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ORIGINAL RESEARCH PAPER

THE INFLUENCE OF LIGHT WAVELENGTH ON THE GERMINATION PERFORMANCE OF LEGUMES

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Light quality is a very important factor affecting plant photosynthesis and growth. The use of light-emitting diode (LED) technology for growth stimulation and crops yield increase became very interesting in the last years. The present study aimed to determine the effect of light exposure on germination power and antioxidant activity of lentil, chickpea and broad bean legumes. Soaked legume seeds were germinated under e4xposure of four monochromatic wavelengths of 405, 500, 700 and 780 nm, respectively provided by batteries of 10 LEDs. Germination process resulted to be favorable to legumes biological value enhancement. LED lighting was the most efficient in lentil germination, with the violet light at 405 nm and red light at 700 nm presenting the best results. The present study showed that not only small seeds can benefit from light exposure (at specific wavelengths), as broad bean germination under light also presented a significant improvement of total protein content and antioxidant activity.

Keywords: monochromatic light, germination, legumes, antioxidant activity

Introduction

Legumes are important raw materials due to the high content of proteins, carbohydrates, dietary fibre, minerals, and vitamins (Liu et al., 2016). Germinated legumes are also an important source of secondary metabolites. Many studies showed that germination is an inexpensive and effective method for increasing the nutritive and nutraceutical quality of legumes (Liu et al., 2013; Świeca and Gawlik-Dziki, 2015; Liu et al., 2016).

The quality of the germinated legumes mainly depends on the quality of the seeds and the germination conditions. Light is one of the essential environmental factors that influence plant growth. It was observed that different light qualities presented distinct effects on bioactive compounds content (Jin et al., 2015; Liu et al., 2015; Deng et al., 2017). In particular, exposure to different light-emitting diodes (LED) wavelengths can induce the synthesis of bioactive compounds and antioxidants,

which in turn can improve the nutritional quality of crops. Similarly, LEDs increase the nutrient contents, reduce microbial contamination, and alter the ripening of postharvest fruits and vegetables (Hasan et al., 2017). Compared to conventional lighting sources, LEDs showed several advantages, such as high energy conversion efficiency, longer life and controllable emission spectrum, along with low thermal energy output and cold emitting temperature (Massa et al., 2008; Liu et al., 2016; Son and Oh, 2015; Thwe et al., 2014).

Plants have multiple photoreception systems which respond different to light quality, thus determining morphogenetic changes in the photosynthesis pathways, and in the metabolic reactions. Two major photoreceptors, phytochromes (absorbs red/far-red-light) and cryptochromes (absorbs blue/ultraviolet A (UV-A) light), are responsible for plant morphological and developmental changes (Hasan et al., 2017). The importance of phytochromes (the red/far-red-light receptors) in mediation of seeds germination has been previously investigated. The blue and green lights are less studied, although there is some evidence that plant photoreceptors can perceive blue and green lights (Goggin and Steadman, 2012). Moreover, it was reported that, together with red light, the blue light is very important to plant growth, being involved in photosynthesis and CO_2 assimilation (Lee et al., 2014). The importance of light from the blue region of visible spectrum (400-500 nm) in photomorphogenesis, like stomatal control which affects water relations and CO_2 exchange, stem elongation, and phototropism was also reported (Massa et al., 2008).

In order for light to be perceived by plant tissues, one of the major conditions was reported to be the presence of water. The mature dry seeds perceive light inefficiently in comparison to the hydrated ones, because photoconversion of phytochrome is inhibited in dehydrated tissues of plant (Goggin and Steadman, 2012; Sineshchekov, 2006). It is likely that these photoreceptors are unable to function in the absence of free water (Goggin and Steadman, 2012). The light reaching the photoreceptors depends upon the presence and type of light-filtering pigments present in the seed coats, such as anthocyanins, condensed tannins, carotenoids, and chlorophyll (Hendricks et al., 1968; Goggin and Steadman, 2012). Light was reported to stimulate the pigment concentration by its effects on the regulatory and structural genes of anthocyanin biosynthesis (Seo et al., 2015).

To the best of our knowledge the available scientific literature is mostly focused on the effects of light quality on plant growth and crops yield, while its influence on the synthesis of functional components in germinated legumes was less studied. Thus, the objective of the present study was to evaluate the effect of different monochromatic LED lighting on the germination efficiency and nutritional functionality of germinated green lentil, broad bean and chickpea legumes.

