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Effects of UV-C light and *Spirulina maxima* seed conditioning on the germination and the physical and nutraceutical properties of lentils (*Lens culinaris*)

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A b s t r a c t. The aim of this research was to evaluate the effects of UV-C light on lentil (*Lens culinaris*) and its conditioning by *Spirulina*. The main findings were: (i) Lentil brightness presented a significant slight variation (9%) when compared to the control (T_0) and UV-C (T_{10}=10 min) irradiated lentil samples. (ii) The total flavonoids tended to increase by 17% at 10 min (49.18 μg mL^{-1}) compared to T_0 (42.07 μg mL^{-1}). (iii) The conditioning of lentils with UV-C (0, 5, and 10 min) and the imbibition in water with *Spirulina* (0, 0.5, 0.75, and 1.5%) generated significant statistical differences ($p \leq 0.05$) in the seedlings. The priming cyanobacteria *Spirulina* improved the physiological quality against damage caused by UV-C radiation. (iv) Morphological changes occurred in the lentils due to radiation, damage in the testa (protective layer on the outside) area (row 1) due to the application of UV-C was found, which increases with higher exposure to radiation. Through the application of UV-C for 10 min the cell wall and protein body were damaged. However, no damage to the starch is visible. (v) FT-IR indicates that the UV-C radiation did not induce any change in the chemical structure of the starch but, decreases in intensity within the range of 3 000-3 600 cm^{-1} indicated differences in their water content, while those between 1 600-1 700 cm^{-1} were attributed to the reorganization of the secondary structure of proteins.

K e y w o r d s: physical methods, UV-C, seed conditioning, degradation, *Spirulina maxima*

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INTRODUCTION

Seed priming using biophysical methods such as lasers, constant and alternating magnetic fields, plasma, vacuum application, sound waves, microwaves, ultrasound, ozone, and ultraviolet radiation have been found to produce diverse bioeffects. Studies using various crops have confirmed, for example, the stimulating effect of bioactive compounds in pre-sowing seeds and in seedlings or plants during their development (Bera *et al*., 2022). Both ionizing and non-ionizing physical methods are promising techniques to use in the seed priming stage in order to improve seed quality parameters (sanitary, physiological, nutritional, genetic), reduce agrochemical use during cultivation and increase the tolerance to stress in agricultural crops (Romero *et al.*, 2021). However, to fulfil a specific purpose or priority, it is necessary to find and optimize the radiation parameters that are used.

Among the different physical methods applied, ultraviolet light which was discovered by Ritter (photon energy: 3.3-2 10^2 eV) and whose components are contained in sunlight; they are classified as UV-A (320-400 nm), UV-B

(280-320 nm) and UV-C (200-280 nm) has proven its effectiveness, although mainly as a germicide (Bera *et al*., 2022). Currently, antimicrobial applications continue to be demonstrated in the food industry, in postharvest products and in agricultural seeds, eliminating or reducing micro-organisms such as bacteria, viruses (including SARS-CoV-2), and fungi (Debnath, 2020). Different artificial sources of UV-C have been used in agriculture and in food product treatment, such as mercury vapour lamps, Xenon lamps and LEDs among other light emitting sources. The applied UV-C light dose may be defined as I x t (J m^{-2}): power density I (W m^{-2}) x exposure time t (s) (Urban *et al*., 2016).

In addition to the aforementioned effect, knowledge regarding its stimulating effect has also been generated. In food, its effect on modifying secondary metabolites has been discovered in several studies. Liu *et al.* (2018) studied the possibility of increasing the flavonoid content of food by using UV-C. They irradiated ripe green tomato fruits (*Solanum lycopersicum*, cv. Wanza 15) with 4 kJ m^{-2} using UV-C lamp tubes (30 W and a spectral peak at 254 nm) stored in the dark and found that UV-C light was effective in increasing flavonoid content. However, an inhibitory effect produced by secondary metabolites was also found in UV-C treated lettuce, peanuts, and curcuma-enriched bread (Hernández *et al*., 2022a). The effect on germination and on the accumulation of secondary metabolites has been evaluated in agricultural seeds. Guajardo-Flores *et al*. (2014) treated black bean (*Phaseolus vulgaris* L.) seeds with UV-C radiation (using 30 W lamps) over a period of 5, 10, 15 and 20 h. Overall, the authors found that UV-C induced stress not only reduced the seed germination time, but also increased the production of secondary metabolites (flavonols, and saponins) from seed coat extracts. The potential of UV-C light to activate secondary metabolites, mainly phenolic compounds, has allowed it to be envisioned as a type of treatment that stimulates the plant's defence system (Urban *et al*., 2016). The secondary metabolites play a role in benefiting human health, but their role in plant defence and physiological performance are being explored on a continuous basis (Poiroux-Gonord *et al*., 2010). Other authors such as Huché-Thélier *et al.* (2016) have noted that the formation of phenolic compounds is directly proportional to UV radiation resistance. The flavonoids, play a key role in protecting plants against photo-deterioration through their antioxidant capacity (Park and Kim, 2015). However, in the case of seeds, as is the case with food and postharvest products, these adverse effects can also be reduced depending on the applied light parameters and their radiation regimes as well as on the characteristics of the biological objects. In bean seeds (*Phaseolus vulgaris*) it has been reported that UV-C decreases the level of phenolic acids and some flavonoids with increasing UV-C radiation time (Hernández *et al*., 2022a) without significantly modifying seed germination. Hence the importance of continuing to explore the effects of UV-C on seed conditioning and its possible degradation of the seed surface. It is also important to continue to apply hybrid treatment methods, which may be a physical method used in conjunction with a biological method. Therefore, in this research, another type of biostimulant such as *Spirulina*, which is known as a type of cyanobacteria, and has been shown to promote seedling growth through its biofertilization and phytostimulant effect (Mógor *et al.*, 2018). In some seeds, the importance of cyanobacteria in their conditioning has been highlighted for its ability to improve their vigour, while other authors have also reported that it has a protective effect against various plant stressors.

With regard to agricultural grains and legume seeds it is necessary to explore further and generate knowledge that can be applied to real-world agricultural problems in order to improve crop establishment at different phenological stages, such as germination. For both methods (UV-C and *Spirulina*) the hormetic doses *i.e.* doses with beneficial effects and correct concentrations in the case of *Spirulina* should be investigated, since like other methods they could also present inhibitory and damaging effects. In this context, the objective has been to evaluate the quantitative effects of UV-C light on lentil (*Lens culinaris* Medik) grains in terms of colour, bioactive compounds (total flavonoids and saponins), germination (treated with UV-C and conditioned with cyanobacteria (S*pirulina*), morphological structure and the spectra of lentils treated with UV-C as determined using scanning electron microscopy and infrared spectroscopy.

