Food Chemistry 345 (2021) 128788

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Laser light as a promising approach to improve the nutritional value, antioxidant capacity and anti-inflammatory activity of flavonoid-rich buckwheat sprouts

Mohammed S. Almuhayawi [a,1,*], Abdelrahim H.A. Hassan [b,1], Mohamed Abdel-Mawgoud [c], Galal Khamis [d], Samy Selim [e,f], Soad K. Al Jaouni [g], Hamada AbdElgawad [h,*]

[a] Department of Microbiology and Medical Parasitology, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia
[b] Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef 62511, Egypt
[c] Department of Medicinal and Aromatic Plants, Desert Research Centre, Cairo, Egypt
[d] Department of Laser Applications in Metrology, Photochemistry and Agriculture (LAMPA), National Institute of Laser Enhanced Sciences, Cairo University, Cairo, Egypt
[e] Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Jouf University, Sakaka, P.O. 2014, Saudi Arabia
[f] Botany Department, Faculty of Science, Suez Canal University, Ismailia, P.O. 41522, Egypt
[g] Hematology/Pediatric Oncology, Yousef Abdulatif Jameel Scientific Chair of Prophetic Medicine Application, Faculty of Medicine, King Abdulaziz University, Jeddah 21589, Saudi Arabia
[h] Botany and Microbiology Department, Faculty of Science, Beni-Suef University, Beni-Suef 62521, Egypt

ARTICLE INFO

Keywords:
Buckwheat sprouts
He–Ne laser
Nutritious metabolites
Antioxidants
Anti-inflammatory

ABSTRACT

Buckwheat sprouts are rich in several nutrients such as antioxidant flavonoids that have a positive impact on human health. Although there are several studies reported the positive impact of laser light on crop plants, no studies have applied laser light to enhance the nutritive values of buckwheat sprouts. Herein, the contents of health-promoting minerals, metabolites and enzymes as well as the antioxidant and anti-inflammatory activities were determined in laser-treated (He–Ne laser, 632 nm, 5 mW) common buckwheat (CBW) and tartary buckwheat (TBW) sprouts. Out of 49 targeted minerals, vitamins, pigments and antioxidants, more than 35 parameters were significantly increased in CBW and/or TBW sprouts by laser light treatment. Also, laser light boosted the antioxidant capacity and anti-inflammatory activities through inhibiting cyclooxygenase-2 and lipoxygenase activities, particularly in TBW sprouts. Accordingly, laser light could be recommended as a promising method to improve the nutritional and health-promoting values of buckwheat sprouts.

1. Introduction

Plant-based foods have been receiving widespread attention in terms of their protective effects against various diseases, in addition to their main roles in providing the essential phytonutrients (Bachiega et al., 2016). In particular, sprouts are regarded as important sources of valuable phytochemicals, such as minerals, vitamins and polyphenols, besides being valuable food additives (Manchali et al., 2012).

Among the highly nutritive sprouts, buckwheat (*Fagopyrum esculentum* Moench.) sprouts have been considered as a big store for a variety of phytochemicals, such as phenolic compounds, unsaturated fatty acids, amino acids, sugars, vitamins and minerals (Kim, Kim, & Park, 2004). Buckwheat (BW) sprouts may serve as a valuable source of dietary carotenoids such as β-carotene, with high antioxidant capacity (Tuan et al., 2013). BW has been classified into two species: common buckwheat (CBW) and tartary buckwheat (TBW). TBW sprouts have been known to contain higher bioactive compounds than CBW sprouts (Kim et al., 2008). In comparison to BW seeds, BW sprouts are rich in flavonoids, particularly rutin, along with orientin, isoorientin, vitexin, isovitexin and quercetrin (Kim et al., 2008; Kim, Kim, & Park, 2004; Lim et al., 2012; Liu et al., 2008), which are being invested on their various health-promoting benefits such as vasodepressor, vasorelaxant, antibacterial, antioxidant and antihypertensive properties (Zhang et al., 2012). In addition, BW sprouts also contain proteins with hypocholesterolemic and hypotensive effects (Chen, Jiao & Ma, 2008). Due to their anthocyanin content, BW sprouts are characterized by their

* Corresponding authors.
E-mail addresses: msalmuhayawi@kau.edu.sa (M.S. Almuhayawi), hamada.abdelgawad@uantwerpen.be (H. AbdElgawad).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.foodchem.2020.128788
Received 23 July 2020; Received in revised form 6 November 2020; Accepted 30 November 2020
Available online 5 December 2020

beautiful pink or red colors, which give them an attractive appearance when used as garnishing for food dishes (Tsurunaga et al., 2013).

Based on the positive effects of some environmental factors and elicitors on the accumulation of bioactive compounds, several techniques have been exploited to improve the nutritional and health-promoting values of many plants (Saleh et al., 2020, AbdElgawad et al., 2015) and sprouts as well (Almuhayawi et al., 2020). In this regard, the application of physical methods, such as laser irradiation, to enhance plant productivity has been a promising approach to assure safety to the environment (Perveen et al., 2010, 2011). According to their uses and applications, laser light could be classified into two types, pulsed and continuous laser. The former has been used in medical applications such as Nd: YAG and XeCl laser, while the latter has been employed in improving crop production such as He-Ne and CO_2 (Perveen et al., 2010, Chen, Yue & Wang, 2005). The changes in plant physiological attributes in response to laser light are based on the effects of light, electromagnetism and temperature (Chen, Yue & Wang, 2005). Under this situation, the plant macromolecules can absorb light at a specific wavelength to trigger photosynthetic activity, resulting in an increased growth rate (Y.-P. Chen et al., 2005). Several studies have dealt with the effect of laser light on enhancing the crop yield and quality in many plants (Asghar et al., 2016; Khamis et al., 2020). For example, laser light has induced a significant increase in the antioxidant capacity and biomass accumulation of sunflower (Perveen et al., 2011).

Although laser light seems to be an effective strategy to enhance the plant growth and accumulation of bioactive compounds, to the best of our knowledge, the effect of laser on improving the nutritional and health-promoting values of sprouts has not been investigated (Perveen et al., 2010, 2011). Thus, the current study aimed at evaluating the laser-enhanced effects on the nutritional and health-promoting bioactive phytochemicals of the two species of BW sprouts, CBW and TBW sprouts. For this purpose, bioactive metabolites, enzyme activities and mineral profile, as well as antioxidant and anti-inflammatory properties were investigated under He-Ne laser treatment in comparison with control. We assume that laser light could improve the bioactive phytochemicals, biological activities and health-promoting values of BW sprouts.

2. Materials and methods

2.1. Plant material and experimental conditions

Seeds of CBW and TBW (*Fagopyrum esculentum* Moench.) were obtained from Field Crops Research Institute, Agricultural Research Centre, Giza, Egypt. The uniform seeds were soaked before laser re-illumination in distilled water for two hours. The seeds were divided into two groups (each group contains 100 seeds) i.e., non-irradiated control group and laser-irradiated group. The light source used in this experiment was the helium-neon (He-Ne) laser equivalent system (equipment whitening, laser II, DMC Equipment Ltd.). Treated seeds were irradiated by a He–Ne laser (632 nm at a power of 5 mW for 5 min and 500 mJ energy from the embryonic area side, beam diameter 1 mm). These conditions were selected following a preliminary experiment for detecting the most optimum laser treatment conditions based on the responses of BW species to laser in terms of fresh weight and antioxidant capacity (Supplementary Material Table 1). The distance between the laser source and the seeds was 12 cm, the laser was perpendicular to the seeds. The irradiated group was under controlled conditions and the whole system was in the dark covered with a box. The experiment was repeated three times.

The pretreated seeds were kept and rinsed in distilled and spread on trays lined with vermiculite and watered for the first three days with Milli-Q water and kept in dark at room temperature. The trays were then transferred and maintained at 25 °C air temperature in a controlled growth chamber under 16 h light/8h dark cycle managed through cool white fluorescent tubes with photosynthetically active radiation (PAR) of 400 μmol m^{-2} s^{-1} and relative humidity of 60% per day. Aquaponic water (100 mL) was poured evenly over the BW seeds in each tray. After 9 days, the sprouts from each tray (a biological replicate) were taken and weighed as fresh mass then frozen in liquid nitrogen and stored at −80 °C for further biochemical analyses. Five biological replicates (each biological replicate was a pooled of 10 plants of the same tray) were used for each measurement.

2.2. Elemental analysis

Elemental analysis was done according to Hamad et al. (2015) by using inductively coupled plasma (ICP-MS, Finnigan Element XR, Scientific, Bremen, Germany).

