Nutrient contents of kale (*Brassica oleraceae* L. var. *acephala* DC.)

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Abstract

Fructose, glucose and sucrose, as the major soluble sugars and citric and malic acids, as the major organic acids, were identified and determined in kale (*Brassica oleraceae* L. var. *acephala* DC., black cabbage) leaves. Fructose was the predominant sugar (2011 mg 100 g^{-1} dry wt) identified, followed by glucose (1056 mg 100 g^{-1} dry wt) and sucrose (894 mg 100 g^{-1} dry wt). The contents of citric and malic acids were at 2213 and 151 mg 100 g^{-1} dry wt in the leaves. The 16:0, 18:2$n-6$ and 18:3$n-3$ fatty acids were the most abundant fatty acids in the leaves. Considering the level of these fatty acids, 18:3$n-3$ was found to be the highest (85.3 μg g^{-1} dry wt), contributing 54.0% of the total fatty acid content. Linoleic acid (18:2$n-6$), being the second most abundant fatty acid was present at 18.6 μg g^{-1} dry wt, contributing 11.8% of the total fatty acid content. In the seed oil of kale, 22:1$n-9$ was the most abundant fatty acid (4198 μg g^{-1} dry wt, 45.7%), with 18:2$n-6$ (1199 μg g^{-1} dry wt, 12.3%) and 18:1$n-9$ (1408 μg g^{-1} dry wt, 14.8%) being the second next most abundant fatty acids. The most abundant amino acid was glutamic acid (Glu) which was present at 33.2 mg g^{-1} dry wt. Aspartic acid, which was the second most abundant amino acid, was present at 27.6 mg g^{-1} dry wt and accounted for 10.2% of the total amino acid content of kale leaf. The amino acid content was assessed by comparing the percentages of the essential amino acids in kale leaf versus those of a World Health Organization (WHO) standard protein. The protein of kale leaf compares well with that of the WHO standard. Only one amino acid, lysine, had a score that fell below 100%; the lysine score of kale leaf was 95%. This study attempts to contribute to knowledge of the nutritional properties of the plant. These results may be useful for the evaluation of dietary information.

Keywords: Sugar; Acid; Fatty acid; Amino acid; Mineral; Kale; Black cabbage; *Brassica oleraceae* var. *acephala*

1. Introduction

Brassicaceous plants represent one of the major vegetable crops grown, worldwide. White cabbage (*Brassica oleraceae* L. var. *capitata* L.) and black cabbage, kale, (*Brassica oleraceae* L. var. *acephala* DC.) are the major cabbages consumed in Turkey. Geographically, the kale (black cabbage) leaves are widely consumed, especially in northern parts of Turkey (in northeast Anatolia) during the winter season. The most common use of kale leaves is for fresh consumption, as soup, in different ways. Part of the crop is occasionally used to make pickles. The early consumption of the plant begins from late autumn to early spring (through December and the following three or four months). The leaves contribute widely to general diets of the people living in the region

in winter season. In fact, some ailments have been attributed to too much consumption of the crop by the people in the region. Besides the leaf benefits, the seed of kale has been used for its oil by mixing the crude oil with some food products (e.g., bread and cake).

Plants possess free sugars, organic acids, amino acids (free and in proteins), lipids and minerals which are natural components of many fruits and vegetables and they play an important role in maintaining fruit and vegetable quality and determining nutritive value in human diet. Amino acids, a class of biologically active compounds present in food and beverages, are important for human nutrition and affect the quality, including taste, aroma and colour (Belitz & Grosch, 1999). Among different substances that constitute fruits and vegetables, amino acids are becoming increasingly important and, for various reasons, their analytical determination is becoming more necessary (Gomis, Lobo, Alvarez, & Alonso, 1990). Lipids are partly responsible for the physical and chemical properties of food and those that are of major nutritional interest are the fatty acid esters. Many lipid properties in food are explained in terms of their fatty acid composition (Blanco-Gomis, Alonso, Cabroles, & Abrado, 2001). Minerals play an important role in the proper development and good health of the human body (Tahvonen, 1993). Fruits and vegetables are an important source of these. The risk of deficiencies and attendant pathologies depends on a number of factors, such as the daily dietary intake, the chemical form of the minerals contained in the food consumed, the technological treatment of the products, the presence of substances that limit or increase the bioavailability of minerals and the physiological state and overall health of the consumer (Barberá & Farré, 1992).

