

# Nutritional Evaluation of an Outstanding Apple Cultivar, 'Göbek' (*Malus communis* L.)

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**Abstract** The purpose of this study was to evaluate physicochemical parameters and nutritional changes in the whole fruit, the peel and the flesh parts in fruit of 'Göbek' apple during three ripening stages over a 20-day period (188 to 208 DAFBs) on the tree. A significant ( $P < 0.01$  or  $0.05$ ) increasing trend for pH, total soluble solids, dry matter, sweetness index, total sweetness index and a decreasing trend for total soluble solids:titratable acidity, titratable acidity, fruit firmness and moisture content were observed through ripening. The nutrient analysis representing the major components in the peel and flesh (respectively) indicates fructose in the range of (ave. 2260.1 and 3486.1 mg/100 g fw), malic acid (ave. 1052.8 - 278.6 mg/100 g fw), palmitic acid (ave. 33.1 - 26.8%) and linoleic acid (ave. 21.1 - 18.23%), potassium (ave. 186.3 and 105.6 mg/100 g fw) and asparagin (ave. 149.3 - 59.8 mg/100 g fw). Among the polyamines analyzed, spermidin was the most abundant polyamine in the peel and flesh in the following range (ave. 281.6 and 124.7 nmol g<sup>-1</sup> fw). The ripening process affected nutrient concentrations in the fruit as the maturity progressed on the tree. 'Göbek' fruit could be an excellent potential source for specific nutrients if it is harvested at different ripening stages.

**Keywords:** *Malus communis*, apple, sugar, polyamine, mineral, fatty acid, amino acid

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## 1. Introduction

There is a growing appreciation and understanding of the link between fruit and vegetable consumption and improved health in humans against degenerative diseases, cancer, cardiovascular diseases, diabetes, pulmonary disorders, and Alzheimer's disease [1,2]. Information regarding the quality and quantity of nutrients or nutraceuticals in fruits and vegetables during the pre- and post-harvest periods is therefore needed if the consumption of these is to exert a protective effect against the development of diseases such as those cited above. The nature and concentration of nutrients and their qualitative and quantitative distribution during the pre- and post-harvest ripening periods or storage have an important impact on the organoleptic properties of fruit in the natural form or their products in terms of market quality [3].

Apple (*Malus* L.) is one of the most economically important fruit crops in the world. It is widely consumed fresh or as apple products (AP) in processed forms such as juices and dried fruit. Both types have been investigated in health-related studies across the world due to their rich

and varied phytochemical contents. The potential of AP phytochemicals to reduce the risk of disease and improve health has attracted the interest of scientists, medical practitioners, and the general public [1,2]. Apples contain several nutrients (sugars, mostly fructose, and organic acids, mostly malic and citric acids) as well as non-nutrient components, including dietary fiber, minerals (mostly K, P, and Ca), vitamins (particularly vitamin C), polyphenols (mostly flavanols) and hydroxycinnamic acids (mostly chlorogenic acid). Moreover, apple is one of the main natural sources of phytochemicals, most of which express relevant antioxidants that have often been evaluated *in vitro* on cultured cells and in animal models [2].

Turkey has 460 apple varieties and is the world's third largest apple producing country, with an annual production of approximately 3.1 million tones [4]. Apple is native to the South Caucasian region, including some parts of Anatolia (North Anatolia, coastal regions of the Black Sea, central and eastern Anatolia and the Lakes region in the south). North Anatolia and its coastal areas have the most available land for good apple production with its humid and temperate climate. The province Gümüşhane in this region produces a large number of

apple varieties and contributes approximately 3.03% of the total apple production in Turkey (approximately 3.903 tonnes/year) [4].

'Göbek' apple (*Malus communis* L.) has been grown for many years by local producers in Gümüşhane province in northeast Anatolia (Turkey). The fruit of the cultivar (cv.) is bright yellow in color and is more widely cultivated and favourable consumed in Gümüşhane and its surroundings in the elsewhere in the region compared to other local varieties. Some pomological properties of the fruit have been recently reported by Şenyurt [4], including water soluble solids content (SS, 14.75%) and 0.44% titratable acidity (TA, 0.44%). The fruit is included in the "sweet" apple category Şenyurt [4]. However, to the best of our knowledge, no previous studies have reported on the nutrient contents in 'Göbek' apple during ripening. Specifically, our aim was to determine changes in nutrient composition (soluble sugars, organic acids, fatty acids, amino acids, and minerals) and polyamine profiles in fruit 'Göbek' apple, as an outstanding Turkish apple cv., during three ripening stages on the tree. To our knowledge an official application of geographical indication registration for the cv. will be launched in the very near future (personal communication, Gümüşhane Directorate of Provincial Food Agriculture and Livestock).

