Major phenolic compounds in processed sea buckthorn leaves

Anne Morgenstern[1,2], Anders Ekholm[2], Petra Scheewe[2], Kimmo Rumpunen[1] (kimmo.rumpunen@slu.se)

[1] *Swedish University of Agricultural Sciences, Department of Plant Breeding, Balsgård, Fjälkestadsvägen 459, 291 94 Kristianstad, Sweden,*

[2]*Hochschule für Technik und Wirtschaft Dresden (University of Applied Sciences), Faculty of Agriculture and Landscape Management, PillnitzerPlatz 2, 01326 Dresden, Germany*

ABSTRACT

Sea buckthorn (*Hippophae rhamnoides* L.) is increasingly being cultivated because of its valuable fruits which are rich in several bioactive compounds. However, also the leaves of sea buckthorn plants are of interest since they have a high content of eg. ascorbic acid and phenolic compounds. In this pilot study leaves of the sea buckthorn cultivar ‘Ljubitelskaja’ were processed in different ways to mimic production of tea leaves. Thus, sea buckthorn leaves were dried, steamed and fermented for preparation of herbal, green and black sea buckthorn “tea” leaves, respectively. Total phenolic com- pounds were investigated in hot water extracts and in ethanol extracts. The content of catechin, epigallocatechin, gallic acid, hydrolyzable tannins I-III, isorhamnetin-3-O-glucoside, isorhamnetin-3-O- rutinoside, kaempferol, kaempferol-3-O-glucoside, procyanidin dimer aglycones, procyanidin mono- mer glycosides, quercetin, quercetin-3-O-glucoside and rutin were investigated only in hot water extracts.

Ethanol extracts had significantly higher contents of total phenolic compounds compared to the water extracts, and the highest content was noticed when herbal (only dried) sea buckthorn leaves were extracted. By hot water the highest content of total phenolic compounds was instead obtained from the green (steamed and dried) sea buckthorn leaves. The content of specific phenolic compounds differed depending on the processing method of the leaves, and on the method for extraction. The sea buckthorn infusion of fermented (and dried) sea buckthorn leaves had the most pleasant taste compared to the infusion of green sea buckthorn leaves. In the infusion of fermented sea buckthorn leaves the grass-like flavour was almost absent and the bitterness was even less than in the infusion of green sea buckthorn leaves.

Key words: Catechin, gallic acid, isorhamnetin, kaempferol, tannins, tea

INTRODUCTION

Sea buckthorn (*Hippophae rhamnoides* L.) is increasingly being investigated as a valuable multipurpose Euroasian plant species because of its good adaptation to marginal soils and harsh climates, and because of its high content of different bioactive compounds (Ruan et al. 2013). Despite that sea buckthorn leaves are known to be a rich source of ascorbic acid and phenolic compounds, and also used for tea brewing, there are only few reports available on specific phenolic compounds in sea buckthorn leaves (Heinäaho et al. 2006, Kumar et al. 2013, Xing 2003, Morgenstern et al. 2014). No report has been found on the effect of different post-harvest treatments of sea buckthorn leaves on the content of phenolic compounds in the extracts of processed leaves. Thus, the aim of this study was to investigate the influence of different processing methods on the content of phenolic compounds of three different sea buckthorn leaf tea preparations: herbal, green, and black tea, extracted by hot water and ethanol.

MATERIALS AND METHODS

Fresh leaves were sampled from annual shoots of the sea buckthorn cultivar `Ljubitelskaja` in the middle of August. Leaves were prepared as green sea buckthorn tea (GST), black sea buckthorn tea (BST) and herbal sea buckthorn tea (HST). For preparation of GST and BST, immediately after harvest fresh leaves were spread on paper towels for 3 h at room temperature. For preparation of GST leaves were then steamed for a few seconds over boiling water, rolled for 30 min and dried for 6 h at 50 °C. For preparation of BST leaves were rolled for 30 min, wrapped up in 1 cm layers in warm (about 45°C) and wet paper towels, fermented in a perforated plastic bag for 5 h at room temperature, and then dried for 60 min at 85 °C. For preparation of HST leaves were cut in strips of 5 to 10 mm directly after harvest and then dried for 6 h at 50 °C in a convection oven.

Phenolic compounds were extracted from intact sea buckthorn tea leaf preparations by hot water (2 min, 90 °C) and from finely ground samples by 50% EtOH.

The content of total phenolic compounds was analyzed by spectrophotometer using Folin-Ciocalteu's reagent and specific phenolic compounds was analyzed by HPLC-MS as described by Morgenstern et al (2014).

