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# CONTENT OF GALLIC ACID IN SELECTED PLANT EXTRACTS

*Magdalena Karamać[1], Agnieszka Kosińska[1], Ronald B. Pegg[2]*

*[1]Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Division of Food Science, Olsztyn, Poland; [2]University of Saskatchewan, Department of Applied Microbiology and Food Science, Saskatchewan, Canada*

Key words: gallic acid, tannase, plant extracts, hydrolysed tannins, green tea, bearberry

Ethanolic extracts of phenolic compounds were prepared from green teas (*Camellia sinensis* L.), bearberry leaves (*Arctostaphylos uva-ursi* L.), hazelnuts (*Corylus avellana*), evening primrose (*Oenothera biennis*) and grape seeds (*Vitis vinifera* L.). All crude extracts were examined for their gallic acid content by HPLC. The bearberry-leaf preparation was also fractionated by a Sephadex LH-20 column chromatographic method with 95% (v/v) ethanol and acetone:water (1:1; v/v) as mobile phases: one fraction consisted of low molecular-weight phenolics and the other of tannins. To all samples tannase was applied in an effort to liberate gallic acid from the phenolic esters and hydrolysable tannins. The content of free gallic acid in the extracts ranged from 1 to 15 mg/g with the highest quantity being found in evening primrose. The content of gallic acid liberated by tannase ranged from 5 to 309 mg/g with the highest content being found in the tannin fraction from the bearberry-leaf extract.

## INTRODUCTION

Gallic acid exists in plant material in the form of free acids, esters, catechin derivatives and hydrolysable tannins (Figure 1). This ubiquitous chemical is one of the most biologically-active phenolic compounds of plant origin. The antioxidant activity of gallic acid and its derivatives has been reported in several studies [Gramza *et al.*, 2005; Karamać *et al.*, 2005; Rice-Evans *et al.*, 1996; Brand-Williams *et al.*, 1995]. Gallic acid has been shown to possess antimicrobial activity against human pathogens (*Staphylococcus aureus*, *Corynobacterium accolans*), a plant pathogen (*Erwinia carotovora*) and human pathogenic yeast (*Candida albicans*) [Fogliani *et al.*, 2005]. The antifungal activity of gallic acid, isolated from *Oenothera biennis* roots, was investigated by Shukla *et al.* [1999]. Methylgallate has demonstrated activity against a number of Gram positive and Gram negative bacteria and fungi [Penna *et al.*, 2001]. The cytotoxic effects of Triphala, an Indian herbal drug, on breast and prostate cancer cells were attributed to gallic acid [Kaur *et al.*, 2005]. Since gallic acid can act as a nucleophile, it can therefore scavenge electrophilic mutagens [Hour *et al.*, 1999].

The aim of the present work was to identify the content of gallic acid in extracts of selected plant species before and after treatment with the enzyme tannase.

## MATERIALS AND METHODS

**Chemicals.** Acetonitrile and acetic acid (HPLC grade) were purchased from Merck (Darmstad, Germany), ethanol and acetone from the P.O.Ch. Company (Gliwice, Poland),

