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Antioxidant activity and polyphenol composition of black mulberry (*Morus nigra* L.) products

Senem Kamiloglu, Ozge Serali, Nihan Unal and Esra Capanoglu*
Faculty of Chemical and Metallurgical Engineering, Department of Food Engineering, Istanbul Technical University, Maslak, Istanbul, Turkey

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Abstract.
BACKGROUND: Today, due to its nutritive value, black mulberry (*Morus nigra* L.) is consumed both as fresh and in processed forms.
OBJECTIVE: In order to investigate the health-related constituents of black mulberry products, total phenolics, flavonoids, anthocyanins, antioxidant capacity and major phenolic compounds were determined for different products including fresh mulberry, dried mulberry, mulberry wine, molasses, ice cream, juice, jam and syrup.
METHODS: Samples were obtained as three replicates, and total phenolics, flavonoids, anthocyanins, antioxidant capacity were determined by spectrophotometric methods, whereas major phenolic compounds were detected using high-performance liquid chromatography (HPLC) coupled with a photodiode array (PDA) detector.
RESULTS: In analyzed samples, chlorogenic acid, rutin and cyanidin-3-*O*-glucoside were confirmed as the major phenolic acid, flavonol and anthocyanin, respectively. In general, fresh mulberry showed higher contents of total phenolics (0.49–57 fold higher), flavonoids (0.02–162 fold higher), anthocyanins (6-12209 fold higher) and antioxidant capacity (0.72–691 fold higher) compared to other products. Total flavonoids and phenolics showed a linear relationship with antioxidant capacity (CUPRAC: $R^2 = 0.9070$ and $R^2 = 0.8959$, respectively), indicating that flavonoids and phenolics were the major contributors to the antioxidant capacity.
CONCLUSIONS: These results provide valuable insights into the biochemical composition of black mulberry and how this may change during fruit processing.

Keywords: Black mulberry (*Morus nigra* L.), phenolic, flavonoid, anthocyanin, antioxidant, HPLC

1. Introduction

The mulberry belongs to the genus *Morus* of the family *Moraceae*. There are 24 species of *Morus* and one subspecies, with at least 100 known varieties. In general, three main types of mulberry are grown for fruit production, including white (*Morus alba*), red (*Morus rubra*), and black (*Morus nigra*) mulberry. Black mulberry (*M. nigra*), which originates in Iran, is one of the important species grown in the Mediterranean countries [1, 2].

Mulberry is an important crop in Turkey and has been cultivated for centuries. About 2.3 million mulberry trees are present in Turkey and approximately 65.000 tons of mulberry fruit are produced annually [3], The main mulberry production areas of Turkey are Black Sea region as well as eastern and central Anatolian regions [4], where the

*Corresponding author: Esra Capanoglu, Faculty of Chemical and Metallurgical Engineering, Department of Food Engineering, Istanbul Technical University, Maslak, Istanbul, Turkey. Tel.: +90 212 2857340; Fax: +90 212 2857333; E-mail: capanogl@itu.edu.tr.

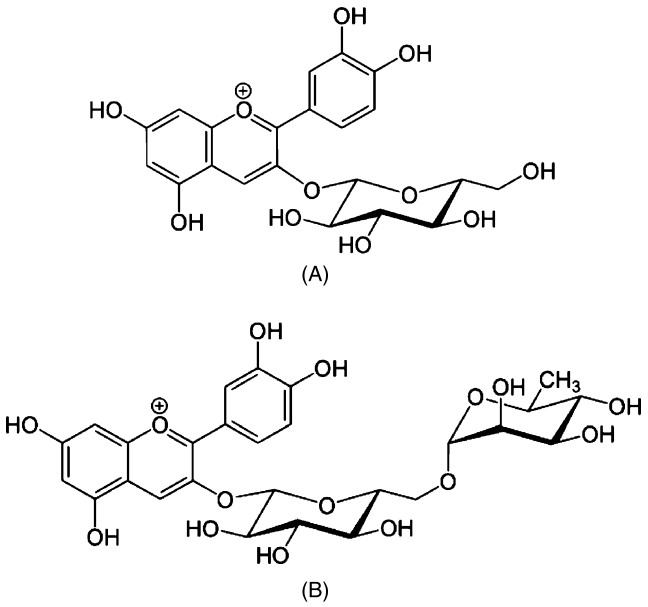

Fig. 1. Chemical structure of (A) cyanidin-3-*O*-glucoside (C3G) and (B) cyanidin-3-*O*-rutinoside (C3R).

harvesting period is between June and August [5]. Moreover, 95% of the mulberry trees grown in Turkey are white (*M. alba*), whereas 3% are red (*M. rubra*) and 2% are black (*M. nigra*) [6].