RESULTS

The resulting sea buckthorn tea leaf preparations following processing of fresh leaves is shown in Figure 1. From the figure it is obvious that the different treatments changed the color and structure of the leaf preparations.

Figure 1. Fresh and processed sea buckthorn leaves, from left to right: steamed and dried (GST), fresh, dried (HST), fermented (BST).

Significantly more total phenolic compounds were extracted by 50% EtOH from HST than from GST and BST (Figure 2). By hot water the highest amount was instead obtained from GST and HST yielded the significantly lowest amount of phenolic compounds.

The content of specific phenolic compounds differed depending on the processing method of the leaves, and on the method for extraction. Quercetin and epigallocatechin were not present in detectable levels in any extracts of GST, BST or HST despite that in a previous study (Morgenstern et al. 2014) it was present in the leaves of the same sea buckthorn cultivar ('Ljublitelskaja') sampled in July the same year (Table 1). In general GST yielded the highest amount of water extractable specific phenolic compounds with a few exceptions. The content of catechin was significantly higher in HST and the content of gallic acid was higher in BST, however this was not significant.

There were also visible differences in colour between the tea brews of GST, BST and HST. The colour of the GST hot water extract was brown, the BST extract was dark brown and the colour of the HST extract was bronze. The tea brews also varied in their taste. The HST brew had a grass-like taste and became bitter. The taste of the GST brew was more like the taste of herbal tea brew, and the bitterness and the grass-like flavour was less pronounced than of the HST brew. The BST brew had the most pleasant taste. The grass-like flavour was almost absent and the bitterness was even less than in GST. The general taste was little stronger and more aromatic than the taste of GST and HST.

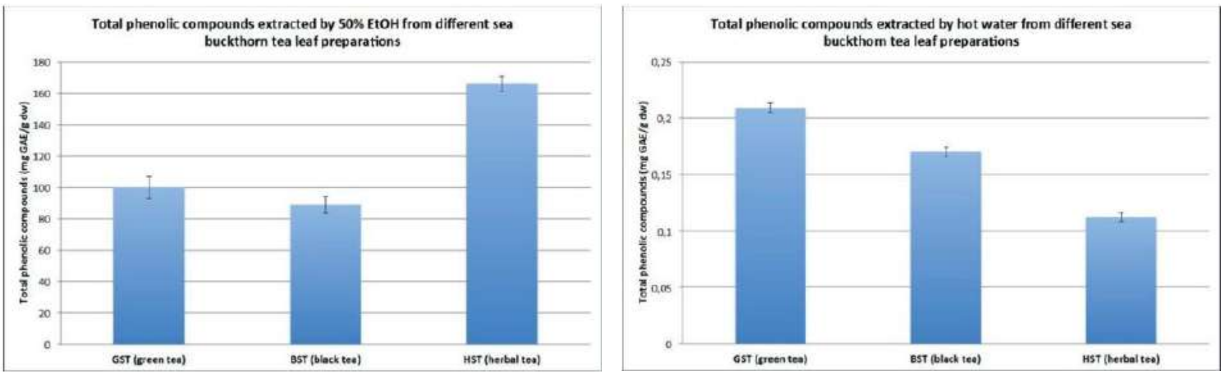

Figure 2. Total phenolic compounds extracted by 50% EtOH of finely ground samples or by hot water from entire leaf samples of different sea buckthorn tea leaf preparations (Av. value and standard deviation, n=3).

Table 1. Content of specific phenolic compounds of three preparations of sea buckthorn leaf tea: green sea buckthorn tea (GST), black sea buckthorn tea (GST), and herbal sea buckthorn tea (GST) when extracted by hot water (values sharing the same letter in a row are not significantly different at $p < 0.05$, n=3, dw = dry weight)

