PAPER

NUTRITIONAL FEATURES OF LEEK CULTIVARS AND EFFECT OF SELENIUM-ENRICHED LEAVES FROM GOLIATH VARIETY ON BREAD PHYSICAL, QUALITY AND ANTIOXIDANT ATTRIBUTES

N. GOLUBKINA*[a], T. SEREDIN[a], T. KRIACHKO[b] and G. CARUSO[c]
[a]Federal Scientific Center of Vegetable Production, Moscow region 143080, Russia,
[b]K.G. Razumovsky Moscow State University of Technologies and Management, 109004 Moscow, Russia
[c]Department of Agricultural Sciences, University of Naples Federico II, Italy
*Corresponding author: Tel: +79031185030
E-mail address: segolubkina45@gmail.com

ABSTRACT

Despite high nutritional value, *A. porrum* leaves are usually wasted. To evaluate the use efficiency of leek selenium fortified leaves in bread production, cultivar Goliath was selected among nine leek varieties based on quality and antioxidant characteristics. Fortified plants attained higher leaf content of Se and polyphenols compared to pseudo-stems. The addition of Se leaves increased bread polyphenol stability during baking, but it resulted in bread porosity decrease compared to both traditionally made bread and bread containing leaf powder of non-fortified plants. About 31 % of the daily required Se is provided by 100 g of bread enriched with Se.

Keywords: *A. porrum* cultivars, bread, polyphenols, porosity, Se-biofortification

1. INTRODUCTION

Among the several vegetables cultivated, the edible parts of *Allium* species are the most widely consumed around the world. The plants belonging to this genus are healthy not only for their nutritional features, but also for their wide biological activity. The latter is greatly attributed to the presence of some substances which *Allium* plants are able to accumulate, such as flavonoids, organosulfur compounds and methylated forms of selenium-containing aminoacids, i.e. selenomethyl selenocystein and γ-gluthamyl selenomethyl selenocystein, showing high antioxidant, cardioprotective and anticarcinogenic properties (IP *et al*., 2000; ADHIKARI, 2012). Notably, *Allium* plants fortified with Se reportedly display higher pharmacological activity than non-fortified ones (IP *et al*., 2000; ADHIKARI, 2012). Moreover, all *Allium* species belong to secondary accumulators of Se, even in the seeds (GOLUBKINA *et al*., 2015), with remarkable tolerance to high concentration of this element; accordingly, consumption of Se fortified *Allium* parts may give additional benefits to human health, taking also into account that Se deficiency has been detected in many countries worldwide (GOLUBKINA and PAPAZYAN, 2006).

Much attention is being paid for the last decade to waste valorization technologies of vegetable processing and to search for new natural sources of biologically active compounds. In this respect, the high content of antioxidants and organic sulfur compounds in *A. porrum* plant parts confer them high protective functions, such as antimicrobial, cardioprotective, hypocholesteremic, hypoglycemic and anticancer activity (RADOVANOVIĆ *et al*., 2015).

However, leek has not been deeply investigated as a potential source of pharmacologically beneficial compounds and, in most cases, pseudo-stems are just consumed whereas the leaf blades are discarded. Moreover, though this species is a natural source of methylated Se-containing amino acids with high anticancer activity, so far only one study concerning soil Se biofortification of leek has been carried out (LAVU *et al*., 2012) and no data on leaf Se accumulation have been reported.

The uneven distribution of elements in the earth crust is the cause of widespread Mg, Ca, Fe, Zn, Cu, I and Se deficiency in quite a few populations (WHITE and BROADLEY, 2009), which leads to numerous attempts to include these elements in bakery products (WIMALAWANSA, 2013; ROSELL *et al*. 2015). Among the latter, bread is largely consumed in most world countries and this justifies the relevant fortification with different nutrients, in order to produce functional food enriched with natural antioxidants, macro and trace elements (DE VALENÇA *et al*., 2017; ALLEN *et al*., 2006). Indeed, the interest in Se is due to its powerful antioxidant, immune-modulating, cardio-protective and anti-carcinogenic properties. It is estimated that the mean levels of wheat Se are 10-550 μg/kg, reaching 3-7 μg/kg in regions with Se deficient soils (several provinces of China) and 70 mg/kg in selenosis areas (India) (TAMAS *et al*., 2010). With the aim to solve the Se-deficiency issue in human organism, the use of Se biofortified cereals (LAZO-VÉLEZ *et al*., 2016; BRYSZEWSKA *et al*., 2007), Se enriched yeast (SCIENTIFIC OPINION, 2008) and bread supplement with Se fortified seedlings (BRYSZEWSKA *et al*., 2005) have been proposed. The main Se chemical form present in the latter products is selenomethionine (TAMAS *et al*., 2010), whereas *Allium* plants may provide more powerful anticarcinogen compounds, such as methylated forms of Se containing aminoacids (IP *et al*., 2000; ADHIKARI, 2012).