Black mulberry (*Morus nigra* L.) is a fruit known not only for its nutritional qualities and its flavor, but also for its traditional use in natural medicine as it has a high content of active therapeutic compounds [7–9]. Recently, mulberry fruit have been reported to exhibit several biological activities such as antidiabetic, antioxidative, antiinflammatory and antihyperlipidemic activities. These biological activities were due to their polyphenol components including anthocyanins present in some of the varieties [10]. Anthocyanins, belonging to the group of flavonoids and being responsible for the orange, red, and blue colors of flowers, fruits, and vegetables, are viewed as natural colorants to replace synthetic ones. Therefore, new sources of these compounds are nowadays desired [11]. Black mulberry have cyanidin-based anthocyanins, particularly cyanidin-3-*O*-glucoside and cyanidin-3-*O*-rutinoside (Fig. 1) [12].

The fruits can be consumed either as fresh or processed. Fresh black mulberry is very perishable and since it softens very quickly, handling during transportation and marketing is very difficult [13]. Furthermore, black mulberry has a brief harvest season, after which fresh fruits can only be stored refrigerated for a maximum of six weeks. Thus, further processing is desirable to allow storage [2]. Mulberry fruit can be processed into many forms such as syrup, jam, pulp, ice cream, vinegar, concentrate, alcohol [14] and several other products. As far as it is known, no previous study evaluated the antioxidant activity and polyphenol composition of different black mulberry products. Given the above, the aim of this study was to examine the total phenolics, flavonoids, anthocyanins, antioxidant capacity and the major phenolic compounds of selected black mulberry products.

2. Materials and methods

2.1. Plant materials

The black mulberry (*Morus nigra* L.) products including fresh mulberry, dried mulberry, mulberry wine, mulberry molasses, mulberry ice cream, mulberry juice, mulberry jam and mulberry syrup were collected at the beginning of 2012 from a local market in Istanbul, Turkey. All the samples were processed in 2011 according to the information presented on their label. For each product, three repetitions were carried out. All samples were ground to a fine powder in liquid nitrogen using a pre-cooled grinder (IKA A11, Germany), and stored at −80°C before analysis.

2.2. Chemicals

In this study, chemicals with analytical purity were used. For extraction and determination of spectrophotometric assays, gallic acid, catechin, ethanol, Folin-Ciocalteu phenol reagent, 1, 1-diphenyl-2-picrylhydrazyl (DPPH), 2, 4, 6-tris (2-pyridyl)-s-triazine (TPTZ) and neocupraine from Sigma-Aldrich Chemie GmbH (Steinheim, Germany); methanol, formic acid, hydrochloric acid, sodium carbonate, sodium nitrite, sodium hydroxide, sodium acetate trihydrate, potassium persulfate, dipotassium hydrogen phosphate, potassium dihydrogen phosphate, copper (II) chloride and ammonium acetate from Merck KGaA (Darmstadt, Germany); 6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox) and aluminum chloride from Fluka Chemie (Buchs, Switzerland); potassium chloride from Riedel-de Haen Laborchemikalien GmbH (Hanover, Germany); ferric chloride from Lachema (Czech Republic) and 2, 2′-azinobis (3-ethylbenzo-thiazoline-6-sulphonic acid) diammonium salt (ABTS) from Applichem GmbH (Darmstadt, Germany) were purchased. The following standards and reagents were used for the quantification of phenolic compounds: gallic acid, cyanidin-3-*O*-glucoside, cyanidin-3-*O*-rutinoside, pelargonidin-3-*O*-glucoside and pelargonidin-3-*O*-rutinoside from Extrasynthese (Genay, France); chlorogenic acid, syringic acid, neochlorogenic acid, caffeic acid and quercetin-3-*O*-glucoside from Fluka; rutin, trifluoroacetic acid and acetonitrile from Sigma-Aldrich. Water used for all analysis was distilled and purified with the water purification system (TKA GenPure, Germany).

