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Ellagic acid content in berries: Influence of domestic processing and storage

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Abstract The content of ellagic acid was determined from the berries of the family *Rosaceae* (strawberry, red raspberry, cloudberry, arctic bramble). Extraction and hydrolysis procedures were optimized and analysis was done with an HPLC method and UV detection. The influence of processing on ellagic acid content was studied in strawberry jam. Strawberries, red raspberries, and strawberry jam were analyzed fresh and after 3, 6, and 9 months of storage in a domestic freezer or refrigerator. Ellagic acid contents after 3 months of storage at –20 °C varied between 31.5 (strawberry 'Senga Sengana') and 68.6 mg/100 g f.w. (arctic bramble). Ellagic acid content in strawberry jam (23.8 mg/100 g f.w.) was 80 % of that in unprocessed strawberries. The content of ellagic acid in strawberries and red raspberries was reduced by 40 % and 30 %, respectively, during the 9 months of storage at –20 °C. The unprocessed berries studied, together with nuts, make the main contribution to the total dietary intake of ellagic acid in Finland.

Keywords Ellagic acid · Berry · Fruit · Processing · Storage · HPLC

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Introduction

Ellagic acid is a dietary hydroxybenzoic acid which may occur in the free form in plants [1]. More commonly, however, it is present in plant vacuoles as hydrolyzable, water-soluble ellagitannins, i.e., esters of glucose with a diphenic acid analog [2–4] (Fig. 1). Interest in ellagic acid has increased during the past few years due to its possible antimutagenic and anticarcinogenic effects [3, 5–10]. Ellagic acid has also shown antioxidative activity as an effective inhibitor of in vitro lipid peroxidation [11]. Ellagic acid has been quantified from berries, fruits, nuts [12–15], as well as from berry and fruit juices [16, 17]. We have recently screened selected flavonoids (kaempferol, quercetin, myricetin) and phenolic acids (*p*-coumaric, caffeic, ferulic, *p*-hydroxybenzoic, gallic and ellagic acids) from 19 wild or cultivated berries [18]. A semi-quantitative method was used to analyze the main phenolic compounds in freeze-dried berry powders [19]. Ellagic acid was the main phenolic compound in the berries of the family *Rosaceae*, genus *Rubus* (red raspberry, arctic bramble, and cloudberry), forming 77–88 % of the phenolic compounds analyzed, and in the genus *Fragaria* (strawberry), where 51 % of the content of phenolic compounds was ellagic acid [18]. Based on this, it can be assumed that these berries contribute a significant amount of ellagic acid to the diet. However, accurate information about the ellagic acid content in these berries is lacking.

Only a small proportion of berries in Finland is consumed fresh because of the short season during which they are available. Thus, most berries are preserved frozen or processed into jams, jellies, juices, desserts, etc. Influence of juice- and wine-making on ellagic acid content has been studied in red raspberries [16] and grapes [20]. Gil et al. [21] analyzed changes in strawberry polyphenolics, including ellagic acid, in response to carbon dioxide treatments during storage at 5 °C. The factors influencing ellagic acid precipita-