The determination of the chemical composition of kale has become an interest of ours due to its extensive consumption in the north of Turkey. The aim of this work was, first to contribute to the scarce knowledge about the free sugars, fatty acids (including seed oil), free amino acids and mineral composition of kale (black cabbage) leaf. Second, after displaying the nutritional profile of the plant, the data would aid knowledge of how important kale leaf is nutritionally for the diet of people who consume it. Furthermore, the marked changes that occur in the sugar, organic acid, amino acid, mineral and fatty acid compositions and profiles of such fresh vegetables can have deleterious effects on their acceptability as a food source. To our knowledge, no information is available on the levels of sugars, fatty acids, proteins (free amino acids) and minerals in kale leaf. For leaf nutrient content, it is important to analyze the seed oil fatty acid composition since some local people have been using the seed crude oil in their diets. Here, higher amounts of erucic acid might be usefully delineated by the present work.

2. Materials and methods

2.1. Plant material

Kale (*B. oleraceae* L. var. *acephala* DC., black cabbage) leaves were harvested from six different fields 5 km away from each other in Trabzon (Turkey) in the winter of 2003. Three mature leaves from each field were harvested and combined. After weighing, all leaf samples were combined, treated with liquid N_2 and stored in a freezer until they were analyzed. Three replicates of extractions and determinations were carried out.

2.2. Sugar and organic acid extraction

For the ethanolic extraction of sugars and organic acids, fresh leaves were treated with liquid N_2 for 5 min and blended in the dark with 95% ethanol for 3–5 min, depending on tissue softness, at maximum speed with a blender. The homogenate was vacuum-filtered through Whatman No. 1 filter paper and the residue washed three times with 80% ethanol. The filtrates were combined and adjusted to 5 ml/g of fresh weight (FW) with ethanol. Henceforth, this is considered the ethanolic extract (Pérez, Olías, Espada, Olías, & Sanz, 1997).

2.3. HPLC analyses for sugars and organic acids

Sugars and organic acids were analyzed in a Hewlett–Packard 1090 liquid chromatograph equipped with a photodiode array detector and a Waters 410 differential refractometer (Milford) connected in series. Data were processed by means of Hewlett–Packard 85-B computing system and a Beckman Analogue Interface Module 406 and a Gold V.711 software, respectively. Isocratic separations of the compounds were made on a stainless steel Ion-300 (300 mm × 7.8 mm, 10 μm) column, containing a cation-exchange polymer in the ionic hydrogen form, with an IonGuard GC801 guard column (Interaction, San Jose, CA), and thermostated at 23 °C. The mobile phase utilized for the elution consisted of a filtered (0.22 μm nylon) and degassed solution of 0.0085 N H_2SO_4 and a flow rate of 0.4 ml/min. UV detection was at 195 and 245 nm, the refractive index detector was used at sensitivity setting 16×, and the injection volume was 20 μl (Pérez et al., 1997).

2.4. Protein and amino acid analysis

Vacuum-dried (25 °C) leaf samples were ground to a fine powder with the aid of a mortar and pestle. Each sample was analyzed in triplicate. Samples of 2–3 mg of each leaf specimen were placed in three 2 ml glass ampoules containing 50 μl of internal standard (norleucine) and 0.35 ml of 6 N HCl. The ampoules were frozen using liquid nitrogen, evacuated, sealed, and then placed

in an oven at 110 °C for 24 h. The ampoules were allowed to cool and were then placed in a vacuum centrifuge to remove acid. Samples were redissolved in 0.4 ml of 1 mM HCl, and a 20 μl aliquot was removed from each ampoule for derivatization. A single set of samples was analyzed separately for cysteine content. These samples were first oxidized with performic acid (Hirs, 1967) at room temperature for 18 h. Performic acid was removed in a vacuum centrifuge and samples were hydrolyzed according to the technique described above.

Tryptophan analysis was performed separately on a single set of samples. Dried leaf specimens were placed in polypropylene tubes and hydrolyzed in 4.2 M KOH containing 1% thiodiglycol (w/v) (Hugli & Moore, 1972) at 110 °C for 18 h. The KOH was neutralized with 4.2 M perchloric acid. The supernatant was then removed and adjusted to pH 3 with dilute acetic acid, and then a 50 μl aliquot was removed for derivatization. Quantitative analysis was performed using a Pierce standard H amino acid calibration mixture supplemented with tryptophan. Norleucine was used as the internal standard in all determinations.

