

Influence of Biodynamic Preparations on the Quality Indices and Antioxidant Compounds Content in the Tubers of Coloured Potatoes (*Solanum tuberosum* L.)

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Abstract

Biodynamic preparations 500 and 501 are plant strengthening agents of biodynamic agriculture method, prepared of manure and powdered quartz. The objective of the present study was to determine effects of biodynamic (BD) preparations 500 and 501 on the quality indices and antioxidant compounds content in the tubers of coloured flesh potatoes. The experiment included two factors: potato cultivar and treatment with BD preparations as field sprays. The experiment was carried out in four replications, in a randomly design. Results showed that application of BD preparations did not influence significantly the contents of dry matter, crude fibre and crude ash in all tested cultivars. Separately used BD preparation 500 increased content of total anthocyanins in tubers of ‘Vitelotte’ and ‘Red Emmalie’ and leucoanthocyanins content in ‘Blue Congo’, but decreased the content of total phenolics in all cultivars. Treatment with BD preparation 501 had significant effect on the contents of total phenolics and total anthocyanins in all cultivars. However, treatment with BD preparation 500 in combination with BD preparation 501 substantially increased the contents of total phenolics and total anthocyanins in all cultivars. Tubers of ‘Vitelotte’ with dark-purple flesh contained significantly more antioxidant compounds than the light-purple and red.

Keywords: coloured potato cultivars, biodynamic agriculture, crude fiber, crude ash, anthocyanins, phenolics

Introduction

Potato is one of the most widely grown vegetables and represents an important source of nutrients in many countries (Leo *et al.*, 2008). It is a balanced food containing high energy, nutritional quality proteins, dietary fibre and minerals (Pełksa *et al.*, 2013; Danilčenko *et al.*, 2014). In current years, more attention is being given to the research of red and purple coloured potato cultivars, due to their antioxidant property, which is related to various polyphenols (Nayak *et al.*, 2011; Jariene *et al.*, 2013). There are number of studies showing that these antioxidants of high free radical scavenging activity may reduce the risk of chronic health diseases and age related neuronal degeneration (Teow *et al.*, 2007; Dai and Mumper, 2010; Khurana *et al.*, 2013).

There are number of reports on the negative effects of using chemicals in agriculture on the quality of vegetables and fruits (Lairon, 2010).

Therefore, in order to preserve the quality of potatoes it is necessary to look for alternative farming methods like biodynamic agriculture. Similar to traditional organic agriculture, biodynamic agriculture eliminates synthetic chemical fertilizers and pesticides. A major difference is that biodynamic farmers use eight specific preparations (Zaller and Kopke, 2004). Biodynamic (hereinafter BD) preparations are plant strengthening agents and they belong to two classes: preparations sprayed directly onto the soil or crops (500 and 501) and preparations added to composting farmyard manure (502-507). The main aim of these preparations is to improve soil and plants quality (Reganold, 1995; Raupp, 1999; Koepf *et al.*, 2001).

Table 1. Description of potato cultivars

Cultivar	'Vitelotte'	'Blue Congo'	'Red Emmalie'
Time of maturity	Medium later	Medium early	Medium early
Colour of flesh	Dark purple	Light purple	Red
Colour of skin	Dark purple	Dark purple	Red
Shape of tuber	Long	Round	Long
Depth of eyes	Deep	Shallow	Shallow

The BD preparation 500 is made from high quality manure, fresh or aged, put in bovine horns, then buried at autumn and dug up in spring, after that it can be stored under controlled conditions for some months. The resulting product is dissolved in water and sprayed on the soil (Brinton, 1997). The other field spray is one made of silicon dioxide and is known as BD preparation 501. It is used to reinforce the plant against pests and diseases and to improve its nutritional properties, flavours and shelf-life. A very small quantity of the 501 is then dissolved in water and sprayed on the plants, mostly at flowering stage (Koepf *et al.*, 2001; Catellani, 2006). These two BD preparations are believed to work synergistically, with BD preparation 500 mainly improving the common soil fertility, and BD preparation 501 being active in enhancing the plant physiological response to the light radiation (Spaccini *et al.*, 2012).