2.3. Pigment analysis

Sprouts were homogenized in acetone using MagNALyser (Roche, Vilvoorde, Belgium, 1 min, 7000 rpm), and centrifuged at 14,000 g (4 °C, 20 min), and the supernatant was filtered (Acrodisc GHP filter, 0.45 μm 13 mm) and analyzed by high-performance liquid chromatography (HPLC, Shimadzu SIL10-ADvp, reversed-phase, at 4 °C) (Thayer & Björkman, 1990). Pigments and carotenoids were separated on a silica-based C18 column (Waters Spherisorb, 5 μm ODS1, 4.6 × 250 mm, and column temperature used was 40 °C). Mobile phase: A) 81:9:10 acetonitrile:methanol:water and solvent B) 68:32 methanol: ethyl acetate. The flow rate of the mobile phase was 1.0 mL/min at room temperature. Chlorophyll *a* and b, beta-carotene and xanthophylls were detected using a diode-array detector (Shimadzu SPD-M10Avp) at four wavelengths (420, 440, 462, 660 nm). Concentrations were determined using the Shimadzu Lab Solutions Lite software and a calibration curve.

2.4. Determination of antioxidant metabolites

Polyphenols and flavonoids were extracted by homogenizing 100 mg of frozen sprouts in 1 mL of 80% ethanol (v/v). After centrifugation at 4 °C for 20 min, the supernatant was used to measure the total phenolic and flavonoid contents. Phenolic content was determined using a Folin–Ciocalteu assay with gallic acid as a standard (Versieren et al., 2017). While flavonoid content was estimated using the modified aluminum chloride colorimetric method, with quercetin as a standard (Hassan, 2012; de Sousa et al., 2019).

Individual phenolic acids and flavonoids were determined following the protocol described by Hamad et al. (2015) and Alam (2016), Fina (2017), Hassan (2012), Naudts (2014) using HPLC (SCL-10A vp, Shimadzu Corporation, Kyoto, Japan). Approximately 50 mg of freeze-dried sprouts were homogenized in an acetone–water solution (4:1 v/v) for 24 h. The Shimadzu HPLC system (SCL-10 AVP, Japan), equipped with a Lichrosorb Si-60, 7 μm, 3 × 150 mm column, diode array detector). 90:10 (v/v) of water-formic acid 85:10:5 (v/v/v) of and acetonitrile/water/formic acid were employed a mobile phase at 0.8 mL/min (flow rate) and the internal standard was 3,5-dichloro-4-hydroxybenzoic. The concentration of each compound was calculated through a calibration curve of the corresponding standard.

Similar to ASC, GSH was extracted in 1 mL of 6% (w/v) *meta*-phosphoric acid at 4 °C and was separated by reversed-phase HPLC coupled with a UV detector (100 mm X 4.6 mm Polaris C18-A, 3 lm particle size; 40 °C, isocratic flow rate: 1 mL min^{-1}, elution buffer: 2 mM KCl, pH 2.5 with *O*-phosphoric acid).

2.5. Vitamin level analysis

Ascorbate and tocopherols were estimated using reversed-phase HPLC methods (Hamed et al. 2017). Ascorbate and tocopherols were mixed with metaphosphoric acid and hexane in MagNALyser, respectively. The homogenized samples were centrifuged at 14,000g (4 °C for 20 min). Ascorbate was extracted in 1 mL of 6% (w/v) meta-phosphoric acid at 4 °C and was separated by reversed-phase HPLC coupled with a

UV detector (100 mm × 4.6 mm Polaris C18-A, 3 lm particle size; 40 °C, isocratic flow rate: 1 mL min^{-1}, elution buffer: 2 mM KCl, pH 2.5 with *O*-phosphoric acid). Tocopherols were separated in Particil Pac column material and measured by HPLC according to Hamed et al. (2017). Tocopherol was separated on Particil Pac 5 μm column material (length 250 mm, i.d. 4.6 mm) and quantified by HPLC (Shimadzu's Hertogenbosch, normal phase conditions), coupled with a fluorometric detector (excitation at 290 nm and emission at 330 nm). The HPLC method with fluorescence detection was applied for the determination of riboflavin and thiamine in ethanol extracts of BW (Sunarić et al., 2020). The separation was performed on Zorbax Eclipse plus C18 solvent saver analytical column (3.0 mm × 150 mm, 3.5 μm) at 30 °C. The mobile phase consisting of 30.0% (v/v) methanol and 70.0% (v/v) 0.005 M NH4Ac (pH 5.0) was isocratically pumped. The flow rate was kept at 0.425 mL/min.

2.6. Determination of total antioxidant capacity

The antioxidant capacities were conducted *in vitro* through ferric reducing antioxidant power (FRAP, μmol trolox/g FW), diphenylpicrylhydrazyl (DPPH, %) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS, μmol trolox/g FW) (Benzie and Szeto, 1999; Casasole et al., 2017; Hamad et al., 2015). About 0.1 g of each BW sprout sample was extracted in 80% ethanol and centrifuged (20 min at 14,000 rpm). Where 0.1 mL of diluted extract was used to determine the antioxidant capacity after mixing with 0.25 mL of FRAP reagent (mixing $FeCl_3$ (20 mM) in acetate buffer (0.25 M, pH 3.6) at room temperature. The ABTS radical was prepared by mixing ABTS with 2.4 mM potassium persulphate, which were allowed to react for 12 h in the dark at room temperature and measure the absorbance at 734 nm. Regarding DPPH, the antioxidant capacity was measured by mixing 0.1 mL of the diluted sprout extracts with 0.25 mL of the DPPH solution. After incubation at room temperature, the absorbance was measured at 517 nm using the spectrometric method, respectively.

2.7. Investigation of enzymatic antioxidants

Peroxidase (POX), ascorbate peroxidase (APX), glutathione peroxidase (GPX) and Superoxide dismutase (SOD) activities were measured according to Fina (2017), Naudts (2014), Hamed et al. (2017), El-Soud et al. (2013) and Sinha et al. (2015). These enzyme activities were determined by homogenizing (MagNA Lyser, Germany) freeze-dried leaves in 1 mL of 50 mM potassium phosphate buffer (pH 7.0) containing 10% polyvinyl pyrrolidone (PVP), 0.25% Triton X-100, 0.001 M polymethylsulfonyl fluoride (PMSF) and 0.001 M ascorbate. POX, APX, GPX and SOD were expressed in μmol oxidized pyrogallol, μmol ascorbate, μmol NADPH and Unit SOD (the amount of enzyme required to inhibit the autooxidation of nitro blue tetrazolium by 50%) per mg (protein) min, respectively. POX activity was determined by the oxidation of pyrogallol. APX activity was measured as the decrease in ascorbate absorbance measured at 240 nm. GPX activity was assayed by measuring the decrease in NADPH absorbance measured at 340 nm. While SOD activity was determined by measuring the inhibition of NBT reduction at 560 nm. All activity measurements were scaled down for semi-high throughput analysis using a microplate reader (Synergy Mx, Biotek Instruments Inc., Germany).

2.8. Determination of lipoxygenase (15-LOX) inhibition

The linoleic acid was used as a substrate and 15-LOX as an enzyme to evaluate the anti-lipoxygenase. The extracts (100 mg/mL of 80% ethanol) of each BW sprout type were subjected to laser light and the control was performed as well, whereas 10 μL from each extract were mixed with 90 μL of 15-LOX (400 U/mL) and kept in dark condition for 5 min at 25 °C. To start the reaction, 100 μL of linoleic acid solution (0.4 mM) were added to each well, then the reaction was maintained in dark condition for 20 min at 25 °C. Afterwards, we added 100 μL of freshly prepared ferrous orange xylenol (FOX) reagent containing (90% methanol, 10 μM $FeSO_4$, 100 μM xylenol orange, 30 mM H_2SO_4). The reaction was incubated for 30 min at 25 °C, then the absorbance was measured at 560 nm. The percentage of inhibition was calculated using the following formula: % inhibition = $100 - ((OD_{sample} - OD_{blank}/OD_{control}) \times 100)$.

Table 1
Fresh weight (g) of and contents of pigments and minerals in control and laser light-treated common buckwheat (CBW) and tartary buckwheat (TBW) sprouts. Data are represented by the means of at least 3 replicates ± standard deviations.

