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Original scientific paper

FATTY ACID AND TRACE ELEMENT COMPOSITIONS OF THE SEEDS OF DIFFERENT *Onobrychis viciifolia* GENOTYPES

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Seed oil contents, fatty acid compositions and mineral contents of twenty *Onobrychis viciifolia* genotypes (sainfoin) were investigated in this study. The fatty acid composition of *O. viciifolia* genotypes had different saturated and unsaturated fatty acids. For saturated fatty acids, *O. viciifolia* genotypes contained palmitic and stearic acids as the major component and contained small amount of myristic and arachidic acids. The major unsaturated fatty acids were identified as oleic, linoleic and linolenic acids. Total saturated fatty acids contents varied between 10.50 and 14.28% and total unsaturated fatty acid contents varied between 85.72 and 89.50%. Co, Mn, Fe, Cu, Ni and Zn were detected in crop seeds in different amounts. The proximate analysis indicated that seed Co contents varied between 13.53 and 114.83 ppm, Mn contents between 28.14 and 97.20 ppm, Fe contents between 113.7 and 277.7 ppm, Cu contents between 7.19 and 12.24 ppm, Ni contents between 1.14 and 19.73 ppm and Zn contents between 26.03 and 52.39 ppm, respectively. The result of this study suggest that further studies are obligatory to invigorate in terms of animal nutrition and phytochemicals of *O. viciifolia*.

Keywords: Legume seeds, *O. viciifolia*, genotypes, fatty acid, trace elements

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INTRODUCTION

Fatty acids consumed through daily diets may contribute to cardiovascular diseases, cancer, diabetes and degenerative diseases (TAPIERO *et al.*, 2002). Excessive unsaturated fatty acids of herbal oils and their ω3 and ω6 fatty acids on the other hand may reduce the negative impacts and have positive impacts on human health (KIRSHENBAUER, 1960). Just because of positive health impacts, several studies have been conducted about fatty acid compositions of various plants (KARATAS, 2013). Minerals are essential component of life and they are required for the function of enzyme systems (OZLU *et al.*, 2012). Low-income rural and urban population groups meet their mineral requirements mostly from plan originated foodstuffs (MONASTERIO *et al.*, 2007). Therefore, researchers conducted mostly to improve nutritional values of plants through cultural practices and genetic selections (WHITE and BROADLEY, 2005).

O. viciifolia hay are quite rich in protein and thus widely used in animal feeding. Grains are also used as concentrate feed (TAN and SANCAK, 2009). Historically, the high-protein sainfoin seeds have only been fed in times of severe feed shortage (BALDINGER *et al.*, 2016). Despite to high protein contents, sainfoin seeds are poor in oils (TARASENKO *et al.*, 2015). However, sainfoin has a great potential to be an alternative oil source with various clinical advantages for human and animal nutrition. Both genetic structure and environmental conditions greatly influence oil quantity and quality of herbal oils (BAENZIGER *et al.*, 2001). Therefore, plant fatty acids should be assessed based on these factors.

Previous studies on complex processing characteristics, chemical composition and pharmacological attributes of sainfoin seeds relieved that sainfoin could reliably be used as an alternative source of protein, dietary fiber and valuable lipids. Such a case than proves that it was possible to conduct research on potential use of sainfoin seeds in food industry. (TARASENKO *et al*., 2015).

The present study was conducted to characterize 20 different sainfoin (*O. viciifolia*) genotypes with regard to fatty acid and trace element compositions.

MATERIALS AND METHODS

Plant samples

In this study, 20 sainfoin (*O. viciifolia*) genotypes from Kahramanmaras, Kayseri, Kirsehir, Yozgat and Sivas provinces of Turkey were used as the plant material (Table 1).

