Eur Food Res Technol (2004) 219:142–144
DOI 10.1007/s00217-004-0927-0

ORIGINAL PAPER

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Enhanced selenium content in buckwheat (*Fagopyrum esculentum* Moench) and pumpkin (*Cucurbita pepo* L.) seeds by foliar fertilisation

Received: 13 February 2004 / Revised: 23 March 2004 / Published online: 6 May 2004

Abstract The fruit and thin-husked seeds of the pumpkin (*Cucurbita pepo* L.) and buckwheat grain (*Fagopyrum esculentum* Moench), both grown in Slovenia, were analysed for selenium (Se) content following foliar application of Se(VI) solution during the period of blooming. Samples were digested by a H_2SO_4-HNO_3-H_2O_2-V_2O_5 mixture and Se determined, based on hydride generation atomic fluorescence spectrometry. The whole procedure from weighing to measuring was carried out in the same Teflon vessel. The detection limit of the method was 0.14 ng g^{-1} solution. Buckwheat seeds from untreated plants contained 47 ng g^{-1} of Se and 394 ng g^{-1} from plants after foliar fertilisation with Se. Pumpkin seeds from untreated plants contained 108 ng g^{-1} of Se, and 381 ng g^{-1} of Se from Se-treated plants, all per lyophilised sample. Se content in lyophilised pumpkin fruit was 15 ng g^{-1} in untreated plants and 20 ng g^{-1} in Se-treated pumpkin plants. It is thus feasible to enhance Se content in buckwheat and pumpkin seeds by foliar fertilisation, making them a rich source of dietary Se and useful as a raw material for enriched food products.

Keywords Pumpkin seed · Buckwheat · Selenium · Deficiency · Foliar application

Introduction

In central Europe, buckwheat (*Fagopyrum esculentum* Moench) and pumpkin (*Cucurbita pepo* L.) are traditional crops, which, because of their high nutritional value and particular culinary attraction, have regained interest. Pumpkin seeds are known for their content of nutritional plant sterols [1] and vitamin E [2], and buckwheat is a low-input plant and an important source of the antioxidant rutin [3, 4, 5].

Buckwheat proteins may prevent gallstone formation more effectively than soy protein products; they may also retard mammary carcinogenesis by lowering serum estradiol, and suppress colon carcinogenesis by reducing cell proliferation [6, 7, 8]. The biological value of buckwheat seed protein is about 90% [9] in comparison to egg proteins (100%) and for pumpkin seed protein is in the range 73–86% [8]. Both kinds of seeds can be used as additives to improve the quality of bread or other products [10, 11, 12, 13, 14].

Pumpkin seeds have been used for centuries in traditional medicine, mainly in cases of problems of the kidney or the urinary tract, and against tapeworms [15, 16]. The seeds can be eaten dry and unprocessed, or roasted and salted.

Buckwheat seeds are an important dietary source of Zn, Cu and Mn [17] and pumpkin seed products are rich in the minerals Ca, K, P, Mg, Mn, Fe and Zn [18]. Both buckwheat and pumpkin are good sources of the vitamin B group [10, 19]. However, both are normally just a moderate source of Se [16, 20].

In several central European countries, for example Poland [21], Austria [22], and Croatia [23], there are regions with Se deficiency in the human diet, due to lack of Se available for plants in the soil. Slovenia is known as a moderately Se-deficient area [16, 24, 25]. Finland, New Zealand and some regions of China are also known for Se deficiency [26, 27]. One solution to this problem is to use Se dietary supplements, although there is real concern over toxicity and over-zealous use of Se supplementary intake, such that in Australia, the use of Se as dietary supplement is restricted [28]. Enrichment of agricultural crops by adding Se fertilisers is, at least partly, an irreversible process; in many parts of the world there is too much Se in the biosphere, which may endanger fish, livestock and

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humans [28, 29]. Until the role and migration of Se in the biosphere is clearly established, it is appropriate to enhance Se content in edible plants by agricultural measures which have less irreversible consequences for the environment, for example foliar application of Se compounds. Even in this case, however, safety measures must be taken, such as protecting agricultural workers applying the spray, and applying foliar fertilisation before the edible parts of the plants develop, to exclude the possibility of food pollution. Foliar application of Se has been used to enhance the Se content in potato [30], rice [31] and soybean [32] but there are, as yet, no reports of foliar or soil fertilisation with Se for buckwheat or pumpkin.

The aim of this study was to evaluate the buckwheat seed and pumpkin seed and fruit from Se foliar-treated plants, and to assess whether they could be used as a better source of the trace element Se than materials from untreated plants.

