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FATTY ACID COMPOSITION OF LEAVES OF FORCED CHICORY (*CICHORIUM INTYBUS* L.)

Lovro Sinkovič[1], Janez Hribar[1], Rajko Vidrih[1], Žarko M. Ilin[2] and Dragan Žnidarčič[3,*]

[1]*Department of Food Science and Technology, Biotechnical Faculty, University of Ljubljana*, Jamnikarjeva 101, 1000 Ljubljana, Slovenia

[2] *Department for Field and Vegetable Crops, Faculty of Agriculture, University of Novi Sad,* Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia

[3]*Department of Agronomy, Biotechnical Faculty, University of Ljubljana,* Jamnikarjeva 101, 1000 Ljubljana, Slovenia

***Corresponding author:** dragan.znidarcic@bf.uni-lj.si

Abstract: The objective of the present study was to determine the composition of fatty acids in leaves of nine chicory cultivars (*Cichorium intybus* L.). The growing practice followed the traditional forcing method of developed roots in a peat to obtain new etiolated vegetative apical buds, known as chicons. The fatty acid content was determined by the extraction of fatty acid methyl esters and analysis by means of gas chromatography. The analysis revealed the following ratios of C16:0, C18:0, C18:1, C18:2 and C18:3 of individual fatty acids. The total fatty acid content in forced chicory leaves ranged from 104 to 644 mg/100 g fresh weight. The highest relative content (64%) is presented by α-linolenic acid, followed by linoleic (44%) and palmitic (21%). An n-6/n-3 polyunsaturated fatty acids ratio of studied forced chicory is below 1.4 and thus, in accordance with the recommended dietary ratio that is close to 1.

Key words: Forced chicory; *Cichorium intybus*; gas chromatography; fatty acid methyl esters; MUFA and PUFA

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INTRODUCTION

A plant cell contains about 5-10% of total lipids by dry weight and most of them are found in the membranes. The major fatty acids present in plants have a carbon chain length of 16 or 18, varying in the number of *cis*-double bounds from 0 to 3 (Ohlrogge and Browse, 1995; Krebsky et al., 1996). Plant lipids consist of five fatty acids, the saturated (SFA) palmitic (C16:0) and stearic (C18:0) acids, monounsaturated (MUFA) oleic acid (C18:1; n-9), and the polyunsaturated (PUFA) linoleic (C18:2; n-6) and α-linolenic (C18:3; n-3) acids (Singh et al., 2005; Žnidarčič and Vidrih, 2009). The regular consumption of SFAs and high ratio of n-6 (omega-6) to n-3 (omega-3) PUFAs is associated with higher risk for chronic diseases, such as cardiovascular dis-

eases and cancer (Staerfl et al., 2011). Two types of PUFAs, n-3 and n-6 fatty acids in foods, have received increasing attention due to reported health benefits for humans (Peterson et al., 2011). Regular consumption of n-3 essential fatty acids is a dietary recommendation to prevent potential diseases, but even more important is the n-6/n-3 ratio currently recommended at less than ten (Vidrih et al., 2009).

In recent years, leafy vegetables belonging to *Cichorium intybus* L. have become widely used in Mediterranean countries as raw salads (Žnidarčič et al., 2011), especially in the winter time when fresh vegetables are limited on the market (Carazzone et al., 2013). α-linolenic acid (C18:3, n-3) is the precursor of long chain n-3 PUFAs, such as eicosapentaenoic and docosahexaenoic acids and it is known to be present in many plants, including chicory (Lavelli et al., 2009). An important sustainable economic production of chicory is the forcing of its developed roots in darkness where, in the absence of photosynthesis, new leaves growth relies on carbon reserves (Casan et al., 2008).

To the best of our knowledge, no investigations have been undertaken to determine the fatty acid composition in different cultivars (red, red-spotted and green sugarloaf) of forced chicory. The objective of the present work was to analyze the fatty acid composition of chicory leaves from several cultivars obtained according to the traditional forcing method in a peat from pre-developed chicory roots. The gas chromatography method with prior fatty acid methyl esters preparation was used for analysis.