Table 1. Codes and abbreviations of Onobrychis viciifolia genotypes

No	Code	Origin	No	Code	Origin
1	K2	Kayseri	**11**	K13	Kahramanmaras
2	K2-2	Kayseri	**12**	K13-2	Kahramanmaras
3	K4-5	Kayseri	**13**	K13-4	Kahramanmaras
4	K5-3	Kayseri	**14**	K16-1	Yozgat
5	K7-1	Kayseri	**15**	K16-2	Yozgat
6	K8	Kirsehir	**16**	K17-5	Yozgat
7	K8-3	Kirsehir	**17**	K18-5	Sivas
8	K8-6	Kirsehir	**18**	K19	Sivas
9	K9-3	Kahramanmaras	**19**	K19-1	Sivas
10	K10-1	Kahramanmaras	**20**	KM-5	Sivas

The seeds supplied from gene banks were sown and propagated under controlled conditions of Kayseri province of Turkey (39°48'N, 38°73'E). Resultant seeds were subjected to relevant analyses.

Oil extraction and preparation of fatty acid methyl esters (FAME)

2g seed material of *Onobrychis viciifolia* genotypes was homogenized in 40mL of chloroform/methanol (2:1) at magnetic stirrer for 1 hour and waited at refrigerator for 1 night. The upper part was removed and placed in a 50mL glass cylinder by filtration. Non-fat impurities such as protein, carbohydrate and amino sugars were removed by washing with 1:8 ratio 0.9% NaCl. The lower layer was collected and the total oil containing chloroform phase was removed glass ball for evaporated under vacuum at 40°C on a rotary evaporator (FOLCH *et al.*, 1957). Fatty acid methyl esters were obtained according to the recommendation of Agilent Technologies with a minor modification of the method described by ICHIHARA *et al.,* (1996). For this, the total oil contained in the glass ball of rotary evaporator was dissolved by shaking with 2mL of n-hexane for one or two minutes. After transferring the mixture to a 5mL capped plastic bottle, added 100µL 2 M KOH in methanol and vortexed for 1 minute, centrifuged for 5 minutes at 3000 rpm. The resulting supernatant was transferred to a 2mL vial and ready for GC analysis.

Capillary GLC

Analysis of individual fatty acid methyl esters was carried out on a gas chromatograph (Agilent Technologies 7890A GC/5975C MS) with a Optima delta -6 brand 60 m x 0.25x 25 µm ID column. The device began to read fatty acids at 120°C, with a ramp rate of 5°C/min, until temperature reached 250°C; a hold time at this temperature was 3 min. Then 2°C/min reached 220°C and still finished the reading process by waiting for 16 minutes at this temperature. The device was run in splitless mode and the injection volume was 1µL. Nitrogen gas was used as carrier gas.

Analysis of Mineral Elements in Seeds

Trace element content of Seeds of *O. viciifolia* genotypes were determined regarding acid digestion procedure. Briefly, seeds were washed and dried for 48 h at 65°C and ground to pass through a 60 mesh screen. Sample preparation was performed using the Mars 5 Microwave Digestion System (CEM, Kamp-Lintfort, Germany). After adding 500 mg 250-350 mg of sample and 10 mL nitric acid (65%), the samples were predigested for a period of 20 min. The basic microwave digestion in this procedure was as follows: temperature–time ramp for 20 min with a final temperature of 180°C (356°F), then 20-30 min hold time at 1200 W for more than six vessels. Following this, the samples were cooled to room temperature and the vessels were uncapped. The clear sample solutions were transferred to a volumetric flask 50 mL and filled with ultrapure water (XING and YENEMAN, 1998). After get digestion, Mn, Co, Fe, Cu, Ni and Zn contents of the seed samples were determined using an ICP OES spectrometer (PerkinElmer, Optima 2100 DV, ICP/OES, Shelton, CT 06484-4794, USA) (MERTENS, 2005).

Statistical Analysis

Experiments were conducted in randomized complete block design with 3 replications. SAS (SAS Inst., 1999) software was used to perform variance analysis on all experimental data

accordance with split plots experimental design. LSD test was used to test the significance of differences among the means.

RESULTS AND DISCUSSION

In this study, oil content and fatty acid compositions of the seeds of 20 different *O. viciifolia* genotypes were determined and the results are shown in Table 2. The genotypes had significant effects on the oil content and fatty acid compositions ($P \leq 0.01$). Oil contents of *O. viciifolia* genotypes varied between 3.18 and 5.11% with the greatest values in K11 (5.11%), K13-2 (4.84%), K19-1 (4.64%) and K9-3 genotypes (4.62%) and the least value in K8 genotype. Gas chromatographic analysis of the oil of *O. viciifolia* revealed that the fatty acid composition was composed of 7 different fatty acids. The carbon numbers of these fatty acids range from 14 to 20. The main components in the seed oils of these genotypes are palmitic, oleic, linoleic and linolenic acids.

