

Comparison of Nutrient Content in Fruit of Commercial Cultivars of Eggplant (*Solanum melongena* L.)

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Eggplant (*Solanum melongena* L.) is one of the most popular common major vegetable crops worldwide. This study evaluated the nutritional content of seven commercial eggplant fruits in terms of fatty acid, mineral, sugar, organic acid, amino acid and polyamine contents. The most abundant fatty acid was linoleic acid (range, 39.14–53.81%, ave. 45%), and the most abundant mineral was K (range, 1556.2–3171.6 mg/kg fw, ave. 2331.9). The major organic acid was malic acid (range, 129.87–387.01 mg/g fw, ave. 157.49), and the major sugar was fructose (range, 1242.81–1379.77 mg/100 g fw, ave. 1350.88). The major polyamine was putrescine (11.54 and 25.70 nmol/g fw, ave. 17.86), and the major amino acid was glutamine (148.4 and 298.75 mg/100 g fw, ave. 219.74). Overall, taking into account the export potential of eggplants, these results may contribute to further studies aiming to improve other nutrient-rich varieties of eggplant in breeding programs.

INTRODUCTION

With their basic/essential nutrients, fruit and vegetables constitute an essential part of a daily diet. One of the most common strategies in evaluating fruits and vegetables for feeding the human population is to profile their nutritional importance by analyzing their basic/essential nutrients (amino acids, fats (fatty acids), carbohydrates, minerals and vitamins). Many human diseases are due to unbalanced diets or malnutrition. A balanced diet is known to be very important for human health. There is therefore increasing interest in profiling fruit and vegetables for potential nutrients in order to improve diets and fight malnutrition.

With over 1.7 million ha of production worldwide, eggplant, *Solanum melongena* L. (fam: Solanaceae), is one of the most important vegetable crops. China is the largest grower of eggplant, producing 17.03 million metric tons/year, while Turkey ranks fourth with 880,000 metric tons/year. Throughout its distribution, eggplant fruit exhibits considerable phenotypic variability with a wide range of ovoid, globular, oblong, semi-long, long and serpentine shapes, weighing from a few grams to more than 1 kg, and being green, white, violet, purple, striped, black or orange or variegated between violet-white and purple-white in color [Okmen *et al.*, 2009].

With its great phenotypic variability, eggplant fruit contributes a major part of the Turkish diet in fresh, dried, preserved, and cooked forms [Okmen *et al.*, 2009]. Interest in the fruit has encouraged growers to produce different cultivars of eggplant. Recent studies of eggplant fruit have revealed that it is a good source of dietary fiber and vitamins (vitamins A, B1 and B6), and provides significant quantities of minerals such as P, K, Ca and Mg [Raigón *et al.*, 2008; Okmen *et al.*, 2009]. Twenty-six eggplant accessions from Turkey were recently studied for their water-soluble antioxidant activity and total phenolic compound content [Okmen *et al.*, 2009]. However, there is a lack of information about the fruit fatty acid, mineral, organic acids, sugar, amino acid and polyamine composition of six common cultivars (Aydın Siyahı, Pala 49, Süper Pala, Kemer 27, Kadife Kemer, Bostan) and one potent local type eggplant (Kadife, Figure 1) [Torun *et al.*, 2015]. This paper addresses also the first investigation of the nutrient content of a local-type eggplant, which is becoming increasingly used. The objective of the study was to determine and compare the fruits of these eggplants in terms of nutritional contents.

MATERIALS AND METHODS

Plant material

The seeds of six common eggplant cultivars (Aydın siyahı, Pala 49, Süper pala, Kemer 27, Kadife Kemer and Topan (Gümüşay)) from Yaprak Tarım Sanayi (Istanbul, Turkey)

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and a local-type eggplant known as “Kadife” by local growers in northeast Anatolia from Turkey were obtained. The plant, with its light purple and white striped fruit, is widely cultivated and consumed in Trabzon and elsewhere in the region (Figure 1). The seeds of the eggplants were grown in soil in the several open fields within and near the campus of Karadeniz Technical University in Trabzon (Turkey) during the summer of 2012, using standard cultivation techniques for eggplant. Eight plants from each type were randomly selected in the fields and the fruits were harvested as they reached commercial market size. The fruit was treated with liquid nitrogen and stored at -80°C for further analyses. All extractions and determinations were performed in triplicate ($n=6$).

Lipid extraction and fatty acid analysis

A conventional method of total lipid extraction as described by Folch *et al.* [1957] was used in triplicate for eggplant fruit. Derivatization of the fatty acids to the methyl esters was performed by adding 500 mL of HCSM (hexane/chloroform/sodium methoxide, 75/20/5, v/v/v, Sigma #403067, Aldrich) solution to the sample vials.

Fatty acid methyl ester (FAME) peaks were identified by comparison against FAME standards. A Hewlett-Packard 5890 Series II gas chromatograph (Hewlett-Packard, Palo Alto, CA, USA) with a flame-ionization detector and a fused-silica capillary column (DB-23, 20 m x 0.18 mm, 0.20 μm film; J&W Scientific, Folsom, CA, USA) was employed. Helium was used as the carrier gas (at 29 psi, 0.7 mL/min at 190°C). The injector temperature was set at 230°C and the detector temperature at 250°C . The oven temperature was initially set at 190°C for 3.8 min, and then raised to 220°C at $16^{\circ}\text{C}/\text{min}$. This was then held for 1 min, and then raised to 240°C at $26^{\circ}\text{C}/\text{min}$ and held again for 2 min. Injector split flow with helium was set at 150–200 mL/min. Detector gas was air set at 345 mL/min, and hydrogen gas was set at 36 mL/min. Detector makeup gas was helium at 35 mL/min.

Amino acid analysis

Fifty milligrams of lyophilized fruit samples in triplicate were weighed and hydrolyzed *in vacuo* in 6 N HCl containing 0.1% (w/v) phenol at 110°C for 24 h. The resultant amino acids were separated and quantified using the Dionex Bi-OLC Chromatographic System (Thermo Fisher Scientific Inc., USA) configured for AAA-Direct analysis according to the manufacturer's instructions (Dionex Corp. Technical Note 50) and previously published methods [Clarke *et al.*, 1999; Jandik *et al.*, 1999; Glew *et al.*, 2003]. For the determination of methionine and cysteine, the samples were first oxidized with performic acid [Hirs, 1964] prior to acid hydrolysis. Tryptophan was determined using the method described by Hugli & Moore [1972]. The reproducibility of the method ranged from 0.6% to 1% for the amino acids reported.