Materials and methods

Legumes germination

Green lentils (*Lens culinaris*), broad beans (*Vicia faba*), and chickpeas (*Cicer arietinum*) were purchased from a local market (Galati, Romania).

Prior to germination, legumes were rinsed with tap water, sanitized by soaking for 15 min with aqueous ethanol solution (70%), and finally rinsed with tap water again. Legumes were then subjected to swelling in tap water under dark conditions, for 7 to 24 h depending on seeds dimensions, adequate to assure the needed humidity for germination. Preliminary tests were performed for each investigated legume type, to establish the optimum swelling time (data not shown), as follows: 24 hours for broad beans, 12 hours for chickpeas, and 7h for green lentils. After soaking, legumes were distributed in aluminium containers (20×10×5cm) and covered with opaque lids provided with small orifices for LEDs. Standard light emitting diodes (LED) were purchased from ROITHNER LASERTECHNIK GmbH, Vienna, Austria. The effect of the four different wavelengths was studied. For each wavelength, 10 lighting units of single monochromatic light were used as follows: violet LEDs (405 nm, 15 mW/20 mA), green LEDs (500 nm, 10 mW/20 mA), red LEDs (700 nm, 10 mW/50 mA) and infrared LEDs (780 nm, 16 mW/50 mA). Seeds germinated under dark conditions were used as controls. The samples were coded as shown in Table 1.

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

Sample code	Applied treatment
$\mathbf{L_0\ B_0\ C_0}$	Native, untreated legumes
$\mathbf{L_D\ B_D\ C_D}$	Samples germinated in darkness
$\mathbf{L_{405}\ B_{405}\ C_{405}}$	Samples germinated in violet light at a wavelength of 405 nm
$\mathbf{L_{500}\ B_{500}\ C_{500}}$	Samples germinated in green light at a wavelength of 500 nm
$\mathbf{L_{700}\ B_{700}\ C_{700}}$	Samples germinated in red light at a wavelength of 700 nm
$\mathbf{L_{780}\ B_{780}\ C_{780}}$	Samples germinated in infrared light at a wavelength of 780 nm

The germination time varied from 24 to 48 h, depending on the size of the seeds. The germinated seeds were counted after each 24 h, and when necessary were further allowed to germinate. Seeds were considered germinated when the radicle broke through the seed coat (Bewley and Black, 1994). The germination process was conducted at 23±2°C under highly humidified conditions (RH > 75%).

At the end of the germination process, germinated seeds were counted to estimate the germination power. Samples were afterwards dried at 55°C for 24 to 30 h in a convection oven (LabTech LDO-030E, Daihan LabTech Co., LTD, Kyonggi-Do, Koreea). Germinated broad beans and chickpeas were dehulled prior to drying. The dried native and germinated legumes were finally ground into flours with particles size lower than 500 μm, using a laboratory mill (WZ-2, Sadkiewicz Instruments, Bydgoszcz, Poland). For assuring samples uniformity in terms of applied treatment and for appropriate comparison of the results, native unprocessed legumes were subjected to swelling and dehulling as well.

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 ash content using SR ISO 2171: 2002 Method (ASRO, 2008); the protein content through the semimicro-Kjeldahl method (Raypa Trade, R Espinar, SL, Barcelona, Spain) using the nitrogen conversion factor of 6.00; and the soluble proteins were determined using the Lowry method described by Patrascu et al. (2011).

Antioxidant activity determination

Extraction

The studied flours were subjected to extraction with 80% methanol solution, under mild stirring conditions (250 rpm) 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 assaying the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity and total phenolics content.

DPPH radical scavenging ability

The overall antioxidant activity of studied legumes was determined with the DPPH method. The DPPH radical scavenging activity was expressed as IC50 values, representing 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. In short, 0.1 mL of extract was mixed with 3.9 mL of 6×10^{-5} M DPPH solution in methanol. The reaction was allowed to take place in the dark for 30 min, and the absorbance at 515 nm was recorded to quantify the remaining DPPH. The maximum DPPH absorbance of the control was determined by using the extraction solvent instead of sample extract.

Total phenolics 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 admixture 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 (FAE)/g of sample.

Total flavonoids content

The flavonoid compounds were extracted in ethanolic solution (0.5 g flours with 9.5 ml ethanol 70%). The total flavonoids content was further determined using a colorimetric method with aluminum chloride (Florea et al., 2009). A volume of 1 ml extract solution was added to 1 ml of 2.5% aluminum chloride solution in distilled water and was diluted to 10 ml with 70% ethanol. After allowing the compounds to react for 30 min, the absorbance was measured at 420 nm. Quercetin was used as reference (10-50µg/mL), and the results were reported as quercetin equivalents (QE) per 1 g of sample.