Parameters	Common buckwheat		Tartary buckwheat	
	Control	Laser-treated	Control	Laser-treated
Growth (Fresh weight, g)	0.12 ± 0.02^{a}	0.15 ± 0.03^{a}	0.09 ± 0.0259^{b}	0.22 ± 0.04^{a}
Pigments (mg/g FW)				
Chlorophyll *a*	1.14 ± 0.40^{a}	1.10 ± 0.10^{a}	1.17 ± 0.36^{a}	1.40 ± 0.39^{a}
Chlorophyll *b*	0.39 ± 0.02^{a}	0.45 ± 0.04^{a}	0.43 ± 0.04^{a}	0.41 ± 0.26^{a}
α-Carotene	0.06 ± 0.01^{b}	0.09 ± 0.002^{ab}	0.06 ± 0.02^{b}	0.12 ± 0.03^{a}
β-Carotene	0.05 ± 0.003^{b}	0.07 ± 0.01^{ab}	0.06 ± 0.001^{b}	0.13 ± 0.57^{a}
Lutein	0.17 ± 0.00^{b}	0.39 ± 0.06^{a}	0.23 ± 0.01^{b}	0.40 ± 0.04^{a}
Neoxanthin	0.01 ± 0.002^{b}	0.02 ± 0.002^{ab}	0.01 ± 0.002^{ab}	0.03 ± 0.01^{a}
Violaxanthin	0.05 ± 0.006^{b}	0.11 ± 0.01^{a}	0.05 ± 0.005^{b}	0.10 ± 0.01^{a}
Minerals (mg/g DW)				
Ca	0.55 ± 0.23^{bc}	0.99 ± 0.21^{b}	0.19 ± 0.07^{c}	1.57 ± 0.12^{a}
Cu	0.23 ± 0.05^{ab}	0.33 ± 0.05^{a}	0.082 ± 0.02^{b}	0.43 ± 0.15^{a}
Fe	0.07 ± 0.01^{b}	0.13 ± 0.02^{a}	0.02 ± 0.00^{c}	0.06 ± 0.01^{b}
K	5.25 ± 0.42^{a}	5.13 ± 0.81^{a}	4.42 ± 1.01^{a}	6.08 ± 1.58^{a}
P	4.10 ± 0.75^{c}	8.17 ± 1.62^{ab}	5.29 ± 1.11^{bc}	9.72 ± 1.32^{a}
Na	2.12 ± 1.25^{a}	2.91 ± 0.31^{a}	2.98 ± 0.15^{a}	3.05 ± 0.81^{a}
Zn	0.59 ± 0.18^{b}	0.96 ± 0.06^{a}	0.21 ± 0.04^{c}	0.77 ± 0.20^{ab}
Mn	0.14 ± 0.03^{b}	0.29 ± 0.05^{a}	0.11 ± 0.02^{b}	0.44 ± 0.10^{a}
Mg	0.65 ± 0.20^{ab}	0.91 ± 0.26^{ab}	0.44 ± 0.15^{b}	1.52 ± 0.64^{a}

Different small letter superscripts (a, b, c…) within a row indicate significant differences between control and laser-treated samples at $p < 0.05$.

2.9. Determination of cyclooxygenase-2 inhibition

A total of three replicates were used from each type of BW sprouts subjected to laser light and the control. The experiments were conducted according to the manufacturer's instructions. The effect of 80% ethanol sprouts extract on cyclooxygenase-2 activity was performed by using the kit of COX assay (Cayman chemical company, Ann Arbor, MI (USA). The microtitre plate (96-well) was covered with plastic film and maintained at room temperature on an orbital shaker for 18 h. According to the manufacturer's instructions, the microtitre plate was incubated in a dark condition for 90 min at 25 °C. Then, the reading of the plate was performed at 420 nm. Calculation of the inhibition of enzyme activity was conducted using the following formula: % inhibition $=100 - ((OD_{sample} - OD_{blank}/OD_{control}) \times 100)$.

2.10. Statistical analyses

Statistical analyses were performed using the SPSS statistical

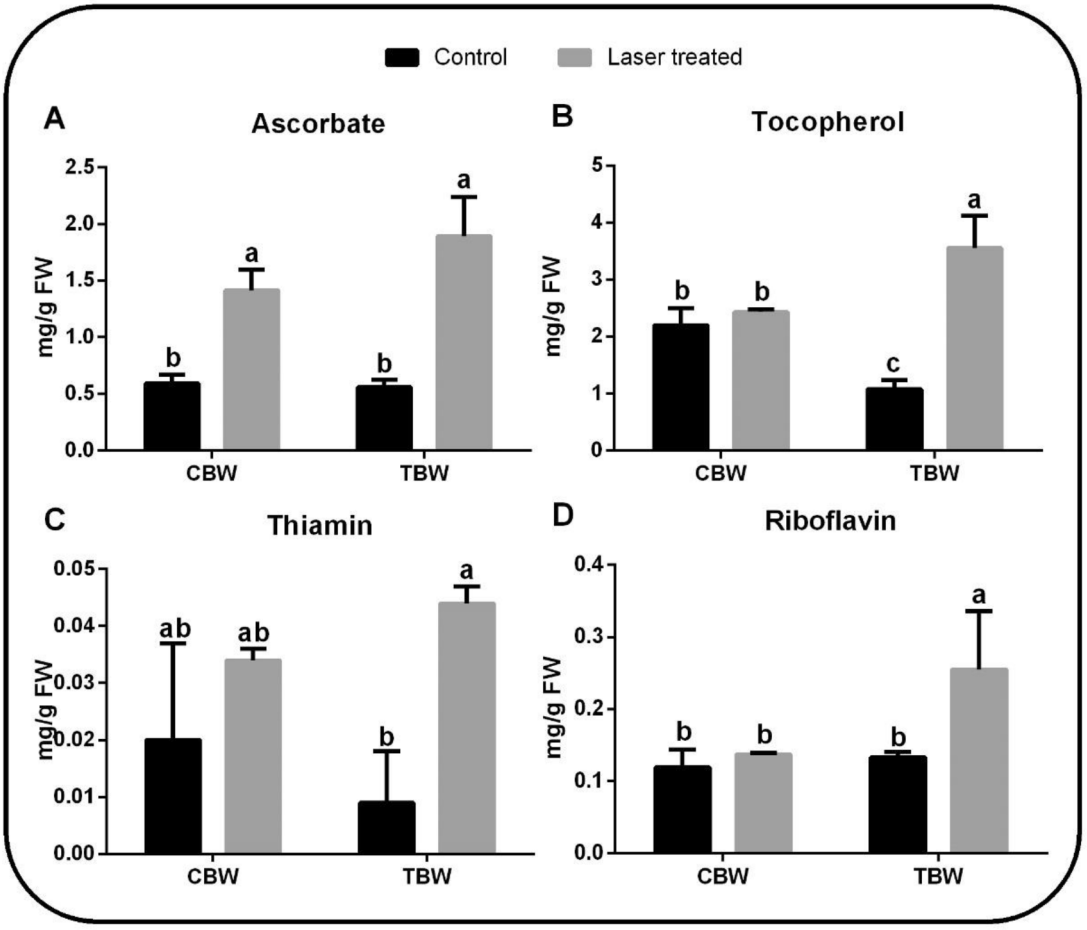

Fig. 1. Vitamin contents in control and laser light-treated common buckwheat (CBW) and tartary buckwheat (TBW) sprouts. A) Ascorbate, B) Tocopherols, C) Thiamin and D) Riboflavin. Data are represented by the means of at least 3 replicates and error bars represent standard deviations. Different small letters above bars indicate significant differences between means at $p < 0.05$.

package (SPSS Inc., Chicago, IL, USA). One-way Analysis of Variance (ANOVA) was applied to all data. Tukey's Test ($P < 0.05$) was carried out as the post-hoc test for mean separations. Each experiment was replicated at least three times (n = 3–5). Hierarchical clustering (Pearson correlation) was generated by the Multi-Experimental Viewer (TM4 software package).

3. Results and discussion

3.1. Laser treatment improved the growth of BW sprouts

At the species level, the traits of sprout growth of CBW were significantly higher than those of TBW. Compared to TBW grown under control condition, CBW showed high dry and fresh weights, larger seed size and high sprouts from its seeds. In agreement with the study of Shin et al. (2010), fresh weight (FW) of CBW was higher under the control condition. **The increased FW of CBW can be explained by larger seeds that tend to do better in germination and growth** (Perveen et al., 2010).

Laser light has been considered as a stimulating factor for seed germination and plant growth. When laser light is absorbed by phytochromes at a certain wavelength, it tends to increase the internal energy of seeds by converting light energy into chemical energy (Chen, Yue & Wang, 2005), which consequently increases cell pumping and enhances the electro potential of biomembranes (Chen, Yue & Wang, 2005). Accordingly, the produced energy is utilized in enhancing the germination rate, inducing the thermodynamic properties, and physiological and biochemical processes. As well, this energy accelerates cell division and improves enzymatic activities such as amylase and protease, all of which that end up with an improved growth and higher plant yields (Asghar, Jamil, Iqbal & Abbas, 2016; Chen, Yue & Wang, 2005; Perveen et al., 2010). Supporting such a concept, the current results showed that the growth (FW) of both laser light treated CBW and TBW sprouts was improved, such improvement was significant ($p < 0.05$) in the case of TBW (Table 1). In addition, TBW sprouts exhibited higher biomass accumulation than CBW sprouts.

In agreement, several studies have previously investigated the enhancing effects of laser light on plant growth and biomass accumulation, such as sunflower (Perveen et al., 2011), *Isatis indogotica* (Chen, Yue & Wang, 2005), fennel and coriander (Osman, El-Tobgy, & El-Sherbini, 2009). Similar to laser light treatment, lower concentrations of NaCl and silicon enhanced the growth of CBW and/or TBW sprouts (Lim et al., 2012, Azad et al., 2020). The observed induction in plant growth after laser light treatment can be explained by that the laser light increased net photosynthetic activity (Perveen et al., 2011, Chen, Yue & Wang, 2005). In this context, the higher the photosynthetic rate, the more the biosynthesis of sugars and organic acids, and thus the higher biomass production (Li et al., 2017).

