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ORIGINAL PAPER

DETERMINATION OF THE CONTENT OF ANTIOXIDANTS AND THE BIOCHEMICAL COMPOSITION OF LEGUME MICROGREENS*

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Abstract

This research was conducted in 2021, in a controlled climate room of the Van Yuzuncu Yil University, Faculty of Agriculture, Department of Field Crops. It was set up according to the Completely Randomized Experimental Design. Total antioxidant activity (TAA), total phenolic (TPC), total flavonoid (TFC), total ascorbic acid (TAC), total chlorophyll (TCHL), chlorophyll a (CHLa), chlorophyll b (CHLb) and total carotenoid (CAR) concentrations and their correlations in sainfoin (Lutfibey), alfalfa (Bilensoy), red clover (Dadas) chickpea (Arda), lentils (Sazak), cowpea (Amazon), black chickpea (local), mung bean (local) and maize (Arifiye) were determined. The highest amounts of TAA (4789.373 mg TE g^{-1} DM) and TPC (791.770 mg GAE 100 g^{-1} DM) were determined in red clover, and the amount of TFC (672.177 mg QE 100 g^{-1} DM) was the highest in maize. The TAA content of the other plants was 6- to 8-fold lower than in red clover and maize. The TAC content of the plants, except alfalfa, red clover and maize, was similar. Cowpea had the lowest values in terms of TAA, TPC and TPC. The highest TCHL (36.632 μg g^{-1} TA FW), CHLa (25.247 μg g^{-1} TA FW), CHLb (11.385 μg g^{-1} TA FW) and CAR (7.015 μg g^{-1} TA FW) were found in lentils. Pigment values of lentils are 75-79% higher than those in the closest plant. All pigment values of the mung bean were at the lowest level and 50% lower than in the closest plant. A negative and insignificant correlation was found among TAA, TPC and TFC with TAC, a positive and significant correlation was determined among TFC with TAA and TPC, and a positive and very important correlation was detected between TPC and TAA. A positive and very significant correlation was found between CAR with all pigment properties, between CHLb with TCHL and CHLa, and between CHLa and TCHL.

Keywords: Fabaceae, microgreens, antioxidants, bioactive compounds, correlations.

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INTRODUCTION

Legumes have an important place in nutrition in many parts of the world owing to their high protein, carbohydrate, dietary fiber and high energy source food content (Bubelova et al. 2018). In addition, legumes are rich in secondary metabolites, such as antioxidants, flavonoids, carotenoids and chlorophyll. These compounds, which cannot be synthesized in the body, have the potential to counteract nutritional deficiencies (Klopsch et al. 2018). In addition, consumption of legumes helps prevent degenerative diseases, such as cancer, thanks to the rich supply of bioactive compounds they ensure (Frede et al. 2017). The antioxidant and bioactive contents of cereals, such as corn, wheat and barley, have been studied extensively (Niroula et al. 2019). Although it is widely consumed (as food and feed), the biochemical potential of some legume species (such as *Medicago, Trifolium* and *Onobrychis* species) is still unknown, as research in this regard mostly focuses on medical and aromatic plants (Peel et al. 2009, Kabtni et al. 2020).

Microgreens with a stem and cotyledon leaves are harvested before the true leaves emerge, when they are 5-10 cm in height depending on the plant. Sprouts and microgreens, which can be consumed in different ways by adding them to sandwiches, salads, soups, desserts and beverages, have become an integral part of the popular diet. The fact that they can be used in many formats allows them to be consumed throughout the year. Legumes are widely used as microgreens (Wojdyło et al. 2020). Some legumes are difficult to digest due to their anti-nutritional substances (Haileslassie et al. 2016). Consumption of these as microgreens, as well as cooked, might be a solution to their anti-nutritional properties (Alonso et al. 2000). However, more dietary research is needed in this regard (Cornara et al. 2016).

The aim of this study was to determine the content of antioxidants and some bioactive substances in Lutfibey sainfoin (*Onobrychis sativa* L.), Bilensoy clover (*Medicago sativa* L.), Dadas red clover (*Trifolium pratense* L.), Arda chickpea (*Cicer arietinum* L.), Sazak lentil (*Lens culinaris* L.), Amazon cowpea (*Vigna unguiculata* L.), mung bean (*Vigna radiata* L. Wilczek) and black chickpea (*Cicer arietinum* L.) legume microgreens and, for comparison, in Arifiye maize (*Zea mays* L.) cereal microgreens, grown in a controlled environment, and to reveal their mutual relationships through the determination of appropriate correlations.

