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Nutritional Value of Crisphead 'Iceberg' and Romaine Lettuces (*Lactuca sativa* L.)

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Abstract

Lettuce (*Lactuca sativa* L.) is one of the most popular vegetables worldwide, but is often viewed as low in nutritional value. However, lettuce contains health-promoting nutrients and biosynthesis of such phytochemicals varies depending on cultivar, leaf color and growing conditions. Studies of such parameters on the nutritional value have not been conclusive because the lettuce samples were collected from heterogeneous growing conditions and/or various developmental stages. In our study nutritional composition was evaluated in the two most popular lettuce types in Western diets, romaine and crisphead 'Iceberg', with red or green leaves grown under uniform cultivating conditions and harvested at the same developmental stage. In the investigated lettuce cultivars, insoluble fiber content was higher ($P \leq 0.05$) in romaine than crisphead lettuces. α-linolenic acid (omega-3 polyunsaturated fatty acid) was the predominant fatty acid and was higher in romaine than crisphead. Iron and bone health-promoting minerals (Ca, Mg and Mn) were significantly higher ($P \leq 0.001$) in romaine. The content of β-carotene and lutein in romaine (668.3 μg g^{-1} dry weight) was ~45% higher than in crisphead (457.3 μg g^{-1}dry weight). For leaf color comparison, red cultivars provided higher amount of minerals (Ca, P, Mn and K), total carotenoids, total anthocyanins and phenolics than green cultivars. Based on our study results, romaine was generally higher in nutrients analyzed, especially red romaine contained significantly higher amount of total carotenoids, total anthocyanins and phenolics. Therefore, romaine type lettuces with red rather than green leaves may offer a better nutritional choice.

Keywords: carotenoid, dietary mineral, fatty acid, insoluble fiber, lettuce, phenolic compound

1. Introduction

Epidemiological studies have reported a correlation between fresh vegetable consumption and reduced risk of chronic diseases (Rodriguez-Casado, 2016). Lettuce (*Lactuca sativa* L.) is one of the most popular vegetables worldwide. In countries with Western diets such as the United States (US), the most serious public health threat is chronic diseases partially due to poor dietary habits (Cordain et al., 2005). Even though salad consumption is increasing, lettuce is regarded as low in nutritional value. However, nutritional value of lettuce greatly varies with lettuce types and depending on lettuce type, and nutrient composition can be equivalent to other "nutritious" vegetables (Kim, Moon, Tou, Mou, & Waterland, 2016).

Lettuce contains several dietary minerals important for human health such as iron (Fe), zinc (Zn), calcium (Ca), phosphorus (P), magnesium (Mg), manganese (Mn), and potassium (K) and other health-promoting bioactive compounds (Kim et al., 2016). Yet, few human clinical studies have investigated lettuce consumption in disease prevention. A case-control study reported an inverse association between colorectal cancer and lettuce consumption (Fernandez, LaVecchia, Davanzo, Negri, & Franceschi, 1997). Investigating potential nutrients in lettuce contributing to reduced colorectal cancer found no relationship to Ca, vitamin E, and folate, while β-carotene showed a significant relationship. Beneficial health properties of lettuce have mainly been attributed to carotenoids and other phytochemicals such as phenolic compounds (López, Javier, Fenoll, Hellin, & Flores, 2014). High quantities of carotenoids (*i.e.* β-carotene and lutein) were reported for several lettuce types including crisphead, butterhead, romaine, green and red leaf lettuces (Mou, 2005; Nicolle et al., 2004). Phenolic acids and anthocyanins were reported in red and green butterhead, crisphead (subtype Batavia), and green and red oak leaf lettuces (García-Macías et al., 2007; Nicolle et al., 2004). In these studies, carotenoids, phenolic acids, and anthocyanin contents varied depending on lettuce types. Although low in fat, α-linolenic acid, an omega-3 polyunsaturated fatty acid, was found to be the major fatty acid in lettuce (Le Guedard, Schraauwers, Larrieu, & Bessoule, 2008; Pereira, Li, & Sinclair, 2001) but to date, fatty acid composition in different lettuce types has not been well investigated.

