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Effect of Cultivar on Chemical Composition of Some Iranian Spinach

[1]F. Erfani, [2]M.R. Hassandokht, [1]A. Jabbari and [3]M. Barzegar
[1]Department of Chemistry, K.N. Toosi Technology University, Tehran, Iran
[2]Department of Horticultural Science, Tarbiat Modares University, P.O. Box 14115-336, Tehran, Iran
[3]Department of Food Science and Technology, Tarbiat Modares University, Tehran, Iran

Abstract: This research was carried out to determine fatty acids, phenolic compounds, oxalic acid, protein, fat, total fiber and mineral elements of Iranian spinach cultivars (Varamin 1, Varamin 2, Varamin 3, Zabol, Kashan, Qom and Zanjan). Results showed that cultivar Zabol had the highest protein (2.59%). The highest fat percentage, phenolic compounds, P, linoleic acid (18:2) and the lowest oxalic acid were found in cultivar Kashan. Cultivar Varamin 2 had the highest total fiber (2.22%). Dry matter, K, Ca, Cu and Zn contents of cultivar Zanjan were higher than the other cultivars. Cultivar Qom had the highest amounts of Mg and Fe. Cultivar Varamin 3 had the highest Na (93.8 mg/100 g fw). Cultivars Qom and Varamin1 had the highest percentage of linolenic acid (18:3). Present results for chemical composition of studied cultivars, were similar to values reported by other researchers.

Key words: Spinach (*Spinacea oleracea* L.), fatty acids, phenolic compounds, mineral elements, oxalic acid, physicochemical properties

INTRODUCTION

Spinach (*Spinacea oleracea* L.) is an important leafy vegetable belongs to chenopodiaceae family. Spinach is probably a native of southwest of Asia (Salunkhe and Kadam, 1998). The origin of spinach has been placed near Iran where it has been cultivated for at least 2000 years (Rubatzky and Yamaguchi, 1997). Spinach is a traditional potherbs, but it is widely used uncooked in salads. It is also processed by canning and freezing (Rubatzky and Yamaguchi, 1997). Spinach is rich in calcium and iron, but the calcium is probably unavailable, as it combines with oxalic acid, leading to the formation of calcium oxalate. Kim and Zemel (1986) found calcium from spinach to be less soluble than that from skim milk. Poor availability of calcium from spinach has been confirmed in humans (Heany *et al*., 1988) and rabbits (Kikunaga *et al*., 1988). Plants, including spinach, contain many antinutritional components, of which oxalate occurs in abundante. Oxalate levels of 658-1760 mg/100 g fw have been reported in spinach (Salunkhe and Kadam, 1998). It is thought that L-ascorbic acid might be metabolized to oxalic acid in oxalate-accumulating plants, including spinach (Yang and Loewcis, 1975). Oxalic acid combines with calcium to form insoluble calcium oxalate as well as reducing dietary magnesium and iron availability. Oxalic acid content also varied among cultivar spinach types, savoy types contain less than smooth types (Rubatzky and Yamaguchi, 1997).

Spinach leaf protein has been shown to have hypocloesterolemic properties (Satoh and Igarashi, 1993).

Spinach leaves are known to contain about 0.6% fat (Salunkhe and Kadam, 1998) and 0.65% fiber (Rubatzky and Yamaguchi, 1997). A notable feature is the presence of high amounts of 18:3 fatty acid (Browse *et al*., 1981).

The biological activities of spinach polyphenols have been reported (Edenharder *et al*., 2001; Gil *et al*., 1999; Lomnitski *et al*., 2000). Hermann (1995) reported antioxidant activity, principally involving the spinach flavonoids, as these constitute the major water-soluble polyphenols found in this vegetable.

Since there was little information about chemical composition of Iranian spinach cultivars, this research was carried out to determine fatty acids, phenolic compounds, oxalic acid, protein, fat, total fiber and mineral elements of these cultivars.

MATERIALS AND METHODS

In 2004, seeds of seven Iranian spinach cultivars including Varamin 1, Varamin 2, Varamin 3, Zabol, Kashan, Qom and Zanjan were collected from the main origin of these cultivars. Seeds of these cultivars were sown in research field. After harvesting, leaves of each cultivar were placed in electric oven in 70°C for 3 days. Mineral compounds including Na, K, Ca, Mg, Fe, Cu, Zn were detrmined by atomic absorbtion (AOAC, 1990) and P was determined using a spectrophotometeric method (Jeffery *et al*., 1989).