3.2. Laser light stimulated higher photosynthetic pigments levels

Laser light could affect the growth of sprouts, either directly through enhancing germination and thermodynamic parameters, or indirectly through affecting the photosynthetic process. In this regards, laser light

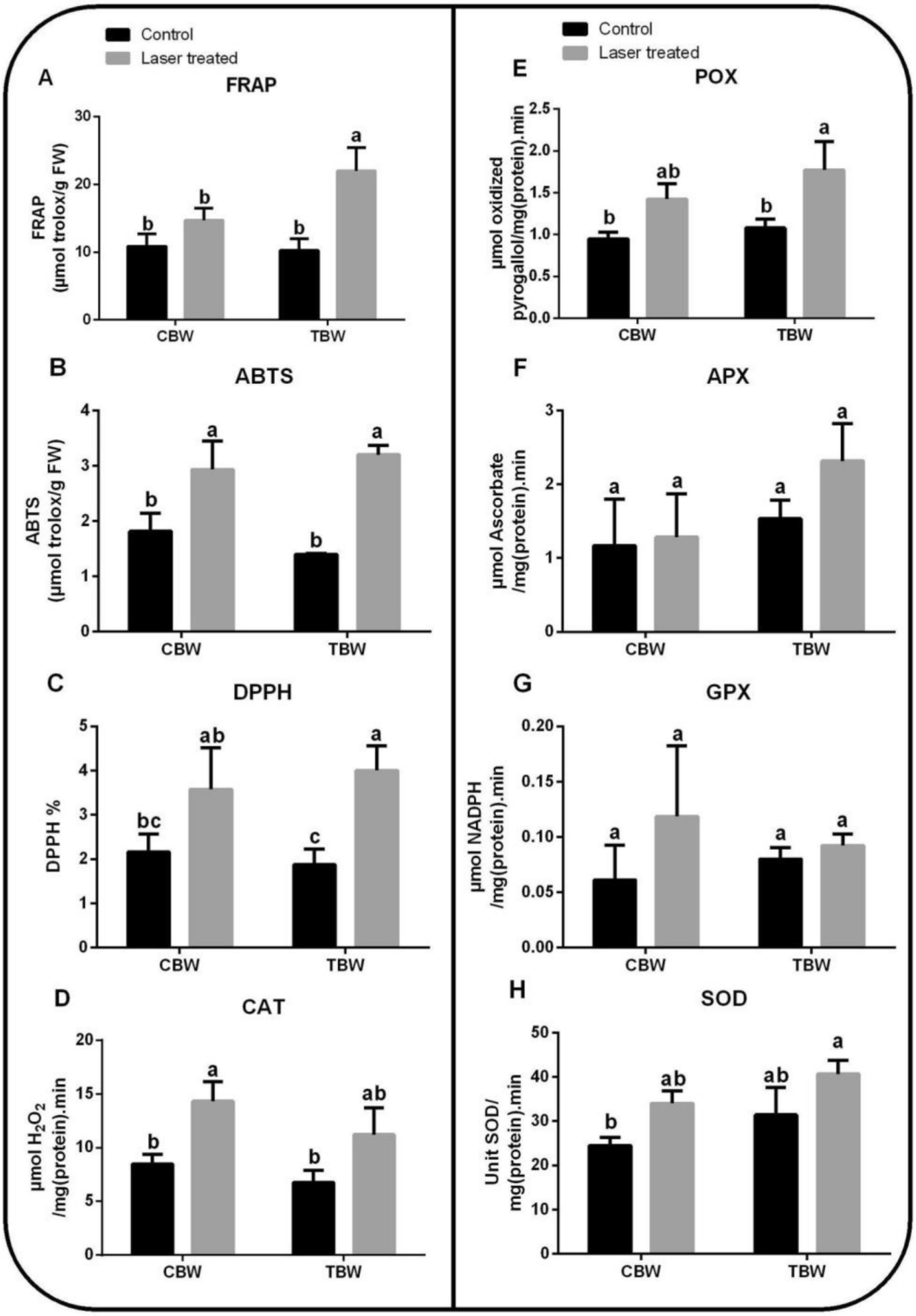

Fig. 2. The overall antioxidant capacity (A-C) and antioxidant enzyme activities (D-H) of control and laser light-treated common buckwheat (CBW) and tartary buckwheat (TBW) sprouts. A) Ferric reducing antioxidant power (FRAP), B) 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and C) Diphenylpicrylhydrazyl (DPPH), D) Catalase (CAT), E) Peroxidase (POX), F) Ascorbate peroxidase (APX), G) Glutathione peroxidase (GPX), and H) Superoxide dismutase (SOD). Data are represented by the means of at least 3 replicates and error bars represent standard deviations. Different small letters above bars indicate significant differences between means at $p < 0.05$.

has been also reported to enhance chlorophyll content (chlorophyll *a* and *b*) in many plants such as *Isatis indogotica* (Chen, Yue & Wang, 2005), soybean (Asghar, Jamil, Iqbal & Abbas, 2016) and sunflower (Perveen et al., 2011). Moreover, the photon energy of laser radiation is absorbed by chlorophyll and directly affects the photosynthetic intensity (Aladjadjiyan, 2007). In the present study, there were insignificant increases in chlorophyll *a* and *b*, but significant elevations in all carotenoids measured in both CBW and TBW sprouts subjected to laser light

versus control ones (Table 1). Supporting our results, previous studies have reported the positive effect of different light sources on chlorophyll accumulation in 9-day-old BW sprouts (Nam, Lim, & Eom, 2018). Carotenoids have been known to play a key role in the photosynthesis process, so carotenoid biosynthesis is supposed to be affected by light quality and intensity (Wu, Gao & Zhang, 2007).

In our study, lutein seems to be the dominant carotenoid, followed by alpha-carotene in all laser-treated and non-treated BW sprouts of both types. It has been previously observed by Tuan et al. (2013) that BW sprouts grown under light conditions had a high content of total carotenoids, whereas lutein and β-carotene were the major carotenoids. The accumulation of carotenoids has been attributed to the up-regulation of the genes involved in carotenoid biosynthesis, which indicates the essential role of light in such process (Tuan et al., 2013). Similar reports have demonstrated that BW sprouts-treated with other elicitors, such as sucrose, had higher contents of β-carotene (Jeong et al., 2018). Carotenoids have been well documented as important nutrients, being a precursor for vitamin A. Therefore, a diet containing carotenoids can help in lowering the risk of many diseases (Tuan et al., 2013).

3.3. Accumulation of vitamins and minerals indicates the high nutritive value of laser light-treated BW sprouts

The deficiency of mineral elements and vitamins has been strongly associated with negative effects on human health. In this sense, sprouts have been known as a rich source of bioavailable mineral elements such as Fe, Zn, Mn, Mg, Cu and Ca (Sun Lim et al., 2001). Therefore, increasing the mineral element contents of sprouts, such as BW sprouts, by employing physical methods such as laser light, might improve the nutritional and health-promoting effects of BW sprouts.

The results of the present investigation have shown that most of the measured elements have significantly increased ($p < 0.05$) in both CBW and TBW sprouts after laser light treatment, in comparison to the non-irradiated sprouts (Table 1). The highest concentrations were recorded for K, P and Na in the laser-treated species. Supporting our results, laser light has previously enhanced the accumulation of K, Ca and Mg in sunflower (Perveen et al., 2011), P and Mo in alfalfa (Cwintal, Dziwulska-Hunek, & Wilczek, 2010) and N, P and K in fennel and coriander (Osman, El-Tobgy, & El-Sherbini, 2009). It was suggested that the energy produced from the absorption of laser light by the plant cell could stimulate plant growth, metabolism and nutrient uptake (Dinani et al., 2019). In this regard, laser light has been assumed to induce changes in mitochondria, which consequently cause an increase in ATP production. Such a process of energy production is necessary for plants to absorb minerals (Dinani et al., 2019; Wu et al., 2007). Higher mineral content in laser light treated *Moringa oleifera* particularly in roots was explained by increased root growth which consequently increased mineral uptake and accumulation (Shafique et al., 2017). Thus, laser light treatment could have pronounced effects on the mineral profile of sprouts through providing high energy needed for the growth of sprout roots which induces mineral uptake.

Concerning vitamins, we demonstrated that laser light exposure significantly enhanced ($p < 0.05$) all targeted vitamins [ascorbate (Vit-C), tocopherol (Vit-E), thiamin (Vit-B1) and riboflavin (Vit-B2)] contents, particularly in TBW sprouts and to less extent in CBW sprouts (Fig. 1). In addition to their nutritive value, vitamins such as ascorbate and tocopherols have high antioxidant activity. Such laser-induced increments in vitamin content could be ascribed to the increased photosynthetic activity, which leads to increased carbon allocation and excess non-structural carbohydrates, that in turn, could be used as precursors for the synthesis of secondary metabolites and antioxidants such as vitamins (Li et al., 2017). Moreover, the stimulation of vitamin and antioxidant production was due to the increasing energy supply of seeds by laser light treatment, which is later transformed into a chemical one and accelerated the vital processes during sprouting such as intensive molecular transformations (Aladjadjiyan, 2007).

Table 2
Contents of phenolics and total glutathione in control and laser light-treated common buckwheat (CBW) and tartary buckwheat (TBW) sprouts. Data are represented by the means of at least 3 replicates ± standard deviations.

