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IMPACT OF DENSITY OF BREEDING ON THE GROWTH AND SOME NUTRACEUTICAL PROPERTIES OF READY-TO-EAT LENTIL (*Lens culinaris*) SPROUTS

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Abstract. Nutritional and nutraceutical quality of sprouts is strongly affected by growth conditions. This study focused on determining the influence of breeding density on seedling growth, phenolics content and some antioxidant capacity of ready-to-eat lentil sprouts. Content of condensed tannins (ranging from 1.77 to 3.16 mg g^{-1} DM) and flavonoids (ranging from 15.13 to 25.08 mg g^{-1} DM) increased with the increasing density of breeding. The contents of the p-hydroxybenzoic and ferulic acids, and (+) catechin decreased with the increasing density of breeding in 3-days-old seedlings. Additionally, the level of quercetin was elevated at a higher degree in sprouts cultivated at density of 1.22 seeds per cm^2 and average 10.42 and 5.91 μg g^{-1} DM for 3- and 4-days-old sprouts, respectively. Metal chelating ability was the highest for sprouts obtained at the lowest density: 92% and 86% for 3- and 4-days-old sprouts, respectively. Fresh mass yield and lipids preventing abilities were negatively affected by density of breeding. It can be concluded that density of breeding plays an important role in design of chemical composition and bioactivity of lentil sprouts.

Key words: antioxidant activity, cultivation conditions, lentil, density of breeding, sprouts

INTRODUCTION

Obtaining adequate nutrients from various plant foods plays a vital role in maintaining normal function of the human body [Zhao 2007]. It is known that sprouted legumes are excellent and low-cost sources of dietary proteins and other nutrients for a large part of the World's population. With recent advances in medical and nutrition sciences new concepts of functional foods sign in trend, such as nutraceuticals, nutritional therapy or phytomedicine [Fang et al. 2002, Ferrari and Torres 2003, Scalbert et al. 2005]

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Reactive oxygen and nitrogen species have been implicated in more than 100 diseases, including heart disease, stroke, arteriosclerosis, diabetes and cancer [Fang et al. 2002, Gawlik-Dziki et al. 2011, Gawlik-Dziki et al. 2012b]. In living organisms various reactive species can be formed by different ways, eg. normal aerobic respiration and some metabolic processes including activity of lipoxygenase, which catalyzes oxygenation of polyunsaturated fatty acids [Gawlik-Dziki et al. 2011].

Plants secondary metabolites, including polyphenolics, have been widely studied and their beneficial influence on human health was confirmed. The beneficial effect comes in part through the antioxidant characteristics of phenolic compounds; therefore, it is important to evaluate their antioxidant activities. Due to the potential significance of phenolic antioxidants for the prevention of a wide range of degenerative physiological processes deepening the knowledge about plant food composition is necessary. In the light of this, a proper selection of food production conditions (e.g. that may lead to increase phenolics content) could be an important aspect [Gawlik-Dziki et al. 2012a, Shetty et al. 2002, Shetty 2004, Randhir and Shetty 2003].

Germination is one of the most common and effective processes for improving the quality of legumes. This process generally improves the nutritional quality of legumes eg. protein and starch digestibility, bioavailability of vitamins and microelements [Ghavidel and Prakash 2007, Świeca et al. 2013]. Additionally, it also increases the functionality of the seeds due to the subsequent enhancement of nutraceutical properties (pro-health abilities such antioxidant, anticancer, antidiabetic, antimicrobial) [Amarowicz et al. 2009, Świeca et al. 2012, Gawlik-Dziki et al. 2012a]. Several reports were found in the literature about the effect of germination method on the nutraceutical value of legumes including soybeans, mung beans or lentils [Shetty et al. 2002, Shetty 2004, Randhir and Shetty 2003, Ghavidel and Prakash 2007, Randhir et al. 2004]. Most studies have been conducted using biotic and abiotic stresses and/or elicitors [Gawlik-Dziki et al. 2012a, Randhir and Shetty 2003, Ghavidel and Prakash 2007, Zhao et al.2005]. These techniques effectively increased production of secondary metabolites and other compounds involved in the plant response to stress [Fujita et al. 2006]. Additionally, the usage of proper conditions of breeding may preserve shelf life of sprouts, reduce food microorganisms without affecting the sensory and nutritional quality [Shetty 2004, Khattak et al. 2007, McCune and Johns 2007].