In the US, crisphead and romaine are the most popular lettuce types, however, their nutritional value has not been extensively evaluated. The United States Department of Agriculture (USDA) nutrient database (2015) includes crisphead and romaine lettuce; however, few bioactive compounds were measured and cultivars with different leaf colors (*i.e.* green and red) were not specified. Nutritional composition is greatly affected by environmental (light, fertilizers, growing conditions) and biological (cultivar, leaf color, developmental stage) factors (Kim et al., 2016). In many studies samples were prepared from lettuces grown under different growing conditions and/or various developmental stages. Thus, the evaluation of the nutritional value in lettuces among different researchers have been often inconsistent. Particularly, the influence of type/cultivar and leaf pigmentation on nutritional value merits further investigation.

Our hypothesis is that genetic difference controlling characteristics of lettuce (type/cultivar) and pigmentation of leaf (red or green) affect phytochemical contents. Although a number of studies reporting nutritional composition of lettuce are available, there are only a few studies comparing both essential and non-essential nutrients of popular lettuce types that are grown under the same cultural conditions. Therefore the objective of this study was to investigate the effect of type/cultivar and leaf color on nutritional value. In our study nutritional composition, including fatty acid, essential dietary minerals, phytonutrients carotenoids and phenolic compounds, and insoluble dietary fiber was evaluated in two most popular lettuce types in the Western diets, crisphead 'Iceberg', and romaine, with red or green leaves grown under uniform conditions.

2. Materials and Methods

2.1 Plant Materials and Growing Conditions

Seeds of green and red cultivars of crisphead (*L. sativa* L. var. *capitata*, 'Ithaca' and 'Red Iceberg') and romaine (*L. sativa* L. var. *longifolia*, 'Coastal Star' and 'Outredgeous') lettuces were sown to plug trays filled with commercial media (Sunshine Mix #1, Sun Gro Horticulture, Agawam, MA), and germinated under a misting system in a greenhouse. Three-week old seedlings were transplanted to 15-cm pots and grown for additional 60 d. A total of four plants were grown for each cultivar with one plant as an individual biological replication. Plants were grown under natural sunlight with supplemental high pressure sodium lighting when the light intensity was below 50 W m^{-2} with 14 h d^{-1} of photoperiod.

Mean greenhouse temperature was 22.6/18.0 ± 1.7/2.5 °C (mean ± SD) day/night, and plants were irrigated as needed with a 20% Hoagland modified nutrient solution #2 (PhytoTechnology Lab., Shawnee Mission, KS). After harvest plants were freeze-dried (VirTis Freezemobile 12SL, SP Scientific, Warminster, PA) and ground to powder, and then stored at -80 °C until analyses.

2.2 Insoluble Fiber Content

Insoluble fiber content was analyzed using neutral detergent method to determine cellulose, hemicellulose, and lignin following the method of Goering and Soest (1970) with modifications. Freeze-dried powdered lettuce (0.5 g) sample were placed into individual fiber filter bag (ANKOM Technology, Macedon, NY) and sealed. Bags were placed in a fiber analyzer (ANKOM^{200} Fiber Analyzers, ANKOM Technology, Macedon, NY) and agitated with 2 L of neutral detergent solution (ANKOM Technology, Macedon, NY) and 4 mL of heat stable α-amylase at 100 °C for 1 h. Neutral detergent solution was drained and samples were washed four times with boiling water (2 L) for 5 min. Bags were rinsed with 100% acetone and then dried in a drying oven at 100 °C overnight. Individual bags were weighed to quantify total insoluble fiber. Each sample was analyzed in duplicate.

2.3 Fatty Acids Analysis

For lipid extraction, a ground sample (50 mg) was mixed with 5 mL of Triz/EDTA buffer and an internal standard (50 µL of nonadecanoic acid) (Nu-Chek Prep, Inc., Elysian, MN). Then, samples were mixed with 20 mL chloroform:methanol:acetic acid solution (2:1:0.015, v/v/v) and centrifuged at 900 g_n for 10 min at 10 °C.