	Content (average ± standard deviation, µg/g dw)		
Phenolic compound	***GST***	***BST***	***HST***
Catechin	101 ± 2.9^{a}	13 ± 0.3^{b}	175 ± 2.9^{c}
Epigallocatechin	0 ± 0	0 ± 0	0 ± 0
Gallic acid	968 ± 229.1^{a}	1287 ± 411.3^{a}	422 ± 86.4^{a}
Hydrolyzable tannin I	2108 ± 402.0^{a}	584 ± 28.0^{b}	1743 ± 163.5^{a}
Hydrolyzable tannin II	92 ± 14.1^{a}	19 ± 0.7^{b}	35 ± 1.7^{b}
Hydrolyzable tannin III	234 ± 1.3^{a}	115 ± 3.7^{b}	190 ± 6.4^{c}
Isorhamnetin-3-*O*-glucoside	55 ± 1.4^{a}	41 ± 1.8^{b}	19 ± 1.0^{c}
Isorhamnetin-3-*O*-rutinoside	229 ± 9.3^{a}	205 ± 8.2^{b}	110 ± 2.1^{c}
Kaempferol	12 ± 2.9^{a}	5 ± 0.3^{b}	4 ± 0.2^{b}
Kaempferol-3-*O*-glucoside	52 ± 22.4^{a}	51 ± 10.3^{a}	50 ± 10.0^{a}
Procyanidin dimer aglycone	0 ± 0	0 ± 0	18 ± 0.6
Procyanidin monomer glycoside	120 ± 5.0^{a}	111 ± 3.5^{a}	47 ± 3.1^{b}
Qercetin	0 ± 0	0 ± 0	0 ± 0
Quercetin-3-*O*-glucoside	46 ± 5.5^{a}	40 ± 5.0^{a}	32 ± 6.4^{a}
Rutin	215 ± 70.3^{a}	223 ± 57.5^{a}	102 ± 1.8^{a}

DISCUSSION

The green colour of fresh sea buckhorn leaves was almost completely preserved in the HST preparation whereas GST and BST lost their green colour to a large extent. This was especially noticeable for BST, which had an even darker colour than GST. The change in structure and colour of the sea buck- thorn leaves following processing is similar to the change that occurs when leaves of the tea plant (*Camellia sinensis*) are being processed.

The fact that 50 % EtOH extracts of finely ground samples yielded more than 400 times the amount of phenolic compounds than hot water extracts of intact leaves shows that sea buckthorn leaves are indeed a valuable source of beneficial phenolic compounds and that grinding may considerably improve extractability.

Total content of phenolic compounds and content of specific phenolic compounds differed between GST, BST and HST due to the different post-harvest processing treatments. This may be ascribed to redox processes in combination with thermal heating causing activation and deactivation of enzymes in the plant material during the production of green, black and herbal tea.

The content of catechin in the hot water extracts of the three sea buckthorn tea preparations BST, GST and HST in this study was 13, 101 and 175 µg/g dry weight, respectively. These values are considerably lower compared to the average catechin contents reported for water extracts of green (240 µg/g dry weight) and black (1670 µg/g dry weight) tea (Peterson et al. 2005). Catechin content was highest in HST, when leaves were only cut and dried, and decreased during processing of GST and BST. This is likely a result of higher enzyme activity in BST during fermentation process when apparently the catechin was converted to more complex proanthocyanidins (condensed tannins). These results differ from ordinary tea production where the black tea usually has the highest content of catechins.

In conclusion, sea buckthorn leaves are very rich in beneficial phenolic compounds, however hot water is only able to extract a minor part of the total amount in the leaves. Processing affects the extractability and is also a possible way to affect the composition of specific phenolic compounds in extracts.

REFERENCES

Heinäaho M, Pusenius J,Julkunen-Tiitto R. 2006. Effects of different organic farming methods on the concentration of phenolic compounds in sea buckthorn leaves. Journal of Agricultural and Food Chemistry 54:7678–7685.

Kumar, MSY, TirpudeRJ, Maheshwari DT, BansalA, Misra K. 2013. Antioxidant and antimicrobial properties of phenolic rich fraction of seabuckthorn (Hippophae rhamnoides L.) leaves in vitro. Food Chemistry 141:3443–3450

Morgenstern A, Ekholm A, Scheewe P, Rumpunen K. 2014. Changes in content of major phenolic compounds during leaf development of sea buckthorn (Hippophaë rhamnoides L.). Agricultural and Food Science 23:207–219.

Peterson J, Dwyer J, Bhagwat S, Haytowitz D, Holden J, Eldridge AL, Beecher G, Aladesanmi J. 2005. Major flavonoids in dry tea. Journal of Food Composition and Analysis 18:487–501.

Ruan C-J, Rumpunen K, Nybom H. 2013. Advances in improvement of quality and resistance in a multipurpose crop: sea buckthorn. Critical Reviews in Biotechnology 33:126–144.

Xing C. 2003. Health protection function and processing technology of sea buckthorn tea. In: Singh, V. (Editor), Sea buckthorn (Hippophae L.) – A multipurpose wonder plant. Vol I: Botany, harvesting and processing technologies. Indus Publishing Company, New Delhi. p. 475–478.