## 2. Materials and Methods

### 2.1 Plant Material

Ten trees of 'Göbek' apple aged 15-20 years in the province of Gümüşhane (Northeast Anatolia, Turkey) were selected. The flowers were considered to be in full bloom (DAFB) on April 20, 2015. Fruits (2 - 3 kg) that reached medium and commercial market size in the early morning were sampled after 188 (24 October, mid ripe), 198 (3 November, ripe) and 208 (13 November over-ripe) days of full bloom at 10-day interval by dividing them into the three distinct maturity stages. The fruits were hand-picked from the top and middle surroundings by turning around the trees 360° and were transferred to the laboratory in cold conditions (1.5 h, ~ 4° C). The fruit samples were immediately peeled. The peel and flesh were separated and treated with liquid nitrogen (-195° C) and stored at -80°C in tightly sealed steel vacuumed containers. Part of the peel and flesh samples were saved for the measurement of fresh and dry weights, and some whole fruits were set aside for visible or physico-chemical parameters. All extractions ( $n = 3$ ) and determinations ( $n = 3$ ) were performed in triplicate ( $\Sigma n = 6$ ).

### 2.2. Determination of Physicochemical Parameters

The whole fruit for each stage was analyzed in terms of both moisture (MC) and dry matter (DM) contents according to the AOAC Method [5] with slight modifications. In brief, 3-4 mm apple slices thick were placed in stainless steel dishes in a vacuum-drying oven for 2 days at 105° C until a constant weight was reached. Both MC and DM values were calculated in terms of weight difference. A MP 220 pH meter (Mettler Toledo)

was used to measure the pH, and a titration protocol with NaOH was used to determine titratable acidity (TA) based on the AOAC method, with slight modification, as described recently elsewhere [6]. Total soluble solids (TSS) expressed as percentages (%) were measured in juice pressed from the whole fruit sample during ripening using a digital refractometer (RE 50 Mettler-Toledo, Tokyo, Japan) at 21°C. Fruit firmness ( $\text{kg}/\text{cm}^2$ ) was measured using a penetrometer (FT-327) with a 11-mm diameter probe from three different areas (top, middle and bottom) of the whole fruit.

### 2.3. Sugar and Organic Acid Analysis

Soluble sugars and organic acids were separated and quantified following the method described by Ayaz et al. [7] using 0.5 g peel and flesh parts of the fruit. An Agilent 1100 HPLC (Palo Alto, CA, USA) device equipped with a quaternary HPLC pump, refractive index detector (RID), micro-vacuum degasser (MVD), thermostatic column compartment (TTC), UV/VIS detector, and a standard micro and preparative autosampler was used for sugar analysis on a Nucleosil C18 Carbohydrate analytical column (250 × 4.0 mm i.d., 10- $\mu\text{m}$  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  $\text{min}^{-1}$ .

Organic acid analysis was performed using the same HPLC equipped with the same instruments/modules. An Ace 5 C18 (Advanced Chromatography Technologies, Aberdeen, Scotland) column (25 cm x 4.6 mm i.d., 10 mm particle size) and potassium phosphate solution (0.02 M, pH 2.04) as mobile phase were used in the elution of organic acids. The flow rate was 2 mL/min and the column temperature was held at 25°C. Organic acids were detected using a HP 1100 Series multivariable wavelength detector of the above HPLC set at 210 nm. Calibration curves of the standard solutions were prepared for each sugar (sucrose, glucose, fructose, maltose, and lactose) and organic acid (malic acid, citric acid and ascorbic acid). Peaks 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. Quantifications were 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.

### 2.4. Polyamine (PA) Extraction and Analysis

Free polyamine extraction and analysis were performed following the method described by Hwang et al. [8] and Petřivalský et al. [9], with minor modifications. Lyophilized peel and flesh parts of the apple fruit samples (150 mg) were extracted with 5% (v/v) trichloroacetic acid (TCA) and derivatized with benzoyl chloride. Different concentrations of polyamine standard solutions were prepared to produce calibration curves for putrescine, cadaverine, spermidine and spermine using the methodology described above [8,9]. Benzoylated derivatives of polyamines from the peel and flesh samples and standards were extracted from reaction mixtures with diethyl ether

(3x15 mL), evaporated to dryness at 50°C under nitrogen gas, and dissolved in 120 µL of initial mobile phase. Next, a 10 µL sample was analyzed using a HPLC (Knauer Smartline-Manager) device 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 water (solvent A) and methanol (solvent B) in the following gradient program; 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. The UV detection wavelength was 227 nm with individual polyamines identified based on their UV-Vis spectra. Retention times were compared to the corresponding polyamine standards given above. A Clarity (DataApex) software was used for the integration of peak areas. The results were expressed as nmol per gram fresh weight (nmol/g fw).

