

GENOTYPIC VARIATION IN NUTRITIONAL AND ANTIOXIDANT PROFILE AMONG ICEBERG LETTUCE CULTIVARS

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ABSTRACT

Regular consumption of fruits and vegetables is associated with reduced risk of chronic diseases and improvement of cognitive health. Lettuce (*Lactuca sativa* L.) is a major salad food, which is widely grown and consumed worldwide. Cultivars-specific information on bioactive composition of crisphead subtype iceberg remains scarce. The present study aimed to profile the nutrition and antioxidant composition of seven new cultivars of iceberg ('Equinos', 'Ice Castle', 'Metalia', 'Num 189', 'Silvinas', 'Ombrinas' and 'Vanguardia'). The head fresh mass of the iceberg cultivars ranged from 485 to 801 g per plant, with the highest values recorded in 'Num 189' and 'Vanguardia'. Across cultivars, K was the predominant macronutrient and was followed by Ca. The highest nitrate content was recorded in 'Equinos' and 'Umbrinas', whereas the lowest value was observed in 'Metalia'. The hydrophilic (HAA) and lipophilic antioxidant activities (LAA) of the selected iceberg cultivars ranged from 4.9 to 13.0 mmol ascorbic acid 100 g⁻¹ dw, and from 6.8 to 12.5 mmol Trolox 100 g⁻¹ dw, respectively. Chicoric acid was the main phenolic acid present in all cultivars, followed by chlorogenic acid and caffeoyl tartaric acid. The highest HAA, LAA, caffeoyl tartaric acid, chicoric acid and total phenolic content were observed in 'Num 189', whereas an opposite trend was recorded for chlorogenic acid. The current findings will improve knowledge of the compositional variation among iceberg cultivars and assist growers in selecting cultivars combining optimal yield with high nutritional value.

Key words: antioxidant activity, functional quality, *Lactuca sativa* L., mineral composition, phenolic acid profile

INTRODUCTION

Epidemiological studies indicate that regular consumption of fruits and vegetables is associated with reduced risk of non communicable chronic diseases, such as cardiovascular disease, type 2 diabetes, obesity, and some types of cancer, but also with improvement of cognitive health [Llorach et al. 2008, Slavin and Lloyd 2012]. Hence in an effort to promote well-being and improve life expectancy, while alleviating

the cost of national health care systems, government health agencies in several countries continuously advocate the consumption of fruits and vegetables [Kim et al. 2016]. The health promoting effects of vegetables have been related to their nutritional composition and particularly to their content of bioactive compounds such as dietary fiber and polyphenols [Kris-Etherton et al. 2002].

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Lettuce (*Lactuca sativa* L.) is a major salad food crop belonging to the *Asteraceae* family, which is widely grown and consumed worldwide [Baslam et al. 2013]. It has garnered a central role in human nutrition as it combines generally pleasing organoleptic properties with a rich content of nutraceutical compounds [Kim et al. 2016]. The health-promoting properties of lettuce have been attributed to minerals, vitamins (B9, C and E) and bioactive terpenoids and polyphenols such as carotenoids, phenolic acids and flavonoids. Its high content of phytonutrients is combined with a low content of dietary fats which makes lettuce an attractive low-calorie food whose consumption is highly suggested within weight-loss dietary plans [Kim et al. 2016]. On the other hand, leafy vegetables are characterized by high nitrate accumulation, thus constituting an important dietary source of nitrate for humans [Parks et al. 2008]. Nitrate *per se* is a non-toxic compound; however its metabolites and reaction products (e.g. nitrite, N-nitroso compounds and nitric oxide) are responsible for adverse health effects associated with the intake of excessive amounts of nitrate (e.g. methaemoglobinaemia and carcinogenesis) [Amr and Hadidi 2001, Parks et al. 2008].

Lettuce comes in a variety of leaf shapes, sizes, textures, head formations and colors, and it is conventionally classified into seven major groups: Butterhead, Crisphead, Cos, Cutting, Stalk, Latin and Oilseed lettuce [Lebeda et al. 2007]. Quantitative and qualitative variation in lettuce bioactive compounds depends on many preharvest factors, such as genotypic and environmental, as well as on factors related to production systems and agronomic practices [Rouphael et al. 2012a]. Genetic material is a key preharvest factor and the major determinant of the biosynthesis, composition and reposition of bioactive compounds [Rouphael et al. 2012a, Colonna et al. 2016]. Considering that Italy is the European leader in leafy vegetables (including lettuce), in terms of cultivated area, production volume and market value [<http://agri.istat.it>], the selection of lettuce cultivars having a pronounced content of phytonutrients is of particular interest for nutritionists, food scientists, consumers as well as growers.