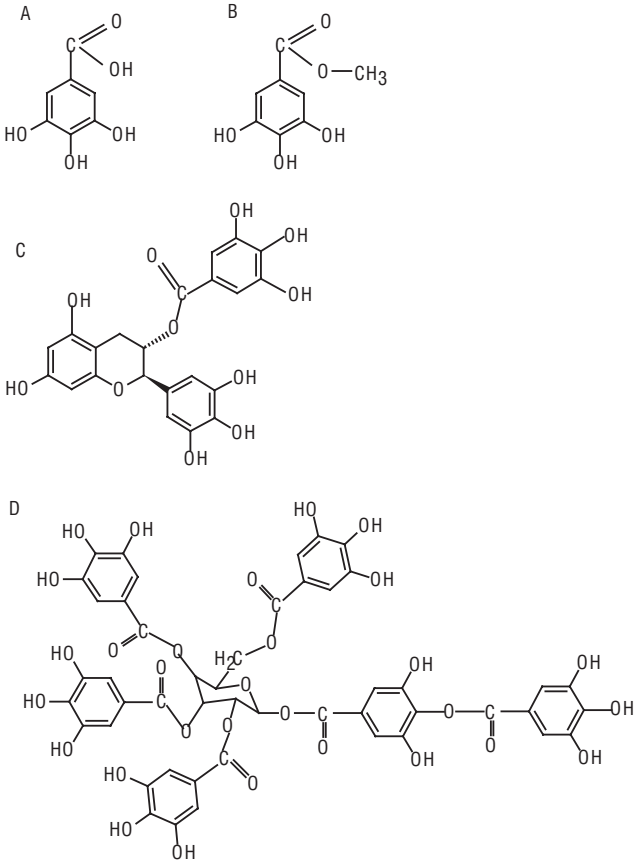


FIGURE 1. Chemical structure of gallic acid (A); gallic acid methyl ester (B); (–)-epigallo-catechin gallate (EGCG) (C); and hydrolysable tannin (D).

Author's address for correspondence: Magdalena Karamać, Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, ul. Tuwima 10, P.O. Box 55, 10-747 Olsztyn, Poland; tel.: (48 89) 523 46 27; fax: (48 89) 524 01 24; e-mail: magda@pan.olsztyn.pl

and tannase-KT50 (50 000 U/g) was acquired as a gift from the Kikkoman Corporation (see **Acknowledgement** for further details).

**Materials.** Green teas (*Camellia sinensis* L.) were bought from local supermarkets in Olsztyn (Poland) and Saskatoon (Canada), dried leaves of bearberry (*Arctostaphylos uva-ursi* L.) were acquired from the Department of Plant Sciences (University of Saskatchewan, SK, Canada), fresh hazelnuts (*Corylus avellana*) were purchased from a local supermarket in Olsztyn, authenticated evening primrose (*Oenothera biennis*) seeds were a gift from the Chair of Plant Technology and Chemistry (Warmia-Masuria University, Olsztyn), and authenticated grape seeds (*Vitis vinifera* L.) were provided from the Chair of Biochemistry (Warmia-Masuria University, Olsztyn).