2.3. Determination of moisture content

Moisture content was determined following the guidelines of the official "Turkish Standard" [15] method TS 1129 ISO 1026. All samples were analyzed in triplicate and average values were reported.

2.4. Extract preparation

Three independent extractions for each sample were carried out according to the method described by Capanoglu et al. [16] with slight modifications. 2 ± 0.01 g of each sample was extracted with 5 ml of 75% aqueous-methanol containing 0.1% (v/v) formic acid in a cooled ultrasonic bath (Azakli, Turkey) for 15 min. The treated samples were centrifuged (Hettich Zentrifugen Universal 32R, UK) for 10 min at 4000 rpm and the supernatant was collected. Another 5 ml 75% aqueous-methanol containing 0.1% (v/v) formic acid was added to the pellet and this extraction procedure was repeated two more times. All four supernatants were combined and adjusted to a final volume of 20 ml. Prepared extracts were stored at -20°C until analysis.

2.5. Determination of Total Phenolic content (TP)

The TP of extracts was determined using Folin-Ciocalteu reagent as described previously by Velioglu et al. [17]. The TP of extracts was expressed as milligrams of gallic acid equivalent (GAE) per 100 g dry weight (dw) of sample. Samples of each extraction were analyzed in triplicate.

2.6. Determination of Total Flavonoid content (TF)

The TF was measured colorimetrically as described by Kim et al. [18]. The TF of extracts was determined by a (+)-catechin standard curve and expressed as milligrams of (+)-catechin equivalent (CE) per 100 g of dw of sample. Triplicate samples were analyzed for each extract.

2.7. Determination of Total Anthocyanin content (TA)

The TA content was determined according to the pH differential method [19]. TA of extracts was expressed as milligrams of cyanidin-3-*O*-glucoside (C3G) equivalent per 100 g of dw of sample. Samples of each extraction were analyzed in triplicate.

2.8. Determination of Total Antioxidant Capacity (TAC)

The TAC was estimated by four different assays. The ABTS, DPPH, FRAP and CUPRAC assays were performed according to Miller and Rice-Evans [20], Karuman and Karunakaran [21], Benzie and Strain [22] and Apak et al. [23], respectively. In all assays, trolox was used as a standard and results were expressed in terms of milligrams of trolox equivalent antioxidant capacity (TEAC) per 100 g of dw of sample. Samples were analyzed in triplicate for each assay.

2.9. HPLC analysis of major phenolic compounds

Major phenolic compounds were determined following the method of Capanoglu et al. [16]. Extracts were filtered through a 0.45 μm membrane filter and analyzed using a Waters 2695 HPLC system with a PDA (Waters 2996) detector. A Supelcosil LC-18 25 cm × 4.60 mm, 5 μm column (Sigma-Aldrich, Steinheim, Germany) was used. The mobile phase consisted of solvent **A**, Milli-Q water with 0.1% (v/v) TFA and solvent **B**, acetonitrile with 0.1% (v/v) TFA. A linear gradient was used as follows: at 0 min, 95% solvent **A** and %5 solvent **B**; at 45 min, 65% solvent **A** and 35% solvent **B**; at 47 min, 25% solvent **A** and 75% solvent **B**; and at 54 min returning to initial conditions. The flow rate was 1 ml/min. Detection was done at 280, 312, 360, and 520 nm. Identification was based on the retention times and characteristic UV spectra. Quantification was done using external standards as well as taking the information from the literature into account. All analyses were performed in triplicate.

2.10. Statistical analysis

Data were collected from three independent extractions and reported as mean ± standard deviation. For multiple comparisons, data were subjected to statistical analysis using SPSS software (version 20.0) for the analysis of variance (ANOVA). Duncan's new multiple range test was used to analyze differences between treatments ($p < 0.05$). The correlation coefficients (R^2) for spectrophotometric assays were calculated by using the Microsoft Office Excel 2011 software (Microsoft Corporation, Redmond, WA).

3. Results

3.1. Moisture content

The moisture content of the samples (Table 1) was found to be significantly different for each product ($p < 0.05$). Thus, in order to eliminate the differences in the moisture contents of black mulberry products, all the results in this study are expressed on dry weight (dw) basis.

