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The lipide soluble vitamin contents of some *Onobrychis* Miller (*Fabaceae*) taxa

Bazı Onobrychis Miller (Fabaceae) Taksonları'nın yağda çözünen vitamin içeriği

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Abstract

The goal of this study is to determine the lipid-soluble vitamin contents in seeds of the some Onobrychis Miller (Fabaceae) taxa by using HPLC. Samples were collected from the natural habitats. Studied materials were dissolved in acetonitrile/methanol (75/25 v/v) and were injected 50 µL to HPLC instrument (Shimadzu, Kyota Japan). According to data obtained from present study showed that *O. hypargyrea*, *O. viciifolia*, *O. caput-galli*, *O. fallax* and *O. oxyodonta* var. *armena* have high lipide-soluble vitamin contents. Present study found that *O. oxyodonta* var. *armena* (1777.27±6.24 µg/g), *O. fallax* (916.0±4.51 µg/g) *O. hypargyrea* (809.7±5.03 µg/g) and *O. viciifolia* (399.7±3.54 µg/g) have highest beta-caroten content. Also, *O. caput-galli* has high beta caroten content (73.3±.94 µg/g). on the other hand, it was found that O. fallax has highest gamma-tocopherol content (1401.2±8.76 µg/g). *O. viciifolia* (574.9±2.35 µg/g), O. *caput-galli* (410.1±4.56 µg/g), O. *oxyodonta* var. a*rmena* (267.7±3.68 µg/g), *O. podporea* (162.5±2.14 µg/g) were the other high gamma tocopherol content. Whereas, retinol, retinol acetate and r-tocopherol contents were found absent or trace amounts in the present study.

Özet

Bu çalışmanın amacı, bazı Onobrychis Miller (Fabaceae) taksonlarının tohumlarındaki yağda çözünen vitamin içeriğini HPLC kullanarak belirlemektir. Doğal yaşam alanlarından örnekler alındı. Çalışılan malzemeler asetonitril / metanol (75/25 h / h) içinde çözüldü ve HPLC cihazına (Shimadzu, Kyota Japonya) 50 µL enjekte edildi. Bu çalışmadan elde edilen verilere göre *O. hypargyrea*, *O. viciifolia, O. caput-galli*, *O. fallax* ve *O. oxyodonta* var. *armena*'nın lipitte çözünen vitamin içeriğinin yüksek olduğunu göstermiştir. Bu çalışma *O. oxyodonta* var. *armena* (1777.27 ± 6.24 µg / g), *O. fallax* (916.0 ± 4.51 µg / g) *O. hypargyrea* (809.7 ± 5.03 µg / g) ve *O. viciifolia* (399.7 ± 3.54 µg / g) en yüksek beta karoten içeriğine sahiptir. Ayrıca, *O. caput-galli* de yüksek beta karoten içeriğine sahiptir (73.3 ±. 94 µg / g). Öte yandan, *O. fallax*'ın en yüksek gamma-tokoferol içeriğine sahip olduğu belirlendi (1401.2 ± 8.76 µg / g). *O. viciifolia* (574.9 ± 2.35 µg / g), *O. caput-galli* (410.1 ± 4.56 µg / g), *O. oxyodonta* var. *armena* (267.7 ± 3.68 µg / g), *O. podporea* (162.5 ± 2.14 µg / g) diğer yüksek gama tokoferol içeriğine sahip taksonlardır. Diğer taraftan bu çalışmada retinol, retinol asetat ve r-tokoferol içerikleri bulunmamakta veya eser miktarda bulunmaktadır.

INTRODUCTION

Onobrychis Miller, is a member of the Fabaceae, includes about 170 perennial and annual species in two subgenera (Aktoklu 1995, Karamian et al. 2012, Avci et al. 2013). The genus distributed in Europe, Asia, North America and Africa (Yildiz et al. 1999, Pavlova and Monova 2000, Kaveh et al. 2019). Turkey is one of the most significant center of the genus and it is represented by 55 taxa which 28 of them are endemic (Duman and Vural 1990, Davis et al. 1988, Aktoklu 2001, Avci and Kaya 2013).

The members of Onobrychis Miller are important agricultural sources as a forage, fodder legume or ornamental (Ranjbar et al. 2010, Carbonero et al. 2011). The species of genus also used to improve the quality of the soil by serving fix atmospheric nitrogen and they contribute to the organic structure of soil with root systems (Ozaslan Parlak and Parlak 2008, Arslan and Ertugrul, 2010, Yildiz and Ekiz 2014). Biochemical studies performed Onobrychis Miller taxa showed that the genus have antioxidant, antibacterial and antifungal effects (Karakoca et al. 2015, Karamanian and Asadbegy, 2016, Bektas et al. 2018). However, these biochemical studies of genus extremely limited. Therefore, it was aimed to contribute the such studies of Onobrychis Miller by determining the lipide-soluble vitamins in this study.

MATERIAL AND METHODS

Collection of plant materials

In the present study, lipid-soluble vitamin contents in mature seeds of the Onobrychis L. taxa were examined. Sample plants were gathered from the natural habitats and details about the materials are explained in table I.