Mineral analysis

Eggplant samples were desiccated for five days to obtain a constant weight and then exposed to microwave digestion. Digestion of the samples and ICP-MS analysis for the elements Ca, Mg, K, Zn and P were performed according to the method described by Glew *et al.* [2005], with slight modi-



FIGURE 1. *Solanum melongena* “Kadife”. A local-type eggplant known as Kadife by local growers in northeast Anatolia with its light purple and white striped fruit.

fications. An official AOAC method [AOAC, 999.10, 2010] using AAS analysis was employed for Fe determination.

Polyamine analysis

Free polyamines were quantified after extraction and benzylation according to Hwang *et al.* [1997] and Petrivalsky *et al.* [2007], with minor modifications. Lyophilized eggplant fruit samples (150 mg) were homogenized in liquid nitrogen and extracted with 5% (v/v) trichloroacetic acid and derivatized with benzoyl chloride. Standard solutions of polyamines were used to construct calibration curves for putrescine, cadaverine, spermidine and spermine using the methodology described above. Benzyolated derivatives were extracted from reaction mixtures with diethyl ether, evaporated to dryness at 50°C under nitrogen, dissolved in 120 μL of initial mobile phase and analyzed by HPLC. A 10 μL sample was analyzed using a Knauer Smartline-Manager HPLC equipped with a Pump 1000, PDA detector 2800, Autosampler, and a GraceSmart™ RP 18 column (5 μm particles; 4.6 x 250 mm). The mobile phase consisted of solvent A (water) and solvent B (methanol) following the gradient program of 55–70% B over 20 min, isocratic 70% B for 5 min, 70–55% B over 1 min, and finally 10 min of equilibration to initial conditions. The column temperature was set at 40°C , and the flow rate was kept at 0.8 mL/min throughout the analysis. UV detection wavelength was 227 nm with individual polyamines identified based on their UV-Vis spectra and retention times compared

to the corresponding standards. Integration of peak areas was performed using Clarity (DataApex) software.

Sugar and organic acid analysis

A known amount of fruit sample (0.5 g) was first defatted and extracted according to Güney *et al.* [2013]. Sugar and organic acid analyses were run on a Agilent 1100 HPLC (Palo Alto, CA, USA) equipped with a quaternary HPLC pump, refractive index detector (RID), micro vacuum degasser (MVD), thermostatic column compartment (TTC), UV/VIS detector, and standard micro and preparative autosampler. Sugar elution was through a Nucleosil C18 Carbohydrate analytical column (250 × 4.0 mm i.d., 10- μ m particle size) with a column temperature of 25°C. The mobile phase was acetonitrile:water (79:21) for isocratic elution at a flow rate of 2 mL/min. Organic acid extraction was performed according to Ayaz *et al.* [2011]. Analysis was performed using an Ace 5 C18 (Advanced Chromatography Technologies, Aberdeen, Scotland) column (25 cm × 4.6 mm i.d., 10 mm particle size). The mobile phase (MP) employed was potassium phosphate solution (0.02 mol/L, pH 2.04). The flow rate of the MP was 2 mL/min with the column temperature held constant at 25°C. Organic acids were detected using a HP 1100 Series multivariable wavelength detector set at 210 nm. Calibration curves of the standard solutions were calculated for each sugar (sucrose, glucose, fructose, maltose, and lactose) and organic acids (malic acid, citric acid and ascorbic acid). They were identified by comparing their retention times to those of authentic standards. Peak areas were quantified using HP ChemStation (Hewlett-Packard, Palo Alto, CA, USA) software. Quantitation was performed by comparing the peak areas with those of the respective external standards. Compounds' areas of peaks were quantified on HP ChemStation (Hewlett-Packard, Palo Alto, CA, USA) software.

RESULTS AND DISCUSSION

Fatty acid contents and compositions in fruits of seven eggplants are summarized in Table 1. Gas chromatography analysis of the fatty acids in the total lipid fraction of the whole fruit samples revealed the presence of six different fatty acids, three of which were saturated, palmitic acid (C16:0) being the major component, while the other three were unsaturated, with linoleic acid being the major component (Table 1). In general, with a few exceptions, there were no great significant differences ($P < 0.05$) among the eggplants in terms of their fatty acid content. The major saturated fatty acid was palmitic acid (C16:0) (ave. 20.8%), and the most abundant unsaturated fatty acid, as well as fatty acid, was linoleic acid (C18:2), with a comparatively high mean of 44.96%; the highest level was determined in Kemer (53.81%) and the lowest in Super Pala (39.14%). The mean content of the second major fatty acid, linolenic acid (C18:3), was 11.63%, ranging from as low as 7.55% in Kadife kemer to a high of 14.59% in Aydın Siyahı. Kadife Kemer represented the highest oleic acid (C18:1) content, at 11.71%, with an average of 7.67% for all cvs. Results have revealed that the present eggplant cvs were rich in unsaturated fatty acids (UFA, ave. 64.3%) and polyunsaturated fatty acids (PUFA, ave. 56.6%) (see details in Table 1).

It has been suggested that diets rich in MUFAs and some PUFAs, especially oleic acid (OA), linoleic acid (LA) and linolenic acid (LN), help reduce or inhibit cardiovascular diseases. The human body is unable to synthesize these fatty acids, while plants have the ability to synthesize C18:3n-3 acid *de novo*. Intensive efforts have therefore been made to improve the lipid/fatty acids profile of fruits and vegetables, with high nutritional values in diets [Kris-Etherton *et al.*, 2001].