3.2. Total Phenolic content (TP)

TP of all samples is shown in Table 1. Among 8 extracts, fresh mulberry showed the highest TP, which was 49% higher than dried mulberry, which had the second highest TP. On the other hand, mulberry syrup showed the lowest TP, which was ∼3 fold lower than mulberry jam, which had the second lowest TP. Besides mulberry molasses and mulberry ice cream, all products were found to be statistically significant from each other ($p < 0.05$). In the literature, higher results of TP have been reported for fresh mulberry [1, 6, 8, 12, 24–26] whereas no previous study reported the TP of other black mulberry products analyzed in this study.

3.3. Total Flavonoid content (TF)

TF of samples were measured colorimetrically (Table 1) and similar to TP, the highest level of TF was found in fresh mulberry, while the lowest was in mulberry syrup. Mulberry wine also had high levels of TF, which was not statistically significant from fresh mulberry ($p < 0.05$). The TF of fresh mulberry have been investigated before,

Table 1
Moisture, total phenolic (TP), total flavonoid (TF) and total anthocyanin (TA) contents of selected black mulberry (*Morus nigra* L.) products

Sample	Moisture (%)	TP (mg GAE/100 g dw)	TF (mg CE/100 g dw)	TA (mg C3G/100 g dw)
Fresh mulberry	87.2 ± 0.5b	1451.4 ± 124.2a	768.7 ± 132.8a	1221.0 ± 42.9a
Dried mulberry	5.7 ± 0.2h	976.4 ± 27.3b	380.0 ± 34.4b	61.3 ± 7.0c
Mulberry wine	89.6 ± 0.1a	737.3 ± 29.7c	751.1 ± 56.2a	26.6 ± 3.5d
Mulberry molasses	17.9 ± 0.2f	402.4 ± 23.5d	84.8 ± 1.7d	2.4 ± 0.1d
Mulberry ice cream	63.6 ± 0.3d	377.9 ± 28.1d	139.8 ± 6.9d	173.1 ± 1.8b
Mulberry juice	85.1 ± 0.3c	266.8 ± 26.6e	205.7 ± 22.8c	19.2 ± 3.9d
Mulberry jam	35.7 ± 1.5e	108.7 ± 9.4f	91.8 ± 3.5d	4.0 ± 0.4d
Mulberry syrup	12.8 ± 1.5g	25.0 ± 4.7g	4.7 ± 0.9e	0.1 ± 0.0d

Data represent average values ± standard deviation of three independent samples. All contents are expressed per 100 g dry weight (dw). Different letters in the columns represent statistically significant differences ($p < 0.05$).

however it is difficult to compare the TF values of this study with the literature since most of the existing reports are based on quercetin equivalents [1] whereas in our study (+)-catechin is used as a standard.

3.4. Total Anthocyanin content (TA)

As shown in Table 1, fresh mulberry had the highest TA, whereas TA levels in mulberry syrup, mulberry molasses, mulberry jam, mulberry juice and mulberry wine were significantly low ($p < 0.05$). Results for fresh mulberry were found to be ∼2 fold higher than those reported by Ozgen et al. [12] and Ercisli et al. [24]. On the other hand, Fazaeli et al. [9] determined higher levels of TA in mulberry juice (16.4 mg C3G/100 ml) compared to the value obtained in this study.

3.5. Total Antioxidant Capacity (TAC)

TAC values (Table 2), measured by using four different methods (ABTS, DPPH, FRAP, CUPRAC), indicated extremely large variations between samples (∼700 fold difference). In general, ABTS and CUPRAC assays gave higher TAC values compared to DPPH and FRAP assays. Moreover, besides CUPRAC assay, ranking of the samples based on their TAC values was found to be as follows: fresh mulberry > mulberry wine > dried mulberry > mulberry ice cream > mulberry molasses > mulberry juice > mulberry jam > mulberry syrup. In case of CUPRAC assay, the only difference in the rankings was the sequence of mulberry ice cream and mulberry juice. Ozgen et al. [12] and

Table 2
Total antioxidant capacity (TAC) of selected black mulberry (*Morus nigra* L.) products