## 2.5. Lipid Extraction and Fatty Acid Analysis

Total lipid extraction of the peel and flesh and fatty acid methyl esters (FAME) analyses were performed according to Ayaz et al. [7] described elsewhere. 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°C/min. This was then held for 1 min, and then raised to 240°C at 26°C/min and held again for 2 min. Injector split flow with helium was set at 150 – 200 mL/min. The detector gas was air set at 345 mL/min, and hydrogen gas was set at 36 mL/min. The detector makeup gas was helium at 35 mL/min. The FAME peaks were identified by comparison against FAME standards.

## 2.6. Mineral Analysis

Both peel and flesh parts in of the fruit of 'Göbek' apple were exposed to microwave digestion for mineral element analysis. Digestion of the samples and AAS or ICP-MS analysis for the elements Fe, Mg, Zn, K, Ca and P were performed according to the official method described by Glew et al. [10], with slight modifications. An official AOAC method [11] using AAS analysis was employed for Fe determination.

## 2.7. Amino Acid (AA) Analysis

Fifty milligrams of peel and flesh samples of the apple fruit were weighed and hydrolyzed in triplicate under vacuum 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 BioLC 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 method [10]. For the determination

of methionine and cysteine, samples were first oxidized with performic acid [12] prior to acid hydrolysis. Tryptophan was determined using the method described by Hugli and Moore [13]. The reproducibility of the method ranged from 0.6% to 1% for the amino acids reported.

## 3. Results and Discussion

During the ripening period in fruit of the 'Göbek' apple, a gradual significant increase was observed in the pH value, total soluble solids (TSS) and DM contents, while a decrease was determined in titratable acidity (TA), moisture content (MC) and fruit firmness (FF) values. All these six physicochemical parameters were significantly correlated ( $P < 0.01$  or  $0.05$ ) and associated with the analyzed nutrient or related components (e.g. polyamines, Table 1 and Table 2, data sets of PCA in Figure 1). It has been indicated that TSS content is a good indicator of sugar content in apples and presumably of sweetness, as reported elsewhere [14]. Similarly, the TSS content in the present apple cv. in this study was significantly strong correlated with the sugars in the peel and flesh and associated with the three stages of fruit ripening (see PCA in Figure 1). Titratable acidity (TA) is considered an important tool for predicting the taste of apples during the assessment of fruit quality, being a distinct preference for acid or sweet tasting apples [14]. Accordingly, the TA values obtained from the present apple cv. were significantly correlated with the sugars rather than with the organic acids. A similar pattern of TSS and TA contents to that in this study was recently reported by Şenyurt [4] for the same apple.

The stage-dependent changes in sugar concentrations in the peel and flesh in the apple fruit investigated in this study are presented in Table 1. Their concentrations increased significantly ( $P < 0.01$  or  $0.05$ ) and regularly on the tree from DAFBs 188 to 208. The quantification of fructose (fru), glucose (glc) and sucrose (suc) concentrations in the present study are not in agreement with previously reported ranges for common apple cvs. ('Golden Delicious', 'Fuji', 'Granny Smith', 'Jonagold', 'Delicious', 'Glockenapfel', etc.) [2,15,16]. Sugar profiles in the pulp and flesh of apple fruit have been described as an important component of chemical composition tables and provided valuable information regarding the authenticity of fruit juices. They also have an effect on the sensory properties and nutritional value of fruit products, and should be determined together with other aspects of chemical composition [16]. We observed a regular increase in the concentrations of the sugars in the two fruit parts during the ripening stages. Ackerman et al. [15] reported that the accumulation of sugars is limited to the conversion of sorbitol translocated from the leaves to the fruit during cell expansion, fru and suc being the favored conversion products. A rapid increase in glc concentrations just before harvest may also be related to starch synthesis and hydrolysis. This phase is characterized by a period of increased respiration during which the sugars and acids are rapidly used as substrates in the metabolic processes [15].