MATERIALS AND METHODS

Plant material

Nine commercial cultivars of chicory (*Cichorium intybus* L.) were studied: five red ('Leonardo',

Table 1. Fatty acid composition of leaf samples at the end of the forced phase in peat.

Fatty acid	mg/100 g fresh weight					
	Palmitic	Palmitoleic	Oleic	Linoleic	α-Linolenic	Total fatty acids
Chicory type/ Cultivar	$C_{16:0}$	$C_{16:1}$	$C_{18:1}$;cis-9	$C_{18:2}$;n-6	$C_{18:3}$;n-3	
Red						
Leonardo	22.17	n.d.[1]	2.56	46.20	33.47	104.41
Mesola	36.59	n.d.	4.83	71.73	111.33	224.47
Verona	77.70	n.d.	7.79	164.31	394.08	643.87
Trevisio	59.52	2.35	6.77	126.13	215.08	409.86
Chioggia	54.24	n.d.	4.73	67.55	157.09	283.61
Red-spotted						
Castelfranco	72.13	13.63	4.93	161.62	283.59	535.90
Green sugarloaf						
Uranus	34.99	n.d.	3.58	57.20	107.32	203.09
Mercurius	47.39	6.10	3.98	67.70	218.13	343.29
Jupiter	31.71	n.d.	2.56	50.50	93.38	178.15

[1]not detected

'Treviso', 'Mesola', 'Verona' and 'Chioggia'), one red-spotted ('Castelfranco') and three green sugarloaf types ('Jupiter', 'Uranus' and 'Mercurius'). The chicory roots were cultivated in the Posavje region (Slovenia) in a moderate soil, with their planting following alfalfa (*Medicago sativa*). Before plowing, 500 kg ha^{-1} Multicomb (13-11-20+microelements) was used. The chicory seedlings were transplanted to the open field at the beginning of August 2012. Additional nutrition was supplied by 400 kg ha^{-1} Multi K (12-0-42+2% MgO) and Multi Cal (15.5-0-0+19% Ca) at a ratio of 3:5. Plants were dug up by hand-fork on 16 November 2012. After harvest, the undercut roots were left to wilt on the field for 5 days according to the traditional method. Before the clean roots were placed for forcing, the leaves were cut to about 2 cm above the root crown. The roots were trimmed to a similar length of approximately 15 cm. The roots were placed in an upright position in a few centimeters of peat in forcing boxes. The boxes were placed on rolling benches in a heated dark glasshouse compartment of the Biotechnical Faculty, Ljubljana. Air temperature was maintained at 10°C during the first forcing period (20 days) and was gradually increased to 15°C during the second period (10 days). After 30 days, the forced leaves were harvested.

Determination of fatty acids

For the experimental analysis, leaves of three randomly chosen plants of each cultivar were used. The leaves were combined in plastic bags and frozen at -20°C until analysis. Fatty acid composition was determined using gas chromatography of fatty acid methyl esters (FAMEs). FAMEs were prepared from the unfrozen leaf samples according to literature (Park and Goins, 1994). In a Hach test tube with a screw cap 100 µL of internal standard solution (heptadecanoic acid, C17:0, Sigma H 3500, lot 097K1029) and approximately 0.1-0.4 g chopped unfrozen leaves of each chicory cultivar were weighed. To the same vial 300 µL of methylene chloride and 3 mL 0.5 M of freshly prepared sodium hydroxide in methanol were added. The test tubes were mixed and heated in a water bath for 60 min at 90°C with occasional

Table 2. Nutritional information regarding forced chicory cultivars derived from raw fatty acids data.