The chloroform layer was collected and filtered through a pre-rinsed 1-phase separation filter paper (Whatman 1-PS, GE Healthcare Bio-Sciences, Pittsburgh, PA). Samples were re-extracted with 10 mL of chloroform:methanol solution (4:1, v/v). Extracted lipids were transmethylated into fatty acid methyl esters (FAMEs) according to Fritsche and Johnston (1990). Briefly, samples were dried under a nitrogen gas at 55 °C and extracted fatty acids were methylated by adding 4 mL of 4% H_2SO_4 in anhydrous methanol and incubated at 90 °C for 1 h. After samples were cooled to room temperature, 3 mL of deionized distilled water and 8 mL of chloroform were added. Samples were centrifuged at 900 g_n for 10 min at 10 °C and chloroform layer was filtered through Na_2SO_4. Samples were dried under a nitrogen gas at 55 °C then dissolved in 2 mL of iso-octane. To analyze FAMEs, a gas chromatography (Varian CP-3800 GC system, Agilent Technologies, Inc., Santa Clara, CA) equipped with an autosampler, a flame ionization detector, and a CP-Sil 88 capillary column (Agilent Technologies, Inc., Santa Clara, CA) was used. Initial temperature was 140 °C and held for 5 min and then increased 4 °C per min to the final temperature of 220 °C. Total running time was 85 min per sample. Carrier gas was nitrogen at 0.4 mL min^{-1} of flow rate. Fatty acids were identified by retention time and quantified using a standard curve made with external standards (16A, Nu-Chek Prep, Inc., Elysian, MN).

2.4 Mineral Content

Lettuce powder (0.5 g) was ashed at 550 °C overnight. Ashed samples were dissolved in 2% nitric acid and adjusted to total volume of 20 mL with deionized distilled water. Mineral content of samples was analyzed by inductively coupled plasma mass spectrometry (Optima 2100DV, Perkin Elmer Corp., Waltham, MA), and each sample was analyzed in triplicate.

2.5 Carotenoid Analysis

Carotenoids were analyzed according to Kopsell, Barickman, Sams, and McElroy (2007). Freeze-dried samples (0.1 g) were re-hydrated in 0.8 mL of ultrapure water for 10 min. Ethyl-β-8'-apo-carotenoate (Carotenature, Lupsingen, Switzerland) was added as an internal standard (0.8 mL). Then, tetrahydrofuran (THF) (2.5 mL) stabilized with 2,6-di-*tert*-butyl-4-methoxyphenol (25 mg L^{-1}) was added. Samples were homogenized then centrifuged at 500 g_n for 3 min. The supernatant was collected and samples were re-extracted in 2 mL of THF as described above. The combined supernatants were evaporated and total volume was adjusted to 5 mL with acetone. Final extracts of 2 mL was filtered through a 0.2-µm polytetrafluoroethylene filter (Model Econofilter PTFE 25/20, Agilent Technologies, Wilmington, DE). A high performance liquid chromatography (Agilent 1200 series, Agilent Technologies, Palo Alto, CA) equipped with a photodiode array detector was used for carotenoid analysis. Carotenoids were identified and quantified using an external standard (ChromaDex Inc., Irvine, CA).

2.6 Total Anthocyanin and Phenolic Analysis

Total anthocyanin and phenolic compounds were extracted using the method of Nicolle et al. (2004) and analyzed following the method of Olsen, Aaby, and Borge (2010) with modifications. Absorbance was measured at 510 and 700 nm using a spectrophotometer (Spectronic® Genesys™ 5, Thermo Fisher Scientific Inc., Waltham, MA). The total anthocyanin content was expressed as cyanidin 3-glucoside equivalent.

To determine total phenolic content, 0.3 mL of extract or gallic acid standard was mixed with 1.5 mL of 0.5 N Folin-Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO) and the mixture was incubated for 5 min. Then 1.5 mL of sodium carbonate solution (0.5 M) was added and the mixture was incubated for 1 h at room temperature in the dark before measuring absorbance at 765 nm using a spectrophotometer (Spectronic® Genesys™ 5, Thermo Fisher Scientific Inc., Waltham, MA). The total phenolic content was determined using a gallic acid standard curve.

2.7 Statistical Analysis

The experimental design was a randomized complete block with four replications (n = 4) per cultivar. Each cultivar within a block was randomly placed. All results were expressed as mean ± standard error. Two-way analysis of variance (ANOVA) was conducted using SAS (SAS 9.2, SAS Institute, Inc., Cary, NC, USA) with lettuce type (crisphead vs. romaine) and leaf color (red vs. green) as the main factors. Fisher's least significant difference test (LSD) was performed at the 95% significance level for mean separation.