Stages	DM (%)	SI [23]	TSI [23]	TSS:TA		
		whole fruit				
188 DAFB	12.58±0.16 a	7115.18 (8386.26) a	4888.46 (5697.21) a	24.46 a		
198 DAFB	16.24±0.23 b	8223.18 (11898.21) b	5530.82 (8095.83) b	50.63 b		
208 DAFB	18.77±0.17 c	9089.21 (10164.04) c	6236.53 (9372.69) c	82.11c		
		Pearson correlation (r), peel (flesh)				
Variables	pH	TA	TSS	FF	MC	DM
Fructose, <i>fru</i>	1.000** (0.972)	-0.973 (-1.000**)	0.971 (1.000**)	-0.988* (-0.998*)	-0.976 (-0.907)	0.999* (0.986)
Glucose, <i>glc</i>	0.989* (0.982)	-0.994 (-0.998*)	0.993* (0.997*)	-1.000* (-1.000*)	-0.941 (-0.926)	0.997* (0.993*)
Sucrose, <i>suc</i>	0.909 (0.974)	-0.985 (-1.000**)	0.986 (0.999*)	-0.969 (-0.999*)	-0.810 (-0.911)	0.935 (0.987)
TS, total sugar <sup>§</sup>	0.987 (0.909)	-0.996* (-0.985)	0.995* (0.986)	-1.000** (-0.968)	-0.936 (-0.809)	0.995* (0.934)
Malic acid, <i>MaA</i>	-0.905 (-0.757)	0.983** (0.898*)	-0.985 (-0.902)	0.966 (0.861)	0.803 (0.614)	-0.931 (-0.799)
Citric acid, <i>CiA</i>	-0.909 (-0.995*)	0.774 (0.936*)	-0.769 (-0.934)	0.821 (0.961)	0.973 (0.995*)	-0.880 (-0.986)
TOA, total organic acid <sup>§</sup>	0.999* (-0.975)	0.976 (1.000*)	-0.974 (-0.999*)	0.990 (0.999*)	0.972 (0.911)	-1.000* (-0.987)
C16:0, palmitic acid	1.000** (0.959)	-0.963 (-1.000**)	0.961 (1.000**)	-0.981 (-0.994*)	-0.983 (-0.885)	0.997* (0.976)
C18:0, stearic acid	0.999* (0.929)	-0.976 (-0.804)	0.974 (0.799)	-0.990* (-0.848)	-0.973 (-0.983)	1.000** (0.902)
C20:0, eicosanoic acid	-0.985 (-0.957)	0.909 (0.851)	-0.906 (-0.847)	0.939 (0.890)	1.000** (0.995*)	-0.972 (-0.935)
C22:0, docosanoic acid	0.784 (-0.963)	-0.916 (1.000**)	0.919 (-1.000**)	-0.881 (0.996*)	-0.646 (0.891)	0.823 (-0.979)
C24:0, tetracosanoic acid	-0.946 (-0.857)	0.997* (0.698)	-0.998* (-0.693)	0.989* (0.752)	0.864 (0.942)	-0.966 (-0.821)
C18:1, oleic acid	0.997* (1.000**)	-0.984 (-0.972)	0.983 (0.970)	-0.995* (-0.987)	-0.962 (-0.977)	1.000** (0.999*)
C20:1, eicosenoic acid	-0.731 (-0.960)	0.534 (1.000**)	-0.527 (-1.000**)	-0.598 (0.994*)	0.851 (0.886)	-0.684 (-0.976)
C18:2, linoleic acid	-0.998* (-0.999*)	0.981 (0.957)	-0.979 (-0.955)	0.993* (0.977)	0.967 (0.987)	-1.000** (-0.995*)
C18:3, linolenic acid	-0.986 (0.160)	0.996* (0.096)	-0.995* (-0.104)	1.000** (0.018)	0.935 (-0.351)	-0.995* (0.094)
∑ MUFA	0.969 (0.995*)	-1.000** (-0.936)	1.000** (0.933)	-0.997* (-0.961)	-0.902 (-0.995*)	0.983 (0.986)
∑ SFA	0.999* (-0.790)	-0.979 (0.608)	0.978 (-0.602)	-0.992* (0.669)	-0.969 (0.895)	1.000** (-0.747)
∑ PUFA	-0.993* (-0.963)	0.991* (1.000**)	-0.990* (-1.000**)	0.998* (0.995*)	0.949 (0.891)	-0.998* (-0.979)
UFA/SFA	-0.970 (0.546)	1.000** (-0.315)	-1.000** (0.307)	0.998* (-0.388)	0.904 (-0.700)	-0.984 (0.489)
Putrescin, <i>put</i>	-0.912 (-0.860)	0.986 (0.962)	-0.987 (-0.964)	0.970 (0.937)	0.813 (0.743)	0.937 (-0.892)
Spermidin, <i>spd</i>	-0.947 (-0.990*)	0.997* (0.993*)	-0.998* (-0.964)	0.989* (0.999*)	0.865 (0.943)	-0.966 (-0.997*)
Spermin, <i>spm</i>	-0.985 (-0.931)	0.997* (0.993*)	-0.996* (-0.994*)	1.000* (0.981)	0.931 (0.841)	-0.994 (-0.953)
Fe, iron	0.976 (0.895)	-0.999* (-0.979)	0.999* (0.981)	-0.999* (-0.960)	-0.913 (-0.790)	0.988* (0.923)
Mg, magnesium	0.981 (0.994*)	-0.899 (-0.989*)	0.896 (0.988*)	-0.931 (-0.998*)	-1.000** (-0.954)	0.966 (0.999*)
Zn, zinc	-0.892 (-0.694)	0.747 (0.489)	-0.742 (-0.482)	0.797 (0.556)	0.963 (0.822)	-0.860 (-0.645)
K, potassium	0.982 (-0.975)	-0.901 (0.887)	0.898 (-0.883)	-0.933 (0.920)	-1.000** (1.000**)	0.967 (-0.958)
Ca, calcium	-0.885 (-0.145)	0.974 (-0.111)	-0.976 (0.119)	0.953 (-0.033)	0.776 (0.337)	-0.914 (-0.079)
P, phosphorus	0.053 (-0.838)	0.202 (0.949)	-0.210 (-0.952)	0.124 (0.921)	-0.249 (0.714)	-0.013 (-0.872)