Statistical analysis

Two independent germination experiments were conducted and all measurements were performed in duplicate. Statistical analysis was performed using Microsoft

Excel Software. The results were reported as mean values together with standard deviations.

Results and discussion

Effect of LEDs on the germination power of legumes

The effect of various monochromatic light exposure on the germination power of some legumes was quantified by determining the percentage of germinated seeds (Figure 1). In case of lentils the germination time 24 h (Figure 1a), while broad beans and chickpeas were allowed to germinate for 48 h. The three studied legumes behaved differently when subjected to the same germination conditions. Thus, a significant increase in germinated seeds was observed for lentils treated under exposures at 405, 700 and 780 nm wavelengths ($p<0.05$) compared to samples germinated under dark conditions. As can be seen from Figure 1a, the most efficient treatment was at 405 nm with 93.65% of germinated seeds, which is by 16% higher in respect to the control. On the other hand, the lowest efficiency was registered for lentil seeds germinated at 500 nm (germination power of 86.83%). However, no significant statistical differences in terms of germination power values were observed when comparing the all lentil samples subjected to monochromatic light exposure ($p>0.05$). Lee et al. (2014) showed that blue and red lights from visible spectrum are the most useful for the photosynthesis. The presence of both red and far red lights was reported as very important for plant growth, with lower red to far red ratios (<3.1) having a positive effect on the *in vitro* flowering of lentils (Mobini et al., 2016).

Similar to lentils, broad beans exposure at 405nm monochromatic light during germination resulted in the highest germination efficiency of 95.56% (Figure 1b), while red light did not affect the process ($p>0.05$) when compared to control sample. Finally, light exposure did not influence the chickpeas germination power ($p>0.05$). Except for the chickpea samples germinated under red light, the germination power of the samples exposed to monochromatic light was slightly lower compared to the control (Figure 1c).

Effect of germination under LEDs on proximal composition of legumes

The proximal composition of the studied legumes subjected to germination under different wavelength exposures is presented in Table 2. The higher moisture content for the control lentil is a consequence of a shorter drying period. Regardless of light presence, germination of all investigated legumes caused the significant increase of the protein content compared to the native seeds ($p<0.05$). This observation complies with the literature, and is most probably 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). The increase of protein content in germinated legumes was also reported by Ghavidel and Prakash (2007). The mechanism of protein biosynthesis during germination was detailed by Bewley and Black (1994). Synthesis of proteins is initiated immediately after appropriate hydration of the seeds, and relies on the information available on the mRNA template associated with proteins in the cells

cytoplasm or nucleus. Regarding the seeds reserves supporting the metabolic pathways specific to germination, Zhao et al. (2018) indicated the high heterogeneity of the biochemical reactions. The processes ensuring the growth of the embryo and radicle protrusion involve different energy reserves varying with the species. Zhao et al. (2018) hypothesized that the major role is usually played by the reserve present in the seeds in the highest amount. Therefore, the changes registered in the present study in the protein content after germination may account on the catabolic reactions involving mainly the starch and fats.