Corresponding Author: Dr. M.R. Hassandokht, Department of Horticultural Science, Faculty of Agriculture, Tarbiat Modares University, P.O. Box 14115-336, Tehran, Iran Tel: +982144196523 Fax: +982144196524

Ash, oxalic acid, percentage of protein and total fiber were determined by methods of the AOAC (1990).

For determining fat, 2 g of dry matter powder were transferred on a filter paper placed in a Büchner funnel and washed using 20 mL of distilled water. Then the samples were dried at 70°C in a vacuum oven. The lipids of samples were extracted into hexane using a solvent extractor. After 6h extraction period, the solvent was evaporated with helping of a rotary evaporator, cooled to room temperature and weighed.

The concentration of phenolic compounds in the extracts was determined according to the Folin-Ciocalteu method (Singh *et al*., 2002) and the results were expressed as tannic acid equivalents per gram dry weight of sample (TAE/gdw). The spinach leaf extracts were dissolved in a mixture of methanol and water (2:1 V/V). Samples 0.2 mL were mixed with 1 mL of 10-fold-diluted Folin-Ciocalteu reagent and 0.8 mL of 7.5% sodium carbonate solution, after the mixture had been allowed to stand for 30 min at room temperature, the absorbance was measured at 765 nm using UNICAM 8620 UV-Vis spectrophotometer. The estimation of phenolic compounds in the extracts was carried out in triplicate and the results were averaged.

For determination of fatty acid profiles, 15 mL methanol-chloroform (1: 2) solvent were added to 1 g of leaf powder. Then 15 mL distilled water was added to this mixture and the mixture was centrifuged in 2500 rpm for 10 min to form two phases. Lower phase was filtered with filter paper and solvent evaporated with nitrogen gas. Finally, 1 μL of methyl ester was injected to gas chromatograph to determine fatty acid profile of samples. Conditions of GC were: column BP×70 (0.22 mm×30 m×0.22 μm) temperature of column, injection port and detector (FID) were 160, 230 and 280°C, respectively. Carrier gas was helium (99.99 from Roham Gas Co., Tehran, Iran) with 20 psi pressure at inlet.

Statistical analysis: All experiments were done in triplicate and means of data were analyzed statistically using analysis of variance and means were compared with Duncan's multiple range test.

RESULTS AND DISCUSSION

Dry matter, ash, protein, fat, total fiber, phenolic compounds and oxalic acid: Dry Matter (DM) content, ash, protein, fat, total fiber and phenolic compounds of Iranian spinach cultivars are presented in Table 1. Dry matter contents of cultivars (cvs.) showed significant difference ($p<0.05$) and cultivar Zanjan had the highest DM (11.31%). Mean dry matter of cvs. (10.23%) was higher than value reported by USDA (2006) (8.6%) Chakraborti *et al*. (1989) (8.5%) and Rubatzky and Yamaguchi (1997) (8.5%). Cultivars with high dry matter content are suitable for processing and they require lower oil for frying than cultivars with lower dry matter. These differences are genetically and also could relate to environmental conditions.

Cultivar Varamin 3 had higher ash than the other cultivars. There was no significant differences between ash of cultivars Varamin 1, Varamin 2 and Kashan ($p<0.05$). Ash contents of these cultivars were higher than values reported by other researchers (USDA, 2006, Rubatzky and Yamaguchi, 1997). Cultivar Zabol had the highest percentage of protein (2.59%) and its amount was lower than value reported by UDSA (2006) (2.86%).

Fat of seven spinach cultivars showed significant difference and the highest fat content was belonged to cultivar Kashan (0.33%). Fat of these cultivars (Table 1) was lower than amounts reported by USDA (2006) and Salunkhe and Kadam (1998).