The lentils are legumes with a high nutritional value, bioactive components, antioxidants, and other phytochemicals that confer pro-health properties and are considered a nutraceutical food, mainly in their sprouted form (Hernández *et al*., 2020). Therefore, enhancing their production as well as their bioactive properties is of great interest for the cause of improving food security and the health of people in developing countries.

MATERIALS AND METHODS

This study was carried out at ESIME-Zacatenco, Mexico City, at an altitude of 2240 m a.s.l., 19.42° North Latitude and 99.12° West Longitude. The plant material used was lentils (*Lens culinaris* Medik.) purchased in Mexico City (Verde Valle ™). In terms of physical dimensions, they had an average weight of 100 g, and the shape, density, and roundness index may be observed in Table 1. S*pirulina* powder was also used as a biostimulatory ingredient in the germination test, it was purchased in Mexico City (Pronat-ultra ™). According to the manufacturer the nutritional information per 100 g is protein = 59 g, fat = 5 g, chlorophyll = 720 mg, sodium = 1549 mg, thiamine and riboflavin = 3 mg, and biotin = 28 mg.

The lentils were treated before planting with a UV-C radiator system (UV-C/RS-Homemade) according to Hernández *et al*. (2020). The system consisted of an array

Table 1. Characteristics of lentil (*Lens culinaris* Medik.)

Brand	Price	Place of purchase (area)	Diameter (mm)		Index of roundness	Lentil shape	Thickness (mm)	Weight 1000 grains (g)	Volume (cm^3)	Density w/v ($g\ cm^{-3}$)
			Polar	Equatorial						
"Verde valle"	$30	Mexico city	5.85	5.75	1.03	Round	2.875	29.8	12.37	0.2407

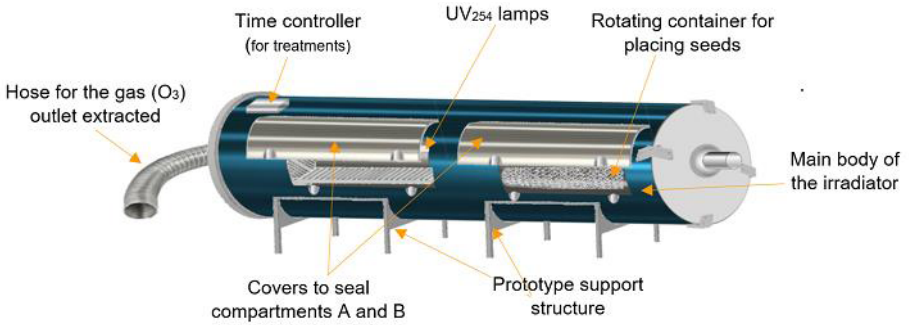


Fig. 1. UV-C radiator system used to irradiate lentil seed in June-July 2021 (Hernández *et al.*, 2022a).

of 4 lamps (UV-C, 254 nm) arranged on the top and bottom of a cylindrical stainless-steel base. When switched on, the lamps emit light into a fixed metal mesh container. The lentil grains were placed in this container (Fig. 1). Various radiation times were applied with durations of 0, 5, and 10 min, they were programmed using a timer. The intensity of the light was measured using Lutron measuring equipment, model UV-C/254 produced a light intensity of 700 W cm^{-2}. The lentil moisture level was evaluated according to AOAC method 925.10 (AOAC, 1990).

The colour of the different lentil treatments was determined using a pre-calibrated handheld colorimeter (FRU WR-10QC, Shenzhen Wave Optoelectronics Technology Ltd, China) with an 8 mm sensor head diameter. The colour parameters corresponding to the uniform CIELAB colour space (L^*, a^* and b^*) were obtained directly from the equipment. L^* indicates lightness (100=white and 0=black), "a" indicates a greenish-reddish hue [negative (-a) (green) to red (+a) (positive)] while "b" indicates a bluish-yellowish hue [negative (-b) (blue) to yellow (+b) (positive)]. The equipment was calibrated using a reference blank L^*=96.65, a^*=-0.03 and b^*=1.86.

Additional colour parameters were determined such as the whiteness index (*WI*), yellowness index (*YI*), tone (h_{ab}) and chrome (C_{ab}). Finally, the colour differences in the CIELAB space were determined between the control lentil grains (T_0 = without radiation) and the lentils treated for 5 ($\Delta E_{0\text{-}5\ min}$), and 10 min ($\Delta E_{0\text{-}5\ min}$), which are calculated as the Euclidean distance between their locations in three-dimensional space as defined by L^*, a^*, and b^*, the respective equations for these calculations are presented in Table 2 (Hernández *et al*., 2022b).

Table 2. Equations for the determination of colour variables

Equation
Tone
$h_{ab} = arctan\,(b^*/a^*)\ C_{ab} = \left[(a^*)^2 + (b^*)^2\right]^{1/2}$
Chrome
$C_{ab} = \dfrac{\left[(a^*)^2 + (b^*)^2\right]^{1/2}}{arctan\,(b^*/a^*)}$
Whiteness index
$WI = 100 - \left[(100 - L^*)^2 + (a^*)^2 + (b^*)^2\right]^{1/2}$
Yellowness index
$YI = \dfrac{142.86 b^*}{L}$
Differences in colour
$\Delta E = \left[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2\right]^{1/2}$

The test used to determine the presence of saponins was the foam test. For this test, lentils (2 mg) from each of the treatments were placed in a test tube with 10 mL of distilled water and incubated in a water bath (CIVEQ thermostat water bath HH2) at 80°C for 30 min. Afterwards, the tube was allowed to cool, shaken vigorously and foam heights were measured (cm) at 0, 30, 60, and 90 min to define the presence of saponins (Aarland, 2015).

Total flavonoids were determined using a spectrometric assay developed by Andary (1990) using 2-aminoethyl diphenyl borate (1% in methanol) as a reagent. Methanolic

extracts were prepared from treated lentils (0, 5 and 10 min) placed in aluminium-lined test tubes, the samples (1 g) were mixed with 10 mL of solvent (pure methanol). The mixtures were incubated for 24 h with periodic gentle agitation and then ultrasound was applied for 15 min. The extracts were recovered and diluted to 50 and 80% in methanol and then mixed with the reagent solution (100 µL per 2 mL of extract). A spectrometric evaluation was performed at 404 nm. The absorbances of the extract were compared with the absorbances of a rutin standard curve (0 to 50 mg mL^{-1}). Subsequently, the quantity of total flavonoids was calculated from the curve and the total flavonoid contents were expressed in terms of µg of rutin equivalents per mL^{-1} of extract.

