ОРИГИНАЛЬНАЯ СТАТЬЯ

EFFECT OF SELENIUM ON BIOCHEMICAL CHARACTERISTICS OF «BLACK GARLIC»

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ABSTRACT. High biological activity of garlic and products of its processing and also selenium enriched garlic determine their popularity among residents of different countries of the world. The aim of the present work was evaluation of biochemical characteristics of "black garlic" produced via long-term heating of bulbs in conditions of high humidity. High content of selenium was shown to improve monosaccharide accumulation more intensively than in non fortified garlic. Long-term heating resulted in less intensive total acidity increase and pH decline in selenium enrich garlic compared to control samples and to slowing of polyphenol accumulation process and increase of antioxidant activity. Distinctive feature of "black garlic" with high selenium content is conservation of conductivity value compared to non fortified garlic whereas the latter demonstrates two time increase of the parameter during "black garlic" ripening.

KEYWORDS: biofortification, selenium, black garlic, biochemical characteristics.

INTRODUCTION

Garlic and products of its processing enjoy constant popularity among the population of different countries of the world due to unique taste and high biological activity (Bayan et al., 2014; Gupta et al., 2015). The attribution of garlic to functional foods is determined by its high antioxidant activity, antimicrobial, anti-carcinogenic, antihypertensive effects, hepato- and cardio protection and also insecticidal properties (Rahman, 2012; Ryu, Kang, 2017).

In the world markets, this plant is represented by fresh bulbs and dried powder, garlic oil (Satyal et al., 2017) and ethanol extract (aged garlic) (Rahman et al., 2012). In recent years, a new product of garlic processing – the so called "black garlic" has appeared on the Korean market and in several other countries (Ryu, Kang, 2017).

Recent studies demonstrated high antioxidant and anti-carcinogenic properties of black garlic produced during long-term storage of bulbs at elevated temperature and humidity (Choi et al., 2014; Ryu, Kang, 2017). Distinctive feature of black garlic is dark color, sweet taste and lack of strong smell typical for ordinary garlic.

During black garlic processing allicin is converted to water soluble antioxidants such as S-allylcystein, tetrahydro- β-carbolines, biologically active alkaloids and flavonoids (Lu et al., 2017). S-Allylcystein is shown to inhibit oxidation processes connected with aging and different diseases (Colin-Gonzalez et al., 2012). Tetrahydro-β-carbolines derivatives extracted from black garlic also possess high antioxidant activity (Sato et al, 2006). Black garlic extracts are shown to possess also antiallergenic, anti-diabetic, anti-inflammatory, hypocholesterolemic, hypo-lipidemic properties (Kim et al., 2011a,b, 2012; Lee et al., 2009; Sato et al., 2006; Colin-Gonzalez et al., 2012).

The main biologically active compounds of garlic are: di- and tri sulfides, allycin and S-containing aminoacids. Fresh and dried garlic contain allycin, di- and tri sulfides. In garlic oil- diallyl- allylmethyl- and dimethyl mono and hexa sulfides are the most important biologically active compounds (Bayan et al, 2014). Ethanol extracts (aged garlic) contains S-allylcystein and S-allyl mercaptocystein. Black garlic is rich with polyphenols, flavonoids, piruvic acid, thiosulfate, S-allylcystein and allyl mercaptocystein (Choi et al., 2014; Ryu, Kang, 2017).

Easiness of sulfur substitution by selenium in biological objects was the basis of production of selenium enriched garlic with high anti-carcinogenic activity (Ip, Ganther, 1992; Ip, Lisk, 1995). Grown in hydroponics garlic fortified with selenium is pro-

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duced in USA as biologically active supplement (Tsuneyishi et al., 2006).

Garlic is known to be a secondary selenium accumulator capable to convert about 78% of inorganic selenium to organic derivatives (Yang et al, 2005). The most important biologically active compounds of such garlic with distinct anti-carcinogenic properties are methylated forms of selenium containing aminoacids and peptides: Se-Met-Se-Cys and γ-glutamyl- Se-Met-Se-Cys (Dong et al., 2001; Fang et al., 2012; Ip, Ganther, 1992; Ip, Lisk, 1995; Yang et al., 2005).