According to some authors the products of biodynamic agriculture are nutritionally superior and they taste better than the conventional foods (Fritz and Kopke, 2000; Jayasree and Annamma, 2006). However, studies on the impact of BP preparations on the potato tuber quality indices are very limited. Therefore, the aim of this study was to establish effects of BD preparations 500 and 501 on some quality traits and antioxidant compounds content in the tubers of coloured potatoes.

Materials and Methods

Growth conditions and plant material

Field experiment was carried out in 2013-2014 in organic farm (Prienu district, Lithuania). When using BD preparations, potatoes were grown applying the traditional potato growing technology (Razukas, 2003). The tubers were planted in May, and harvested in September. The field experiment was carried out in four replications, arranged randomly. The overall field size of experiment was 17.5 m², whereas the size of the accounting field was 10 m². The main soil properties were as follows: soil pH_{KCl} 6.86-6.92, content of total nitrogen 0.126-0.142%, available phosphorus 166.1-181.8 mg kg⁻¹ and available potassium 207.6-248.8 mg kg⁻¹.

The experiment included two factors: potato cultivar ('Vitelotte', 'Blue Congo' and 'Red Emmalie', with the characteristics presented in Table 1) and treatment with BD preparations as field sprays. There were four treatments to evaluate the effectiveness of BD preparations:

1. Control (BD preparations were not used).
2. BD preparation 500 (the soil was sprayed two weeks before the planting of tubers, 1% solution).
3. BD preparation 501 (two times early in the morning potato plant leaves were sprayed with 0.5% solution, in the VIII and IX stages of organogenesis).
4. BD preparation 500 in combination with BD preparation 501 (two weeks before the planting of the tubers the soil was sprayed with BD preparation 500 with 1% solution, and two times early in the morning potato leaves were sprayed with BD preparation 501 with 0.5% solution in the VIII and IX stages of organogenesis).

BD preparations 500 and 501 were purchased in the Biodynamic Preparations Centre, Germany.

Chemical analyses

Chemical potato tubers analyses were conducted in the Laboratory of Food Raw Materials, Agronomic and Zootechnical Investigation of Aleksandras Stulginskis University and in the laboratory of Immanuel Kant Baltic Federal University (Kaliningrad, Russia). Each laboratory sample comprised 5 kg of tubers.

Dry matter, crude fibre and crude ash analyses

Dry matter in the tubers was determined by drying samples at temperature of 105 °C to the constant weight (LST ISO 751:2000); content of crude fibre was determined by the method of Heneberg-Shtoman (Methodenbuch-VDLUFA, 1983-1999); content of crude ash was determined by combustion at 550 °C.

Total phenol analyses

The total content of phenolic compounds was determined using the spectrophotometric method. An analytical reaction was a positive reaction of Berlin blue solution, which was obtained from a mixture of ferrous iron and potassium hexacyanoferrate (K₃Fe(CN)₆). The content of phenolic compounds was calculated based on light absorbance of the obtained solution at the wavelength 720 nm. The solutions of gallic acid were used as a standard.

The plant samples were homogenized in acidulated 96-degree ethanol (20:1), the homogenate was centrifuged at 4500 rpm for 30 min (Gupta and Verma, 2011). The optical density of the solutions was determined using a spectrophotometer "SF-2000" (ZAO OKB Spectr).

Anthocyanin and leucoanthocyanin analyses

The concentration of the anthocyanin pigments was determined spectrophotometrically in 1% hydrochloric acid aqueous extract at wavelength 510 nm, after the homogenate was centrifuged at 4500 rpm for 30 min. The content of anthocyanin was calculated based on light absorbance of the obtained solution at the wavelength 657 nm. Anthocyanin content was calculated from the amount of cyanidin-3,5-diglucoside (Chupakhina *et al.*, 2010). The absorption of these pigments was determined using a spectrophotometer "SF-2000" (ZAO "OKB SPECTRUM", Russia). Content of leucoanthocyanin was measured according to Yashin *et al.* (2007) method.

Soil analyses

Soil analyses were conducted at the Laboratory of Food Raw Materials, Agronomic and Zootechnical Research of Aleksandras Stulginskis University. Soil pH_{KCl} was established by the potentiometric method in 1N KCl extract. The content of total nitrogen in the soil was established by the Kjeldahl method. The content of available phosphorus was determined by the CAL method using a spectrophotometer as well available potassium content using a flame photometer.

