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EFFECTS OF PULSED LIGHT TREATMENT ON GERMINATION EFFICIENCY OF PULSES

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Abstract

Germination tests were performed on chickpeas, broad beans and green lentils, with the aim of identifying the processing conditions that allow improving germination efficiency and bioactivity profile of the germinated pulses. Germination experiments were carried out under different lighting conditions. The preliminary treatment of the soaked pulses through pulsed light of different fluency values before germination was also tested. Regardless of the investigated pulses, the dark or light regime had no significant influence on the total germination efficiency. Anyway, the light had a positive effect on the germination rate of chickpeas in the first 24 hours. A more pronounced increase of the antioxidant capacity was observed for samples germinated under dark conditions. The investigated pulses reacted differently to the pulsed light stimuli. Only in the case of lentils an increase of the percentage of germinated seeds was observed after the pulsed light treatment. Regarding the synthesis of both proteins and antioxidant compounds, the most promising results were registered for germinated broad beans after the pulsed light treatment at fluence of 43.2 J/cm^2.

***Key words**: lentil, chickpea, broad beans, light pulses, germination.*

INTRODUCTION

Pulses are remarkable sources of valuable nutrients such as high quality proteins, vitamins, minerals and fibers (Bassett et al., 2010). However, the nutritional value of pulses can be compromised because of the presence of trypsin inhibitors, vicilin, covicilin, and tannins, and because of the reduced digestibility of proteins and starch. Different studies showed the possibility of enhancing protein and starch digestibility, together with reducing the content of phytic acid, tannins, lectins and protease inhibitors through germination (Ghavidel and Prakash, 2007; Freitas et al., 2007; Khandelwal et al., 2010; Gan et al., 2017). Germination is recognized as an inexpensive process for increasing the nutritional value of crops (Cáceres et al., 2014; Gan et al., 2017). The germination process refers to embryo development, when partial hydrolysis of the starch, proteins, hemicellulose and cellulose occurs, leading to important transformations in the morphological structure and seeds composition. The efficiency of the germination process is influenced by seeds quality and hydration prior to germination, and environmental factors such as presence of light, temperature, humidity and oxygen (Seo et al., 2009). The importance of light in the germination is twofold, being the source of both energy and information, in terms of photoperiodicity (day/night), phototropism (light direction) and photomorphogenesis (quantity and quality of light). Seeds are provided with sophisticated equipment to monitor and determine the extent to which the environmental parameters are suitable for germination and subsequent plant growth (Seo et al. 2009). Thus the interaction between light and hormonal signals plays an essential role in the control of germination process. Many studies have investigated the effect of different regions of the electromagnetic radiation field (static magnetic field, type of illumination and pulses light) on the germination efficiency of small seed crops (Lindig-Cisneros and Zedler, 2001; Rajendra et al., 2005; Seo et al., 2009; Shine et al., 2011; Vashisth and Nagarajan, 2010). Anyway, the knowledge on the effect of

light on pulses germination efficiency and nutritional value is scarce.

The present study aimed to determine the effect of the pulsed light on the germination efficiency and antioxidant properties of different pulses under light and dark conditions. Three pulses were considered in the study, namely broad bean (*Vicia faba*), chickpea (*Cicer arienatum*) and green lentils (*Lens culinaris*).

MATERIALS AND METHODS

Materials

Commercial broad beans (*Vicia faba*), chickpeas (*Cicer arienatum*) and green lentils (*Lens culinaris*) retailed on the local market (Galati, Romania) were used in the study.

Germination process

Prior to germination, the beans were rinsed with tap water, sanitized by soaking for 15 min with aqueous ethanol solution (70%), and finally rinsed with tap water again. The beans were then subjected to swelling in tap water under dark conditions, for a period of time adequate to assure the needed humidity for germination. Preliminary tests (results not shown) were performed, for each investigated legume type, to establish the optimum swelling time, as follows: 24 hours for broad bean, 12 hours for chickpea, and 7-8 h for green lentils. Afterward, beans were subjected to germination at 22 ± 2°C using the automatic Easy Green germinator, equipped with fog generator.