The Pico-tag system (Waters, Milford, MA) was used for quantification of amino acids. Aliquots were dried after hydrolysis, mixed with 10 μl of redrying solution (ethanol:water:triethylamine, 2:2:1), and dried again. The samples were then reacted with 20 μl of phenylisothiocyanate (water:ethanol:triethylamine:phenylisothiocyanate, 7:1:1:1) at room temperature for 20 min (Cohen & Straydom, 1988), and then excess reagent was removed in a vacuum centrifuge. Derivatized samples were dissolved in 0.1 ml of 0.14 M sodium acetate that had been adjusted to pH 6.4 with acetic acid. A 10 μl aliquot was injected into the column for analysis. A Waters C_{18} column (3.9×300 mm) was used with conditions described by Buzzigoli et al. (1990) in order to obtain complete resolution of tryptophan and the ornithine produced as a result of alkaline hydrolysis of arginine. Analysis of all other amino acids was conducted using a Waters C18 column (3.9×150 mm) with gradient conditions described by Bidlingmeyer, Cohen, and Tarvin (1984). Egg white lysozyme was used as the control protein.

2.5. Lipid extraction

Samples of finely ground powder of leaves (1 g) in triplicate were weighed and extracted with chloroform:methanol (2:1, v/v) (Chamberlain, Nelson, & Milton, 1993) and a saline solution (NaCl, 0.9%) was added at a rate of 20% of the extraction volume. The mixture was shaken and centrifuged (IEC HN-SII Benchtop Centrifuge, International Equipment Company, Needham Heights, MA, USA) at 1000 rpm for 5 min to allow phase development. The bottom (organic) layer was collected and filtered. The total extracted lipid material was recovered after the solvent was removed in a stream of nitrogen. The samples were dissolved in anhydrous chloroform. Sixteen microgrammes of triheptadecanoin (containing 3 molecules of heptadecanoic acid; 17:0) and a 0.1 ml aliquot of the lipid sample were transferred to a 15 ml teflon-lined screw tube. Fatty acid methyl esters were obtained using 14% (w/v) boron trifluoride (BF_3) in methanol (Morrison & Smith, 1964).

After removing the solvent by nitrogen gas, the sample was mixed with 0.5 ml of the BF_3 reagent and placed in a warm bath at 100 °C for 30 min. After cooling, saline solution (NaCl, 0.9%) and hexane were added and the fatty acid methyl esters were extracted into the hexane. A mixture of known fatty acid methyl ester standards was used to calibrate the gas chromatograph and to identify the fatty acid methyl ester peaks.

2.6. Gas chromatography of fatty acid methyl esters

Aliquots (1–2 μl) of the hexane solution containing the fatty acid methyl esters were analyzed using a Hewlett–Packard 5890 Series II gas chromatograph (Hewlett–Packard, Palo Alto, CA) equipped with a fused-silica capillary column (Omegawax; 30 m × 0.32 mm ID, Supelco, Bellefonte, PA) and a flame–ionization detector.The injector temperature was set at 200 °C, detector at 230 °C, oven at 120 °C initially, then 120–205 °C at 4 °C/min, and then held at 205 °C for 18 min. The carrier gas was helium (99.999%) and the flow rate was approximately 50 ml/s. Electronic pressure control in the constant flow mode was used. The fatty acids are reported as the averages of three determinations conducted on three independent assays. The internal standard (heptadecanoic acid, 17:0) and a calibration mixture of fatty acid standards (GLC-68, Nu-Check, Elysian, MN, USA) were used to identify and quantify the fatty acids in the various lipid extracts.