Sample	TAC (mg TEAC/100 g dw)			
	ABTS	DPPH	FRAP	CUPRAC
Fresh mulberry	7475.6 ± 740.0a	2114.7 ± 104.3a	3536.7 ± 106.0a	6092.3 ± 637.0a
Dried mulberry	2686.3 ± 238.3c	580.0 ± 23.8c	1016.2 ± 26.7c	2749.4 ± 304.1c
Mulberry wine	4338.8 ± 784.0b	1039.5 ± 82.9b	1683.1 ± 35.4b	4020.6 ± 93.2b
Mulberry molasses	1311.2 ± 144.8de	368.5 ± 21.2de	458.2 ± 28.1e	1218.4 ± 110.3d
Mulberry ice cream	1729.3 ± 134.1d	402.0 ± 12.1d	886.1 ± 20.3d	1161.6 ± 84.3d
Mulberry juice	670.5 ± 231.8ef	313.4 ± 21.3e	456.5 ± 35.3e	1347.5 ± 32.0d
Mulberry jam	333.3 ± 87.5f	94.0 ± 4.8f	146.6 ± 3.4f	491.3 ± 32.0e
Mulberry syrup	44.3 ± 7.9f	10.8 ± 0.8g	19.4 ± 2.7g	77.3 ± 5.0f

Data represent average values ± standard deviation of three independent samples. All contents are expressed per 100 g dry weight (dw). Different letters in the columns represent statistically significant differences ($p < 0.05$).

Table 3
The correlation coefficients (R^2) between spectrophotometric assays

Variable	TP	TF	TA	ABTS	DPPH	FRAP	CUPRAC
TP	1.0000						
TF	0.7252	1.0000					
TA	0.6013	0.3787	1.0000				
ABTS	0.8698	0.8349	0.6984	1.0000			
DPPH	0.8420	0.7913	0.7931	0.9629	1.0000		
FRAP	0.8478	0.7774	0.8117	0.9663	0.9901	1.0000	
CUPRAC	0.8959	0.9070	0.6169	0.9557	0.9488	0.9353	1.0000

TP: Total phenolic content, TF: Total flavonoid content, TA: Total anthocyanin content.

Gundogdu et al. [14] measured lower TAC values in fresh mulberry (approximately one third of our results) compared to our ABTS results. Similarly, lower FRAP and DPPH results have been reported in fresh mulberry by Ercisli et al. [24] and Stefanut et al. [26], respectively. As far as it is known, no previous study evaluated the antioxidant capacity of black mulberry and its products using the CUPRAC method.

3.6. Relationship between TP, TF, TA and TAC

The correlation coefficients (R^2) for spectrophotometric assays are shown in Table 3. Among all four TAC assays, the highest correlation was demonstrated between DPPH and FRAP ($R^2 = 0.9901$), followed by ABTS and FRAP ($R^2 = 0.9663$), and ABTS and DPPH ($R^2 = 0.9629$). This is quite unexpected since the capacity measured by FRAP or CUPRAC method does not always correlate well with that for radical scavenging [27]. TF and TA showed a low correlation ($R^2 = 0.3787$) indicating that the flavonoid content of the investigated samples is not limited to anthocyanins. On the other hand, either TF or TP and TAC (especially in case of CUPRAC) of samples showed a linear relationship with high correlation coefficients. These results imply that phenolics and flavonoids were the major contributors to the antioxidant capacity of the examined black mulberry products. High positive correlation between TP and TAC in the black mulberry was also confirmed previously [1].

3.7. Major phenolic compounds

The major phenolic compounds present in the black mulberry products are reported in Tables 4, 5 and 6. Up to 12 phenolic compounds were detected in analyzed samples, belonging to three groups of phenolics including

Table 4
Phenolic acid contents of selected black mulberry (*Morus nigra* L.) products

Sample	Chlorogenic acid (mg/100 g dw)	Gallic acid (mg/100 g dw)	Syringic acid (mg/100 g dw)	Neochlorogenic acid (mg/100 g dw)	Caffeic acid (mg/100 g dw)
Fresh mulberry	144.5 ± 9.5a	12.0 ± 0.5d	10.4 ± 0.4a	8.1 ± 0.3a	3.0 ± 0.2a
Dried mulberry	46.7 ± 4.1c	2.1 ± 0.1f	2.1 ± 0.0c	2.2 ± 0.1c	0.3 ± 0.0d
Mulberry wine	85.6 ± 0.7b	19.5 ± 0.3c	nd	4.2 ± 0.1b	0.7 ± 0.0b
Mulberry molasses	14.1 ± 0.3d	10.3 ± 0.2e	0.7 ± 0.0d	2.3 ± 0.2c	nd
Mulberry ice cream	36.3 ± 0.7c	1.6 ± 0.0f	nd	5.2 ± 0.4b	0.8 ± 0.1b
Mulberry juice	83.7 ± 13.9b	22.0 ± 0.5b	2.6 ± 0.4b	7.3 ± 1.4a	0.5 ± 0.0c
Mulberry jam	6.4 ± 0.2de	28.5 ± 0.9a	2.0 ± 0.1c	2.4 ± 0.0c	0.2 ± 0.1d
Mulberry syrup	0.3 ± 0.0e	Nd	nd	0.1 ± 0.0d	0.1 ± 0.0de