Parameters	Common buckwheat		Tartary buckwheat	
	Control	Laser-treated	Control	Laser-treated
Phenolic compounds (mg/100 g FW)				
Total phenols	5.82 ± 1.0^{b}	12.19 ± 1.98^{a}	6.52 ± 1.09^{b}	10.14 ± 3.45^{ab}
Total flavonoids	1.34 ± 0.40^{b}	3.72 ± 0.92^{ab}	1.99 ± 0.55^{b}	5.46 ± 1.69^{a}
Phenolic acids (mg/100 g FW)				
Galic acid	0.02 ± 0.0^{b}	0.04 ± 0.0^{ab}	0.029 ± 0.0^{b}	0.055 ± 0.0^{a}
Caffeic acid	0.8 ± 0.01^{a}	1.20 ± 0.4^{a}	1.20 ± 0.1^{a}	1.50 ± 0.8^{a}
Ferulic acid	0.34 ± 0.0^{b}	0.6 ± 0.1^{ab}	0.4 ± 0.07^{b}	0.85 ± 0.1^{a}
Protocatechuic acid	0.046 ± 0.01^{a}	0.038 ± 0.0^{a}	ND	ND
p-Coumaric acid	1.2 ± 0.2^{b}	2.08 ± 0.3^{ab}	1.6 ± 0.3^{b}	1.9 ± 0.5^{a}
Sinapic acid	ND	ND	ND	0.001 ± 0.0^{a}
Flavonoids (mg/100 g FW)				
Catechin	0.97 ± 0.5^{a}	1.6 ± 0.8^{a}	1.21 ± 0.070^{a}	2.28 ± 1.3^{a}
O-hydroxydaidzein	0.03 ± 0.00^{b}	0.52 ± 0.1^{a}	ND	ND
Luteolin	0.05 ± 0.00^{b}	0.15 ± 0.002^{a}	0.07 ± 0.01^{b}	0.17 ± 0.0^{a}
Apigenin	0.1 ± 0.02^{c}	0.22 ± 0.00^{b}	0.14 ± 0.01^{bc}	0.39 ± 0.01^{a}
Quercetin	0.8 ± 0.001^{a}	1.1 ± 0.001^{a}	1.1 ± 0.0016^{a}	1.8 ± 0.00^{a}
Isoquercetrin	0.04 ± 0.02^{a}	0.11 ± 0.06^{a}	0.05 ± 0.01^{a}	0.04 ± 0.01^{a}
Ellagic acid	0.08 ± 0.004^{a}	0.24 ± 0.01^{a}	0.1 ± 0.005^{a}	0.3 ± 0.016^{a}
Velutin	ND	0.01 ± 0.01^{b}	0.06 ± 0.04^{ab}	0.18 ± 0.1^{a}
Vitexin	4.0 ± 0.7^{b}	6.9 ± 0.9^{b}	0.7 ± 0.0^{ab}	0.46 ± 0.0^{a}
Isovitexin	7. 0 ± 0.2^{a}	1. 1 ± 0.08^{a}	0. 1 ± 0.05^{a}	0.17 ± 0.01^{a}
Orientin	1.1 ± 0.00^{b}	3.0 ± 0.01^{a}	0.2 ± 0.0^{b}	0.4 ± 0.06^{a}
Isoorientin	0. 9 ± 0.0^{b}	2. 1 ± 0.0^{a}	ND	ND
Rutin	497 ± 3.8^{d}	749 ± 11^{c}	845 ± 33^{b}	1022 ± 27^{a}
Glutathione (mg/gFW)				
Total glutathione	0.09 ± 0.007^{b}	0.12 ± 0.03^{ab}	0.09 ± 0.01^{b}	0.17 ± 0.01^{a}

ND: not detectable.
Different small letter superscripts (a, b, c…) within a row indicate significant differences between control and laser-treated samples at $p < 0.05$.

In this regard, BW sprouts have been considered as a rich source of Vit-B1, B2, B6, C and E (Kim, Kim, & Park, 2004; Zhang et al., 2012). Generally, TBW contains higher concentrations of Vit-B (Bonafaccia, Gambelli, Fabjan, & Kreft, 2003) and tocopherol (Kim, Kim & Park, 2002) than CBW. Moreover, light conditions increased Vit-C levels in both TBW and CBW sprouts (Kim et al., 2006). Similarly, laser light has been revealed to promote the ASC contents in soybean (Asghar, Jamil, Iqbal & Abbas, 2016). It was also reported that seed germination stimulated a high level of Vit-C and B1 content in TBW. Furthermore, it has been found that BW sprouts treated with other elicitors, such as sucrose, had higher contents of tocopherol than the control sprouts (Jeong et al., 2018), while the elicitation with both sucrose and $CaCl_2$ effectively enhanced the accumulation of vitamin C and E (Sim et al., 2020).

3.4. Laser light improved the antioxidant activity of BW sprouts, particularly TBW

High antioxidant activities as indicated by increased DPPH, FRAP and ABTS levels were recorded for BW sprouts (Zych-Wężyk & Krzepiłko, 2012). Similarly, we found that BW sprouts showed high overall antioxidant capacity, which was further induced by laser treatment. That enhancement was significant ($p < 0.05$) in the case of DPPH, FRAP

and ABTS in TBW sprouts and ABTS in CBW sprouts (Fig. 2). Similarly, the application of other biophysical treatments, such as UV irradiation (>300 nm for 24 h at room temperature) increased the DPPH activity of 7th days old BW sprouts by about 1.6 fold (Tsurunaga et al., 2013). In this context, TBW had a higher antioxidant capacity than CBW (Liu et al., 2008). Moreover, BW sprouts-treated with sucrose had significantly higher DPPH and ABTS antioxidant potential than control sprouts (Jeong et al., 2018).

3.5. Laser light induced the production of antioxidant metabolites in BW sprouts

The elevated levels of polyphenolic compounds, especially flavonoids, in BW sprouts are being reflected on their higher antioxidant activities (Lee et al., 2016). Sprouting is means of increasing active primary and secondary metabolites as seeds undergo several physiological and morphological changes. Active phytochemicals such as phenols and flavonoids increase gradually during sprouting processes in BW species, however, the number and concentration of such metabolites may differ due to genetic differences and expression of the biosynthesis-related gene (Rauf et al., 2019). For instance, TBW sprouts contain higher flavonoids, e.g. rutin content, than that of CBW sprouts (Rauf et al., 2019).

The present results revealed that the non-laser treated sprouts of both CBW and TBW had almost similar total phenols and flavonoids contents

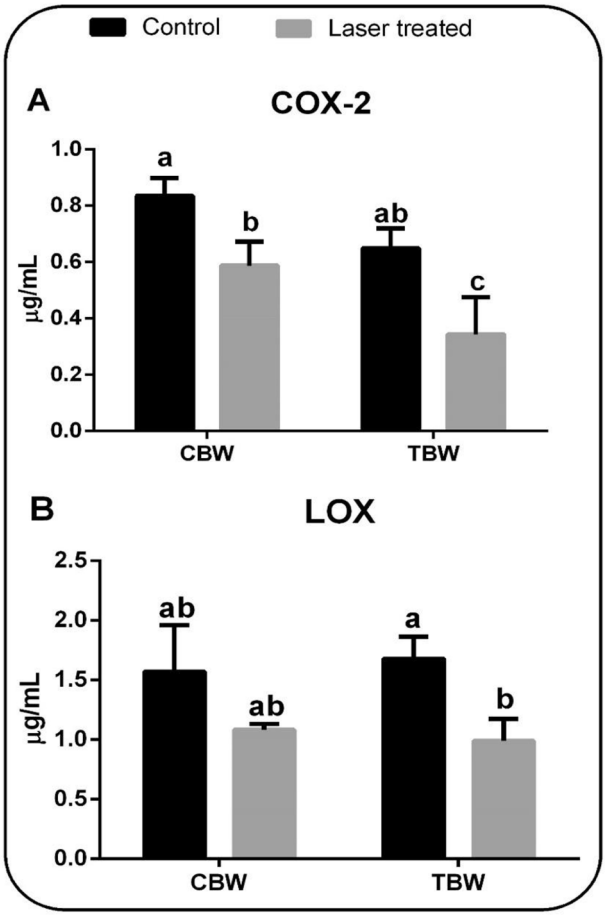

Fig. 3. The anti-inflammatory activity of control and laser light-treated common buckwheat (CBW) and tartary buckwheat (TBW) sprouts represented by A) Cyclooxygenase-2 (COX-2) and B) Lipoxygenase (LOX) activities. Data are represented by the means of at least 3 replicates and error bars represent standard deviations. Different small letters above bars indicate significant differences between means at $p < 0.05$.

(Table 2), the result which is in line with other previous studies (Kim et al., 2008). Meanwhile, the total phenols and total flavonoids were significantly enhanced under laser light treatment in both sprout types ($p < 0.05$), whereas TBW sprouts had higher flavonoid and lower phenolic contents than CBW sprouts, in response to laser exposure (Table 2). This also concurs with the reported enhancing effects of laser light on the total phenolic content of some plants such as soybean (Asghar, Jamil, Iqbal & Abbas, 2016) and sunflower (Perveen et al., 2011). Similarly, the application of other elicitors, such as methyl jasmonate (Kim, Park, & Lim, 2011) and silicon biostimulant (Azad et al., 2020), to BW sprouts resulted in significant increases in the total amount of phenolics and antioxidant capacity.