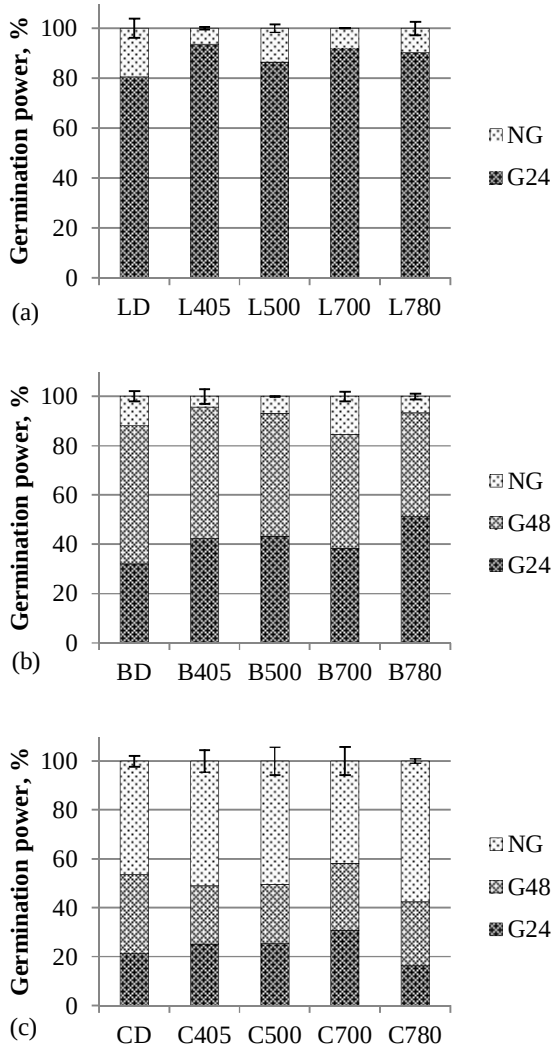

Figure 1. The effect of LEDs treatment on germination performance of lentils (a), broad beans (b) and chickpeas (c). NG – non-germinated seeds, G24 – seeds germinated after 24 h, G24 – germinated after 48 h

For all studied legumes the presence of monochromatic exposure during germination ensured the synthesis of higher protein contents compared to control samples germinated under total darkness ($p<0.05$). In case of lentils, germination under light exposure determined the increase of the protein content by ~12% compared to the seeds germinated in dark (L_{405} and L_{780}), and by ~18% in case of broad bean (B_{500}). Chickpea germination under mentioned conditions did not significantly affect the total protein content when compared to the control samples germinated under dark (N_D).

The mineral content of broad beans and chickpeas was not affected by the germination process ($p>0.05$), while in case of lentils a slight decrease of the mineral content could be seen (Table 2). Other studies also 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).

Table 2. Effect of germination under different LEDs on the proximate composition of lentils (L), broad beans (B) and chickpeas (C)

Sample	Moisture, g/100g	Ash, g/100g DM	Proteins, g/100g DM	Soluble proteins, g/100g protein
L_0	13.03±0.01	3.03±0.06	21.25±0.09	11.88±0.12
L_D	7.15±0.04	2.86±0.12	22.28±0.02	9.74±0.15
L_{405}	3.85±0.19	2.93±0.05	24.91±0.15	8.13±0.00
L_{500}	3.86±0.22	2.75±0.19	25.36±0.04	6.63±0.89
L_{700}	4.94±0.03	2.93±0.12	23.43±0.41	6.87±0.00
L_{780}	3.76±0.04	2.79±0.03	24.93±0.78	7.54±0.32
B_0	8.14±0.17	4.17±0.20	27.44±0.76	9.12±0.03
B_D	6.15±0.15	4.09±0.61	28.14±0.00	8.96±0.19
B_{405}	5.47±0.00	4.31±0.02	31.61±0.23	8.70±0.66
B_{500}	6.24±0.30	4.22±0.06	33.43±0.01	9.64±0.00
B_{700}	4.56±0.17	4.19±0.02	32.63±0.18	9.42±0.71
B_{780}	6.43±0.33	4.40±0.21	31.06±1.11	13.36±0.63
C_0	8.60±0.01	2.70±0.01	21.16±0.35	6.56±0.06
C_D	7.89±0.04	2.69±0.02	22.66±0.05	7.85±0.10
C_{405}	4.14±0.28	2.29±0.01	23.20±0.54	3.55±0.64
C_{500}	4.44±0.06	2.51±0.02	23.65±0.57	3.75±0.58
C_{700}	4.02±0.16	2.40±0.07	23.67±0.10	3.39±0.39
C_{780}	4.41±0.04	2.51±0.01	23.80±0.03	2.78±0.00

Results represent mean values of two replicates ± standard deviations

Effect of germination under LEDs on the antioxidant properties of legumes

The effect of germination under monochromatic light exposure on the overall antioxidant profile of studied legumes was first assessed as the DPPH radical scavenging activity (Figure 2). Generally, germination process was found to be 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). Our results indicated that the presence of light exposure during germination improved the DPPH radical scavenging ability of lentils and broad beans ($p<0.05$), whereas in case of chickpeas no significant differences were obtained compared to the control sample, germinated under dark ($p>0.05$). In particular, wavelength of 405 nm was the most effective in case of lentils, with an IC50 value of 10.9±0.04 mg, closely followed by red wavelength of 700nm (Figure 2a). Other studies also reported that light is effective in improving the antioxidant properties of the sprouted seeds of pea lentil, wheat and radish (Liu et al., 2016; Samuolienė et al., 2011).