MATERIAL AND METHODS

This research was conducted in a controlled plant growing room of the Van Yuzuncu Yil University, Faculty of Agriculture, Field Crops Research Laboratory, in 2021.

Herbal material

Onobrychis sativa (Lutfibey), *Medicago sativa* (Bilensoy) and *Trifolium pratense* (Dadas) were obtained from the Erzurum Eastern Anatolia Agricultural Research Institute, *Cicer arietinum* (Arda), *Lens culinaris* (Sazak) and *Vigna unguiculata* (Amazon) originated from the GAP İnternational Agricultural Research and Training Center, *Vigna radiata* (local) was grown by Siirt farmers, *Zea mays* (Arifiye) came from the Sakarya Corn Research Institute and *Cicer arietinum* (local) originated from Iraq.

Establishment of the experiment and production of microgreens

The layout of the trial and the methods applied followed NIROULA et al. (2019). The growth substrate consisting of sterile peat, cocopite (coconut shell) and perlite mixture was obtained from a certified commercial company. It was placed by pressing it gently to cover 2/3 of the 500 cc plastic chalets and then the seeds were sown by scattering. The seeds were then covered with the same mixture in 2 cm thickness and pressed gently. The prepared material was placed in a fully controlled climate cabinet at 21±2/17±2°C temp., 50-60% humidity and 16/8 light / dark periods. A Fujika 60-watt spiral fluorescent lamp was used for lighting. The cultivated material was irrigated daily by spraying with a sufficient amount of pure water. After the microgreens reached sufficient maturity, mung bean and cowpea were cut on day 7, chickpeas, black chickpeas, maize and lentils on day 11, and sainfoin, alfalfa and red clover on day 20 by cutting with sterile scissors from the junction where the soil and stem part met.

Total chlorophyll, chlorophyll a, chlorophyll b, carotenoid values were measured in fresh weight (FW), and total phenolic, flavonoid, ascorbic acid and antioxidant amounts were measured in dry matter (DM).

Determination of total antioxidant activity (mg TE g^{-1} DM) (Ferric Reducing Antioxidant Power, FRAP)

A 0.2 g sample of the leaves of a test plant was placed in a homogenizer with 5 ml of methanol, and then centrifuged at 12000 rpm for 15 minutes, after which the supernatant was removed. Later, 300 mM acetate buffer (pH 3.6), 10 mmol L^{-1} 2,4,6-tripyridyl-s-triazine (TPTZ), prepared by dissolving in 40 mM HCl, and 20 mmol L^{-1} $FeCl_3 \cdot 6H_2O$ solutions were made, respectively, while 10 FRAP reagent was prepared by mixing in a ratio of 1:1. The mixture prepared for analysis with 2850 µL of the FRAP reagent was diluted 20 times with methanol, next an amount of 150 µL of a sample was mixed and kept at room temperature for 1 hour. The resulting ferrus tripyridyltriazine complex was measured at 593 nm in the spectrophotometer and the results were reported as mg Trolox g^{-1} (Lutz et al. 2011). The trolox concentration was studied in the range 0-500 ppm.

Determination of total phenolic content (mg GAE 100 g^{-1} DM)

The method developed by modifying the Folin-Ciocalteu spectrophotometric method as specified by Obanda, Owuor (1997) was used to determine the total phenolic compound content. The Folin-Ciocalteu solution was diluted in a ratio of 1:10. For saturated sodium carbonate (20%) solution, 20 g of sodium carbonate were dissolved in distilled water, replenished to 100 ml, and then filtered after being left overnight. Gallic acid stock solution (500 μg ml^{-1}) was prepared by dissolving 50 mg of gallic acid in 100 ml of pure water. For gallic acid working solution, each portion of 500 μg ml^{-1} stock solution was prepared in 5 ml measuring flasks, obtaining 9 separate solutions with a concentration varying between 0-55 μg ml^{-1}. 150 μl of each of the gallic acid working solutions (9) at different concentrations were mixed with 150 μl of Folin-Ciocalteu solution. After 5 min, 300 μl sodium carbonate were added to the mixture, vortexed and diluted with 2400 μl water. After that, the mixture was kept in the dark for 60 min, the absorbance value of the blue color formed was read on a spectrometer at 725 nm wavelength. The calibration curve was obtained by plotting the absorbance readings against these different concentrations of gallic acid and calculated as mg GAE 100 g^{-1}.