The higher dry matter in leek leaves compared with pseudo-stems simplifies the leaves dehydration process and gives wide chances to leaves powder utilization both as a spice and a supplement in functional food production with remarkable level of Se natural antioxidants.

Indeed, in recent years research has been frequently focused on the use of leaves and peel to produce powders with high antioxidants content and to the utilization of such powders in functional food preparation (FERREIRA *et al*., 2015; SONIA *et al*., 2016; ODUNLADE *et al*., 2017; LAKSHMI *et al*., 2017). However, so far leaves powder from Se enriched plants has never been used for these purposes, and in the case of *Allium* species this approach can also improve human organism protection against numerous diseases in addition to the increase of Se and other antioxidants consumption (GONZÁLEZ-MORALES *et al*., 2017).
The aims of the present research were: comparison of leek cultivars in terms of antioxidant and element composition in leaves and pseudo-stems; assessment of the effects of Se biofortification on quality characteristics of the most antioxidant-containing cultivar; evaluation of the efficiency of leek leaves powder use in the production of bread fortified with selenium-enriched and non-enriched leek leaves.

2. MATERIAL AND METHODS

2.1. Crop trials

Research was carried out on leek (*A. porrum* L.) at the experimental fields of Federal Scientific Center of Vegetable Production, in Odintsovo (Moscow, Russia, 55°39.51'N, 37°12.23'E) in 2015 and 2016 on a clay-loam soil, with pH 6.8, 2.1 % organic matter, 108 mg kg^{-1} N, 450 mg kg^{-1} P_2O_5, 357 mg kg^{-1} K_2O, exchangeable bases sum as much as 95.2%. Mean temperature and relative humidity values from May to October were 13.0, 16.1, 19.8, 18.6, 12.3, 6.4°C and 59.1, 63.8, 69.7, 72.4, 79.1, 81.0 % respectively. The experimental protocol was based on the comparison between nine cultivars (Goliath, Summer breath, Premier, Casimir, Kalambus, Campus, Vesta, Giraffe, Bandit), using a randomized complete block design with three replicates.
The sowing was performed on 5 December in 8 x 8 cm trays and the plantlets were transplanted in open field on 14 May, spaced 15 cm along the rows, the latter being 40 cm apart. Prior to planting, ploughing at 30 cm depth, hoeing at 15 cm and fertilization with 180 kg ha^{-1} N, 80 P_2O_5 and 120 K_2O were practiced; during the crops, 40 kg ha^{-1} N were supplied in three times at two-week intervals and, just in the last N application 7 kg ha^{-1} of P_2O_5 and of K_2O were also provided.
A further experimental trial was carried out in 2016 and 2017, assessing the effects of Se biofortification on leek pseudo-stem and leaf quality, by spraying the plants from June to August, once a week, with a total sodium selenate dose of 75 mg m^{-2}; cultivar Goliath was used, as it had showed the best quality indicators among the nine varieties compared in the previous year.
In either research, commercially ripe plants were harvested at mid-October and on samples taken in each plot the total, pseudo-stem and leaf blade weights were determined. Further plant samples were collected, gently washed with water to remove surface contaminants and dried with filter paper. Pseudo-stems and leaves were separated, cut with plastic knife, dried to constant weight and homogenized. The resulting powders were subjected to laboratory analysis and further used for bread production.

2.2. Bread production trial

Production of bread was achieved in 2016 and 2017 with three different processing procedures: bread obtained upon addition of leaves powder from leek selenium fortified plants to dough; bread obtained upon addition of leaves powder from leek non-fortified plants; traditionally-made bread.

Two hundred g of wheat flour with 90 μg selenium /kg d.w., 1.5 g salt and 8 g leek leaves powder were placed in a Kenwood dough mixer (Model A 907 D) set at highest speed and mixed for 1 min; control samples were not supplemented with leaves powder. Then, a suspension of 5 g yeast in 120 mL of water was added and the mixture was further run at high speed for 2 minutes. The dough was later kneaded on the kneading table, rounded into balls by hand and placed in lightly greased fermentation bowl in fermentation cabinet. The dough was then proofed for thirty minutes, baking was done at 250°C for 15 minutes and, next, the baked bread was allowed to cool at room temperature before performing determinations.
Baking was achieved in triplicate.