3. Results and Discussion

3.1 Insoluble Fibers

Fiber consumption is low in the US (Dahl & Stewart, 2015). Fresh lettuce (100 g) can provide up to 10% of the daily recommended intake of fiber for adults (Institute of Medicine, 2002). Eichholz, Förster, Ulrichs, Schreiner, and Huyskens-Keil (2014) reported the dietary fiber in lettuce consisted mainly of insoluble fiber, with 80% as cellulose. In our study, insoluble fiber content depended on lettuce type (Figure 1, Table 1). Insoluble fiber content was higher ($P \leq 0.05$) in the romaine than in the crisphead cultivars. The USDA (2015) reported fiber content of 21 mg g^{-1} FW (420 mg g^{-1} DW) in romaine lettuce and 12 mg g^{-1} FW (240 mg g^{-1} DW) in 'Iceberg' lettuce, but total fiber, rather than insoluble fiber, was measured and leaf color of these cultivars were not mentioned. Eichholz et al. (2014) reported lower fiber content in red butterhead 'Teodore' than in green butterhead 'Wiske', suggesting leaf color can affect fiber content depending on lettuce type. Our study showed no significant difference in insoluble fiber content between green and red pigmented cultivars of romaine and crisphead lettuces. Based on the results, fiber intake in the diet may be increased by choosing romaine lettuce types over others. Among lettuce cultivars examined in our study, insoluble fiber content was higher in romaine than crisphead lettuce types. This is significant since crisphead ('Iceberg') is the most commonly used lettuce in fast food restaurants (Mulabagal et al., 2010).

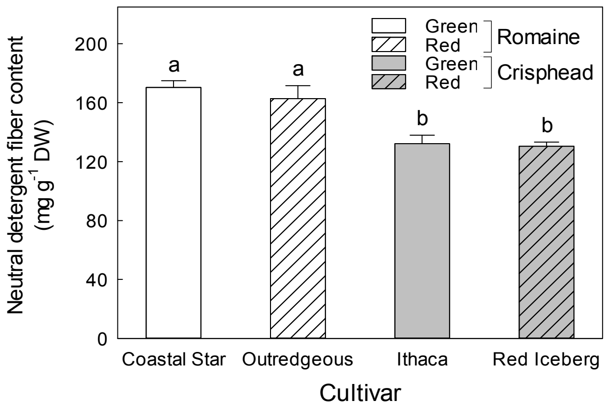

Figure 1. Neutral detergent fiber content in green and red cultivars of romaine (*L. sativa* L. var *longifolia*) and crisphead (*L. sativa* L. var *capitata*) lettuces

Note. Vertical bars are standard errors of the means with four replications (n = 4). Different letters indicate significant difference at $P \leq 0.05$ by Fisher's LSD.

Table 1. ANOVA results for neutral detergent fiber, total anthocyanin and total phenolic contents in green and red cultivars of romaine (*L. sativa* L. var *longifolia*) and crisphead (*L. sativa* L. var *capitata*) lettuces

Source of variation	Insoluble fiber	Total anthocyanin	Total phenolic
Type (T)	*	***	***
Color (C)	NS	***	***
T × C	NS	***	***

Note. ^{NS}, *, *** Nonsignificant or significant at $P \leq 0.05$ or 0.001, respectively.

3.2 Fatty Acid Profile

Major fatty acids present in romaine and crisphead lettuces were the essential fatty acids, α-linolenic acid (ALA) and linoleic acid (LA), which comprise over 75% of the total fatty acids (Table 2). Similarly, the major fatty acids reported for 'Trocadéro' butterhead lettuce (Le Guedard et al., 2008) and Cos romaine lettuce (Pereira, Li, & Sinclair, 2001) were ALA and LA. In our study, lettuce type, but not leaf color influenced total fatty acids content. Green romaine had greater ($P \leq 0.01$) ALA content than crisphead cultivars. The USDA (2015) reported higher ALA for romaine compared to crisphead ('Iceberg'). However, leaf color of these types were not identified. It has been reported that low omega-3 to omega-6 PUFA intake contributes to the increased burden of chronic diseases (Innis, 2014). Modern Western diets typified by the US are low in omega-3 PUFAs. Based on our results, green romaine lettuce provides a higher amount of omega-3 PUFA due to its higher ALA content. Although lettuce provides only a minor contribution to omega-3 PUFA intake in the human diet due to its low fat content, consumption of green romaine would fulfill some of the omega-3 fatty acid requirement.