Selenium enriched garlic is known to be more effective in inhibition of breast adenocarcinoma than selenium enriched yeast thanks to high concentrations of γ-glutamyl- Se-Met-Se-Cys , demonstrating high anti-cancer protection effect (Ip et al., 2000). Besides this garlic rich with selenium inhibits toxic effect of heavy metals: such as As, Cd, Hg and Sn (Zhao et al., 2013)

Due to high concentrations of sulfur garlic preparations: garlic oil and aged garlic,- produced from garlic with high selenium content are also rich with selenium (Satyal et al., 2017; Rahman et al., 2012). At the same time it should be noted that black garlic has never been produced earlier.

The aim of the present study was production and evaluation of biologically active compounds of selenium fortified black garlic.

MATERIALS AND METHODS

Biofortification of garlic with selenium. Garlic Dobrinya cultivar was grown in Moscow region (55°39′23″ N, 37° 12′43″ E), on a clay-loam soil, with pH 6.8, 2.1% organic matter, 108 $mg \cdot kg^{-1}$ N, 450 $mg \cdot kg^{-1}$ P_2O_5, 357 $mg \cdot kg^{-1}$ K_2O, exchangeable bases sum as much as 95.2%. Plot size is 0.5 × 5 m with five fold repeatability.

Selenium was introduced to soil with liquid fertilizers Fertika lux (N1.6; P205 2.0; K20 2.7; Fe 0,01; B 0,002; Cu 0,001; Mn 0,01; Mo 0,002; Zn 0,001%) using the total dose of 75 mg of sodium selenate per m^2. To intensify the process of biofortification and exclude selenium toxicity selenium administration was achieved during 2.5 months (June-August) once a week using a solution of 7.5 mg of sodium selenate in 25 ml of Fertika fertilizer diluted to 10 litre of water per 2 m^2.

Black garlic processing. 0.5 kg of garlic fortified and not fortified with selenium was placed into a chamber with constant humidity (90%) and temperature (70 ºC). Three weeks later garlic was removed from a chamber, cloves were cleaned from shell and homogenized. The resulting samples were stored at +4 ºC up to the beginning of the analysis.

Selenium content was determined using fluorimetric analysis (Alfthan, 1984), using a sample of reference-standard – lyophilized cabbage with selenium content 150 μg/kg d.w.

The level of antioxidant activity was determined according to a method (Maximova et al., 2001) based on titration of potassium permanganate solution with garlic extract in acidic media. The results were expressed as Gallic acid equivalent per 100 g acid of fresh weig.

Mono- and disaccharides were determined using cyanide method (Kidin 2008).

Titratable acidity (TA) and organic acids. TA was measured using 20 ml of the whole water extract (1:1), titrated to pH 8.1 using 0.1 N NaOH (GOST 1999).

Total polyphenols. The concentration of total polyphenols in each sample was determined in 70 % ethanol extract, using the Folin-Ciocalteu colorimetric method, according to (Sagdic et al., 2011) on Unico 2804 UV (USA) spectrophotometer. The phenolic contents were calculated by using a calibration curve of gallic acid constructed with five concentrations of this compound (0–90 μg/mL). Phenolic contents were expressed as milligrams of Gallic acid equivalents per 100 gram of fresh weight (mg GAE/100 g).

Conductivity of water garlic extracts were determined using TDS-3 conductometer (Russia)

Statistical analysis (five replicates) was achieved using mean and standard deviation values. The mean separations were performed through the Duncan multiple range test, with reference to 0.05 probability level, using SPSS software version 21.

