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Impact of 50 Hz magnetic field on the content of polyphenolic compounds from blackberries

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Blackberries are fruits abundant in bioactive compounds which may be extracted from the matrix by several strategies. Different physical or (bio)chemical pre-treatment of samples might favour the extraction of specific compounds. Little is known about the magnetic field influence on anthocyanins and phenolics extraction, which determined the purpose of this work. Fresh wild and cultivated blackberries were exposed to homogenous 50 Hz frequency magnetic field of 3 mT for up to 12 h. The results showed a significant increase of total anthocyanins and antioxidant activity of all samples exposed up to six hours, compared to control sample. Longer exposure time to magnetic field determined a decrease of total anthocyanins content of wild blackberries. The recovery of high amounts of valuable bioactive compounds using the investigated non-thermal strategy renders the technique useful for applications in food, pharmaceutical or cosmetic industry.

Keywords: Blackberry, Low-frequency magnetic field, Anthocyanins, Phenolics

INTRODUCTION

Apart from their nutritional profile, berry fruits – in particular from *Rubus* species, are abundant in valuable chemical compounds, mainly of polyphenolic structure, such as flavonoids / anthocyanins [1,2]. These compounds are powerful antioxidant biomolecules known for their human health benefits [3].

Considering the bioactive compounds and the health promoting properties of blackberries [4], a lot of research has been conducted toward their valorisation in innovative and value-added products for food or pharmaceutical industry. The main focus for such applications is the recovery of high amounts of biologically active compounds extracted by different, conventional or non-conventional extraction techniques. At the same time, it is known that fresh berries are susceptible to microbial contamination. Several techniques have been proposed for their preservation [5]: (i) chemical (chlorine-based surface disinfectants, ozone, edible coatings); (ii) physical (temperature, ionizing radiation, UV), or (iii) biological (non-pathogenic microorganisms, essential oils, plant extracts). Thermal treatments (heat) are very popular for food preservation, but may lead to degradation of various thermolabile compounds, in particular anthocyanins. Based on the great demand for healthy foods with required quality characteristics, new mild preservation techniques have emerged as useful tools for enzyme and microbial inactivation, such as pulse electric field, high pressure, microwave, ultrasound, X-ray, or magnetic field [6].

Application of magnetic field (static or oscillating) has been taken into consideration as non-thermal new technology of foods preservation, showing the advantage of maintaining the sensory and nutritional quality of the product, because of the small temperature increase (below 5^{o}C), and of no adverse effects compared to other types of irradiation. Both inhibitory and stimulatory effects were observed on microorganisms exposed to magnetic field [7]. The effect depends on the magnetic field strength and frequency, or time of pulse duration. Low-frequency oscillation magnetic field (10 mT) was successfully applied for inactivation of *Escherichia coli* [8], while low-frequency static magnetic field (40 mT) was found useful to inhibit wine yeast [9]. The mechanism of microbial inactivation by exposure to magnetic fields has not been completely elucidated, but it is considered that membrane integrity and DNA is affected [6]. Little is known about the impact of magnetic field on the extraction and recovery of bioactive compounds from plant foods. It is known that physical processes such as ultrasound or high-voltage pulsed electric fields may significantly increase the extraction of phenolics and anthocyanins [10,11]. The literature is scarce in studies regarding the evaluation of the nutritional composition and/or content of phytochemicals in plant foods which have been exposed to magnetic field. Several studies reported higher enzymatic

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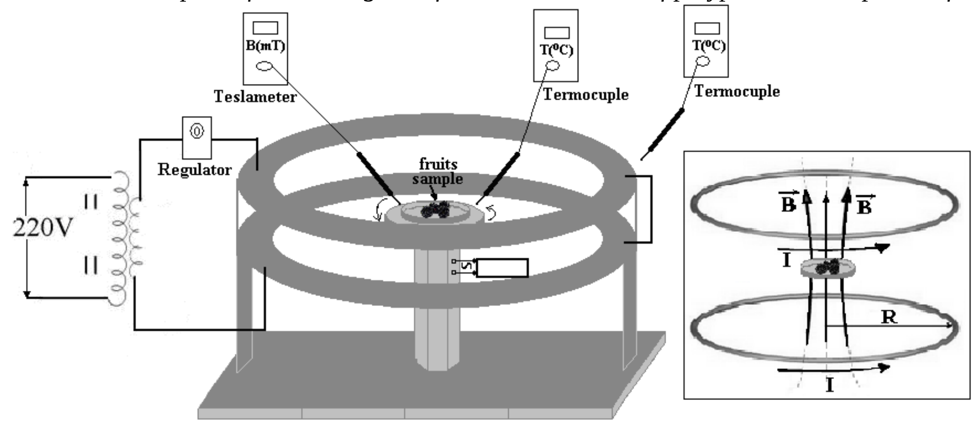