Parameter	Relative ratio (wt %)					PUFA/SFA	n-6/n-3
Chicory type/ Cultivar	SFA[1]	MUFA[2]	PUFA[3]	n-3	n-6		
Red							
Leonardo	21.24	2.45	76.31	32.06	44.25	3.59	1.38
Mesola	16.30	2.15	81.55	49.60	31.95	5.00	0.64
Verona	12.07	1.21	86.72	61.20	25.52	7.19	0.42
Treviso	14.52	2.22	83.25	52.48	30.78	5.73	0.59
Chioggia	19.13	1.67	79.21	55.39	23.82	4.14	0.43
Red-spotted							
Castelfranco	13.46	3.46	83.08	52.92	30.16	6.17	0.57
Green sugarloaf							
Uranus	17.23	1.76	81.01	52.84	28.16	4.70	0.53
Mercurius	13.80	2.94	83.26	63.54	19.72	6.03	0.31
Jupiter	17.80	1.43	80.77	52.42	28.35	4.54	0.54

[1]saturated fatty acids, [2]monounsaturated fatty acids, [3]polyunsaturated fatty acids

mixing. The reaction mixture was quickly cooled and 3 mL of 14% boron trifluoride in methanol were added and the test tubes were heated again for another 10 min at the same temperature. The reaction mixture was cooled and 3 mL of 10% sodium chloride solution and 1.0 mL hexane were added. The reaction mixture was mixed vigorously by Vortexer for 1 min and centrifuged at 4 000 xg for 10 min. The hexane phase with dissolved methyl esters of fatty acids was transferred to a dark vial and frozen at -20°C until analyzed by gas chromatography.

The solution of FAMEs was quantified on an Agilent 6890N gas chromatograph with flame ionization detector, equipped with an Agilent 7683 series autosampler (Agilent Technologies Inc., Wilmington, DE, USA). Separation was carried out on column SPB PUFA; 30 m×0.25 mm×0.2 μm column (SUPELCO). The following conditions were used to separate and detect the FAMEs: column temperature 210°C, injector temperature 250°C, detector temperature 260°C, injection volume 1 μL, helium was used as the carrier gas at a flow rate of 1 mL min^{-1}. The resultant data was processed by computer using GC Chem Station software. Identification of fatty acids was carried out using a reference standard mixture of methyl esters of higher fatty acids (Lipid standard Sigma 189-19). GC analysis of chicory samples detected the following fatty acids: C 16:0, C 16:1, C 18:1, C 18:2 and C 18:3. The amounts of each of fatty acid were calculated from the areas of the internal standard (heptadecanoic acid, C 17:0).

Data analysis

Correlations among five fatty acids (%) and the total fatty acid content (mg/100 g FW) in nine chicory cultivars were calculated by PROC CORR procedure in SAS ver. 9.1. The principal component analysis (PCA) based on five fatty acids (%) and the total fatty acid content (mg/100 g FW) in nine chicory cultivars was performed using PROC PRINCOMP in SAS. The biplot was constructed by two principal components showing cultivars and analyzed variables (as vectors).

RESULTS AND DISCUSION

Following the method described by Garces and Mancha (1993), the composition of individual fatty acid and total fat contents of chicory cultivars at the end of forcing phase is presented in Table 1. Five different fatty acids were identified and quantified. The most abundant fatty acid was omega-3 α-linolenic (C 18:3) with range from 90 to 390 mg/100 g FW, except for cv. 'Leonardo' (33.47 mg/100 g FW), in which linoleic acid (C 18:2) predominated. The highest concentration of α-linoleic acid was determined in the red cv. 'Verona' (394.08 mg/100 g FW), followed by red-spotted cv. 'Castelfranco' (283.59 mg/100 g FW) and green sugarloaf cv. 'Mercurius' (218.13 mg/100 g FW). The second most represented was omega-6 linoleic (C 18:2) fatty acid with content from 50 to 160 mg/100 g FW. The highest concentrations were found in cv. 'Verona' (164.31 mg/100 g FW), 'Castelfranco' (161.2 mg/100 g FW) and 'Treviso' (126.13 mg/100 g FW). The total fatty acid (FA) concentrations of the chicories under study ranged from 100 to 640 mg/100 g FW. Cultivars with high levels of total FA content (>400 mg/100 g FW) were 'Verona', 'Castelfranco' and 'Treviso'. There are no data available for total FA contents of chicory, while previous studies on etiolated Belgian endive reported lower values (Krebsky et al., 1996). Clapham et al. (2005) studied chicory forages (cv. 'Forage Feast', 'Lacerta' and 'Puna') and reported total FA contents (26.7 to 62.8 mg/g dry weight) similar to ours.