Table 2. Fatty acid composition of green and red cultivars of romaine (*L. sativa* L. var *longifolia*) and crisphead (*L. sativa* L. var *capitata*) lettuces

Type	Color (Cultivar)	α-Linolenic acid (18:3n-3)	Linoleic acid (18:2n-6)	Palmitic acid (16:0)	Stearic acid (18:0)	Oleic acid (18:1n-9)	Total
		-----------mg g^{-1} DW-----------					
Romaine	Green (Coastal Star)	12.9±0.4a[z] (58.0%)[y]	4.5±0.2a (20.3%)	3.7±0.1a (16.5%)	0.8±0.0a (3.5%)	0.5±0.1a (1.7%)	22.2±0.7a
	Red (Outredgeous)	11.3±1.0ab (58.4%)	4.1±0.3a (21.4%)	3.1±0.2a (15.8%)	0.5±0.1a (2.8%)	0.3±0.1a (1.7%)	19.3±1.2a
Crisphead	Green (Ithaca)	7.3±1.1c (47.1%)	4.2±0.3a (28.1%)	3.0±0.4a (19.4%)	0.5±0.2a (3.0%)	0.3±0.1a (2.5%)	15.2±1.8a
	Red (Red Iceberg)	8.7±2.1bc (53.4%)	3.9±0.6a (24.8%)	2.7±0.4a (17.1%)	0.3±0.1a (2.2%)	0.4±0.1a (2.5%)	16.0±3.0a
Significance							
Type (T)		**	NS	NS	NS	NS	*
Color (C)		NS	NS	NS	NS	NS	NS
T × C		NS	NS	NS	NS	NS	NS

Note. [z] Data are presented as mean ± standard error (n = 4). Mean separation within columns by Fisher's LSD at $P \leq 0.05$; [y] Relative ratio of each fatty acid to the total fatty acid content; [NS], [*], [**] Nonsignificant or significant at $P \leq 0.05$ or 0.01, respectively.

3.3 Dietary Minerals

Inadequate intake of Zn and Fe is a concern in vegetarian diets (Hunt, 2003), in part due to a relatively low bioavailability of these minerals in plants compared to meat. Lettuce (100 g fresh weight) provided only 1-4% of the recommended Zn intake of 8-11 mg/day for adults (Kim et al., 2016). In our study, there was no significant difference in Zn content regardless of lettuce type and leaf color (Table 3). Fe content was higher ($P \leq 0.001$) in the romaine than the crisphead cultivars. Similarly, the USDA (2015) reported higher Fe content in romaine (9.7 μg g^{-1} FW) compared to crisphead ('Iceberg') lettuce (4.1 μg g^{-1} FW). Mou and Ryder (2004) also reported higher Fe content in romaine than in crisphead. Fresh lettuce (100 g) depending on lettuce type can provided up to 18% of recommended Fe intake of 8-18 mg/day for adults (Institute of Medicine, 2005; Kim et al., 2016).

Vegetarians may be at greater risk of low bone density and bone fracture due to low Ca in vegetarian diets (Tucker, 2014). The Ca content was higher ($P \leq 0.05$) in romaine than crisphead lettuces (Table 3). There was an interaction of lettuce type and leaf color with higher ($P \leq 0.05$) Ca content in the green than red crisphead cultivars, but not romaine lettuce cultivars. Another important mineral for bone health, P was higher ($P \leq 0.01$) in red than in green romaine and crisphead cultivars. Koudela & Petříková (2008) analyzed five cultivars of leaf lettuces with different leaf colors and reported a significant influence of cultivar on Ca content; however, influence of pigmentation on Ca content was not analyzed. Good-quality vegetarian diets are high in Mg and Mn which are also important to bone health (Tucker, 2014). In our study, romaine had significantly higher Mg content than crisphead lettuces. Red 'Outredgeous' romaine had higher Mn content than crisphead lettuces (Table 3). Based on our results, minerals important for bone health were generally higher in the romaine than the crisphead lettuces studied.