Crisphead subtype iceberg is highly commercialized in Italy as a fresh-cut or minimally processed

leafy vegetable and extensively cultivated under both open field and greenhouse conditions. Despite iceberg lettuce is one of the most consumed and sensorially appreciated salad vegetable, a systematic nutritional characterization of its cultivars is still lacking in the literature.

In this framework, the aims of the current study were: (1) to depict the nutritional and antioxidant profile of seven iceberg lettuce cultivars by focusing on mineral composition, antioxidant capacity as well as on total polyphenols and phenolic acid content, and (2) to assess the associations between these nutritional traits. With several cultivars profiled for the first time, these findings will allow a better understanding of the variation in nutritional quality of a wide collection of iceberg cultivars. The results will also assist growers in selecting suitable cultivars of optimal yield and quality thereby increasing their crop's profitability.

MATERIALS AND METHODS

Plant material, experimental design and sampling. The experiment was conducted in spring 2015 at the experimental farm of 'Il Giardino di Getsemani' – Latina, central Italy (latitude 41°47'N, longitude 12°90'E, altitude, 21 m), on clay soil (26% sand, 26% silt, 48% clay), with a pH of 7.6, electrical conductivity of 0.4 dS m⁻¹, organic matter of 2.4% (w/w), total N at 0.14%, available P at 120 mg kg⁻¹, and exchangeable K at 256 mg kg⁻¹. Seven iceberg lettuce cultivars: 'Equinos', 'Ice Castle', 'Metalia', 'Num 189', 'Silvinas', 'Ombrinas' and 'Vanguardia' were selected from different seed suppliers (Nuhmens, Rijk Zwaan and Syngenta). These cultivars were selected as the most representative of commercial iceberg cultivars used in Italy.

Lettuce plantlets at the stage of three-to-four true leaves were transplanted to the open field on 15 April at a crop density of 80,000 plants ha⁻¹. An experimental design of Randomized Complete Blocks was applied, with treatments replicated three times. Each experimental unit consisted of a 12 m² plot area. Base-dressing fertilizer containing 11% N, 22% P₂O₅ and 16% K₂O was broadcast at a dosage of 700 kg ha⁻¹ and incorporated mechanically into the soil. Additional

top-dressing fertilizer (90 kg ha⁻¹ N and 110 kg ha⁻¹ K₂O) was applied biweekly through mini-sprinklers using NH₄NO₃ and KNO₃ as sources of N and K.

Plants were harvested during the last week of May at the same physiological age, expressed as the standard accumulation of growing degree days after transplanting. For the determination of antioxidant activities, phenolic acid profile and total phenolic content, a representative fraction of each lettuce head was instantly frozen in liquid nitrogen and stored at -80°C until used. Total and marketable yield was determined on sampling areas of 4 m² from the center of each experimental unit plot.

Dry matter and mineral content analyses.

The leaf dry matter (DM) content was determined by oven drying at 65°C to constant weight, according to the official method 934.01 of the Association of Official Analytical Chemists [AOAC 2005]. Dried leaves were ground in a Wiley Mill (IKA Werke MF 10 Basic, Germany) to pass through a screen of 841 microns, and then portions of the dried tissues were analyzed for the following elements: K, Ca, Mg, Na and nitrate nitrogen (N-NO₃). Nitrate content was determined on aqueous extracts of the dried leaf samples, based on the cadmium reduction method [Sah 1994]. The absorbance of the solution was determined at 550 nm wavelength using a Hach DR 2000 spectrophotometer (Hach Co., Loveland, Colorado, USA), and N-NO₃ was expressed as mg kg⁻¹ fresh weight (fw) on the basis of the original sample dry matter content.

Potassium, Ca, Mg and Na were extracted from 250 mg dried leaf samples with deionized water at 80°C in a shaking water bath for 10 min (ShakeTemp SW22, Julabo, Seelbach, Germany). The resulting solution was filtered, diluted, and analyzed by ion chromatography using an ICS-3000, Dionex system (Dionex, Sunnyvale, CA, USA). A conductivity detector with IonPac CG12A guard column and IonPac CS12A analytical column were used for the analysis of minerals.

