol glycosides quercetin-3-*O*-rutinoside and isorhamnetin-3-*O*-glucoside also decreased significantly in ET samples when packed in aluminium foil bags compared to packing in paper bags. No significant differences was observed for the other flavonoids except for quercetin-3-*O*-glucoside, which was found to be higher in plastic bags compared to storage in paper and aluminium foil bags (Table 2). The tendency to a higher content of polyphenols in paper and plastic bags compared to aluminium foil bags could be due to loss of water by evaporation through the bags made of plastic and paper (Table 2) as aluminium foil bags have the lowest diffusion coefficient for water in comparison to paper and plastic bags. Also the diffusion coefficient for oxygen, and hence oxidation of polyphenols, which is highest for paper and plastic bags compared to aluminium foil bags may explain some of the differences in the content of polyphenols observed between the different packing materials.

As shown in Table 3 significant differences were found between storage times for 3-*O*-caffeoylquinic acid, 3-*O*-*p*-coumaroylquinic acid, 5-*O*-caffeoylquinic acid, 1,5-di-*O*-caffeoylquinic acid, 3,4-di-*O*-caffeoylquinic acid and quercetin-3-*O*-glucoside. The content of 3-*O*-caffeoylquinic acid was highest immediately after processing (control sample) and lowest after storage for 21 months. However, no clear trend occurred for this compound and

Table 2. Content of phenolic acids and flavonoids in packed elderflower tea using different packaging materials (averages of cultivar and storage time; n = 132).

Compound	Paper	Plastic	Aluminium foil
		(mg g^{-1} ET)[a]	
3-*O*-Caffeoylquinic acid	1.42 ab[b]	1.38 b	1.35 b
3-*O*-*p*-Coumaroylquinic acid	0.99 a	0.93 b	0.89 c
5-*O*-Caffeoylquinic acid	13.50 a	13.00 b	12.90 b
5-*O*-*p*-Coumaroylquinic acid	1.30 ab	1.28 b	1.31 a
1,5-Di-*O*-caffeoylquinic acid	16.80 a	16.70 a	15.90 b
3,4-Di-*O*-caffeoylquinic acid	1.31 b	1.40 a	1.37 a
3,5-Di-*O*-caffeoylquinic acid	1.11 a	1.06 a	1.09 a
4,5-Di-*O*-caffeoylquinic aicd	0.71 a	0.71 a	0.72 a
Total content of phenolic acids	37.10 a	36.40 a	35.50 b
Quercetin-3-*O*-rutinoside	19.30 a	19.00 ab	18.90 b
Quercetin-3-*O*-glucoside	1.63 b	1.71 a	1.62 b
Quercetin-3-*O*-glucoside-6"-acetate	1.08 a	1.03 a	1.07 a
Kaempferol-3-*O*-rutinoside	3.28 a	3.27 a	3.24 a
Isorhamnetin-3-*O*-rutinoside	4.88 a	4.85 a	4.78 a
Isorhamnetin-3-*O*-glucoside	0.75 a	0.72 ab	0.70 b
Total content of flavonoids	31.00 a	30.60 ab	30.30 b

[a] ET = elderflower tea;
[b] Statistical different averages are separated using different letters, $P < 0.05$.

Table 3. Average content of phenolic acids and flavonoids in elderflower tea (ET) samples (in mg g^{-1} ET) during storage (averages of cultivar and packing material; n = 132).