The calculated nutritional information as relative ratio (wt %) of studied chicory cultivars are reported in Table 2. The major fatty acid present in lipids of chicory plants was omega-3 α-linolenic (C 18:3), ranging from 32 to 64%, followed by omega-6 linoleic (C 18:2; 20-44%) and palmitic (C 16:0; 12-21%), which is the only saturated fatty acid (SFA). Palmitoleic (C 16:1) and oleic (C 18:1) fatty acids are less present in analyses samples and together represent a minor group of monounsaturated fatty acids (MUFA; 1-4%). α-linolenic and linoleic acid represent the group of polyunsaturated fatty acids (PUFA), ranging from 76 to 87%. The PUFA/SFA ratio ranged from 4 to 7, while the n-6/n-3 ratio of the analyzed chicory cultivars was below 1, except for cv. 'Leonardo' (1.38). Previous investigations done on wild species of *Cichorium intybus* reported slightly lower values found for n-6/n-3 ratio (Vardavas et al., 2006; Morales et al., 2012). The balance of n-6 and n-3 fatty acids is important for homeostasis, normal development, decreasing the risk for coronary heart disease, gene expression and is a determinant of health; therefore the appropriate recommended n-6/n-3 ratio is close to 1 (Simopoulos, 2002, 2008). The dietary recommendations for human health to increase the consumption of fish or n-3 rich vegetables remain (Russo, 2009), and chicory, with a good balance of n-6 and n-3 fatty acids, proved to be a beneficial vegetable in this respect.

Table 3. Pearson's correlation coefficients among five fatty acids (%) and total fatty acid content in nine forced chicory cultivars.

No.	Variable	Variable					
		1	2	3	4	5	6
1	Palmitic acid (%)		ns	*	ns	*	**
2	Palmitoleic acid (%)	-0.531		*	ns	ns	ns
3	Oleic acid (%)	0.767	-0.668		*	*	*
4	Linoleic acid (%)	0.559	-0.258	0.714		***	ns
5	α-Linolenic acid (%)	-0.748	0.305	-0.787	-0.965		ns
6	Total fatty acids (mg/100 g FW)	-0.875	0.456	-0.744	-0.428	0.613	

The significance of the correlations is indicated as follows: ***, significance at the 0.1% nominal level; **, significance at the 1% nominal level; *, significance at the 5% nominal level; ns, not significant.

Table 4. Component loadings of the five fatty acids (%) and total fatty acid content on the first three principal components.

No.	Variable	Principal component					
		PC1		PC2		PC3	
1	Palmitic acid (%)	0.902	***	0.159	ns	0.297	ns
2	Palmitoleic acid (%)	-0.613	ns	-0.629	ns	0.462	ns
3	Oleic acid (%)	0.936	***	0.093	ns	-0.156	ns
4	Linoleic acid (%)	0.798	*	-0.550	ns	-0.231	ns
5	α-Linolenic acid (%)	-0.899	**	0.431	ns	0.037	ns
6	Total fatty acids (mg/100 g FW)	-0.831	**	-0.253	ns	-0.455	ns
	Eigenvalue	4.20		0.98		0.59	
	% of Variance	70.02		16.36		9.79	

The significance is indicated as follows: ***, significance at the 0.1% nominal level; **, significance at the 1% nominal level; *, significance at the 5% nominal level; ns, not significant.

As presented in Table 3, out of 15 pairwise correlations between five fatty acids (%) and the total fatty acid content (mg/100 g FW) in nine forced chicory cultivars, eight were proven significant at $P<0.05$, out of which seven could be considered as strong ($r>0.70$ or $r<-0.70$). The strong positive correlations ($r>0.70$; $P<0.05$) were observed between oleic acid and both palmitic ($r=0.767$) and linoleic acid ($r=0.714$). The strong negative correlations ($r<-0.79$; $P<0.05$) were observed between palmitic acid and both α-linolenic acid ($r=-0.748$) and the total fatty acids ($r=-0.875$), between oleic acid and both α-linolenic acid ($r=-0.787$) and the total fatty acids ($r=-0.744$), and between linoleic and α-linolenic acid ($r=-0.965$).