Analysis of hydrophilic and lipophilic antioxidant activities. Two different cation assays were applied on lyophilized samples to measure the antioxidant activity of the hydrophilic (HAA) and lipophilic (LAA) fractions. For the HAA assay, samples were

extracted with distilled water and their antioxidant activity was measured with the N,N-dimethyl-p-phenylenediamine (DMPD) method [Fogliano et al. 1999] and expressed as mmol of ascorbic acid 100 g⁻¹ dry matter (DM). The LAA assay was performed on samples extracted with methanol and measured with the 2,2'-azinobis 3-ethylbenzothiazoline-6-sulfonic acid ABTS method [Pellegrini et al. 1999] and expressed as mmol of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) per 100 g DM. Both HAA and LAA were determined by UV-Vis spectrophotometry based on the extracts absorbance at 505 and 734 nm, respectively. External standard (ascorbic acid/Trolox) calibration curves within linear absorbance limits were used for quantification in each assay.

Determination of total phenolics. Total phenolics were quantified in methanol extracts using the Folin-Ciocalteu procedure [Singleton et al. 1999] and gallic acid as an external standard. A 100 µl aliquot of the supernatant was combined with 500 µl of Folin-Ciocalteu's reagent (Sigma Aldrich Inc, St Louis, MO, USA) and 400 µl of 7.5% sodium carbonate/water (w/v). The assay mixture was obtained inside centrifuge tubes, shaken in an IKA® Vortex Genius 3 (IKA®-Werke GmbH & CO.KG, Germany) for 15 s and then allowed to stand for 30 min at room temperature. Absorption was measured after 30 min at 765 nm using a UV-Vis spectrophotometer, and the result was expressed as mg gallic acid (Sigma Aldrich Inc, St Louis, MO, USA) per 100 g dry weight using an external standard calibration curve.

Analysis of phenolic acids by HPLC. All reagents and solvents used were of HPLC grade, purchased from Merck (Darmstadt, Germany). Phenolic acids standards used for identification and quantification were obtained from Sigma (Sigma –Aldrich, Milano, Italy). The extraction of phenolic acids from lettuce was performed as described by Llorach et al. [2008] with brief modifications. Briefly, 12 ml of methanol/water/formic acid (25/24/3) were added to 0.4 g of freeze-dried samples. The mixture was sonicated for 30 min. After the ultrasounds-assisted extraction, the samples were centrifuged (4000 rpm for 30 min at 4°C) and the supernatant was collected. A further centrifugation at 14800 rpm for 15 min at 4°C was

applied before HPLC analysis. The chromatographic separation of phenolic acids was carried out using a C18 reversed phase column (Prodigy 5 μm ODS-3 250 \times 4.6 mm, Phenomenex, Torrance, CA), according to the method described by Llorach et al. [2008], with slight modifications.

The HPLC system consisted of two binary pumps (LC-20A, Shimadzu, Kyoto, Japan), equipped with an UV-Vis detector (SPD20A, Shimadzu, Kyoto, Japan) and an auto-sampler (SIL-20A, Shimadzu, Kyoto, Japan). The mobile phases were: water with 5% of formic acid (A) and methanol (B). The flow rate was 1 $\text{cm}^3 \text{min}^{-1}$ and the following gradient of solvent B was applied: (min)/(%B) (0/5), (25/40), (32/40), (37/5), (43/5). Twenty microliters of extract were injected and the analysis was performed in duplicate. The UV chromatogram was recorded at 330 nm. Three main phenolic acids (caffeoyl tartaric acid, chlorogenic acid and chicoric acid) were identified (by LC-MS), and quantified (by HPLC) as chicoric acid equivalents (fig. 1).

Statistical analysis. Analysis of variance (ANOVA) and Duncan's multiple range tests with significance at $p \leq 0.05$ were performed using the SPSS 20 software package (www.ibm.com/software/analytics/spss). Quality traits were subjected to principal component analysis (PCA), to explore relationships among variables and treatments, and also to determine which traits were the most effective in discriminating between cultivars. The PCA outputs include variable loading to each selected component and treatment component scores.