In our study, sodium (Na) content in lettuces ranged from 0.4-0.8 mg g^{-1} DW (20-40 μg g^{-1} FW) which is low based on the daily recommended intake of Na of 1.2-1.5 g/day for adults (Kim et al., 2016). Reducing Na and increasing K intake lowers the risk of hypertension (Adrogué & Madias, 2014). Red 'Outredgeous' romaine had the highest ($P \leq 0.05$) K content among the different lettuce cultivars evaluated. And mineral analysis within the same lettuce types revealed significantly higher amount of K as well as P content in red than green cultivars. While the role of leaf pigmentation on mineral content has not been intensively studied, our result indicated that leaf color might be associated with certain mineral compositions of romaine and crisphead lettuces. The mineral content of our study was generally lower than the result by Baslam, Morale, Garmendia, and Goicoechea (2013) or similar or higher in Zn and Mg content, respectively, reported by Lyons, Goebel, Tikai, Stanley, and Taylor (2015), indicating that mineral content in lettuce can greatly vary depending on lettuce type, cultivar, and cultural conditions.

Table 3. Mineral content in green and red cultivars of romaine (*L. sativa* L. var *longifolia*) and crisphead (*L. sativa* L. var *capitata*) lettuces

Type	Color (Cultivar)	Zn	Fe	Ca	P	Mg	Mn	Na	K
		---------μg g^{-1} DW--------		----------------mg g^{-1} DW--------------			--μg g^{-1} DW--	---------mg g^{-1} DW---------	
Romaine	Green (Coastal Star)	24.6±2.7a[z]	38.8±2.1a	9.2±0.3a	2.5±0.1bc	5.5±0.1a	19.0±0.5ab	0.8±0.2a	21.9±0.8b
	Red (Outredgeous)	25.1±0.7a	37.6±4.1a	9.1±0.3a	3.0±0.3a	5.8±0.2a	21.8±1.5a	0.7±0.1a	28.3±0.7a
Crisphead	Green (Ithaca)	22.5±3.0a	26.8±2.2b	8.1±0.4b	2.4±0.2c	5.0±0.2b	14.9±0.6c	0.5±0.1a	18.9±0.2c
	Red (Red Iceberg)	26.1±4.9a	27.9±2.0b	6.5±0.1c	2.9±0.2ab	4.6±0.1b	16.5±0.9bc	0.4±0.0a	21.9±0.6b
Significance									
Type (T)		NS	***	***	NS	***	***	*	***
Color (C)		NS	NS	*	**	NS	*	NS	***
T × C		NS	NS	*	NS	*	NS	NS	*

Note. [z] Data are presented as mean ± standard error (n = 4). Mean separation within columns by Fisher's LSD at $P \leq 0.05$; ^{NS}, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01 or 0.001, respectively.

3.4. Carotenoids

Several carotenoids including β-carotene have been studied for their ability to reduce risk of chronic diseases. Oxygenated carotenoids (xanthophylls) have been studied for its protective effects against macular degeneration (Cooper, Eldridge, & Peters, 1999). Some carotenoids such as β-carotene have a provitamin A activity with β-carotene to retinol (animal form of vitamin A) conversion rate ranging 3.6-28.1 by weight in humans (Tang, 2010). In our study β-carotene, lutein, and antheraxanthin were the major carotenoids ranging from 63.2% of total carotenoids in green romaine 'Coastal Star' to 70.6% in red crisphead 'Red Iceberg' (Table 4). The β-carotene and lutein were higher ($P \leq 0.01$) in red 'Outredgeous' romaine compared to the crisphead lettuces. According to the USDA database (2015), romaine contained a higher amount of β-carotene (52.3 μg g^{-1} FW) compared to crisphead ('Iceberg') lettuce (3.0 μg g^{-1} FW). It was difficult to make a direct comparison since the values in USDA database were reported from two studies, and growing conditions, leaf color, and cultivar were not provided.

Biosynthesis of carotenoids is regulated by light. Differences in carotenoid content in lettuce types was suggested to be related to head structure. Crisphead lettuce forms a closed head that obstructs light penetration into the head. In contrast, romaine lettuce has an open head structure which allows more light penetration, resulting in higher amount of carotenoids accumulated (Mou, 2005; Mou & Ryder, 2004). In these studies carotenoid contents were higher in green than red cultivars. In another study, the contents of carotenoids were evaluated in two differently pigmented lettuce cultivars; 'Blonde of Paris Batavia' (green) and 'Oak Leaf' (Pérez-López, Miranda-Apodaca, Muñoz-Rueda, & Mena-Petite, 2015). The carotenoids were higher in red than in green cultivar by 130%. In our study red leaf 'Outredgeous' contained the highest amount of total carotenoids ($P \leq 0.05$), and there was no difference between leaf colors within the same lettuce type. Of the major carotenoids measured in our study, there was an interaction of type and color on antheraxanthin content. Red romaine was higher ($P \leq 0.001$) in antheraxanthin than in green cultivars.