2.3. Volume, weight and specific volume of loaves produced

Loaf volume was measured by small seeds displacement method described by Khalil et al. (2000). Loaf was placed in a container of known volume where onion seeds were run until the container was full. The volume of seeds displaced by the loaf was considered as the loaf volumes, which were measured in a graduated cylinder. The weight of the loaf was determined using a sensitive weighing balance and the specific volume of the loaf was assessed by averaging the loaf volume with loaf weight. The specific volume was calculated according to the equation:

$$\text{specific volume } (cm^3/g) = \text{loaf volume}/\text{loaf weight}$$

2.4. Bread porosity

Bread porosity was determined according to GOST procedure (2001). Four cylindrical grooves from fresh bread were made with a volume of 27 (±0.5) cm each and weighed simultaneously. The porosity (%) was calculated by the following formula:

$$\text{porosity } (\%) = [(V\text{-}m/1.31):V] \times 100$$

where V is the total volume of bread grooves in cm^3; m is the mass of bread grooves in g; 1.31 is the density of non-porous mass of breadcrumb.

2.5. Dry matter content

The dry matter content in leaves and pseudo-stems of *A. porrum* as well as in bread samples was assessed after dehydration of the fresh samples in oven at 70°C, until they reached constant weight.

2.6. Selenium content

Se content in leek leaves and pseudo-stems as well as in bread samples was analyzed using the fluorimetric method previously described for tissues and biological fluids (ALFTHAN, 1984). The method includes digestion of dried homogenized samples via heating with a mixture of nitric-chloral acids, subsequent reduction of Se^{+6} to Se^{+4} with a solution of 6 N HCl, and formation of a complex between Se^{+4} and 2,3-diaminonaphtalene. Se concentration was assessed in triplicate by recording piazoselenol fluorescence value in hexane at 519 nm λ emission and 376 nm λ excitation. The results precision was checked using a reference standard-lyophilized cabbage at each determination with 150 μg/Kg Se concentration (Institute of Nutrition, Russia).

2.7. Potassium content

Potassium content in leek pseudo-stems was assessed using AAS technique on Shimatsu 7000 spectrophotometer (Japan) after dry ashing of 2 g leek pseudo-stems and leaves at 420°C, and dissolution of the residues in 15 ml of 3% nitric acid (ANALYTICAL METHODS, 1996).

2.8. Total soluble solids (TSS) and sugars

Determination of total soluble solids was carried out in water extracts of leek leaves and pseudo-stems using TDS-3 conductometer (Russia).
Monosaccharides were determined using ferricyanide colorimetric method, based on the reaction of monosaccharides with potassium ferrycianide (SWAMY, 2008). Total sugars were analogically determined after acidic hydrolysis of water extracts with 20% hydrochloric acid. Fructose was used as an external standard.

2.9. Polyphenols

The concentrations of total phenolics in each sample of leaves, pseudo-stems and bread were determined in 70 % ethanol extract (1 hour at 80 °C) using the Folin-Ciocalteu colorimetric method, according to GOLUBKINA *et al.* (2017) by 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 dry weight (mg GAE/100 g d.w.).

2.10. Ascorbic acid

Ascorbic acid content in leek leaves and pseudo-stems was assessed by visual titration of fresh plant extracts in 6% trichloracetic acid with Tillmans reagent (AOAC, 2012). Five grams of fresh leek leaves were homogenized in porcelain mortar with 5 ml of 6% trichloracetic acid and quantitatively transferred to measuring cylinder. The volume was brought to 80 ml using trichloracetic acid, and the mixture was filtered through filter paper 15 min later. The ascorbic acid concentration was determined from the amount of Tillmans reagent, which went into titration of the sample.

2.11. Antioxidant activity

The antioxidant activity of leek leaves and pseudo-stems as well as bread samples was assessed using redox titration method (MAXIMOVA *et al*., 2001), via titration of 0.01 N $KMnO_4$ solution with ethanolic extracts of leaves, pseudo-stems and bread samples. Reduction of $KMnO_4$ to colorless Mn^{+2} in this process reflects the amount of antioxidants dissolvable in 70 % ethanol. The values were expressed in mg GAE/100 g d.w. The use of $KMnO_4$ acidic solution is known to be successfully used for the determination of *Ocimum basilicum* antioxidant potential (SRIVASTAVA *et al*., 2015) and antioxidant capacity of serum (ZHAN *et al*., 2014).

2.12. Statistical analysis

Data were processed by analysis of variance and mean separations were performed through the Duncan multiple range test, with reference to 0.05 probability level, using SPSS software version 21. The data expressed as a percentage were subjected to angular transformation before processing.

3. RESULTS AND DISCUSSION

3.1. Leek quality parameters

From the evaluation of nutritional indicators of nine leek cultivars, it arose that cultivar Goliath had significantly higher content of antioxidants, monosaccharides and potassium compared to the other varieties (Table 2). Notably, Goliath pseudo-stems attained 1.9 to 3.8 fold higher ascorbic acid, 1.6-2.6 fold polyphenols and 1.3-1.8 fold Se; leaf polyphenols concentration was 1.3-1.6 times higher. These results suggest that among the nine cultivars examined Goliath contains the highest content of polyphenols not only in pseudo-stems but also in leaves which are usually discarded, though their high potential benefits. Moreover, *A. porrum* leaves proved to be better sources of polyphenols than pseudo-stems in all cultivars. Conversely, selenium distribution between leaves and pseudo-stems in unfortified plants is less nutritionally important due to the low concentrations of this element.