Table 1. Localities of collected plant samples

Taxa	Section	Region	Locality	Altitude
O. hypargyrea Boiss.	*Hymenobryhis*	B2, Kutahya	Usak Gediz road Abide bridge locality	690 m
O. viciifolia Scop.	*Onobrychis*	B2, Usak	From Usak to Banaz 7th km	100m
O. cappadocica Boiss.	*Hymenobryhis*	B7, Elazig	Firat University Campus, Faculty of Engineering locality	1060 m
O. podporea Širj.	*Onobrychis*		Usak Gediz road 30. km	740m
O. caput-galli (L) Lam	*Lophobrychis*	B2, Manisa	3 km from Kula to Alasehir, Kula dam lake locality	731 m
O. galegifolia Boiss.	*Hymenobryhis*	B7, Elazig	Elazig-Harput road	1230m
O. fallax Freyn & Sint. ex Freyn var. *fallax*	*Onobrychis*	B7, Elazig	Firat University Campus, Faculty of Engineering locality	1060 m
O. oxyodonta Boiss.var. *armena* (Boiss. & Huet) Aktoklu	*Onobrychis*	B2, Usak	Usak between Akarca	972m

Extraction of plant materials

1 g seed used to analyse the lipide-soluble vitamin contents. The seeds are finely ground in a mill and were then extracted with hexane/isopropanol (3:2 v/v) (Hara and Radin, 1978). Extracts were centrifuged at 10.000 g for 5 minutes and filtered. The solvent was then removed on a rotary evaporator at 40°C. After that lipid-soluble vitamins were extracted based on the method of Sánchez-Machado (2002) with minor modifications. The experiment was repeated three times.

Chromatographic analysis and quantification of lipid-soluble vitamins

Seed materials were dissolved in acetonitrile/methanol (75/25 v/v) and were injected 50 µL to HPLC instrument (Shimadzu, Kyota Japan). Supelcosil TM LC18 (250 x 4.6 mm, 5 mm, Sigma, USA) was used as column. The mobile phase was acetonitrile/methanol (75/25 v/v) and the elution was performed at a flow-rate of 1 ml/min. The temperature of analytical column was maintained at 40 °C. Detection was conducted at 320 nm for retinol (vitamin A) and retinol acetate, and 215 nm for δ-tocopherol, vitamin D2 and D3, α-tocopherol, α-tocopherol acetate, 235 nm for vitamin K1. Identification of the individual vitamins were performed by frequent comparison with authentic external standard mixtures analyzed under the same conditions. Class Vp 6.1 software assisted at workup of the data (Yilmaz et al. 2007). The results of analysis were expressed as µg/g for samples.

RESULTS

The lipide-soluble vitamin contents of studied Onobrychis species were given in table 2.

Table 2. The lipide-soluble vitamin contents of studied *Onobrychis* species

Taxa	Lipide-soluble vitamins (µg/g)									
	Beta carotene	Gamma tocopherol	R-tocopherol	D2	D3	a-tocopherol	a-tocopherol acetate	K1	Retinol	Retinol acetate
O.hypargyrea	809.7±5.03	33.3±.97	0.7±0.05	3.3±0.2	51.2±1.12	22.2±2.64	10.2±0.97	4.9±0.24	0.4±0.15	0.3±0.01
O. viciifolia	399.7±3.54	574.9±2.35	1.4±0.06	0.3±0.1	64.2±2.28	1.8±0.4	0.8±0.02	5.2±0.33	0.2±0.11	0.4±0.03
O. cappadocia	-	-	0.1±0.01	2.7±0.5	55.4±0.97	0.9±0.02	0.2±0.01	6.3±0.52	1.0±0.12	0.5±0.01
O. podporea	-	162.5±2.14	0.3±0.01	1.1±0.3	39.3±0.75	8.0±0.7	-	3.7±0.21	0.4±0.05	0.6±0.02
O. caput-galli	73.3±0.94	410.1±4.56	-	-	36.7±1.1	0.4±0.01	0.6±0.02	-	0.3±0.07	0.3±0.03
O. galegifolia	-	-	3.0±0.7	15.0±1.1	55.2±2.1	6.1±0.3	-	-	0.9±0.08	0.7±0.03
O. fallax	916.0±4.51	1401.2±8.76	-	1.8±0.08	66.7±1.2	2.0±0.05	1.9±0.74	1.8±0.13	0.8±0.03	0.9±0.03
O. oxyodonta var. armena	1777.2±6.24	267.7±3.68	-	-	58.3±1.04	2.0±0.04	0.6±0.1	1.6±0.1	0.4±0.06	0.6±0.01