RESULTS AND DISCUSSION

Among different methods of plants biofortification with selenium (ground introduction, foliar application, hydroponics) we have chosen soil supplementation with sodium selenate. On one hand this is connected with relatively small leaves surface of garlic and strong water repellent properties of leaves due to high content of wax that significantly reduces the efficiency of plants foliar fortification with selenium. On the other hand conditions of hydroponics are not intended for large scale garlic production, whereas ground application does not possess these drawbacks. To improve the efficiency of selenium fortification we used long-term application of sodium selenate diluted solution (2.5 months). Compared to other chemical forms of selenium selenate is known to provide the highest levels of selenium accumulation (Poldma et al., 2012).

In these conditions selenium fortification level of garlic reached 16.7 times whereas selenium concentration in cloves was equal to 1.5 mg/kg d.w. (Table 1). It is known that the main chemical form of selenium in garlic with selenium concentration less than 333 mg/kg d.w. is γ-Glu-Se-Met-Se-Cys, while at higher concentrations the predominant selenium form is Se-Met-Se-Cys (Kotrebai et al., 2000). Thus obtained selenium enriched garlic may be considered as a good source of natural anti-carcinogen - γ-Glu-Se-Met-Se-Cys. Selenium enriched garlic is known to demonstrate higher anti-carcinogen activity than non fortified samples (Ip, Ganther, 1992; Ip, Lisk, 1995), despite the fact that selenium supplementation decreases allycin level (Ghasemi et al., 2015).

As can be seen from Table 1 selenium biofortification resulted in the 30 % increase of bulb mass and significantly decrease of cloves number that is in good agreement with the literature data (Poldma et al, 2012). Control plants did not differ much from that of selenium fortified one on the dry matter content.

Published data indicate that garlic cultivar and place of vegetation do no effect much the biochemical parameters of processed black garlic (Choi et al., 2014). Changes in cloves color as a result of high temperature and humidity is due to the reaction of non-enzymatic browning of *Allium* plants (Choi et al., 2014). The intensity of such a process depends on temperature and duration of garlic maturation. Optimal time of black garlic processing at 70 °C and high humidity is considered to be 3 weeks when a product contains the highest levels of antioxidants and anti-carcinogen activity.

We have shown for the first time that selenium content in garlic bulbs significantly affects the biochemical characteristics of black garlic. As can be seen from Fig. 1 cloves of selenium enriched garlic possess lighter color than non fortified ones.

These preparations differ significantly by their biochemical characteristics. Thus, black garlic demonstrated twice higher content of total sugar compared to non processed bulbs with several times

Table 1. ***Bulb parameters, dry matter and selenium content in garlic***

Parameter	Control	Selenium enriched garlic	*p*<
Mean bulb weight, g	34.5±2.1	45.0±2.3	0.001
Number of cloves	11±1	8±1	0.001
Dry matter content, %	41.9±0.1	39.1±0.1	0.01
Selenium content, μg/kg d.w.	90±7	1500±35	0.0001

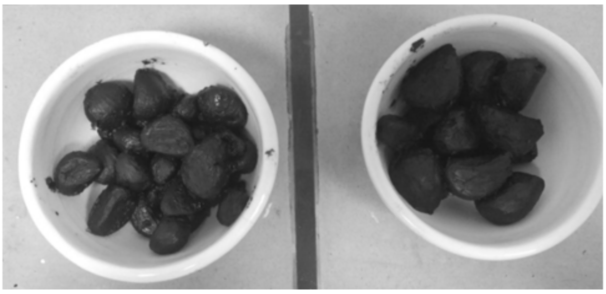
a) *b*)

Fig. 1. *Black garlic obtained from non fortified (a) and selenium fortified (b) plants*