Data represent average quantities ± standard deviation (determined by HPLC-PDA at 280 and 312 nm) of three independent samples. All contents are expressed per 100 g dry weight (dw). Different letters in the columns represent statistically significant differences ($p < 0.05$). nd, not detected.

Table 5
Flavonol contents of selected black mulberry (*Morus nigra* L.) products

Sample	Rutin (mg/100 g dw)	Quercetin-3-*O*-glucoside (mg/100 g dw)	Quercetin derivative (mg/100 g dw)
Fresh mulberry	206.2 ± 4.9a	9.8 ± 0.7a	15.1 ± 0.7a
Dried mulberry	66.0 ± 2.2b	7.6 ± 0.5b	0.9 ± 0.1cd
Mulberry wine	27.2 ± 1.9b	7.5 ± 0.5b	2.4 ± 0.5b
Mulberry molasses	nd	nd	nd
Mulberry ice cream	10.1 ± 0.5d	1.2 ± 0.0d	0.6 ± 0.0cde
Mulberry juice	9.9 ± 0.7d	3.5 ± 0.5c	1.2 ± 0.0c
Mulberry jam	11.1 ± 0.4d	1.9 ± 0.1d	0.2 ± 0.0de
Mulberry syrup	0.6 ± 0.0e	0.2 ± 0.0e	nd

Data represent average quantities ± standard deviation (determined by HPLC-PDA at 360 nm) of three independent samples. All contents are expressed per 100 g dry weight (dw). Different letters in the columns represent statistically significant differences ($p < 0.05$). nd, not detected.

Table 6
Anthocyanin contents of selected black mulberry (*Morus nigra* L.) products

Sample	Cyanidin-3-*O*-glucoside (mg/100 g dw)	Cyanidin-3-*O*-rutinoside (mg/100 g dw)	Pelargonidin-3-*O*-glucoside (mg/100 g dw)	Pelargonidin-3-*O*-rutinoside (mg/100 g dw)
Fresh mulberry	704.1 ± 20.4a	572.1 ± 22.5a	27.8 ± 2.1a	1.4 ± 0.2b
Dried mulberry	12.6 ± 1.8c	5.4 ± 0.6c	0.7 ± 0.1c	nd
Mulberry wine	8.8 ± 1.3c	4.5 ± 0.4c	nd	nd
Mulberry molasses	0.1 ± 0.0c	nd	nd	nd
Mulberry ice cream	122.3 ± 4.8b	77.6 ± 2.5b	14.5 ± 0.1b	1.8 ± 0.3a
Mulberry juice	2.2 ± 0.6c	3.9 ± 0.7c	0.5 ± 0.1c	nd
Mulberry jam	0.1 ± 0.0c	nd	nd	nd
Mulberry syrup	0.0 ± 0.0c	nd	nd	nd

Data represent average quantities ± standard deviation (determined by HPLC-PDA at 520 nm) of three independent samples. All contents are expressed per 100 g dry weight (dw). Different letters in the columns represent statistically significant differences ($p < 0.05$). nd, not detected.

phenolic acids, flavonols and anthocyanins. In our samples, chlorogenic acid, rutin and cyanidin-3-*O*-glucoside were confirmed as the major phenolic acid, flavonol and anthocyanin, respectively.