Determination of total flavonoid content (mg QE 100 g^{-1} DM)

The total flavonoid content was determined according to the method developed by Quettier et al. (2000). 2 ml of 2% $AlCl_3$ were added to 2 ml of extract and kept at room temperature in the dark for 1 hour. The total flavonoid content of the extracts was measured on a spectrophotometer at 415 nm wavelength by performing 2 parallel runs on each sample, and calculated in mg QE 100 g^{-1} by using the calibration curve prepared using standard quercetin.

Determination of ascorbic acid content (mg 100 g^{-1} DM)

2 g of a fresh plant sample were homogenized in an Ultra-Turrax (Wise-Tis® homogenizer, HG 15A) with 20 ml of oxalic acid (0.4%) and kept at 5°C in a circular shaking oven (ACMI 006) for 24 hours. It was then centrifuged at 5000 rpm for 10 minutes. The supernatant was used for an ascorbic acid assay. Ascorbic acid was determined spectrophotometrically (AOAC, 1990). Absorbance values were determined spectrophotometrically at 520 nm by adding 400 μL 0.4% oxalic acid and 4.5 ml (30 ppm) 2,6-dichlorophenolindophenol solution into 100 μl of the supernatant. The amount of ascorbic acid in the samples was calculated in mg 100 g^{-1} with the help of the calibration curve drawn with pure ascorbic acid.

Pigment analysis

Determination of photosynthetic pigments was performed according to Lichtenthaler (1985). Namely, 0.2 g (200 mg) of a fresh plant sample was

extracted with 10 mL of 80% acetone and centrifuged at 4600 rpm for 15 minutes. The absorbance values of the aliquots taken after centrifugation at 662, 652, 645 and 470 nanometer (nm) wavelengths were determined on a spectrophotometer (PG T60 UV-VIS) and recorded. Total chlorophyll, chlorophyll a, chlorophyll b and carotenoid concentrations were calculated from the formulas developed by Lichtenhaler, Welburn (1985) given below:

total chlorophyll (μg g^{-1} FW) = (27.8 x A652);
chlorophyll a (Chl a, μg g^{-1} FW) = (11.75 x A662) - (2.350 x A645);
chlorophyll b (Chl b, μg g^{-1} FW) = (18.61 x A645) - (3.960 x A662);
carotenoid (Car, μg g^{-1} FW) = [(1000 x A470) - (2.270 x Chl a)] - - (81.4 x Chl b 227^{-1});

where: FW – fresh weight, A662 – absorbance reading at 662 nm wavelength, A652 – absorbance reading at 652 nm wavelength, A645 – absorbance reading at 645 nm wavelength, A470 – absorbance reading at 470 nm wavelength.

Statiscal analysis

The data of the research were subjected to analysis of variance (ANOVA) according to the Completely Randomized Experimental Design. Statistical calculations were made using the program COSTAT (version 6.303). The differences between the means were determined according to the least significant differerence (LSD 0.05) comparison method and relationships between the analyzed properties were determined with the Pearson correlation method.

RESULTS AND DISCUSSION

The contents of TAA, TPC, TFC and TAC of the legumes examined in the study are given in Table 1. The differences between the averages were found to be significant for all the properties examined.

TAA activities (mg TE g^{-1} DM FRAP)

Total TAA activities ranged from 394.563±8.125 to 4789.373±12.497 mg TE g^{-1} DM. The highest TAA was determined in the red clover and the lowest TAA values were in Arda chickpea and Amazon cowpea, which are in the same group (Table 1).

Many methods are used to determine TAA activities and results may vary according to these methods. In addition, the properties of the examined material (grain, dry extract, sprout, microgreen etc.) affect the TAA activities. It is therefore impossible to make straightforward comparisons. In addi-

Table 1

Total antioxidat activity, total phenolics and total flavonoids in legumes

Genotypes	TAA (mg TE g^{-1} DM)	TPC (mg GA 100 g^{-1} DM)	TFC (mg QE 100 g^{-1} DM)	TAC (mg 100 g^{-1} DM)
Sainfoin (Lutfibey)	765.406±9.781*d**	512.325±40.555*b*	397.269±10.332*d*	No Measurement
Alfalfa (Bilensoy-80)	990.417±14.555*c*	327.885±0.555*d*	366.273±11.070*e*	9.775±1.056*d*
Red clover (Dadas)	4789.373±12.497*a*	791.770±10.000*a*	472.915±23.985*b*	11.587±0.483*c*
Mung bean (Siirt-Local)	554.886±5.888*f*	402.885±12.225*c*	111.661±7.380*h*	13.577±0.633*a*
Chickpea (Arda)	394.563±8.125*h*	285.882±5.063*e*	227.528±0.922*g*	13.014±0.620*ab*
Cowpea (Amazon)	410.813±7.500*h*	219.550±7.780*f*	113.137±5.904*h*	12.451±0.479*bc*
Maize (Arifiye)	4357.917±19.094*b*	813.995±32.225*a*	672.177±10.701*a*	9.423±0.464*d*
Lentils (Sazak)	742.500±4.375*e*	497.330±2.220*b*	418.672±8.118*c*	12.592±0.521*abc*
Black chickpea (Iraq-Local)	518.427±8.534*g*	398.995±2.775*c*	271.808±3.690*f*	13.577±0169*a*
CV (%)	0.713	3.145	3.437	5.036
$LSD_{0.05}$	10.711	14.847	11.647	0.648