Material and methods

Seeds of buckwheat (*Fagopyrum esculentum* Moench) cv. Siva, and pumpkin (*Cucurbita pepo* L.) cv. Štajerska golica with thin-husked (naked) seeds, were grown on a field in the vicinity of Ljubljana (Slovenia) in 2002. Plants in flower were treated once by spraying the leaves in the beginning of flowering with a water solution containing Se in the form of Na selenate at 1 mg Se L^{-1}. Untreated plants were grown in the same location besides the treated plants, on the same soil, and separated to avoid contamination with solution.

For laboratory analysis, seeds and pumpkin fruit (lyophilised after the removal of seeds and the outer 15 mm thick part) were milled and homogenised with an agate Fritsch mill, Pulverisette 7.

Se determination was based on digestion of a sample by a H_2SO_4-HNO_3-H_2O_2-V_2O_5 mixture and detected by hydride generation atomic fluorescence spectrometry (HG-AFS) as described in [33, 34].

Sample (0.150–0.200 g) was weighed in a 50 mL Teflon tube, to which was added 0.5 mL conc. H_2SO_4 and 1.5 mL HNO_3. The tube was closed and heated for 60 min at 130 °C in an aluminium block. H_2O_2 (2 mL) was added to the cooled solution, heated for 10 min at 115 °C, and finally a further 2 mL of H_2O_2 was added and the solution heated for 10 min at 115 °C.

After digestion, the solution was cooled to room temperature, 0.1 mL V_2O_5 in H_2SO_4 added and the vessel heated for approximately 20 min at 115 °C. Se (VI) was reduced to Se (IV) following the addition of 2.5 mL conc. HCl and heating at 100 °C for 10 min. Samples were diluted to 10 g or 50 g, depending on the expected Se content of the samples. Se was detected by HG-AFS under optimal conditions [34]. Standard solutions were prepared in the same acid media as samples. Each sample was analysed in quadruplicate.

The complete procedure was carried out in the same Teflon vessel. The lower detection limit was <0.14 ng g^{-1} solution and the linearity of determination between 0.5 and 30 ng g^{-1}. The method was tested with reference materials with similar matrices to that of our samples, Wheat Gluten NIST 8418, 2580±190 ng Se g^{-1}, and Corn Bran NIST 8433, 45±8 ng Se g^{-1}. There was good agreement between our results (2469±246 ng Se g^{-1} and 45±8 ng Se g^{-1}) and the certified values.

Results and discussion

The Se contents of the buckwheat samples are presented in Table 1. The Se content in buckwheat seeds grown on plants treated with foliar Se fertilisation was about 8.5-fold higher than that of the untreated control plants. The Se content in pumpkin seed (Table 2) was also higher (about 3.5-fold) than the control. Se was more concentrated in seeds (about 380 ng g^{-1} lyophilised sample) than in the fruit (about 20 ng g^{-1} lyophilised sample) of treated plants. The Se content of untreated control plants was slightly higher than in samples collected in an oil mill 2 years earlier in northeastern Slovenia, which is more deficient in Se than central Slovenia [16]. The content of Se in pumpkin seeds from untreated plants was within the range of that in cereals from a Se-deficient region [27]. The content of Se in the sample of canned pumpkins from the USA, about 4 ng g^{-1} [35], is higher than our result for pumpkin fruit.

Table 1 Se content in buckwheat seeds (mean±SD)

Sample	Treatment	Sample	Lyophilised sample
		ng g^{-1}	ng g^{-1}
Seed	Control	43±5	47±5
	Se treatment	367±28	394±30

Table 2 Se content in pumpkin fruit and seeds (mean±SD)

Sample	Treatment	Sample	Lyophilised sample
		ng g^{-1}	ng g^{-1}
Fruit	Control	0.7±0.1	15±1
	Se treatment	1±0.3	20±6
Seed	Control	105±6	108±6
	Se treatment	369±14	381±15

According to Chen et al. [31], Se content in rice rose from 71 ng g^{-1} to 471 ng g^{-1} when subjected to foliar treatment with selenite, and to 640 ng g^{-1} with selenate. In soybean, foliar application of Se resulted in increases in seeds from 55 to 1,126–1,211 ng g^{-1} [32].

No toxic effects were observed on Se-treated plants, which developed normally, and no morphological differences were observed between Se-treated and untreated plants.

The recommended dietary intake for Se is 55 μg per day [36]. Thus, a considerable part of this could be covered by pumpkin seeds from pumpkins and buckwheat subjected to foliar fertilisation with Se.

In conclusion, foliar application of Se fertiliser is feasible and effective in buckwheat and pumpkin. This Se is, in this case, additional to other essential nutrients and trace elements contained in buckwheat and pumpkin seeds.

Acknowledgements This research was supported by the Ministry of Education, Science and Sports of Slovenia, through the project V4–0385 “Development of methods for testing the quality of plant and animal products in accordance with internationally recognised methods for their official control”, project J4–6476–0481–04/4.03 and program P4–0085.

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