From the group of phenolic acids, chlorogenic, gallic, syringic, neochlorogenic and caffeic acids were determined (Table 4). Results showed that besides gallic acid content, fresh mulberry was significantly the richest sample in phenolic acids, whereas in terms of gallic acid content mulberry jam was found to be the highest ($p < 0.05$). On the other hand, mulberry syrup showed the lowest phenolic acid content ($p < 0.05$); in fact gallic and syringic acids were not detected in mulberry syrup. The presence of neochlorogenic acid in fresh mulberry was also confirmed by Pawlowska et al. [11] Furthermore, the amounts of chlorogenic, gallic, syringic and caffeic acids in this study were quite lower than the fresh mulberry results stated by Gundogdu et al. [14]. Next to the phenolic acids determined in our study, Zadernowski et al. [28] also identified gentisic, protocatechuic, salicylic, vanilic, *m*-coumaric, *o*-coumaric, *p*-coumaric, 3, 4-dimethoxycinnamic, ferulic and hydroxycaffeic acids in fresh mulberry.

The following flavonol compounds were determined: rutin, quercetin-3-*O*-glucoside (Q3G) and a quercetin derivative (calculated in terms of Q3G) (Table 5). Among the 8 extracts, fresh mulberry showed significantly the highest flavonol content ($p < 0.05$), while mulberry molasses contained none of the flavonols that are identified in this study. For fresh mulberry, lower levels of rutin and Q3G have been detected compared to those reported previously [11, 14]. In addition to the flavonols that we have identified in our study, presence of kaempferol-3-*O*-rutinoside has also been reported [11].

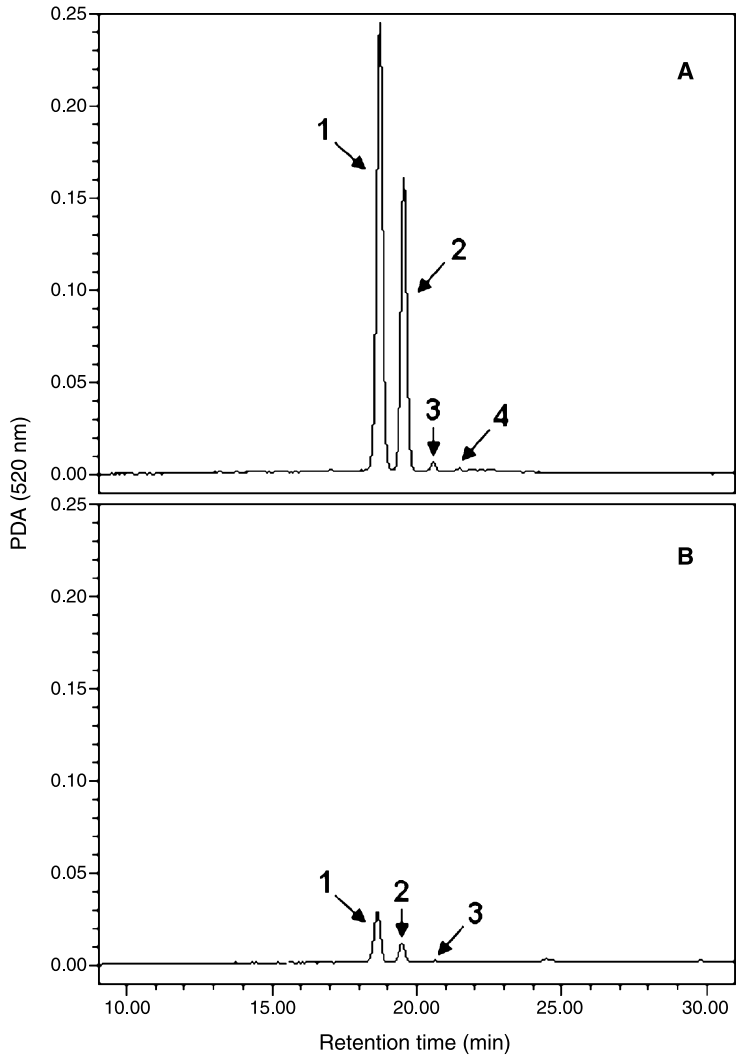

Fig. 2. HPLC chromatograms (PDA, recorded at 520 nm) of extracts of (A) fresh mulberry and (B) dried mulberry. Numbers refer to the major anthocyanins identified: 1, cyanidin-3-*O*-glucoside (C3G); 2, cyanidin-3-*O*-rutinoside (C3R); 3, pelargonidin-3-*O*-glucoside (P3G); 4, pelargonidin-3-*O*-rutinoside (P3R).

