Impact of germination conditions on antioxidant properties and protein content in lentils (Lens culinaris) of Ukrainian cultivars

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Abstract

Introduction. Changes in the free radical scavenging activity, the yield of phenolic compounds, ascorbic acid and proteins content in lentil seeds of two Ukrainian cultivars were determined during the germination period under different light conditions.

Materials and methods. Were studied the effect of germination on two Ukrainian varieties of lentils namely Luganchanka and Svitanok. The total phenolics content (TPC) in samples was determined using Folin-Ciocalteu assay, the ascorbic acid level was determined by a colorimetric assay, free radical scavenging activity was elucidated using DPPH assay, changes of proteins content were found out according to Kjeldahl`s method.

Results and discussion. Results of the research have shown that during germination in seeds highly increased levels of the total phenolics, ascorbic acid and free radical scavenging activity, especially under day-night photoperiod light conditions. The proteins content in seeds at the end of germination was a little lower comparing with ungerminated seeds under both of light conditions.

The highest rise of TPC was fixed in lentils var. Luganchanka on the 3–4th germination day. The increase was about 65%.

A similar situation was with changing of ascorbic acid level. It was higher 6 times if compare germinated and ungerminated samples of lentils var. Luganchanka, obtained under day-night conditions.

The antiradical activity of germinated seeds was the highest at the end of the germination process. However, germinating after the 5th day under studied conditions is unacceptable, because the rotting of sprouts happened. The lowest increase near 60% had germinated materials of var. Svitanok that were obtained in dark conditions. The highest increase of antioxidant activity was near 550% in samples var. Luganchanka after germinating in day-night conditions.

Conclusions. Our results demonstrate that germinated lentil seeds both cultivars have advantages over non-germinated and can be useful in functional food technologies.

Introduction

Legumes play an important role in human nutrition in many diets. Lentil is one of the most valuable species of legumes. Its seeds are an excellent source of protein, rich in important vitamins, minerals, dietary fiber. According to recent studies [1–5], lentil could be considered as a prophylactic and therapeutic functional ingredient due to it sizable content of essential micronutrients as well as phytochemicals. These components can provide anti-inflammatory, hepatoprotective, antioxidant effects, lead to the prevention of heart diseases, diabetes, DNA damage and other disorders [4, 5].

Germination is inexpensive and effective bioengineering process that can highly increase the nutritional value of lentil seeds by intensive synthesis of bioactive compounds [6, 7], desirable changes in the nutrient availability [8] and decreasing of anti-nutritional components (phytic acid, tannins) [9]. During the germination period, the content of low-molecular weight antioxidants such as polyphenols [10, 11], ascorbic acid, tocopherols grows up, that provides increasing the antioxidant properties of seeds [7, 12]. The qualitative and quantitative composition of the functional components in germinated seeds depends on many variables, which includes soaking time, humidity, temperature, germination time, presence of chemical elicitors [13, 14] and other biotic and abiotic factors [15, 16]. Numerous studies have shown that the content of the synthesized components also depends on seeds variety and places of plant cultivation [17].

The aim of our study was to determine the dynamic of free radical scavenging activity changes, the yield of total phenolic compounds and ascorbic acid in lentils of Ukrainian cultivars depending on the germination time, light conditions (dark or day-night photoperiod) and the plant variety. Also changes of the crude protein content, as one of the main component of lentil seeds were monitored.

Materials and methods

Raw materials of two Ukranian varieties of lentil (*Lens culinaris*) namely Luganchanka and Svitanok were used in the study. Plants had been grown under the same environmental conditions in the Mykolaiv region, Ukraine. Seeds were collected during summer and autumn months of 2014 and handpicked to ensure usage of unbroken material.

Germination was done according to procedure written below**.** Dry seeds were cleaned and disinfected with 1% potassium hypochlorite for 5 min, washed with distilled water to neutral pH, soaked in distilled water for 4 h at 20 ºC and placed in Petri dishes with an adsorbent paper. Seeds were germinated for 5 days at 20 ºC in two different light conditions: in dark (D) and day-night (DN) (day – 16 h, night – 8 h) photoperiod. Seeds were kept moist by spraying them with distilled water. The germination process was done in triplicate. The percentage of germinated seeds was evaluated.

Experimental parameters of seeds were assayed every day during the period of germination. Before that, seeds were air-dried at 35 ºC for 8 h and milled.

The total phenolics content (TPC) of extracts was determined using Folin-Ciocalteu assay [18]. Seeds and sprouts were subjected to extraction using 70% aqueous-ethanolic solution. The ratio of raw material : extractant was 1:10. The extraction process had been continued for 4 h at room temperature with intense shaking. Extracts were centrifuged at 4000 rpm for 15 min and filtered through the filter paper. Supernatants were used for further analysis.