A distinctive feature of cultivar Goliath was the high proportion of monosaccharides, accounting for 60.3 % of the total sugar amount in pseudo-stems compared to 18.2-41.9 % in the other varieties (Table 1). Moreover, Goliath pseudo-stems accumulated 2.2 to 11 fold more potassium (Fig. 1). Interestingly, positive correlations relevant to pseudo-stems were detected between polyphenols and K, Se and polyphenols, Se and K ($r = +0.96$; $r = +0.97$ and $r = +0.97$ respectively, at $P \leq 0.01$) and a negative correlation between leaves and pseudo-stems Se content ($r = - 0.88$ at $P \leq 0.01$). Due to the high nutritional value of cultivar Goliath, this variety was chosen in order to assess the effect of Se biofortification on leek quality and antioxidant features.

Table 1. Quality and antioxidant indicators of nine leek cultivars.

	Dry matter %	Ascorbic acid mg/100 g	Polyphenols mg GAE/100 g d.w.		Se μg/kg d.w.		Sugars %	
							monosaccharides	total
	plant	pseudo-stems	pseudo-stems	leaves	pseudo-stems	leaves	pseudo-stems	pseudo-stems
Goliath	12.35±0.4^{e}	13.0±0.7^{a}	683±56^{a}	964±72^{a}	107±7^{a}	14±1^{d}	3.8±0.3^{b}	6.3±0.4^{e}
Premier	15.20±0.5^{d}	8.9±0.5^{b}	432±34^{b}	650±21^{c}	80±5^{b}	65±3^{b}	4.4±0.3^{a}	10.5±0.7^{c}
Bandit	15.32±0.6^{d}	6.2±0.4^{c}	394±26^{bc}	647±26^{c}	75±5^{b}	48±2^{c}	3.5±0.2^{c}	8.6±0.5^{d}
Kalambus	17.64±0.6^{c}	4.5±0.4^{e}	319±19de	731±46^{b}	72±4^{b}	74±3^{b}	3.9±0.2^{b}	10.3±0.7^{c}
Cazimir	18.86±0.6^{c}	5.7±0.5^{cd}	284±20^{e}	728±53^{b}	60±3^{c}	76±4^{ab}	2.8±0.2^{d}	10.7±0.7^{c}
Giraffe	20.51±0.8^{b}	5.1±0.4^{d}	331±19^{d}	665±41^{bc}	73±4^{b}	49±2^{c}	3.4±0.2^{c}	11.0±0.7^{c}
Camus	21.36±0.7^{b}	4.5±0.3^{e}	347±21^{cd}	684±43^{bc}	64±3^{c}	81±5^{ab}	2.5±0.2^{e}	12.2±0.8^{b}
Vesta	23.39±0.8^{a}	4.5±0.3^{e}	301±19de	740±55^{b}	69±3^{bc}	84±5^{a}	2.6±0.2^{de}	14.3±0.8^{a}
Summer breath	24.28±0.9^{a}	5.4±0.4^{cd}	329±20^{d}	616±42^{c}	72±3^{b}	72±4^{b}	2.8±0.2^{d}	15.1±0.9^{a}
M	18.77	6.86	317	686	74,7	62,5	3.3	11.1
SD	3.24	2.69	71	37	8,4	17	0.6	2.1
CV (%)	17.3	39.3	22,4	5,4	11,2	27,2	18.2	18.9
Concentration range		4.5-16.9	284-683	616-964	60-107	14-84	2.5-4.4	6.3-15.1

Within each column, means followed by different letters are significantly different according to Duncan test at P<0.05.

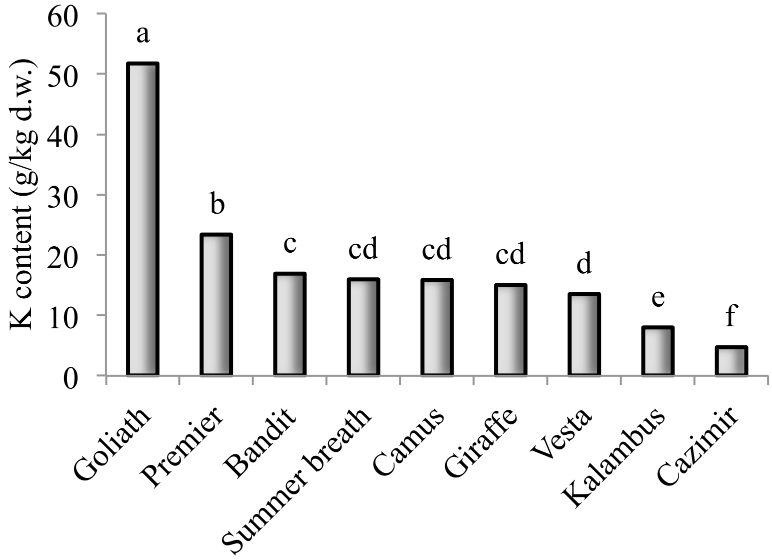

Figure 1. Intervarietal differences in K accumulation by leek pseudo-stems. Means followed by different letters are significantly different according to Duncan test at P<0.05.

