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Short communications

INFLUENCE OF DIFFERENT EXTRUSION TEMPERATURES ON THE STABILITY OF ELLAGIC ACID FROM RASPBERRY SEEDS

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ABSTRACT: Production of raspberry products leaves large amounts of seeds which are considered as by-product or waste. These seeds are rich source of ellagic acid and about 88% of the total ellagic acid content in raspberries comes from the seeds.
This study investigates the influence of extrusion process at different temperatures on the content of ellagic acid in "Willamette" raspberry seeds. The extrusion was performed on a Brabender single-screw laboratory extruder and at three temperature regimes: 140, 160 and 200°C. HPLC/DAD analysis was used to determine and quantify the content of ellagic acid in the extruded samples. Ellagic acid content was quantified by calculation using a calibration curve established from standard ellagic acid.
The content of ellagic acid in raspberry seeds was found to be 286.54 μg/g. Use of different extrusion temperatures did not have any impact on the stability of ellagic acid from „Willamete" raspberry seeds, i.e. did not make signifficant diferences in the content of the ellagic acid. These findings indicated that raspberry seeds may be suitable for the high temperature food processing.

Key words: *extrusion, raspberry seeds, ellagic acid, corn grits, value-added product*

INTRODUCTION

Serbia is one of the largest producers of raspberries with export at about 78000 tons of fresh raspberries in 2011 (Dimić et al., 2012). Production of raspberry products, such as jams, juices, concentrates leaves large amounts of raspberry seeds which are under-exploited or wasted (Couto et al., 2008). The berry seed material removed as a waste by-product contains health-beneficial, bioactive compounds (Nile et al., 2014), which are also known as natural antioxidants (Szajdek et al., 2008; Van Hoed et al., 2011).

Raspberry seeds contain a lot of antioxidants such as phenols, flavonoids, antocyanins, vitamins, etc. (Rios de Souza et al., 2014). Godjevac et al. (2009), found that raspberry seeds contain a lot of polyphenolic compounds such as ellagic acid, gallic acid, procyanidin dimer, sanguiin, roshenin, and some others (Godjevac et al., 2009). Previous studies reported that raspberry seeds contain about 87.8% of the total ellagic acid present in raspberries (Daniel et al., 1989). Ellagic acid is found in raspberries in three dif-

ferent forms: as ellagitannins, as free ellagic acid and as ellagic acid glycosides (Zafrilla et al., 2001).

Different studies have shown that ellagic acid has antimicrobial, antimutagenic, antioxidant and anti-inflammatory properties (Khanduja et al., 1999; Wang et al., 2000; Mullen et al., 2002; Kähkönen et al., 2001; Puupponen-Pimiä et al., 2001).

One of the most important functions of ellagic acid is the ability to "adjust" cell physiology on biochemical, physiological and molecular level. This is the result of its phytophenolic structure, which has very similar biological effect as signal molecules, and can influence behavior of genes that activate and inactivate proteins and enzymes (Mates et al., 1999).

Due to the phenol ring and hydroxyl groups, ellagic acid is very effective antioxidant as it has the ability to inhibit the oxidative free radical reactions with vital biomolecules (Rice-Evans et al., 1997).

Also, the antioxidant properties of ellagic acid prevent the damage caused by oxidation of the DNA, which proved to be important in the prevention of some types of cancer and, in addition, ellagic acid prevents the carcinogenic substances to be incorporated into DNA, and thus exerts its antimutagenic properties (Halliwell et al., 1999; Teel et al., 1986).

One of the most important effects of ellagic acid is the anticancer effect and one of the main reasons for the anticancer effect is modulation of the toxin metabolism, and prevention of carcinogenesis induced by these compounds (Mitscher et al., 1996; Zhang et al., 1993; Seeram et al., 2006).

Ellagic acid also inhibits mutagenesis induced by aflatoxin B1 (Soni et al., 1997). Also, ellagic acid has hepatoprotective cha-racteristics. It inhibits the effect of CCl_4, decontaminates the bloodstream (Bravo et al., 1998; Singh et al., 1999), reduces the effect of alpha-amylase and alpha-glucosidase, and prevents diabetic conditions (Valdimarsdottir et al., 2003).