Myristic acid was detected between 0.19% and 0.36% in other genotypes (K2, K2-2, K4-5, K5-3, K8, K8-3, K8-6, K13, K13-4, K16-1, K17-5, K19 and KM-5) except for K7-1, K9-3, K10-1, K13-2, K16-2, K18-5 and K19-1 genotypes. From the data presented it could be seen that the highest myristic acid was found in KM-5 genotype, while the lowest percentage was found in K2-2 and K19 genotypes. These results agree with finding of BAGCI *et al*. (2004), in which ranging from 0.2% to 0.3% in *O. altissima, O. hypargyrea* and *O. huetiana*. The same researchers have found 0.9% of myristic acid in *O. major*. On the other hand, the values we obtained about myristic acid were found to be lower than those obtained by some research (VIANO *et al.*, 1995; BAKOGLU *et al*., 2009; KARATAS, 2013). It is thought that this is due to the different species used in the research.

It was evident from our results that in the seed oil of studied *O. viciifolia* genotypes, palmitic acid and stearic acid were major saturated fatty acids. The palmitic acid was at the highest level in K8-6 (12.53%), K13 (12.03%) and lowest level in K19-1 (10.11%), K10-1 (10.50%) and K7-1 (10.55%). The palmitic acid values we have acquired were lower than those acquired by VIANO *et al*. (1995) as 32.7%, KOCAK *et al*. (2011) as 17.95% and KARATAS (2013) as 22.67%; but higher than those of BAGCI *et al*. (2004) as 4.9-8.8% and BAKOGLU *et al*. (2009) as 8.95%.

Stearic acid, the second major saturated fatty acid component, was found only in the K2-2 (2.83%) and K5-3 (2.67%) genotypes of the studied genotypes. These results were in agreement with BAGCI *et al*. (2004) who reported that stearic acid was detected from *O. major, O. huetiana* and *O. altissima* as 2.1, 2.2 and 1.8%, respectively. On the other hand, some research reported that stearic was found to be 6.13% in *O. saxatilis* (VIANO *et al*., 1995), 4.82% in *O. fallax* (BAKOGLU *et al*., 2009), 4.79% in *O. crista-galli* (KOCAK *et al*., 2011) and 5.87% in *O. armena* (KARATAS, 2013). The values found by these researchers were higher than our values.

The results in Table 2 also indicate that the contents of oleic acid were detected between 29.00% and 37.87%. Oleic acid was the highest unsaturated fatty acid (USFA) in KM-5 genotype (37.87%) and K9-3 genotype (36.76%). The lowest percentage of oleic acid was found in K2-2 genotype. While the values we obtained for oleic acid were higher than the values of some researches (VIANO *et al*., 1995; BAGCI *et al*., 2004; KOCAK *et al*., 2011 and KARATAS, 2013), they were lower than the values of BAKOGLU *et al*. (2009) as 52.56%. Many studies on fatty acid compositions of Fabaceae have similarly reported that oleic acid is the major contributor to TUSFA content (UZUN *et al*., 2007 and AYAZ *et al*., 2009).

Linoleic acid, an unsaturated ω6 fatty acid with two double bonds, was ranged from 15.13-23.88%, which was lower than those given by BAGCI *et al*. (2004) as 31.5-51.8% and KOCAK *et al*. (2011) as 59.4% and higher than those given by VIANO *et al*. (1995) as 12.9% and KARATAS (2013) as 15.09%. The highest linolenic acid was obtained in K7-1 genotype with 41.22%, while the lowest linolenic acid was obtained in K9-3 genotype with 31.84%. The values we obtained for linolenic acid, an essential ω3 fatty acid, were higher than the values of some scientists (VIANO *et al*., 1995; BAGCI *et al*., 2004; KOCAK *et al*., 2011 and KARATAS, 2013). Arachidic acid was found in all genotypes except for K2, K2-2, K5-3, K7-1, K9-3, K10-1, K16-2 and K18-5 genotypes (Table 2). The arachidic acid was ranged 0.34-0.94%, which was agreement with those results given by VIANO *et al*. (1995), BAGCI *et al*. (2004) and KOCAK *et al*. (2011).