TAA – total antioxidant activity, TPC – total phenolic content, TFC – total flavonoid content, TAC – total ascorbic acid content;

* There is no significant difference between values shown with the same letter in the same column. Values are given with standard deviation.

tion, some studies (Kobus-Cisowska et al. 2019, Tkacz et al. 2019) determined that TPC and other bioactive compounds affect the TAA potential.

Although the tested plants belong to the same family, the TAA or TAC capacities vary considerably between species. In another study (Zhao et al. 2014), TAC values in 10 different legume ranged from 116±3e to 721±51a, the highest being in lentil and the lowest – in baby lima bean.

In a study similar to ours (Sushama et al. 2011), TAA activities of 30 legumes varied between 8.5 - 16.5 μmol FRAP g^{-1}, the highest was in (brown) cowpea and the lowest was in (white) lablab bean. In another study (Wojdyło et al. 2020), TAA capacities were determined to be 0.1 mMol Trolox 100 g^{-1} FW and less in legume sprouts, and 1.2±0.3 mMol Trolox 100 g^{-1} FW (FRAP) in green pea microflora. In a study conducted on dry grain extracts (Djordjevic et al. 2011), the TAA capacities of legumes were 8.34±0.54 - 24.98±0.58 nmol Fe2+ mg^{-1} d.e. and the highest TAA activity was determined in mung beans. In another study conducted with the FRAP

method (Sreeramulu et al. 2009), the highest TAA activities varied between 16.21-471.71 μmoles g^{-1} and were determined in finger millet (*Eleusine cora cana*) and Rajmah (*Phaseolus vulgaris*). In another study (Ghoora et al. 2020), TAA activities were detected between 7.0±0.5 - 38.7±2.0 μmol Fe2 + g^{-1} FRAP in 10 salad plant microphilis. In a study conducted with a Randox kit (Amarowicz et al. 2004), TAA activities in the seed extracts of 7 legumes varied between 0.30±0.02 - 1.76±0.13 μmol Trolox mg^{-1}, the highest in adzuki bean and the lowest in pea. In another experiment by the same researchers (Amarowicz et al. 2009), TAA activities were determined to be 0.68±0.03 μmol Trolox® eq. mg^{-1} in red lentyl acetonic crude extract, and 5.85 μmol Trolox® eq. mg^{-1} in tannin fraction. The TAA activity in corn (*Zea mays*) was 68.29±21.90 μmoles FRAP g^{-1} (Sreeramulu et al. 2009).

TPC content (mg GAE 100 g^{-1} DM)

The TPC content varied between 219.550±7.780 - 813.995±32.225 mg GAE 100 g^{-1} DM (Table 1). The highest TPC was found in Arifiye maize and red clover, which were in the same group, and the lowest TPC was in the Amazon cowpea.

The TPC content changes depedning on the maturity phase, genetic traits and environmental factors. In addition, there is no uniform method suitable for the complete extraction of phenolic compounds from plant materials, and the available methods vary in terms of the extractability of phenolic compounds, type of solvent used, degree of polymerization, extraction time and temperature (Sushama et al. 2011).

Our findings are higher than the amounts of total polyphenols in microgreen lentil, black medick, mung beans sprouts and in green peas determined by others (Wojdylo et al. 2020), but are similar to results reported from studies on 30 legumes (Sushama et al. 2011) and 10 legumes (Sreeramulu et al. 2009). Our results are lower than data from a study on 10 legume extracts (Zhao et al. 2014) or legume dry extracts (Djordjevic et al. 2011) and 7 legume seeds (Amarowicz et al. 2004). What all these studies have in common (except Sushama et al. 2011) is that lentil (*Lens culinaris*) has the highest amounts of TPC.

In their study, Djordjevic et al. (2011) showed that the highest amount of TPC in legumes was in lentil, and legumes are the main antioxidant source in foods with an abundant amount of TPC in their grains. In our study, the highest values were determined in Arifiye maize (*Zea mays*) and red clover (*Trifolium pratense*), followed by Sazak lentil (*Lens culinaris*).