In recent literature there is lack of information concerning the influence of density of breeding on nutritional quality and antioxidant abilities of legumes sprouts. There are some evidences confirming that this factor may play a key role in yielding and designing of chemical composition of legumes seeds [Biswas et al. 2002, Njoku and Muoneke 2008, Turk and Tawaha 2002, Miguélez Frade and Valenciano 2005].

The aim of this study was to determine the influence of density of breeding on the seedling growth, changes of phenolics content and some antioxidant activities of ready-to-eat lentil sprouts.

MATERIAL AND METHODS

Lentil seeds var. Tina were purchased from the PNOS S.A. in Ozarów Mazowiecki, Poland. Seeds were sterilized in 1% (v/v) sodium hypochloride for 10 min, then drained and washed with distilled water until they reached neutral pH. They were placed in distilled water and soaked by 6 hours at 25°C. Seeds were germinated 4 days in a growth chamber on the Petri dishes (ø 125 mm) lined with absorbent paper in darkness. The density test was designed with rates of seeding as follows: 100, 150 and 200 seeds per plate (0.82, 1.22 and 1.63 seeds per cm^2, respectively). Sprout samples were collected at different time points (3 and 4 days) – sprouts ready-to-eat. For each day and growth conditions sprouts were gently collected, rapidly frozen and kept in polyethylene bags at -20°C for analysis. For each test, three replicates were taken out for analysis.

The experiments tended to analyze seedlings growth parameters such as length (hypocotyls and roots), weight and the correlation of dry weight to wet weight. Lentil sprouts samples from various stages of germination were measured for both their dry and fresh weight. Sprouts were freeze-dried. The fresh/dry weight ratio was determined as an amount of fresh mass obtained from 1g of dormant seeds during germination.

Sprouts were freeze-dried and grounded in a labor mill, and sieved (60 mesh). Sprouts flours were stored at 4°C. Extraction procedure was conducted according to Xu and Chang [2007]. Briefly, lentil flours (0.2 g in triplicate) were extracted three times with 4 ml of acetone/water/ hydrochloric acid (70:29:1, v/v/v). After centrifugation (10 min 6400 g) fraction were collected, combined and used in further analysis.

The amount of total phenolics was determined using Folin-Ciocalteau reagent [Singleton et al. 1974] and expressed as a gallic acid equivalent per g of dry weight (DM). Total flavonoids content was determined according to the method described by Lamaison and Carnat [1990] and expressed as a quercetin equivalent per g of dry weight (DM. Condensed tannins content was determined according to the method described by Sun et al. [1998] and expressed as a (+) catechin equivalent (GAE) per g of dry weight (DM). *Additionally,* qualitative – quantitative analysis of polyphenols was performed with a Varian ProStar HPLC System separation module (Varian, Palo Alto, CA) equipped with Varian ChromSpher C18 reverse phase column (25 mm × 4.6 mm) and ProStar DAD detector according to Świeca et al [2012]. Spectrum analysis and comparing their retention times with those of the standard compounds identified phenolic compounds in a sample. Metal chelating ability was determined by the method of Guo et al. [2001]. The lipids preventing ability was determined as the degree of inhibition on the hemoglobin-catalyzed peroxidation of linoleic acid according to Groupy et al. [2007]. Lipoxygenase activity was determined according to Axelrod [1981]. Briefly, the reaction mixture contained 2.45 ml M/15 phosphate buffer, 20 µl enzyme solution (167 $U \cdot ml^{-1}$), 50 µl of inhibitor solution. After preincubation of the mixture at 30°C for 10 min, the reaction was initiated by adding 80 µl 2.5 mmol/L linoleic acid. One unit of LOX activity was defined as an increase in absorbance of 0.001 per minute at 234 nm.

All experimental results were mean ±S.D. of three parallel measurements (n = 9) and data were evaluated by using one-way analysis of variance post-hocTukey's test. The p values < 0.05 were regarded as significant.

RESULTS AND DISCUSSION

Density of breeding seems to be one of the most important factor determining sprouting yield, as well as other important nutritional and nutraceutical properties of low processed food. Despite this fact there are no comprehensive studies regarding a density of breeding during sprouts production. In these studies some physical properties of sprouts, phenolic content and some potentially health beneficial activities were taken into account as determinants of sprouts quality.