In order to determine the impact on germination power and the accumulation of biologically active compounds in the investigated pulses, the following germination conditions were considered in the study: the pulsed light treatment of the soaked beans and the dark/light regime during germination. The pulsed light treatment, with fluence values of 19.2 J/cm^2 and 43.2 J/cm^2,was applied immediately after completing the swelling step. Afterwards, the pulsed light treated samples were subjected to germination under dark (germination under total (24/24h) darkness) or light (germination under daylight (12h/24h)) regime. The study was conducted in September 2017, when the daylight regime is of approximately 12 h. Light pulses were generated by an IFP xenon type lamp with the following characteristics: electromagnetic field (λ) of 200-1000 nm and impulse regime of 10^{-1} - 10^{-4} s, as indicated by Turtoi and Nicolau (2007).

The germination time varied with the type of legume; the broad beans and chickpeas were germinated for 48 h, while green lentils were allowed to germinate for 24 h. The broad beans and chickpeas sprouted after the first 24 h of germination were counted, and allowed then to germinate for additional 24 h. At the end of the germination step all sprouted beans were counted to estimate the germination power. The germinated samples were afterwards dried at 55°C for 24 - 30 h in a convection oven (LabTech LDO-030E, Daihan Lab Tech Co., LTD, Kyonggi-Do, Koreea). Germinated broad beans and chickpeas were dehulled prior to drying. The dried native and germinated beans were finally grinded into flours with particles size lower than 500 μm using a laboratory mill (WZ-2, Sadkiewicz Instruments, Bydgoszcz, Poland).

For each legume considered in the study, the control sample consisted of beans processed through swelling, dehulling, drying and grinding, under the same conditions as mentioned before. This type of processing of the control samples was considered necessary to assure the uniformity of the samples. In this respect López-Amorós et al. (2006) stated that swelling process leads to a significant decrease of total phenols content. Finally, 21 samples were obtained and coded as shown in Table 1.

Proximate composition

The proximate composition of the flours obtained from native and germinated legumes was determined as follows: the moisture content using the AACC 44-51 method (AACC International, 2010); the protein content through the semimicro-Kjeldahl method (Raypa Trade, R Espinar, SL, Barcelona, Spain) using the nitrogen conversion factor of 6.00; the fiber content through the AOAC Official Method 962.09, using Gerhardt Fibertech equipment (C. Gerhardt GmbH & Co. KG); and the ash content using SR ISO 2171: 2002 Method (ASRO, 2008).

Table 1. Codification of the lentil (L), broad bean (B) and chickpea (C) samples subjected to germination under different conditions

Sample code	Applied treatment
$L_0B_0C_0$	Control samples
L_1 B_1 C_1	Samples germinated in darkness (24/24h)
L_2 B_2 C_2	Samples germinated in daylight (12/24h)
L_3 B_3 C_3	Samples treated with light pulses of 43.2 J/cm^2 fluence and germinated in darkness (24/24h)
L_4 B_4 C_4	Samples treated with light pulses of 43.2 J/cm^2 fluence and germinated in daylight (12/24h)
L_5 B_5 C_5	Samples treated with light pulses of 19.2 J/cm^2 fluence and germinated in darkness (24/24h)
L_6 B_6 C_6	Samples treated with light pulses of 19.2 J/cm^2 fluence and germinated in daylight (12/24h)

Extraction for determination of total phenolics and antioxidant activity

The studied flours were subjected to extraction with 80% methanol solution, while stirring for 2 h at room temperature, using a magnetic stirrer. The mixture was then centrifuged at 9690×g for 10 minutes (Martinez Villaluenga et al., 2009). The supernatant was collected for further determinations.

Determination of total phenolic content

The Folin-Ciocalteu method was used to determine the concentration of total phenolic compounds. A volume of 0.2 ml extract solution was mixed with Folin-Ciocalteu reagent (1.5 mL, previously diluted with water 1:10, v/v). After 10 min of resting period at room temperature, 1.5 mL of 60 g/L sodium carbonate was added. The ad mixture was let to rest for another 90 min and then the absorbance was read at 725 nm. The total phenolic compounds were quantified and expressed as mg ferulic acid equivalents (FA)/g of sample.

Determination of antioxidant activity by DPPH method

The ability of the investigated samples to scavenge DPPH free radicals was determined by mixing 0.1 mL of extract with 3.9 mL of 6×10^{-5} M solution of DPPH in methanol. After the reaction was allowed to take place in the dark for 30 min, the absorbance at 515 nm was recorded to quantify the remaining DPPH. A control was prepared using solvent instead of sample extract and was used to measure the maximum DPPH absorbance. The DPPH radical scavenging activity was expressed as IC50 values, corresponding to the amount of antioxidant (mg of legume flours) necessary to decrease the absorbance by 50% (López-Amorós et al., 2006). Thus smaller IC50 values are related to higher antioxidant activities. All samples were prepared and measured separately in duplicate.