Compound	Months of storage								P[a]
	0	3	6	9	12	15	18	21	
3-*O*-Caffeoylquinic acid	2.07 d[b]	1.33 b	1.28 b	1.18 a	1.34 b	1.30 b	1.42 c	1.15 a	**
3-*O*-*p*-Coumaroylquinic acid	1.06 de	1.00 cd	0.94 c	0.72 a	0.94 c	0.87 b	1.11 e	0.87 b	***
5-*O*-Caffeoylquinic acid	13.0 ab	13.1 b	13.4 bc	13.4 bc	12.6 a	12.5 a	13.8 c	13.1 b	***
5-*O*-*p*-Coumaroylquinic acid	1.29 bcd	1.29 bcd	1.24 ab	1.36 d	1.23 b	1.20 a	1.46 e	1.33 cd	ns
1,5-Di-*O*-caffeoylquinic acid	15.7 abc	16.3 bc	16.5 c	20.6 d	15.4 ab	15.0 a	16.4 c	15.6 abc	*
3,4-Di-*O*-caffeoylquinic acid	1.31 bcd	1.40 d	1.51 e	1.71 f	1.18 ab	1.15 a	1.36 cd	1.25 abc	*
3,5-Di-*O*-caffeoylquinic acid	0.60 a	1.14 b	1.22 b	1.22 b	1.17 b	1.08 b	1.22 b	1.05 b	ns
4,5-Di-*O*-caffeoylquinic acid	0.87 c	0.63 a	0.70 b	0.58 a	0.62 a	0.73 b	0.74 b	0.72 b	ns
Total content of phenolic acids	35.9 bcde	36.1 cd	36.8 de	40.9 f	34.5 ab	33.9 a	37.5 e	35.1 bc	***
Quercetin-3-*O*-rutinoside	17.6 ab	18.5 bc	19.5 de	22.6 f	18.8 cd	17.5 a	19.6 e	18.5 bc	ns
Quercetin-3-*O*-glucoside	1.58 abc	1.67 cd	1.86 e	1.58 abc	1.77 d	1.59 abc	1.73 cd	1.52 a	**
Quercetin-3-*O*-glucoside-6"-acetate	1.09 cd	1.07 bc	1.24 d	1.08 c	1.09 c	1.02 abc	0.99 ab	0.93 a	ns
Kaempferol-3-*O*-rutinoside	3.38 d	3.36 d	3.37 d	3.35 d	3.28 cd	3.15 b	3.23 bc	2.99 a	ns
Isorhamnetin-3-*O*-rutinoside	4.73 bcd	5.03 ef	4.94 de	5.14 f	4.78 c	4.51 a	4.93 cde	4.62 ab	ns
Isorhamnetin-3-*O*-glucoside	1.02 g	0.63 bc	0.65 bc	0.68 c	0.82 e	0.60 b	0.90 f	0.48 a	ns
Total content of flavonoids	29.4 ab	30.3 b	31.5 d	34.4 e	30.4 bc	28.4 a	31.4 cd	29.1 ab	ns

0 (no storage);
[a] P < 0.05 *; 0.01 **; 0.001 ***;
[b] Statistical different averages are separated using different letters, P < 0.05.

the values for storage at 3, 6, 12, and 15 months were not significant different. A significant decrease occurred for 3-*O*-*p*-coumaroylquinic acid with the lowest value after 9 months and highest immediately after processing and after 18 months of storage. For 5-*O*-caffeoylquinic acid, the maximum and minimum content was found after 18 and 15 months of storage, respectively. For 1,5-di-*O*-caffeoylquinic acid, 3,4-di-*O*-caffeoylquinic acid, and total phenolic acids maximum content was observed after 9 months of storage whereas for quercetin-3-*O*-glucoside the maximum content was observed after 6 month of storage (Table 3). An increase in the concentration of specific phenolic acids and flavonoids during storage may be explained by enzymatic hydrolysis from glycoside precursors and a decrease in the concentration of these polyphenols may be due to oxidation and/or enzymatic degradation (Es-Safi et al. 2007).

