Chapter 14

Impact of Organic Fertilizers on Phenolic Profiles and Fatty Acids Composition: A Case Study for *Cichorium intybus* L.

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Additional information is available at the end of the chapter

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Abstract

Radicchio (*Cichorium intybus* L.) is an increasingly appreciated leafy vegetable that exhibits great diversity in appearance, including different colored leaves, rosettes, or heads. Varieties of radicchio ('Treviso', 'Verona' 'Anivip', 'Castelfranco', and 'Monivip') commonly produced in Slovenia were investigated for their phenolic and fatty acid profiles. Plants were grown under organic and/or mineral fertilizer managements in greenhouse conditions. High-performance liquid chromatography analysis was used to study phenolic compounds in radicchio leaf samples. Thirty-three phenolic compounds were quantitatively evaluated. Significant differences were found between varieties and across different fertilizer managements. The total phenolic amount (TPA) was found in a wide range from 58 to 403 mg/100 g fresh weight (FW). Between varieties, the highest TPA was observed for var. 'Treviso' (300 mg/100 g FW) and the lowest TPA was observed for var. 'Castelfranco' (125 mg/100 g FW). The main phenolic compounds in radicchio leaves were represented by phenolic acids, chlorogenic acid and cichoric acid, respectively. The fatty acid levels of radicchio leaf samples were determined by the chromatographic analysis of fatty acid methyl esters using gas chromatography with flame ionization detector. The analysis revealed the amounts of C16:0, C18:0, C18:1n9, C18:2n6, C18:3n3, and C20:0 fatty acids. The total fatty acid levels varied from 170 to 500 mg/100 g FW. The highest fatty acid quantity was represented by C18:3n3 (≤63%) followed by C18:2n6 (≤45%) and C16:0 (≤24%). All radicchio samples had a ratio of *n*-6/*n*-3 essential fatty acids below 1 and thus in accordance with the current dietary guidelines. Among different fertilizer managements, the highest total fatty acid levels were found for organic fertilizer (384 mg/100 g FW).

Keywords: fatty acids, fertilizers, GC-FID, HPLC-DAD, phenolic compounds, radicchio

1. Introduction

Radicchio (*Cichorium intybus* L.; Asteraceae) is a popular salad vegetable in the Mediterranean region, and its usage is increasing in Europe. Other cultivated types of this species are Italian chicory, French endive, witloof, sugarloaf, and succory. It has been known since 1616, when it was first mentioned in Germany. Cultivation began in England in 1886 and later in 1926 also France. There is a discussion about whether radicchio should be classified as a root or a leafy vegetable crop. It can be produced for leaves, rosettes, or heads with a wide range of colors [1]. Radicchio is typically consumed as a raw vegetable in various fresh, mixed, or garnished salads [2,3]. Its popularity among consumers and its nutritional characteristics have great potential for growth in the local markets as well as in the international ones. Most radicchio varieties thrive best during cooler, moist weather and do not tolerate high temperature. Radicchio is a leafy vegetable that can withstand low temperatures, which gives it an advantage for consumption in the winter time of the year when the supply of fresh leafy vegetable in the market is limited [4]. In addition, radicchio represents a plant with several medicinal properties and effects [5].

Vegetable production in many countries depends on high-input systems to maximize yield and product quality, while they try to achieve low production costs, which keep local products competitive in international markets [6]. Conventional high-input farming system is often associated with problems, such as nitrate leaching and ground water pollution, degradation of soil structure, and pesticide contamination [7–10]. The answers to problems associated with conventional practices are alternative cropping systems. Over the past decade, criteria have been developed, which define organic crop production requirements [11]. Now, there exist several national systems of designated requirements to have vegetable products marketed as » organically produced«. In fresh vegetable market, organically grown products of reasonable quality are readily available, but their price is usually much higher compared to those grown by the other than organic manner [12,13].

The polyphenol compositions of vegetable depend on several factors. It is influenced by genetic as well as environmental factors, such as temperature, light, moisture, and the nutritional status of the soil in which the vegetable is grown [14,15]. It is also influenced by the growing manner, phase of maturity, postharvest managements, and storage conditions. Moreover, many vegetables are processed before they are used for consumption. Processing methods, such as cooking and canning, can also influence the polyphenol composition of the vegetable. Regular consumption of vegetables is proven to be associated with lower risks of various types of modern diseases, such as chronic or cardiovascular diseases [7,16].

Polyphenols are organic compounds widely distributed in vegetables. All phenolic compounds have an aromatic ring that contains various attached substituent groups, such as hydroxyl, carboxyl, and methyl groups, and often other nonaromatic ring structures. Phenolics differ from lipids in higher solubility in water and lower solubility in nonpolar organic solvents. These properties greatly aid in the separation of phenolics from one another and from other compounds. Many phenolics arise from the shikimic acid pathway and its subsequent reactions. Among these are cinnamic, *p*-coumaric, caffeic, ferulic, chlorogenic, protocatechuic, and gallic phenolic acids. They are important not because they are abundant in uncombined (free) form but because they are converted into several derivatives besides proteins. These derivatives include phytoalexins, coumarins, lignin, and various flavonoids, such as the anthocyanins [17]. Chlorogenic acid is widely distributed in various parts of many plants and usually occurs in easily detectable quantities. Both chlorogenic and protocatechuic acids have special functions in disease resistance of plants. Gallic acid is important because of its conversion to gallotannins, which are heterogeneous polymers containing numerous gallic acid molecules connected in various ways to one another, to glucose, and to other sugars [18].

Of the various classes of naturally occurring compounds based on the flavonoid skeleton, flavone and flavonols are collectively the most abundant group. The distinction between flavones and flavonols, which are 15 carbon compounds, is an arbitrary one, as flavonols are simply a class of flavone in which the 3-position is substituted by a hydroxyl group [19]. Anthocyanins are present as glycosides, usually containing one or two glucose or galactose units attached to the hydroxyl group in the central ring or to that hydroxyl group at the 5-position of the A ring. When the sugars are removed, the remaining parts of the molecules, which are still colored, are called anthocyanidins. Anthocyanins are soluble and reasonably stable, whereas anthocyanidins produced on acid hydrolysis are insoluble in water, unstable to light, and rapidly destroyed by alkali [20]. Flavones and flavonols are easier to identify than anthocyanins because they are more stable [21]. Several polyphenols, such as derivatives of hydroxycinnamic acids (HCA), flavonoids, and anthocyanins [4,6,22–27], previously determined in radicchio leaves are presented in **Table 1**.