Statistical analysis

Two independent germination experiments were conducted and all measurements were performed in duplicate. Statistical analysis was performed using Microsoft Excel Software. Correlation analysis was performed to identify potential relationships between germination process and antioxidant activity of germinated pulses. The results are reported as mean values together with standard deviations.

RESULTS AND DISCUSSION

Influence of light and pulsed light treatment on the germination of pulses

The influence of light and pulsed light treatment on the germination process was quantified by determining the percentage of germinated seeds, and the results are shown in Figure 1. Regardless of the investigated sample, the light or dark regime during the germination operation had no significant influence on the germination power ($p>0.05$). Anyway, in the case of chickpea and broad bean samples, a significantly higher percentage ($p<0.05$) of seeds germinated under light regime was observed after the first 24 hours (samples C2 in Figure 1b and B2 in Figure 1c). Similar observation regarding the lack of influence of different light conditions on the percent of germination have been reported by Martín-Cabrejas et al. (2008) when investigating the soybean andnon-conventional legume slike cowpea, jack bean, mucuna, and dolichos.

An increase of the germination power was noticed in case of all investigated legume samples treated with pulsed light at high fluence value. Regardless of the light condition

during germination, the increase of the germination performance was significant ($p<0.05$) only in case of the lentil sample subjected to pulsed light treatment at fluence of of 43.2 J/cm^2, when the germination power reached 94% (samples L3 and L4 in Figure 1a).

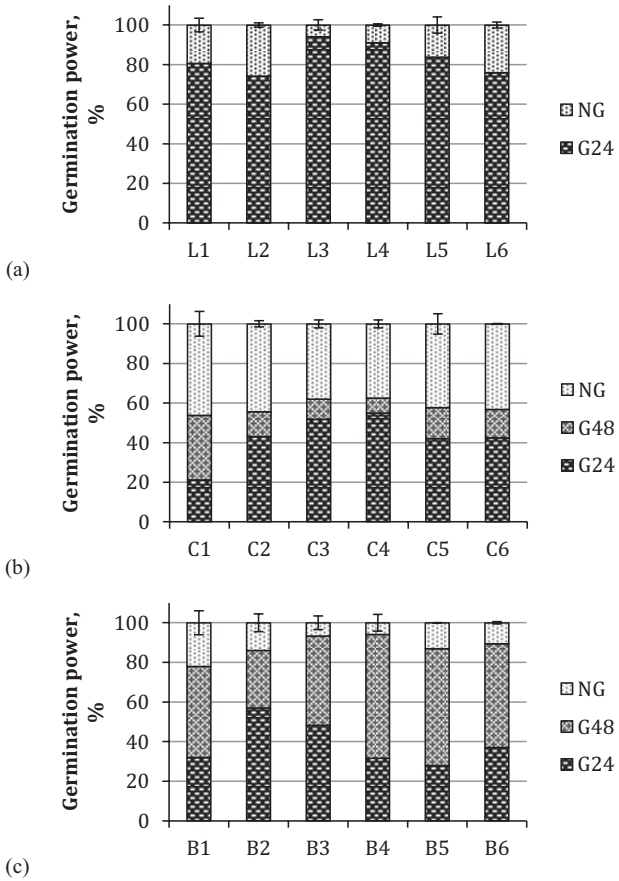

Figure 1. Germination performance of lentil (a), chickpea (b) and broad bean (c) at different light conditions.
NG - non-germinated seeds, G24 – percent of seeds germinated after 24 h, G48 – percent of seeds germinated after 48 h

The proximal chemical composition of the lentil, chickpea and broad bean samples germinated under different light conditions after eventual pulsed light treatment is presented in Table 2. The highest protein contents were determined for broad bean samples, whereas lentil and chickpea had similar protein contents. When compared to the controls, one can see that germination ensured the significant increase of the protein content in case of all studied samples ($p<0.05$). Regardless of the light conditions during lentil and chickpea germination, the most significant increase of the protein content was observed for the samples treated with light pulses of 43.2 J/cm^2 fluence. The protein content increase during germination was higher in case of lentils (from 21.25±0.09 g/100g DM corresponding to L_0, up to 26.74±0.28 g/100g DM corresponding to L_4) compared to chickpeas (from 21.16±0.35 g/100g DM corresponding to C_0, up to 24.73±0.09 g/100 g DM corresponding to C_3). On the other hand, germination without prior pulsed light treatment appeared to be more efficient in terms of increasing the protein content of the broad beans. Germination carried out under daylight conditions (12/24 h) resulted in the protein content increase from 29.94±0.41 g/100 g DM (B_0) to 36.21±0.21 g/100 g DM (B_2). Our results are in agreement with Yu-Wei