Phenolic compounds ranged from 60.69 to 98.82 mg TAE/100 g dw and the highest amount was belonged to cultivar Kashan (Table 1). Flavonoids are typical phenolic compounds and powerful chain-breaking antioxidants (Prat, 1992). The possible presence of flavonoid-like compounds in spinach was first reported by Weatherby and Cheng (1943). Flavonoids have multiple biological activities including potent anti-allergic and anti-inflammatory and antiviral reactions, which may result, at least in part, from their antioxidant and free radical-scavenging abilities (Kahl, 1991; Middleton and Candaswami, 1993; Van Acker *et al*., 1996; Santos *et al*., 1998).

Cultivars showed significant difference for total fiber. Cultivar Varamin 2 had the highest amount of total fiber (2.22%). This value was similar to the amount reported by USDA (2006) and much higher than value reported by Rubatzky and Yamaguchi (1997).

The variation in these traits could originate from the spinach cultivar and agro-climatic as well as environmental conditions.

The average of oxalic acid was 85.55 mg/100 g fw over a range 184.14±0.62 mg/100 g fw (for cv. Varamin 3) to 53.31±0.81 mg/100 g fw (for cv. Kashan) (Table 1). Cultivars Zabol, Qom, Kashan, Varamin 1 and Varamin 2 had lower oxalic acid ($p<100$ mg/100 g fw) than the other cultivars. Amount of oxalic acid was one third of value reported by Salunkhe and Kadam (1998) (658-1760 mg/100 g fw). Oxalic acid to calcium ratio ranged from 0.74 for Qom and Kashan cultivars to 2.12 for Varamin 3 cultivar. This ratio was <1 in cultivars Kashan, Qom, Varamin 2 and Zanjan. Since oxalic acid is an antinutritional component of some vegetables such as spinach, celery, purslane, so

Table 1: Comparison of some analytical properties of different cultivars of Iranian spinach*

Cultivars	Dry matter (%)	Ash (%)	Protein (%)	Fat (%)	Total fiber (%)	Phenolic compounds (mg TAE/100 g dw)	Oxalic acid mg/100 g fw	Oxalic acid/calcium
Zabol	10.19±0.4ab	1.56±0.44d	2.59±0.04a	0.12±0.002g	2.03±0.04b	74.81±0.49e	63.6±0.8d	1.1
Kashan	10.08±0.1ab	1.99±0.40a	1.80±0.00e	0.33±0.004a	2.05±0.03b	70.74±0.18f	53.3±0.8g	0.7
Qom	10.83±0.3ab	1.75±0.42b	2.52±0.02b	0.22±0.001e	1.80±0.04e	81.89±0.06d	57.9±0.8e	0.7
Varamin 1	8.97±0.5b	1.65±0.01c	2.27±0.03c	0.24±0.003b	2.22±0.02a	60.69±0.07g	81.6±0.9c	1.2
Varamin 2	10.43±0.4ab	2.03±0.30a	2.28±0.01c	0.23±0.002d	1.64±0.04f	85.88±0.05c	55.7±0.8f	0.9
Varamin 3	10.79±0.6ab	2.06±0.94a	1.80±0.01e	0.19±0.002f	1.87±0.02d	87.19±0.31b	184.1±0.6a	2.1
Zanjan	11.31±0.4a	1.81±0.28b	2.17±0.06d	0.23±0.001c	1.97±0.01c	98.82±0.28a	102.6±0.6b	0.9
Mean	10.23	1.83	2.20	0.22	1.94	80	85.6	1.1

* Values are mean±SD of triplicate measurements, values with the same letter(s) are not significant different ($p<0.05$)

Table 2: Comparison of mineral elements of Iranian spinach cultivars (mg/100 gfw)*