Once the seeds were treated with UV-C radiation, they were primed using cyanobacteria (*Spirulina*) *i.e.* soaked in water with *Spirulina* at different concentrations (0, 0.5, 0.75 and 1.5 g) in 100 ml of water for two hours. The pH of the water and *Spirulina* were determined with a pH meter Milwaukee (MW100 PRO-pH meter). After that, the lentils were dried on absorbent paper, and subsequently sown. The seeds were placed on sterilized blotting paper and moistened with 10 ml of purified water, paper rolls were formed and stored in plastic bags according to the experimental design. Germination was allowed to proceed with alternating cycles of light and dark according to the environmental conditions, with an average daytime temperature and humidity of 23˚C and 32%. The germinated seeds were counted every 24 h until germination became uniform (five days). The physiological variables evaluated in the experiment were the number of emerged seedlings (G), the fresh weight of seedlings, fresh weight of the roots, dry weight of seedlings. The criterion for evaluating germination was the rupture of the seed and the appearance of the radicle, with a length equal to or greater than 2 mm. The fresh weight in grams was only measured for the normal root (RW), the aerial part (fwap) and the whole seedling (fws) on a digital balance. For the determination of the dry weight (DW), the seedlings were dried in a Hamilton Beach White food dehydrator at a temperature of 54°C for 24 h. Finally, the dry weight in grams of the seedlings was measured on a precision scale (balance model VE-1000- Velab® electronic balance).

The morphological changes in the lentil seed control and UV-C treatments (5 and 10 min) were analysed using a scanning electron microscope (JEOL JSM-6010LA, Tokyo, Japan) at high vacuum. Before the analysis, whole lentil seeds were cut in half and placed in an aluminium sample holder, they were fixed with carbon tape, the samples were coated with gold nanoparticles. The micrographs were obtained at 1000x, 3 zones were analysed; testa which is the seed surface, the sclerenchyma zone for the purposes of visualizing the sclereids and the parenchyma where the starches, cell wall and protein body are located. The analysis conditions for the equipment were a 15 kV electron accelerating voltage.

An FT-IR (4000-400 cm^{-1}) was performed on the dried control and on the irradiated lentil samples (T_0, T_5 and T_{10}). Each of the samples (lentil powder) were ground to a fine powder using a mortar and pestle. Subsequently, about 1% of the sample and 99% of the KBr were added, the mixture was ground and homogenized again (in total 150 mg were used). Finally, using a conventional pellet die and maintaining the pressure at 10 metric tons for 1 min after applying a vacuum, a pellet was created and used for the FT-IR measurement. Thereafter, the samples were analysed using a NICOLET brand FT-IR spectrometer, model 6700 (resolution: 2 cm^{-1}, 200 scans). The measurements of the FT-IR spectra were performed in transmission mode using KBr disks at room temperature.

The variables evaluated in lentils due to their exposure to radiation varied according to the applied times of the exposure and were compared using analysis of variance ($p \leq 0.05$) (ANOVA) followed by the least significant difference test (LSD) and the 5% probability level was used to compare the different treatments. A data compilation and all test calculations were performed using SAS software (SAS Institute, 2008).

A PCA was applied to the experimental data obtained from the radiated lentil grains (0, 5 and 10 min) for the variables: a) L^*, b^*: bluish-yellowish, *WI*: whiteness index, Tone, G24, G48: germination at 24, 48 h (G24, G48), RW: fresh root weight, WAP: weight of aerial part and DW: dry weight; b) foam height from the test for saponins at 0, 30, 60 and 90 min (Hs0, Hs30, Hs60 and Hs90), total flavonoids, A_{405}: absorbance at 405 nm, A_{500}: absorbance at 500 nm. An analysis was performed using R Project software version 0.10-47, with R Commander and the program factoMiner and Fitopac (2.1).

RESULTS AND DISCUSSION

Among the colour parameters evaluated (L^*, a^* and b^*, whiteness and yellowness indexes, hue and chroma); L^*, b^*, *WI* and hue underwent no significant statistical changes when comparing the samples treated with UV-C radiation (5 and 10 min) and the control samples (without radiation), (Table 3). Although there was a slight tendency towards modification, this was negligible. For the variable L^*, the lentil grains (T_0 = 0 min of radiation) presented the lowest value (48.7) when compared with lentils treated by 5 and 10 min of radiation (T_5 and T_{10}), these treatments produced values of 51.08 and 53.5. The samples of lentil from T_{10} presented a slight tendency to produce an increase with respect to the value obtained in the control lentil (not irradiated-T_0), but these changes are not significant. As may be observed in Table 3 (column 1), the L^* values also show a tendency to increase, but without a significant increase for

Table 3. Parameters related to colour, saponins, flavonoids and the absorbance of lentils treated with UV-C (0, 5 and 10 min). Means with different letters in a column are statistically different ($p \leq 0.05$) they varied according to the UV-C radiation treatment (0, 5 and 10 min) applied. NS: there was no significant difference, (DMS, $\alpha = 0.05$). *, **Significant at 5 and 1% probability. *L**: brightness, *a**: greenish-reddish and *b**: bluish-yellowish. *YI*, yellowness index, *WI*, whiteness index. Foam height at 0, 30, 60 and 90 min (Hs0, Hs30, Hs60 and Hs90). Absorbance at 405 and 500 nm (A_{405}, A_{500}) and nm: nanometres

UV-C Radiation	L	*a**	*b**	*YI*	*WI*	Tone	Chrome	Hs0	Hs30	Hs60 (cm)	Hs90	Flavonoids (mg_{RE} mL^{-1})	$A_{405\ nm}$	$A_{500\ nm}$
0	48.70 b	9.6 a	21.28 a	62.58 a	43.54 a	23.35 ba	56.67 a	1.0 b	1.0 b	0.75 b	0.75 b	42.07 b	1.8 a	0.71 a
5	51.08 ba	8.96 a	21.17 a	59.39 a	45.88 a	22.99 b	58.51 a	2.05 a	1.7 a	1.6 a	1.5 ba	47.61 a	1.76 a	0.81 ba
10	53.50 ba	9.41 a	23.05 a	61.65 a	47.21 a	24.90a	61.21 a	2.6 a	2.6 a	2.1 a	2.0 a	49.18 a	1.62 b	0.85 a
LSD	2.4	1.29	1.48	4.07	2.1	1.6	1.77	0.63	0.6	0.91	0.97	4.56	0.11	0.11
C.V.	4.47	12.98	6.32	6.2	4.31	6.4	8.12	7.81	8.83	14.03	16.04	4.35	3.02	6.15
R^2	0.8	0.44	0.6	0.53	0.79	0.55	0.46	0.98	0.97	0.96	0.94	0.94	0.94	0.94
Significance	0. NS	0.57 NS	0. NS	0.25 NS	0. NS	0.0 NS	0.26 NS	0.01*	0.03*	0.04*	0.06 NS	0.02*	0.02*	0.05*

the times used in this research. The higher *L** value, means a lower value of nutraceutical content. The *L** colour parameter is related to the anthocyanin content according to some authors (Hernandez *et al*., 2022b). Therefore, in this research, the metabolites related to this variable did not undergo significant changes, *i.e.* its antioxidant potential was not modified.