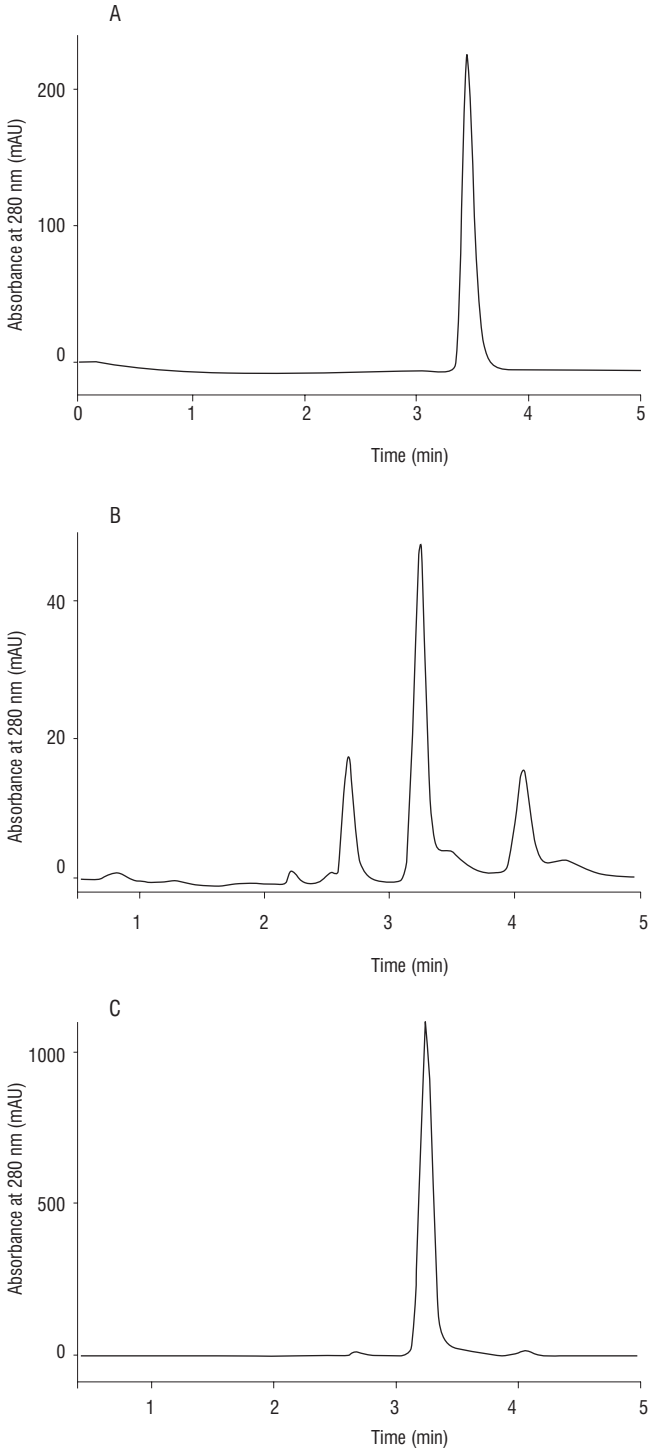


FIGURE 2. HPLC chromatograms of the bearberry-leaf extract. The chromatograms represent free standard (A); free gallic acid (B); and phenolic acids liberated from soluble esters and glycosides with tannase (C).

**Preparation of crude extracts.** Crude extracts of green teas and bearberry were prepared from 95% (v/v) ethanol, whereas those of hazelnuts, evening primrose, and grape seeds using 80% (v/v) acetone according to Amarowicz *et al.* [1995]. It should be emphasized, however, that hazelnuts, evening primrose and grape seeds were first defatted with hexanes using a Soxhlet apparatus before the preparation of extracts.

**Column chromatography.** Separation of low-molecular weight phenolic compounds from tannins for the crude extract of bearberry was achieved according to the method described by Strumeyer & Malin [1975]. Briefly, a 2-g portion of the crude extract was suspended in 20 mL of 95% (v/v) ethanol and loaded onto a chromatographic column (5×40 cm) packed with Sepahdex LH-20 that had been equilibrated with 95% (v/v) ethanol. Low molecular-weight phenolic compounds were eluted from the column using 1 L of 95% (v/v) ethanol, while tannins remained at the top. To recover the tannins, the mobile phase's polarity was increased and the column was washed with 500 mL of 50% (v/v) acetone. Organic solvents were evaporated using a Büchi Rotavapor/Water bath (Models EL 131 and 461, respectively, Brinkmann Instruments [Canada] Ltd., Mississauga, ON) and the tannin fraction recovered with 50% acetone was then lyophilized.

**Enzymatic liberation of bound gallic acid.** To 4 mL of a water solution containing 1–5 mg of the extracts examined 1 mL of tannase solution in citrate buffer (50 mmol/L, pH 5.5) containing 50 $\mu$g of enzyme was added. After 15 min of incubation at 30°C, the sample's pH was adjusted to 2 with 2 mol/L HCl [Sharma *et al.*, 2000]. Then, liberated gallic acid was extracted 5 times into 6 mL of anhydrous diethyl ether. The organic solvent was removed using the Rotavapor and the residue was dissolved in 2 mL of methanol. The sample was then ready for HPLC analysis. To determine the content of free gallic acid in the samples, the buffer devoid of tannase was used.

**HPLC analysis of gallic acid.** Gallic acid was analysed using a Shimadzu HPLC system (Shimadzu Corp., Kyoto, Japan) consisting of a LC-10AD pump, SCL 10A system controller and SPD-M 10A photodiode array detector. Chromatography of the phenolic acids was achieved using a prepacked LiChrospher 100 RP-18 column (4×250 mm, 5 $\mu$m; Merck). The mobile phase comprised water-acetonitrile-acetic acid (88:10:2; v/v/v) [Amarowicz & Weidner, 2001] and was delivered at a rate of 1 mL/min. Detection was monitored at 280 nm.

All results of this work are an average of two independent determinations.

## RESULTS AND DISCUSSION

Representative chromatograms for the analysis of the bearberry-leaf extract are depicted in Figure 2. The method employed offered base line separation of gallic acid with a retention time of 3.23 min.

Using tannase, the content of free gallic acid and that liberated from plant extracts are presented in Table 1. The content of free gallic acid in the extracts so examined ranged from 1 to 15 mg/g with the highest quantity being found in the crude extract of evening primrose. The content of gallic acid liberated by tannase ranged from 5 to 309 mg/g with the highest amount determined in the tannin fraction of the bearberry-leaf extract (*i.e.* 116–309 mg/g).