Phenolic acids	Flavonoids	Anthocyanins
gallic acid	luteolin 7-O-glucuronide	cyanidin 3-O-glucoside
protocatechuic acid	apigenin	cyanidin 3-O-rutinooside
caftaric acid (caffeoyl tartaric acid)	apigenin glucuronide	pelargonidin 3-O-glucoside
chlorogenic acid	apigenin 7-O-arabinoside	peonidin 3-O-glucoside
caffeic acid	quercetin 3-O-glucuronide	malvidin 3-O-glucoside
cichoric acid (dicaffeoyl tartaric acid)	quercetin 3-O-galactoside	cyanidin 3-malonylglucoside
	quercetin 3-O-rhamnoside	delphinidin 3-O-(6″ malonyl)-glucoside
	quercetin malonyl glucoside	cyanidin 3-O-(6″ malonyl)-glucoside
	kaempferol 3-O-glucoside	pelargonidin
	kaempferol 3-O-glucuronide	peonidin
	methyl quercetin glucuronide	malvidin
	kaempferol malonyl glucoside	
	methyl quercetin glucoside	
	isorhamnetin 3-O-glucuronide	
	isorhamnetin 7-O-glucuronide	

Table 1. Phenolic compounds reported in radicchio leaves from scientific data.

Lipids are derived from long-chain fatty acids and alcohols or closely related derivatives. They are water-insoluble components of cells that can be extracted by nonpolar solvents. In various parts of the plants, mostly in the cell membranes, are small amounts of lipids (~2%). In higher plants, the predominant fatty acid residues consist of palmitic, oleic, linoleic, and stearic acid.

Fatty acids with <12 and >20 carbon atoms are less common in nature [28]. The most common fatty acids in plants are those containing 16 or 18 carbon atoms. These include saturated palmitic (C16:0) and stearic (C18:0) acids, monounsaturated oleic acid (C18:1n9), polyunsaturated linoleic acid with two double bonds (C18:2n6), and linolenic acid with three double bonds (C18:3n3) [29]. When the carbon atoms in the hydrocarbon chain of a fatty acid hold their full complement of hydrogen, they are described as saturated. Where two adjoining carbon atoms in the hydrocarbon chain of a fatty acid each lack a hydrogen atom, a double bond forms between them. The fatty acid is then said to be unsaturated. The term polyunsaturated fatty acid (PUFA) is accepted as referring to those fatty acids that contain two or more carbon-carbon double bonds within the hydrocarbon chain [30]. Particular PUFAs, which the human system can employ as building blocks while being unable to synthesize them, have been classed as essential fatty acids. The *n*-3 (ω-3, omega-3) PUFAs found in plants refer to a number of health benefits [31]. The most common and most important PUFA is linolenic acid, which is known as a precursor of the long-chain fatty acids (eicosapentaenoic and docosahexaenoic) [32]. Modern agriculture and food industrialization are associated with large changes in the structure of contemporary Western diets. The intake of *n*-6 fatty acids has enlarged during evolution, and the intake of *n*-3 fatty acids has been reduced. Consequently, the *n*-6/*n*-3 ratio increased from 1 to 10 or, in some places, even up to 20 or even 25. These differences in food consumption led to increased risk of numerous modern diseases [33].

Over the past decade, radicchio has become popular for cultivation and consumption in different regions of the world. Scientific literature has revealed that radicchio plants contain important compounds with biological activity and several vitamins and minerals [4,18,34–36]. The effects of fertilizer managements (organic, mineral) on the phenolic and fatty acid profiles in different radicchio varieties (red, red-spotted, green) are poorly discussed in scientific data. This chapter discusses the effect of fertilizers (organic, mineral, and combination) on the total phenolics, the main phenolic classes, and the fatty acids levels of five *C. intybus* varieties. High-performance liquid chromatography (HPLC) was used for the analysis of phenolic compounds and their classes and gas chromatography (GC) was used for the determination of fatty acid levels.

2. Materials and analytical methods

2.1. Selection of plant material and fertilization experiment

The experiment was carried out in 2012 under the controlled conditions of the central research greenhouse at Biotechnical Faculty (46°04′N, 14°31′W; 320 m a.s.l.). The commercial radicchio varieties were included in our research: red (‘Treviso’, ‘Verona’, and ‘Anivip’), red-spotted

('Castelfranco'), and green ('Monivip'). Photos of individual radicchio variety are shown in **Figures 1** to **3**.

Figure 1. Var. 'Anivip' (left) and var. 'Monivip' (right). Photo: D. Žnidarčič.

Figure 2. Var. 'Treviso' (left) and var. 'Castelfranco' (right). Photo: D. Žnidarčič.

Figure 3. Var. 'Verona.' Photo: D. Žnidarčič.

Fertilizer treatment	Fertilizer name	N/P/K	Application details	Mark
Unfertilized	/	/	Watering	CONT
Single basal organic	Plantella Organik	3/3/2	67.5 g/7 L soil	ORG1
Single basal organic	Stallatico Pallettato	3/3/3	45 g/7 L soil	ORG2
Water soluble mineral	Kristalon Blue	19/6/20	Irrigation with 9 g/100 L	MIN1
Single basal mineral	Entec perfect	14/7/17	7.9 g/7 L soil	MIN2
Combination of organic and mineral fertilizer	Plantella Organik + Kristalon Blue	3/3/2 + 19/6/20	Plantella Organic 3.5 g /7 L soil + after 1 month irrigation with 3.5 g/L Kristalon Blue	ORG1+MIN1

Table 2. Fertilizer managements used to set up the pot experiment.

The growing experiment in controlled conditions included two mineral fertilizers, two organic fertilizers, a combination of one organic and one mineral fertilizer, and the control (no added fertilizer). In each of the five radicchio varieties, the same six fertilizer managements were applied as presented in **Table 2** in the following design: unfertilized control (CONT), two organic fertilizers (ORG1 and ORG2), two mineral fertilizers (MIN1 and MIN2), and combination of organic and mineral fertilizer (ORG1+MIN1). The experiment consisted of 30 plastic pots filled up with 7 L of soil with application of the selected fertilizers. Sowing was performed on 30 January 2012. Then, the pots were placed in the greenhouse and irrigated appropriately. Water-soluble mineral fertilizer (MIN1) was applied through the irrigation solution containing water and MIN1. The sampling of developed leaves was performed on 10 June 2012. A few leaves from each pot were lyophilized and powdered using a ball mill before analysis. The dry matter content of radicchio leaves varied from 6.8% to 14.8%.