RESULTS AND DISCUSSION

Head Fresh Mass and Yield. The head fresh mass per plant of the selected iceberg cultivars ranged from 485 to 801 g, with the highest values recorded in 'Num 189' and 'Vanguardia' (tab. 1). The highest fresh total and marketable yields were also observed for 'Vanguardia', while the cultivar 'Umbrinas' exhibited the lowest fresh leaf production. The marketable yield of the remaining iceberg cultivars was in the following order: 'Num 189' > 'Equinos' = 'Ice Castel' = 'Silvinas' > 'Metalia' (tab. 1). The fresh leaf production of the iceberg cultivars was consistent

with the findings of Ryder [1999], who reported head fresh mass values ranging from 300 to 750 g per plant. The head size is a primary visual criterion for the consumer in making purchasing decisions, and is also important for growers, since the priority is to pack a fixed number of heads per carton when harvesting [Maboko et al. 2015].

Table 1. Effect of iceberg lettuce cultivars on head fresh mass, total and marketable yields

Cultivar	Head fresh mass (g plant ⁻¹)	Total yield (t ha ⁻¹)	Marketable yield (t ha ⁻¹)
Equinos	713 b	57.1 b	47.9 c
Ice Castle	725 b	58.1 b	47.8 c
Metalia	642 c	51.4 c	44.5 d
Num 189	782 a	62.6 a	51.9 b
Silvinas	700 b	56.0 b	49.0 c
Umbrinas	485 d	38.8 d	35.3 e
Vanguardia	801 a	64.1 a	56.9 a
Significance	**	**	*

Values are the means of three replicate samples; means within columns separated using Duncan's multiple range test, $P = 0.05$; *, ** – significant at $P < 0.05$ or 0.01, respectively

Leaf dry matter, mineral composition and nitrate content. The iceberg/crisphead lettuce group is known for its more succulent texture and higher water content than other lettuce types. In fact, the dry matter content recorded for the seven select cultivars was characteristically low and ranged from 4.0% to 5.1% (tab. 2), in line with the value (4.4%) reported by the USDA National Nutrient Database. The highest leaf dry matter content was observed in 'Metalia' and 'Umbrinas', whereas the lowest values were recorded in both 'Equinos' and 'Vanguardia' (tab. 2).

Vegetables significantly contribute toward human daily allowance of dietary vitamins and minerals required for maintaining a good health and metabolic functions [Kim et al. 2016]. Levander [1990] reported that vegetables contribute normally by 35%, 7% and 24% to the human dietary intake of total K, Ca and Mg, respectively. Increased intake of K, Ca and

Table 2. Effect of iceberg lettuce cultivars on dry matter percentage (DM), macronutrient composition, sodium and nitrate contents of iceberg lettuce

Cultivar	DM (%)	K (mg g ⁻¹ dw)	Ca (mg g ⁻¹ dw)	Mg (mg g ⁻¹ dw)	Na (mg g ⁻¹ dw)	NO ₃ -N (mg kg ⁻¹ fw)
Equinos	4.2 bc	25.1 c	3.0 b	1.5	0.4	1183 a
Ice Castle	4.5 b	34.9 b	4.7 a	1.8	0.6	1059 b
Metalia	5.1 a	33.8 b	4.6 a	1.7	0.6	740 c
Num 189	4.8 ab	39.2 a	4.5 a	1.6	0.5	1024 b
Silvinas	4.6 b	43.5 a	4.7 a	1.5	0.7	749 c
Umbrinas	5.1 a	38.3 a	3.1 b	1.5	0.5	1223 a
Vanguardia	4.0 c	40.1 a	4.6 a	1.6	0.5	1030 b
Significance	*	**	*	n.s.	n.s.	**

Values are the means of three replicate samples; means within columns separated using Duncan's multiple range test, $P = 0.05$; n.s. – nonsignificant, *, ** – significant at $P < 0.05$ or 0.01 , respectively