Extrusion cooking is one of the most important food processing technologies, and over the years, it has become the major processing method for food and feed industries (Brennan et al. 2011).

Extrusion process has been used to produce pasta products and ready to eat breakfast cereals, baby foods, snack foods, texturized vegetable protein, pet foods, dried soups and dry beverage mixes (Singh et al. 2010).

Also, it improves digestibility and nutrients bioavailability compared to conventional cooking. In addition to these properties, extrusion cooking is preferred over conventional cooking techniques because of its ability to develop range of products with distinct textural advantages including expansion, crispiness and general mouthfeel, being versatile, high productivity, low operating costs, energy efficiency and shorter cooking times (Brennan et al., 2011).

The main objective of this study was to investigate the stability of ellagic acid from “Willamette” raspberry seeds through the extrusion process.

MATERIAL AND METHODS

Materials

Corn grits and milled “Willamette” raspberry seeds were used for this study. “Willamette” raspberry seeds were milled on a laboratory mill “Glen Mills, C/11/1, Clifton, NJ, 07012” to granulation of 150 μm, and mixed with corn grits so that the ratio was 10% of the seed and 90% of the grits.

The extract of this mixture was analyzed on the HPLC to quantify the amount of ellagic acid. Prior to the extrusion, the moisture content of the mixture was set to 18%. After that, HPLC method was used to determine ellagic acid content in the extruded products.

Extrusion process

A single-screw laboratory extruder (Model GNF 1014/2, Type 110513, Brabender, Germany) was used for the extrusion process. The diameter of the die was 2

mm. The temperature of the feeding zone was 50 °C, the metering zone was adjusted to 100 °C, and the die zone was adjusted to three temperatures: 140 °C, 160 °C and 200 °C throughout the process.

The extruder was capable of a screw speed ranging from 0-250 rpm, so the screw speed was adjusted to 150 rpm for the appropriate treatment. Once the processing was completed, the extrudates were stored in cool and dry place prior to further analyses.

HPLC/DAD analysis of ellagic acid

Samples were prepared as follows: the samples were homogenized and aliquots of 1.5 g were weighed and transferred into a 100 ml Erlenmeyer flask and diluted in 40 ml of 70% aqueous ethanol. The mixtures were ultrasonicated for 10 minutes. Then, they were filtered, made up to 50 ml with ethanol and ultrasonicated for another 10 minutes.

Before quantification by HPLC-DAD, the samples were filtered through a 0.45 µm membrane filter. Samples were analyzed using an Agilent 1260 series HPLC (Agilent Technologies, Santa Clara, CA, USA), using C18 column (4.6 mm×50 mm, 1.8 µm particles).

Injection volume was 5 µl and the temperature was at 30 °C. Solvent A was 1% formic acid and solvent B was acetonitrile. The used gradient was as follows: 0–10 min, 10% of B in A; 10–25 min, 15–50% of B in A; 25–30 min, 50–80% of B in A; 30–35 min, 10% of B in A.

In the tested raspberry samples, good purity and separation were achieved using this gradient at flow rate 0.5 mL/min. The HPLC equipment was used with a diode array detector (DAD). Ultraviolet–visible spectra (ranging from 190 to 540 nm) were recorded for all peaks. Triplicate analyses were performed for each sample.

Ellagic acid was detected at 260 nm, and identified according to peak retention time and UV/Vis spectra, which were compared with those of the standard. The quantities of ellagic acid were based on peak areas, and expressed as mg/g.

Statistical analysis

Statistica 10.0 Software (Statsoft Inc., 2010, Tulsa, Oklahoma) was used for statistical data processing using one-way ANOVA. The comparison of mean values was performed by Tukey- test. Differences were considered significant if $P<0.05$.

RESULTS AND DISCUSSION

HPLC/DAD analysis was performed to determine potential ellagic acid content in pure corn grits, and the result was negative. Results of HPLC/DAD analysis of the ellagic acid content in raspberry seeds, corn grits and non-extruded sample wich contain 10% of raspberry seeds in corn grits are shown in Table 1.

Results of HPLC analysis of the extruded products are shown in Table 2. The results show that the concentration of the ellagic acid in samples extruded at 140 °C is 7.585 µg/g, in samples extruded at 160 °C is 5.533 µg/g, and in samples extruded at 200 °C is 6.618 µg/g.