TFC content (mg QE 100 g^{-1} DM)

The TFC content was between 111.661±7.380 - 672.177±10.701 mg QE 100 g^{-1} DM (Table 1). The highest TFC was determined in Arifiye maize and the lowest TFC values were in red clover and Amazon cowpea, which were in the same group.

TFC are abundant in all legume groups; they play a key role in the germination of seeds (Samanta et al. 2011) and in the formation of the root nodule system for rhizobium bacteria (Kabtni et al. 2020). It has been determined (Złotek et al. 2015) that the TFC content increases significantly with sprouting compared to its content in the seed.

Our total TFC results are higher than the TFC content determined in 10 salad plant microgreens (Ghoora et al. 2020) and in the raw and cooked samples of 7 commercial lentils (Bubelova et al. 2018). Our data are similar to results reported by Bubelova et al. (2018) obtained from germinated samples of 7 commercial lentils (34.8±1.65 - 55.2±2.74 mg RE kg^{-1} DM), or by Klopsch et al. (2018), who determined their concentrations in pea microgreens and leaves (1.38 - 3.31 and 3.84 - 4.37 mg g^{-1} FW, respectively). Our results are lower than given by Kabtni et al. (2020), who analyzed the seed and leaf extracts of *Medicago minima* (10.35 mg RE g^{-1} DM), or by Van Hung (2016), who studied 7 barley varieties (112.3±23 - 241.3±62.2 μg g^{-1}).

The TAC (vitamin C) content (mg 100 g^{-1} DM)

The TAC content varied between 9.423±0.464 and 13.577±0169 mg 100 g^{-1} DM. The highest value was found in black chickpea and mung bean in the same group, and the lowest value was in Arifiye maize. Because the TAC content in sainfoin could not be measured, it not excluded from this part of the study.

The TAC results from our study are higher than given by Wojdyło et al. (2020), who determined TAC in green pea microgreen (8.1±0.1 mg 100 g^{-1} FW), and in black medick and mung beans sprouts (6.2±0.0 and 4.7±0.2 mg 100 g^{-1} FW, respectively), and by XIAO et al. (2015) in bull's blood beet (75.9±1.7 mg kg^{-1}). The current results are similar to the ones reported by Kyriacou et al. (2019), who determined TAC in coriander, jute, purple bacillus, and swiss chard (130.9±19, 123.5±1.3, 134.7±3.0 and 127.8±1.4 mg kg^{-1} FW, respectively), and in red amaranth (126.1±24.0 mg kg^{-1}) reported by Xiao et al. (2015). The results of this study are lower than the quantities measured in lentil shoots (46.4±0.2 mg 100 g^{-1} FW) – Wojdyło et al. (2020) and in commercial microgreens of golden pea tendrils and pea tendrils (25.1±0.7 and 50.5±0.9 mg 100 g^{-1} FW, respectively) – Xiao et al. (2012).

TAC (vitamin C) is a vitally important antioxidant which, when taken regularly, reduces oxidative stress, reduces unwanted enzymatic oxidation reactions, and is essential for collagen synthesis in humans (Xu et al. 2005, Xiao et al. 2012, Kyriacou et al. 2019). While TAC is not found in soybean, buckwheat and mung bean seeds, it increases significantly during the germination and shrinking phase (Xu et al. 2005). However, the literature shows that the TAC level peaks in the germination stage a few days after planting, but is not very high in shoots and microgreens (Xiao et al. 2012). This situation varies depending on many factors, such as a variety, cultivation style, stress conditions, harvest, storage, irrigation, fertilization (Kyriacou et al. 2019).

CHL and CAR contents

The pigment contents of the legumes examined in the study are given in Table 2. Accordingly, the differences between the averages were found to be significant for all the properties examined.