Table 2. Fatty acid composition of studied samples (%)

Genotypes	CO	14:00	16:00	18:00	18:01	18:02	18:03	20:00	TSFA	TUSFA
K2	3.44^{j}	0.26^{c}	11.95^{c}	0.00c	34.34^{d}	19.88^{fgh}	33.57^{o}	0.00^{h}	12.21^{h}	87.79i
K2-2	3.33^{k}	0.19^{h}	10.95^{j}	2.83^{a}	29.00^{r}	23.88^{a}	33.15^{p}	0.00^{h}	13.97^{b}	86.03^{o}
K4-5	4.28^{e}	0.20^{gh}	11.95^{c}	0.00^{c}	31.39^{k}	18.56^{klm}	37.40^{f}	0.50^{e}	12.65^{f}	87.35^{k}
K5-3	3.87^{h}	0.29^{b}	11.32^{g}	2.67^{b}	29.94^{q}	19.70^{f-i}	36.08^{j}	0.00^{h}	14.28^{a}	85.72^{p}
K7-1	3.88^{h}	0.00i	10.55^{m}	0.00^{c}	30.23^{p}	18.00^{lm}	41.22^{a}	0.00^{h}	10.55^{p}	89.45^{a}
K8	3.18^{l}	0.22^{def}	11.79^{d}	0.00^{c}	31.04^{l}	21.25^{cd}	34.76^{k}	0.94^{a}	12.95^{d}	87.05^{m}
K8-3	4.42^{d}	0.21^{efg}	11.26^{h}	0.00^{c}	32.74^{g}	20.74^{de}	34.71^{k}	0.34^{g}	11.81^{l}	88.19e
K8-6	3.75^{i}	0.24^{cd}	12.53^{a}	0.00^{c}	32.89^{f}	21.43^{c}	32.29^{q}	0.62^{b}	13.39^{c}	86.61^{n}
K9-3	4.62^{c}	0.00^{i}	11.33^{g}	0.00^{c}	36.76^{b}	20.07^{fg}	31.84^{r}	0.00^{h}	11.33^{m}	88.67^{d}
K10-1	5.11^{a}	0.00^{i}	10.50^{n}	0.00^{c}	30.53^{o}	19.48^{ghi}	39.49^{c}	0.00^{h}	10.50^{p}	89.50^{a}
K13	4.11^{f}	0.23^{de}	12.03^{b}	0.00^{c}	32.25^{h}	17.94^{m}	37.05^{g}	0.50^{e}	12.76^{e}	87.24^{l}
K13-2	4.84^{b}	0.00^{i}	10.99^{j}	0.00^{c}	31.86^{i}	20.25^{ef}	36.51^{h}	0.39^{f}	11.38^{m}	88.62^{d}
K13-4	4.13^{f}	0.23^{def}	11.66^{f}	0.00^{c}	34.27^{e}	19.21^{ij}	34.10^{n}	0.53^{d}	12.42^{g}	87.58^{j}
K16-1	4.27^{e}	0.21^{fgh}	10.87^{k}	0.00^{c}	34.52^{c}	19.26^{hij}	34.21^{m}	0.93^{a}	12.01^{k}	87.99^{f}
K16-2	4.38^{d}	0.00^{i}	12.07^{b}	0.00^{c}	34.55^{c}	19.12^{ijk}	34.26^{m}	0.00^{h}	12.07^{j}	87.93^{g}
K17-5	3.89^{h}	0.24^{cd}	11.73^{e}	0.00^{c}	31.73^{j}	15.13^{n}	40.67^{b}	0.50^{e}	12.47^{g}	87.53^{j}
K18-5	4.22^{e}	0.00^{i}	10.71^{l}	0.00^{c}	32.24^{h}	19.50^{ghi}	37.55^{e}	0.00^{h}	10.71^{n}	89.29^{c}
K19	4.00^{g}	0.19^{h}	10.60^{m}	0.00^{c}	30.84^{n}	23.21^{b}	34.60^{l}	0.56^{c}	11.35^{m}	88.65^{d}
K19-1	4.64^{c}	0.00^{i}	10.11^{o}	0.00^{c}	30.95^{m}	19.63^{jkl}	38.81^{d}	0.50^{e}	10.61^{o}	89.39^{b}
KM-5	4.12^{f}	0.36^{a}	11.20^{i}	0.00^{c}	37.87^{a}	13.68^{f-i}	36.32^{i}	0.57^{c}	12.13^{i}	87.87^{h}
Sig. Deg.	**	**	**	**	**	**	**	**	**	**
LSD	0.067	0.022	0.052	0.012	0.067	0.638	0.047	0.019	0.055	0.053