In general, antioxidant phenols and flavonoids individuals, such as rutin has been shown to be the principal contributor to the antioxidative capacity of BW sprouts, where a strong correlation has been found between rutin content and DPPH radical scavenging activity (Tsurunaga et al., 2013). According to our results, catechin was quantified as the most prominent phenolic acid (about 100% increase in comparison to control sprouts), followed by p-coumaric acid (80% increase compared with untreated sprouts); while rutin was the predominant flavonoid in both BW sprouts (3-fold change comparing to control sprouts), treated and non-treated with laser light (Table 2). There were significant increments in the majority of all the detected phenolic acids and flavonoids following laser treatment. In TBW sprouts, sinapic acid and isovitexin were found to appear in response to laser irradiation, while protocatechuic acid and O-hydroxydaidzein were not detected even after laser treatment. Similarly, in CBW sprouts, velutin was stimulated to appear under laser light treatment, while isoorientin was not detected following laser exposure. So, laser light might act like a stress factor that promotes the appearance of some phenolic compounds. In agreement with our results, some of the detected compounds here have been previously identified in CBW sprouts, such as chlorogenic acid, catechin, orientin, vitexin, isovitexin, rutin and quercetin, while only rutin, along with low concentrations of quercetin were detected in TBW sprouts as reported by Kim et al. (2008). Rutin was reported as the major flavonoid and a determinant factor of the nutritional value of BW sprouts, being higher in TBW sprouts than in CBW sprouts (Kim et al., 2008), also it was reported to increase in 9-old day BW sprouts (Nam et al., 2018). While, no significant differences were reported for vitexin and isovitexin contents of 9-old day BW sprouts grown under different light conditions (Nam et al., 2018). The presence of these antioxidant flavonoids such as quercitrin reportedly depends on environmental factors, such as light intensity (Kim, Kim, & Park, 2004). Similarly, it has been observed that BW sprouts treated with certain concentrations of NaCl significantly increased the accumulation of phenolic compounds such as isoorientin, orientin, rutin, and vitexin, and consequently their antioxidant activity (Lim et al., 2012). Furthermore, methyl jasmonate-treated CBW sprouts have been reported to exhibit higher levels of antioxidant capacity and phenolic compounds, such as chlorogenic acid, catechin, isoorientin, orientin, rutin, vitexin, and quercitrin (Kim et al., 2011). The elicitation of BW sprouts with sucrose and $CaCl_2$ (Sim et al., 2020), indole-3-acetic and gibberellic acids (Park et al., 2017), as well as silicon biostimulant (Azad et al., 2020) have resulted in increases in flavonoids and phenolic compound contents.

Additionally, the present results also showed significant increments in the activities of total GSH in the laser-treated BW sprouts of the two species comparing with controls (Table 2). Meanwhile, laser-treated TBW sprouts displayed a higher total GSH than that of CBW sprouts.

In a broader sense, several reports have highlighted the positive effects of various physical factors and light sources, such as light-emitting diodes (LEDs) and UV, on the production of secondary metabolites, especially flavonoids in BW sprouts (Lee et al., 2014; Tsurunaga et al., 2013; Tuan et al., 2013). It has been also found that BW sprouts exposed to LED light increased the accumulation of rutin and cyanidin 3-O-rutinoside (Thwe et al., 2014). Moreover, the application of LED light together with l-phenylalanine, to TBW sprouts has been reported to

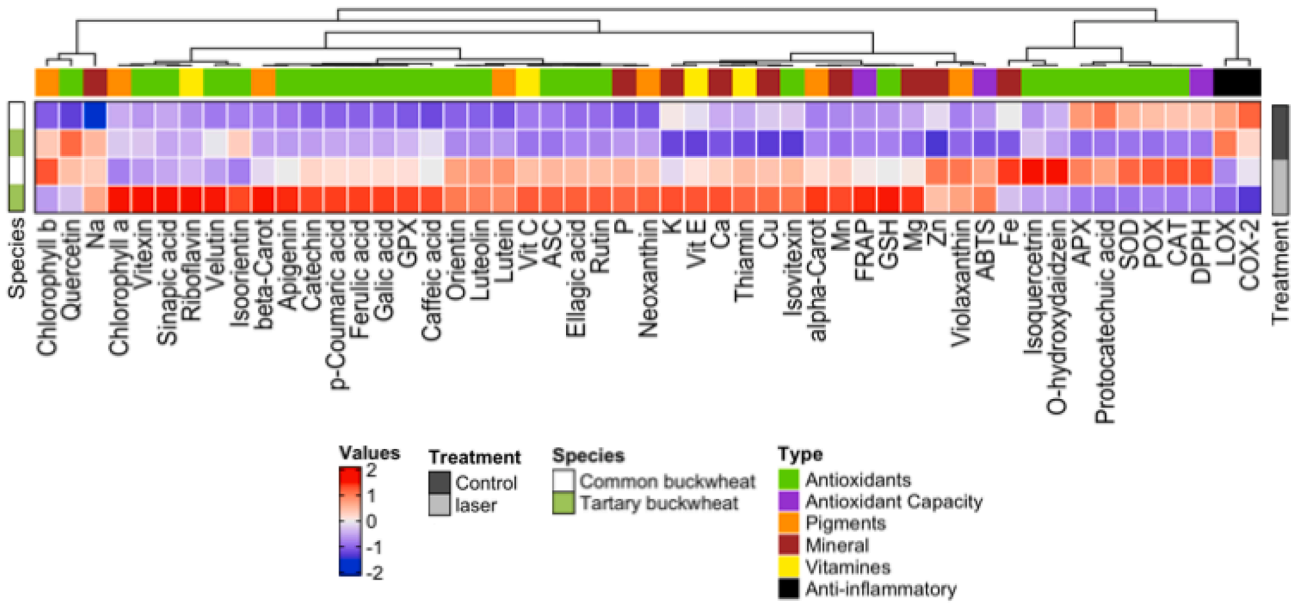

Fig 4. Species-specific responses of common and tartary buckwheat sprouts to the effect of laser light treatment on the nutritional and health-promoting characteristics. The measured parameters are represented by antioxidant metabolites and enzymes, overall antioxidant capacity, contents of pigments, minerals and vitamins and anti-inflammatory activity. Data are represented by the means of at least 3 replicates.

increase their contents of phenolic compounds such as chlorogenic acid and rutin (Seo et al., 2015). Such increments in flavonoid contents have been ascribed to the increased enzymatic activities of phenylalanine ammonia-lyase (PAL) and tyrosine ammonia-lyase (TAL) that are incorporated in the phenylpropanoid pathway (Lim et al., 2012). Following the same principle, treatment of BW sprouts with elicitors such as sucrose (Jeong et al., 2018) or methyl jasmonate (Kim et al., 2011) could significantly increase the flavonoid contents and antioxidant potential of BW sprouts.

3.6. Laser light enhanced the activities of antioxidant enzymes in BW sprouts

In addition to the laser irradiation role in the induction of antioxidant metabolites, it has been employed to stimulate the activity of antioxidant enzymes (Chen & Wang, 2003). Herein, we found that all the measured antioxidant enzymes were markedly stimulated in the two species irradiated with the laser, being much higher in TBW sprouts (Fig. 2). In agreement with our results, laser light has been reported to trigger the activities of POX, APX, SOD and CAT in wheat (Qiu, Liu, Tian, & Yue, 2008), and SOD, POX, APX and CAT in soybean (Asghar, Jamil, Iqbal & Abbas, 2016). Also, the use of other physical methods such as microwave irradiation was reported to increase CAT and SOD activities in BW sprouts (Wang, Wang & Guo, 2018). Furthermore, it has been observed that treatment of BW sprouts with sucrose significantly enhanced the enzymatic activities of CAT, GR, SOD, and GPX (Jeong et al., 2018).

3.7. High antioxidants increased the anti-inflammatory activity of laser-treated BW sprouts

Rutin was reported to have a wide range of biological properties, such as anti-inflammatory, antitumor, antibacterial, antiprotozoal and antiviral activities (Calabro et al., 2005). Therefore, BW sprouts have been well known for their health-promoting values.

To test the effect of laser treatment on the anti-inflammatory activity of BW sprouts, we measured cyclooxygenase (COX-2) and lipoxygenase (LOX) activities, which are known as inflammatory markers. Herein, we determined the COX-2 and LOX activities of laser light treated and non-treated BW sprouts (Fig. 3). Apparently, laser light led to a significant reduction in the activities of COX-2 and LOX of both CBW and TBW sprouts, which indicates an enhancement in the anti-inflammatory activities. Similarly, BW sprouts have previously exerted strong anti-inflammatory effects, by inhibiting some inflammatory mediators such as COX-2 (Karki, Park, & Kim, 2013), and inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-a (TNF-a) (Karki, Park, & Kim, 2013). The anti-inflammatory properties of BW sprouts have been basically ascribed to their rich content of phenolic compounds particularly rutin (Karki, Park, & Kim, 2013), and probably quercetin, which was reported to inhibit low-density lipoprotein (LDL)-induced inflammatory events by modulating TLR4- and TLR2-induced NF-κB activation (Bhaskar, Shalini & Helen, 2011).