In relation to the colour parameter *b**, a slight non-significant increase was found when comparing the irradiated lentil seeds (T_0) with respect to the control grains (Table 3, column 2). The type of lentil used in this research has a lower value than some lentils found in other studies (37.60, 37.57) (Jiao *et al*., 2012), higher *b** values have come to be associated with a higher carotenoid content. The *b** values of distinct types of carotenoids were found to range from 12.74 – 52.37 (Meléndez-Martínez *et al*., 2022). In this research a slight tendency for an increase in *b** does not guarantee changes in the carotenoid content.

With regard to the calculated variable of the whiteness index (*WI*), no significant statistical differences were obtained when comparing the mean values of each of the lentil conditions, those irradiated at 0, 5 and 10 min. These variables are a consequence of the changes obtained in the parameters *L** and *b**, as shown in Table 2, *WI* is a function of these variables. The value of this colour attribute has a slight tendency to increase as the radiation time increases, with the lowest value being found in the lentil without exposure to UV-C radiation (43.54), this was followed by the value in the lentils treated for 5 (45.88) and 10 (47.21) min. Since these value changes are not significant, it is not possible to claim that there are meaningful changes or that these changes would indicate a variation in the bioactive elements of lentils.

The hue (tone) is another colour variable that did not present any significant statistical difference ($p \leq 0.01$) for the lentils depending on the time of exposure to radiation. This variable is a function of the *a**, *b** and chroma components, although the values of *a**, *YI* and Chrome did not have any significant statistical differences. When the mean tone values were compared, the highest tone value was found at T_{10} (24.9) and the lowest at T_0 (23.35). Finally, for colour difference (ΔE) between T_0 - T_5 and T_0 - T_{10} values of 2.21 and 5.11 were obtained, with only slight changes mainly due to the *L** value in the lentil samples.

Another aspect of the colour parameter results of the lentil samples is shown in Fig. 2, where the "xy" coordinates are plotted to the location of the region of the colour tone. The "x" axis is the relative proportion of red and "y" is the relative proportion of green. In Fig. 2, the "xy" coordinates for the lentil group T_0 (x = 0.396 - 0.398 and y = 0.375 - 0.377), T_5 (x = 0.394 - 0.396 and y = 0.377 - 0.380) and T_{10} (X = 0.398 - 0.4 and y = 0.382 - 0.383) are observed. Since there are no significant colour changes, it is necessary to zoom in to appreciate the different colour coordinates of the lentils.

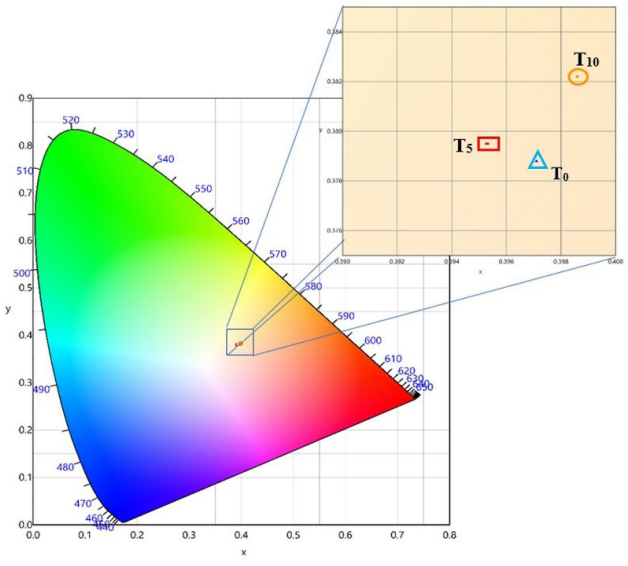


Fig. 2. "xy" coordinates of the tone in the CIE chromatic triangle (neutral achromatic point x = 0.333, y = 0.333) – T_0 (control samples), T_5 and T_{10} (samples irradiated for 5 min and 10 min).

The column height of the foam column formed by the different treatments and measured at 0, 30 and 60 min shows significant statistical differences at the 1 and 5% probability. A higher column value is associated with a higher saponin value, which increases in both treatments by more than 100% with respect to the column height measured for the control lentils (Table 3, columns 8, 9, and 10). Other authors, *e.g*. Guajardo-Flores *et al*. (2014) demonstrated that UV-C light modified the saponin content of the seed coat extracts. Among the various phytochemicals, saponins have a great potential to protect against chronic diseases. The increase in stress tolerance has been associated with the improvement in both primary and secondary metabolites. Likewise, in the present investigation using UV-C, it is possible that the saponins were modified.

Table 3 (column 12) shows that the total amount of flavonoids had a slightly tendency to increase (no significant statistical difference), when the control lentils are compared with the lentils treated with both treatments at 5 and 10 min. These increases were found to be 13 and 17%, at 5 (47.61 mg_{RE} mL^{-1}) and 10 min (49.18 mg_{RE} mL^{-1}) in relation to the control sample T_0 (42.07 mg_{RE} mL^{-1}). The changes in flavonoid composition and total content have been associated with the protective mechanisms of the plant against stresses caused by environmental conditions, such as UV light (Urban *et al.*, 2016), thus, a positive correlation between flavonoid biosynthesis and abiotic stress has been shown in several studies. The UV-C treatment may be a viable technique with which to increase the level of phytochemicals by finding the optimal combination of radiation parameters (light intensity, exposure time, number of radiation regimes, distance from the light emitting source with respect to the object of study, sample characteristics, *etc*.). In the case of human consumption, the existence of flavonoids in lentils means that they may be classified as a nutraceutical food, and if the amount is increased, this benefits the potential consumer. In this study, the amount of flavonoids did not change significantly. Therefore, the challenge is to increase their levels which would be good for the plant stage, as well as the stage at which the plant is consumed by the general population in their food.

According to the analysis of variance and comparison of means between the treatments established in the germination test, UV-C radiation (0, 5, and 10 min) and imbibition in water with *Spirulina* at concentrations S_0, S_1, S_2, S_3, and S_4 (0, 0.5, 0.75, and 1.5 g), significant statistical differences were found between the variables of the seedling ($p \leq 0.05$) and seedling ($p \leq 0.01$) aerial portion fresh weight, and the seedling dry weight ($p < 0.01$).