C14:0 Myristic acid; C16:0 Palmitic acid; C18:0 Stearic acid; C18:1 Oleic acid; C18:2 Linoleic acid; C18:3 Linolenic acid; C20:0 Arachidic acid; TSFA: Total saturated fatty acid; TUSFA: Total unsaturated fatty acid; **: P≤0.01; Sig. Deg.: Significant degree; LSD: Least significant difference

Total saturated fatty acid (TSFA) of studied *O. viciifolia* genotypes was between 10.50% and 14.28% (Table 2). From the table presented it could be seen that the highest TSFA was found in K5-3 genotype, while the lowest percentage was found in K10-1 genotype. Total unsaturated fatty acid (TUSFA) of studied *O. viciifolia* genotypes was between 85.72% and 89.50%. K10-1 genotype has highest level of TUSFA; also in the K7-1 genotype (89.45%), K19-1 genotype (89.39%) and K18-5 genotype (89.29%). The lowest percentages of TUSFA were found in K5-3 genotype. The values we acquired related to TSFA and TUSFA were agree with BAGCI *et al*. (2004) and BAKOGLU *et al*. (2009) and disagree with VIANO *et al*. (1995), KOCAK *et al*. (2011) and KARATAS (2013).

Table 3. Mineral content of studied samples (ppm)

Genotypes	Mn	Co	Fe	Cu	Ni	Zn
K2	61.62^{e}	166.97^{a}	133.40^{j}	7.19^{k}	12.95^{e}	33.52^{ef}
K2-2	55.90^{f}	41.10^{jk}	138.00^{j}	8.64^{hij}	14.81^{d}	43.60^{b}
K4-5	55.23^{f}	46.83^{i}	131.00^{j}	9.80^{d-g}	18.08^{b}	31.79^{g}
K5-3	43.58^{i}	23.57^{n}	193.10^{h}	9.45^{fgh}	19.73^{a}	27.96^{i}
K7-1	38.34^{k}	22.13^{n}	113.70^{k}	9.24^{ghi}	18.38^{b}	31.34^{g}
K8	52.34^{g}	31.36^{l}	151.50^{i}	9.03^{ghi}	16.38^{c}	28.12^{i}
K8-3	46.26^{h}	13.53^{o}	219.30^{fg}	7.74^{jk}	6.51^{h}	37.03^{d}
K8-6	42.48^{ij}	28.90^{m}	214.40^{g}	8.52^{ij}	6.04^{h}	29.71^{h}
K9-3	35.01^{l}	22.03^{n}	212.60^{g}	10.42^{cde}	2.48^{k}	31.37^{g}
K10-1	28.14^{m}	54.00^{g}	229.20^{ef}	10.55^{bcd}	3.62^{j}	32.28^{fg}
K13	42.26^{ij}	57.20^{f}	237.60^{e}	11.34^{ab}	2.21^{k}	32.09^{fg}
K13-2	41.76^{j}	39.40^{k}	238.20^{e}	12.24^{a}	5.05^{i}	34.42^{e}
K13-4	46.63^{h}	87.20^{d}	260.90^{cd}	11.39^{ab}	4.60^{i}	52.39^{a}
K16-1	97.20^{a}	105.47^{c}	254.60^{d}	12.17^{a}	6.34^{h}	36.95^{d}
K16-2	73.07^{c}	114.83^{b}	265.70^{bc}	10.88^{bc}	11.67^{f}	38.73^{c}
K17-5	94.23^{b}	46.03^{i}	277.70^{a}	10.36^{c-f}	10.86^{g}	40.19^{c}
K18-5	61.99^{e}	71.53^{e}	274.10^{ab}	8.67^{hi}	4.27^{ij}	34.17^{e}
K19	65.63^{d}	50.63^{h}	274.60^{ab}	8.43^{ij}	1.14^{l}	36.23^{d}
K19-1	53.25^{g}	88.50^{d}	272.30^{ab}	9.51^{e-h}	6.46^{h}	26.03^{j}
KM-5	45.45^{h}	41.60^{j}	222.30^{fg}	10.27^{c-f}	1.93^{k}	36.21^{d}
Sig. Deg.	**	**	**	**	**	**
LSD	1.44	1.88	10.73	0.91	0.78	1.50