The presence of light during germination appeared to have a different effect on the antioxidant activity of broad beans compared to lentils. Most probably, the light related activation mechanism of specific metabolic pathways is linked to the size of the seeds. Thus, in case of broad beans, the long-wave lighting generated a better response. The best results were obtained for broad bean samples germinated in infrared (B_{780}, Figure 2b). However, the IC50 values obtained for the broad bean samples germinated under LEDs with wavelengths of 780, 700 and 500 nm were fairly close.

Regarding the total phenolic content, a group of compounds known to have a great contribution to the antioxidant activity of the vegetable products, no significant correlation with the DPPH radical scavenging ability of the samples could be established. Analyzing the results presented in Figure 3, one can see that germination process caused the significant increase of the total phenolics content of all studied legumes ($p<0.05$). However, although significantly different among them ($p<0.05$), values obtained for the total phenolic content of germinated legumes did not resulted to be always improved by the germination under light exposure conditions. In case of broad beans and chickpeas, because of the large size of the seeds, the light should overpass more barrier coating and pigments to activate the photoreceptors (Goggin and Steadman, 2012; Hendricks et al., 1968). Light is essential for both plant growth and activating the mechanisms that lead to the improvement of their polyphenolic and antioxidant profile.

The highest increase of the total phenolic content was obtained for lentils (Figure 3a), the most efficient germination treatment relying on the use of light at 405 and 700 nm wavelengths. In this respect, some studies indicate that the optimum light is in the blue and red light ranges (Lee et al., 2014; Cosgrove, 1981; Kasajima et al., 2008). Moreover, Lee et al. (2014) reported increased total phenolic contents of Tartary buckwheat sprouts as a result of the exposure to the blue and red exposure. The total phenolic content values obtained for lentils germinated under green and infrared light resulted to be similar to the native seeds ($p>0.05$).

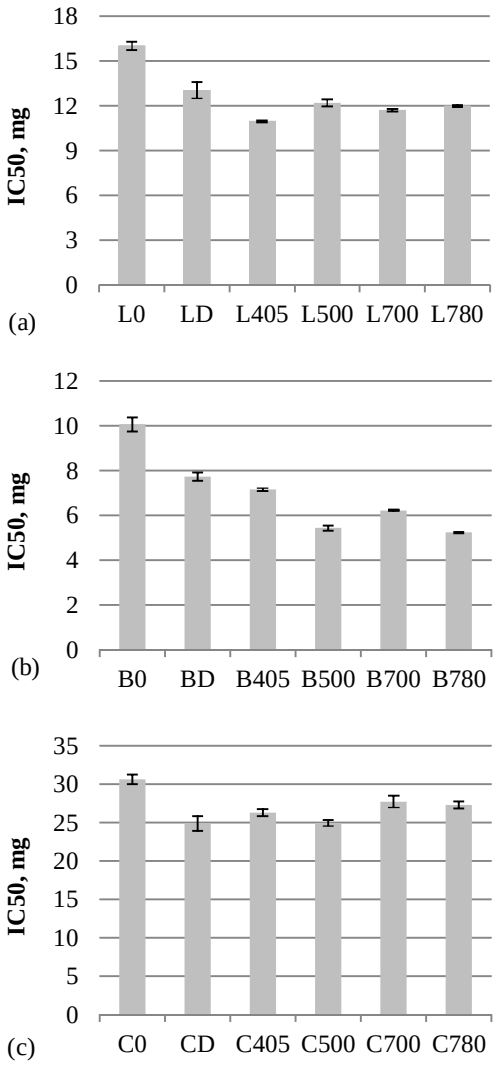

Figure 2. Effect of germination under different LED conditions on the DPPH scavenging activity of lentils (a), broad beans (b) and chickpeas (c)

It is known that flavonoids play important roles in the plant UV protection, and defense against insects and microbes attacks. Beggs and Wellmann (1994) reported that the biosynthesis of flavonoids in plants is highly dependent on phtyochrome mediated reactions as well as B and UV-B photoreceptors, although in case of legumes the DNA itself was stated to act as a photoreceptor for flavonoid synthesis stimulation, especially isoflavonoids.

Total flavonoid content as a trigger of antioxidant activity in studied legumes was also determined and presented in Figure 4. Light exposure during germination

resulted in increased values of total flavonoids for all three studied legumes ($p<0.05$). In agreement with our results, Kim et al. (2007) and Lee et al. (2014) reported significant increase of the flavonoid content of Tartary buckwheat sprouts treated with different wavelengths. All four wavelengths used for legumes treatment in our study caused the significant increase of the flavonoids content in germinated lentil samples ($p<0.05$). In case of broad beans and chickpeas, significant higher flavonoid contents were obtained only for samples exposed to wavelength of 780nm (Figure 4b and c).