Mg has been associated with lowering of blood pressure, bone health and reduced risk of osteoporosis [Sacks et al. 1998, Soetan et al. 2010, Gupta and Gupta 2014]. In the current experiment, the content of minerals in leaf iceberg lettuce cultivars ranged as follows: K (25.1–43.5 mg g⁻¹ dw), Ca (3.0–4.7 mg g⁻¹ dw), Mg (1.5–1.8 mg g⁻¹ dw) and Na (0.5–0.7 mg g⁻¹ dw), with K being the most abundant macronutrient (tab. 2). The lowest K content was recorded in 'Equinos', whereas, the lowest Ca content was observed in the cultivars 'Equinos' and 'Umbrinas'. Our findings on the Ca and Mg contents of iceberg cultivars tested were proximate to those reported by the National Nutrient Database for Standard References [USDA 2015; 4–8 mg g⁻¹ and 1.4–2.8 mg g⁻¹, respectively]. On the contrary the values recorded for K and Na contents were not in line with those reported by the USDA Database [2015]. This disagreement could be attributed to variation in cultivars, different growing practices and prevailing environmental conditions. Interestingly, the projected contribution of Na from iceberg is relatively low (mean 0.55 mg g⁻¹ dw). Low content of Na in vegetables is considered a positive nutritive trait, since high Na intake is associated with hypertension and cardiovascular diseases risk [Krummel 2000].

Concerning the undesirable components such as nitrates, the highest values were recorded in 'Equinos' and 'Umbrinas' (tab. 2). The nitrate content of

the remaining iceberg cultivars descended in the following order: 'Ice Castle' = 'Num 189' = 'Vanguardia' > 'Metalia' = 'Silvinas'. Moreover, the nitrate contents of the seven iceberg cultivars ranged between 740 and 1223 mg kg⁻¹. This finding was in accordance with Raczuk et al. [2014] who reported for iceberg lettuce a range of 125–1580 mg kg⁻¹ fw, that was far lower than nitrate content recorded on other lettuce types such as cos and butterhead (> 4000 mg g⁻¹ fw) [Raczuk et al. 2014]. Moreover the nitrate content found were below the maximum limit of 2000 mg kg⁻¹ fw imposed by the European Community for iceberg type lettuce grown in the open air irrespective of the season [European Commission 2011].

Antioxidant activity, total polyphenols and phenolic acid profile. The antioxidant activity, total phenols content and phenolic acid profile in lettuce leaves appears to be strongly influenced by genetic factors (tab. 3), as previously demonstrated for several baby leaf vegetables [Colonna et al. 2016] and leaf artichoke [Rouphael et al. 2012b, Colla et al. 2013]. In the current study, the HAA and LAA of the selected iceberg cultivars ranged from 4.9 to 13.0 mmol ascorbic acid 100 g⁻¹ dw, and from 6.8 to 12.5 mmol Trolox 100 g⁻¹ dw, respectively (tab. 3). The highest HAA was observed in the cultivars 'Equinos', 'Ice Castle', 'Num 189' and 'Vanguardia', whereas the highest level of LAA was recorded in 'Num 189'

(tab. 3), indicating that ‘Num 189’ could be considered a good source of natural antioxidants.

Quantitative but not qualitative differences were observed in the phenolic acid profile among cultivars. Particularly, three main caffeic acid derivatives were identified in the seven iceberg cultivars analyzed (fig. 1). Chicoric acid (dicafeoyltartaric acid; DCTA) was the main phenolic acid present in all cultivars, followed by chlorogenic acid (5-caffeoylquinic; 5-CQA) and caffeoyl tartaric acid (CTA; tab. 3). These findings are in agreement with previous com-

positional studies on lettuce [Zhao et al. 2007, Llorach et al. 2008]. Among cultivars, ‘Num 189’ was richer in CTA and DCTA content, which is in agreement with its higher total phenolic content and antioxidant activity than the other cultivars. The total phenolic content, encompassing plant secondary metabolites with well known beneficial properties against oxidative stress and age-related neurodegeneration [Slavin and Lloyd 2012], ranged from 9.2 to 11.8 mg gallic acid 100 g⁻¹ dw, with the highest values recorded in ‘Num 189’ (tab. 3).

Table 3. Effect of iceberg lettuce cultivars on hydrophilic (HAA) and lypophylic (LAA) antioxidant activities, mono-caffeoyl tartaric, chlorogenic and chicoric acids and total phenols (TP) content