**: P≤0.01; Sig. Deg.: Significant degree; LSD: Least significant difference

Mineral contents of *O. viciifolia* genotypes are provided in Table 3. Six elements (Co, Mn, Fe, Cu, Ni and Zn) were detected in the crop seeds in different amounts. The genotypes had significant effects on the mineral contents ($P \leq 0.01$). There were highly significant differences in mineral contents of the genotypes ($P < 0.01$). Co contents of *O. viciifolia* genotypes varied between 13.53 and 114.83 mg/kg, Mn contents between 28.14 and 97.20 mg/kg, Fe contents between 113.7 and 277.7 mg/kg, Cu contents between 7.19 and 12.24 mg/kg, Ni contents between 1.14 and 19.73 mg/kg and Zn contents between 26.03 and 52.39 mg/kg. These findings related to iron and nickel was in agreement with the findings of BAKOGLU *et al*. (2009), which found that 4.62 ppm of nickel and 136.88 ppm of iron were found in *O. fallax*. On the other hand, the same researches detected low manganese (30.04 mg/kg), copper (2.55 mg/kg) and zinc (14.48 mg/kg) from our findings (BAKOGLU *et al*., 2009).

In conclusion, it was found that all *O. viciifolia* genotypes studied in this research had high TUSFA content and that K10-1 and K7-1 genotypes had highest TUSFA content among them. Oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3) were major unsaturated fatty acid in twenty studied *O. viciifolia* genotypes. In addition, palmitic acid (C16:0) was found a major saturated fatty acid component in the studied twenty genotypes. Moreover, K2-2 and K5-3 genotypes had similar fatty acid compositions. *O. viciifolia* genotypes exhibited quite different characteristics with regard to their mineral contents. K16-2 genotype was found to be prominent for Co content, K16-1 for Mn, K17-5 for Fe, K13-2 for Cu, K5-3 for Ni and K13-4 for Zn.

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KOMPOZICIJE MASNIH KISELINA I MIKROELEMENTA SEMENA RAZLIČITIH *Onobrychis viciifolia* GENOTIPOVA

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Izvod

U ovom istraživanju ispitivani su sadržaji ulja u semenu, kompozicije masnih kiselina i mineralni sadržaj dvadeset genotipova esparzete *Onobrichis viciifolia*. Sastav masnih kiselina genotipova *O. viciifolia* imao je različite zasićene i nezasićene masne kiseline: od zasićenih masnih kiselina, genotipovi *O. viciifolia* sadrže palmitinsku i stearinsku kiselinu kao glavnu komponentu i malu količinu mirističkih i arahidnih kiselina, dok su glavne nezasićene masne kiseline oleinska, linolna i linolenska kiselina. Sadržaj ukupnih zasićenih masnih kiselina varirao je između 10,50 i 14,28%, a ukupni sadržaj nezasićenih masnih kiselina varirao je između 85,72 i 89,50%. Co, Mn, Fe, Cu, Ni i Zn detektovani su u semenu useva u različitim količinama. U neposrednoj analizi utvrđeno je da se sadržaj Co u semenu varira između 13,53 i 114,83 ppm, sadržaj Mn između 28,14 i 97,20 ppm, sadržaj Fe između 113,7 i 277,7 ppm, sadržaj Cu između 7,19 i 12,24 ppm, sadržaj Ni između 1,14 i 19,73 ppm i sadržaj Zn između 26.03 i 52.39 ppm. Rezultati ovog istraživanja ukazuju na neophodnost daljih istraživanja esperzete za ishranu životinja i fitohemikalija.

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