Statistical analysis

The experimental data were statistically processed by ANOVA, software STATISTIKA 7.0 (StatSoft, USA). Means and standard deviations of the data were calculated. Tukey's HSD test (p<0.05) was applied to estimate significance of differences.

Table 2. The influence of biodynamic (BD) preparations on some quality indices of tubers of coloured potato cultivars (% d.m.)

Treatments	Quality indices		
	Dry matter	Crude fiber	Crude ash
		‘Red Emmalie’	
Control (without BD preparations)	17.96±0.69 ^b	2.20±0.06 ^b	5.45±0.19 ^b
BD preparations 500	19.63±0.71 ^a	2.47±0.13 ^a	6.17±0.22 ^a
BD preparations 501	19.80±0.78 ^a	2.22±0.10 ^b	6.23±0.23 ^a
BD preparation 500 in combination with BD preparation 501	18.87±0.64 ^b	2.26±0.09 ^{ab}	6.14±0.25 ^a
		‘Blue Congo’	
Control (without BD preparations)	20.56±0.73 ^a	1.99±0.09 ^a	4.84±0.13 ^a
BD preparations 500	19.33±0.87 ^a	2.03±0.08 ^a	4.85±0.11 ^a
BD preparations 501	19.60±0.69 ^a	2.15±0.10 ^a	5.06±0.15 ^a
BD preparation 500 in combination with BD preparation 501	20.63±0.93 ^a	2.21±0.14 ^a	4.93±0.17 ^a
		‘Vitelotte’	
Control (without BD preparations)	26.17±0.93 ^a	1.85±0.07 ^b	4.95±0.15 ^a
BD preparations 500	26.27±0.86 ^a	2.17±0.13 ^a	5.25±0.16 ^a
BD preparations 501	26.71±0.98 ^a	2.15±0.15 ^a	5.29±0.21 ^a
BD preparation 500 in combination with BD preparation 501	27.69±1.09 ^a	2.20±0.08 ^a	5.11±0.17 ^a

Note: Mean values ±standard deviation (x ± SD).

^{ab} – Different letter superscripts in the same column indicate significant differences among samples (*p* < 0.05)

Table 3. The influence of biodynamic (BD) preparations on the contents of antioxidant compounds in tubers of coloured potato cultivars

Treatments	Antioxidant compounds		
	Total phenolics (mg g ⁻¹ d.m.)	Total anthocyanins (mg 100 g ⁻¹ d.m.)	Leucoanthocyanins (mg 100 g ⁻¹ d.m.)
		‘Red Emmalie’	
Control (without BD preparations)	2.37±0.07 ^b	0.91±0.02 ^d	85.55±2.99 ^{bc}
BD preparations 500	1.98±0.06 ^c	1.36±0.05 ^b	87.68±2.45 ^b
BD preparations 501	2.59±0.07 ^a	1.19±0.03 ^c	82.90±2.26 ^c
BD preparation 500 in combination with BD preparation 501	2.69±0.08 ^a	1.52±0.06 ^a	93.54±3.11 ^a
		‘Blue Congo’	
Control (without BD preparations)	2.44±0.09 ^b	0.98±0.05 ^b	28.47±1.03 ^c
BD preparations 500	1.53±0.10 ^c	1.00±0.04 ^b	39.58±1.41 ^b
BD preparations 501	3.17±0.15 ^a	1.00±0.05 ^b	43.65±1.68 ^a
BD preparation 500 in combination with BD preparation 501	2.95±0.11 ^a	1.17±0.08 ^a	46.51±1.56 ^a
		‘Vitelotte’	
Control (without BD preparations)	8.42±0.10 ^b	5.39±0.09 ^c	297.60±5.71 ^{ab}
BD preparations 500	7.76±0.09 ^c	6.53±0.13 ^a	289.74±5.43 ^b
BD preparations 501	8.64±0.08 ^a	6.23±0.11 ^b	288.69±6.02 ^b
BD preparation 500 in combination with BD preparation 501	8.66±0.10 ^a	6.59±0.16 ^a	308.95±6.53 ^a

Note: Mean values ±standard deviation (x ± SD).