Fig. 1. Magnetic field exposure design setup used for blackberry samples irradiation.

activity in seedlings originating from wheat seeds exposed to low- frequency alternating magnetic field [12] or improved respiration in sugar beet seeds treated with magnetic field [13].

The aim of the present paper was to investigate the changes induced in total anthocyanins content and total antioxidant activity in blackberry fruits (wild and cultivated) exposed to low-frequency magnetic field (50 Hz sinusoidal magnetic field, 3 mT).

EXPERIMENTAL

Plant material and reagents

The experimental study was carried out using two types of blackberry samples grown in Romania: *Rubus sulcatus Ves*t, harvested from spontaneous flora of Şanta area, Sălişte (Sibiu County) and *Rubus fruticosus L.*, Thornfree variety harvested from Târgovişte (Dâmboviţa County) cultivated area. The moisture content of the samples was determined at 105°C using the moisture analyser (MAC 210 - RADWAG, Poland). Chemical reagents of analytical grade without further purification were used.

Magnetic field treatment

Samples were exposed to magnetic field using a laboratory Helmholtz coil system formed of two coils (each 1000 turns of 1 mm copper wire) with 260 mm diameter each, mounted coaxially and placed at a mean distance of 130 mm from each other (Fig. 1). The coil system was powered through a power terminal of 50 Hz sinusoidal voltage (220V) equipped with variable transformer, for generation of a vertical homogenous magnetic field with 3mT of magnetic flux density in the centre of the coil system. A low-frequency field analyser (NARDA EFA-300) was used as no significant variations of the magnetic field value were detected within the central zone (10 cm diameter) of the Helmholtz coil system. During the exposure, no temperature variation was registered. The vertical magnetic field background was measured (0.13±0.01μT) using the C.A.40 Gaussmeter. The magnetic field treatment of samples was performed by placing a 9 cm diameter Petri dish containing the same number of fruit samples of similar weight in the centre of the Helmholtz coil system. Blackberry samples were exposed to a homogenous 50 Hz magnetic field with 3 mT magnetic flux density, at room temperature (22.0 ± 0.5°C) at different exposure times (between 1 and 12 h).

The magnetic field dose was expressed using the following formula:

$$D = \frac{B^2}{2\mu_0} \cdot t \qquad (1)$$

where B[T] is the magnetic flux density, t[s] is the exposure time under magnetic field and μ_0 is the magnetic permeability of free space ($4\pi \cdot 10^{-7}\,H/m$).

The applied magnetic doses ranged between 12.89 kJ·s/m^3 and 154.69 kJ·s/m^3.

Extraction and assay of total anthocyanins

Extraction of anthocyanins was performed immediately after the magnetic treatment, in 70 % (V/V) ethanol solution. Untreated samples were used for comparison studies. The total anthocyanins content in the extracts was determined spectrophotometrically by the pH differential method [14]. The Specord 200Plus UV–Vis spectrophotometer (Analytik Jena, Germany) was used. The results were expressed as mg Cyn–3–O–G equivalents 100 g^{-1} DM.

Total antioxidant activity

The total antioxidant activity in the blackberry extracts was measured by determination of total phenolics according to the Folin-Ciocâlteu method [15] and was expressed as mg gallic acid equivalents GAE 100 g^{-1} DM.

Statistical analysis

All experiments were done in duplicate. The results were presented as mean values ± standard error. The statistical analysis of the experimental data was carried out using the Systat v.10 software. The statistical model for several regression curves was done using the Statistics v.7.0 software.