Cultivar	HAA (mmol asc. acid 100 g ⁻¹ dw)	LAA (mmol Trolox 100 g ⁻¹ dw)	Caffeoyl tartaric acid (mg 100 g ⁻¹ dw)	Chlorogenic acid (mg 100 g ⁻¹ dw)	Chicoric acid (mg 100 g ⁻¹ dw)	TP (mg gallic acid 100 g ⁻¹ dw)
Equinos	12.4 a	7.6 c	10.9 d	28.6 d	59.2 e	10.4 b
Ice Castle	12.4 a	9.8 b	6.6 e	29.0 d	68.1 c	11.0 b
Metalia	9.8 b	6.8 d	21.8 b	38.7 b	63.4 d	9.2 c
Num 189	12.0 ab	12.5 a	86.7 a	23.5 e	93.0 a	11.8 a
Silvinas	9.9 b	10.4 b	6.1 e	39.4 b	73.3 b	11.3 b
Umbrinas	4.9 c	8.8 c	10.7 d	45.3 a	68.1 c	11.2 b
Vanguardia	13.0 a	8.5 c	13.0 c	33.4 c	72.6 b	9.2 c
Significance	***	***	***	***	***	*

Values are the means of three replicate samples; means within columns separated using Duncan’s multiple range test, $P = 0.05$; *, *** – significant at $P < 0.05$ or 0.001, respectively

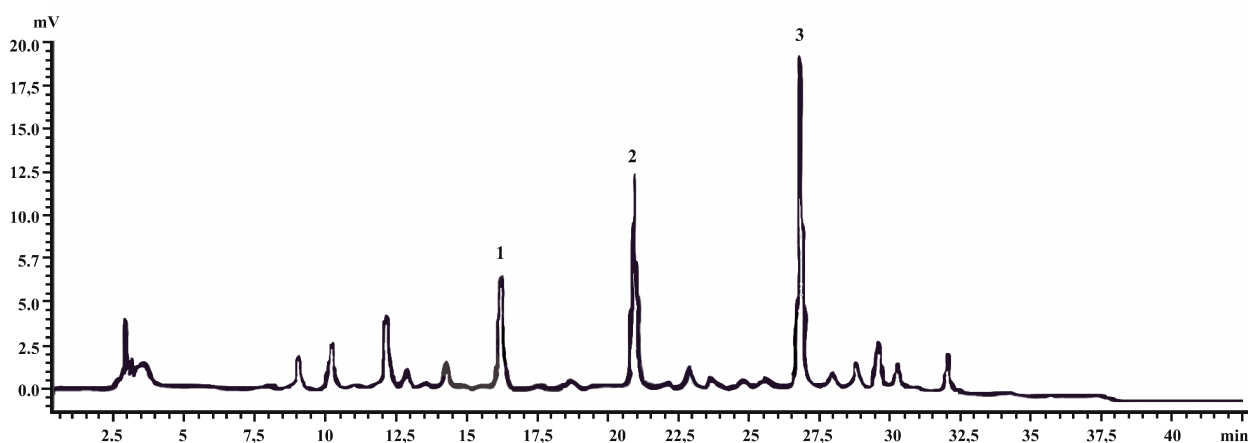


Fig. 1. HPLC chromatogram of iceberg cultivar ‘Metalia’; detection was performed at 330 nm; peaks are as follow: (1) caffeoyltartaric acid (CTA), (2) chlorogenic acid (5-CQA) and (3) chicoric acid (dicafeoyltartaric acid) DCTA

Principal component analysis. Principal component analysis (PCA) was performed to interpret the variation in the nutritional composition of iceberg cultivars. The first three PCs were associated with eigen values higher than 1 and explained 85.0% of the total variance, with PC1 accounting for 40.9% and PC2 for 32.0% (tab. 4). PC1 was correlated positively and strongly (>0.6) with head mass, Ca, LAA, caffeoyl tartaric and chicoric acid, whereas PC2 was positively correlated with dry matter, K and Na, and negatively correlated with HAA (tab. 4). The loading plot in fig. 2A illustrates the relations among nutritional traits. For instance, variation in Na was most closely aligned to that of K content, and variation in TP content was more strongly correlated to K (i.e. narrower angle between the corresponding vectors) rather than Na content. Similarly, LAA was more strongly correlated to DCTA content than to Ca and CTA. The two PCs score plot separates and categorizes

treatment populations into four groups, enabling interpretation of results on the basis of all parameters examined (fig. 2B). The upper right quadrant in the positive side of PC1, included ‘Silvinas’ and ‘Num 189’. These cultivars, in particular ‘Num 189’ were mostly characterized by high mineral composition (K and Ca), DCTA and CTA contents but also high LAA and TP. The cluster in the lower right quadrant represents iceberg cultivars (‘Ice Cristal’ and ‘Vanguardia’) characterized by high head mass, Mg content and HAA. The upper and lower left quadrants (negative side of PC1), which clustered three iceberg cultivars (‘Umbrinas’, ‘Metalia’ and ‘Equinos’), depicted the treatments with the highest nitrate (lower quadrant) and highest DM and 5-CQA levels (upper quadrant). The results of the PCA may provide the basis for a more in-depth approach to elucidate the effects of genetic variation on the head mass and nutritional quality of iceberg.