Other carotenoids detected in our lettuce samples were neoxanthin and violaxanthin (~13-18%). There was a significant effect of lettuce type, but not leaf color. Romaine was higher in neoxanthin ($P \leq 0.01$) than in crisphead lettuce. Violaxanthin was higher ($P \leq 0.05$) in red romaine than in the green crisphead, but no difference was found in cultivars differing leaf colors within the same type. Baslam et al. (2013) analyzed one romaine and two crisphead type lettuces and reported significantly higher levels of both neoxanthin and violaxanthin in romaine lettuce. Advantages of higher consumption of neoxanthin and violaxanthin in humans is unknown. We detected only small quantities of zeaxanthin and α-carotene at 1-1.8% of the total carotenoid content. Among the lettuce types evaluated in our study, total carotenoid content was highest in red 'Outredgeous' romaine lettuce.

Table 4. Carotenoid content in green and red cultivars of romaine (*L. sativa* L. var *longifolia*) and crisphead (*L. sativa* L. var *capitata*) lettuces

Type	Color (Cultivar)	α-Carotene	β-Carotene	Lutein	Antheraxanthin	Neoxanthin	Violaxanthin	Zeaxanthin	Total
		----------	----------	----------	---------- μg g^{-1} DW ----------	----------	----------	----------	----------
Romaine	Green (Coastal Star)	16.5±2.9a[z] (1.4%)[y]	280.6±7.8ab (23.6%)	338.5±11.1ab (28.5%)	132.5±5.1c (11.1%)	215.2±6.6a (18.1%)	184.2±6.5ab (15.5%)	22.0±1.1a (1.8%)	1189.4±38.0b
	Red (Outredgeous)	16.0±2.2a (1.0%)	344.2±35.1a (20.7%)	373.4±34.7a (22.5%)	450.8±44.8a (27.1%)	228.5±23.8a (13.8%)	226.2±27.7a (13.6%)	21.4±5.3a (1.3%)	1660.4±167.3a
Crisphead	Green (Ithaca)	12.7±1.3a (1.3%)	193.3±9.8c (19.1%)	220.3±14.3c (21.8%)	296.7±18.9b (29.3%)	135.6±8.4b (13.4%)	138.8±8.5b (13.7%)	14.4±0.9a (1.4%)	1011.7±60.6b
	Red (Red Iceberg)	12.2±0.6a (1.0%)	232.0±18.0bc (18. 7%)	268.9±21.2bc (21.7%)	374.2±19.1ab (30.2%)	160.6±12.6b (13.0%)	177.7±12.9ab (14.3%)	13.5±1.6a (1.1%)	1239.1±84.4b
Significance									
Type (T)		*	**	**	NS	**	*	*	*
Color (C)		NS	NS	NS	***	NS	NS	NS	*
T × C		NS	NS	NS	**	NS	NS	NS	NS

Note. [z] Data are presented as mean ± standard error (n = 4). Mean separation within columns by Fisher's LSD at $P \leq 0.05$; [y] Relative ratio of each carotenoid to the total carotenoid content; ^{NS}, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

3.5 Total Phenolic and Anthocyanin

Total phenolic and anthocyanin contents were significantly higher in romaine than in crisphead lettuce in both green and red cultivars (Figure 2, Table 1). Biosynthesis of phenolic compounds might be light dependent as suggested by Tsormpatsidis et al. (2008). As discussed above, romaine lettuces have an open head structure that allows greater light penetration compared to crisphead lettuces. Therefore, romaine lettuce could accumulate higher levels of phenolic compounds including anthocyanins.