2.2. Extraction and identification of phenolic compounds

Radicchio powder was mixed with the solvent 5% formic acid in methanol, which contained flavone as an internal standard. For extraction, an ultrasonic bath at 4°C for 30 min was used. After centrifugation, a 10 μL aliquot of supernatant was injected into the HPLC system. For analysis, reverse-phase HPLC coupled with a diode array detector (DAD) was used. The phenolic compounds were separated on Nucleosil C18 analytical column (250 cm × 4 mm; 3 μm) and eluted using 5% formic acid and HPLC-grade methanol at a constant flow rate. The gradient profile has been flowing to the protocol previously published for the analysis of complex polyphenol mixtures [37].

The DAD was scanning from 250 to 600 nm with four discrete channels. Phenolics were gathered into five classes and monitored at related wavelengths: unknown phenolic compounds (UPCs; 280 nm), HCAs and flavones (320 nm), flavonols (350 nm), and anthocyanins (540 nm). The quantification of each phenolic compound was carried out using the internal standard manner. The phenolic compounds in the radicchio leaves separated by HPLC are presented in **Table 3**. They were classified based on the absorbance spectra [38] and the comparison to representatives [39]. Chlorogenic and caftaric acids were confirmed by previously identified standards [40].

Compound name/acronym	Peak no.	R_t (min)	UVλ_{max}(nm)	Phenolic class
HCA 1	1	18.2	318, 322	Monomeric hydroxycinnamic acid
Caftaric acid (caffeoyl tartaric acid)	2	19.3	330	Monomeric hydroxycinnamic acid
Benzoic acid derivative (protocatechuic acid)?	3	28.3	286, 290, 334, 338	Flavone
HCA 2	4	35.8	322	Monomeric hydroxycinnamic acid
HCA 3	5	41.7	330	Monomeric hydroxycinnamic acid
UPC 1	6	42.6	262	Unknown phenolic compound
Chlorogenic acid	7	43.3	326	Monomeric hydroxycinnamic acid
HCA 4	8	53.2	326	Monomeric hydroxycinnamic acid
Gallic acid derivative 1	9	61.4	262, 266	Unknown phenolic compound
Gallic acid derivative 2	10	65.5	262	Unknown phenolic compound
HCA 5	11	66.2	310	Monomeric hydroxycinnamic acid
UPC 2	12	75.7	262	Unknown phenolic compound

Compound name/acronym	Peak no.	R_t (min)	UVλ_{max}(nm)	Phenolic class
HCA 5	13	84.9	326	Monomeric hydroxycinnamic acid
Cichoric acid (dicaffeoyl tartaric acid)	14	100.5	330	Oligomeric hydroxycinnamic acid
HCA 6	15	104.2	330	Oligomeric hydroxycinnamic acid
HCA 7	16	112.1	330	Oligomeric hydroxycinnamic acid
HCA 8	17	114.4	322	Oligomeric hydroxycinnamic acid
UPC 3	18	115.1	262, 266	Unknown phenolic compound
Gallic acid derivative 3	19	126.5	262	Unknown phenolic compound
HCA 9	20	131.5	326	Oligomeric hydroxycinnamic acid
Kaempferol or quercetin derivative 1	21	140	262, 346, 350	Flavonol
Kaempferol or quercetin derivative 2	22	141.7	262, 346	Flavonol
Kaempferol or quercetin derivative 3	23	146.3	262, 346, 350, 354	Flavonol
ANTHO 1	24*	147.1	278, 518, 522	Anthocyanin
HCA 10	25	149	318, 326	Oligomeric hydroxycinnamic acid
Apigenin or luteolin derivative	26	149.5	262, 338	Flavone
UPC 4	27	149.6	262, 266	Unknown phenolic compound
UPC 5	28	155.2	262	Unknown phenolic compound
UPC 6	29	159	262, 266	Unknown phenolic compound
FLAVONOL 1	30	159.5	262, 346	Flavonol
FLAVONOL 2	31	160.3	262, 342, 346	Flavonol
Gallic acid derivative 4	32	161	262	Unknown phenolic compound
FLAVONOL 3	33	164.1	262, 266, 342, 346	Flavonol

Not detected in var. 'Anivip', 'Castelfranco', and 'Monivip'.

Table 3. Phenolic compounds in the radicchio leaves separated by HPLC.

2.3. Determination of fatty acid levels

Fatty acid levels were analyzed using GC with prior prepared fatty acid methyl esters. In the protocol [41], NaOH and BF_3 in methanol were used for transesterification and heptadecanoic acid (C17:0) was used as an internal standard for the quantification of fatty acids. The solution of fatty acid methyl esters was quantified on the GC (Agilent 6890N, USA) with flame ionization detector (FID). At the constant flow rate, the separation was performed on a column for analyses of PUFAs as fatty acid methyl esters. The identification and quantification of fatty

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acids were carried out using a reference standard mixture of methyl esters of greater fatty acids regularly before the samples. The following fatty acids were detected in the radicchio plants: C16:0, C18:0, C18:1n9, C18:2n6, C18:3n3, and C20:0 (**Table 4**).

3. Results and discussion

3.1. Phenolic profiles

Thirty-three main phenolic compounds obtained using HPLC detection were selected in all five studied radicchio varieties from six fertilizer managements. Those were grouped according to their absorbance spectra and retention times to UPCs, HCAs, flavonols, flavones, and anthocyanins (**Table 4**). All chromatograms of radicchio samples were similar, but the areas of individual peaks varied considerably. An example of chromatogram for var. 'Castelfranco' is presented in **Figure 4**. Anthocyanins, which are quite unstable, were found in minor quantities in only few radicchio samples.