3.2. Fortification of leek cultivar Goliath with Se

The data reported in Table 2 suggest that the enrichment of leek cultivar Goliath with selenium decreased plant weight due to leaf reduction, and significantly increased pseudo-stem occurrence to the total plant weight (+ 49.5%). Moreover, the leaves of selenium fortified *A. porrum* plants contained significantly higher contents of both dry matter and antioxidants compared to pseudo-stems. Notably, the contents of all the antioxidants detected were significantly higher in leaves than in pseudo-stems, i.e. 3.86 fold for ascorbic acid, 1.35 fold for polyphenols and 3.2 fold for Se. Total soluble solids value was also higher in leaves than in pseudo-stems (1.2 fold).

Table 2. Effect of Se application on yield, quality and antioxidant indicators of *A. porrum* cultivar Goliath.

	Se fortified plants		Ratio between fortified plants and non-treated control	
	pseudo-stems	leaves	pseudo-stems	leaves
Mean weight (g per plant)	196±15^{a}	200±9^{a}	1.1$^{n.s.}$	0.68*
Dry matter (%)	11.3±0.3^{b}	13.5±0.3^{a}	1.2*	1.38*
Ascorbic acid (mg/100 g f.w.)	15.0±0.6^{b}	57.9±0.8^{a}	1.15$^{n.s.}$	1.04$^{n.s.}$
Polyphenols (mg GAE/100 g d.w.)	1106±88^{b}	1494±76^{a}	1.62*	1.55*
Total sugars (%)	6.9±0.4	-	1.10$^{n.s.}$	-
Monosaccharides (%)	5.9±0.4	-	1.55*	-
Total soluble solids (mg/g)	70.0±2.7^{b}	83.2±3.6^{a}	1.27*	0.72*
Se content (μg/kg d.w.)	1451±50^{b}	4645±40^{a}	16.1*	332*
Potassium content (g/kg d.w.)	52.8±5.1^{a}	28.5±2.5^{b}	1.02$^{n.s.}$	1.04$^{n.s.}$

n.s. means no statistically significant differences between fortified and control plants;
*statistically significant at P≤0.05; within Se fortified plants, values along the rows followed by different letters are significantly different at P≤0.05.

According to the described data, leek foliar biofortification with Se performed on cultivar Goliath is beneficial to leek production, as it led to the increase of pseudo-stem yield as well as polyphenols and selenium concentration.
The investigations of LAVU (2013) on leek upon soil Se supply showed that the lower initial Se concentration in non-fortified pseudo-stems the higher the fortification level. The same phenomenon was observed in the present work: the fortification value reached 16.1 in pseudo- stems with high initial Se content (107 μg/kg), whereas it was as much as 332 in leaves with low initial Se concentration (14 μg/kg).
The uneven distribution of biologically active compounds in *A. porrum* plants was characterized by higher content of dry matter, ascorbic acid, polyphenols and total soluble solids in leaves, compared to pseudo-stems of selenium enriched plants. Similar distribution of these compounds was recorded in control plants, suggesting the nutritional importance of leek leaves, which are unfortunately discarded in the common farming practice. The antioxidant content increase in leaves and pseudo- stems as a result of selenium fortification is in agreement with the previously reported stimulating effect of selenium absorption on plant antioxidant defense (GOLUBKINA, 2016).

3.3. Supplementation of bread with leaves powder from Se fortified leek plants

Recent investigations have revealed that 4% supplementation of vegetable leaves power to wheat flour is optimal for producing functional bread (ODUNLADE *et al.*, 2017).
Physical and biochemical characteristics of bread enriched with *A. porrum* leaves powder recorded in our research are reported in Table 4. The results suggest peculiar changes in bread quality upon supplementation of unfortified or Se fortified leaves to flour (Table 3).

Table 3. Physical, quality and antioxidant characteristics of bread enriched with *A. porrum* leaves powder.