Table 2

Total chlorophyll, chlorophyll a, chlorophyll b and total carotenoids in legumes

Genotypes	TCHL (μg g^{-1} TA FW)	CHLa (μg g^{-1} TA FW)	CHLb (μg g^{-1} TA FW)	CAR (μg g^{-1} TA FW)
Sainfoin (Lutfibey)	23.645±0.061*c**	17.390±0.146*c*	6.255±0.084*c*	4.200±0.055*ef*
Alfalfa (Bilensoy-80)	26.617±0.157*b*	19.439±0.035*b*	7.177±0.121*b*	5.232±0.018*b*
Red clover (Dadas)	22.964±0.180*d*	16.980±0.083*d*	5.984±0.096*d*	4.365±0.037*de*
Mung bean (Siirt-Local)	8.423±0.035*i*	6.000±0.065*g*	2.424±0.031*g*	1.769±0.004*h*
FChickpea (Arda)	19.568±0.123*h*	14.451±0.043*f*	5.117±0.166*f*	4.523±0.077*d*
Cowpea (Amazon)	21.094±0.131*f*	15.396±0.090*e*	5.698±0.041*e*	4.041±0.006*f*
Maize (Arifiye)	20.525±0.290*g*	15.491±0.209*e*	5.033±0.081*f*	3.756±0.006*g*
Lentils (Sazak)	36.632±0.318*a*	25.247±0.224*a*	11.385±0.094*a*	7.015±0.380*a*
Black chickpea (Iraq-Local)	22.445±0.322*e*	16.830±0.365*d*	5.616±0.043*e*	4.838±0.061*c*
CV (%)	0.956	1.025	1.546	2.903
$LSD_{0.05}$	0.214	0.167	0.094	0.128

TCHL – total chlorophyll, CHLa – Chlorophyll a, CHLb – Chlorophyll b, CAR – total carotenoid content;

* There is no significant difference between values shown with the same letter in the same column. The values for the properties are given with the standard deviation.

The TCHL content varied between 8.423±0.035 and 36.632±0.318 μg g^{-1} TA FW. It was the highest in Sazak lentil and the lowest in mung bean. The CHLa content varied between 6.000±0.065 and 25.247±0.224 μg g^{-1} TA FW. It was the highest in Sazak lentil and the lowest in mung bean. The CHLb content varied between 2.424±0.031 and 11.385±0.094 μg g^{-1} TA FW. The highest was found in Sazak lentil and the lowest in mung bean. The CAR content varied between 3.756±0.006 - 7.015±0.380 μg g^{-1} TA FW. The highest was determined in Sazak lentil and the lowest in Arifiye maize.

Microgreens and the color of sprouts are important factors affecting the quality and consumer choice. Generally, microgreens contain more chlorophyll and carotenoids than sprouts do (Wojdylo et al. 2020).

When our findings concerning TCHL are compared with results reported in similar research (Wojdylo et al. 2020), they are higher than in mung beans sprouts (6.0 μg g^{-1} FW), similar to ones in black medick sprouts (14.3 μg g^{-1} FW), and lower than in lentil sprouts and green pea microgreen (108.5 and 522.7 μg g^{-1} FW). Unlike in our study, the amount of TCHL determined by Wojdylo et al. (2020) was generally higher because chlorophyll was included in pheophytin with a ′ and b ′ isomers. According to another study (Ghoora et al. 2020), our TCHL values are lower than the microgreens of 10 plants (29.5±0.1 - 80.0±0.2 mg 100 g^{-1} FW) used extensively in raw feeding.

The results of CHLa according to similar studies (Wojdylo et al. 2020) are higher than in black medick and mung beans sprouts (10.5±0.7 - 3.9±0.1 μg g^{-1} FW, respectively) and lower than in lentil sprout and green peas microgreen (77.6±1.5 - 288.3±3.6 μg g^{-1} FW, respectively). Likewise, according to another study (Ghoora et al. 2020), our CHLa values are lower than in microgreens (16.9±0.1 - 45.8±0.3 mg 100 g^{-1} FW). When compared with similar studies (Wojdylo et al. 2020), our CHLb values are higher than in black medick and mung beans sprouts (1.6±0.1 - 1.0±0.2 μg g^{-1} FW, respectively) but lower than in lentil sprouts and green pea microgreens (16.1±0.6. - 157.8±2.4 μg g^{-1} FW, respectively). Similarly, according to another study (Ghoora et al. 2020), our CHLb values are lower than in the microgreens (11.2±0.8 - 34.2±0.1 mg 100 g^{-1} FW). CHLa is the basic photosynthetic pigment, and plants can photosynthesize even without CHLb. However, CHLb allows the plant to photosynthesize by absorbing a wider wavelength (Wojdylo et al. 2020).

The CAR values are lower than determined by Wojdylo et al. (2020) in legumes, and by Klopsch et al. (2018) in pea (135-264 μg g^{-1} FW) and lupene microgreen (133-249 μg g^{-1} FW). In these studies, other components were included in the CAR content (lutein, ß-carotene, neoxanthin, violaxanthin, etc.). Differences in the CAR content in microgreens are closely related to the spectral quality and light intensity during the cultivation and harvest phases, as well as the differences arising from the species and variety (Kopsell et al. 2012, Samuolienė et al. 2017). Another finding of our study is that CHLa and CHLb values are higher than the CAR level. This was also determined by Mroczek-Zdyrska et al. (2016) and Klopsch et al. (2018), who concluded that CHLa values were higher than CHLb and CAR values.