3.8. Species-specific response to laser light treatment

The hierarchical clustering data in Fig. 4 confirms that there was an obvious sprout species-specific response to the effect of laser light treatment. TBW sprouts showed a more prominent response to the positive effect of laser light than CBW sprouts. The variations among the two species could be ascribed to the ontogeny and species diversity. In agreement, relative to CBW sprouts, TBW showed higher levels of bioactive compounds (Kim et al., 2008). For instance, TBW contains higher tocopherol (Kim, Kim & Park, 2002) and Vit-B (Bonafaccia, Gambelli, Fabjan, & Kreft, 2003) content as well as overall antioxidant capacity (Liu et al., 2008) than CBW.

4. Conclusions

Our findings illustrated the potentiality of using laser light treatment as a successful way to produce BW sprouts with high nutritional and health-promoting values. The accumulation of vitamins and minerals and the induction of antioxidant and anti-inflammatory activities by laser treatment could make laser-treated BW sprouts, particularly TBW, an excellent dietary source. Our results, combined with those previously obtained from the application of other biophysical methods in enhancing plant productivity, could effectively support the use of laser irradiation as a promising approach for increasing the bioactive metabolites and health-promoting values of sprouts.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This project was funded by the Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah, Under grant No. (D-209-140-1441). The authors, therefore, gratefully acknowledge DSR technical and financial support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2020.128788.

References

AbdElgawad, H., De Vos, D., Zinta, G., Domagalska, M. A., Beemster, G. T. S., & Asard, H. (2015). Grassland species differentially regulate proline concentrations under future climate conditions: An integrated biochemical and modelling approach. *New Phytologist, 208*(2), 354–369.

Aladjadjiyan, A. (2007). The use of physical methods for plant growing stimulation in Bulgaria Journal of Central European. *Agriculture, 8*(3), 369–380.

Alam. (2016). The protective role of Ulva lactuca against genotoxic and biochemical effects induced by γ-irradiation in rats. *International Journal of Pharmaceutical Sciences Review and Research, 37*(2), 40–48.

Almuhayawi, M., AbdElgawad, H., Al Jaouni, S., Selim, S., Hassan, A. H. A., & Khamis, G. (2020). Elevated CO_2 improves glucosinolate metabolism and stimulates anticancer and anti-inflammatory properties of broccoli sprouts. *Food Chemistry*, 127102.

Asghar, T., Jamil, Y., Iqbal, M., & Abbas, M. (2016). Laser light and magnetic field stimulation effect on biochemical, enzymes activities and chlorophyll contents in soybean seeds and seedlings during early growth stages. *Journal of Photochemistry and Photobiology B: Biology, 165*, 283–290.

Azad, M. O. K., Park, B. S., Adnan, M., Germ, M., Kreft, I., Woo, S. H., & Park, C. H. (2020). Silicon biostimulant enhances the growth characteristics and fortifies the bioactive compounds in common and Tartary buckwheat plant. *Journal of Crop Science and Biotechnology*, 1–9.

Bachiega, P., Salgado, J. M., de Carvalho, J. E., Ruiz, A. L. T. G., Schwarz, K., Tezotto, T., et al. (2016). Antioxidant and antiproliferative activities in different maturation stages of broccoli (Brassica oleracea Italica) biofortified with selenium. *Food Chemistry, 190*, 771–776.

Benzie, I. F., & Szeto, Y. T. (1999). Total antioxidant capacity of teas by the ferric reducing/antioxidant power assay. *Journal of Agricultural and Food Chemistry, 47*(2), 633–636.

Bhaskar, S., Shalini, V., & Helen, A. (2011). Quercetin regulates oxidized LDL induced inflammatory changes in human PBMCs by modulating the TLR-NF-κB signaling pathway. *Immunobiology, 216*(3), 367–373.

Bonafaccia, G., Gambelli, L., Fabjan, N., & Kreft, I. (2003). Trace elements in flour and bran from common and tartary buckwheat. *Food Chemistry, 83*(1), 1–5.

Calabro, M. L., Tommasini, S., Donato, P., Stancanelli, R., Raneri, D., Catania, S., et al. (2005). The rutin/β-cyclodextrin interactions in fully aqueous solution: Spectroscopic studies and biological assays. *Journal of Pharmaceutical and Biomedical Analysis, 36*(5), 1019–1027.

Casasole, G., Raap, T., Costantini, D., AbdElgawad, H., Asard, H., Pinxten, R., et al. (2017). Neither artificial light at night, anthropogenic noise nor distance from roads are associated with oxidative status of nestlings in an urban population of songbirds. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 210*, 14–21.

Chen, Y. P., & Wang, X. L. (2003). Study on biochemistry role of He–Ne laser pretreatment seeds of Isatis indogotica. *Chinese Journal of Lasers, 27*, 543–547.

Chen, Y.-P., Yue, M., & Wang, X.-L. (2005). Influence of He–Ne laser irradiation on seeds thermodynamic parameters and seedlings growth of Isatis indogotica. *Plant Science, 168*(3), 601–606.

Chen, Z.-Y., Jiao, R., & Ma, K. Y. (2008). Cholesterol-lowering nutraceuticals and functional foods. *Journal of Agricultural and Food Chemistry, 56*(19), 8761–8773.

Cwintal, M., Dziwulska-Hunek, A., & Wilczek, M. (2010). Laser stimulation effect of seeds on quality of alfalfa. *International Agrophysics, 24*(1), 15–19.

de Sousa, A., Saleh, A. M., Habeeb, T. H., Hassan, Y. M., Zrieq, R., Wadaan, M. A., et al. (2019). Silicon dioxide nanoparticles ameliorate the phytotoxic hazards of aluminum in maize grown on acidic soil. *Science of The Total Environment, 693*, Article 133636.

Dinani, H. J., Eslami, P., Mortazaeinezhad, F., & Ghahrizjani, R. T. (2019, May). Effects of laser radiation on the growth indicators of Kelussia odoratissima Mozaff. Medical plant. In 2019 Photonics North (PN) (pp. 1–8).

Fina. (2017). UV-B inhibits leaf growth through changes in growth regulating factors and gibberellin levels. *Plant Physiology, 174*(2), 1110–1126.

Hamad, I., Abdelgawad, H., Al Jaouni, S., Zinta, G., Asard, H., Hassan, S., et al. (2015). Metabolic analysis of various date palm fruit (Phoenix dactylifera L.) cultivars from Saudi Arabia to assess their nutritional quality. *Molecules, 20*(8), 13620–13641. https://doi.org/10.1007/s12206-017-0606-2.

Hamed, S. M., Zinta, G., Klöck, G., Asard, H., Selim, S., & AbdElgawad, H. (2017). Zinc-induced differential oxidative stress and antioxidant responses in Chlorella sorokiniana and Scenedesmus acuminatus. *Ecotoxicology and Environmental Safety, 140*, 256–263.

Hassan. (2012). Effect of different growth conditions on certain biochemical parameters of different cyanobacterial strains. *Malaysian Journal of Microbiology, 8*(4), 266–272.

Jeong, H., Sung, J., Yang, J., Kim, Y., Jeong, H. S., & Lee, J. (2018). Effect of sucrose on the functional composition and antioxidant capacity of buckwheat (Fagopyrum esculentum M.) sprouts. *Journal of Functional Foods, 43*, 70–76.

Karki, R., Park, C.-H., & Kim, D.-W. (2013). Extract of buckwheat sprouts scavenges oxidation and inhibits pro-inflammatory mediators in lipopolysaccharide-stimulated macrophages (RAW264. 7). *Journal of Integrative Medicine, 11*(4), 246–252.

Khamis, G., Hassan, M., Morsy, M., Ibrahim, M. A., Abd-Elsalam, R. M., El Badawy, S. A., et al. (2020). Innovative application of helium-neon laser: Enhancing the germination of Adansonia digitata and evaluating the hepatoprotective activities in mice. *Environmental Science and Pollution Research*, 1–12.

Kim, S. L., Kim, S. K., & Park, C. H. (2002). Comparisons of lipid, fatty acids and tocopherols of different buckwheat species. *Food Science and Biotechnology, 11*(4), 332–336.

Kim, S.-J., Kawaharada, C., Suzuki, T., Saito, K., Hashimoto, N., Takigawa, S., et al. (2006). Effect of natural light periods on rutin, free amino acid and vitamin C contents in the sprouts of common (Fagopyrum esculentum Moench) and tartary (F. tataricum Gaertn.) buckwheats. *Food Science and Technology Research, 12*(3), 199–205.

Kim, S.-J., Zaidul, I. S. M., Suzuki, T., Mukasa, Y., Hashimoto, N., Takigawa, S., et al. (2008). Comparison of phenolic compositions between common and tartary buckwheat (Fagopyrum) sprouts. *Food Chemistry, 110*(4), 814–820.