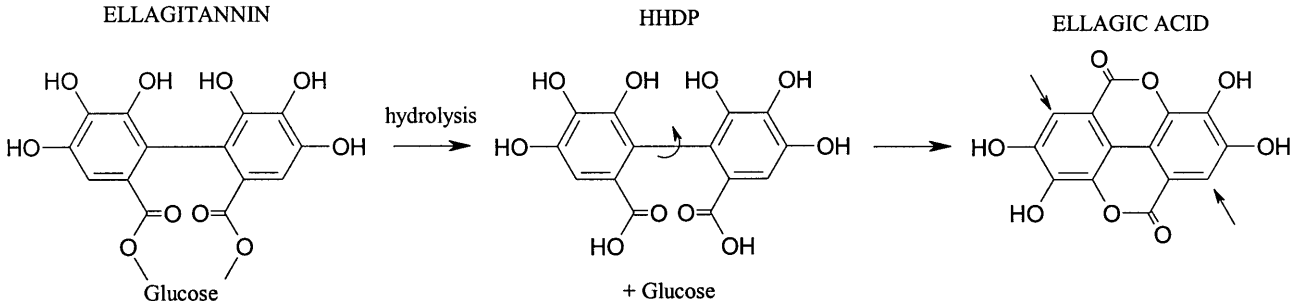

Fig. 1 Hydrolysis of ellagitannin produces hexahydroxydiphenic acid (HHDP), which is spontaneously lactonized to ellagic acid [2, 3]. The unsubstituted carbons of ellagic acid, susceptible to electrophilic attack, are indicated by the *arrows*

tion in muscadine grape juice during storage have been studied by Boyle and Hsu [22] and Garrido et al. [23]. No studies on the effects of jam-cooking or storage in a freezer on ellagic acid content (as ellagitannins) in berries are available.

The purpose of this study was to investigate the amount of ellagic acid in frozen strawberries, red raspberries, cloudberries, and arctic brambles by using an optimized extraction and hydrolysis procedure combined with an HPLC method and UV detection. Also, the effect of jam-cooking on ellagic acid content of strawberry was studied. In addition, the content of ellagic acid was measured in fresh strawberries, red raspberries, and strawberry jam, and after 3, 6, and 9 months of storage in a domestic freezer or refrigerator.

Materials and methods

Berry samples. Cultivated strawberries (*Fragaria x ananassa* 'Jonsok' and 'Senga Sengana'), red raspberries (*Rubus idaeus* 'Ottawa' and 'Muskoka'), arctic brambles (*Rubus arcticus* 'Pima' and 'Mespi'), as well as wild red raspberries (*Rubus idaeus*) and cloudberries (*Rubus chamaemorus*) were from the same batches as those used in a previous study [24]. Berries were collected from eastern Finland in July–August, 1997. A sample of wild cloudberries was also picked from western Finland. The berries were frozen in 100-g batches, stored at –20 ± 2 °C and analyzed after 3 months. Fresh strawberries ('Jonsok') were stored at + 5 °C or, for comparison, at room temperature (+22 °C) and analyzed within 24 h, and after 3, 6, and 9 months of storage at –20 ± 2 °C.

Two batches of strawberry jam were prepared by cooking 1500 g of fresh strawberries ('Jonsok') with 750 g of sugar for 30 min. Ellagic acid content in the jam was studied after 0, 3, 6, and 9 months of storage at +5 ± 1 °C or at –20 ± 2 °C.

Extraction and hydrolysis. The frozen berries or jam (100 g) were thawed in a microwave oven and crushed in a food processor. The berry or jam sample (5 g) was diluted with purified water to 15 ml, and 25 ml of methanol was added. Moreover, 10 ml of 6 mol/l HCl was added (final HCl concentration 1.2 mol/l). The mixture was refluxed for 20 h at 85 ± 5 °C. The extract was allowed to cool and was then filtered. A 10-ml portion of the filtrate was evaporated to dryness using a rotary evaporator and a 35 °C water bath. The residue was dissolved in 2 ml methanol and filtered through a 0.45-μm filter compatible with organic solvents (Cellulose acetate, Lida, USA) prior to injection to the HPLC system.

In a preliminary experiment, extraction and hydrolysis times of 2, 3, 4, 6, 16, 20, 40, and 46 h were tested at 85 ± 5 °C in 50 % aqueous methanol and 1.2 mol/l HCl. All analyses were carried out in duplicate. We also tested the extraction and hydrolysis procedure modified from that of Daniel et al. [12] for strawberry. Here 5 g of homogenized strawberries were refluxed with 50 ml of 100 % methanol for 2 h at 85 ± 5 °C. The extract was filtered and a 20-ml portion was evaporated to dryness using a rotary evaporator and a 35 °C water bath. The residue was dissolved in 5 ml of methanol and 5 ml of 4 mol/l trifluoroacetic acid and the mixture was refluxed for 2 h at 85 ± 5 °C. The mixture was evaporated to dryness as described before and the residue was dissolved in 5 ml methanol and filtered as above prior to injection to the HPLC system.