The conditions of our study and the light source used (white fluorescent bulb) were effective in lowering chlorophyll and especially carotenoid content compared to similar studies. Although it is possible to achieve up to 10% efficiency under white fluorescent lamp lighting (Hamamoto,Yamazaki 2011), blue light used in similar studies has a positive effect of 16-33% on photosynthetic and carotenoid pigments (Samuolienė et al. 2017). Also, LED luminous illumination significantly increased pigment levels (Da-Wei et al. 2011, Hasperué et al. 2016).

Pigment ratios

The ratios of pigments examined in the study are given in Table 3. The differences between the means of all ratios were found to be significant.

Table 3

Chlorphyll a and total chlorophyll, chlorphyll b and total chlorophyll, chlorophll a and chlorophyll b, total chlorophyll and total carotenoid ratios in legumes

Genotypes	CHLa $TCHL^{-1}$	CHLb $TCHL^{-1}$	CHL (a b^{-1})	TCHL CAR^{-1}
Sainfoin (Lutfibey)	0.735±0.004 *bc**	0.265±0.004 *cd*	2.781±0.061 *bc*	5.631±0.060 *a*
Alfalfa (Bilensoy-80)	0.730±0.003 *c*	0.270±0.003 *c*	2.709±0.041 *c*	5.087±0.013 *d*
Red clover (Dadas)	0.739±0.002 *b*	0.261±0.002 *d*	2.838±0.032 *b*	5.261±0.003 *c*
Mung bean (Siirt-Local)	0.712±0.005 *d*	0.288±0.005 *b*	2.476±0.059 *d*	4.761±0.009 *e*
Chickpea (Arda)	0.739±0.006 *b*	0.261±0.006 *d*	2.826±0.100 *b*	4.327±0.047 *f*
Cowpea (Amazon)	0.730±0 *c*	0.270±0 *c*	2.702±0.003 *c*	5.220±0.024 *cd*
Maize (Arifiye)	0.755±0 *a*	0.245±0 *e*	3.078±0.008 *a*	5.466±0.164 b
Lentils (Sazak)	0.689±0 e	0.311±0 *a*	2.218±0.001 *e*	5.230±0.238 *cd*
Black chickpea (Iraq-Local)	0.750±0.005 *a*	0.250±0.005 *e*	2.997±0.088 *a*	4.641±0.125 *e*
CV (%)	0.457	1.244	1.766	1.855
$LSD_{0.05}$	0.003	0.003	0.0482	0.094

* There is no significant difference between values shown with the same letter in the same column. The values for the properties are given with the standard deviation.

The CHLa $TCHL^{-1}$ ratios ranged from 0.689 to 0.750. The highest ratio occurred in black chickpea and the lowest was in Sazak lentil. The CHLb $TCHL^{-1}$ ratios ranged from 0.250 to 0.311. The highest one occurred in Sazak lentil and the lowest was in black chickpea. The CHL (a b^{-1}) ratios ranged from 2.218 to 2.997. The highest ratio occurred in black chickpea and the lowest one was in Sazak lentil. TCHL CAR^{-1} ratios ranged from 4.327 to 5.631. The highest ratio was determined in sainfoin and the lowest one was in Arda chickpea.

Our CHLa $TCHL^{-1}$ results are similar to the ones given by Wojdylo et al. (2020) in lentil (0.715) and black medick (0.734) sprouts, but higher than in mung bean sprouts (0.650) and green pea microgreens (0.552). Our CHLb $TCHL^{-1}$ results are similar to the ones given by the cited researchers for

green pea microgreen (0.302) and higher than in the sprouts of lentil (0.148), black medick (0.112) and mung beans (0.167).

While the CHL (a b^{-1}) ratio is high in plants that benefit from sunlight and heat, this ratio is lower under artificial light conditions (Niroula et al, 2019). Our CHL (a b^{-1}) findings are higher than provided by Niroula et al. (2019) for grain microgreens (2.11±0.04) and higher than given by Wojdylo et al. (2020) for green pea microgreens (0.187) and lower than in lentil (4.819), black medick (6.562) and mung bean (3.9) sprouts. Accordingly, in a study conducted under open field conditions (Wakeham 2013), the ratio of CHL (a b^{-1}) was higher than these values (3.14). Nirolua et al. (2021) determined that the CHL (a b^{-1}) ratio varied between 2.27-2.65 (similar to our findings) and decreased with the growth period, also noting a correlation of this ratio with growth periods in dark and light. The researchers attributed their findings to new CHLa formed in the first period to gradually transforming into CHLb in the later period. Nath et al. (2013) also reported that as the leaves were aging, there was a gradual decrease in the CHL (a b^{-1}) ratio.