The nine mineral concentrations (mg/kg fw) in the eggplant fruit are given in Table 1. The mineral concentrations varied significantly ($P < 0.05$) among the studied cvs. The minimum and maximum levels were very similar for Cu, Zn, Fe and Mn, while the range was much higher for the other five minerals, P, Ca, Mg, K and Na (Table 1). The highest K concentration (ave. all cvs. 2331.87) was detected in Kemer 27 (3171.56), following Topan (1556.2). The second most abundant element of the eggplants was Mg (ave. all cvs. 283.34), phosphorus (ave. 97.85) and Ca (88.89), respectively. These results confirm previous findings that eggplant fruit contains high concentrations of K, Mg, Ca and P [Flick *et al.*, 1978; Kowalski *et al.*, 2003; Raigón *et al.*, 2008; Chinedu *et al.*, 2011; Das *et al.*, 2011; Amadi *et al.*, 2013; Arivalagan *et al.*, 2012; 2013]. The published values (mg/kg fw) for the four major minerals in eggplant fruit cited above range from 28 to 2318.3 (ave. 1421.5) for K, 12.7 to 140 (ave. 91.2) for Mg, 46.6 to 255 (ave. 141.3) for Ca and 37.2 to 484.5 (ave. 233) for P. However, the average values and ranges for concentrations of K (1556.2–3171.6, (ave. 2331.9)) and Mg (107.8–722, 283.3) were higher than those reported in the literature. The ranges of Cu, Zn and Fe concentrations in the literature (0.3–2.2, 1.1–2.5 and 1.8–23.6 mg/kg) are compatible with this study. Interestingly, the Na concentration in this study averaged 13.61 and varied between 9.9 and 18.8 mg/100 g, whereas the published values for the same mineral in other eggplants average 854 and vary between 106 and 1595.4. The published data for Na levels seem suspiciously high.

Minerals are important constituents of the human diet as they serve as co-factors for many physiological and metabolic processes. Potassium is the most abundant intracellular cation in the body and magnesium is an important co-factor of many regulatory enzymes (*e.g.* kinases), and is fundamental in energy transfer reactions (*e.g.* ATP and creatine phosphate, *etc.*). Adequate dietary intake of Fe, Z and Cu is essential for human health. More than 2 billion people worldwide are anemic, and this can be largely attributed to Fe deficiency. Iron is an essential component of body systems involved in the utilization of oxygen. Iron deficiency during childhood and adolescence impairs physical and mental development [Oski, 1993; Grantham-McGregor & Ani, 2001]. Zinc is required for protein and carbohydrate metabolism and plays a central role in the immune system [Shankar & Prasad, 1998]. Signs of zinc deficiency include poor prenatal development, growth retardation, mental retardation, reproductive failure, dermatitis and loss of appetite [Pelkonen *et al.*, 2008]. The adult body contains approximately 80 mg Cu, mainly stored in the liver, followed by brain and muscle. Cytochrome C oxidase, superoxide dismutase (SOD), lysyl oxidase and tyrosine oxidase are the major enzymes which require Cu for

TABLE 1. Fatty acid and mineral composition in fruit of seven eggplant cultivars grown in Turkey.

Cultivar/Compound	Kadife	Aydin Siyahı	Kadife Kemer	Kemer 27	Topan	Super Pala	Pala	Average	Range (min.-max.)
Fatty acid* (%)									
C16:0	21.93 ± 0.79 ^{bc}	23.40 ± 0.05 ^c	16.63 ± 0.31 ^a	17.30 ± 0.05 ^a	20.69 ± 0.68 ^b	23.08 ± 0.15 ^c	22.41 ± 0.65 ^c	20.8	16.6–23.40
C18:0	11.74 ± 0.77 ^a	12.05 ± 0.05 ^{ab}	13.36 ± 0.48 ^{bc}	14.40 ± 0.03 ^c	11.97 ± 0.38 ^{ab}	12.55 ± 0.14 ^{ab}	11.39 ± 0.38 ^a	12.5	11.39–14.40
C18:1	7.42 ± 0.47 ^{bc}	7.18 ± 0.08 ^b	11.71 ± 0.09 ^c	3.04 ± 0.03 ^a	9.10 ± 0.05 ^d	8.00 ± 0.02 ^c	7.27 ± 0.075 ^b	7.7	3.04–11.71
C18:2	41.92 ± 2.49 ^a	41.32 ± 0.11 ^a	48.81 ± 0.28 ^b	53.81 ± 0.05 ^c	47.29 ± 0.63 ^b	39.14 ± 0.00 ^a	42.45 ± 0.11 ^a	45	39.14–53.81
C18:3	13.94 ± 1.29 ^b	14.59 ± 0.09 ^b	7.55 ± 0.17 ^a	8.90 ± 0.00 ^a	7.59 ± 0.21 ^a	14.52 ± 0.03 ^b	14.30 ± 0.33 ^b	11.63	7.55–14.59
C20:0	1.64 ± 0.13 ^a	1.47 ± 0.01 ^a	1.46 ± 0.06 ^a	2.55 ± 0.01 ^c	2.06 ± 0.06 ^b	1.62 ± 0.02 ^a	1.47 ± 0.06 ^a	1.8	1.47–2.55
SSFA [†]	35.31	36.92	31.44	34.25	34.72	37.24	35.27	35.0	31.44–36.92
SUFA [‡]	63.28	63.09	68.06	65.75	63.98	61.66	64.02	64.3	61.66–65.75
SMUFA [§]	7.42	7.18	11.71	3.04	9.10	8.0	7.27	7.7	3.04–11.71
SPUFA [¶]	55.86	55.91	56.36	62.71	54.88	53.66	56.75	56.6	53.66–62.71
UFA/SFA [¶]	1.8	1.7	2.2	1.9	1.8	1.7	1.8	1.8	1.7–2.2
Element ^{***} (mg/kg fresh weight)									
Cu	0.20 ± 0.01 ^a	0.26 ± 0.03 ^a	0.57 ± 0.00 ^b	0.55 ± 0.07 ^b	0.23 ± 0.01 ^a	0.25 ± 0.06 ^a	0.21 ± 0.01 ^a	0.3	0.20–0.57
Zn	2.19 ± 0.25 ^b	2.15 ± 0.15 ^b	2.24 ± 0.08 ^b	2.63 ± 0.12 ^c	1.80 ± 0.13 ^a	2.39 ± 0.23 ^b	2.12 ± 0.20 ^{bc}	2.2	1.80–2.63
Fe	2.06 ± 0.11 ^a	2.77 ± 0.12 ^c	3.28 ± 0.01 ^d	3.71 ± 0.07 ^e	2.29 ± 0.14 ^b	2.37 ± 0.04 ^b	2.02 ± 0.02 ^a	2.6	2.02–3.7
P	99.78 ± 1.86 ^a	116.23 ± 0.26 ^c	236.03 ± 5.47 ^d	269.59 ± 1.93 ^e	97.74 ± 0.66 ^a	109.21 ± 2.44 ^b	97.85 ± 2.50 ^a	146.6	97.74–269.59
Ca	93.12 ± 3.36 ^{cd}	37.31 ± 0.54 ^a	40.27 ± 0.21 ^a	55.33 ± 19.72 ^b	31.14 ± 0.83 ^a	104.63 ± 2.98 ^c	88.89 ± 3.16 ^c	64.4	31.14–104.63
Mg	140.56 ± 0.85 ^{bc}	130.80 ± 0.29 ^b	591.24 ± 22.59 ^d	721.96 ± 4.71 ^e	107.84 ± 4.68 ^a	156.37 ± 4.85 ^c	134.59 ± 0.44 ^b	283.3	107.84–721.96
Mn	0.90 ± 0.07 ^{ab}	1.01 ± 0.13 ^{bc}	1.40 ± 0.05 ^d	1.57 ± 0.07 ^e	0.76 ± 0.06 ^a	1.10 ± 0.11 ^c	0.89 ± 0.03 ^{ab}	1.1	0.76–1.57
K	2420.98 ± 84.13 ^{abc}	1844.93 ± 3.76 ^{ab}	2360.13 ± 5.14 ^{abc}	3171.56 ± 1213.53 ^c	1556.2 ± 5.04 ^a	2650.41 ± 91.98 ^{bc}	2318.54 ± 9.19 ^{abc}	2331.9	1556.2–3171.56
Na	17.02 ± 0.08 ^d	13.27 ± 1.67 ^c	8.65 ± 0.29 ^a	9.86 ± 0.22 ^a	11.42 ± 1.46 ^b	18.83 ± 0.58 ^e	16.24 ± 0.04 ^d	13.6	9.86–18.83