RESULTS AND DISCUSSION

By exposure of fresh wild and cultivated blackberries to 50 Hz magnetic field (MF) at 3 mT of magnetic flux density for relatively short periods – 1, 2, 4 and 6 h – there was an increase of total anthocyanins content (TAC) compared to control (untreated sample), as shown in Fig. 2.

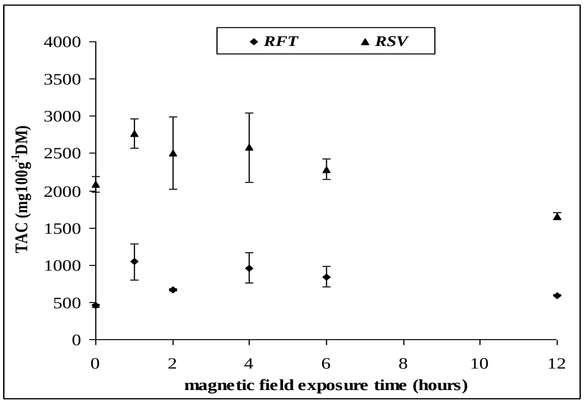

Fig. 2. Comparative total anthocyanins content (**TAC**) (mg 100 g^{-1}DM) changes in wild blackberries *Rubus sulcatus* Vest (**RSV**) and cultivated blackberries *Rubus fruticosus* Thornfree variety (**RFT**), exposed to magnetic field of 3 mT. Values are expressed on dry mass (DM) basis, as mean ± standard error of two replicates.

The MF pre-treatment caused an increase in the level of anthocyanins by 9 – 33% in wild blackberries (*Rubus sulcatus Vest*) and by 30 – 129% in cultivated ones (*Rubus fruticosus* Thornfree variety) depending of irradiation time. The highest level was recorded at 1 h, both in wild and cultivated blackberries. Long MF exposure (12 h) caused a decrease of TAC in wild blackberries by 20% compared to control.

As previously reported [16], wild berries contain much higher amounts of anthocyanins compared to cultivated ones. The present investigation showed that wild blackberries (*Rubus sulcatus Vest*) contained a 4.5 times higher concentration of anthocyanins (2085.64 mg 100 g^{-1} DM) than cultivated ones (*Rubus fruticosus* Thornfree variety) (454.96 mg 100 g^{-1} DM). TAC was expressed on dry mass basis, in order to have homogenous results to be compared. These values are within the range of TAC of blackberries measured with the same method reported by other authors [17].

The regression analysis revealed a third order polynomial dependence between the TAC and the MF energy dose (D). The regression model (R^2= 0.93) for wild blackberry is presented in Fig. 3. The total antioxidant activity as measured by total phenolics content (TPC) of fresh wild and cultivated blackberries exposed to 50 Hz MF of 3 mT of magnetic flux density, increased up to 63% compared to control.

The TPC changes with exposure time (1 - 12 h) are comparatively presented in Fig. 4. The MF pre-treatment generated an increase in the level of TPC of wild blackberries by 5 - 27% and of cultivated blackberries by 6.5 - 63% in relation to the exposure time. The highest TPC of both wild and cultivated blackberry samples was recorded after 1 h.

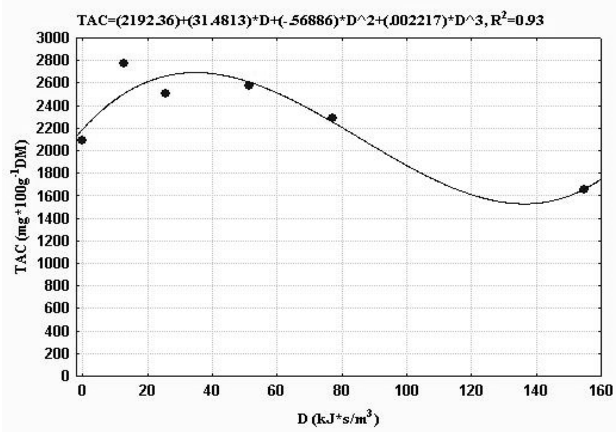

Fig. 3. Dependence between the total anthocyanins content (**TAC**) (mg 100 g^{-1}DM) of wild blackberries *Rubus sulcatus* Vest (**RSV**) and the magnetic field energy dose (**D**) (kJ s/m^3). Values are expressed on dry mass (DM) basis, as mean of two replicates.