It was found that O. hypargyrea, O. viciifolia, O. caput-galli, O. fallax and O. oxyodonta var. armena have high lipide-soluble vitamin content based on results of this study (table 2). Present study showed that O. oxyodonta var. armena (1777.27±6.24 µg/g), O. fallax (916.0±4.51 µg/g) O. hypargyrea (809.7±5.03 µg/g) and O. viciifolia (399.7±3.54 µg/g) have quite highest beta-caroten content. O.caput-galli has high beta caroten content (73.3±.94 µg/g). It was found that O. fallax has highest gamma-tocopherol content (1401.2±8.76 µg/g). In addition to, O. viciifolia (574.9±2.35 µg/g), O. caput-galli (410.1±4.56 µg/g), O. oxyodonta var. armena (267.7±3.68 µg/g), O. podporea (162.5±2.14 µg/g) high gamma-tocopherol content. Furthermore, O. hypargyrea has low gamma tocopherol content (33.3±.97µg/g) while O. cappadocica and O. galegifolia don't have gamma tocopherol content. Furthermore, present study showed that O. taxa have D3 vitamin content between 66.7±1.2 µg/g (O. fallax) and 36.7±1.1 µg/g (O. caput-galli). A-tocopherol content of studied O. species range from 0.4±0.01 µg/g (O. caput-galli) to 22.2±2.64 µg/g (O. hypargyrea). Also, O. hypargyrea has high a-tocopherol content 10.2±0.97 µg/g among studied O. species. Moreover, K1 content of O. species varied from 1.6±0.1 µg/g (O. oxyodonta var. armena) from 6.3±0.52 µg/g (O. cappadocia) except for O. caput-galli and O. galegifolia which don't have K1 content. Retinol and retinol acetate contents of O. species found lowest or trace amounts in the present study.

DISCUSSION

Legumes are consumed high levels especially Asia, Africa and South America (Frias et al. 2005) and studies showed that legumes have complex carbohyrates, vitamins, fibers, polyphenols (Oboh 2006, Amarowicz and Pegg 2008). These bioactive compounds play significant role many diseases such as cancer, diabetes (Frias et al. 2005, Arslan, 2017). Lipide-soluble phytonutrients such as carotenoids and tocopherols have been reported to inhibit the risk of cardiovascular, cancer, eye patologies and diabetes (Monge-Rajos and Campos 2011, Nadeau et al. 2013). Also, they have important roles in anti-inflammatory processes and immune system by scavenging cells against free radical damages (McDowell 2000, Chou et al. 2007, Fernandez-Marin et al. 2014).

Beta-carotene is considered to be pro-vitamin which has the ability to be converted into vitamin A (Hojer et al. 2012). Beta-carotene, is considered to be pro-vitamins because they have the ability to be converted into vitamins (vitamin A or retinol) by the animal (Hojer et al. 2012). On the other hand, vitamin E is, a lipophilic structure and major constituent of cell membrane (Kappus and Diplock 1992), externally intaken in foods or supplements because it isn't generating by humans (Berman and Brodaty 2004). Tocopherols have protective role against free radical damages in cells by interrupting the chain reactions (Bramley et al. 2000). Present study showed that some of studied Onobrychis species have highest beta-carotene and gamma-tocopherol contents. A study done Wyatt et al. (1998) showed that all of the legumes analyzed showed the presence of γ-tocopherol in relatively high levels, with the exception of black beans. Fernandez-Marin et al. (2014) found that of all tocopherols, γ-tocopherol was the most abundant isoform in all species, apart from Vigna and Arachis, where δ−tocopherol and α-tocopherol were the main isoforms, respectively. Also, they found that total carotenoids were between 0.9±0.2 µg/g and 17.7±2.2 µg/g (Fernandez-Marin et al. 2014). Another study done by Boschin and Arnoldi (2011) showed that legume seeds have 0.3-2.99 mg/100 g tocopherol content. It was reported that legumes have contain only γ-tocopherols (86.1–146.8 mg/kg) study done by Cho et al. (2007). Also, Cho et al. (2007) determined the carotene content of legumes is 9.2±10 mg/kg). El-Qudah (2014) identified legumes including Vicia, Lens, Phaseolus and Cicer have appreciable amounts of carotenoid. However, Mamatha et al. (2011) found that studied legumes including Phaseolus, Vigna, Lens and Cicer have lowest a-and b-carotene contents. A-tocopherol content of O. was found between 22.2±2.64 µg/g and 1.8±0.4 µg/g while K1 content of O. was found between 1.6±0.1 µg/g and 10.2±0.97 µg/g (except for O. caput-galli and O. galegifolia which don't have K1 content) in present study. Arslan (2017) indicated that legumes include K vitamin together with vitamin B1, B2, B6, vitamin C, vitamin E. Furthermore, it was found that studied O. species have high D3 content (66.7±1.2-36.7±1.1 µg/g) in this study. Sahin et al. (2009) found that Lathyrus taxa, the other genus of legumes, have high vitamin D3. Also, they determined that Lathyrus has high δ-tocopherol, α-tocopherol, α-tocopherol acetate contents (Sahin et al. 2009). On the other hand, present work demonstrated that r-tocopherol, retinol, retinol acetat, vitamin D2 (except for O. galegifolia) contents of O. has lowest. Similarly, Sahin et al. (2009) found that retinol, retinol acetate, vitamin D2 were trace amounts in their work.

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