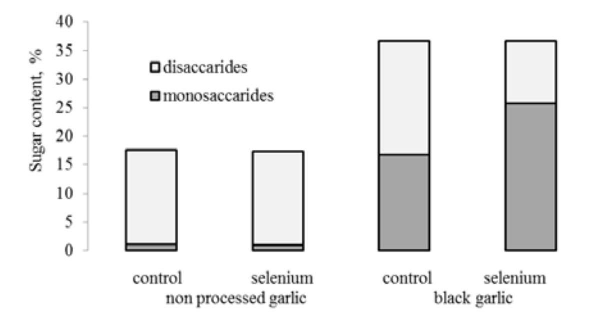

Fig. 2. *Sugar content in black garlic*

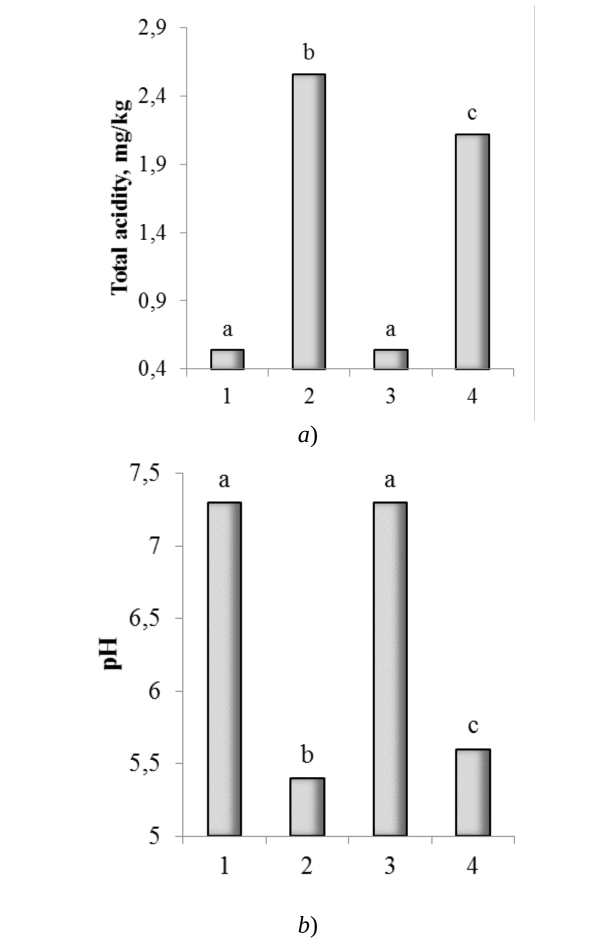

Fig. 3. *Titratable acidity (a) and pH (b) changes in non fortified and selenium fortified garlic:*
1 – raw non fortified garlic,
2 – black garlic with low concentration of selenium,
3 – selenium enriched garlic,
4 – black garlic with high concentration of selenium.
Values with similar indexes do not differ statistically (p > 0.05)

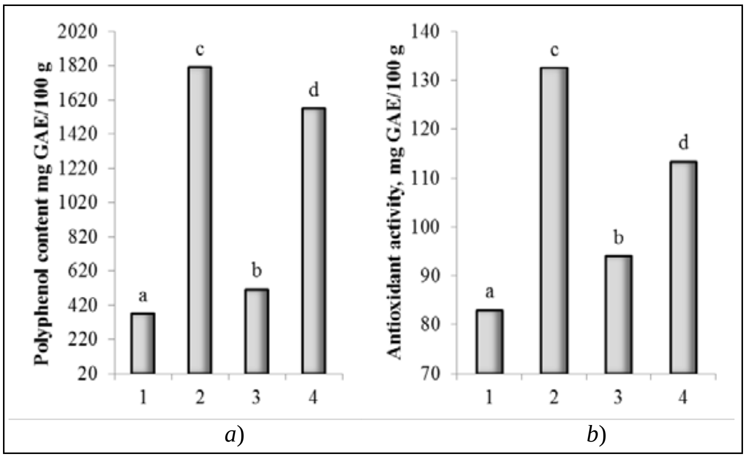

Fig. 4. *Parameters of antioxidant activity of non fortified and fortified with selenium garlic:*
1 – raw non fortified garlic,
2 – black garlic with low concentration of selenium,
3 – selenium enriche garlic,
4 – black garlic with high concentration of selenium.
Values with similar indexes do not differ statistically (p > 0.05)

The value of titratable acidity during black garlic processing increased 3.1–4.8 times and accordingly pH value decreased by 30–35 % that is in good agreement with literature data (Choi et al., 2014). (Fig. 3). On the other hand significantly less changes of theses parameters in processing of selenium fortified black garlic (Fig. 3) indicate the slowdown process of black garlic formation due to selenium influence.