and Wang (2015) who reported significant increase of the protein content from 25.18% to 28.56% when germinating the broad beans. The increase of the protein content is the result of synthesis of cell constituents and enzymes, on the account of degrading other constituents of the cells (Yu-Wei and Wang, 2015; Lee and Karunanithy, 1990). Moreover an improvement of the protein quality was reported in case of germinating *Glycine* and *Phaseolus* beans; the total essential amino acids increased by 76% and 52%, respectively (Lee and Karunanithy, 1990).

Table 2. Effect of germination under different conditions on the proximate composition of lentils, chickpeas and broad beans

Sample	Moisture g/100g	Ash g/100g DM	Proteins, g/100g DM	Fibers, g/100g DM
L_0	13.03±0.01	3.03±0.06	21.25±0.09	4.85±0.22
L_1	7.15±0.04	2.86±0.12	22.28±0.02	3.91±0.12
L_2	8.43±0.05	3.00±0.07	24.70±0.01	4.37±0.01
L_3	7.41±0.08	2.97±0.08	25.47±0.40	4.00±0.00
L_4	6.21±0.13	2.93±0.07	26.74±0.28	4.46±0.30
L_5	6.52±0.35	2.80±0.25	25.96±0.62	3.04±0.20
L_6	6.32±0.18	3.02±0.19	24.54±0.44	3.85±0.40
C_0	8.60±0.01	2.70±0.01	21.16±0.35	1.28±0.17
C_1	7.89±0.04	2.69±0.02	22.66±0.05	0.90±0.11
C_2	7.49±0.05	2.69±0.08	22.93±0.35	0.86±0.03
C_3	7.26±0.05	2.73±0.06	24.73±0.09	0.89±0.45
C_4	5.67±0.25	2.68±0.12	24.54±0.00	0.64±0.18
C_5	6.22±0.00	2.62±0.01	23.96±0.82	1.87±0.50
C_6	6.73±0.17	2.62±0.10	24.21±0.43	1.19±0.00
B_0	8.14±0.17	4.17±0.20	29.94±0.41	1.78±0.06
B_1	6.15±0.15	4.09±0.61	35.83±0.64	2.06±0.51
B_2	6.69±0.22	4.30±0.40	36.21±0.21	2.44±0.16
B_3	5.89±0.15	4.35±0.48	33.69±0.20	1.96±0.23
B_4	8.74±0.18	4.56±0.19	34.84±0.16	1.45±0.22
B_5	6.60±0.00	4.31±0.38	32.47±0.21	1.84±0.09
B_6	6.73±0.15	4.51±0.07	33.32±0.23	1.98±0.19

Results represent mean values of two replicates ± standard deviations

Among the conventional techniques used for food processing, germination is one the few processes that ensures significant increase in nutritional value by increasing the bioavailability of nutrients, vitamins, biominerals and other biologically active substances. Studies conducted by Ghavidel and Prakash (2007) on lentils, chickpeas, black eye beans and Indian green beans highlighted the fact that germination leads to the reduction of lipid content due to the use as energy source, for supporting specific cellular processes, and the increase of protein and thiamine contents through biosynthesis.

The ash content of the investigated legumes was not affected by germination (Table 2). Regardless of the preliminary treatment and light conditions during germination, only the broad beans showed insignificant increase of the ash content. Our results are in agreement with the observations of Yu-Wei and Wang (2015) on the ash content evolution during germination of different pulses and legumes. Anyway, other studies indicated the reduction of the mineral content in the germinated seeds, mainly as a consequence of solubilization and loss of these compounds in the soaking step (Lee and Karunanithy, 1990; Ghavidel and

Prakash, 2007). A number of antinutritive factors that exist in the raw materials diminish or even disappear during germination, allowing for a more efficient biological utilization of the nutrients. The increase of the bioavailability of iron and calcium from legumes during germination was reported by Ghavidel and Prakash (2007), as well as the reversed relationship with tannins, fibers and phytic acid contents.