Statistical analysis showed that there were no significant differences among the mean contents of ellagic acid. So it can be concluded that extrusion temperature does not have significant influence on ellagic acid content in extruded products.

Additionally, it can be seen that the ellagic acid in extruded products is very stable and it was not degraded, which is in consistence with previous research of Li et al. (2013).

Figures 1 and 2 show peaks of ellagic acid in the samples extruded at 200 °C and in the sample of raspberry seeds. Chromatograms of ellagic acid in the samples extruded at 140 and 160 °C were similar to that in Figure 1.

Retention times for the sample extruded at 140 °C was 17.938 min whereas for the sample extruded at 160 °C, the retention time was 17.944 min. The sample extruded at 200 °C has retention time at 17.956 min.

The retention time of ellagic acid in raspberry seeds was 18.041 min, and the retention time of non-extruded sample with 10% of raspberry seeds in corn grits was 17.942 min. Positions of signals were confirmed using analytical standard of the ellagic acid.

Table 1.
Concentration of ellagic acid in raspberry seeds, corn grits and mix of 10% raspberry seeds in corn grits

Sample	Concentration (μg/g)
Raspberry seeds	286.54 ± 2.1944 [a]
Corn grits	n.d. [b]
10% raspberry seeds in corn grits	7.22 ± 1.9575 [c]

[a,b,c] *Values are means of three determinations of ± SD. Values in each row with different superscript are significantly different (p<0.05)*
** Tukey's test*
** p<0.05*
n.d. not detected

Table 2.
Concentration of ellagic acid in the products extruded at different temperatures

Temperature (°C)	Concentration (μg/g)
140	7.585 ± 2.3335[a]
160	5.533 ± 2.2368[a]
200	6.618 ± 2.1053[a]

[a]*Values are means of three determinations of ± SD. Values with the same superscript are not significantly different (p<0.05)*
**Tukey's test*
**p<0.05*

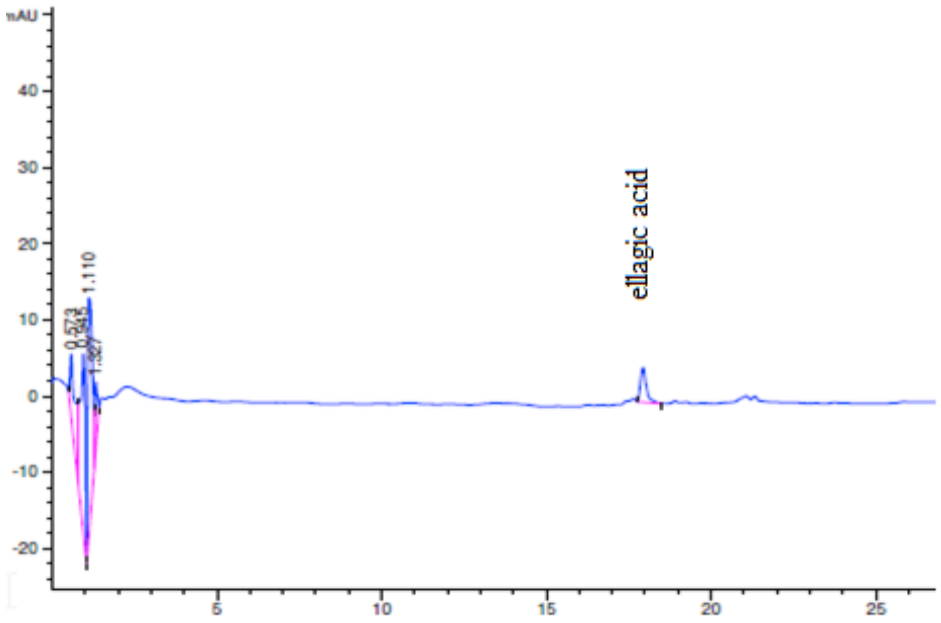

Figure 1. Chromatogram of ellagic acid extracted from the sample extruded at 200 °C