Both, germination as well as density of breeding changed morphology of sprouts. Generally, a tendency to reduce seedlings size and weight with increasing plant-breeding density was observed. Hypocotyls length (ranging from 16 mm to 39 mm) and roots length (from 26 mm to 50 mm) decreased with the increasing plant concentration. Furthermore, the mass of 10 sprouts and the fresh/dry weight ratio also decreased with increasing density of breeding (tab. 1). Generally, a tendency to reduce seedlings size and weight with increasing plant breeding density was observed. These observations are in agreement with those concerning agronomical and economical analyses of plant population density of field cultivated legumes plants eg. cowpea, faba bean, blackgram, soybean and lentil [Njoku and Muoneke 2008, Turk and Tawaha 2002, Enyi 1973].

Table 1. Fresh/dry weight ratio, size and weight of lentil sprouts as affected by density of breeding

Sprouts	Density of breeding	Hypocotyls (mm)	Root (mm)	10 sprout mass (g)	Fresh / Dry weight ratio
3-days-old	100* (0.82)**	23 ±0.7^{c}	34 ±1.3^{c}	1.61 ±0.1^{a}	3.35 ±0.1^{a}
	150 (1.22)	18 ±1.3^{a}	28 ±0.3^{a}	1.49 ±0.2^{ab}	3.09 ±0.2^{a}
	200 (1.63)	16 ±1.4^{a}	26 ±3.9^{a}	1.45 ±0.1^{b}	2.88 ±0.4^{a}
4-days-old	100 (0.82)	40 ±5.0^{b}	50 ±3.9^{b}	1.65 ±0.1^{a}	4.33 ±0.3^{c}
	150 (1.22)	34 ±0.2^{b}	45 ±0.1^{b}	1.61 ±0.2^{ab}	3.73 ±0.1^{b}
	200 (1.63)	30 ±1.3^{d}	38 ±1.6^{d}	1.52 ±0.1^{b}	3.38 ±0.1^{a}

Means in the same column followed by different letters are significantly different at $P < 0.05$
* – seeds per plates; ** – seeds per cm^2

Phenolics content and composition of food is very often linked with its nutraceutical potential. Phenols have been widely studied and their beneficial influence on human health was confirmed [Zhao 2007, Fang et al. 2002, Ferrari and Torres 2003, Scalbert et al. 2005]. Therefore qualitative and quantitative analysis of sprout polyphenols was performed in order to determine the conditions providing an increase of their level. Antioxidant potential of phenolics is bound with: a) radicals scavenging abilities; b) ability to chelate metal transition ions; c) reducing power; d) prevention of lipids and other biomolecules; e) inhibition of prooxidant enzymes; f) activation of enzymatic defense system [Amarowicz et al. 2009, Świeca et al. 2012, Gawlik-Dziki et al. 2012a, Gawlik-Dziki et al. 2012c, Ghavidel and Prakash 2007]. The general trend of phenolics in lentil sprouts was a steady decline during germination (tab. 2). As it could be ob-