Parameter	Additives		
	No additive control	***A. porrum* leaves powder**	**Se-enriched *A. porrum* leaves powder**
Dry matter (%)	62.4±1.0^{ab}	60.4±1.0^{b}	64.5±1.1^{a}
Se content (μg/kg d.w.)	90±1^{b}	90±1^{b}	266±18^{a}
Total soluble solids (mg/g d.w.)	18.5±0.3^{c}	19.7±0.3^{b}	20.5±0.4^{a}
AOA (mg GAE/100g d.w.)	-	3.3±0.2^{b}	5.8±0.2^{a}
Polyphenols content (mg GAE/100g d.w.)	-	3.2±0.1^{b}	5.6±0.2^{a}
Specific volume (cm^3/g)	2.23±0.08^{a}	2.04±0.07^{b}	1.86±0.08^{c}
Bread porosity (%)	67.5±0.8^{a}	64.3±0.9^{b}	61.8±0.8^{c}
Colour	White	Light green	Light green

Along each row, values followed by different letters are statistically different according to Duncan test at P≤0.05.

As far as bread sensory attributes are concerned, the three products did not differ in terms of odor and flavor, whereas the bread supplemented with leek leaves powder showed a light green color, which did not vary between the samples treated with Se-fortified or non-fortified leaves. From a practical point of view, this unusual color may be preferred by consumers.

Notably, bread supplementation with selenium enriched leek leaves powder greatly differs from use of inorganic forms of selenium in bread production. Indeed, selenium salts (selenates and selenites) are known to be highly toxic and particularly dangerous upon overdosing. On the other hand, plant treatment with selenium inorganic salts allows to convert the latter to organic selenium derivatives of amino-acids and proteins, and in fact this process is named biofortification. As far as *Allium* species are concerned, such biofortification results in production of methylated forms of selenium-containing aminoacids showing remarkable anticancer activity (IP *et al.*, 2000; ADHIKARI, 2012) which is significantly higher than that associated to the selenomethionine present in selenium enriched yeast (GOLUBKINA and PAPAZYAN, 2006)

Notably, the incorporation of leek leaves powder with high Se concentration into dough resulted in the highest values of Se content, total soluble solids, polyphenols and antioxidant activity of the final product. One hundred grams of such bread contain about 17 μg Se, which accounts for 31% of the required selenium consumption (Dietary Reference Intakes, 2000).

Se losses during bread baking were low and did not exceed 3%, which is consistent with previous investigation results (LYONS *et al.*, 2005; ROSELL *et al.*, 2015; GARVIN *et al.*, 2011) about the stability of Se compounds during baking of bread from Se enriched wheat flour. Moreover, leek polyphenol stability during baking is higher in Se enriched product than under non fortified leaves use (93.7% vs 84.2% respectively). This phenomenon may reflect the well-known antioxidant protective effect of Se (GOLUBKINA and PAPAZYAN, 2006).

The results of the present work also prove the effect of Se supplementation on bread porosity and specific volume, the latter parameters decreasing according to the following sequence: control > bread with leaves powder from non fortified leek > bread supplemented with Se enriched leek leaves powder. The decrease in bread porosity and specific volume was reported in previous studies carried out on bakery products fortified with leaves powder from different plants by ODUNLADE *et al.* (2017). These authors explained that this phenomenon is the consequence of gluten concentration decrease due to the replacement of the flour portion containing leaves powder. Unfortunately, such a statement is not exhaustive for describing the phenomenon relevant to the effect of Se enriched leaves on bread porosity and specific volume; it is just indicative that using wheat with high Se content in bread production leads to 10% decrease of final product porosity (GARVIN *et al.*, 2011), which is consistent with the 8.9% decrease relevant to Se fortified leek utilization detected in the present study. This peculiarity is presumably associated with Se, as the porosity decrease in the case of ordinary leek powder addition results in smaller changes of this parameter (4.7 %).

One of the reasons connected to dough rheological properties decrease in case of Se-enriched leaves powder use, compared to dough with non-fortified leaves and control dough, may be the increased concentration of plant polyphenols under selenium fortification. Indeed, despite their high nutritional value, polyphenols are known to cause changes in dough rheology via interaction with proteins, resulting in the decrease of enzymes and yeast activity and thus worsening bread porosity (WANG *et al.*, 2007). In particular, gallate and hydroxylate benzol groups of polyphenols form noncovalent bonds with amino-, hydroxyl- and carboxyl groups of proteins (HUANG *et al.*, 2004; ROSELL *et al.*, 2015).

Another factor possibly affecting dough porosity is the high level of total soluble solids (ROSELL *et al.*, 2015), which is increased in bread supplemented with *A. porrum* leaves powder, especially with high selenium content.

4. CONCLUSIONS

The experimental investigation carried out on leek in northern Europe allows to draw interesting remarks regarding both plant Se fortification and bread production using Se-enriched leaves. In this respect, Goliath was identified as the best responsive cultivar to Se application for obtaining nutrient-added pseudo-stems and its leaves powder was successfully mixed with dough during the bread making process. The mentioned practice is aimed to valorize both leek crop waste such as leaf blades and a widely consumed daily food such as bread. As arisen from this research, Se supply to *A. porrum* plants entails beneficial effects to human organism as a consequence of the use of either a fresh vegetable or a functional food.