Chemicals. Ellagic acid was purchased from Sigma Chemical Co. (St. Louis, MO, USA). The standard was dissolved in methanol. Methanol (Lab-Scan, Dublin, Ireland) and acetonitrile (Rathburn, Walkerburn, Scotland) were of HPLC grade. Formic acid (Merck, Darmstadt, Germany) and hydrochloric acid (Riedel-deHaën, Seelze, Germany) were of analytical grade.

Apparatus. The HPLC system used for UV quantification was a Hewlett-Packard (Waldbronn Analytical Division, Germany) instrument with a 1050 Series quaternary pump, an autosampler, and a variable wavelength detector. For the identification of ellagic acid in berries, an HPLC apparatus consisting of a Hewlett-Packard 1050 Series pumping system, an injector, and a 1040 M Series II diode array UV-vis detector was used.

Chromatographic systems. Ellagic acid was quantified and identified by HPLC on a LiChroCART column (125 × 3 mm I.D., Purospher RP-18e, 5 μm) protected with a LiChroCART guard column (4 × 4 mm I.D., Purospher RP-18e, 5 μm) (Merck, Darmstadt, Germany). Solvent A was 1 % formic acid and solvent B was acetonitrile. The gradient was first optimized using a mixture of phenolic acids (*p*-coumaric, caffeic, ferulic, and ellagic acids) and flavonol standards (kaempferol, quercetin, and myricetin). The reason for testing both phenolic acids and flavonols together was that, in our earlier studies, ellagic acid and myricetin were not adequately separated from each other in some elution systems [19]. After testing with hydrolyzed strawberry extract, the final gradient was: 0–10 min, 10–13 % of B in A; 10–20 min, 13–41.5 % of B in A; 20–25 min, 41.5–70 % of B in A; 25–28 min, 70–10 % of B in A; 28–35 min, 10 % of B in A. By using this gradient (flow rate 0.5 ml/min), the best purity and separation was achieved for ellagic acid peak in strawberry (Fig. 2). UV detection (260 nm) was used to quantify ellagic acid in berries. Peak identity and purity were confirmed using a diode array detector to record on-line UV spectra of ellagic acid from the samples. Peaks were considered pure when there was a correspondence of >950 among the spectra. Determinations were carried out in duplicate.

Analytical quality control. To study the within-laboratory repeatability (within-day precision), ellagic acid content of frozen strawberry and red raspberry samples were analyzed six

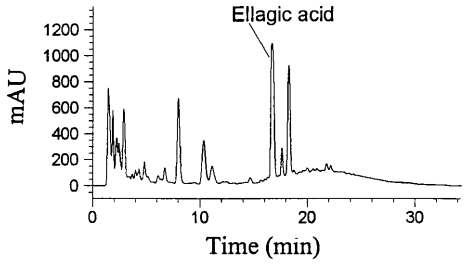

Fig. 2 Typical chromatogram of a strawberry extract in 1 % formic acid/acetonitrile gradient. Detection at 260 nm; flow rate 0.5 ml/min

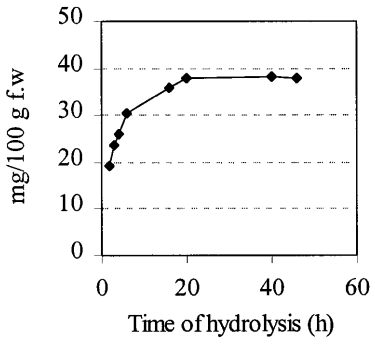

Fig. 3 Influence of time on ellagic acid yield in a strawberry sample when extracted and hydrolyzed at 85 °C in 50 % methanol and 1.2 mol/l HCl. Each *point* represents mean of duplicate analyses

times within one day. The within-laboratory reproducibility (day-to-day precision) was studied in duplicate from a freeze-dried cloudberry sample on six different days during a period of eight months. The control sample was stored at –18 °C between the analyses.