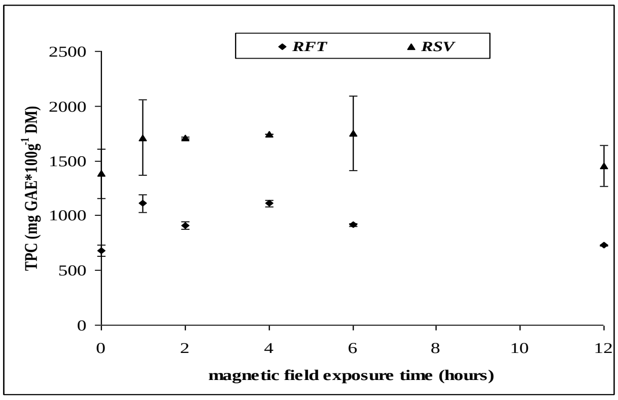

Fig. 4. Comparative total phenolics content (TPC) (mg GAE 100 g^{-1} DM) changes in wild blackberries *Rubus sulcatus Vest (RSV)* and cultivated blackberries *Rubus fruticosus* Thornfree variety (RFT), exposed to magnetic field of 3 mT. Values are expressed on dry mass (DM) basis, as mean ± standard error of two replicates.

The TPC of wild blackberries (*Rubus sulcatus Vest*) was 2 times higher than that of cultivated ones (*Rubus fruticosus* Thornfree variety), which indicates the higher total antioxidant activity of wild blackberries. TPC was expressed on dry mass basis, in order to have homogenous results to be compared. TPC of blackberries was reported to vary from 114 to 1056 mg 100 g^{-1} FW [4] depending on variety, environmental factors and methods of analysis.

Fig. 5 indicates the polynomial dependence between the TPC of the wild blackberries and the MF energy dose (D), resulted from the regression analysis (the coefficient of determination R^2= 0.93). By calculation of the correlation coefficients, a positive linear correlation between TPC and TAC levels was found for cultivated blackberries (R= 0.9572; p= 0.002) (Fig. 6).

Enhanced levels of TAC and TPC of blackberries were obtained for samples exposed to homogeneous magnetic field (50 Hz, 3 mT) for relatively short times (1-6 h) compared to control. A tendency of TAC decrease was registered in samples exposed to magnetic field for longer periods (12 h).

To our knowledge, there is a scarcity of such studies regarding the influence of magnetic fields on total phenolics and anthocyanins level in fruits. Lipiec *et al.* [18] reported a 1.5-fold increase of TPC in sprouts of naked oat (cv. Akt) under the treatment with oscillating magnetic field pulses of 10 × 3 T. Most of the previous studies reported the effects of magnetic field on the growth of various plants. Esitken [19] studied the influence of 50 Hz magnetic field with magnetic induction of 96, 192 and 384 mT, respectively, on raspberry (*Camarosa* variety) during its growth in a greenhouse. Their results showed a positive influence on plant growth and fruit production. In another study, the pre-treatment of seeds of *Artemisia sieberi* with MF of 200 mT for 20 min before germination, led to a considerable increase in the concentration of polyphenols and antioxidant activity of plant shoots, compared to control [20].

The mechanism by which magnetic fields impact living cells is still unclear, but most hypotheses refer to the change in the Ca^{2+} balance, antioxidant enzymes and different metabolic processes [21,22].

Another hypothesis regarding the mechanism of interaction of low-frequency magnetic fields with the absorbing material is based on the induction of electric fields, their distribution being related to the electrical conductivity of tissues [23].