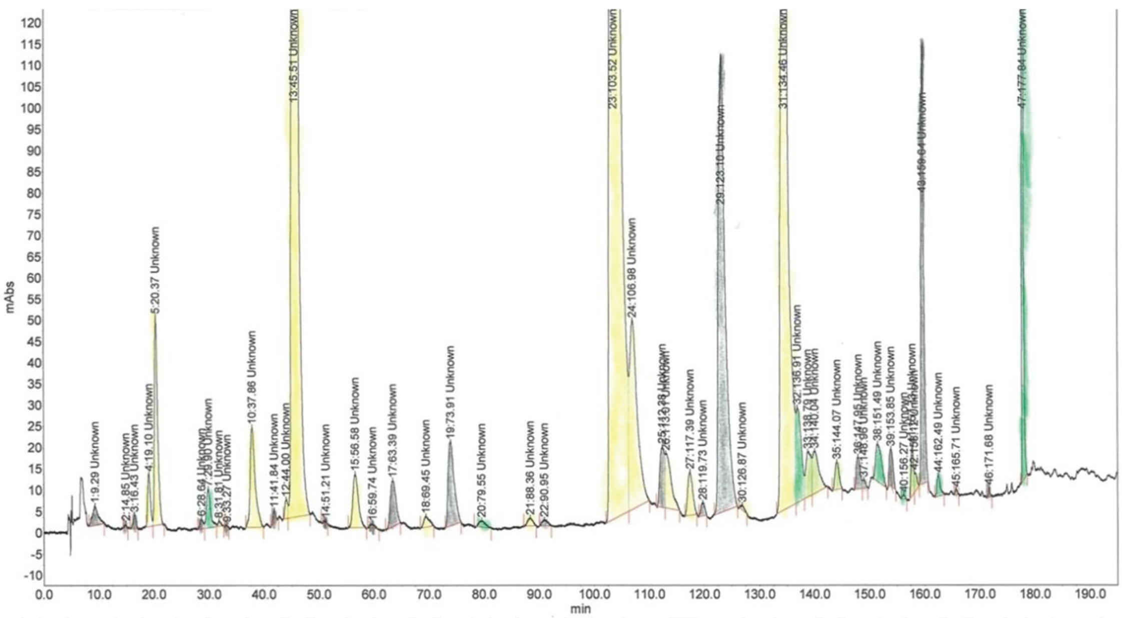

Figure 4. Chromatogram of var. 'Castelfranco' from unfertilized management obtained using HPLC analysis.

The phenolic profile data were comparable to former reports, which also found that chlorogenic and cichoric acids are the main phenolic compounds in radicchio leaves [4,6,26,42]. The total phenolic amount (TPA) in the analyzed radicchio leaves under different fertilizer managements varied from 58 to 403 mg/100 fresh weight (FW; **Figure 5**). The results showed large differences between the varieties as well when comparing different fertilizer managements. The average levels over all different fertilizer managements for individual variety showed significantly greater TPA for var. 'Treviso' (300 mg/100 g FW) followed by var. 'Verona' (181 mg/100 g FW), var. 'Monivip' (146 mg/100 g FW), var. 'Anivip' (135 mg/100 g

FW), and var. 'Castelfranco' (125 mg/100 g FW). The red colored var. 'Treviso' showed two times greater TPA in comparison to red-spotted or green radicchio varieties. A high TPA for var. 'Treviso' was reported by D'evoli et al. [34].

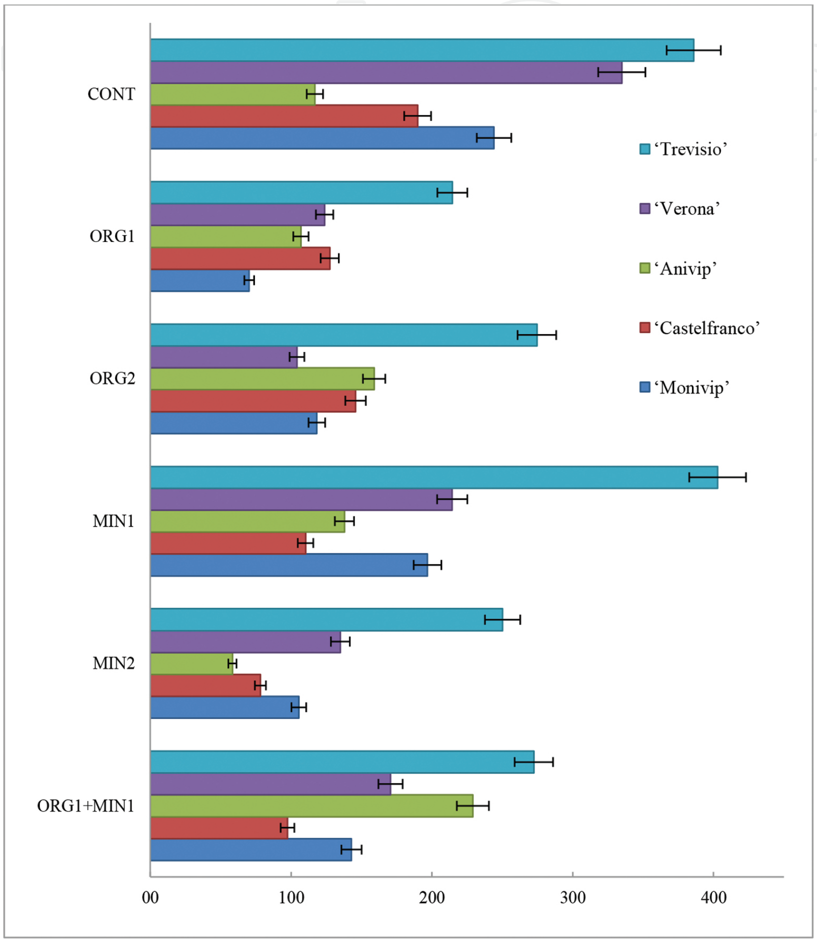

Figure 5. TPA (as mg/100 g FW) in the radicchio leaves among varieties and different fertilizer managements.

Across different managements, the highest TPA was seen for unfertilized (CONT) treatment (254 mg/100 g FW) followed by MIN1 (213 mg/100 g FW), combination of ORG1+MIN1 (183 mg/100 g FW), ORG2 (160 mg/100 g FW), ORG1 (129 mg/100 g FW), and MIN2 (126 mg/100 g FW). Significantly greater TPAs were seen for the radicchio varieties grown under unfertilized management and those with mineral fertilizer (MIN1). Crecente-Campo et al. [43] have reported that the organic or conventional cultivation system did not affect the TPA but only the antioxidant compounds. Vinha et al. [10] found greater TPA for organically grown vegetables, whereas Mitchell et al. [8] obtained only greater amounts for quercetin and kaempferol. According to Oliveira et al. [9], organic manner resulted in greater TPA and vitamin C. Some other studies [44,45] reported that the enzyme phenylalanine ammonia-lyase is involved in the biosynthesis of phenolics and is regulated by nitrogen. In general, the

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availability of soil nitrogen strongly impacts the synthesis of several phenolic compounds [46]. In relation to nitrogen fertilization, the response of radicchio varieties differs, as high and low nitrogen demanding varieties were previously reported [47].