\*significance at  $P < 0.05$ , \*\*significance at  $P < 0.01$ , <sup>§</sup>sum of individual components, SI; sweetness index, TSI; total sweetness index (calculation based on the data of individual sugar analysis using HPLC using the following equations;  $SI = (1.00 \times [\text{glucose}]) + (2.30 \times [\text{fructose}]) + (1.35 \times [\text{sucrose}])$  and  $TSI = (1.00 \times [\text{sucrose}]) + (0.76 \times [\text{glucose}]) + (1.50 \times [\text{fructose}])$ , peel (flesh) were according to Magwaza and Opara [23]. Abbreviation; DAFB; days after full bloom, total saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA), unsaturated (UFA) and unsaturated/saturated (UFA/SFA) fatty acids, TSS; total soluble solids, FF; fruit firmness, MC; moisture content, DM; dry matter, n.d.; not detected

**Table 2. Changes in amino acid concentrations (mg 100 g<sup>-1</sup> fw) in the peel and flesh parts in the fruit of 'Göbek' (*Malus communis* L.) apple during ripening on the tree. Values represent the mean ± SD of three separate extractions and determinations. Analysis of variance (one-way ANOVA) was used for comparisons between means within the peel and flesh. Values with the same letter within a row are not significantly different at  $P < 0.05$**

Amino acid	Peel			Flesh		
	188 DAFB	198 DAFB	208 DAFB	188 DAFB	198 DAFB	208 DAFB
Ala, alanine	10.71±0.10 a	12.76±0.08 b	15.75±0.04 c	4.47±0.12 c	2.92±0.07 a	3.23±0.12 b
Gly, glycine	14.29±0.12 a	16.51±0.06 a	22.18±2.91 b	5.09±0.11 b	4.30±0.00 a	4.33±0.07 a
Val, valine	9.80±0.03 a	13.37±0.03 b	14.99±0.17 c	3.88±0.03 b	3.02±0.02 a	3.00±0.02 a
Leu, leucine	15.98±0.15 a	20.97±0.08 b	25.56±0.14 c	7.00±0.06 a	5.57±0.04 b	2.68±0.10 c
Ile, isoleucine	9.84±0.03 a	13.38±0.02 b	14.92±0.01 c	3.98±0.07 c	2.93±0.16 b	2.68±0.11 a
Thr, threonine	7.09±0.01 a	13.51±0.15 b	24.30±0.14 c	3.57±0.20 a	3.41±0.20 a	5.34±0.19 c
Ser, serine	7.06±0.16 a	7.52±0.02 b	11.06±0.06 c	2.50±0.11 b	1.72±0.16 a	1.74±0.02 a
Pro, proline	21.30±0.12 a	34.20±0.03 b	40.21±0.01 c	13.81±0.02 a	14.01±0.17 a	19.16±0.12 b
Arg, arginine	9.35±0.22 a	9.65±0.05 b	11.35±0.09 c	3.91±0.23 b	2.92±0.15 a	2.67±0.03 a
Asp, aspartic acid	108.07±0.07 a	129.50±0.05 b	210.28±0.22 c	53.34±0.17 a	61.95±0.05 b	64.25±0.04 c
Met, methionine	7.50±0.00 c	6.44±0.02 b	5.25±0.15 a	3.98±0.98 c	3.43±0.05 a	3.84±0.04 b
Glu, glutamic acid	85.20±0.92 c	48.05±2.10 a	66.27±0.75 b	36.95±2.63 c	9.23±0.12 a	21.61±0.11 b
Phe, phenylalanine	9.82±0.68 a	12.90±0.17 b	15.46±0.4 c	4.11±0.11 b	3.39±0.06 a	3.22±0.08 a
Lys, lysine	24.56±0.19 a	26.89±0.66 b	30.5±0.83 c	13.2±0.02 c	11.18±0.14 a	11.91±0.00 b
His, histidine	1.26±0.07 b	1.06±0.11 a	3.08±0.02 c	1.02±0.11 c	0.08±0.03 a	0.65±0.03 b
Tyr, tyrosine	5.85±0.27 a	7.52±0.28 b	9.48±0.07 c	1.55±0.02 a	1.66±0.11 a	1.58±0.09 a
∑AA	347.68	374.23	520.64	112.36	131.72	151.89
∑EAA	84.59	107.46	130.98	39.72	32.93	32.67
∑EAA (%)	24.33	28.71	25.16	35.35	25	21.51
∑NEAA	263.09	266.77	389.66	72.64	98.79	119.22
∑NEAA (%)	75.67	71.29	74.84	64.65	75	78.49
Phe + Tyr	16.91	26.41	39.74	7.68	6.80	8.56