Cyanidin-3-*O*-glucoside (C3G), cyanidin-3-*O*-rutinoside (C3R), pelargonidin-3-*O*-glucoside (P3G) and pelargonidin-3-*O*-rutinoside (P3R) were identified from the group of anthocyanins (Table 6). Only fresh mulberry and mulberry ice cream was found to contain all these four anthocyanins. Moreover, fresh mulberry was significantly the richest sample in anthocyanins ($p < 0.05$), while the anthocyanin content in other products were quite low. In particular, Fig. 2 shows the anthocyanins identified in extracts from fresh and dried mulberry. As shown in the figure, ~40–105 fold lower amounts of C3G, C3R and P3G were determined in dried mulberry compared to fresh mulberry and in fact P3R was not even detected. In previous studies, the main anthocyanins of black mulberry have also been identified as C3G and C3R (Fig. 1) [11, 26, 29–32]. However, in this study, the amount of C3G determined for fresh mulberry was found to be lower than those stated by Ogawa et al. [31] and Pawlowska et al. [11], whereas higher results were reported here for C3R compared to Perez-Gregorio et al. [32]. Besides the anthocyanins identified in this study, some other anthocyanins have been reported in fresh mulberry including cyanidin-3-*O*-sophoroside [13, 29] and cyanidin-3-*O*-halavtoside [31].

4. Discussion

Epidemiological studies indicate that diets rich in fruit and vegetables are associated with a lower risk of several degenerative diseases. However, the health-promoting capacity of fruit and vegetables strictly depends on their processing history. Although the products examined in this study were not produced from the same raw material, still the obtained results give some ideas concerning the effect of processing. There are several studies on the antioxidant capacity or polyphenol composition of fresh black mulberry [1, 6, 8, 11–14, 24–26, 28, 29, 31] as well as black mulberry juice [9, 33] and black mulberry wine [34]; however, so far no studies have examined the antioxidant activity of other black mulberry products reported in this study. Overall the results demonstrated the complexity of the biochemical composition of black mulberry, how this may be modified upon fruit processing and the potential relevance of these changes to fruit quality.

Considering the TP results, despite the fact that the examined fresh mulberry was lower than those reported in the literature, still it had high phenolic content, comparable to some other berries including blueberry [35] and raspberry [36]. However, Folin–Ciocalteu method was not able to reflect the amount of phenolics in black mulberry products when compared to the HPLC results, hence, is not recommended for determining phenolic compounds as a single method. Due to the lack of specificity of this method for phenolic compounds, the presence of other non-phenolic reducing compounds may affect the accuracy of the assay. Therefore, to obtain more accurate results, HPLC analysis of individual phenolic compounds should also be performed as was done in this study.

Polyphenols act as antioxidants by scavenging free radicals or limiting their formation [27, 37]. Many antioxidant capacity assays have been developed, but only a few are rapid and reliable methods. Nevertheless, the measurement of antioxidant activities, in the case of multifunctional or complex multiphase systems cannot be evaluated satisfactorily by a single method. The principles of the methods vary greatly depending on the radical that is generated or the time of reaction. Even ABTS and DPPH methods which are based on the same principle can show several important differences in their response to antioxidants. Therefore, it is highly recommended to apply different methods evaluating the antioxidant activities of food materials to obtain the full picture [38, 39]. All antioxidant assays applied in this study have been widely used to determine the antioxidant capacities of fruits and vegetables as they require relatively standard equipment and deliver fast and reproducible results.

HPLC analysis of individual phenolic compounds allowed determination of the detailed alteration of each compound as a result of processing. The highest loss was observed in case of anthocyanins. Previously, exposure of heat and light to the stability of mulberry (*Morus alba* L.) anthocyanins was evaluated [40] and in agreement with the findings in this study, anthocyanin content decreased significantly after heat or light exposure. Similarly, in another study, alcoholic fermentation was found to decrease the anthocyanin levels in mulberry, while the antioxidant activity was not affected [31]. In our study, we also encountered similar findings, as mulberry wine was found to contain the highest antioxidant capacity, following fresh mulberry. On the other hand, alcohol generated during fermentation may also contribute to antioxidant activity by providing a better extractability of the phenolic compounds.

In conclusion, commercialization of black mulberry as a fresh fruit is not possible due to its short shelf life and the difficulties in harvesting. Moreover, consumers may prefer black mulberry products for their antioxidant potential since their nutritional characteristics are preserved to certain degree after processing. In further studies, it would be interesting to focus on the bioavailability of these black mulberry products.

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