Peculiarities in the increase of antioxidant activity as a result of black garlic processing also prove the lower intensity of black garlic formation in the presence of high selenium concentration (Fig. 4).

Thus Fig.4 data indicate that elevated temperature and humidity increase polyphenol levels in non fortified garlic 6.3 times whereas in selenium fortified garlic this parameter increases only 2.6 times. Similarly increase of AOA was more intensive in non fortified garlic (3.5 times) than in selenium fortified one (1.5 times)

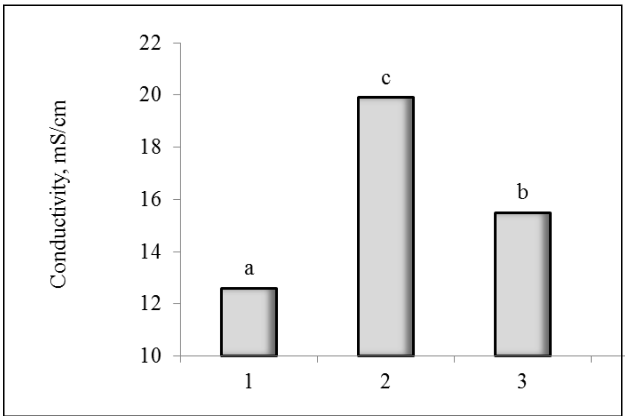

Fig. 5. *Conductivity of non fortified and selenium fortified garlic:*
1 – raw non fortified garlic,
2 – black garlic with low concentration of selenium,
3 – selenium enriched garlic,
4 – black garlic with high concentration of selenium.
Values with similar indexes do not differ statistically (p >0.05)

higher concentration of mono-saccarides in selenium enriched samples (Fig. 2). This phenomenon results in significant changes of garlic taste increasing sweet perception. Fig. 2 data demonstrate that high concentration of selenium in non processed garlic stimulates the formation of mono-saccarides, thus changing the mono/di saccarides ratio from 0.84 in non fortified black garlic to 2.3 in black garlic with high selenium concentration (1.5 mg Se/kg d.w.).

Special attention should be paid to conductivity values of garlic tissues. As can be seen from Fig. 5 this parameter increases 1.6 times only in non fortified black garlic with low selenium content whereas black garlic with high concentration of selenium possesses the same conductivity value as fresh garlic cloves. It seems obvious that the phenomenon is connected not only with changes in total acidity but also with the formation of water soluble forms of minerals present in garlic.

CONCLUSION

Thus the present results indicate that biochemical characteristics of black garlic may vary significantly depending on the content of selenium. Furthermore utilization of selenium enriched garlic in processing of black garlic results in lower values of antioxidants than in non fortified black garlic.

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ВЛИЯНИЕ СЕЛЕНА НА БИОХИМИЧЕСКИЕ ПОКАЗАТЕЛИ ЧЕРНОГО ЧЕСНОКА

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РЕЗЮМЕ. Высокая биологическая активность чеснока и продуктов его переработки, а также чеснока, обогащенного селеном, определяют их неизменную популярность среди населения разных стран мира. Целью настоящей работы было установление биохимических показателей «черного чеснока», полученного долговременным нагреванием обогащенного селеном чеснока в условиях высокой влажности. Установлено, что высокое содержание селена способствует более интенсивному образованию моносахаров в процессе «созревания» «черного чеснока», менее интенсивному увеличению кислотности и снижению pH, а также замедлению процесса накопления полифенолов и возрастания антиоксидантной активности. Отличительной особенностью «черного чеснока», содержащего высокие концентрации селена, является сохранение величины проводимости в процессе изготовления, в то время как проводимость «черного чеснока», полученного из не обогащенного селеном чеснока, оказалась в 2 раза выше, чем исходного чеснока.

КЛЮЧЕВЫЕ СЛОВА: селен, обогащение, черный чеснок, биохимические характеристики.