2.7. Mineral analysis of leaf

Leaf samples (500 mg) were weighed and refluxed for 18 h at 150 °C with 15 ml of concentrated HNO_3 and 2.0 ml of 70% $HClO_4$. Samples were heated at 120 °C until all solvent was evaporated and the remaining material was dissolved in 10 ml of 4.0 M HNO_3 containing 1% (v/v) $HClO_4$. Fruit concentrations of 31 minerals (Al, Ba, Ca, Cu, Fe, K, Li, Mg, Mn, Na, Ni, P, Sr, Ti, Zn, Ag, As, Be, Cd, Cr, La, Mo, Pb, Sb, Se, Sn, Te, Tl, V, Y and Zr) were analyzed. Al, Cr, Cu, Mo and Zn in the digest were determined by inducively-coupled plasma-mass spectrometry using a VG PlasmaQuad Type I instrument with a plasma forward power of 1.35 kW, a spray chamber temperature of 15 °C, and a solution uptake rate of 0.6 ml/min. The other elements were determined using inducively-coupled plasma atomic emission spectroscopy (ICP-AES) under conditions

described by Yazzie, Vanderjagt, Pastuszyn, Okolo, and Glew (1994). The R_r ICP instrument was operated at 1.1 kW with a reflected power of less than 5 W and a specimen flow rate of 1.2 ml/min. The height of the specimen above the work coil was 15 mm. Selenium was analyzed by stabilized temperature graphite furnace atomic absorption spectrometry. An internal standard was prepared by adding a known amount of pure analyte to a solution, and the assay of the internal standard was used as a quality check for the mineral analysis. The samples were compared to a NIOSH tomato leaves standard analyzed by ICP-AES at the same time as the samples. The coefficient of variation for mineral analysis was 1.2%. The data presented are the means of three separate extractions and determinations.

3. Results and discussion

3.1. Sugar and organic acid contents of leaf

Fructose, glucose and sucrose as the major soluble sugars, and citric and malic acids as the major organic acids were detected in kale leaves. Fructose was the most abundant sugar (2011 mg 100 g^{-1} dry wt), followed by glucose (1056 mg 100 g^{-1} dry wt) and sucrose (894 mg 100 g^{-1} dry wt). The total sugar content, defined as the sum of fructose, glucose and sucrose, was 3961 mg 100 g^{-1} dry wt. With regard to major organic acids, the contents of citric and malic acids of the leaf were 2213 and 151 mg 100 g^{-1} dry wt, respectively. Total acid content of the leaf, defined as the sum of free of citric and malic acids, was estimated 2364 mg 100 g^{-1} dry wt. The glucose/fructose (G/F) and malic/citric acid ratios were found to be 0.5 and 0.1, respectively (Table 1).

The contents of fructose and glucose as free sugars in broccoli have been reported (King & Morris, 1994). The type and concentration of free sugars influence the flavour of *Brassica* products. Rosa, David, and Gomes (2001) reported that in white cabbage and Portuguese cabbage, fructose and glucose were the most dominant sugars (48.8–56.9% and 29.6–35.5%), while sucrose accounted for 8.5–20.5% of the total sugars.

Table 1
Major soluble sugars and organic acids of kale (*Brassica oleraceae* L. var. *acpehala* DC.) leaf[a]

Compound	mg 100 g^{-1} dry wt
Fructose	2011 ± 34.9
Glucose	1056 ± 16.2
Sucrose	894 ± 42.8
G/F[b]	0.5
∑Sugar[c]	3961
Citric acid	2213 ± 94.9
Malic acid	151 ± 14.9
MA/CA[d]	0.1
∑Acid[e]	2364

[a] Values are expressed as the means ± SD of three separate extractions and determinations.
[b] G/F: glucose/fructose ratio.
[c] Total sugar is the sum of sucrose, glucose and fructose.
[d] MA/CA: malic acid/citric acid ratio.
[e] Total acid is the sum of citric and malic acid.

3.2. Fatty acid contents of leaf and seed

In the plant, 18 different fatty acids were found in the leaves and 20 different fatty acids were found in the seeds. Some of the fatty acids, such as 12:0, 20:4$n-3$, and 20:5$n-3$, were not detected in the leaves, while the last two acids were present in the seed. As for the major fatty acids, 16:0, 18:2$n-6$ and 18:3$n-3$ were the most abundant fatty acids in the leaves. Considering the levels of these fatty acids, 18:3$n-3$ was found to be the highest (85.3 μg g^{-1} dry wt), accounting for 54.0% of the total fatty acid content. Linoleic acid (18:2$n-6$), being the second most abundant fatty acid was present at 18.6 μg g^{-1} dry wt, contributing 11.8% of the total fatty acid content. The 16:3 acid was estimated to be 15.7 μg g^{-1} dry wt, comprising 10% of the total (Table 2). Total leaf fatty acid content, calculated as the sum of the individual fatty acids, was 158 μg g^{-1} dry wt and the total leaf saturated fatty acid content was 29.9 μg g^{-1} dry wt, and the unsaturated fatty acid content was 129 μg g^{-1} dry wt, contributing 18.9% and 81.3% μg g^{-1} dry wt, respectively, of the total fatty acid content (Table 2).