Ungerminated seeds were prepared as noted above to serve as a control.

Briefly, 0.1 ml of the extract or ultra-pure water or gallic acid standard solution (0-0.2 mg/ml) was diluted with 1 ml of ultra-pure water, 0,1 ml of Folin-Ciocalteu reagent and left standing at room temperature for 5 min. Then 2 ml of 2% sodium carbonate was added and the mixture was incubated at room temperature for 20 min. The absorbance was measured at 765 nm with the spectrophotometer BioMate 5 (Thermo electron corporation, USA). Gallic acid solution was used for the construction of a calibration curve as a standard. The total phenolic content was expressed as mg gallic acid equivalent (GAE) per 100 g dry weight of seeds.

Ascorbic acid was extracted with ultra-pure water acidified 2% meta-phosphoric acid according to our latest procedure [19] and determined by a colorimetric assay [20].

Free radical scavenging activity of aqueous and 70% aqueous-ethanolic extracts was determined using DPPH assay based on the activity of the stable 1,1-diphenyl-2-2-picrylhydrazyl (DPPH) free radical as described by Brand-Williams et al. [21].

Briefly, the alcoholic solution of DPPH (1.8 ml) was added to 0.2 ml of extracts obtained from plant materials. Samples were incubated in the dark place at room temperature for 30 min. The decrease in absorbance was measured at 517 nm by UV-VIS spectrophotometer for all samples. The absorbance of the DPPH radical solution without antioxidant was measured as the control (Ac). The percentage of inhibition of the DPPH radical by samples was calculated according to the equation:

$$\% \text{ Inhibition} = [(Ac-As)/Ac]\ 100,$$

where: Ac - absorbance of the control, As - absorbance of the solution of DPPH radical with extract or standard after the reaction.

Blank samples contained 1.8 ml of ethanol and 0.2 ml of plant extract; control sample contained 1.8 ml of 0.04 mM DPPH and 0.2 ml of ethanol. The synthetic antioxidant ascorbic acid was used as a standard. Antiradical activity of samples was presented as ascorbic acid equivalent capacity and expressed as mM of the ascorbic acid equivalent (AAE) antioxidant capacity per 1 g of seeds dry weight (mM AAE/g DW).

Total nitrogen was determined according to Kjeldahl`s method [22]. Crude protein was calculated as Nitrogen content multiply on 6,25.

Experimental results were expressed as mean ±standard deviation of three replicates. Where applicable, the data were subjected to one-way analysis of variance (ANOVA) and the differences among samples were determined using the statistical analysis system Statgraphics. P-value of < 0.05 was regarded as significant.

Results and discussion

The dynamic of the lentil sprouts length changing were evaluated under different germination conditions (Table 1).

Sprouts that had been obtained under day-night photoperiod light conditions were significantly longer than sprouts had grown in the dark. They were stronger and thicker. Their green color was conditioned by the photosynthesis process.

The sprout's length of the var. Luganchanka and var. Svitanok under the same germination conditions did not differ significantly.

Table 1

The length of sprouts grown under different conditions [mm]

Germination day	Lentil seeds varieties			
	Luganchanka		Svitanok	
	Light condition			
	D	DN	D	DN
1	3.5 ±0.15	4 ±0.2	3 ±0.18	4 ±0.22
2	13 ±0.85	38 ±1.8	11.5 ±0.7	32 ±1.65
3	19 ±1.05	52 ±3.5	18 ±1.0	50 ±3.8
4	25 ±1.4	68 ±4	24 ±1.5	65 ±4
5	34 ±1.8	97 ±5.5	32 ±2	89 ±4.5

After the 5th day of germination under both of conditions, the rotting of sprouts happened. Therefore, germination after the 5th day under these conditions is unacceptable.

Results have shown that under day-night photoperiod it occurs the increasing of the TPC level (Figure 1).