Cultivars	Na	K	Ca	Mg	P	Fe	Cu	Zn
Zabol	37.0±0.9f	610.9±3.9g	59.1±0.06g	57.9±0.4f	51.5±0.5e	2.03±0.002f	0.15±0.02c	1.19±0.001g
Kashan	68.6±0.8c	1015.9±10.3b	72.2±0.05d	78.9±0.1c	83.8±0.5a	3.76±0.002b	0.16±0.02b	1.92±0.001c
Qom	91.4±1.1b	762.1±4.3d	77.7±0.06c	113.6±0.3a	76.4±0.4b	3.82±0.003a	0.07±0.02g	1.89±0.002d
Varamin 1	55.0±0.4e	696.0±3.0e	70.4±0.08e	58.0±0.3f	41.5±0.4g	2.85±0.00e	0.10±0.02d	1.97±0.002b
Varamin 2	68.8±1.3c	682.1±3.1f	61.3±0.07f	71.8±0.5e	42.7±0.5f	3.44±0.002d	0.09±0.02e	1.45±0.000f
Varamin 3	93.8±0.9a	874.9±5.2c	86.6±0.06b	78.1±0.5d	72.7±0.5c	3.58±0.001c	0.08±0.02f	1.76±0.003e
Zanjan	62.3±1.1d	1068.1±6.0a	111.6±0.03a	97.9±0.1b	60.5±0.5d	1.94±0.001g	0.19±0.02a	2.52±0.000a
Mean	68.1	815.7	77.0	79.5	61.3	3.06	0.12	1.81

* Values are mean±SD of triplicate measurements, values with the same letter(s) are not significant different ($p<0.05$)

determination of this compound is very important (Salunkhe and Kadam, 1998). Oxalic acid is combined with calcium and form calcium oxalate (Hesse and Siener, 1997). Dietary oxalate combines with calcium, magnesium and iron and reduces availability of these mineral elements (Bohn *et al*., 2004). The oxalate content increases with growth (Yamanaka *et al*., 1983), an increase in air temperature and light intensity (Limongelli *et al*., 1974), but it is less influenced by the type of fertilizer used (Van Maercke, 1973). It is thought that L-ascorbic acid might be metabolized to oxalic acid in oxalate-accumulating plants, including spinach (Yang and Loewcis, 1975).

Mineral elements analysis: Table 2 shows mineral elements of studied spinach cvs. Sodium contents of leaves ranged from 93.82 to 37.02 mg/100 g fw. The highest and lowest amount of sodium were found in Varamin 3 and Zabol cultivars, respectively. The sodium content of cultivar Varamin 3 (93.82 mg/100 g fw) was higher than amount reported by USDA (2006) (79 mg/100 g fw) and lower than Fordham (1993), Holland *et al.* (1991) (140 mg/100 g fw) and Rubatzky and Yamaguchi (1997) (110 mg/100 g fw). Mean of potassium in all cultivars (815.71 mg/100 g fw) was higher than amount reported by USDA (2006) (558 mg/100 g fw), Fordham (1993), Holland *et al.* (1991) (500 mg/100 g fw) and Rubatzky and Yamaguchi (1997) (605 mg/100 g fw). The highest amount of potassium was found in cultivar Zanjan (1068 mg/100 g fw) and it was about two times of the amount reported by other researchers (Fordham, 1993; Holland *et al*., 1991; Rubatzky and Yamaguchi, 1997). The highest amount of calcium (112.16 mg/100 g fw) was higher than amount reported by Rubatzky and Yamaguchi (1997) (107 mg/100 g fw), USDA (2006) (99 mg/100 g fw) and lower than Fordham (1993), Holland *et al.* (1991). Magnesium content ranged from 113.58 mg/100 g fw in Zabol to 57.85 mg/100 g fw in Qom cvs. The mean amount of magnesium was higher than value previously reported by Rubatzky and Yamaguchi (1997), the same as USDA (2006) and lower than value published by Fordham (1993) and Holland *et al.* (1991). The highest amount of phosphorus was found in cultivar Kashan and it was higher than values reported by USDA (2006), Fordham (1993), Holland *et al.* (1991) and Rubatzky and Yamaguchi (1997). The iron ranged from 3.82 mg/100 g fw for Qom to 1.94 mg/100 g fw for Zanjan cultivar with average value of 3.06 mg/100 g fw and it was higher than value presented by USDA (2006), Fordham (1993), Holland *et al.* (1991) and Rubatzky and Yamaguchi (1997). Copper content of leaf varied in different cultivars and the highest amount was found in cultivar Zanjan (0.19 mg/100 g fw). It was more than values reported by USDA (2006) (0.13) and Holland *et al.* (1991) (0.04). Zinc content of leaf ranged from 2.52 mg/100 g fw (cv. Zanjan) to 1.19 mg/100 g fw (cv. Zabol). The mean content of zinc was more than three times of amount reported by USDA (2006) and Fordham (1993). Differences in mineral composition of studied cultivars mainly relate to genetic and agro-climatic and environmental conditions.