Gas chromatographic analysis of the fatty acids in the total lipid fraction of the kale seed revealed the presence of 20 different fatty acids, 13 of which were unsaturated and 7 saturated (Table 2, Fig. 1). Erucic acid (22:1$n-9$) was the most abundant (4198 μg g^{-1} dry wt, 45.7%), and 18:2$n-6$ (1199 μg g^{-1} dry wt, 12.8%) and 18:1$n-9$ (1408 μg g^{-1} dry wt, 14.8%) acids were the second most abundant fatty acids in the seed determined. It was reported that the seeds of some *Brassica* species varied in fatty acid contents. For example, 18:1 acid was 45.6% in *Brassica napus*, 18:2 acid was 23.5% in *Brassica juncea*, 18:3 acid was 33.3% in *Brassica elongata*, 22:1 acid was 37.6% in *Brassica rapa* and 32.6% in *Brassica nigra* (Goffman, Thies, & Velasco, 1999).

Research in human nutrition and health has shown a relationship between diet and the increasing frequency of lifestyle diseases among the populations in industrialized countries (Skjervold, 1993). The nutritional deficiency is mainly due to the low content of $n-3$ (omega-3) fatty acids in the diet and Horrobin (1993) and Innis (1996) have shown the dietary importance of these essential fatty acids and their relationship to human health. The polyunsaturated fatty acids, linoleic acid (C18:2$n-6$) and α-linolenic acid (C18:3$n-3$), are essential to the human diet because they cannot be

Table 2
Fatty acid composition in leaf and seed of kale (*Brassica oleraceae* L. var. *acpehala* DC.)[a]

Fatty acid	Leaf ($\mu g\ g^{-1}$ dry wt)	% of total	Seed ($\mu g\ g^{-1}$ dry wt)	% of total
14:0	0.70 ± 0.12	0.44	6.80 ± 0.59	0.08
14:1	0.55 ± 0.10	0.34	3.58 ± 0.00	0.02
15:0	0.33 ± 0.08	0.20	3.59 ± 2.94	0.04
16:0	18.7 ± 0.45	11.8	488 ± 3.80	5.03
16:1	0.51 ± 0.34	0.32	21.7 ± 1.56	0.24
16:3	15.7 ± 0.78	10.0	9.22 ± 7.44	0.11
18:0	5.92 ± 0.43	3.74	142 ± 10.6	1.65
18:1*n* − 9	3.38 ± 0.18	2.14	1408 ± 1082	14.8
18:1*n* − 7	1.10 ± 0.21	0.69	120 ± 93.3	1.24
18:2*n* − 6	18.6 ± 0.87	11.8	1199 ± 952	12.3
18:3*n* − 3	85.3 ± 3.61	54.0	746 ± 605	7.68
20:0	0.72 ± 0.18	0.45	69.4 ± 49.6	0.76
20:1*n* − 9	0.81 ± 0.09	0.51	818 ± 610	8.78
20:2*n* − 6	0.34 ± 0.24	0.21	57.0 ± 448	0.59
20:3*n* − 3	0.50 ± 0.08	0.31	9.45 ± 7.60	0.09
20:4*n* − 3	–	–	5.56 ± 0.00	0.04
20:5*n* − 3	–	–	11.9 ± 0.00	0.08
22:0	0.71 ± 0.25	0.44	56.1 ± 41.8	0.62
22:1*n* − 9	1.50 ± 0.40	0.94	4198 ± 3102	45.7
24:0	2.82 ± 0.05	1.78	15.5 ± 16.5	0.25
TFAT[b]	158		9389	
TS[c]	30.0	18.9	783	8.46
TUS[d]	129	81.3	8607	91.6
US/S[e]	4.28	0.43	11.0	10.8

[a] Values are expressed as the means ± SD of three separate extractions and determinations.
[b] Total fatty acid is the sum of individual fatty acids identified and determined.
[c] Total saturated fatty acids.
[d] Total level of unsaturated fatty acids.
[e] Ratio of unsaturated fatty acids to saturated fatty acids.

synthesized by humans (Innis, 1996; Sinclair, 1990). Kale leaf and seed contain these essential fatty acids, and the combined levels of linoleic acid and α-linolenic acid make up ~66% of the total fatty acid content at 104 $\mu g\ g^{-1}$ dry wt for leaf and ~20% (19,459 $\mu g\ g^{-1}$ dry wt) for the seed (Table 2, Fig. 1). Even though the levels of these two essential fatty acids in kale leaf and seed are not as high as those in other well-known sources, such as olive oil, soya oil, hazelnut, walnut and peanut, consuming the leaf during the winter (on preferably the seed) would satisfy some of the omega-3 fatty acid requirement.