Class	Quantity (mg/100 g fresh weight)					
	No fertilizer	Organic fertilizer		Mineral fertilizer		Combination
	CONT	ORG1	ORG2	MIN1	MIN2	ORG1+MIN1
Hydroxycinnamic acids						
Treviso	345.91 ±17.30 aA	195.67 ±9.78 dA	257.97 ±12.90 bA	361.64 ±18.08 aA	231.91 ±11.60 cA	232.87 ±11.64 cA
Verona	268.73 ±13.44 aB	100.08 ±5.00 dB	82.71 ±4.14 eD	176.76 ±8.84 bB	107.98 ±5.40 dB	131.80 ±6.59 cC
Anivip	100.98 ±5.05 cdE	94.18 ±4.71 dB	134.17 ±6.71 bB	108.03 ±5.40 cC	46.89 ±2.34 eE	190.28 ±9.51 aB
Castelfranco	159.81 ±7.99 aD	104.21 ±5.21 cB	115.28 ±5.76 bC	93.91 ±4.70 dC	60.74 ±3.04 eD	88.12 ±4.41 dE
Monivip	207.62 ±10.38 aC	59.02 ±2.95 eC	86.89 ±4.34 dD	165.82 ±8.29 bB	81.74 ±4.09 dC	109.76 ±5.49 cD
Flavonols						
Treviso	10.05 ±0.50 cB	4.38 ±0.22 eB	10.10 ±0.50 cB	19.95 ±1.00 aA	7.88 ±0.39 dA	11.85 ±0.59 bB
Verona	13.03 ±0.65 aA	3.14 ±0.16 dC	3.73 ±0.19 cdD	11.64 ±0.58 bB	4.33 ±0.22 cC	13.74 ±0.69 aA
Anivip	5.19 ±0.26 dE	2.89 ±0.14 eC	6.31 ±0.32 cC	7.89 ±0.39 aC	5.42 ±0.27 dB	6.87 ±0.34 bC
Castelfranco	6.53 ±0.33 bD	4.11 ±0.21 cB	11.68 ±0.58 aA	3.94 ±0.20 cD	4.26 ±0.21 cC	1.72 ±0.09 dE
Monivip	7.85 ±0.39 aC	5.23 ±0.26 cA	2.80 ±0.14 eE	7.00 ±0.35 bC	4.37 ±0.22 dC	5.73 ±0.29 cD
Flavones						
Treviso	1.33 ±0.07 aB	0.28 ±0.01 eB	0.69 ±0.03 dC	1.22 ±0.06 bA	1.06 ±0.05 cA	1.08 ±0.05 cB
Verona	1.89 ±0.09 aA	0.29 ±0.01 eB	0.81 ±0.04 cB	1.20 ±0.06 bA	0.60 ±0.03 dC	1.15 ±0.06 bB
Anivip	0.96 ±0.05 bC	0.15 ±0.01 eC	0.61 ±0.03 cD	1.07 ±0.05 aB	1.06 ±0.05 aA	0.40 ±0.02 dC
Castelfranco	0.97 ±0.05 bC	0.32 ±0.02 eB	1.17 ±0.06 aA	0.54 ±0.03 cC	0.50 ±0.02 cD	0.40 ±0.02 dC
Monivip	1.35 ±0.07 aB	0.73 ±0.04 bA	0.44 ±0.02 dE	0.62 ±0.03 cC	0.78 ±0.04 bB	1.36 ±0.07 aA
Unknown phenolic compounds						
Treviso	28.63 ±1.43 aB	14.18 ±0.71 dC	4.14 ±0.21 fD	19.07 ±0.95 cB	8.86 ±0.44 eD	26.67 ±1.33 bB
Verona	48.53 ±2.43 aA	20.27 ±1.01 cA	15.64 ±0.78 dC	23.68 ±1.18 bA	21.83 ±1.09 bcA	22.90 ±1.15 bC
Anivip	9.77 ±0.49 dD	9.75 ±0.49 dD	17.91 ±0.90 cB	20.90 ±1.04 bB	5.00 ±0.25 eE	31.51 ±1.58 aA
Castelfranco	22.59 ±1.13 aC	18.81 ±0.94 bB	17.67 ±0.88 bB	11.90 ±0.59 cC	12.76 ±0.64 cC	7.19 ±0.36 dD
Monivip	27.27 ±1.36 aB	5.30 ±0.27 dE	28.13 ±1.41 aA	23.40 ±1.17 bA	18.62 ±0.93 cB	26.03 ±1.30 aB

The average values (n=3) with different lowercase letters in a row are significantly different (P<0.001; differences between the fertilizers), and those with different uppercase letters in a column are significantly different (P<0.001; differences between the varieties).

Table 4. Phenolic classes in the leaves of radicchio varieties derived from different fertilizer managements.

The main classes of phenolic compounds (as mg/100 g FW) among radicchio varieties from different fertilizer managements are presented in **Table 4**. Statistical analysis showed significant differences between both fertilizer managements and the varieties for all of these main classes. HCAs were the greatest represented group of phenolic compounds in radicchios with a range of 60% to 95% followed by unknown phenolics, flavonols, and flavones (**Figure 6**).

Phenolic acids (specifically HCAs) were further on grouped according to their retention times as monomeric (<100 min) and oligomeric (>100 min). HCAs are mostly represented by chlorogenic and cichoric acid in all radicchio samples (**Table 5**). The levels of HCAs varied in a wide range from 47 to 362 mg/100 g FW (**Table 4**). The higher levels of total HCAs were found in var. 'Treviso,' up to two times more than the mean value, whereas var. 'Castelfranco' had the lowest amounts of HCAs. The analysis showed that radicchios contribute a smaller amount of monomeric (27%) in comparison to oligomeric HCAs (56%). Data showed that, across radicchio varieties, var. 'Treviso' had greater total HCA amount compared to other varieties (**Table 4**).

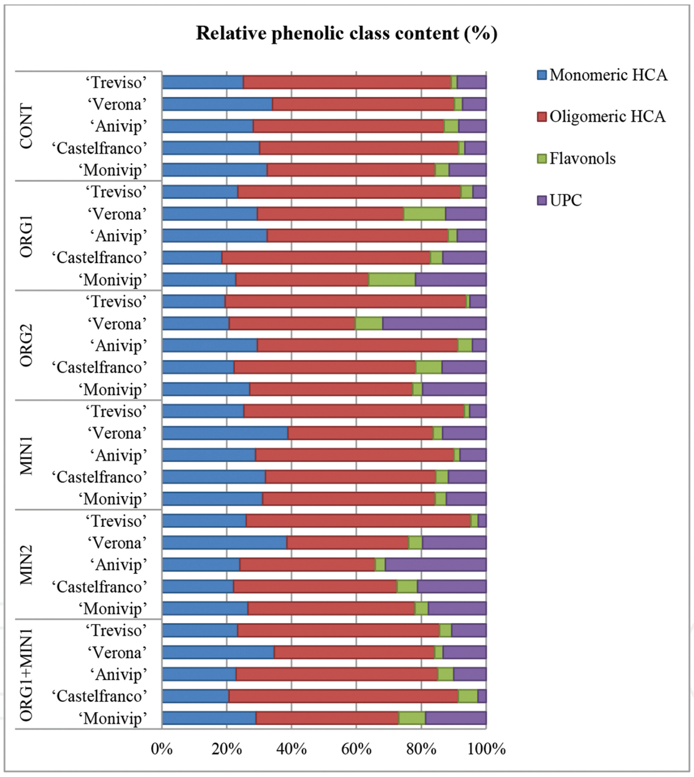

Figure 6. Relative phenolic class contents in leaves of radicchio varieties from different fertilizer managements.