Amino acid	Pearson correlation (r), peel (flesh)					
	pH	TA	TSS	FF	MC	DM
Ala, <i>alanine</i>	0.999* (-0.720)	-0.952 (0.873)	0.950 (-0.876)	-0.973 (0.831)	-0.990* (0.569)	0.993* (-0.764)
Gly, <i>glycine</i>	0.981 (-0.819)	-0.900 (0.938)	0.897 (-0.941)	-0.932 (0.908)	-1.000* (0.691)	0.966 (-0.856)
Val, <i>valine</i>	0.965 (-0.849)	-1.000** (0.955)	1.000** (-0.957)	-0.996* (0.929)	-0.894 (0.728)	0.980 (-0.882)
Leu, <i>leucine</i>	0.997* (-0.990*)	-0.984 (0.923)	0.983 (-0.920)	-0.995* (0.950)	-0.962 (0.998*)	1.000** (-0.979)
Ile, <i>isoleucine</i>	0.962 (-0.923)	-1.000** (0.990*)	1.000** (-0.992*)	-0.995* (0.977)	-0.889 (0.829)	0.978 (-0.947)
Thr, <i>threonine</i>	0.996* (0.855)	-0.940 (-0.695)	0.937 (0.690)	-0.964 (-0.750)	-0.994* (-0.940)	0.987 (0.819)
Ser, <i>serine</i>	0.934 (-0.926)	-0.813 (0.942)	0.808 (-0.944)	-0.856 (0.912)	-0.986 (0.698)	0.908 (-0.861)
Pro, <i>proline</i>	0.966 (0.906)	-1.000** (-0.769)	1.000** (0.764)	-0.997* (-0.817)	-0.897 (-0.972)	0.981 (0.876)
Arg, <i>arginine</i>	0.946 (-0.927)	-0.832 (0.992*)	0.828 (-0.993*)	-0.873 (0.979)	-0.991* (0.835)	0.922 (-0.950)
Asp, <i>aspartic acid</i>	0.964 (0.856)	-0.864 (-0.959)	0.861 (0.961)	-0.901 (-0.934)	-0.997* (-0.737)	0.944 (0.888)
Met, <i>methionine</i>	-1.000** (-0.193)	0.972 (0.436)	-0.970 (-0.443)	0.988 (0.364)	0.976 (-0.004)	-0.999* (-0.257)
Glu, <i>glutamic acid</i>	-0.463 (-0.507)	0.673 (0.709)	-0.678 (-0.715)	0.613 (0.652)	0.279 (0.328)	-0.521 (-0.563)
Phe, <i>phenylalanine</i>	0.994* (-0.923)	-0.989* (0.990*)	0.988 (-0.991*)	-0.997* (0.976)	-0.954 (0.829)	0.999* (-0.946)
Lys, <i>lysine</i>	0.998* (-0.588)	-0.947 (0.774)	0.944 (-0.779)	-0.969 (0.722)	-0.992* (0.418)	0.991* (-0.640)
His, <i>histidine</i>	0.847 (-0.341)	-0.684 (0.569)	0.679 (-0.575)	-0.740 (0.502)	-0.935 (0.149)	0.810 (-0.402)
Tyr, <i>tyrosine</i>	1.000** (0.212)	-0.969 (-0.453)	0.967 (0.460)	-0.985 (-0.382)	-0.979 (-0.015)	-0.764 (0.276)
∑AA	0.947 (0.999*)	-0.834 (-0.977)	0.830 (0.975)	-0.875 (-0.991)	-0.992 (-0.971)	0.924 (1.000*)
∑EAA	0.999* (-0.855)	-0.978 (0.959)	0.976 (-0.961)	-0.991 (0.934)	-0.971 (0.737)	1.000* (-0.888)
∑EAA (%)	0.125 (-0.945)	-0.373 (0.997*)	0.381 (-0.998*)	-0.299 (0.988)	0.072 (0.863)	0.191 (-0.965)
∑NEAA	0.903 (0.992)	-0.764 (-0.991)	0.759 (0.990)	-0.812 (-0.999*)	-0.970 (-0.949)	0.872 (0.998*)
∑NEAA (%)	-0.125 (0.945)	0.373 (-0.997*)	-0.381 (0.998*)	0.299 (-0.988)	-0.072 (-0.863)	-0.191 (0.965)
Phe + Tyr	0.999* (0.546)	-0.955 (-0.315)	0.953 (0.307)	-0.976 (-0.388)	-0.988 (-0.700)	0.994 (0.489)

\* significance at  $P < 0.05$ , \*\* significance at  $P < 0.01$ .