The PCA based on five fatty acids (%) and total fatty acid content (mg/100 g FW) in nine forced chicory cultivars revealed that the first three principal components explained as much as 96.17% of the total variation (Table 4). All variables except palmitoleic acid were strongly ($r>0.70$ or $r<-0.70$) correlated with the first principal component that explained 70.02% of the total variation. The strong positive correlations were observed between the first principal component and palmitic, oleic and linoleic acid, while strong negative correlations were observed between the first principal component and both α-linolenic acid and total fatty acid content. None of the variables showed correlation with either the second or the third principal components. The strongest correlation was found between the second principal component and palmitoleic acid ($r=-0.629$), although non-significant ($P=0.07$).

The biplot constructed by two principal components showing cultivars and the analyzed variables (as vectors) is presented in Fig. 1. The first principal component axis separated the cultivars with higher percentages of palmitic, oleic and linoleic acid ('Leonardo' and 'Mesola') from the cultivars characterized by an above-average percentage of α-linoleic acid and total fatty acid content ('Mercurius', 'Verona' and 'Castelfranco'). On the second principal component axis, the cultivar 'Castelfranco', characterized by an above-average percentage of palmitoleic acid, was separated

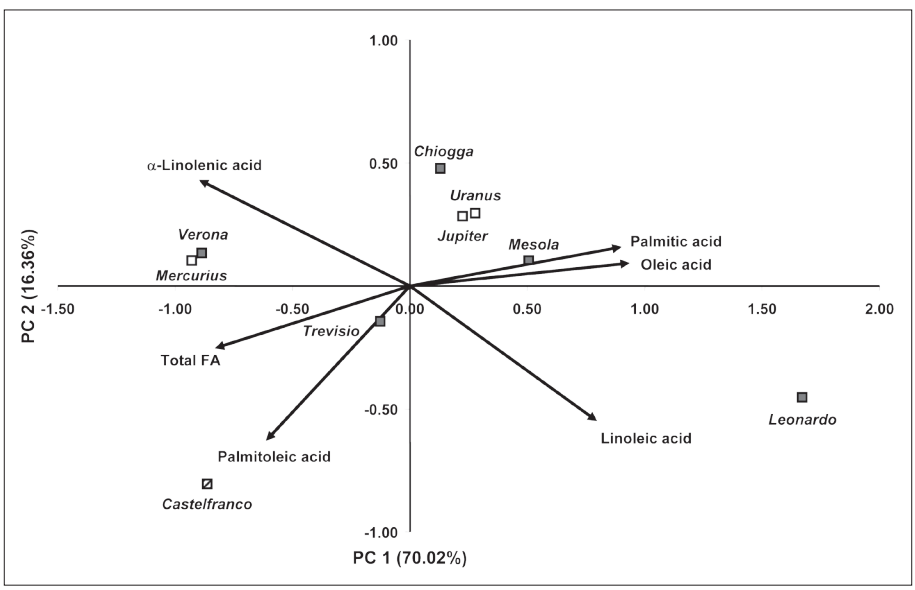

Fig. 1. Biplot of the PCA based on five fatty acids (%) and total fatty acid content in nine forced chicory cultivars

from the rest of the cultivars. Based on five fatty acids (%) and the total fatty acid content, the analyzed cultivars belonging to the same type (sugarloaf, red-spotted and red) did not tend to group together, e.g. the cultivars 'Verona' and 'Mercurius', although belonging to different chicory types grouped closely together, while 'Verona' and 'Leonardo', both red-headed, had clearly different fatty acid profiles and grouped on the opposite sides along the first principal component axis.

CONCLUSIONS

The present analysis demonstrated that the majority of fats in forced chicory leaves consisted of unsaturated fatty acids, among which are main α-linolenic (C 18:3, n-3) and linoleic acid (C 18:2, n-6). Saturated fatty acids are represented mainly by palmitic acid (C 16:0). The forced chicory showed itself to be a good source of essential α-linoleic fatty acid with an appropriate n-6/n-3 ratio. Although the fatty acid content in vegetables is rather low, they have an important nutritional value due to their favorable fatty acid balance.

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