Values represent the mean ± SD of three separate extractions and determinations. An analysis of variance (SPSS version 11.5, one-way ANOVA) was used for comparisons among the means. Values with the same letter within a row are not significantly different at $P < 0.05$.

*C16:0; Palmitic acid, C18:0; Stearic acid, C18:1; Oleic acid, C18:2; Linoleic acid, C18:3; Linolenic acid, C20:0; Arachidic acid (Eicosanoic acid), [†]SFA; total saturated fatty acids, [‡]UFA; total unsaturated fatty acids, [§]MUFA; total monounsaturated fatty acids, [¶]PUFA; total polyunsaturated, [¶]UFA/SFA; unsaturated fatty acids/saturated fatty acids.

*** Cu: copper, Zn: zinc, Fe: iron, P: phosphorus, Ca: calcium, Mg: magnesium, Mn: manganese, K: potassium, Na: sodium.

TABLE 2. Organic acids, sugars, and polyamines content in fruit of seven eggplant cultivars grown in Turkey.

Cultivar	Organic acids (mg/100 g fresh weight)			
	Ascorbic acid	Citric acid	Malic acid	Total organic acids*
Kadife	7.69 ± 1.21 ^a	18.98 ± 0.80 ^a	129.87 ± 6.78 ^a	156.54
Aydın Siyahı	11.09 ± 1.46 ^{ab}	21.63 ± 0.35 ^c	181.06 ± 19.46 ^b	213.78
Kadife Kemer	10.69 ± 2.84 ^{ab}	20.03 ± 1.88 ^{abc}	142.49 ± 7.41 ^a	173.21
Kemer 27	11.68 ± 2.04 ^b	19.09 ± 0.58 ^a	135.55 ± 8.42 ^a	166.32
Topan (Gümüşay)	9.24 ± 1.28 ^{ab}	19.57 ± 0.76 ^{ab}	174.01 ± 10.46 ^b	202.82
Super Pala	11.74 ± 1.33 ^b	21.22 ± 0.26 ^{bc}	167.75 ± 8.78 ^b	200.71
Pala 49	10.87 ± 1.82 ^{ab}	21.07 ± 1.11 ^{bc}	171.12 ± 8.20 ^b	203.06
Mean	10.43 ± 3.58	20.23 ± 0.55	157.49 ± 4.36	188.1
Range (min.-max.)	7.69–11.74	18.98–21.63	129.87–387.01	156.54–213.78
	Sugars (mg/100 g fresh weight)			
	Fructose	Glucose	Sucrose	Total sugars*
Kadife	1242.81 ± 28.55 ^a	1300.74 ± 38.39 ^a	385.03 ± 25.66 ^a	2928.57
Aydın Siyahı	1392.40 ± 39.90 ^b	1327.86 ± 47.49 ^a	493.80 ± 14.77 ^{bc}	3214.07
Kadife Kemer	1379.77 ± 50.94 ^b	1305.76 ± 69.54 ^a	461.81 ± 53.91 ^{bc}	3147.34
Kemer 27	1366.20 ± 68.84 ^b	1292.44 ± 48.41 ^a	474.28 ± 27.50 ^{bc}	3132.93
Topan (Gümüşay)	1340.83 ± 13.14 ^b	1275.50 ± 15.55 ^a	503.08 ± 30.33 ^c	3119.41
Super Pala	1375.95 ± 53.80 ^b	1293.48 ± 43.42 ^a	433.15 ± 38.53 ^{ab}	3102.59
Pala 49	1358.17 ± 71.07 ^b	1281.09 ± 52.69 ^a	453.32 ± 43.31 ^{bc}	3092.58
Mean	1350.88 ± 21.03	1297.59 ± 16.29	108.74 ± 3.06	2435.10
Range (min.-max.)	1242.81–1379.77	1275.50–1327.86	91.46–119.50	2928.57–3214.07
	Polyamines (nmol/ g fresh weight)			
	Putresin (put)	Spermidin (spd)	Spermin (sp)	Total polyamines*
Kadife	25.70 ± 8.76 ^b	1.33 ± 0.37 ^a	n.d.	27.03
Aydın Siyahı	15.95 ± 1.00 ^a	1.60 ± 0.09 ^b	n.d.	17.55
Kadife Kemer	14.93 ± 2.47 ^a	1.78 ± 0.25 ^{bc}	n.d.	16.71
Kemer 27	15.81 ± 2.13 ^a	0.98 ± 0.08 ^{ab}	n.d.	16.79
Topan (Gümüşay)	25.25 ± 4.98 ^b	1.42 ± 0.54 ^{ab}	n.d.	26.67
Süperpala	13.75 ± 1.54 ^a	2.29 ± 0.17 ^c	n.d.	16.04
Pala 49	11.54 ± 1.36 ^a	2.25 ± 0.45 ^c	n.d.	13.79
Mean	17.86 ± 6.33	1.63 ± 0.52	n.d.	19.49
Range (min.-max.)	11.54 – 25.70	0.98 – 2.29	n.d.	12.52–27.99