Kim, S.-L., Kim, S.-K., & Park, C.-H. (2004). Introduction and nutritional evaluation of buckwheat sprouts as a new vegetable. *Food Research International, 37*(4), 319–327.

Kim, H. J., Park, K. J., & Lim, J. H. (2011). Metabolomic analysis of phenolic compounds in buckwheat (Fagopyrum esculentum M.) sprouts treated with methyl jasmonate. *Journal of Agricultural and Food Chemistry, 59*(10), 5707–5713.

Lee, L.-S., Choi, E.-J., Kim, C.-H., Sung, J.-M., Kim, Y.-B., Seo, D.-H., et al. (2016). Contribution of flavonoids to the antioxidant properties of common and tartary buckwheat. *Journal of Cereal Science, 68*, 181–186.

Lee, S.-W., Seo, J. M., Lee, M.-K., Chun, J.-H., Antonisamy, P., Arasu, M. V., et al. (2014). Influence of different LED lamps on the production of phenolic compounds in common and Tartary buckwheat sprouts. *Industrial Crops and Products, 54*, 320–326.

Li, P., Li, H., Zong, Y., Li, F. Y., Han, Y., & Hao, X. (2017). Photosynthesis and metabolite responses of Isatis indigotica Fortune to elevated [CO2]. *The Crop Journal, 5*(4), 345–353.

Lim, J.-H., Park, K.-J., Kim, B.-K., Jeong, J.-W., & Kim, H.-J. (2012). Effect of salinity stress on phenolic compounds and carotenoids in buckwheat (Fagopyrum esculentum M.) sprout. *Food Chemistry, 135*(3), 1065–1070.

Liu, C.-L., Chen, Y.-S., Yang, J.-H., & Chiang, B.-H. (2008). Antioxidant activity of tartary (Fagopyrum tataricum (L.) Gaertn.) and common (Fagopyrum esculentum Moench) buckwheat sprouts. *Journal of Agricultural and Food Chemistry, 56*(1), 173–178.

Manchali, S., Murthy, K. N. C., & Patil, B. S. (2012). Crucial facts about health benefits of popular cruciferous vegetables. *Journal of Functional Foods, 4*(1), 94–106.

Nam, T. G., Lim, Y. J., & Eom, S. H. (2018). Flavonoid accumulation in common buckwheat (Fagopyrum esculentum) sprout tissues in response to light. *Horticulture, Environment, and Biotechnology, 59*(1), 19–27.

Naudts. (2014). Future climate alleviates stress impact on grassland productivity through altered antioxidant capacity. *Environmental and Experimental Botany, 99*, 150–158.

Osman, Y. A. H., El-Tobgy, K. M. K., & El-Sherbini, E. S. A. (2009). Effect of laser radiation treatments on growth, yield and chemical constituents of fennel and coriander plants. *Journal of Applied Sciences Research, 5*, 244–252.

Perveen, R., Ali, Q., Ashraf, M., Al-Qurainy, F., Jamil, Y., & Raza Ahmad, M. (2010). Effects of different doses of low power continuous wave He–Ne laser radiation on some seed thermodynamic and germination parameters, and potential enzymes involved in seed germination of sunflower (Helianthus annuus L.). *Photochemistry and Photobiology, 86*(5), 1050–1055.

Perveen, R., Jamil, Y., Ashraf, M., Ali, Q., Iqbal, M., & Ahmad, M. R. (2011). He-Ne laser-induced improvement in biochemical, physiological, growth and yield characteristics in sunflower (Helianthus annuus L.). *Photochemistry and Photobiology, 87*(6), 1453–1463.

Qiu, Z.-B., Liu, X., Tian, X.-J., & Yue, M. (2008). Effects of CO2 laser pretreatment on drought stress resistance in wheat. *Journal of Photochemistry and Photobiology B: Biology, 90*(1), 17–25.

Rauf, M., Yoon, H., Lee, S., Lee, M. C., Oh, S., & Choi, Y. M. (2019). Evaluation of sprout growth traits and flavonoid content in common and tartary buckwheat germplasms. *Plant Breeding and Biotechnology, 7*(4), 375–385.

Saleh, A. M., Abdel-Mawgoud, M., Hassan, A. R., Habeeb, T. H., Yehia, R. S., & AbdElgawad, H. (2020). Global metabolic changes induced by arbuscular mycorrhizal fungi in oregano plants grown under ambient and elevated levels of atmospheric CO_2. *Plant Physiology and Biochemistry, 151*, 255–263.

Shafique, H., Jamil, Y., ul Haq, Z., Mujahid, T., Khan, A. U., Iqbal, M., & Abbas, M. (2017). Low power continuous wave-laser seed irradiation effect on Moringa oleifera germination, seedling growth and biochemical attributes. *Journal of Photochemistry and Photobiology B: Biology, 170*, 314–323.

Shin, D. H., Kamal, A. H. M., Suzuki, T., Yun, Y. H., Lee, M. S., Chung, K. Y., et al. (2010). Reference proteome map of buckwheat (Fagopyrum esculentum and Fagopyrum

tataricum) leaf and stem cultured under light or dark. *Australian Journal of Crop Science, 4*, 633–641.
Sim, U., Sung, J., Lee, H., Heo, H., Jeong, H. S., & Lee, J. (2020). Effect of calcium chloride and sucrose on the composition of bioactive compounds and antioxidant activities in buckwheat sprouts. *Food Chemistry, 312*, Article 126075.
Sinha, A. K., AbdElgawad, H., Zinta, G., Dasan, A. F., Rasoloniriana, R., Asard, H., et al. (2015). Nutritional status as the key modulator of antioxidant responses induced by high environmental ammonia and salinity stress in European sea bass (Dicentrarchus labrax). *PLoS ONE, 10*(8), Article e0135091.
El-Soud, W. A., Hegab, M. M., AbdElgawad, H., Zinta, G., & Asard, H. (2013). Ability of ellagic acid to alleviate osmotic stress on chickpea seedlings. *Plant Physiology and Biochemistry, 71*, 173–183.
Sun Lim, K., Young Koo, S., Jong Jin Hwangl, S. K. K., Han Sun, H., & Park, C. H. (2001). Development and utilization of buckwheat sprouts as functional vegetables. Fagopyrum, 18(49), 4.
Sunarić, S., Pavlović, D., Stanković, M., Živković, J., & Arsić, I. (2020). Riboflavin and thiamine content in extracts of wild-grown plants for medicinal and cosmetic use. *Chemical Papers, 74*(6), 1729–1738.
Thayer, S. S., & Björkman, O. (1990). Leaf Xanthophyll content and composition in sun and shade determined by HPLC. *Photosynthesis Research, 23*(3), 331–343. https://doi.org/10.1007/BF00034864.
Thwe, A. A., Kim, Y. B., Li, X., Seo, J. M., Kim, S. J., Suzuki, T., … Park, S. U. (2014). Effects of light-emitting diodes on expression of phenylpropanoid biosynthetic genes and accumulation of phenylpropanoids in Fagopyrum tataricum sprouts. *Journal of Agricultural and Food Chemistry, 62*(21), 4839–4845.
Tsurunaga, Y., Takahashi, T., Katsube, T., Kudo, A., Kuramitsu, O., Ishiwata, M., et al. (2013). Effects of UV-B irradiation on the levels of anthocyanin, rutin and radical scavenging activity of buckwheat sprouts. *Food Chemistry, 141*(1), 552–556.
Tuan, P. A., Thwe, A. A., Kim, Y. B., Kim, J. K., Kim, S.-J., Lee, S., et al. (2013). Effects of white, blue, and red light-emitting diodes on carotenoid biosynthetic gene expression levels and carotenoid accumulation in sprouts of tartary buckwheat (Fagopyrum tataricum Gaertn.). *Journal of Agricultural and Food Chemistry, 61*(50), 12356–12361.
Versieren, L., Evers, S., AbdElgawad, H., Asard, H., & Smolders, E. (2017). Mixture toxicity of copper, cadmium, and zinc to barley seedlings is not explained by antioxidant and oxidative stress biomarkers. *Environmental toxicology and Chemistry, 36*(1), 220–230.
Wang, S., Wang, J., & Guo, Y. (2018). Microwave irradiation enhances the germination rate of tartary buckwheat and content of some compounds in its sprouts. *Polish Journal of Food and Nutrition Sciences, 68*(3), 195–205.
Wu, J., Gao, X., & Zhang, S. (2007). Effect of laser pretreatment on germination and membrane lipid peroxidation of Chinese pine seeds under drought stress. *Frontiers of Biology in China, 2*(3), 314–317.
Zhang, Z.-L., Zhou, M.-L., Tang, Y., Li, F.-L., Tang, Y.-X., Shao, J.-R., et al. (2012). Bioactive compounds in functional buckwheat food. *Food Research International, 49*(1), 389–395.
Zych-Wężyk, I., & Krzepiłko, A. (2012). Determination of total phenolic compound content and antioxidant properties of edible buckwheat sprouts. *Ecological Chemistry and Engineering A, 19*(4–5), 441–449.