Interestingly, the level of 22:1*n* − 9 (omega-9 or 13-docosenoic acid) acid, also called erucic acid, was found to be the highest polyunsaturated acid at 45.7% (4198 $\mu g\ g^{-1}$ dry wt) in kale seed. Erucic acid is the *cis*-isomer of brassidic acid (*trans*-13-docosenoic acid), derived from rape seed, wallflower seed or mustard seed, as an intermediate to make compounds such as triglycerides, erucamides, amines, behenic acid, behenyl alcohol, erucyl alcohol, a wide range of erucic acid metallic salts and esters, brassylic acid and pelargonic acid. Uses of erucic include lubricants, heat-transfer fluids, surfactants, slip agents, emolients, cosmetics and coatings. It is also used in polyesters, plastics and nylons (Lühs & Friedt, 1994). *Brassica* has become one of the worldwide most important source of vegetable oil. In general, erucic acid content in the genus *Brassica* varies with the allelic constitution of the genotype, differences in the ploidy level, the genetic background and environmental impact. *B. oleraceae*, normally displays a 22:1 content ranging from 28% to 63% (Lühs, Voss, Seyis, & Friedt, 1999). Present results show that the seed oil of variety *B. oleraceae* var. *acephala* contained 22:1 at 45.7%. It has been reported that natural variation for high 22:1 content is limited in *B. napus* to a level of about 55–60%, while some accessions of related *Brassica* species possess 60% of 22:1 acid and even more in their seed oil (Lühs & Friedt, 1995).

3.3. Amino acid content of leaf

Table 3 summarizes the contents of 18 of the amino acids commonly found in proteins. The protein content (i.e., the sum of individual amino acids) was 271 mg g^{-1}

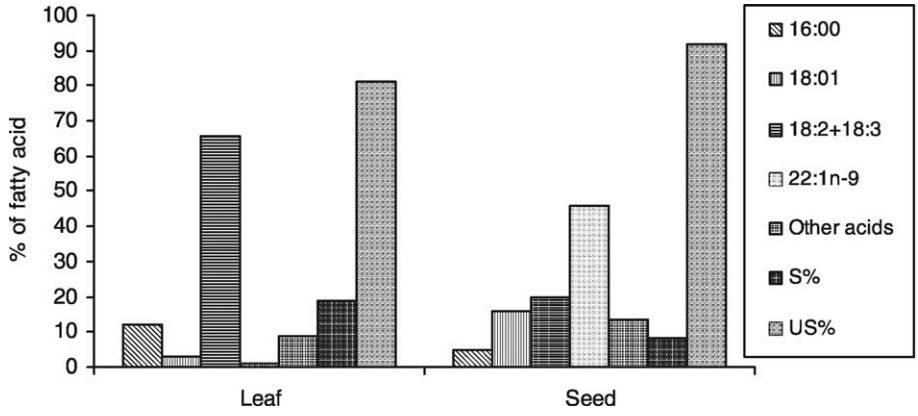

Fig. 1. Comparison of percentage level of abundant fatty acid of the leaf and seed of kale (other acids are the sum of 14:0, 14:1, 15:0, 16:1, 18:0, 20:0, 20:1*n* − 9, 20:2*n* − 6, 20:3*n* − 3, 20:4*n* − 3*, 20:5*n* − 3*, 22:0 and 24:0 acids, *detected in the seed, but not in the leaf).