Values represent the mean ± SD of three separate extractions and determinations. An analysis of variance (SPSS version 11.5, one-way ANOVA) was used for comparisons among the means. Values with the same letter within a column are not significantly different at $P < 0.05$.

*the total comprise individual identified and quantified component.

their activity. Signs of Cu deficiency include anemia, vascular complications, osteoporosis and neurological manifestations [cited in detail by Arivalagan *et al.*, 2013].

HPLC analyses were performed to determine the presence of three common organic acids – ascorbic acid (AA), citric acid (CA), and malic acid (MA), the latter being the most

abundant (Table 2). AA concentration (mg/100 g) in all cvs averaged 10.43 (range 7.69–11.74), CA averaged 20.2 (range 18.98–21.63) and MA averaged 157.49 (range 129.87–387.01). These findings are similar to reports by Mori *et al.* [2013] who determined high concentrations of the major acids (CA and MA), ranging from 7 to 21 and 90 to 190 mg/100 g,

TABLE 3. Amino acid composition (mg/100 g fw) in fruit of seven eggplant (*Solanum melongena* L.) cultivars grown in Turkey.

Amino acid ^d / Cultivar	Kadife	Aydım Sıyahı	Kadife Kemer	Kemer 27	Topan (Gümüüşay)	Super Pala	Pala 49	Average	Range (min.–max.)
Ala	34.92 ± 0.25c	46.19 ± 0.12e	30.46 ± 0.14 ^a	34.61 ± 0.13b	39.33 ± 0.14d	52.70 ± 0.14g	48.35 ± 0.15f	40.94 ± 0.05	30.46 – 52.70
Arg	108.86 ± 0.44g	54.43 ± 0.11 ^e	28.84 ± 0.21b	23.32 ± 0.22a	43.02 ± 0.13c	60.70 ± 0.35 ^f	52.04 ± 0.13d	53.03 ± 0.12	23.32 – 108.86
Asp	131.58 ± 0.80b	223.85 ± 0.56 ^e	137.57 ± 0.17c	142.13 ± 0.26d	83.86 ± 0.13a	220.27 ± 0.43 ^f	203.71 ± 0.28e	163.28 ± 0.06	83.86 – 223.85
Cys	4.40 ± 0.61a	5.04 ± 0.21a ^b	4.68 ± 0.24a	5.47 ± 0.05b ^c	4.41 ± 0.10a	5.94 ± 0.53c	4.73 ± 0.30a	4.95 ± 0.21	4.40–5.94
Glu	186.45 ± 0.27c	255.38 ± 0.63e	148.4 ± 0.22a	162.32 ± 0.62b	211.74 ± 1.00d	298.75 ± 0.73g	275.13 ± 0.25f	219.74 ± 0.29	148.4–298.75
Gly	34.07 ± 0.22c	36.38 ± 0.15c	22.53 ± 4.57a	27.98 ± 0.11b	29.37 ± 0.12b	43.56 ± 0.25d	36.28 ± 0.10c	32.88 ± 1.67	22.53–43.56
His	13.97 ± 0.23f	12.14 ± 0.18e	9.12 ± 0.11a	10.76 ± 0.16d	9.49 ± 0.26b	17.13 ± 0.11g	10.42 ± 0.16c	11.86 ± 0.06	9.12–17.13
Ile	34.21 ± 0.33d	37.36 ± 0.13e	25.60 ± 0.39a	28.93 ± 0.27b	30.86 ± 0.29c	45.91 ± 0.16g	40.89 ± 0.26f	34.82 ± 0.09	25.60–45.91
Leu	48.01 ± 0.19c	60.85 ± 0.11e	40.59 ± 0.26a	43.76 ± 0.97b	49.51 ± 0.27d	72.33 ± 0.37f	63.29 ± 0.14g	54.05 ± 0.29	40.59–72.33
Lys	75.59 ± 0.46d	98.41 ± 0.26f	63.45 ± 0.25b	70.94 ± 0.11c	62.76 ± 0.10a	107.6 ± 0.44g	85.38 ± 0.12e	80.59 ± 0.15	62.76–107.6
Met	19.90 ± 0.18e	17.43 ± 0.13c	15.36 ± 0.22a	16.53 ± 0.09b	15.64 ± 0.10a	21.09 ± 0.23f	18.83 ± 0.18d	17.82 ± 0.06	15.36–21.09
Pro	36.10 ± 0.22d	39.63 ± 0.30e	27.28 ± 0.25a	29.38 ± 0.12b	32.19 ± 0.14c	44.42 ± 0.24g	40.17 ± 0.11f	35.60 ± 0.07	27.28–44.42
Ser	23.97 ± 0.25b	32.40 ± 0.17d	21.97 ± 0.17a	23.63 ± 0.09b	26.38 ± 0.07c	38.04 ± 1.00g	34.89 ± 0.23f	28.76 ± 0.32	21.97–38.04
Phe	33.18 ± 0.18c	41.83 ± 0.14e	28.47 ± 0.08a	30.93 ± 0.34b	33.99 ± 0.96d	48.16 ± 0.38f	41.57 ± 0.09e	36.88 ± 0.31	28.47–48.16
Thr	20.60 ± 0.13c	25.23 ± 0.46d	15.52 ± 0.18a	18.05 ± 0.40b	20.29 ± 0.10c	29.99 ± 0.24f	26.74 ± 0.22e	22.34 ± 0.13	15.52–29.99
Tyr	20.75 ± 0.27d	25.43 ± 0.47f	16.29 ± 0.35a	17.58 ± 0.24b	19.27 ± 0.14c	30.33 ± 0.43g	24.43 ± 0.15e	22.01 ± 0.13	16.29–30.33
Trp	6.58 ± 0.62a	7.76 ± 0.52b	7.37 ± 0.39a ^b	7.89 ± 0.38b ^c	6.79 ± 0.49a	9.20 ± 0.52d	8.66 ± 0.24c ^d	7.75 ± 0.12	6.58–9.20
Val	42.31 ± 0.23d	48.27 ± 0.17e	35.31 ± 0.11a	39.18 ± 0.33c	36.22 ± 0.26b	59.69 ± 0.35g	50.04 ± 0.30f	44.43 ± 0.08	35.31–59.69
Phe + Tyr	53.93	67.26	44.76	48.51	53.26	78.49	66.00	58.89	44.76–78.49
Met+Cys	24.30	22.47	20.04	22.00	20.05	27.03	23.56	22.78	20.04–27.03
ΣEAA ⁱⁱ	294.35	349.28	240.79	266.97	265.55	411.10	345.82	310.54	240.79–349.28
ΣNEAA ⁱⁱ	581.10	718.73	438.01	466.42	489.57	794.70	719.73	601.18	438.01–719.73
ΣAA ^{iv}	875.45	1068.00	678.80	733.39	755.12	1205.80	1065.55	911.73	678.80–1205.80
ΣProtein ^v	1.00 ± 0.05 ^c	1.21 ± 0.05 ^d	0.98 ± 0.02 ^{bc}	0.93 ± 0.03 ^b	0.85 ± 0.02 ^a	1.39 ± 0.01 ^f	1.28 ± 0.02 ^e	1.09 ± 0.02	0.85–1.39