Figure 3 shows the seeds which were not moistened in *Spirulina* (0% concentration, S_0) and those exposed to it for a 24, 48 and 96 h duration (after which the germination test was established) the lowest number of seedlings emerged at T_0 followed by the lentils irradiated for 5 and 10 min (T_5 and T_{10}). Over time, the number of germinated control lentils and radiated lentils became more uniform in quantity. Other studies reported negative effects on germination due to UV-C radiation, such as those studied by Lazim and Nasur (2017), who applied UV-C radiation for 30 and 60 min on sorghum (*Sorghum bicolour* L.) seeds and found a negative effect on the germination rate. It is worth noting that the dose of UV-C radiation which promotes hormesis (*i.e*. the beneficial effect of low doses of UV-C radiation on plant products) is a particular characteristic of each type of seed. In this research, although there was no statistically significant difference in terms of germination rate, it was found that there was a tendency for it to increase in UV-C-treated lentils as compared to the control lentils (without radiation).

A trend that should be widely explored is the combination of physical methods used to modify germination and other variables related to physiological, sanitary, nutritional and/or genetic quality. Studies have already been conducted combining magnetic field with laser treatment or magnetic field with UV-C treatment (Lazim and Nasur, 2017). There are still a whole series of methods which could be combined and applied within different windows of beneficial values that could serve to improve some quality attribute of the seed and likewise of the final product that reaches the consumer, now more than ever the consumption of foods containing nutraceutical substances is required, as is the case with lentils. The use of nutraceutical elements for use as biostimulatory elements is also a trend that should be employed. In this research, *Spirulina* was used in this way.

In relation to the germination of lentils soaked in *Spirulina* at different concentration percentages, it was found that: the fresh weight of the aerial part (fwap) and seedlings (fws) show a similar behaviour pattern, for T_0 and

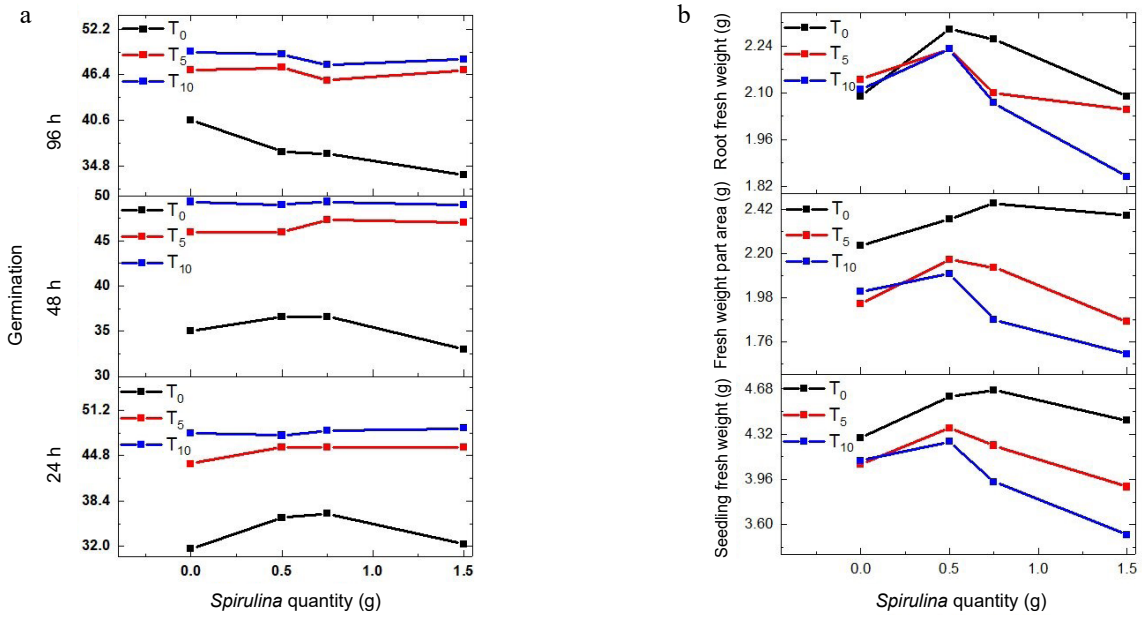


Fig. 3. Physiological variables of lentils at T_0, T_5 and T_{10} (0, 5 and 10 min) and a *Spirulina* concentration at (0, 0.5, 0.75 and 1.5%).

T_5 the values tend to increase (0.5 and 0.75 g) and then tend to decrease at the 1.5 g concentration. The lowest values were found for the T_5 and T_{10} radiated lentils when compared to the T_0 control seeds, for both variables (fwap and fws). For the fresh weight of the aerial part (fwap) of the seedlings from non-irradiated lentils T_0 (column b, image 2) presents the highest values for all concentrations of S*pirulina* (S_0, S_1, S_2, S_3, S_4), which has higher values with respect to the control by 6, 9 and 7%. It is possible to observe the highest value at a concentration of 0.75% of *Spirulina* (T_0, S_2), being the lowest value of fwap for T_0, S_0 (No radiation and not priming in *Spirulina*). For T_5, the highest increases were 11 and 9% for S_1 and S_2 (0.5 and 0.75% *Spirulina* concentration) and there was a 5% decrease for S_3 (1.5 g of *Spirulina*). In the case of T_{10}, there was only a 5% increase with S_1 and decreases of about 7% and 15% for S_2 and S_3, respectively.

In the case of seedling fresh weight (fws) from T_0 (column b, image 3) it presented a behaviour pattern similar to that of fwap, increasing its values with respect to the control by 7, 9 and 3%, respectively for S_1, S_2 and S_3. It is possible to observe the highest value in S_1 and the lowest one in S_0. For T_5, the highest increases were 7 and 4% for S_1 and S_2 (0.5 and 0.75% of *Spirulina* concentration) and there was a decrease of 5% for S_3 (1.5% of *Spirulina*). In the case of T_{10}, there was only a 3% increase with S_1 and decreases of about 6 and 15% for S_2 and S_3, respectively. As may be seen, with increasing UV-C radiation, the percentage of fws decreases. Likewise, there is a decrease in fws with increasing *Spirulina* concentration. Thus, *Spirulina* improves the fwap and fws variables, although this is modified with the increase in UV-C radiation.

It is necessary to adjust the concentrations of *Spirulina* so that the physiological improvement of the lentil is maintained under UV-C radiation. The higher the exposure to radiation, the lower the concentration of *Spirulina* that should be used. Other authors have used *Spirulina* to improve germination and other plant growth variables. In this way, *Spirulina* helps to improve physiological quality in the face of damage caused by UV-C radiation. It has also been shown that UV-C radiation, in combination with certain concentrations of *Spirulina,* may have applications to meet certain agricultural needs, and serve as a biostimulant, but also to inhibit weed growth in crops, according to the concentration of S*pirulina* and radiation parameters. Lazim and Nasur (2017) applied UV-C radiation treatment and found no significant effects on the germination percentage and root length of the seedlings, while the treatment showed a significantly negative impact on germination speed, seedling length and the number of leaves. Thus, there is still a wide range of possibilities to investigate in order to find the benefits of these biophysical methods in crop conditioning and improvement. In the present research concerning lentils, favourable effects on growth were found, but also unfavourable effects were discovered as a function of the duration of UV-C radiation and the concentration of *Spirulina* that was used. UV-C light degrades the surface layer of the seed, and stresses it. In some cases, this situation may be disadvantageous to the seed. In others, the treatment could be applied, for example, when the seed has water absorption problems. UV-C radiation could be used to facilitate more rapid absorption.