^{abcd} – Different letter superscripts in the same column indicate significant differences among samples (*p* < 0.05)

Results

Dry matter content

The content of dry matter in potato tubers is one of the main indices of quality in determining the processing ability of the product. According to Hassanpanah *et al.* (2011) the content of dry matter in potato tubers can fluctuate from 13.1 to 36.8% with an average of 24%. In the present study we found that, in comparison with the control, separately used BD preparations 500 and 501 substantially increased content of dry matter in ‘Red Emmalie’ tubers. However, separately and in combination used BD preparations did not affect the content of these substances in tubers of ‘Blue Congo’ and ‘Vitelotte’ (Table 2). Kjellenberg and Grandstedt (2005) after 33 years of research concluded that using of biodynamic preparations had positive influence on the accumulation of dry matter in cereal.

The highest content of dry matter was observed in ‘Vitelotte’ tubers when treated with BD preparation 500 in combination with BD preparation 501 (27.69%). The lowest dry matter content was recorded for the control (17.96 %) in the case of ‘Red Emmalie’ tubers (Table 2).

Crude fibre content

Another important component of potato tubers is dietary fibre, which consists of cellulose, hemicelluloses, pectins and lignin, as well as other substances resistant to digestive enzymes (Mazurczyk, 2005). Accumulation of crude fibre is a heritable character, but is also affected by a number of environmental factors. The study revealed that BD preparations 500 and 501 used separately or in combination had unequal impact on the content of crude fibre in tubers of the cultivars tested. The content of these compounds in ‘Vitelotte’ tubers was found essentially higher for all biodynamic treatments compared with the control. Also, separately application of BD preparation 500 increased the content of crude fibre in ‘Red Emmalie’ tubers (Table 2). Langenkämper *et al.* (2006) studied the content of ash and fibre in wheat grown in different farming systems (organic, biodynamic and intensive). However, no essential differences of the content of crude fiber were found.

Crude fibre content varied insignificantly among potato cultivars from 1.85% (for ‘Vitelotte’, when cultivated without BD preparations) to 2.47% (for ‘Red Emmalie’, when cultivated with BD preparation 500) (Table 2).

Crude ash content

According to literature data, mean content of crude ash in potato tubers is 4.41% (Lister and Munro, 2000). We found that BD preparations 500 and 501 used separately or in combination had significantly influenced the content of crude ash only in potato tubers of 'Red Emmalie' (Table 2). The application of BD preparations 500 and 501 had no significant effect on the content of crude ash in tubers of 'Vitelotte' and 'Blue Congo'. The crude ash content in the cultivars ranged from 4.84% to 6.23%. The highest content (6.23%) showed 'Red Emmalie' tubers when the plants were cultivated with BD preparation 501. The least content (4.84%) was observed in tubers of 'Blue Congo' when the plants were cultivated without BD preparations (control treatment) (Table 2).

Total phenolic content

Navarre *et al.* (2011) reported that coloured potatoes showed high variability in total phenolic content, ranging from 1.8 to 11.0 mg g⁻¹ d.m. The present study revealed that BD preparations 500 and 501 used separately or in combination had unequal impact on the content of total phenolics for all potato cultivars. Separately used BD preparation 501 and combination of BD preparations 500 and 501 significantly increased the content of total phenolics in tubers of all cultivars (Table 3).

There are some reports in literature that optimization of nutrition with silicon increases the phenolic compounds and stress enzymes in plant tissues (Sivanesan and Park, 2014). However, separately used BD preparation 500 decreased the content of these compounds in tubers of all cultivars (Table 3). It could be associated with a greater impact of this treatment on the preparation of vegetative parts of the plant growth and root system development (Spaccini *et al.* 2012). Maciel *et al.* (2011) found that the biodynamically grown mangoes showed the highest content of phenolic compounds. In another study on effect of BD preparations on grape quality, Reeve *et al.* (2005) generally found no differences and only in one year of their research the content of total phenolics and anthocyanins in biodynamically-cultivated grapes was higher.