Values represent the mean ± SD of three separate extractions and determinations. An analysis of variance was (SPSS version 11.5, one-way ANOVA) used for comparisons among the means. Values with the same letter within a row are not significantly different at P < 0.05.

ⁱAbbreviations 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; ΣEAA: total essential amino acids (mg/100g fw); ΣNEAA: total non essential amino acids (mg/100g fw) sum of individual amino acids. ⁱⁱΣAA: total amino acids (mg/100gfw) sum of individual amino acids. ⁱⁱⁱΣAA: total amino acids (mg/100gfw) sum of individual amino acids. ^{iv}ΣAA: total amino acids (mg/100gfw) sum of individual amino acids. ^vΣprotein: total extractable protein. TEAA: His+Ile+Leu+Lys+Met+Phe+Thr+Trp+Val.

respectively. Although the minimum CA and MA concentrations in our study were 2.7- and 1.4-fold higher than those published by Mori *et al.* [2013], the maximum concentrations of both major acids in our study were more or less the same (21.63 ± 1.83 and 181.1 ± 9.93) (Table 2).

Differences have also been observed among cultivars in terms of soluble sugar content in eggplant fruit [Hanson *et al.*, 2006; Amadi *et al.*, 2013; Mori *et al.*, 2013]. The major soluble sugars analyzed in this study were fructose, glucose and sucrose (Table 2). Fructose content (mg/100 g) in the cvs ranged from 1242.81 to 1379.77 (ave. 1350.88), glucose content ranged from 1275.50 to 1327.86 (ave. 1297.59) and sucrose content ranged from 91.46 to 119.50 (ave. 108.74). Except for the concentration of the three sugars in Kadife, both fructose and glucose concentrations differed significantly among the studied cvs. Values for the three major soluble sugar concentrations in the literature range from 74 to 1700 for sucrose, from 102 to 1370 for glucose and from 50 to 1500 for fructose [Mori *et al.*, 2013]. Our findings agree with the published data concerning the soluble sugar concentrations in eggplant fruit [Mori *et al.*, 2013; Amadi *et al.*, 2013].

Polyamine contents (nmol/g) in fruits of the cvs are summarized in Table 2. No highly significant ($P < 0.05$) variations were determined in their contents among the seven cvs. Two PAs were detected in the fruits, putrescine being the most abundant, averaging 17.86, and spermidine the least abundant, averaging 1.63. No spermine was found in the eggplant cvs. The cultivar Kadife with 25.70 and Topan with 25.25 had the highest concentrations of putrescine, while the concentrations in the remaining five cvs. ranged from 11.54 to 15.95, averaging 14.40. Spermidine concentrations in the cvs. were markedly lower than putrescine concentrations. The content (ave. 1.63) in the fruits varied between 0.98 and 2.29 (Table 2). Similarly, Rodriguez *et al.* [1999] reported the presence of putrescine and spermidine, two of the main polyamines, not only throughout fruit maturation but also in the matured eggplant cv., in "Black Nite" at levels varying between 2 and 20 nmol/g. Although they measured an abrupt rise in putrescine and spermidine on the ninth day (17.44 and ~6) in mature fruit (after the 11th stage, commercial size), their levels were still very low, ranging between ~2 and 3. Extremely high and low polyamine levels, especially for putrescine, have also been reported in the literature. The published putrescine levels (nmol/g) in other fruits range from 150.9 to 1588.2 for orange, 1.48 to 2268.9 for mandarin/orange, 30.4 to 274.5 for pear, 0.57 to 19.29 for apple, 1.02 for grape, and 2.04 for grapefruit [cited in Kalac & Krausova, 2005; Larqué *et al.*, 2007]. The authors reported spermidine and spermine levels in those fruits of (spd; spm, nmol/g fw); 0.90–66.8 and 0.40–243.7, 1.24–30.98 and 0.01–14.83, 14.32–523.24 and 1.98–243.7 and 1.03–19.3, 0.41 and 0.1–0.45, respectively.