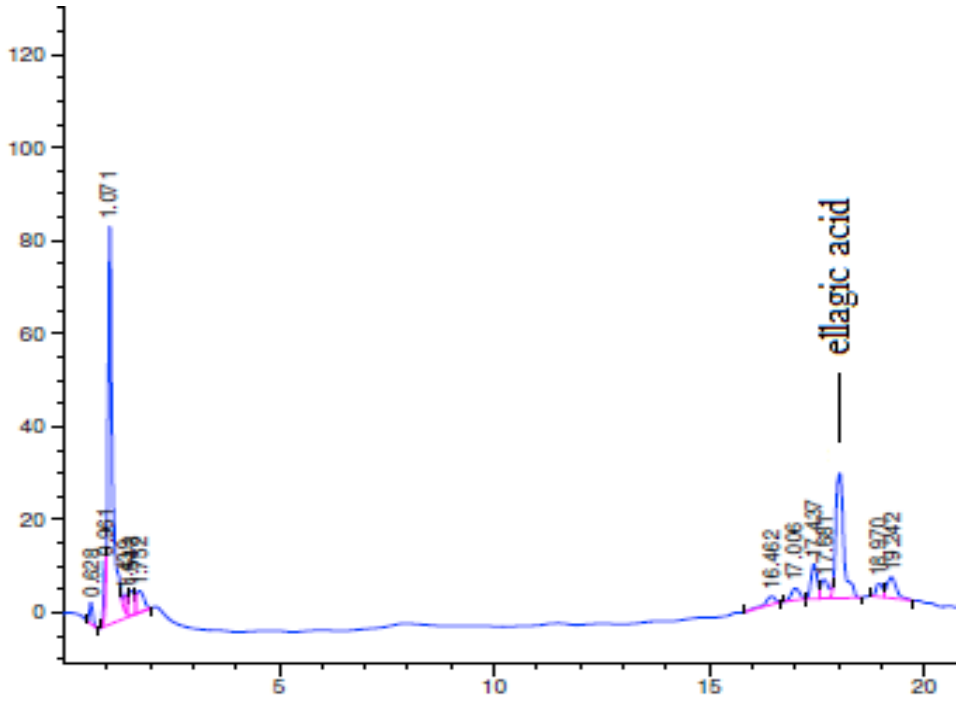

Figure 2. Chromatogram of ellagic acid extracted from raspberry seeds

CONCLUSIONS

Our study found that the raspberry seeds were signifficant source of ellagic acid. The amount of ellagic acid found in „Willamette“ raspberry seeds was 286.54 µg/g.

It was established that ellagic acid was stable up to 200 °C and no signifficant differences in ellagic acid content were found in the non-extruded samples and samples extruded at different temperatures, so raspberry seeds can be implemented in food products requiring higher temperatures during processing.

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УТИЦАЈ РАЗЛИЧИТИХ ТЕМПЕРАТУРА ЕКСТРУДИРАЊА НА СТАБИЛНОСТ ЕЛАГИНСКЕ КИСЕЛИНЕ ИЗ СЕМЕНА МАЛИНЕ

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Сажетак: Производња прехрамбених производа од малине оставља велику количину семена које се третира као нуспроизвод или отпад. Семе малине је богат извор елагинске киселине. Око 88% укупног садржаја елагинске киселине у малинама се налази у семену.
Овај рад се бави истраживањем ефеката процеса екструзије на различитим температурама на садржај елагинске киселине у семену малина сорте "Willamette". Екструдирање је изведено на једнопужном лабораторијском екструдеру и на три температурна режима: 140 °C, 160 °C и 200 °C. Аналитичка техника HPLC/DAD је коришћена за одређивање и квантификацију садржаја елагинске киселине у екструдираним узорцима. Квантификација елагинске киселине одређена је помоћу калибрационе криве добијене од аналитичког стандарда елагинске киселине.
Одређен је садржај елагинске киселине у узорку семена малине сорте „Willamete“ и он износи 286,54 μg/g. Ефекти различитих температура екструдирања на стабилност елагинске киселине из семена малине сорте „Willamete“ нису направили значајну разлику у садржају елагинске киселине, што семе малине сорте „Willamete“ чини погодним за производњу хране на високим температурама.

Кључне речи: *екструдирање, семе малине, елагинска киселина, кукурузна крупица, прозиводи са додатом вредношћу*

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