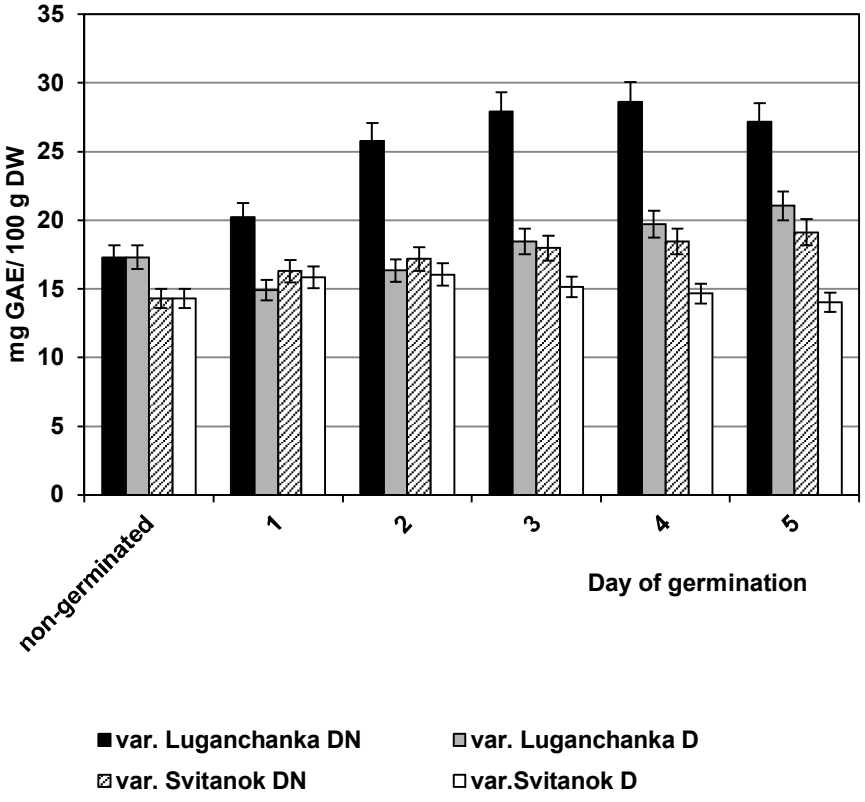

Figure 1. The total phenolics content of germinated seeds obtained under different light conditions (D, DN)

The visible increase of TPC during germination time had lentil sprouts var. Luganchanka, which were growing under the day-night photoperiod condition. The maximum value of total phenolics in this seeds was on the 3–4th germination days and it was higher than in ungerminated material on 65%. Obtained results are in agreement with those were given in similar studies [10, 23]. Sprouts of lentil seeds var. Svitanok demonstrated a decrease of TPC in darkness and increase under the day-night photoperiod condition.

Investigated samples, which were germinated under the day light, demonstrated more significant increase of the TPC level in comparison to sprouts obtained in dark conditions. This can be explained by the fact that different light conditions influence on activating prolin-linked phentose phosphate and shikimate pathways for phenols synthesis [24].

In the similar studies [25] were shown that the level of TPC in lentil sprouts during germination period was in a steady decline. The distinction in results can be explained by different cultivars and environment conditions in regions of cultivation of plants. On the other hand, authors didn't take into consideration changes of moisture content in the investigating samples during germination.

As in the case of polyphenols, better production of ascorbic acid was under the day-night photoperiod condition that in the dark. But in contrast to polyphenols a maximal increase of the ascorbic acid content was observed on the 1st day of germination (Figure 2).

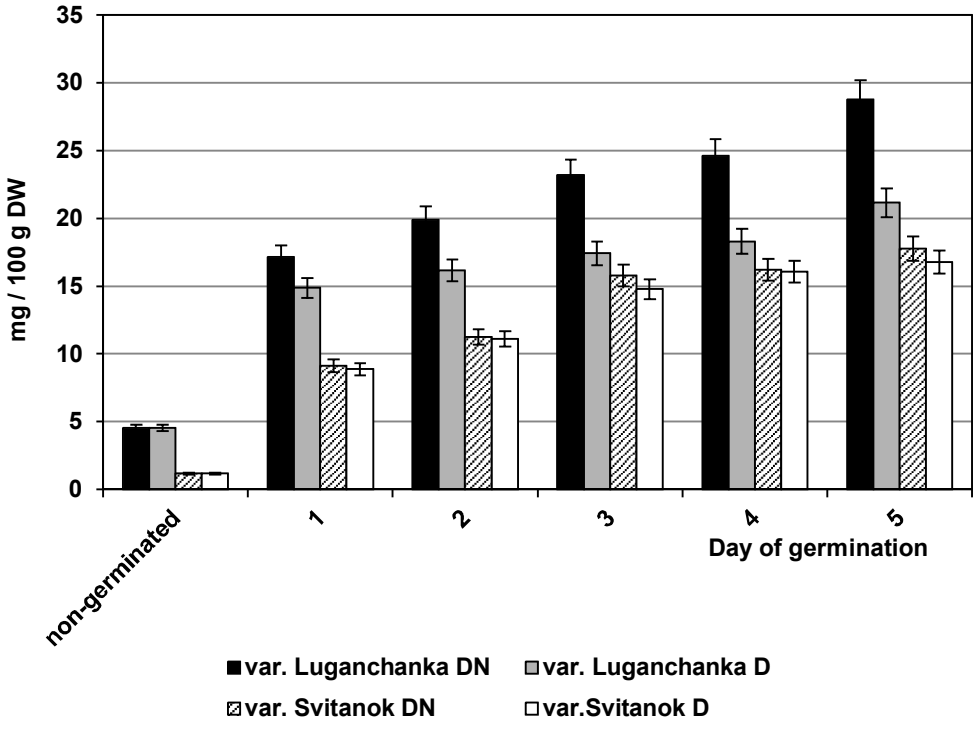

Figure 2. Ascorbic acid content in samples obtained under different light condition (D, DN)