Polyamines are widespread in living organisms and play numerous roles in health and disease [Bardocz *et al.*, 1995; Kalac & Krausova, 2005; Ali *et al.*, 2011]. They are known to play essential roles in cell proliferation, regeneration and differentiation, cell cycle regulation, gene expression, structural changes in RNA, *etc.* Metabolic requirements for polyamines are particularly high in rapidly growing tissues during growth and development, and also in tumors. Polyamine levels are

high in young and metabolically active tissues. Elderly people require a greater intake of dietary polyamine due to their decreased ability to synthesize these endogenously [cited in detail by Bardocz *et al.*, 1995; Kalac & Krausova, 2005; Ali *et al.*, 2011].

Table 3 summarizes the free amino acids commonly found in proteins of eggplant fruit. The amount (mg/100 g) of amino acids varied significantly ($P < 0.05$) among the cvs. Glutamine (ave. 219.74), asparagine (ave. 163.28), and lysine (ave. 80.59) were the three most abundant amino acids in the eggplant cvs. Glutamine was the highest in Super Pala (298.75), following Pala (275.1) and Aydın Siyahı (255.38). Oppositely, high concentration of asparagine was for Aydın Siyahı (223.85), following Super Pala (220.27) and Pala 49 (203.71). Only two of the 18 amino acids, Cys and Trp, had low concentrations, averaging 4.95 and 7.75, respectively. Depending on the individual amino acid concentration, a great variation in total amino acid (Σ AA, ave. 911.73), nonessential amino acid (Σ NEAA, ave. 601.18) and essential amino acid (Σ EAA, ave. 310.54) content of the fruits were found. Reported values of Σ EAA in the Japanese and Bangladesh eggplant cvs was 114.04 mg/100 g (ave.) ranging between 62.7 and 221.44 mg/100 g [Mori *et al.*, 2013]. Total protein (Σ P) content of the eggplant fruits was approximately 1.1 g/100 g, varying significantly ($P < 0.05$, 1.39–0.85 g/100 g) (Table 3). These findings agree with data reported for the three major amino acids in eggplant fruit [Flick *et al.*, 1978; Dahingo *et al.*, 1982–83; Adeyeye & Adanlawo, 2011; Mori *et al.*, 2013]. The published glutamine values for eggplant fruit averaged 37.07 mg/100 g fw [Flick *et al.*, 1978; Mori *et al.*, 2013]. In the present study, average concentration of aspartic acid was 163.23 mg/100 g (range, 83 - 223.85). In contrast, Flick *et al.* [1978] and Mori *et al.* [2013] reported lower levels of glutamine and asparagine than in the present study, averaging 19.68 and 17.26, and 54.46 and 7.40, mg/100 g, respectively. No detectable level of asparagine was found, in agreement with Flick *et al.* [1978]. We also determined considerable levels of lysine (ave. 80.59) and arginine (ave. 50.03) than that of the values reported for Japanese and Bangladeshi eggplant cvs [Mori *et al.* 2013]. Comparisons based on the present and published data showed ranges of glutamine and aspartic acid from 17.35 to 298.75 and 15.36 to 223.85, respectively [Flick *et al.*, 1978; Mori *et al.*, 2013].

CONCLUSION

Our findings and those in the literature reveal a considerable variation in the concentrations of nutrients both within and among countries and cultivars. It has been postulated that environmental conditions and genotype-variety can influence the composition of eggplant fruit including dry matter, content of phenolics, minerals, and amino acids. In general, the current findings largely agree with the published literature. In that respect, the varieties, cultivars and germplasm cited above can be compared with the present eggplant cvs., which have a great export potential. Consequently, such nutritional comparisons among eggplants *via* selection and breeding programs can lead to materials with improved nutritional content, especially amino acids and minerals. When the number of eggplant cultivars, varieties, and germplasms reported

in the literature are considered, sufficient potential genetic variation exists to allow plant breeders to select on the basis of targeted nutritional or antioxidant features.