In order to find possible important correlations that have an impact on the content of individual polyphenols in ET the data from the present full factorial storage experiment was subjected to classical factor analysis that resulted in extraction of three non-correlated factors (principal components (PC) or eigenvectors) named PC1, PC2, and PC3 with eigenvalues 7.0, 4.1 and 1.2, respectively. The principal components PC1, PC2, and PC3 explained 48.2, 27.9 and 8.2 % of the data variation, respectively. PC1 and PC2 with an accumulated percentage of 76.1 % showed that most of the data variation could be explained by these factors. The two-dimensional factor plot of PC1 versus PC2 shown in Fig. 1, illustrates which of the measured parameters are closely correlated and contribute to the data variation. From Fig. 1 it can be observed that genotype is the main contributor to the variation in the content of phenolic acids and flavonol glycosides in ET samples and hence is the major contributor to the concentration differences observed between the ET samples. Fig. 1 also shows that storage time and packing material only contribute very little to the variation in the content of polyphenols in ET samples and therefore storage and packing material only have a relatively small effect on the content of phenolic acids/flavonol glycosides in tea samples. Multiple linear regression analysis using normalized data with forward selection of genotype, packing materials and storage time and the interactions genotype×storage time, genotype×packing materials and storage time×packing materials in the full factorial storage experiment (Table 4) confirmed the results of the PCA analysis (Fig. 1). The multiple regression analysis showed that all phenolic acids and flavonoids were affected significantly by genotype except for 4,5-di-*O*-caffeoylquinic acid. The concentration of 3-*O*-caffeoylquinic acid and 3,4-di-*O*-caffeoylquinic acid decreased significantly with storage time, while the content of 3-*O*-*p*-coumaroylquinic acid increased. The regression analysis also showed that the content of 5-*O*-*p*-coumaroylquinic acid and 5-*O*-caffeoylquinic acid in ET depended on the packing material being highest in paper bags (Table 2), whereas packing material did not

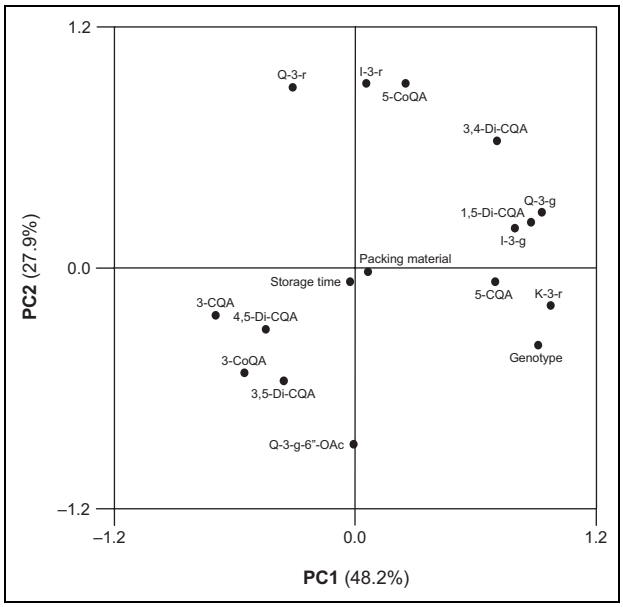


Fig. 1. Two-dimensional factor plot (bi-plot) of PC1 versus PC2 showing the correlation between cultivar (genotype), storage time and packing materials and individual polyphenols in elderflower tea. CQA = caffeoylquinic acid, CoQA = *p*-coumaroylquinic acid, I-3-r = isorhamnetin-3-*O*-rutinoside, I-3-g = isorhamnetin-3-*O*-glucoside, K-3-r = kaempferol-3-*O*-rutinoside, Q-3-r = quercetin-3-*O*-rutinoside, Q-3-g = quercetin-3-*O*-glucoside, Q-3-g-6"-OAc = quercetin-3-*O*-glucoside-6"-acetate.

affect the content of the other phenolic acids (Table 4). The interactions between genotype×storage time, genotype×packing materials and storage time×packing materials had no significant effect on the content of phenolic acids (Table 4). This indicates that packing material has no or only a minor effect on the content of phenolic acids in ET during storage in accordance with the PCA analysis (Fig. 1). On the basis of the total content of phenolic acids during storage it was concluded that the highest level of phenolic acids was obtained using ET processed from 'Samyl' packed in paper bags (Table 1 and 2). Kaempferol-3-*O*-rutinoside decreased with storage time, whereas storage time and packing materials and the various interactions had no significant effect on the content of the other flavonoids and total flavonoids (Table 4). The decrease of kaempferol-3-*O*-rutinoside in ET samples during storage may be due to enzymatic degradation (Es-Safi et al. 2007). Based on the total content of flavonoids in ET samples, it was concluded that the highest level of flavonoids was obtained using ET processed from the cultivar 'Sampo' regardless of packing material (Table 1 and 2).