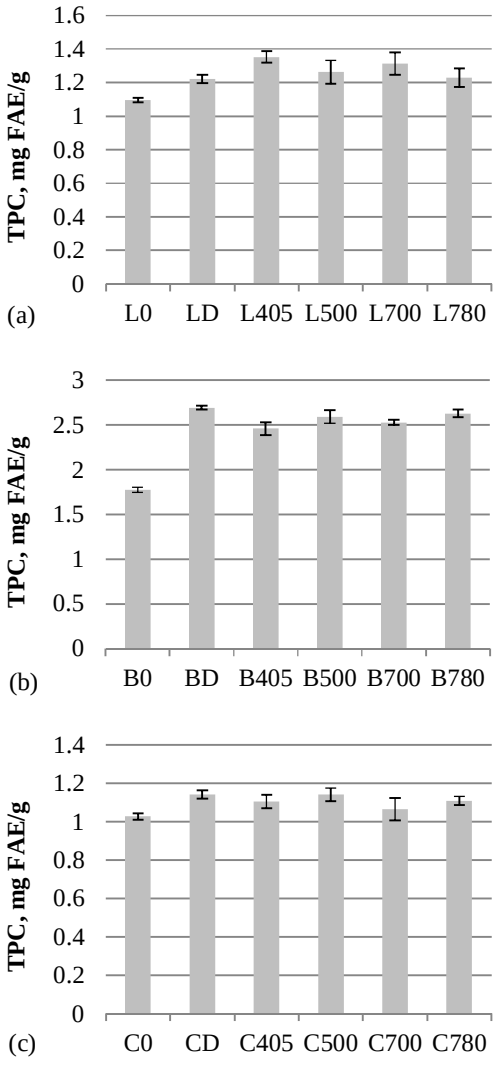

Figure 3. Effect of germination under different LED lighting on the total phenolics content of lentils (a), broad beans (b) and chickpeas (c)

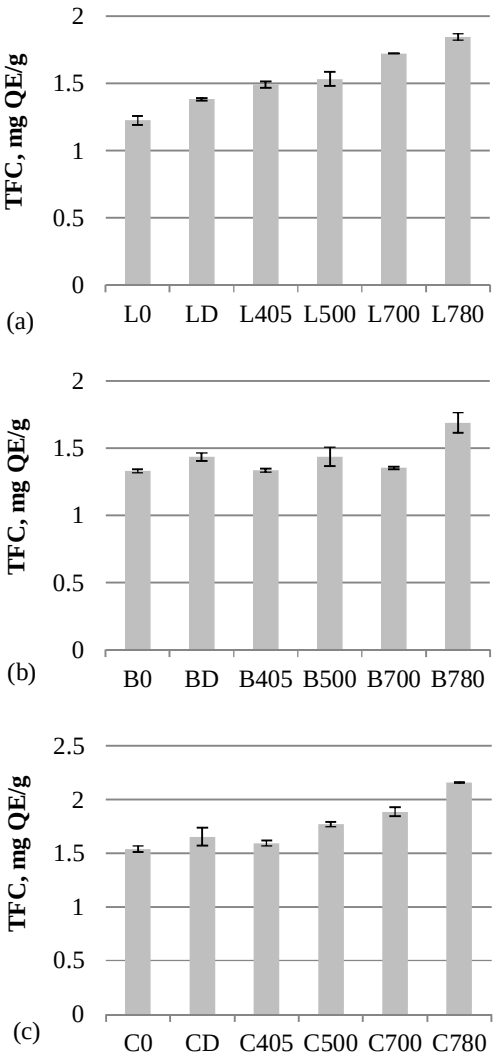

Figure 4. Effect of germination under different wavelengths on the total flavonoids content of lentils (a), broad beans (b) and chickpeas (c).

Conclusions

The present study followed the effects of light exposure during germination on both small seed and big seed legumes. In case of lentils, the most effective treatment in terms of germination power and biosynthesis of biological active compounds was based on the use of monochromatic light of 405 nm wavelength, while for broad beans wavelength of 780 nm ensured the best results. Poor results in terms of total protein content and antioxidant activity obtained for germinated chickpeas are more likely to be attributed to its low germination power than to the

effect of light exposure. Further studies are to be conducted in order to provide a more complete picture on the effect of legumes exposure during germination to lights of different wavelengths on their nutritional value.

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