Figure 4 shows damage to the testa area (images of row 1) due to the application of UV-C, which increases with higher exposure to radiation. It has been established

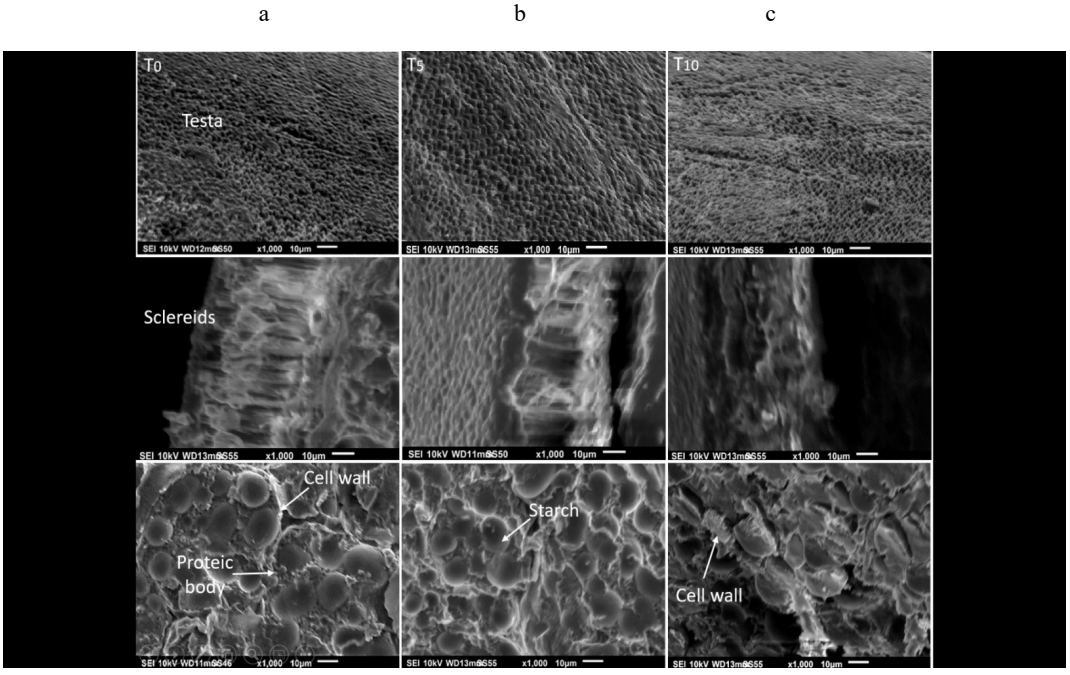


Fig. 4. Micrographs of lentils treated with UV-C radiation: a) 0 min, b) 5 min, and c) 10 min.

that the testa can be modified by various factors such as storage conditions, temperature, humidity, and light. Nasar-Abbas *et al.* (2009) studied the seeds of fava beans (*Vicia faba* L.) and found that exposure to artificial light and storage at elevated temperatures accelerates the darkening of testa colour. In the present study, changes in testa conditions were observed with increasing radiation, it was also associated with a change in colour. In the area of the sclereids in the control treatment (T_0), the structures remained intact, but when radiation was applied for 10 min (T_{10}), these structures were no longer defined. In the parenchyma zone of the cotyledon, the reserve materials, starches, and protein body, as well as the cell wall, were observed.

Therefore, in this research, the application of UV-C for a time of 5 min could be recommended since the results show no relevant changes in the lentil tissue as compared to an application time of 10 min.

The FT-IR spectra of the lentil samples are presented in Fig. 5. It is possible to observe that the spectra obtained are similar. The strong peak at 3 400-3 294 cm^{-1} was attributed to the OH functional group and the O-H stretching of the polymeric compound. The peak at 2 800-3 000 cm^{-1} was attributed to the amount of amylose and amylopectin inside the samples, the C-H groups and CH_2 deformation. The FT-IR results are divided into four stages, region I 400-1 500 cm^{-1} (fingerprint region), region II 1 500-2 000 cm^{-1} (double bonds, carbonyl group), region III 2 000-2 500 cm^{-1} (triple bonds, alkynes), and region IV 2 500-4 200 cm^{-1} (carboxyl groups, alcohol and water, O-H stretching region, the absorption of single bonds) (Chua *et al*., 2019).

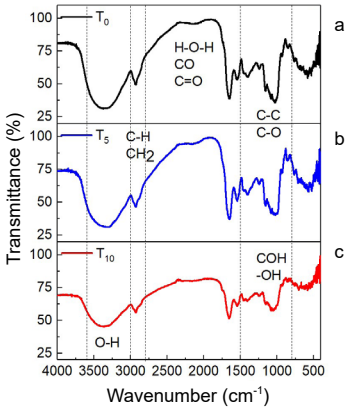


Fig. 5. FTIR spectra of the lentil samples.

The vibration of amylose and amylopectin originates in region I of the fingerprint. A peak may be observed around at 900 cm^{-1}, it was attributed to glycosidic bonds, this is attenuated in the T_{10} sample, which is the one with the longest irradiation exposure time. Also, in the 2 800-3 000 cm^{-1} region it may be observed that the irradiated starches are modified, as the hydroxyl radicals formed through water radiolysis rapidly attack the hydrogen of any C-H bonds and liberate the hydrogen atom from the bond (Kizil *et al*., 2002). This indicates that due to the induced radiation, the water content in the T_{10} sample is lower than that of T_5 and T_0. The results of the moisture content tests were 9.175 ± 0.007, 9.095 ± 0.021, and 8.870 ± 0.028 for lentil T_0, T_5 and T_{10}, respectively. The treatments that were statistically analysed were significantly different, T_{10} produced the lowest value.

The band located between 1 700-1 500 cm^{-1} corresponds to the presence of C = O functional groups, CO stretching and the COO double bond stretching of the deprotonated carboxylate (Chua *et al*., 2019). This suggests that this band arises from the vibrations of the adsorbed water molecules in the non-crystalline region. In particular, the band at around 1 650 cm^{-1} was ascribed to the H-O-H bending vibration due to water being absorbed in the amorphous regions of starch (Gul *et al*., 2016), thereby strengthening the argument that there is a lower water content in the T_{10} sample than in the other two due to its lower level of transmittance. Absorption bands at around 1 650 cm^{-1} are also characteristic of amide I groups, due to the peptide group that is present in the lentil proteins.