Total phenolic content was significantly higher in red compared to green cultivars of romaine and crisphead (Figure 2, Table 4). Llorach, Martínez-Sánchez, Tomás-Barberán, Gil, and Ferreres (2008) reported higher total phenolic content in red oak leaf and lollo rosso than in green crisphead ('Iceberg') and romaine lettuces. Liu et al. (2007) also reported higher total phenolic content in red compared to green cultivars of romaine lettuces as well as leaf type lettuces. We evaluated anthocyanin content since this subgroup of phenolic compound is the main plant pigment responsible for the red color in lettuce (Ferreres, Gil, Castañer, & Tomás-Barberán, 1997). Total anthocyanin content in red 'Outredgeous' romaine and crisphead 'Red Iceberg' was 3418.2 and 442.5 μg g^{-1} DW, respectively (Figure 2). No anthocyanin was detected in green cultivars of either type lettuce. Similarly, anthocyanin in red oak leaf and lollo rosso lettuce was reported, but anthocyanin was not detected in green cultivars of crisphead ('Iceberg') and romaine (Llorach et al., 2008). Based on the results, red pigmentation was indicative of total anthocyanin and phenolic content. There was also an interaction between leaf color and lettuce type (Table 4). Total phenolic and anthocyanin was significantly higher in red 'Outredgeous' romaine than crisphead 'Red Iceberg' (Figure 2). Red lettuce is increasing in popularity due to public perception that red color is associated with greater health benefits. Among lettuces evaluated in our study, choosing red leaf lettuce such as 'Outredgeous' romaine lettuce may result in greater intake of anthocyanin and phenolic compounds which have been associated with diverse health benefits (Kris-Etherton et al., 2002).

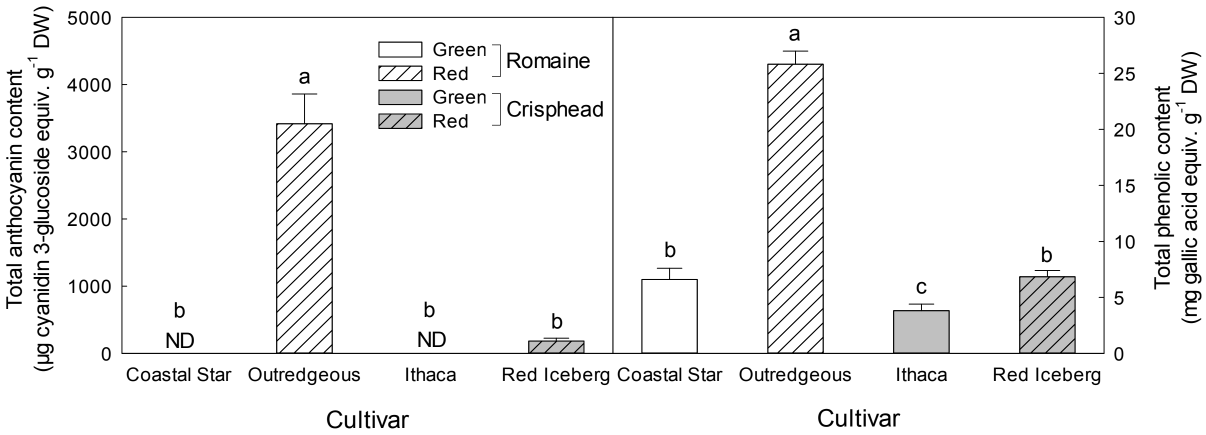

Figure 2. Total anthocyanin and phenolic contents in green and red cultivars of romaine (*L. sativa* L. var *longifolia*) and crisphead (*L. sativa* L. var *capitata*) lettuces

Note. Vertical bars are standard errors of the means with four replications (n = 4). Different letters indicate significant difference at $P \leq 0.05$ by Fisher's LSD.

4. Conclusions

Although lettuce is considered low in nutritional value, the results of the current study showed that nutrient content depended on lettuce type, as well as the morphological factor of leaf color. Generally, the crisphead lettuces were lower in all nutrients analyzed compared to romaine lettuces. Romaine cultivars contained higher amount of insoluble fiber and the essential fatty acids, ALA and LA, which comprise over 75% of the total fatty acids regardless of leaf color. Red cultivars had higher phenolic content than green cultivars with the highest amount in red 'Outredgeous' romaine lettuce. The results of this study provide comparative nutrient data of several popularly consumed lettuce cultivars that were grown under the same growing condition. This information will assist consumers to make food choices that provide higher nutritional value.

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