Each week, a new standard curve with freshly prepared standards (4, 60, and 600 ng in 1 µl of methanol) from stock solution was determined. The stock solution (1000 µg/ml) was prepared by dissolving ellagic acid first in dimethyl sulfoxide and then in methanol (1 : 4); the stock solution was stable for at least two months at –18 °C.

Recoveries were measured from strawberry and strawberry jam by spiking prior to analysis the berry samples in extraction solutions with pure ellagic acid at the level of 50–100 % of the measured content. Recoveries were determined in triplicate.

Calculation of intake. The dietary intake of ellagic acid was calculated by using food consumption data obtained from the Finnish Household Survey 1990 [25]. This survey records the purchase of foods (250–300 food items) for the household. The population of the survey comprises all private households in the country, from which a random sample of 12,000 households is drawn. In 1990 the final sample size was 8258 households. Meals eaten outside the household are not recorded in detail. In the Finnish Household Survey, the consumption of unprocessed strawberries and cloudberries is recorded as individual values but that of red raspberries and arctic brambles is included in the group of other garden berries or wild berries.

Statistical analysis. Statistical analysis was done by one-way analysis of variance (The Student Edition of Minitab for Windows). Values of $P<0.05$ were considered statistically significant.

Results and discussion

Extraction and hydrolysis

Optimal recoveries of hydrolyzed ellagic acid were achieved by 20-h extraction and hydrolysis time at 85 ± 5 °C in 50 % aqueous methanol and 1.2 mol/l HCl (Fig. 3). When using 20-h extraction and hydrolysis time, 50 % more ellagic acid was measured from the strawberry sample compared to the sample extracted and hydrolyzed for 2 h (38.0 and 19.4 mg/100 g f.w., respectively). We also tested the extraction and hydrolysis procedure modified from that of Daniel et al. [12] for strawberry, but the amount of ellagic acid obtained with this procedure (5.5 mg/100 g f.w.) was less than 15 % of that obtained with the method chosen here.

Validation of the method

Precision of the method used in this study was good. Coefficients of variation (CV) for the repeatability of ellagic acid analysis from strawberry and red raspberry samples were 5.3 % and 6.0 %, respectively. The CV for the within-laboratory reproducibility in freeze-dried cloudberry sample was 10 %. Rommel and Wrolstad [16] reported considerably higher variation in ellagic acid measurements with replicate analyses (>28 %), most likely due to low solubility of the external standard (ellagic acid) in ethanol under neutral or acidic conditions. In the present study, the ellagic acid standard was first dissolved in dimethyl sulfoxide and then in methanol (1 : 4). Recoveries of the ellagic acid standard from strawberry and strawberry jam were 80 % and 85 %, respectively. According to Mangels et al. [26], recoveries over 80 % are acceptable. In the calculation of the final results the recoveries were not taken into account.

Ellagic acid contents in selected berries

We have recently reported the flavonol contents of 25 edible berries [24] and the influence of processing on the content of quercetin in berry products [27]. Table 1 shows the ellagic acid content in some of the same berries. Ellagic acid contents after 3 months of storage in a freezer varied from 31.5 (strawberry 'Senga Sengana') to 68.6 mg/100 g f.w. (arctic bramble), being considerably higher than the flavonol content in these (1.5 and 3.1 mg/100 g, respectively) or any other Finnish berries studied, the highest flavonol content being found in cranberry (26.3 mg/100 g f.w.) [24].