By using tannase, between 55 and 155 mg of gallic acid were released from 1 g of the green tea extracts. The high content of gallic acid being liberated by tannase in the green tea preparations is due to the presence of catechins; these are mainly in the form of gallic acid esters and include (-)-epicatechin gallate (ECG) and (-)-epigallocatechin gallate (EGCG) [Gramza *et al.*, 2005]. The presence of hydrolysable tannins (1,4,6-tri-O-gallolyl-$\beta$-D-glucose and 1-O-gallolyl-4,6-(-)-hexahydroxy-diphenoyl-$\beta$-D-glucose) in green tea has been reported by Nonaka *et al.* [1984]. Epicatechin-3-O-gallate was purified from grape seeds by da Silva *et al.* [1991]. The high content of tannins in evening primrose has been noted by Amarowicz *et al.* [2000]. The presence of hydrolysed tannins in bearberry leaves has been observed by Pegg *et al.* [2005]. For the ethanol fraction of the bearberry-leaf extract, tannase was able to liberate gallic acid from the esters associated with arbutin [Pegg *et al.*, 2005]. Amarowicz *et al.* [2005] have reported the content of tannins in acetone extract of hazelnuts.

TABLE 1. Content of free gallic acid and gallic acid liberated after treatment with tannase in selected plant extracts (mg/g extract).

| Plant material | Free gallic acid | Gallic acid liberated by tannase |
|---|---|---|
| Green tea (*Camellia sinensis* L.) | | |
| Extract No. 1 | 4 | 97 |
| Extract No. 2 | 4 | 88 |
| Extract No. 3 | 4 | 89 |
| Extract No. 4 | 3 | 55 |
| Extract No. 5 | 4 | 79 |
| Extract No. 6 | 5 | 70 |
| Extract No. 7 | 5 | 155 |
| Extract No. 8 | 5 | 94 |
| Bearberry-leaf (*Arctostaphylos uva-ursi* L.) | | |
| Extract No. 1 | 1 | 21 |
| Extract No. 2 | 2 | 42 |
| Extract No. 3 | 2 | 46 |
| Extract No. 4 | 1 | 44 |
| Extract No. 5 | 2 | 59 |
| Ethanol fraction | 2 | 5 |
| Tannin fraction No. 1 | 3 | 116 |
| Tannin fraction No. 2 | 3 | 275 |
| Tannin fraction No. 3 | 4 | 181 |
| Tannin fraction No. 4 | 4 | 207 |
| Tannin fraction No. 5 | 5 | 309 |
| Hazelnut (*Corylus avellana*) | 1 | 5 |
| Evening primrose (*Oenothera biennis*) | 15 | 36 |
| Grape seeds (*Vitis vinifera L.*) | 2 | 16 |

## CONCLUSIONS

The data presented in this note confirm the presence of gallic acid in plant material, and the fact that it generally exists in the form of esters and hydrolysable tannins. The application of the enzyme, tannase, has been found useful in the analysis of gallic acid. Tannase treatment of plant material has great potential for the preparation of gallic acid-rich preparations for the use in functional foods and nutraceuticals.

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## ZAWARTOŚĆ KWASU GALUSOWEGO W EKSTRAKTACH ROŚLINNYCH

***Magdalena Karamać[1], Agnieszka Kosińska[1], Ronald B. Pegg[2]***

*[1]Oddział Nauki o Żywności, Instytut Rozrodu Zwierząt i Badań Żywności Polskiej Akademii Nauk w Olsztynie;*
*[2]Wydział Mikrobiologii Stosowanej i Nauki o Żywności, Uniwersytet Sakatchewan, Saskatoon, Kanada*

Ekstrakty związków fenolowych otrzymano z zielonej herbaty, liści mącznicy lekarskiej, orzechów laskowych, wiesiołka i nasion winogron. Z ekstraktu mącznicy lekarskiej wydzielono frakcje niskocząsteczkowych związków fenolowych i tanin stosując chromatografię kolumnową na żelu Sephadex LH-20 z 95% (v/v) etanolem i z układem aceton-woda (1:1; v/v) jako fazami ruchomymi. Do uwolnienia kwasu galusowego związanego estrowo i glikozydowo zastosowano taninazę. Zawartość wolnego kwasu galusowego w analizowanych ekstraktach wahała się od 1 do 15 mg/g. Ekstrakt z wiesiołka zawierał najwięcej wolnego kwasu galusowego. Zawartość związanego kwasu wynosiła od 5 do 309 mg/g. Najwięcej zawierały go frakcje taninowe z ekstraktu z liści mącznicy lekarskiej.