Table 4. Eigen values, relative and cumulative proportion of total variance, and correlation coefficients for each quality trait with respect to the three principal components

Principal components	PC1	PC2	PC3
Eigen value	4.1	2.8	1.6
Percentage of variance	40.9	32.0	12.1
Cumulative variance	40.9	72.9	85.0
Eigen vectors ^a			
Head mass	0.790	-0.543	-0.175
Dry matter	-0.246	0.682	-0.021
Nitrate	-0.325	-0.378	0.774
Ca	0.710	0.158	-0.656
K	0.430	0.704	-0.171
Mg	0.297	-0.233	-0.464
Na	0.335	0.602	-0.494
Hydrophilic antioxidant activity	0.626	-0.737	-0.212
Lipophylic antioxidant activity	0.795	0.376	0.424
Caffeoyl tartaric acid	0.659	0.107	0.480
Chlorogenic acid	-0.697	0.607	-0.276
Chicoric acid	0.821	0.338	0.368
Total phenols	0.338	0.483	0.626

Boldface factor loadings are considered highly weighed

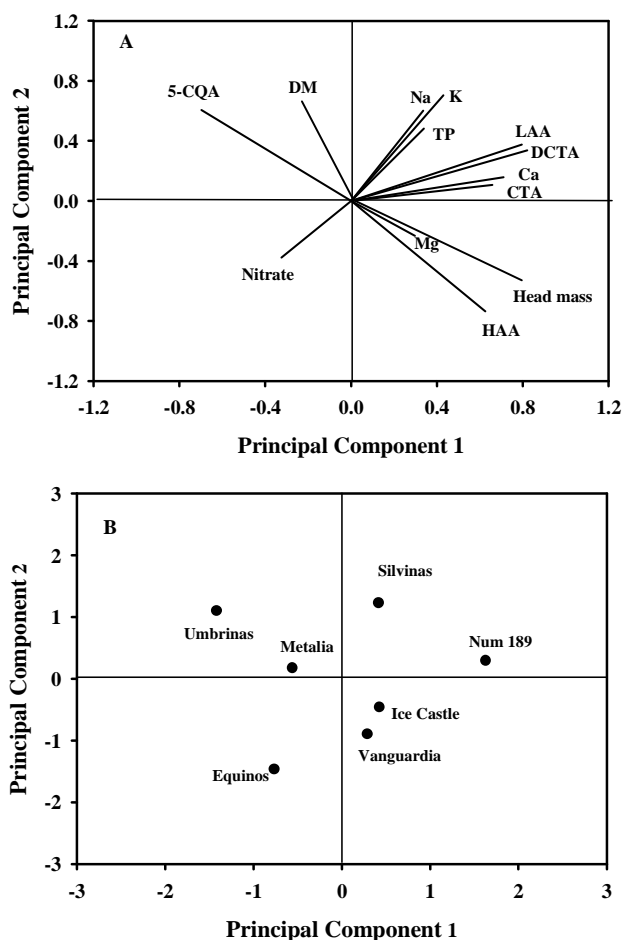


Fig. 2. (A) Loading plot and (B) component scores of the first and second principal components after PCA analysis on nutritional traits in seven iceberg lettuce; DM – dry matter; HAA – hydrophilic antioxidant activity; LAA – Lipophylic antioxidant activity; TP – total phenols; CTA – caffeoyl tartaric acid; 5-CQA – chlorogenic acid; DCTA – chicoric acid

CONCLUSIONS

Consumers, nutritionists and also growers have become more interested and demanding for nutraceutical foods that promote well-being and reduce the risk of diseases. Data showed that the nutritional and antioxidant profiles of the cultivars ‘Num 189’ (higher LAA, CTA, DCTA and TP), ‘Silvinas’ (high mineral composition) and ‘Metalia’ (higher 5-CQA)

make them good candidates for health promoting diets. Our data also demonstrated that the fresh yield and phytochemicals in iceberg are significantly influenced by genotypic constitution, thus indicating that specific cultivars should be selected to attain an optimal profile of phytonutrients.

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