The 1 400 cm^{-1} peak was attributed to the characteristic combination of the O-H bending of the COH group and CH-- bending of the alkenes. The peaks between 1 000-1 200 cm^{-1} were associated with the stretching of C-C and C-O in the aromatic compounds of galactose, rhamnose, galacturonic acid and the -OH of polysaccharide (Chua *et al*., 2019; Gul *et al*., 2016).

Lentil protein is an excellent source of essential amino acids, particularly leucine, lysine, threonine, and phenylalanine (Khazaei *et al*., 2019). Lysine is a protein that contain an amine group and one carboxyl group. Absorption bands that are two primary features of this protein, amide I bands are observed at 1 600-1 700 cm^{-1}, amide I region, which occurs primarily because of the C=O stretch vibrations of the peptide linkages, which is the most excellent sensitive spectral region with which to study the secondary structure of protein (Muhammad *et al.*, 2013).

As shown in Fig. 5, no shifts in band wavelength were observed for the different treatments with respect to the control, which indicated that UV-C radiation did not affect the secondary structure of the lentil protein, as observed in lentil protein isolates exposed to high pressure (Ahmed *et al.*, 2019). No changes were observed in the Amide II and III regions, but there was some attenuation of the Amide I signal of the T_{10} treatment, which was not present in the T_5 one (Fig. 5). This behaviour may be attributed to the reorganization of the secondary structures of the globular proteins of lentils elicited by radiation. Previous studies were performed on egg white protein and showed the disappearance of the peak at 1 653 cm^{-1} caused by the tendency of the protein to adopt a more ordered state and also the induction of cross-lining after prolonged UV irradiation (Kuan *et al.*, 2011). Although the treatment applied in this study was not as severe as the one used in that study (10 min *vs*. 120 min), it is possible that given the intrinsic differences among the proteins of each sample, the lentil protein would have proved more susceptible and thus showed changes at an earlier stage.

For the principal component analysis (a) a variance in components 1 and 2 of 74.82 and 25.18% was found (Fig. 6a). For the second analysis (b), the variance or inertia was 96.27 and 3.73% (Fig. 6b). Thus, between components 1 and 2, for each case a total of almost 100% of the relevant data was obtained. These figures show both the percentage of variance and the eigenvalue for each of the components for the two analyses performed. Figure 7 represents the PCA for the variables evaluated in treatments T_0, T_5 and T_{10}: a) colour and germination and b) total flavonoids, foam height and absorbance measurements. For each of the analyses, two principal components (PCs) were used. Figure 7a shows the formation of two clusters (I: T_0, and T_5, and II: T_{10}). It should be noted that there is a positive correlation between the colour variables *L**, *b**, tone and the physiological variables (G24, G48 and DW). The parameters b* and tone have almost no correlation with WAP (angle of 90°), and L has a negative correlation with WAP (angle between vectors of more than 90°) and a slight positive correlation with RW (angle of 70°). It is possible to observe that the luminosity (L) and whiteness index (*WI*) are closely correlated with germination (G24 and G48), thereby forming an angle between the vectors representing the variables of less than 45°. In this sense, T_0 and T_5 show more similarity with regard to the behaviour of the colour and germination variables, in contrast to T_{10}, which shows the

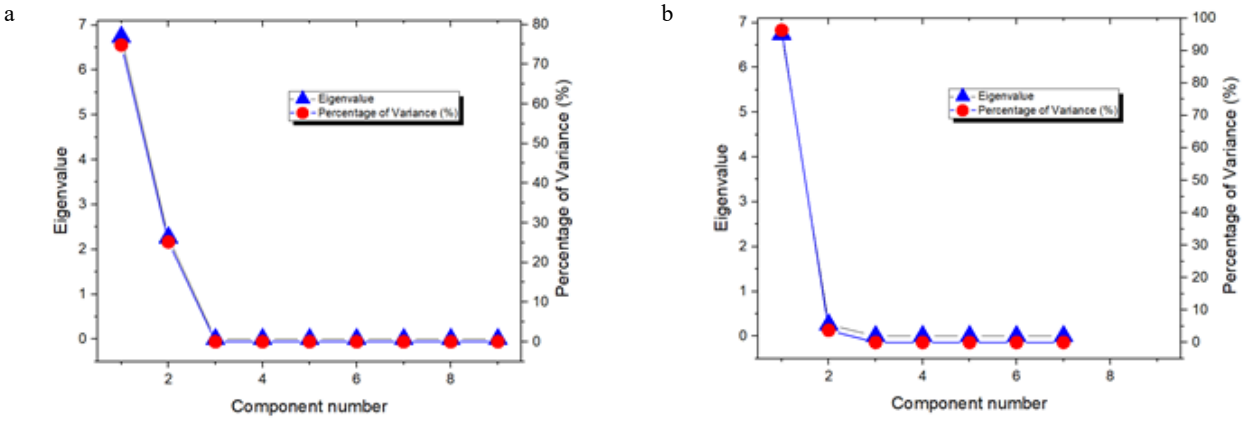
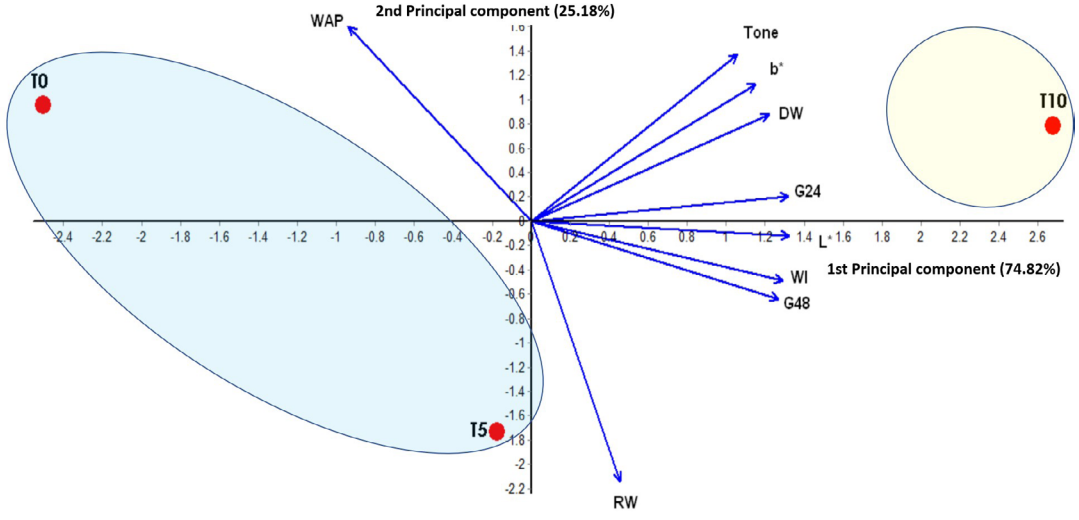