The main identified monomeric HCAs were caftaric and chlorogenic acids, whereas the most represented oligomeric was cichoric acid. Cichoric acid was best represented and accounted for 43% of total HCAs, whereas chlorogenic acid with 28% and caftaric acid with 3% were present in lesser quantities (**Table 5**). All three phenolic acids together represent up to 74% of the total HCAs in radicchio samples (**Figure 6**). The HCA quantities were as follow: cichoric acid (16–190 mg/100 g FW), chlorogenic acid (14–89 mg/100 g FW), and caftaric acid (1–14 mg/

100 g FW). Those levels are in accordance with earlier reports, revealing that the caftaric, chlorogenic, and cichoric acids are the most abundant HCAs in radicchio varieties [4,6,22–26].

HCA	Quantity (mg/100 g fresh weight)					
	No fertilizer	Organic fertilizer		Mineral fertilizer		Combination
	CONT	ORG1	ORG2	MIN1	MIN2	ORG1+MIN1
Cichoric acid						
Treviso	186.37 ±9.32 aA	120.11 ±6.01 cA	134.80 ±6.74 bA	190.12 ±9.51 aA	128.89 ±6.44 bcA	133.56 ±6.68 bcA
Verona	123.71 ±6.19 aB	34.61 ±1.73 cB	36.79 ±1.84 cB	65.58 ±3.28 bB	37.72 ±1.89 cB	62.75 ±3.14 bB
Anivip	58.46 ±2.92 aE	15.51 ±0.78 eD	37.94 ±1.90 bB	26.87 ±1.34 dD	26.09 ±1.30 dC	33.47 ±1.67 cE
Castelfranco	70.99 ±3.55 aD	24.10 ±1.21 eC	43.55 ±2.18 cB	34.27 ±1.71 dD	27.08 ±1.35 eC	53.79 ±2.69 bC
Monivip	103.57 ±5.18 aC	27.41 ±1.37 dC	41.51 ±2.08 cB	49.88 ±2.49 bC	40.36 ±2.02 cB	42.61 ±2.13 cD
Chlorogenic acid						
Treviso	85.38 ±4.27 aA	33.95 ±1.70 cA	39.15 ±1.96 cA	80.39 ±4.02 aA	48.90 ±2.45 bA	52.83 ±2.64 bA
Verona	89.54 ±4.48 aA	31.51 ±1.58 dB	23.80 ±1.19 eC	77.09 ±3.85 bA	47.61 ±2.38 cA	47.84 ±2.39 cB
Anivip	28.79 ±1.44 cD	23.67 ±1.18 dC	32.52 ±1.63 bB	29.07 ±1.45 cC	14.25 ±0.71 eC	41.95 ±2.10 aC
Castelfranco	44.41 ±2.22 aC	17.72 ±0.89 dD	25.29 ±1.26 cC	32.25 ±1.61 bC	15.62 ±0.78 dC	17.26 ±0.86 dE
Monivip	72.57 ±3.63 aB	18.02 ±0.90 eD	24.78 ±1.24 dC	53.31 ±2.67 bB	20.23 ±1.01 eB	36.85 ±1.84 cD
Caftaric acid						
Treviso	8.52 ±0.43 cB	11.98 ±0.60 bA	11.29 ±0.56 bA	14.29 ±0.71 aA	11.50 ±0.57 bA	8.46 ±0.42 cA
Verona	9.18 ±0.46 aA	5.60 ±0.28 bB	3.45 ±0.17 dB	1.86 ±0.09 eCD	4.37 ±0.22 cB	4.52 ±0.23 cB
Anivip	1.86 ±0.09 dE	2.36 ±0.12 cC	3.45 ±0.17 aB	2.38 ±0.12 cC	1.34 ±0.07 eD	2.76 ±0.14 bD
Castelfranco	3.32 ±0.17 bD	2.35 ±0.12 cC	3.92 ±0.20 aB	1.43 ±0.07 dD	1.39 ±0.07 dD	1.56 ±0.08 dE
Monivip	4.88 ±0.24 aC	1.44 ±0.07 dD	3.47 ±0.17 cB	4.00 ±0.20 bB	3.77 ±0.19 bcC	3.78 ±0.19 bcC

The average values (*n*=3) with different lowercase letters in a row are significantly different ($P<0.001$; differences between the fertilizers), and those with different uppercase letters in a column are significantly different ($P<0.001$; differences between the varieties).

Table 5. HCA levels in the radicchio leaves.

Both flavonols and flavones are chemosystematic markers found in tribe Cichorieae of the Asteraceae family [27]. Total flavonol amounts of studied radicchio varieties were found in the range of 1.7 to 20 mg/100 g FW (**Table 4**). The flavonols represented below 10% of TPA for most of the radicchio samples, except for var. 'Verona' ORG1 (13%) and var. 'Monivip' MIN1 (14%). Flavones represented only small concentrations ranging up to 2 mg/100 g FW (**Table 4**). Arabbi et al. [48] found similar amounts of flavonoids ranging from 18 to 38 mg/100 g FW.