Table 2. Phenolics profile of lentil sprouts as affected by density of breeding

Compound (μg g^{-1} DM)	3-days-old			4-days-old		
	density of breeding			density of breeding		
	100* (0.82)**	150 (1.22)	200 (1.63)	100 (0.82)	150 (1.22)	200 (1.63)
p-hydroxybenzoic acid	3.90 ±0.1^{c}	1.93 ±0.1^{b}	1.83 ±0.1^{b}	3.10 ±0.1^{a}	3.15 ±0.1^{a}	2.90 ±0.1^{a}
(+)-catechin	144.28 ±3.5^{d}	82.22 ±0.5^{c}	69.84 ±2.2^{c}	139.94 ±1.6^{b}	135.36 ±8.1^{ab}	103.62 ±3.5^{a}
tannic acid	6.07 ±1.4^{ab}	8.93 ±0.3^{ab}	11.23 ±1.8^{c}	2.10 ±0.0^{b}	2.38 ±0.4^{b}	2.61 ±0.3^{a}
caffeic acid	11.29 ±2.8^{a}	11.21 ±3.3^{c}	7.53 ±0.1^{b}	12.41 ±0.1^{a}	12.08 ±0.6^{a}	10.73 ±0.6^{d}
chlorogenic acid	30.46 ±1.4^{c}	27.75 ±2.8^{c}	15.09 ±1.7^{b}	19.12 ±0.3^{a}	18.32 ±0.7^{a}	17.94 ±0.8^{ab}
syryngic acid	Nd.	Nd.	Nd.	0.38 ±0.2^{a}	0.30 ±0.3^{a}	0.30 ±0.0^{a}
p-coumaric acid	3.26 ±0.7^{a}	2.58 ±0.00^{a}	3.11 ±0.4^{a}	4.46 ±0.3^{b}	5.09 ±0.0^{c}	5.34 ±0.0^{d}
ferulic acid	90.84 ±2.7^{d}	75.92 ±0.5^{b}	76.74 ±1.5^{b}	65.34 ±1.8^{c}	69.09 ±1.4^{a}	70.16 ±2.6^{a}
synapic acid	5.39 ±1.0^{a}	2.42 ±0.2^{e}	1.84 ±0.1^{d}	6.23 ±1.9^{abc}	7.45 ±0.1^{c}	6.74 ±0.0^{b}
daidzein	0.13 ±0.1^{a}	0.11 ±0.0^{ac}	0.22 ±0.1^{d}	0.06 ±0.0^{bc}	0.06 ±0.0^{b}	0.11 ±0.0^{abcd}
luteolin	0.49 ±0.1^{a}	0.49 ±0.1^{a}	0.43 ±0.2^{a}	0.98 ±0.1^{b}	1.76 ±0.1^{c}	1.08 ±0.1^{b}
genistein	0.53 ±0.1^{a}	0.57 ±0.1^{a}	0.56 ±0.1^{a}	0.74 ±0.1^{b}	0.83 ±0.1^{b}	0.96 ±0.1^{c}
quercetin	5.21 ±0.3^{c}	10.42 ±2.4^{e}	1.47 ±1.1^{ab}	2.65 ±0.7^{a}	5.91 ±0.2^{d}	1.61 ±0.0^{b}
kaempferol	0.14 ±0.1^{a}	0.12 ±0.1^{a}	0.40 ±0.5^{a}	0.10 ±0.1^{a}	0.05 ±0.1^{a}	1.11 ±0.1^{b}
naryngenin	0.25 ±0.1^{a}	0.18 ±0.1^{c}	0.27 ±0.1^{a}	0.52 ±0.1^{b}	0.86 ±0.2^{d}	0.38 ±0.01^{b}
TPC mg g^{-1} DM	41.20 ±3.3^{a}	39.17 ±2.2^{a}	40.92 ±1.9^{a}	41.72 ±0.9^{a}	43.94 ±3.1^{a}	40.80 ±1.9^{a}
TFC mg g^{-1} DM	15.80 ±1.1^{a}	17.83 ±2.0^{abc}	20.43 ±2.3c^{d}	15.13 ±1.3^{a}	17.15 ±0.7^{b}	25.08 ±2.1^{d}
CTC mg g^{-1} DM	2.02 ±0.1^{a}	2.24 ±0.3^{ab}	3.16 ±0.1^{d}	1.77 ±0.2^{c}	2.41±0.2^{a}	2.62±0.2^{b}

Means in the same line followed by different letters are significantly different at $P < 0.05$
TPC – total phenolics content, TFC – flavonoids content, CTC – condensed tannins content
* – seeds per plates; ** – seeds per cm^2, Nd. – not detected

served from Table 2 density of breeding significantly influences the qualitative and quantitative phenolics composition of sprouts. Content of condensed tannins (ranging from 1.77 to 3.16 mg g^{-1} DM for 4-days-old-100 density and for 3-days-old-200 density, respectively) and flavonoids (ranging from 15.13 to 25.08 mg g^{-1} DM for 4-days-old-100 density and for 4-days-old-200 density, respectively) increased with the increasing density of breeding. However, there was no significant effect of density of breeding on the total phenolics content. The HPLC results indicated the presence of eight hydroxycinnamic and hydroxybenzoic acids and seven flavonoids. It was found that ferulic, and chlorogenic acids were the dominant components of phenolic acids fraction of sprouts. The contents of the *p*-hydroxybenzoic and ferulic acids decreased with the increasing density of breeding in 3-days-old seedlings. Also, in 3-days-old sprouts case various conditions of germination led to significant influence on the contents of chlorogenic and sinapic acids, a significant decrease was observed with the increasing density of breeding. Similarly, in the sprouts obtained in cultivations with higher density a marked reduction of (+)-catechin level was determined. Additionally, the level of quercetin was elevated at a higher degree in sprouts cultivated in density – 150 (1.22 seeds per cm^2), and average 10.42 and 5.91 μg g^{-1} DM for 3- and 4-days-old sprouts, respectively. In other phenolics case there were no relationships between growth conditions and their level. Data concerning phenolics profile of lentil seedlings are in agreement with those obtained in several previous studies [Ghavidel and Prakash 2007, Amarowicz et al. 2010, Dueñas et al. 2002, Dueñas et al. 2007, Troszyńska et al. 2011, Świeca et al. 2012)]. In recent literature there is no information concerning an influence of density of breeding on the polyphenolics profile of sprouts. There are some evidences that high plant population density may cause a biotic stress conditions – competition [Gillet 2008, Berger et al. 2008]. A significant elevation of flavonoids and condensed tannins levels seem to confirm this thesis. An increase of flavonoids content under biotic stress was also observed by Zhao et al. [2005], Fujita et al. [2006] and Torres et al. [2006]. On the other hand high levels of cell wall precursors (eg. ferulic acid) determined in 3-days-old sprouts cultivated at the lowest density may indicate a high vigour of these sprouts.