Previously, Maas et al. [15] reported large differences (from 43 to 464 mg/100 g dry weight) in ellagic acid contents among 35 strawberry cultivars and selections. We found relatively small but statistically significant differences between the two cultivars of straw-

Table 1 Ellagic acid content (mg/100 g, fresh weight) in fresh and frozen berries as means ± SD of duplicate assays

Berry	Fresh (24 h at +5 °C)	Frozen (at –20 °C)		
		3 months	6 months	9 months
Strawberry				
'Jonsok'	40.3 ± 7.5[a]	38.4 ± 1.9[a]	30.5 ± 3.6[b]	24.9 ± 0.2[b]
'Senga'	–	31.5 ± 1.1[b]	–	–
Red raspberry				
'Ottawa'	70.8 ± 2.8[a]	63.8 ± 3.5[b]	61.6 ± 0.8[b]	49.9 ± 1.2[c]
'Muskoka'	–	50.8 ± 0.8[c]	–	–
wild	–	66.2 ± 5.1[b]	–	–
Cloudberry (wild)				
from eastern Finland	–	60.6 ± 3.2[a]	–	–
from western Finland	–	55.9 ± 5.1[a]	–	–
Arctic bramble				
'Pima and Mespi'	–	68.6 ± 4.6	–	–

[a, b, c]Values for each berry marked by the same letter are not significantly different at $P>0.05$

berries ('Jonsok' and 'Senga Sengana') and red raspberries ('Ottawa' and 'Muskoka') (Table 1). In wild red raspberries, the ellagic acid content was similar to that in the cultivar 'Ottawa'. The location where the berries were picked appeared to have no significant effect on ellagic acid levels in cloudberries (55.9 and 61.6 mg/100 g).

In our previous studies done with a semi-quantitative HPLC method [18], 170 and 40 mg/100 g of ellagic acid were found in freeze-dried, seedless red raspberry and strawberry samples, respectively. Compared to our previous study [18], the apparent amount of ellagic acid in the current study, analyzed per fresh weight, was considerably higher both in red raspberries (50.8, 63.8, and 66.2 mg/100 g f.w.) and strawberries (31.5 and 38.4 mg/100 g), considering that the percentage of water in the samples was approximately 87 % and 89 %, respectively [28]. This is explained by the fact that the extraction and hydrolysis time used in our previus procedure (2 h) was not particularly optimized for ellagic acid as in the preset study (20 h) (Fig. 3). The previous method was developed for screening of flavonols and phenolic acids and was a compromise between efficient hydrolysis and non-destructiveness (especially for hydroxycinnamic acids) [19].

Effect of processing

Ellagic acid content in hydrolyzed strawberry jam samples was 23.8 mg/100 g f.w., being 80 % of that in unprocessed strawberries (Table 2). Dilution of the jam with sugar and evaporation of water during the cooking process were taken into account in the calculations. A similar decrease (18 %) was observed in quercetin content during strawberry jam preparation [27]. The loss of ellagic acid and quercetin may be partly due to the oxidation reactions that are increased when the membrane integrity of the cells is lost during cooking and mixing [29]. Differing from our procedure, Zafrilla et al. [30] analyzed the content of free ellagic acid (without hydrolysis) in strawberries, red raspberries, and jams. The content of free ellagic acid increased in strawberries and red raspberries by 150 % during jam-cooking [30]. This increase was related either to a release of hexahydroxydiphenic acid from ellagitannins, which is transformed to ellagic acid (Fig. 1), or to an easier extractability of this compound from processed products due to the degradation of the cell structures. The apparent increase in free ellagic acid content continued during jam storage [30].