Fig. 6. Principal component analysis of the characteristic variables of lentil (*Lens culinaris* Medik.) treated with UVC radiation (0, 5 and 10 min) a) colour and germination and b) total flavonids, saponins and absorbance.

a



b

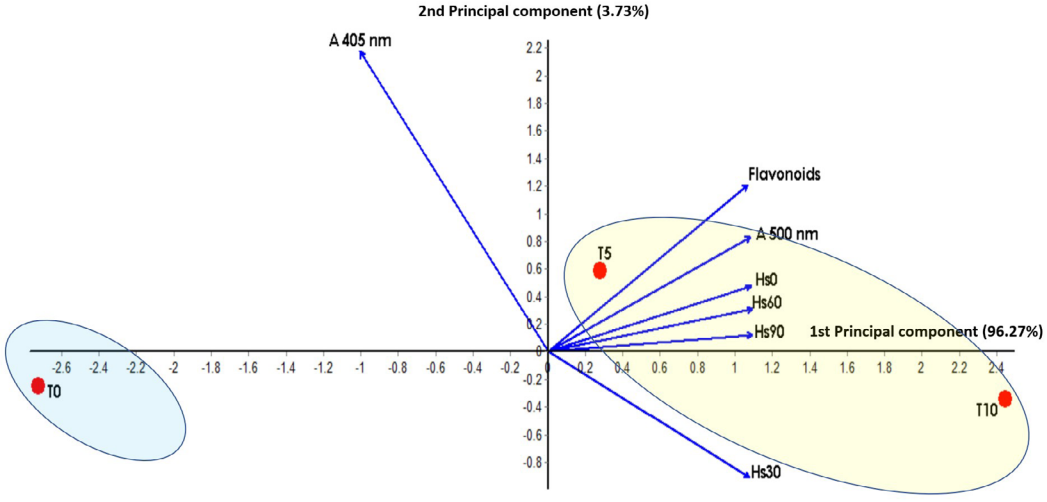


Fig. 7. Percentage of variance and eigenvalue as a function of the component number in the analysis of PCA for the variables evaluated in treatments T_0, T_5 and T_{10}: a) colour and germination and b) total flavonoids, foam height and absorbance.

dissimilarity between these treatments. That is, the colour variables (*L**, *b**, *WI*, and tone) and germination (G24 and G48), tend to be modified by UV-C light irradiation with a duration of 10 min.

Figure 7b shows the two groups that formed due to the behaviour of the lentil variables that were evaluated for each of the treatments applied (I. T_0 and II. T_5 and T_{10}). The similarities in group II are mainly due to the behaviour of the variables: Hs0, Hs30, Hs60, Hs90, A500, total flavonoids which are precisely the variables that contribute most to the existence of some differentiation between the treated (T_5 and T_{10}) and untreated lentils (T_0). The closest correlations between the variables representing the similarities of this group are between Hs0, Hs60 and Hs90 (they form an angle between their vectors of less than 30°) and total flavonoids and A_{500}. In this sense, it may be observed that through the evaluated variables and certain principal components it is possible to distinguish the changes in the seed due to UV-C radiation. The behaviour of the variables is similar between T_5 and T_{10}, when compared with the T_0 control.

Figure 8 shows the heat map which corresponds to the magnitude of the variables obtained. It visually identifies the colour variables that have a higher degree of variation according to the lentil treatment (L, *WI*, Tone, Chrome) and less variation (*YI*). In the case of variables related to bioactive compounds, it is possible to observe that the one with the highest degree of variation was flavonoids. Finally, in relation to the variables related to physiological qualities, the one with the highest degree of variation due to UV-C

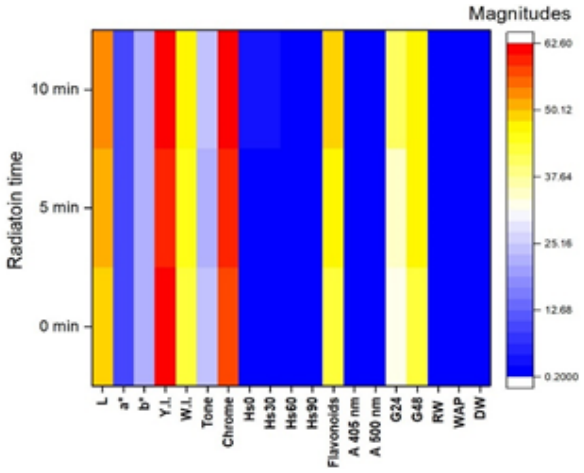


Fig. 8. Heat map of magnitudes of the variables assessed in lentils.

treatments was germinated at 24 h, followed by the one that germinated at 48 h. In this way, UV-C radiation may serve to support the cultivation of crops in agriculture to the extent that the respective instrumentation may be incorporated into the different phenological stages. In this research, observations were made in the seed conditioning stage, and the importance of incorporating biostimulants of bioactive substances such as *Spirulina* to improve the condition of the seedling is also highlighted.

CONCLUSIONS

In this research, with reference to the proposed goals, it was concluded that:

1. The colour parameters *L**, whiteness index, *b** and hue (tone) had a slight tendency to change (without any significant statistical differences), when comparing the evaluated treatments, the greatest increase occurred at T_{10} (10 min of UV-C exposure), of 10, 8, 8 and 7%, respectively when compared to the untreated seeds T_0. Likewise, the greatest difference in the colour difference parameter was also found between T_0 and T_{10}.

2. Total flavonoids had a slight tendency to increase (without any significant statistical differences) by 13 and 17% at irradiation times 5 (47.61) and 10 min (49.18) compared to the control sample T_0 (42.07).

3. As for saponins, a higher value of foam column generated by the lentils is obtained, which is increased by the treatments (5 and 10 min of UV-C exposure) by more than 100% compared to the control samples.

4. In the conditioning of lentils using *Spirulina* to improve the physiological quality of the seeds against UV-C radiation damage, it was found that in the case of the application of UV-C radiation (0, 5, and 10 min) and the imbibition of water with *Spirulina* at concentrations S_0, S_1, S_2, S_3, and S_4 (0, 0.5, 0.75, and 1.5% of concentration), there were significant statistical differences between the variables of the seedling ($p < 0.05$) and seedling ($p < 0.01$) aerial part fresh weight, and seedling dry weight ($p < 0.01$).

5. Morphological changes occur in the lentil due to radiation. By applying UV-C for 10 min, the cell wall and the protein body are damaged by the radiation effect, however, no damage to the starch is visible.

6. FT-IR, indicates that the UV-C radiation used in this experiment did not induce changes in the chemical structure of the carbohydrates or induce the formation of any new compounds. However, decreases in absorption intensity in the bands at 3 000-3 600 cm^{-1} and between 1 600-1 700 cm^{-1} indicate that longer radiation times induced differences in water content and also in the reorganization of the secondary protein structure, respectively.

Conflict of interest. The authors declare no conflict of interest.

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