There are many reports confirming that antioxidant potential is positively correlated with polyphenols content [Gawlik-Dziki et al. 2011, Amarowicz and Troszyńska 2003, Fernandez-Orozco et al. 2008]. Based on this fact we hypothesized that changes of phenolics caused by different growth condition (density of breeding) may have some effects on the nutraceutical potential of sprouts. Thus, antioxidant potential of sprouts, abilities to metal ions chelating and to inhibit a lipid peroxidation, were determined. The highest metal chelating properties were determined for sprouts cultivated at the lowest breeding density (0.82 seed per cm^2). Extracts obtained from 3- and 4-days-old sprouts were able to sequestrate about 92% and 86% of metal ions, respectively. Similarly, taking into account inhibition of lipid peroxidation it should be noted that studied ability decreased with the increasing density of breeding (fig. 1). The highest abilities to prevent lipids were determined for 3- and 4-days-old sprout – 58% and 56%, respectively. Effect of changed cultivation conditions (density of breeding) was also clearly visible on the sprouts ability to inhibit lipoxygenase activity. There was no significant effect for 3-days-old seedlings, however, using a low-density cultivation allowed to improve this ability in the 4-days-old sprouts. Concluding, fresh mass yield and ability to prevent

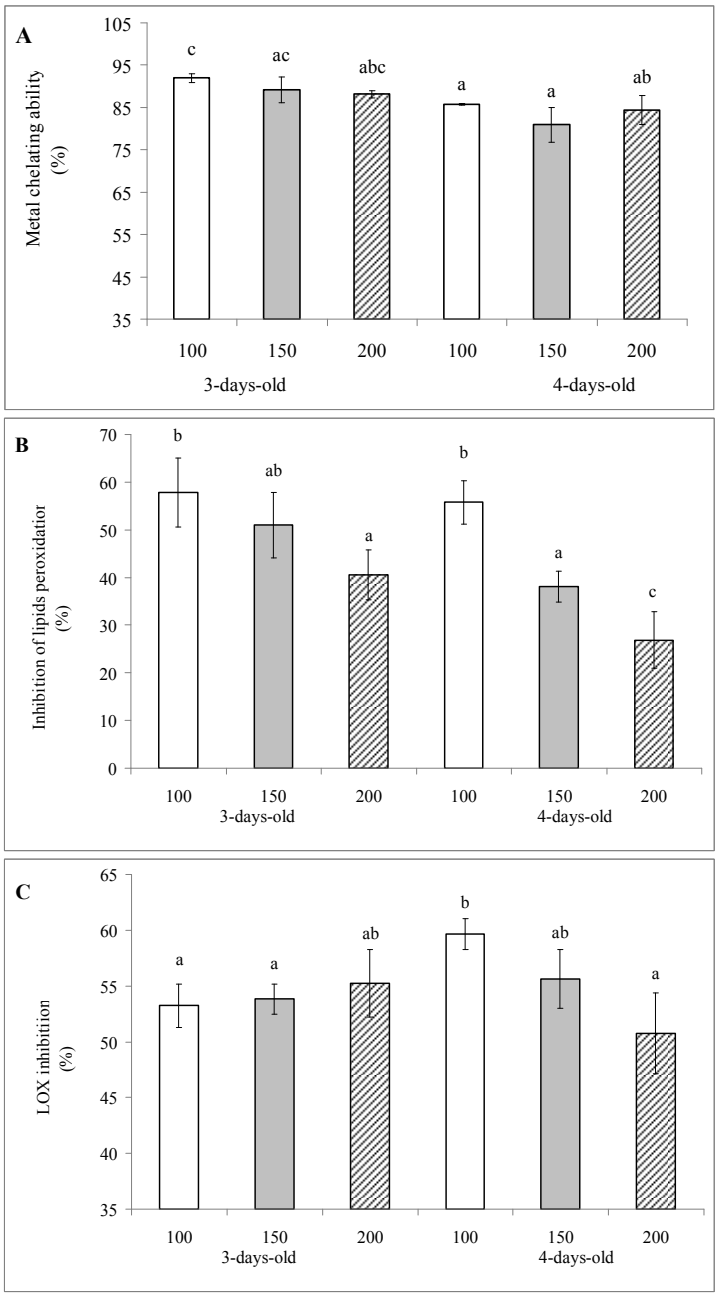

Fig. 1. Antioxidant activities of ready-to-eat lentil sprouts as affected by density of breeding. A – metal chelating ability, B – inhibition of lipids peroxidation, C – inhibition of lipoxygenase activity. Means followed by different letters are significantly different at $P < 0.05$

lipids against oxidation were negatively correlated with density of breeding. It should be also noted that the highest nutraceutical potential was determined for 4-days-old sprouts cultivated at the lowest density (100–0.8 seed per cm^2) (fig. 1).

Based on numerous literature data the fact that germination and conditions of this process modify quality of low-processed food is indisputable [Ghavidel and Prakash 2007, Fujita et al. 2006, Fernandez-Orozco et al. 2008]. This is the reason why intensification of desirable properties of food elicitation by biotic and abiotic factors is applied [Gawlik-Dziki et al. 2012a, Shetty et al. 2002, Shetty 2004, Randhir and Shetty 2003, Ghavidel and Prakash 2007]. However, in all cited studies the influence of population density (plants concentration) was omitted. On the strength of the arguments provided by agricultural and horticultural studies [Njoku and Muoneke 2008, Miguélez Frade and Valenciano 2005, Enyi 1973] we assumed that this factor may also play an important role during sprouts production.