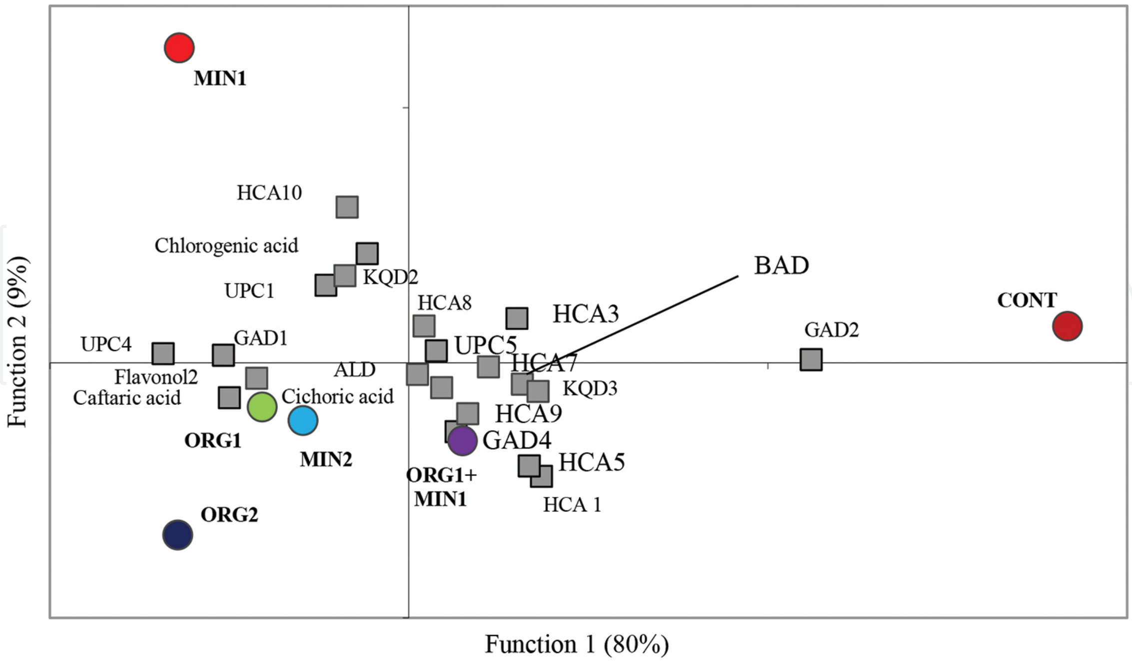

Figure 7. LDA plot for 30 radicchio samples according to six fertilizer managements. For legend, see **Table 3**.

Multivariate data analysis by principal component analysis (PCA) and linear discriminant analysis (LDA) was used for plotting the radicchio samples based on their phenolic compounds. All 60 peaks were included in the analysis. Using PCA, 21 phenolic compounds were selected as the most discriminating variables: 10 HCAs, 3 UPCs, 3 gallic acid derivatives, 3 flavonols, 2 flavones, and a protocatechuic acid (**Table 3**). The LDA scores of the data (30 samples, 21 variables) for first two functions are plotted in **Figure 7**. It should be emphasized that ORG1 and MIN2 fertilizer managements are characterized by slow nitrogen release [49].

3.2. Composition of fatty acids

The levels of the individual and total fatty acids (mg/100 g FW) of radicchio leaf samples are shown in **Table 6**. Data show significant differences for different varieties and fertilizer managements. Using GC analysis, the following fatty acids were identified and quantified: saturated fatty acids (SFAs) C16:0, C18:0, and C20:0; monounsaturated fatty acid (MUFA); and PUFAs C18:1n9, C18:2n6, and C18:3n3. Linolenic acid (C18:3n3) was represented the most and accounted for 48% to 63% of total fatty acids amount, whereas linoleic acid (C18:2n6) accounted for 16% to 30% and palmitic acid (C16:0) for 14% to 24% (**Table 6**). Stearic (C18:0) and oleic (C18:1n9) fatty acids were less abundant (<5%), and the smallest levels were found for arachidonic acid (C20:0; i.e., <1%). The total fatty acid levels ranged from 173 to 503 mg/100 g FW (**Table 6**). In comparison to other varieties, var. 'Castelfranco' showed greater levels of total fatty acid levels. Between fertilizer managements, there were significantly better total fatty acid levels when the organic fertilizers were used (ORG1 and ORG2). Obtained data are well in accordance to those for forage radicchios [50]. Blanckaert et al. [51] reported almost similar amounts for fatty acid levels of the *Cichorium* '474.'

Fatty acid	Unfertilised	Combination	Organic		Mineral		Sign.
	CONT	ORG1+MIN2	ORG1	ORG2	MIN1	MIN2	
C16:0; saturated							
Treviso	43.55 dC	42.22 eD	53.16 aC	49.94 bC	46.54 cD	39.96 fC	***
Verona	44.04 bC	31.82 dE	38.06 cD	46.36 abD	47.42 aD	39.75 cC	***
Anivip	71.93 aA	59.59 bcC	69.74 aB	66.73 abB	74.49 aA	57.24 cB	**
Castelfranco	60.30 dB	68.64 cA	81.50 aA	72.30 bA	71.79 bC	72.19 bA	***
Monivip	77.12 aA	64.86 bB	51.08 dC	66.28 bB	65.13 bC	59.45 cB	***
Sign.	***	***	***	***	***	***	
Average of all cultivars	59.39 ab	53.43 c	58.71 b	60.32 ab	61.08 a	53.72 c	***
C18:0; saturated							
Treviso	4.76 bC	4.24 cD	5.12 aB	5.27 aB	3.86 dE	3.93 dC	***
Verona	4.84 abC	3.00 cE	4.29 bC	5.44 aB	4.87 abD	4.69 abC	**
Anivip	4.78 edC	4.94 dC	7.87 aA	5.56 cB	6.79 bA	4.29 eC	***
Castelfranco	6.02 eB	6.53 cdA	8.25 aA	6.74 cA	6.46 dB	7.01 bA	***
Monivip	7.15 aA	5.72 bB	4.65 cC	7.00 aA	5.71 bC	5.93 bB	***
Sign.	***	***	***	***	***	***	
Average of all cultivars	5.51 b	4.89 d	6.04 a	6.00 a	5.54 b	5.17 c	***
C18:1n9; unsaturated							
Treviso	7.13 cBC	7.90 bC	5.49 dD	10.79 aC	5.17 eD	5.46 dC	***
Verona	7.76 bB	4.88 cE	5.26 cD	9.15 aD	7.16 bC	3.50 dD	***
Anivip	6.57 eC	11.22 dA	16.85 aB	15.10 bA	14.42 bA	13.16 cB	***
Castelfranco	8.56 dA	9.29 dB	24.90 aA	13.81 bB	11.84 cB	12.48 cB	***
Monivip	6.52 dC	5.70 eD	7.47 cC	8.61 bD	5.42 eD	15.17 aA	***
Sign.	***	***	***	***	***	***	
Average of all cultivars	7.31 f	7.80 e	11.99 a	11.49 b	8.80 d	9.95 c	***
C18:2n6; unsaturated							
Treviso	59.37 bB	58.07 cD	49.40 fD	77.52 aC	50.17 eD	55.01 dD	***
Verona	63.57 bB	45.29 dE	54.60 cC	72.02 aD	49.84 cdD	32.09 eE	***
Anivip	62.57 cB	100.62 aA	94.58 abB	97.68 abB	90.52 bB	99.71 abB	***
Castelfranco	77.89 dA	75.46 dB	127.46 aA	111.23 bA	98.67 cA	109.32 bA	***
Monivip	58.52 cdB	65.49 bC	55.42 dC	77.74 aC	61.61 cC	74.43 aC	***
Sign.	**	***	***	***	***	***	
Average of all cultivars	64.38 d	68.99 c	76.29 b	87.24 a	70.16 c	74.11 b	
C18:3n3; unsaturated							