We found that the highest total phenolics content was characteristics for the tubers of 'Vitelotte' when cultivated with combination of BD preparations 500 and 501 (8.66 mg g⁻¹ d.m.), while the lowest for the tubers of 'Blue Congo' when cultivated with BD preparation 500 (1.53 mg g⁻¹ d.m.). 'Vitelotte' tubers had significantly higher content of total phenolics than 'Blue Congo' and 'Red Emmalie' (Table 3). This can be explained by a more intense flesh colour of 'Vitelotte' tubers. According to literature, dark blue- or purple-fleshed potatoes show higher total phenolic content compared to light blue- or purple and red-fleshed ones (Jansen and Flamme, 2006; Reddivari *et al.*, 2007).

Total anthocyanins content

Anthocyanins (classified as flavonoids) are secondary plant metabolites responsible for blue, purple or red hues in vegetables and fruits. Low content of anthocyanins in yellow- and white-flesh potato cultivars was reported, but they are the main source of antioxidants in purple and red cultivars (Andre *et al.*, 2009).

The current results showed that treatment with BD preparations 500 in combination with BD preparation 501 significantly influenced accumulation of anthocyanins in tubers of all cultivars compared to the control ones (Table 3). These pigments content increased 1.7 times in 'Red Emmalie' and 1.2 times in 'Blue Congo' and 'Vitelotte' tubers. Separately used BD preparations 500

and 501 essentially increased the content of total anthocyanins only in 'Red Emmalie' and 'Vitelotte' tubers (Table 3). Heimler *et al.* (2012) studied the effect of BD preparations on 'Batavia' lettuce quality, and also established that the total anthocyanins content was higher in plants from biodynamic farming. According to the authors, the reason may be associated with stress conditions in biodynamic farming or to different microbial environment.

It was also observed that 'Vitelotte' tubers accumulated the highest amount of total anthocyanins in treatment with combination of BD preparations 500 and 501 (6.59 mg 100 g⁻¹ d.m.) (Table 3). It can be explained by the fact that this cultivar has dark-purple flesh practically in the entire cross-section. The lowest amount of these compounds was determined in 'Red Emmalie' tubers (0.91 mg 100 g⁻¹ d.m.) when the plants were cultivated without BD preparations (Table 3). Previous studies on antioxidant compounds in purple-, red-, yellow and white-flesh potatoes also showed that dark-blue or purple-flesh cultivars contained significantly more anthocyanins than other cultivars (Hamouz *et al.*, 2011; Nayak *et al.*, 2011).

Leucoanthocyanins content

Potatoes are considered a good source of antioxidants, such as leucoanthocyanins.

These flavonoids help to strengthen blood vessels, increasing the tone and elasticity of capillary walls and help to quench free radicals (Tsao *et al.*, 2006). The BD preparations had different effect on the content of leucoanthocyanins in the cultivars (Table 3). Separately and in combination used BD preparations 500 and 501 substantially increased the content of leucoanthocyanins in 'Blue Congo' tubers. The content of these compounds in tubers of 'Red Emmalie' was higher after treatment with BD preparations 500 in combination with BD preparation 501 compared to the control. However, BD preparations had no influence on the leucoanthocyanins content in 'Vitelotte' tubers (Table 3).

Basically, the greatest content of all the leucoanthocyanins was established for the biodynamic treatment, when the plants were treated with combination of BD preparations 500 and 501 (308.95 mg 100 g⁻¹ d.m. for the tubers of 'Vitelotte') and the lowest in the control in the case of tubers of 'Blue Congo' (28.47 mg 100 g⁻¹ d.m.) (Table 3).

Conclusion

BD preparations 500 and 501 had no significant effects on the contents of dry matter, crude fibre and crude ash in tubers of all tested potato cultivars of coloured flesh. However, BD preparation 500 decreased the content of total phenolics in all cultivars, but increased the content of total anthocyanins in tubers of 'Vitelotte' and 'Red Emmalie' and the content of leucoanthocyanins in 'Blue Congo'. BD preparation 501 had significant effect on the content of total phenolics (in all cultivars), total anthocyanins (in 'Vitelotte' and 'Red Emmalie') and leucoanthocyanins (in 'Blue Congo'). Application of BD 500 in combination with BD 501 substantially increased the contents of total phenolics and total anthocyanins in all tested cultivars.

The results revealed that 'Vitelotte' tubers, with dark-purple flesh, contained substantially more total phenolics, total anthocyanins and leucoanthocyanins than the light-purple and red potato cultivars.

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