CONCLUSION

Taking into account results obtained in these studies it can be concluded that population density is a very important factor design of the chemical composition of lentil sprouts. Lentil sprouts are a good source of dietary polyphenolics with a high antioxidant activity but different plant concentration during germination significantly influences on their chemical composition and antioxidant potential. The results show clearly that density of breeding population density should be taken into account during production of the low-processed food.

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WPŁYW GĘSTOŚCI HODOWLI NA WZROST I WYBRANE WŁAŚCIWOŚCI NUTRACEUTYCZNE KIEŁKÓW SOCZEWICY (*Lens culinaris*)

Streszczenie. Warunki hodowli ściśle determinują wartość odżywczą i potencjał nutraceutyczny kiełków. Celem pracy było określenie wpływu gęstości hodowli na wzrost, zawartość związków fenolowych oraz poziom wybranych aktywności antyoksydacyjnych kiełków soczewicy. Zawartość tanin skondensowanych (od 1,77 do 3,16 mg g^{-1} suchej masy) oraz flawonoidów (od 15,13 do 25,08 mg g^{-1} suchej masy) wzrastała wraz ze wzrostem gęstości hodowli. W przypadku 3-dniowych kiełków, zawartość kwasu *p*-hydroksybenzoesowego i ferulowego oraz (+)-katechiny ulegała obniżeniu wraz ze wzrostem gęstości hodowli. Ponadto, oznaczona zawartość kwercetyny była największa w kiełkach otrzymanych przy zagęszczeniu 1,22 nasion/cm^2 i wynosiła odpowiednio 10,42 i 5,91 μg g^{-1} suchej masy dla kiełków 3- i 4-dniowych. Zdolność do chelatowania jonów metali była największa w kiełkach otrzymanych z hodowli o najmniejszym zagęszczeniu i wynosiła odpowiednio 92 i 86% dla kiełków 3- i 4-dniowych. Wzrastająca gęstość hodowli niekorzystnie wpływała na przyrost świeżej masy oraz zdolność do hamowania utlenienia lipidów prze kiełki. Podsumowując, można stwierdzić, że zagęszczenie hodowli podczas kiełkowania istotnie wpływa na skład chemiczny i bioaktywność kiełków soczewicy.

Słowa kluczowe: aktywność antyoksydacyjna, warunki hodowli, soczewica, gęstość hodowli, kiełki

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