The ascorbic acid content in sprouts of var. Luganchanka was significantly higher than in var. Svitanok. Also the differences in the vitamin C content in materials prepared under the darkness and day-night conditions in var. Luganchanka was differ significantly, however, this indexes for var. Svitanok was not considerable.

The research of two types of extracts from lentil sprouts (aqua (A) and aqueous-ethanolic (AE)) on their antiradical activity has showed dynamics similar to results previously described (Table 2).

Table 2

Free radical scavenging activity of aqueous-ethanolic and aqueous extracts of lentil seeds [mM AAE/g DW]

Germination day	Varieties of lentil seeds							
	Luganchanka				Svitanok			
	Light condition							
	D		DN		D		DN	
	Types of extracts from lentil seeds and sprouts							
	AE	A	AE	A	AE	A	AE	A
1	34,85 ±1,02	19,91 ±0,86	42,56 ±2,61	25,56 ±1,08	22,18 ±1,89	11,87 ±0,57	23,45 ±1,09	14,93 ±0,66
2	39,43 ±1,71	27,99 ±1,12	54,33 ±1,99	34,19 ±1,78	24,56 ±1,64	14,12 ±0,82	25,11 ±1,34	19,61 ±0,87
3	46,78 ±2,89	34,34 ±1,76	68,48 ±1,66	41,04 ±2,27	25,17 ±1,34	18,19 ±0,63	27,12 ±1,08	23,11 ±1,32
4	54,61 ±1,99	38,12 ±1,96	76,92 ±2,47	52,47 ±2,51	23,52 ±2,22	21,16 ±1,21	30,02 ±1,46	27,08 ±1,41
5	52,46 ±2,13	41,82 ±2,26	78,63 ±3,49	61,71 ±2,87	21,18 ±2,79	22,88 ±1,02	31,41 ±1,62	28,12 ±1,22
non-germinated	31,79 ±2,01	11,07 ±0,63	31,79 ±2,01	11,07 ±0,63	19,77 ±0,46	6,67 ±0,28	19,77 ±0,46	6,67 ±0,28

During the germination process the antiradical activity level of sprouts gradually increased. As in the case with the content of phenols and ascorbic acid, higher activity had sprouts which were cultivated under the day-night photoperiod condition.

By contrast to changes in levels of low-molecular compounds with antioxidant activity, the level of crude protein content in sprouts was decline during the germination period (Table 3).

Bigger protein value had lentil of var. Luganchanka. On the 7th germination day the protein content in both varieties were almost equal. Samples of lentil sprouts which were obtained in the darkness had a less the protein content.

Reducing of the protein amount during germination can be explained by their biotransformation in structural components of sprout and using as an energy source for growth.

Table 3

Crude protein content in seeds and sprouts during germination [g/100g]

Germination day	Varieties of lentil seeds			
	Luganchanka		Svitanok	
	Light condition			
	D	DN	D	DN
1	33,87 ±0,89	34,17 ±1,84	28,96 ±1,21	29,32 ±1,12
2	33,18 ±1,76	32,13 ±0,99	28,33 ±1,08	29,06 ±1,33
3	31,96 ±1,12	30,83 ±1,51	28,01 ±1,67	28,61 ±0,89
4	28,08 ±0,87	29,77 ±0,78	27,69 ±0,87	28,08 ±1,19
5	27,87 ±0,81	29,1 ±1,43	27,17 ±1,33	27,95 ±1,34
non-germinated	35,21 ±1,56	35,21 ±1,34	30,76 ±0,91	30,76 ±1,64

Conclusions

Results have shown that the germination process provided increasing the content of total phenolics, ascorbic acid, and antiradical activity in investigated samples of lentil seeds.

Maximum values of experimental indexes were determined in seeds that were germinated under day-night light conditions.

During the germination period, the content of low molecular weight components with antioxidant activity was highly increased; whereas the content of such valuable component as protein was a little decreased. Definitely, germinated seeds can be used as sources of valuable components for preparing functional foods and supplements for oxidation-associated diseases prevention.

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