Abbreviations: DAFB; days after full bloom, total (AA), essential (EAA) and non-essential (NEAA) amino acids (sum of individual amino acids), TSS; total soluble solids, FF; fruit firmness, MC; moisture content, DM; dry matter.

A gradual significant decrease was observed in the concentrations of malic acid (MaA) and citric acid (CiA) in the peel and flesh throughout ripening (188 - 198 DAFBs). No measurable level of CiA and ascorbic acid (AA) was detected in the peel at the final stage of the ripening period (Table 1). Studies have concluded that MaA is the principal organic acid in apple fruits and that its content is highly correlated with TA [14,15]. In terms of CiA and AsA, these were present in lower quantities, the level of AsA being much lower compared to MaA [14]. We had similar organic acid profiles in the fruit of 'Göbek' apple in the present study. However, the present apple cv. had a higher MaA concentration than those reported in the literature by Veberic and Stampar [17, ave. 835 mg/100 g of FW] and Nour [14, ave. 919 mg/100 g]. Hecke [18] compared MaA concentrations of 7 integrated (737 - 1408 mg/100 g fw) and 11 organically grown apples (953 - 1780 mg/100 g fw). Our findings for the acid in the 'Göbek' apple were within these ranges. Those authors [17,18] observed no measurable level of CiA in the apple cvs investigated. Malic acid concentrations, as well as citric acid, in the peel and flesh declined regularly during ripening in 'Göbek' apple, as reported by Ackermann et al. [15] during ripening and storage in fruit of 'Glockenapfel' apple. That study attributed the decrease to the dilution effect caused by mass increase during the cell growth phase. However, this decrease might also be due to increased respiration, since MaA is the principal metabolic substrate with sugars [15].

### 3.1. Effect of Ripening on Polyamine Concentrations

Table 1 indicates that spermidine (spd) is the major polyamine in both the peel and flesh in fruit of 'Göbek'

apple and its concentration of decreased gradually over the three weeks of ripening (188 - 208 DAFBs), ranging between 411.32 and 188.35 nmol/g fw (ave. 281.6) in the peel and 174.58 and 79.77 nmol/g fw (ave. 124.7) in the flesh. Similarly, concentrations of putrescine (put) and spermine (spm) in the fruit followed the same consistent decreasing trend during ripening of the fruit.

### 3.2. Effect of Ripening on Fatty Acid Concentrations

Ripening on the tree from DAFBs 188 to 198 DAFBs significantly affected ( $P < 0.05$ ) the concentrations of fatty acids. The major saturated fatty acid (SFA) was palmitic acid (ave. 33.1%), and the major unsaturated fatty acid was linoleic acid in the peel (ave. 21.1%, range 24.03 - 18.17) and flesh (ave. 18.2%, range 20.61 - 15.59), followed by oleic acid (Table 1). Our findings confirmed earlier reports that palmitic acid and linoleic acid are the dominant fatty acids in the whole fruit of eight apples [16], constituting 70-80% of the total fatty acids in the fruits. Comparison of fatty acids in surface lipids of 'Champion', 'Golden delicious' and 'Granny Smith' apple varieties have revealed the presence of palmitic acid, stearic acid, linoleic acid and oleic acid as the principal fatty acids in the peel and flesh parts [19]. However, findings of the concentration of the present apple cv. agree in part with those of the concentrations reported in the literature [19].

### 3.3. Effect of Ripening on Mineral Concentrations

Concentration changes of six mineral elements (Table 1) in the fruit differed significantly ( $P < 0.05$ ), not only in the peel and flesh, but also through the stages of ripening in

each case. Potassium (K) was the most abundant mineral element, with a gradual increase in the flesh (ave. 186.3 mg/g fw, range 174.70 - 201.72) and a decrease in the flesh (ave. 105.6 mg/g fw, range 122.71 - 81.33) in fruit of the 'Göbek' apple during ripening. This was followed by a slight but significant decrease in Ca concentrations. Concentrations of Zn and P stayed more or less the same in the two fruit parts, while the concentrations of Fe and Mg increased significantly during the ripening periods (Table 1). Apples are considered a good source of dietary minerals [14]. In accordance with previous reports [2,14,20,21,22,23], K, Ca and Mg were the main minerals present at high concentrations in the present apple cv. In contrast to the above studies, our phosphorus (P) values were very low in the present apple fruit.