Table 3
Amino acid composition in kale (*Brassica oleraceae* L. var. *acpehala* DC.) leaf[a]

Amino acids	mg g^{-1} dry wt	% of total
Cys	3.4 ± 1.2	1.3
Asp	27.6 ± 0.8	10.2
Glu	33.2 ± 1.1	12.2
Ser	13.8 ± 0.7	5.1
Gly	13.1 ± 0.5	4.8
His	6.4 ± 0.3	2.4
Arg	20.6 ± 0.8	7.6
Thr	13.9 ± 0.4	5.1
Ala	14.6 ± 0.5	5.8
Pro	17.5 ± 0.7	6.5
Tyr	12.5 ± 0.8	4.6
Val	17.1 ± 0.6	6.3
Met	6.0 ± 1.9	2.2
Ile	12.8 ± 0.4	4.7
Leu	20.3 ± 0.6	7.5
Phe	14.6 ± 0.4	5.4
Trp	8.9 ± 1.3	3.3
Lys	15.0 ± 0.5	5.5
∑Protein[b]	271	

Abbreviations of amino acids: cys, cysteine; asp, asparagine; glu, glutamine; ser, serine; gly, glycine; his, histidine; arg, arginine; thr, threonine; ala, alanine; pro, proline; tyr, tyrosine; val, valine; met, methionine; ile, isoleucine; leu, leucine; phe, phenylalanine; trp, tryptophan; lys, lysine.

[a] Values are expressed as the means ± SD of three separate extractions and determinations.

[b] Total protein is the sum of individual amino acids.

Table 4
Essential amino acid composition of kale (*Brassica oleraceae* L. var. *acpehala* DC.) leaf, compared with WHO[a] "ideal protein"

Amino acids	% of total AA	Protein	WHO ideal protein
Thr	5.1	150	3.4
Val	6.3	180	3.5
Ile	4.7	168	2.8
Leu	7.5	114	6.6
Trp	3.3	300	1.1
Lys	5.5	95	5.8
Met + Cys	3.5	140	2.5
Phe + Tyr	10.0	159	6.3

Abbreviations of amino acids: thr, threonine; val, valine; ile, isoleucine; leu, leucine; trp, tryptophan; lys, lysine; met + cys, methionine + cysteine; phe + tyr, phenylalanine + tyrosine.

[a] WHO (1985).

dry wt. The most abundant amino acid was glutamate (33.2 mg g^{-1} dry wt), contributing 12.2% of the total amino acid content. Aspartic acid, which is the second most abundant amino acid, was present 27.6 mg g^{-1} dry wt, comprising 10.2% of the total amino acid content. The contents of some amino acids were in lower quantities, ranging between ~3 and ~9 mg g^{-1} dry wt (~1% and ~3% of the total) in cysteine, histidine, methionine and tryptophan (Table 3).

With regard to the nutritional quality of kale leaf, the amino acid content was assessed by comparing the percentages of the essential amino acids with those of a World Health Organization (WHO) (FAO/WHO/UNU, 1985) standard protein, as shown in Table 4; the protein of kale leaf compares well with that of the WHO standard. Only one amino acid, lysine, had a score that fell below 100% (Table 4).

3.4. Mineral content of leaf

Concentrations of plant macro- and micronutrients and other elements are given in Table 5. Among the analyzed macronutrients in the leaf, the concentration of Ca was the highest (19.7 mg g^{-1} dry wt). The K content was 13.5 mg g^{-1} dry wt, while the most abundant micronutrient, Fe, was determined to be 72.6 μg g^{-1} dry wt. However, Mn and Zn were the second most abundant micronutrients found in the leaf, at 53.5 and 39.4 μg g^{-1} dry wt, respectively. Remarkably, the leaf contained a large amount Sr, 252 μg g^{-1} dry wt, higher than both macro- and micronutrients. Al at 29.3 μg g^{-1} dry wt was found to be the second major mineral. It seems that kale leaf is rich in Sr (252 μg g^{-1} dry wt), Fe (72.6 μg g^{-1} dry wt), Mn (53.5 μg g^{-1} dry wt) and Zn (39.4 μg g^{-1} dry wt) (Table 5).