In addition to jam-cooking, the influence of juicing on the content of ellagic acid or other phenolic compounds has been studied in berries. In strawberry and red raspberry juices, negligible amounts of ellagic acid were reported by Daniel et al. [12]. Experimental and commercial red raspberry juices had the mean total concentrations of 28 and 52 μg/l of ellagic acid, respectively (base-hydrolyzed samples) [16]; however, the content of ellagic acid in the corresponding berries was not reported. Only 15 % of quercetin originally present in the berries was extracted into the lingonberry and black currant juices after common domestic juice-making methods [27] because the skins containing most of the flavonols [13] are removed by filtering.

Table 2 Ellagic acid content (mg/100 g fresh weight) in fresh strawberry jam and after storage as means ± SD of duplicate assays

	Fresh	3 months	6 months	9 months
Jam stored at +5 °C	23.8 ± 3.7[a]	24.0 ± 1.8[a]	25.4 ± 3.5[a]	21.0 ± 1.7[a]
Jam stored at –20 °C	23.8 ± 3.7[b]	19.6 ± 1.2[b]	23.4 ± 0.8[b]	20.2 ± 1.0[b]

[a, b]Values in each row marked by the same letter are not significantly different at $P>0.05$

Effect of storage

The post-harvest temperature (+5 °C or +22 °C for 24 h) had no apparent effect on ellagic acid content (40.3 and 38.2 mg/100 g, respectively) in fresh strawberries (hydrolyzed samples). Gil et al. [21] studied the content of free ellagic acid (analyzed without hydrolysis) in strawberries during 10-days post-harvesting at 5 °C in modified atmospheres. The content of free ellagic acid was significantly increased in strawberries during storage, although this was delayed at elevated CO_2 concentrations. This increase could be explained by the degradation of ellagitannins.

During the 9 months of storage at –20 °C, a statistically significant reduction in ellagic acid content of strawberries (40 %) and red raspberries (30 %) was observed (Table 1). Hexahydroxydiphenic acid may be released from ellagitannins during the storage and/or thawing leading to spontaneous formation of ellagic acid [30] (Fig. 1). Free ellagic acid may act as an antioxidant in berries due to its metal chelating capacity and ability to react with free radicals [11] resulting in a reduction in its total amount during storage and/or thawing. In strawberry jams stored at +5 °C or at –20 °C for 9 months, no statistically significant decrease in ellagic acid content was observed (Table 2), probably due to the fact that the (antioxidative) reactions of ellagic acid had already occurred during jam-making.

Ellagic acid intake

The total annual consumption of unprocessed strawberries and cloudberries in 1990 in Finland was 4.00 and 1.24 kg/person, respectively [25]. The mean ellagic acid content (Table 1) in strawberries and cloudberries after 3 months storage was 35.0 and 58.3 mg/100 g, respectively; thus these two berries alone provided 5.8 mg ellagic acid/day. This dietary intake is close to that reported for adults (5.2 mg/day) in a Bavarian subgroup of the German national food consumption survey [31]. In that study, berries (strawberry, red raspberry) and nuts (walnut) provided 38 % and 54 %, respectively, of the ellagic acid intake. The ellagic acid contents in walnuts, red raspberries and strawberries were 740, 65.0, and 22.3 mg/100 g, respectively [31]. In Finland, the total annual consumption of nuts (mainly peanuts) in 1997 was 0.92 kg/person [32].

The intake of ellagic acid calculated in this study was over four times higher than that of flavonols from berries (1.24 mg/day) and not much lower than the total intake of flavonols from meals eaten at home in Finland (7.28 mg/day) [24]. Because the ellagic acid (and flavonol) data were obtained from berries collected during one growing season only, our estimates must be considered tentative. However, the unprocessed berries studied, together with nuts, apparently make the main contribution to the total dietary intake of ellagic acid in Finland.

Precise knowledge about ellagic acid content of berries and about the influence of processing and storage on it is important in assessing the effects of ellagic acid on human health and disease. However, more information about the liberation of ellagic acid from ellagitannins in the gut [33] and about its absorption and metabolism should also be made available for assessing the health promoting potential of berries as dietary sources of ellagic acid [34].

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