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

REFERENCES

AOAC. 2012. The Official Methods of Analysis of the Association of Official Analytical Chemists International, 2012.22 Vitamin C, USA.

Adhikari P. 2012. Biofortification of Selenium in broccoli (*Brassica oleracea* L. var. italica) and onion (*Allium cepa* L.). Master Thesis in Plant Science. Norway.

Alfthan G. 1984. A micromethod for the determination of selenium in tissues and biological fluids by single-test-tube fluorimetry. Anal. Chim. Acta 65:187. DOI: doi.org/10.1016/S0003-2670(00)85199-5

Allen L., de Benoist B., Dary O. and Hurrell R. (eds). 2006. Guidelines on food fortification with micronutrients. WHO.

Analytical methods for atomic absorption spectroscopy. 1996. Perkin-Elmer corporation. 299 pp.

Bryszewska M.A., Ambroziak W., Diowksz A., Baxter M.J., Langford N.J. and Lewis D.J. 2005. Changes in the chemical form of selenium observed during the manufacture of a selenium-enriched sourdough bread for use in a human nutrition study. Food Addit. Contam. 22:135. DOI: doi.org/10.1080/02652030500037787

Bryszewska M.A., Ambroziak W., Langford N.J., Baxter M.J., Colyer A. and Lewis D.J. 2007. The effect of consumption of selenium enriched rye/wheat sourdough bread on the body's selenium status. Plant Foods Hum. Nutr. 62:121. DOI: doi.org/10.1007/s11130-007-0051-y

de Valença A.W., Bake A., Brouwer I.D. and Giller K.E. 2017. Agronomic biofortification of crops to fight hidden hunger in sub-Saharan Africa. Global Food Security 12:8. DOI: doi.org/10.1016/j.gfs.2016.12.001

Dietary Reference Intakes. 2000. Vitamin C, Vitamin E, Selenium, and Carotenoids. Institute of Medicine, Food and Nutrition Board National Academy Press, Washington, DC.

Ferreira M.S.L., Santos M.C.P., Moro T.M.A., Basto G.J., Andrade R.M.S. and Gonçalve É.C.B.A. 2015. Formulation and characterization of functional foods based on fruit and vegetable residue flour. J. Food Sci. Technol. 52:822. DOI: doi.org/10.1007/s13197-013-1061-4

Garvin D.F., Hareland G., Gregoire B.R. and Finley J.W. 2011. Impact of wheat grain selenium content variation on milling and bread baking. Cereal Chem. 88:195. DOI: doi.org/10.1094/CHEM-05-10-0076

Golubkina, N.A. 2016. Prospects of onion (*Allium cepa* L.) fertilization by selenium. Mini-review. Trace Elements in Med. 17:4. DOI: doi.org/10.19112/2413-6174-2016-17-1-4-9

Golubkina N.A., Kosheleva O.V., Krivenkov L.V., Dobrutskaya H.G., Nadezhkin S. and Caruso G. 2017. Intersexual differences in plant growth, yield, mineral composition and antioxidants of spinach (*Spinacia oleracea* L.) as affected by selenium form. Sci. Hort. 225:350. DOI: doi.org/10.1016/j.scienta.2017.07.001

Golubkina N. A., Nadezhkin S. M., Agafonov A. F., Kosheleva O. V., Molchanova A. V., Russo G, Cuciniello A. and Caruso G. 2015. Seed oil content, fatty acids composition and antioxidant properties as affected by genotype in *Allium* cepa L. and perennial onion species. Adv. Hort. Sci. 29(4):199-206.

Golubkina, N.A. and Papazyan, T.T. 2006. Selenium in nutrition. Plants, animals, human beings. Moscow: Pechatny gorod. 265 pp.

González-Morales S., Pérez-Labrada F., García-Enciso E.L., Leija-Martínez P., Medrano-Macías J., Dávila-Rangel I.E., Juárez-Maldonado A., Rivas-Martíne E.N. and Benavides-Mendoza A. 2017. Selenium and sulfur to produce *Allium* functional crops. Molecules 22. DOI: doi.org/10.3390/molecules22040558.

GOST 5669-96. 2001. Bakery products. Method for determination of porosity. MKC 67.

Huang H.H., Kwok K.C. and Liang H.H. 2004. Effect of tea polyphenols on the activity of soybean trypsin inhibitors and trypsin. J. Agric. Food Chem. 84:121-6.