Our TCHL CAR^{-1} results are higher than obtained in a study on legumes by Wojdylo et al. (2020), but similar to the TCHL CAR^{-1} ratios (4.38-4.68) determined in microgreen under etiloation (Niroula et al. 2021). The cited researchers stated that this ratio decreased rapidly with de-etiolation. Our ratios are lower than in obtained in another study under low light conditions (Niroula et al. 2019).

Correlations

Correlations (Pearson correlation) of TAA and bioactive compounds are given in Table 4, and correlations between pigment properties are given in Table 5. According to Table 4, there is a positive and very important correlation between TAA with TPC, a positive and significant correlation with TFC and a negative and insignificant correlation with TAC. This is similar to the results of similar studies (Mikulajová 2007, Sreeramulu et al. 2009, Marathe et al. 2011, Niroula et al. 2021). According to our research, there is a positive and significant correlation between TPC with TFC, and a negative and insignificant correlation with TAC. There is also a negative and significant correlation between TFC and TAC.

The results of this study are similar the data provided by some researchers (Mikulajová 2007, Sreeramulu et al. 2009, Marathe et al. 2011, Niroula et al. 2021), who determined a positive and significant relationship between TAA (FRAP) and TPC (r = 0.872, r = 0.91, r^2 = 0.95, r = 0.918, respectively).

According to Table 5, there is a positive and very significant correlation betweenTCHL and CHLa, CHLb and CAR. There is a positive and very significant correlation between CHLa and CHLb as well as CAR. There is also a positive and very important correlation between CHLb and CAR.

The results of Niroula et al. (2021) are similar to ours, and a significant correlation was determined between TCHL and CAR and vitamin C (r > 0.92) as well as between TPC and TAA (r > 0.91). Likewise, in another

Table 4

Correlation of TAA and bioactive compounds

	TPC	TFC	TAC
TAA	0.909***	0.762*	-0.064
TPC		0.848**	-0.235
TFC			-0.374
TAC			-

*** and ** there is very significant correletion
* there is significant correlation

Table 5

Correlation of pigment properties

Pigment	CHLa	CHLb	CAR
TCHL	0.995***	0.979***	0.969***
CHLa		0.955***	0.966***
CHLb			0.9478***
CAR		-	

*** there is very significant correletion

study (Niroula et al. 2019), there was a significant positive correlation between CAR and TCHL (r = 0.99), a significant and positive between CHLa with TCHL and CAR (r = 0.99), and a highly significant and positive correlation between CHLb with TCHL, CAR and CHLa (r = 0.99). Contrary to those results, a study by Samuolienė et al. (2017) demonstrated a moderately negative correlation between CHLa and CHLb under blue light doses.

CONCLUSIONS

Red clover has the highest total antioxidant activity (4789.373 mg TE g^{-1}) and phenolic content (together with maize) (791.770 mg GAE 100^{-1} g^{-1}). The total phenolic content of red clover and maize was by 46% to 73% higher than in the other legumes. Maize (672.177 mg QE 100 g^{-1}) had the highest total flavonoid content. The total ascorbic acid (vitamin C) content was similar in most plants except alfalfa, red clover and maize. Cowpea had the lowest total antioxidant acitivity, total phenolics and flavonoid content.

Lentil had the highest total chlorophyll (36.632 µg g^{-1} TA), chlorophyll a (25.247 µg g^{-1} TA), chlorophyll b (11.385 µg g^{-1} TA) and carotenoid (7.015 µg g^{-1} TA). Mung bean had low amounts of total chlorophyll (8.423 µg g^{-1} TA), chlorophyll a (6.000 µg g^{-1} TA), chlorophyll b (2.424 µg g^{-1} TA) and carotenoid (1.769 µg g^{-1} TA). In fact, the pigment values of mung bean are 50% lower than in the second pigment poorest legume.

There are negative and insignificant correlations of total antioxidant, total phenolic and total flavonoid contents with total ascorbic acid content. There are positive and significant relationships of total antioxidant with total phenolic content and total flavonoid content, and a positive and significant relationship of total phenolic content and total antioxidant activity. There are positive and very important correlations of chlorophyll b with total chlorophyll and chlorophyll a, chlorophyll a with total chlorophyll, and total carotenoid content with all pigment properties.

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.

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