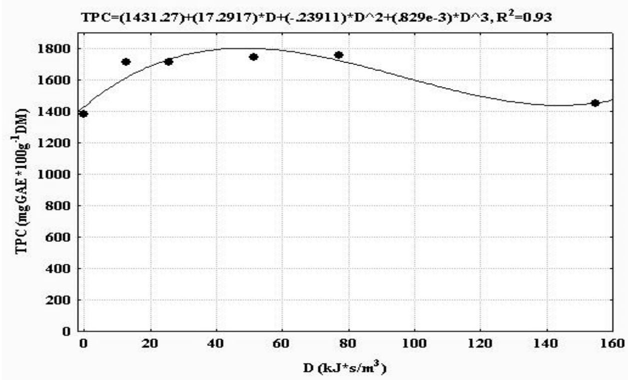

Fig. 5. Dependence between the total phenolics content (**TPC**) (mg GAE 100 g^{-1} DM) of wild blackberries *Rubus sulcatus Vest (**RSV**)* and the magnetic field energy dose (**D**) (kJ s/m^3). Values are expressed on dry mass (DM) basis, as mean of two replicates.

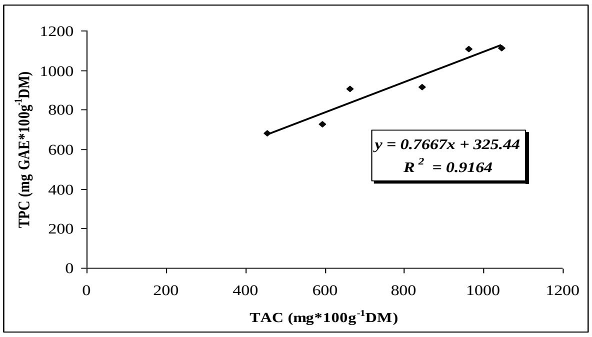

Fig. 6. Linear correlation between **TPC** (mg GAE 100 g^{-1} DM) and **TAC** (mg 100 g^1 DM) of cultivated blackberries *Rubus fruticosus* Thornfree variety (**RFT**).

As water is dominant in all living cells, unusual impact of the magnetic field on the water could explain some of the reported effects since the energy of the extremely low MF (50 Hz) is much lower than the energy required for breaking the bonds of water molecules. Nevertheless, at present, there is no clear mechanism of influence of low MF on water. Some researchers have noticed changes in the electrical conductivity of water due to the action of 50 Hz MF [24]. An increase of water evaporation due to exposure to weak static MF (15 mT) was also reported [25]. However, there are quite a lot of controversial results in the literature on this subject.

Regarding the possible mechanism by which the MF increased the levels of anthocyanins and the antioxidant activity in the hereby investigated fresh blackberries, probably membrane permeability processes are involved favouring the release of intracellular biologically active compounds (enhancement of extraction).

CONCLUSIONS

The results of our study showed that magnetic field exposure produced changes in the content of main antioxidant compounds of polyphenolic structure in fresh blackberries, either wild or cultivated. The treatment of blackberry fruits with low magnetic field (50 Hz, 3 mT) for up to six hours resulted in the increase of total anthocyanins and phenolics content, which was more pronounced in cultivated species. Long exposure time (12 h) determined a decrease of total anthocyanins content of wild blackberries. In all experiments, wild blackberries showed higher concentrations of the investigated phytochemical compounds compared to cultivated ones, for both MF irradiated and non-irradiated samples.

Our findings may be used for the improvement of extraction of polyphenolic compounds from berry fruits using pre-treatment with MF.

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ВЛИЯНИЕ НА 50 Hz МАГНИТНО ПОЛЕ ВЪРХУ СЪДЪРЖАНИЕТО НА ПОЛИФЕНОЛНИ СЪЕДИНЕНИЯ В КЪПИНИ

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(Резюме)

Къпините са плодове, богати на биоактивни вещества, които могат да се екстрахират от матрицата по различни методи. Различната физична или (био)химична обработка на пробите може да благоприятства екстракцията на специфични вещества. Влиянието на магнитно поле върху екстракцията на антоцианини и феноли е малко известно, което определи целта на настоящата работа. Пресни диви и култивирани къпини са изложени на хомогенно магнитно поле от 3 mT и честота 50 Hz за срок до 12 часа. Установено е значително нарастване на общите антоцианини и на антиоксидантната активност на всички проби, изложени за срок до 6 ч в сравнение с контролната проба. По-дълга експозиция на магнитно поле води до понижаване на общото съдържание на антоцианини в дивите къпини. Добиването на големи количества ценни биоактивни вещества с изолзване на изследвания нетермичен подход прави тази техника подходяща за прилагане в хранителната, фармацевтичната и козметичната индустрия.