### 3.4. Effect of Ripening on Amino Acid Concentrations

Both the peel and flesh contributed 16 amino acids at different concentrations (Table 2). In general, concentrations of most of the amino acids in the peel exhibited an increasing trend as fruit ripening progressed, except for the level of methionine (met, Table 2). Concentrations of amino acids in the peel were significantly higher ( $P < 0.05$ ) than those in the flesh part. Analysis revealed no regular trend in the levels of amino acids in the flesh, with values increasing, decreasing, or even remaining constant throughout the ripening. Asparagine (asp) was the predominant amino acid in the peel (ave. 149.3 mg/100 g fw, range 108.07 - 210.28) and in the flesh flesh (ave. 53.18 mg/100 g fw, range 33.34 - 64.25), followed by glutamine (glu) (ave. 66.5 and 25.93 mg/100 g fw). Alanine, asp, serine (ser) and glu were the major amino acids determined in eight apple cvs. from China [16]. We also identified asp and glu as the principal amino acids in the peel and flesh parts in the present apple cv., and partly in agreement with that study, prolin (pro) and lysine (lys) emerged as the second major amino acids in comparison to the Chinese apple cvs. [16]. Very much earlier, asp and glu, followed by ser and alanine (ala) or phenylalanine (phe), were also quantified as the most abundant amino acids in fruit of 'Glockenapfel' apple variety [15].

### 3.5. Principal Component Analysis (PCA) and Pearson Correlation ( $r$ )

The principal components (PCs) of sugars and organic acids explained 100.00% of total variation, where PC1 accounts for 94.49% of the variance separating sugars through 198 and 208 DAFBs at the right upper and lower quadrants with positive loadings and PC2 accounts for 5.51% with organic acids at 188 DAFB at the left upper and lower quadrants with negative loadings (Figure 1A). The sugars in the peel and flesh were significantly strong correlated (positively,  $P < 0.01$  or 0.05) with pH, TSS and DM, and were negatively correlated with TA and FF. The organic acids (MaA and CiA) exhibited high but insignificant correlations ( $P < 0.05$ ) with the physicochemical parameters, with two exceptions, pH and MC contents in the flesh (for detail see Table 1).

PCs were also extracted from the data for the quantities of the three polyamines (PA) (put, spm and spd) compared

with the six physicochemical parameters of the fruit. In this case, PC confirmed that the polyamine profiles, had positive loadings at the right upper and lower quadrants on PC1 with 96.84% of the total variance (Figure 1B). Only spermidin was closely associated with the physicochemical parameters and correlated ( $P < 0.05$ ) with pH, TA, TSS, FF and DM in the fruit, either negatively or positively (Table 1).

Figure 1C shows that the data set for PCA of fatty acids explained 100.00% of total variation, where PC1 accounts for 86.82% of the variance and PC2 for 13.18%. The PC1 having positive scores of C16:0, C18:0 and C18:1 fatty acids in the peel and flesh parts at the right upper and lower quadrants was associated with 198 and 208 DAFBs and strongly correlated ( $P < 0.01$  or 0.05) with pH, TA, DM and TSS. They were all associated and Negative loadings of fatty acids in the peel and flesh at the left upper and lower quadrants were available for C18:2 or other acids with TA, FF and MC on PC2 that were closely associated and strong correlated (negatively,  $P < 0.01$  or 0.05) with TA, FF and MC measured for the first ripening stage, 188 DAFB (Table 1).

The PCs extracted from the element analysis data compared with the six physicochemical parameters are shown in Figure 1D. The PC1 accounts for 78.97% of the total variation (100.00%), with positive loadings of P, K, Mg and Fe in the peel and flesh, with pH, DM and TSS at the left upper and P, Ca, K and Zn in the peel and flesh with TA, FF and MC at the left lower quadrants through 208 and 198 and 188 DAFBs, respectively. The Fe, Mg and K concentrations in the peel and flesh were significantly (positively or negatively) strong correlated with pH, TA, TSS, FF, MC and DM ( $P < 0.01$  or 0.05) (Table 1).

The fifth dataset for PCA represented the quantity of amino acids in the peel and flesh compared with the values of the six physicochemical parameters of the fruit. Their PCs explained 100.00% of the total variation, where PC1 accounts for 79.45% of the variance and PC2 for 20.55% with 188 DAFB (Figure 1E). Nearly all amino acids more or less strongly correlated (positively or negatively,  $P < 0.01$  or 0.05) with at least one or more of the physicochemical parameters measured in the fruit, while the remaining correlations were insignificant (Table 2).

## 4. Conclusion

The majority and maximal availability of essential nutrients in fruit of present apple cv. in the present study have been affected by the ripening process, being more nutrient-dense at earlier stages (188 - 198 DAFBs). 'Göbek' apple may represent a more convenient source of these nutrients if it is harvested at different ripening stages. These findings might be translated into a more profitable agronomic exploitation of this outstanding fruit by using more standardized and sophisticated horticultural procedures instead of manual collection with hooks and bags. However, further studies are now needed to evaluate the changes in nutrients after storage under controlled atmospheric conditions and to compare phenolic status with antioxidant capacities during pre- and postharvest ripening periods.

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