The dietary fibre content varied with the investigated sample, pulsed light treatment and presence of light during germination (Table 2). Lentils had the highest amount of fibers (4.85±0.22 g/100 g DM), followed by broad beans (1.78±0.06 g/100 g DM) and chickpeas (1.28±0.17 g/100 g DM). Legumes germination resulted in the overall decrease of the dietary fiber content, except for the broad beans germinated under light without prior pulsed light treatment (Table 2). Concerning the fate of the fibers during legumes germination, Ghavidel and Prakash (2007) reported the increase of the total and soluble fiber contents, and significant reduction of the insoluble fiber fraction. Previous studies report on the significant variation of the dietary fiber fractions in different legumes with the germination conditions (Martín-Cabrejas et al., 2003).

Influence of light and pulsed light treatment on the antioxidant properties of germinated pulses

Germination is one of the cheapest processes which is effective in improving the profile of the biologically active compounds, and the bioavailability of the nutrient components in the seeds (Cáceres et al., 2014; Gan et al., 2017), thus allowing to obtain products with high nutritional value. Germination is therefore an alternative to controlled food fortification by the addition of nutritive compounds obtained through chemical methods.

The effect of different environmental conditions during the legume germination process on the synthesis of antioxidant compounds has been estimated by determining the antiradical activity (DPPH RSA) and the amount of total phenols. The results obtained for lentil, chickpea and broad bean samples are shown in Figures 2, 3 and 4.

The antioxidant properties of the lentil samples varied with the pulsed light treatment and presence of light during germination (Figure 2).

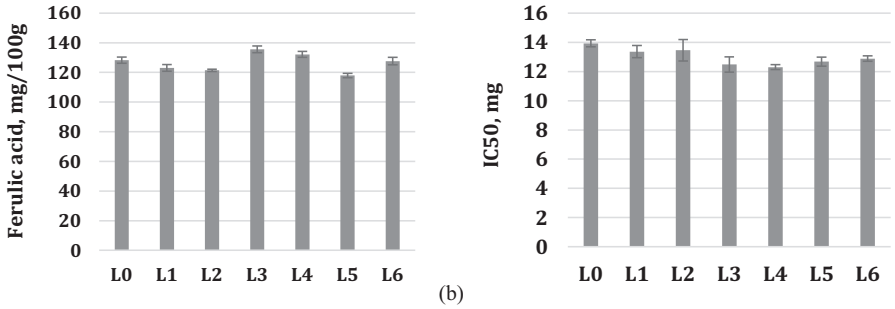

Figure 2. Effect of germination under different conditions on the total phenolic content (a) and antioxidant activity (b) of lentils

The highest total phenolic content and the lowest IC50 value were recorded for the L3 sample, treated with light pulses with fluency of 43.2 J/cm^2 and germinated in the dark. On the other hand, lentils treatment with pulsed light of lower fluence (19.2 J/cm^2) caused the most important decrease of the antioxidant properties when germination was carried out in the dark (L5 in Figure 2).

Regardless of the pulsed light treatment and light conditions, germination had a positive effect on the antioxidant capacity of chickpeas ($p<0.05$). As in case of lentil sample, the highest antioxidant properties (high content of phenolic compounds and low IC50 value) were recorded for the chickpea sample germinated in the dark, after treatment through pulsed light with fluency of 43.2 J/cm^2(C3 in Figure 3).

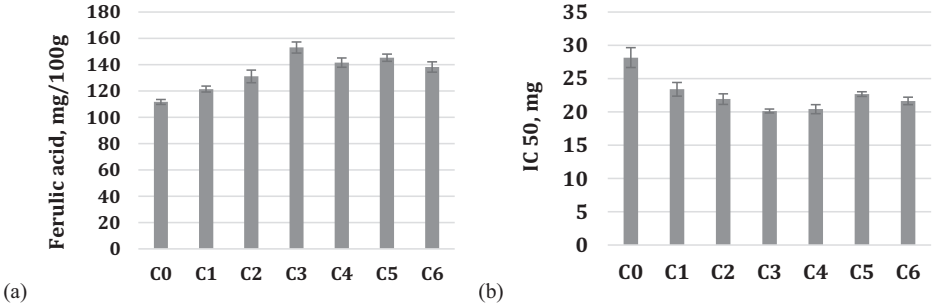

Figure 3. Effect of germination under different conditions
on the total phenolic content (a) and antioxidant activity (b) of chickpeas

Regarding the influence of the light/dark regime during germination, one can see in Figure 3b that chickpea samples germinated in the presence of natural light (C2) had slightly higher antioxidant capacity compared to the sample germinated under dark (C1). Anyway, no significant differences were observed between IC50 values of C1 and C2 ($p>0.05$). On the other hand, samples treated with pulsed light, for both fluence values considered in the experiment, developed higher contents of total phenols in the course of germination under dark (C3 and C5) compared to the daylight conditions (C4 and C6).