Fatty acid profiles: Eight fatty acids were identified in leaves of seven spinach cvs. The fatty acid profiles of different cultivars were presented in Table 3. The detected fatty acids in these cultivars were: palmitic (C16:0), palmitoleic (C16:1), hexadecatrienoic (C16:3), stearic

Table 3: Fatty acid profiles of different cultivars of Iranian spinach (%)*

Cultivars	C16:0	C16:1	C16:3	C18:0	C18:1	C18:2	C18:3	C22:1	Unsaturated/ saturated
Zabol	21.48±0.55c	7.66±0.12b	4.16±0.15cd	1.27±0.05bc	7.56±0.09d	18.87±0.22c	37.92±0.49b	1.08±0.03d	3.39
Kashan	19.65±0.67d	4.22±0.26e	3.79±0.17d	1.05±0.04c	6.88±0.19d	23.89±0.13a	39.02±1.35b	1.32±0.12c	3.82
Qom	21.35±0.46c	6.30±0.31d	4.34±0.07c	1.47±0.04b	9.63±0.35b	15.33±0.42d	40.28±0.37a	1.31±0.06c	3.38
Varamin 1	17.87±0.30e	4.52±0.23e	4.55±0.15b	1.65±0.04b	8.53±0.08c	19.90±0.77b	41.10±0.69a	1.89±0.06b	4.12
Varamin 2	23.08±1.40b	7.02±0.08c	0.18a±5.01	1.28±0.53bc	6.90±0.88d	20.44±0.08b	36.27±0.26c	0.00e	2.91
Varamin 3	22.03±0.27bc	7.19±0.25c	2.78±0.08e	1.49±0.07b	8.65±0.25c	20.30±0.73b	34.41±0.14d	3.16±0.03a	3.25
Zanjan	28.54±0.42a	8.54±0.14a	2.70±0.03e	2.29±0.19a	12.37±0.34a	13.66±0.21e	28.66±0.56e	3.22±0.07a	2.24
Mean	22	6.49	3.21	1.5	8.64	18.91	36.81	1.71	3.38

*Values are mean±SD of triplicate measurements, values with the same letter(s) are not significant different ($p<0.05$)

(C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3) and erusic acid (C22:1). We could not confirm the presence of hexadecadienoic acid (C16:2) which found by Murcia *et al.* (1992). Linolenic acid was determinated to be the predominant fatty acid in seven spinach cvs. Its amount ranged between 28.7-41.1%. Significant differences were observed between the fatty acids of different cvs. The highest amounts of palmitic, palmitoleic and oleic acid were found in cultivar Zanjan (28.54, 8.54 and 12.37%, respectively). These amounts were higher than values previously reported by Murcia *et al.* (1992). The mean amount of linoleic acid (omega-6) of all studied samples was higher than amount reported by Murcia *et al.* (1992) (13.5%). The amounts of linolenic acid (omega-3) of studied cvs. was about two times of linoleic acid (omega-6). The amount of linolenic ranged from 41.10 (cv. Varamin 1) to 28.66 (cv. Zanjan). The average amounts of linolenic acid (36.81%) was lower than value (53%) reported by Murcia *et al.* (1992).

It has been confirmed that the unsaturated fatty acids were predominant in all cultivars. However, linolenic acid was the main fatty acid in this research.

The unsaturated/saturated acid ratio was generally high (Table 3). The highest and lowest ratios were 4.12 (Varamin 1) and 2.24 (cv. Zanjan). These ratios were lower than values previously reported by Murcia *et al.* (1992).

CONCLUSIONS

The results of this study showed that most of the cultivars had good nutritional values. The studied cvs. are good sources of DM, fat, total fiber and protein. The mineral content variation are high, especially those of Na, K, Ca, P, Fe, Cu and Zn. The differences were observed in nutrient composition between studied cultivars correspond to high genetic diversity found between studied cvs. This variability in nutrient can be useful for selecting specific cultivars for special purposes. The studied cultivars had high levels of phenolic compounds and therefore high antioxidant activity. All of studied cultivars had a few amounts of oxalic acid, an antinutritional component and considerable amounts of unsaturated fatty acids, especially linolenic acid (omega-3).

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