Based on the present study it can be concluded that (**i**) the phenolic acids and flavonoids in ET is relatively stable and only to a minor degree undergo enzymatic and or chemical reactions, (**ii**) the genotype more or less determines the content of polyphenols in ET, and only to minor extent is dependent on the packing material and storage time and (**iii**) the selection of the right genotype is important for the production of ET with a high content of polyphenols.

Table 4. Results from multiple linear regression analysis using normalized data with forward selection of variables (F = 4.0).

Compound	Regression coefficient for			Regression coefficient for interaction between		
	genotype	storage time	packing materials	genotype and storage time	genotype and packing materials	packing materials and storage time
3-*O*-Caffeoylquinic acid	-0.56***[a]	-0.23**	ns	ns	ns	ns
3-*O*-*p*-Coumaroylquinic acid	-0.32***	0.18*	ns	ns	ns	ns
5-*O*-Caffeoylquinic acid	0.64***	ns	0.23***	ns	ns	ns
5-*O*-*p*-Coumaroylquinic acid	0.63***	ns	0.23***	ns	ns	ns
1,5-Di-*O*-caffeoylquinic acid	0.71***	ns	ns	ns	ns	ns
3,4-Di-*O*-caffeoylquinic acid	0.41***	-0.17*	ns	ns	ns	ns
3,5-Di-*O*-caffeoylquinic acid	-0.30***	ns	ns	ns	ns	ns
4,5-Di-*O*-caffeoylquinic aicd	ns	ns	ns	ns	ns	ns
Total content of phenolic acids	0.68***	ns	0.15*	ns	ns	ns
Quercetin-3-*O*-rutinoside	-0.65***	ns	ns	ns	ns	ns
Quercetin-3-*O*-glucoside	0.76***	ns	ns	ns	ns	ns
Quercetin-3-*O*-glucoside-6"-acetate	0.38***	ns	ns	ns	ns	ns
Kaempferol-3-*O*-rutinoside	0.97***	-0.080***	ns	ns	ns	ns
Isorhamnetin-3-*O*-rutinoside	-0.34***	ns	ns	ns	ns	ns
Isorhamnetin-3-*O*-glucoside	0.66***	ns	ns	ns	ns	ns
Total content of flavonoids	ns	ns	ns	ns	ns	ns

[a] Probability for coefficient(s), * P < 0.05, ** P < 0.01, *** P < 0.001; ns, non significant.

Conclusion

The content of phenolic acids and flavonoids in ET varied significantly between the investigated cultivars (genotypes) 'Sampo', 'Sambu' and 'Samyl', whereas the effect of packing materials and storage time was found to be of minor importance. Therefore, the overall conclusion of the present study is that phenolic acids and flavonoids in ET are relatively stable and that the content of polyphenols in ET largely is determined by the choice of cultivar. However, previously we have shown that paper bags are clearly less preferable than aluminium and plastic packing materials in order to maintain a good flavour of ET during storage (KAACK and CHRISTENSEN 2008). The best choice of packing material for ET therefore seems to be aluminium foil bags.

Acknowledgement

The authors wish to thank Mr. Kim G. Vitten and Mrs. Lone Borum for their excellent technical assistance and the industrial companies Bottle Green Drinks Company, Thorncroft Vineyard Ltd., Steirische Beerenobst-genossenschaft, Beerenfrost Kuelhaus GmbH, Fyns Bærdyrkerforening, Rynkeby Foods, and Dansk Erhvervsfrugtavl for their financial and technical support. We also thank EU 'project no. FAIR CT98 9653, Elderopt) and the Danish Ministry of Food, Agriculture and Fisheries (J. nr. 93s-2466-Å01-01432) for their financial support.

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Received April 13, 2010 / Accepted October 14, 2010

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