In case of broad beans, germination resulted in significant increase of the total phenols content for all studied variants (Figure 4a). The total phenolic content was not significantly influenced by the dark/light regime during germination ($p>0.05$), but rather by the prior treatment through pulsed light ($p<0.05$). The highest value of the total phenols content was recorded for B4 sample, treated with pulsed light with fluency of 43.2 J/cm^2.

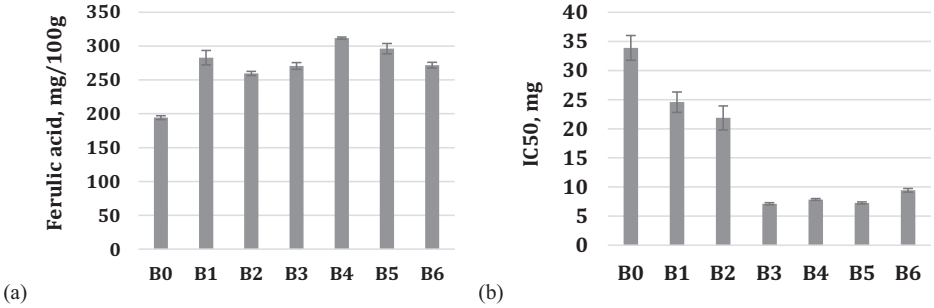

Figure 4. Effect of germination under different conditions
on the total phenolic content (a) and antioxidant activity (b) of broad beans

Among the three investigated pulses, the most significant increase of the antiradical activity, when compared to the control sample, was obtained in case of the germinated broad beans ($p<0.05$). The greatest impact on antiradical capacity, i.e. the minimum value of IC50, was recorded for the broad bean sample treated with light pulses with fluency of 43.2 J/cm^2 and germinated under dark conditions (B3). However, when considering the light/dark regime during the germination process, no significant influence on the antioxidant activity of the broad bean samples ($p>0.05$) was observed (Figure 4b). The favorable effect of germination on the antioxidant capacity of legumes was previously reported by Duenas et al. (2009) and Gharachorloo et al. (2013).

The phytochemical profile of the seeds of different origins is significantly improved by germination. Considering the involvement of free radicals in the occurrence and progress of different diseases, particular attention was paid

to studying the influence of germination on the antioxidant pool. Intensification of particular metabolic processes during germination results in the accumulation of various compounds that form redox systems including B vitamins in their structure. Various seeds may have very low or even non-detectable amounts of vitamin C, but this compound may accumulate significantly during germination by *de novo* synthesis (Gan et al., 2017). The accumulation of tocopherols, the presence of superoxide dismutase and the increased catalase activity contribute to the antioxidant potential of germinated seeds. Moreover, the increased amounts of free SH groups have also been reported to play important role in metabolic mechanisms. The most important biological functions of phytochemical compounds in the germinated seeds are antioxidant, anti-inflammatory, antidiabetic, antibacterial and antitumor effects (Hayat et al., 2014; Gan et al., 2017). Many of these effects of germinated seeds have been associated with the accumulation of biologically active compounds such as polyphenols, leading to the gradual orientation of nutritionists towards the widespread use in the diets of germinated cereals and legumes. Moreover, this trend complies with the traditional medicine recommendations on the great importance of germinated seeds for human health.

CONCLUSIONS

The results obtained in the present work indicate that the composition and the antioxidant properties of green lentils, chickpeas and broad beans can be modulated through germination under different conditions. Germination efficiency was not significantly affected by the dark or light regime. All germinated pulses accumulated higher amounts of proteins compared to the raw materials. The most significant increase of the antioxidant capacity of the germinated pulses was observed in case of the samples preliminary treated through pulsed light at high fluence value of 43.2 J/cm^2, followed by germination underdarkness in case of chickpeas and broad bens, or under daylight conditions in case of lentils.

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