Grace, Craighed, and Watt (2000) reported that three kale crops of New Zealand were deficient in Mn, Zn, Se, and I. Remarkably, in Turkish kale, when compared to

Table 5
Mineral composition of kale (*Brassica oleraceae* L. var. *acpehala* DC.) leaf[a]

Mineral	Content
Macronutrient	*mg g^{-1} dry wt*
Ca	19.7 ± 0.6
Mg	2.4 ± 0.4
Na	1.7 ± 0.3
K	13.5 ± 0.7
P	5.73 ± 0.9
Micronutrient	*μg g^{-1} dry wt*
Co	0.2 ± 0.0
Cu	5.1 ± 0.1
Fe	72.6 ± 1.3
Mn	53.5 ± 1.9
Zn	39.4 ± 1.2
Other elements	
Al	29.3 ± 0.4
As	0.7 ± 0.2
Ba	15.9 ± 0.0
Cd	0.1 ± 0.0
Cr	2.6 ± 0.5
Pb	0.2 ± 0.0
Li	0.1 ± 0.0
Mo	2.9 ± 0.1
Ni	2.0 ± 0.0
Sr	252 ± 9.1
Ti	0.4 ± 0.0

[a] Results are expressed as the means ± SD of three separate extractions and determinations.

New Zealand kale, Mn (53.5 mg g^{-1} dry wt) and Zn (39.4 mg g^{-1} dry wt) were present in higher amounts while Se and I were not detected in Turkish kale.

Most of these elements are essential activators for enzyme-catalyzing reactions. For example, Mn plays a structural role in the chloroplast membrane system and may be responsible for colour, taste and smell, and a cofactor for fatty acids, DNA and RNA synthesis (Gibbs, 1978). Fe and Cu may exist as Fe and Cu proteins. Iron is an essential activator for enzyme–catalysing reactions involving chlorophyll synthesis and for ferrodoxin nitrate reductase (Bowling, 1976). Potassium is an essential nutrient and has an important role in the synthesis of amino acids and proteins (Malik & Srivastava, 1982). Ca and Mg play a significant role in photosynthesis, carbohydrate metabolism, nucleic acids, and binding agents of cell walls (Russel, 1973). Calcium is also the major component of bone and assists in teeth development (Brody, 1994). Zn is an essential micronutrient and is associated with a number of enzymes, especially those for synthesis of ribonucleic acids (Oser, 1979). The contents of trace elements in plants are low; in terms of biological activity they are critical. However, when they are incorporated into mineral complexes, their ability is enhanced (Shkolnik, 1984). Other inorganic elements which may contribute to biological processes, but which have not been established as essential, are barium, bromine, cadmium, lead and lithium (Macrae, Robinson, & Sadler, 1993a). Cadmium and lead are best known for their toxicological properties (Macrae, Robinson, & Sadler, 1993b). Lithium is another element that benefits human; it has been used effectively in the treatment of manic depressive disorders. There is evidence to suggest that Li is also an essential element (Macrae et al., 1993b).

Boron, chromium, manganese, nickel, tin, vanadium, molybdenum, arsenic, lithium, aluminium, strontium, cesium and silicon are regarded as new trace elements in the sense that they have only recently been considered essential in human diets (FAO/WHO, 1973; FAO/WHO/UNU, 1985; WHO, 1989a, 1989b). There is evidence that strontium (Sr) is an essential trace mineral. It is similar to calcium and is necessary for proper bone growth (in calcified tissues) and prevention of dental caries. However, Sr occurs in relatively large concentrations in bones and teeth, where it is thought to replace a fraction of the calcium in hydroxyapatite crystals It has been found that Sr may confer a protective effect on certain energy-producing structures within the cell. Not to be confused with radioactive strontium 90, Sr is stable and one of the least toxic trace elements. Non-radioactive strontium occurs naturally in food. This mineral is apparently quite safe, even with long-term administration at doses hundreds of times greater than the usual dietary intake (Nielsen, 2000; Schauss, 1995). Noteworthy, we have found that kale leaf contains Sr at 252 μg g^{-1} dry wt, much higher than the levels of macro- and micronutrients such as Ca (12.7-fold), K (18.6-fold), Fe (3.6-fold) and Zn (6.4-fold) present in the leaf.

This work constitutes a nutritional report on kale (black cabbage) which is extensively consumed by the local people of northeastern Anatolia (Turkey). Besides the leaf chemical composition, the fatty acid composition of kale seed crude oil was also reported. Like other *Brasssica* species, variety 'Acephala' has a high level (45.7%) of erucic (22:1) acid. Further studies on the other chemical constituents, including other genotypes and cultivars, of kale and seed may enable food technologists to select *B. oleraceae* var. *acephala* with improved nutritional quality. However, some climatic and agronomic changes can occur and need to be examined. This type of information could provide local populations (where kale, black cabbage, is a native species in their flora or under cultivation) with a basis for food choices.

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