Fatty acid	Unfertilised	Combination	Organic		Mineral		Sign.
	CONT	ORG1+MIN2	ORG1	ORG2	MIN1	MIN2	
Treviso	173.17 cB	154.52 dC	197.75 aC	189.77 bC	140.90 eE	127.69 fC	***
Verona	188.05 aB	129.16 bD	147.12 bE	191.62 aC	175.60 aD	91.98 cD	***
Anivip	250.41 bA	187.66 deB	213.95 cdB	238.56 bcA	316.64 aA	162.02 eB	***
Castelfranco	242.55 cA	246.32 bcA	253.40 aA	233.67 dB	250.50 abB	244.30 bcA	***
Monivip	234.30 abA	246.70 aA	161.34 dD	234.73 abAB	232.15 bcC	221.10 cA	***
Sign.	**	***	***	***	***	***	
Average of all cultivars	217.70 a	192.87 b	194.71 b	217.67 a	223.16 a	169.42 c	***
C20:0; saturated							
Treviso	0.26 bB	n.d.	0.94 aB	0.59 abB	0.67 abD	0.62 abB	*
Verona	0.52 bcB	0.15 cC	0.60 bcC	0.83 abAB	0.79 abcC	1.25 aA	*
Anivip	1.12 aA	0.46 cB	1.16 aA	0.96 abAB	0.98 abB	0.70 bcAB	**
Castelfranco	0.80 eAB	1.08 bcA	1.22 aA	0.98 cdAB	1.17 abA	0.93 dAB	***
Monivip	1.27 aA	1.02 bA	0.86 cB	1.20 aA	1.18 aA	0.73 dAB	***
Sign.	*	***	***	Ns	***	Ns	
Average of all cultivars	0.80 a	0.54 b	0.96 a	0.91 a	0.96 a	0.85 a	***
Total fatty acid levels							
Treviso	288.25 cB	266.96 dC	311.86 bC	333.89 aD	247.31 eE	232.66 fD	***
Verona	308.78 abB	214.31 dD	249.93 cE	325.42 aE	285.68 bD	173.26 eE	***
Anivip	397.38 bcA	364.47 cdB	404.15 bcB	424.58 bB	503.84 aA	337.11 dC	***
Castelfranco	396.12 cA	364.47 cA	496.73 aA	438.73 bA	440.44 bB	446.24 bA	***
Monivip	384.88 abA	364.47 abA	280.82 cD	395.57 aC	371.20 bC	376.82 abB	***
Sign.	**	***	***	***	***	***	
Average of all cultivars	355.08 c	328.51 d	348.70 c	383.64 a	369.69 b	313.22 e	***

Sign.: levels of significance, ***$P \leq 0.001$; **$P \leq 0.01$; *$P \leq 0.05$; Ns, not significant.
The average values (n=3) with different lowercase letters in a row are significantly different ($P<0.001$; differences between the fertilizers), and those with different uppercase letters in a column are significantly different ($P<0.001$; differences between the varieties).

Table 6. Fatty acid levels (mg/100 g FW) of radicchio varieties produced with different fertilizer managements.

The nutritional information of radicchio varieties for most optimal fertilizer management (ORG2), which signified the uppermost total fatty acid levels, is presented in **Table 7**. PUFAs represent the range from 79% to 81% of total fatty acid levels, SFAs the range from 16% to 19%, and MUFAs the range <3.6%. The ratio of n-6/n-3 fatty acids was below 0.48 for all radicchio varieties. Simopoulos [52] reported that past human diets had a ratio of n-6/n-3 fatty acids near 1, whereas modern Western diets have that ratio much higher (up to 20). The optimal ratio of *n*-6/*n*-3 fatty acids is believed to be from 1 to 4 [33,52]. Schreck et al. [53] found a higher ratio of *n*-6/*n*-3 fatty acids for the lettuce seedlings, whereas some prior readings on wild *Cichorium* leaves showed much lower values [54,55]. All analyzed radicchio varieties had the ratio at values are considered as optimal and fully in agreement with current nutritional recommendations [56].

Variety	Relative ratio (wt. %)					PUFA/SFA	*n*-6/*n*-3
	SFA	MUFA	PUFA	*n*-3	*n*-6		
Treviso	16.72	3.23	80.05	56.83	23.4	4.79	0.41
Verona	16.18	2.81	81.01	58.88	22.39	5.01	0.38
Anivip	17.25	3.56	79.2	56.19	23.23	4.59	0.41
Castelfranco	18.24	3.15	78.61	53.26	25.57	4.31	0.48
Monivip	18.83	2.18	78.99	59.34	19.95	4.19	0.34

Table 7. Nutritional information of different radicchio varieties derived from organic fertilizer (ORG2) management.

4. Conclusions

The phenolic profiles and distribution of classes of the five analyzed radicchio varieties (three red, one red-spotted, and one green) produced by different fertilizer managements under greenhouse conditions are extensively diverse. The analysis of phenolic profiles using HPLC allowed the identification and quantification of prevalent compounds. In the radicchio leaves, the predominant phenolic compounds are cichoric and chlorogenic acids. The phenolic distribution in radicchio leaves is very predisposed by both variety and fertilizer use. The highest TPAs were found for the unfertilized samples followed by the management with the water-soluble mineral fertilizer and the combination of organic and mineral fertilizers. The analysis of fatty acid profiles in radicchio leaves using GC determined six fatty acids. The main fatty acids consist of polyunsaturated linolenic acid (C18:3n3) and linoleic acid (C18:2n6). The main SFA was palmitic (C16:0). Significantly higher fatty acid levels among the fertilizer managements were seen for organic fertilizers. Radicchio seems to have an excellent nutritious balance of essential fatty acids. In summary, the phenolic and fatty acid profiles of radicchio are highly influenced by growing conditions and indicate considerable dietary and nutritional value due to its bioactive phytochemicals.

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