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REFERENCES

- Adeyeye E.I., Adanlawo I.G., Amino acid composition of the ripe fruits of *Solanum aethiopicum* and *Solanum macrocarpon*. Int. J. Pharm. Bio. Sci., 2011, 2, 39–51.
- Ali M.A., Poortvliet E., Stromberg R., Yngve A., Polyamines: total daily intake in adolescents compared to the intake estimated from the Swedish nutrition recommendations objectivied (SNO). Food Nutr. Res., 2011, 55, 5455.
- Amadi B., Onuoha N., Amadi C., Ugbo A., Elemental, amino acid and phytochemical constituents of fruits of three different species of eggplant. Int. J. Med. Arom. Plants, 2013, 3, 200–203.
- AOAC, 999.10, 2010: Official Methods of the Association of Official Analytical Chemists. 15th Edition. Washington, DC, USA.
- Arivalagan M., Bhardwaj R., Gangopadhyay K.K., Prasad T.V., Sarkar S.K., Mineral composition and their genetic variability analysis in eggplant (*Solanum melongena* L.) germplasm. J. Appl. Bot. Food Qual., 2013, 86, 99–103.
- Arivalagan M., Gangopadhyay K.K., Kumar G., Bhardwaj R., Prasad T.V., Sarkar S.K., Roy A., Variability in mineral composition of Indian eggplant (*Solanum melongena* L.) genotypes. J. Food Compos. Anal., 2012, 26, 173–176.
- Ayaz F.A., Torun H., Özel A., Col M., Duran C., Sesli E., Colak A., Nutritional value of some wild edible mushrooms from the Black Sea region (Turkey). Turk. J. Biochem., 2011, 36, 213–221.
- Bardócz S., Duguid T.J., Brown D.S., Grant G., Pusztai A., White A., White A., Ralph A., The importance of dietary polyamines in cell regeneration and growth. Br. J. Nutr., 1995, 73, 819–828.
- Chinedu S.N., Olasumbo A.C., Eboji O.K., Emiloju O.C., Arinola O.K., Dania D.I., Proximate and phytochemical analysis of *Solanum aethiopicum* L. and *Solanum macrocarpon* L. fruits. Res. J. Chem. Sci., 2011, 1, 63–71.
- Clarke A.P., Jandik P., Rocklin R.D., Liu Y., Avdalovic N., An integrated amperometry waveform for the direct, sensitive detection of amino acids and amino sugars following anion-exchange chromatography. Anal Chem., 1999, 71, 2774–2781.
- Dahingo M.Jr., Rafols E.D., Laspinas V., Lau H.K.F., Boyde T.R.C., Amino acid composition of vegetables and fruits from the Philippines. Bull. Phil. Biochem. Soc., 1982–83, 5, 31–39.
- Das S., Raychaudhuri U., Falchi M., Bertelli A., Braga P.C., Das D.K., Cardioprotective properties of raw and cooked eggplant (*Solanum melongena* L.). Food Func., 2011, 2, 395–399.
- Flick G.J., Burnette F.S., Aung L.H., Ory R.L., Angelo A., Chemical composition and biochemical properties of mirlitons (*Sechium edule*) and purple, green, and white eggplants (*Solanum melongena*). J. Agric. Food Chem., 1978, 26, 1000–1005.
- Folch J., Lees M., Sloane Stanley G.H., A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem., 1957, 226, 497–509.
- Glew R.H., Ayaz F.A., Sanz C., Vander Jagt D.J., Huang H.S., Chuang L.T., Strnad M., Changes in sugars, organic acids and amino acids in medlar *Mespilus germanica* L. during fruit development and maturation. Food Chem., 2003, 83, 363–369.
- Glew R.S., Vanderjagt D.J., Chuang L.T., Huang Y.S., Millson M., Glew R.H., Nutrient content of four edible wild plants from West Africa. Plant Foods Hum. Nutr., 2005, 60, 187–193.
- Grantham-McGregor S., Ani C., A review of studies on the effect of iron deficiency on cognitive development in children. J. Nutr., 2001, 131, 649–668.
- Güney D., Bak Z.D., Aydinoglu F., Turna I., Ayaz F.A., Effect of geographical variation on the sugar composition of the oriental beech (*Fagus orientalis* Lipsky). Turk. J. Agric. For., 2013, 37, 221–230.
- Hirs C.W.H., Performic acid oxidation. Meth. Enzymol., 1964, 1, 197–199.
- Hanson P.M., Yang R.Y., Tsou S.C.S., Ldesma D., Engle L., Lee T.C., Diversity in eggplant (*Solanum melongena*) for superoxide scavenging activity, total phenolis, and ascorbic acid. J. Food Comp. Anal., 2006, 19, 594–600.
- Hugli T.E., Moore S., Determination of the tryptophan content of proteins by ion exchange chromatography of alkaline hydrolysates. J. Biol. Chem., 1972, 247, 2828–2834.
- Hwang D.F., Chang S.H., Shiua C.Y., Chai T.J., High-performance liquid chromatographic determination of biogenic amines in fish implicated in food poisoning. J. Chromatogr. B, 1997, 693, 23–30.
- Jandik P., Clarke A., Avdalovic N., Andersen D.C., Cacia J., Analyzing mixtures of amino acids and carbohydrates using bi-modal integrated amperometric detection. J. Chromatogr. B, 1999, 732, 193–201.
- Kalač P., Krausová P., A review of dietary polyamines: formation, implications for growth and health and occurrence in foods. Food Chem., 2005, 90, 219–230.
- Kowalski R., Kowalska G., Wierciński J., Chemical composition of fruits of three eggplant (*Solanum melongena* L.) cultivars. Folia Hort., 2003, 15, 89–95.
- Kris-Etherton P.M., Daniels S.R., Eckel R.H. et al., AHA conference proceedings, Summary of the scientific conference on dietary fatty acids and cardiovascular health. Conference summary from the nutrition committee of the American Heart Association. Circulation, 2001, 103, 1034–1039.
- Larqué E., Sabater Molina M., Zamora S., Biological significance of dietary polyamines. Nutrition, 2007, 23, 87–95.
- Mori T., Umeda T., Honda T., Zushi K., Wajima T., Matsuzoe N., Varietal differences in the chlorogenic acid, anthocyanin, soluble sugar, organic acid, and amino acid concentrations of eggplant fruit. J. Hortic. Sci. Biotech., 2013, 88, 657–663.
- Okmen B., Sigva H.O., Mutlu S., Doganlar S., Yemencioğlu A., Fray A., Total antioxidant activity and total phenolic contents in different Turkish eggplant (*Solanum melongena*) cultivars. Int. J. Food Prop., 2009, 12, 616–624.

30. Oski F.A., Iron deficiency in infancy and childhood. *N. Engl. J. Med.*, 1993, 329, 190–193.
31. Pelkonen R., Alfthan G., Järvinen O., Element concentrations in wild-edible mushrooms in Finland. *The Finnish Environment*, 2008, Vol. 25, Helsinki, p. 21
32. Petřivalský M., Brauner F., Luhová L., Gagneul D., Šebela M., Aminoaldehyde dehydrogenase activity during wound healing of mechanically injured pea seedlings. *J. Plant Physiol.*, 2007, 164, 1410–1418.
33. Raigón M.D., Prohens J., Muñoz-Falcon J.E., Nuez F., Comparison of eggplant landraces and commercial varieties for fruit content of phenolics, minerals, dry matter and protein. *J. Food Comp. Anal.*, 2008, 21, 370–376.
34. Rodriguez S.C., López B., Chaves A.R., Changes in polyamines and ethylene during the development and ripening of eggplant fruits (*Solanum melongena*). *J. Agric. Food Chem.*, 1999, 47, 1431–1434.
35. Shankar A.H., Prasad A.S., Zinc and immune function: the biological basis of altered resistance to infection. *Am. J. Clin. Nutr.*, 1998, 68(suppl.), 447–463.
36. Torun H., Kolcuoğlu Y., Ayaz F.A., Colak A., Glew R.H., Characterization of polyphenol oxidase during three ripening stages of an eggplant (*Solanum melongena* L.) fruit: a local type in northeast Anatolia. *Turk. J. Biochem.*, 2015, 40, 44–50.

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