Ip C., Birringer M., Block E., Kotrebai M., Tyson J.F., Uden P.C. and Lisk D.J. 2000. Chemical speciation influences comparative activity of selenium-enriched garlic and yeast in mammary cancer prevention. J. Agr. Food Chem. 48:2062. www.scholarworks.umass.edu/chem_ faculty_pubs/ 1046

Khalil A.H., Mansour E.H. and Dawood F.M. 2000. Influence of malt on rheological and baking properties of wheat-cassava composite flours. Lebens Wissen Technol. 33:159. DOI: doi.org/10.1006/fstl.1999.0629

Lakshmi N.B., Suneetha W.J., Maheswari K., Kumari B.A. and Prabhakar B.N. 2017. Antioxidant potential of rice bran and vegetable waste powders incorporated extrudates. The Pharma Innovation J. 6:12.

Lavu R.V., Du Laing G., Van de Wiele T., Pratti V.L., Willekens K., Vandecasteele B. and Tack F. 2012. Fertilizing soil with selenium fertilizers: impact on concentration, speciation, and bioaccessibility of selenium in leek (*Allium ampeloprasum*). J. Agr. Food Chem. 60:10930. DOI: doi.org/10.1021/jf302931z.2012.

Lavu R.V.S. 2013. Biofortification, speciation and bioaccessibility of selenium in food and feed crops. PhD Thesis, Ghent University, Ghent, Belgium.

Lazo-Vélez M.A. 2016. Selenium-enriched germinated wheat, soybean and huauzontle and their effects in bread properties and colon cancer. Thesis of doctor of biotechnology. Monterrey Nuevo León.

Lyons G., Genc Y., Stangoulis J., Palmer L. and Graham R. 2005. Selenium distribution in wheat grain, and the effect of postharvest processing on wheat selenium content. Biol. Trace Elem. Res. 103:155. DOI: doi.org/10.1385/BTER:103:2:155.

Maximova T.V., Nikulina I.N., Pakhomov V.P., Shkarina H.I., Chumakova Z.V. and Arzamastsev A.P. 2001.Method of antioxidant activity determination. RF Patent № 2.170,930 July 20.

Odunlade T.V., Famuwagun A.A., Taiwo K.A., Gbadamosi S.O., Oyedele D.J. and Adebooye O.C. 2017. Chemical composition and quality characteristics of wheat bread supplemented with leafy vegetable powders. J. Food Qual. DOI: doi.org/10.1155/2017/9536716.

Radovanović B., Mladenović J., Radovanović A., Pavlović R. and Nikolić V. 2015. Phenolic composition, antioxidant, antimicrobial and cytotoxic activities of *Allium porrum* L. (Serbia) extracts. J. Food Nutr. Res. 3:564. DOI: doi.org/10.12691/jfnr-3-9-1.

Rosell C.M., Bajerska J. and El Sheikha A.F.(eds). 2015. Bread and its fortification: nutrition and health benefits. CRC Press.

Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) on a request from the Commission on Selenium-enriched yeast as source for selenium. 2008. Eur. Food Safety Authority J. 766:1.

Sonia N.S. Mini C. and Geethalekshmi P.R. 2016. Vegetable peels as natural antioxidants for processed foods – A review. Agr. Rev. 37:35. DOI: doi.org/10.18805/ar.v37i1.9262.

Srivastava S., Adholeya A., Conlan X.A. and Cahill D.M. 2015. Acidic potassium permanganate chemiluminescence for the determination of antioxidant potential in three cultivars of *Ocimum basilicum*. Plant Foods Hum. Nutr. 70(4). DOI: doi.org/10.1007/s11130-016-0527-8.

Swamy P.M. 2008. Laboratory manual on biotechnology Rastogi Publications, 617 pp.

Tamas M., Mandoki Z.S. and Csapo J. 2010. The role of selenium content of wheat in the human nutrition. A literature review. Acta Universitatis Sapientiae Alimentaria 3:5.

Wang R., Zhou W. and Isabelle M. 2007.Comparison study of the effect of green tea extract on the quality of bread by instrumental analysis and sensory evaluation. Food Res. 40:470-9.

White P.J. and Broadley M.R. 2009. Biofortification of crops with seven mineral elements often lacking in human diets-iron, zinc, copper, calcium, magnesium, selenium and iodine. New Phytol. 182:49. DOI: doi.org/10.1111/j.1469-8137.2008.02738.x.

Wimalawansa S.J. 2013. Food fortification programs to alleviate micronutrient deficiencies. J. Food Proc. Technol. 4:257. DOI: doi.org/10.4172/2157-7110.1000257.

Zhan M.G., Liu N. and Liu H. 2014. Determination of the total mass of antioxidant substances and antioxidant capacity per unit mass in serum using redox titration. Bioinorg. Chem. Appl. Article ID 928595.
DOI: doi.org/ 10.1155/2014/928595.

Paper Received June 12, 2018 Accepted October 23, 2018