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Effect of Temperature and Light (UV, IR) on Flavonol Content in Radish and Alfalfa Sprouts

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In this study, the concentrations of four flavonols (morin, myrycetin, quercetin and kaempferol) were determined in radish and alfalfa sprouts. Sprouts were germinated in total darkness and various temperatures (4°C, 20°C, 30°C) and light conditions (UV, IR) According to germination conditions, different amounts of flavonols in sprouts were observed. Total content of flavonols varies within 55.39-216.20 $\mu g \cdot g^{-1}$ and 382.16-721.05 $\mu g \cdot g^{-1}$ dry weight for radish and alfalfa sprouts, respectively. A highest concentration of flavonols was determined in sprouts germinated in darkness and 20°C. The results indicated that alfalfa sprouts are a rich source of kaempferol – 580 μg in 1g of lyophilized product.

Key words: Flavonols, radish, alfalfa, sprouts, functional food.

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Many epidemiological, laboratory and clinical trial data indicate that a plant-based diet can reduce the risk of chronic disease, the so called "civilization diseases" (WHO/FAO 2002, 2003).

Recently functional foods containing physiologically-active components either from plant or animal sources are gaining ground, which probably may enhance human health (INTERNATIONAL FOOD INFORMATION COUNCIL 2004).

The most important plant components of functional food are: lycopene, catechins, glucosinolanes and flavonoids. Dietary flavonoids have shown many biological properties that may account for the prevention of many diseases. They may reduce the risk of cancer, heart disease, asthma, and stroke (CHUN *et al.* 2004; BIRT *et al.* 2001; CALZUOLA *et al.* 2004; LAKO 2004; STEIN *et al.* 1999). Moreover they have anti-inflammatory and anti-allergic properties (DI CARLO *et al.* 1999; HARBORNE & WILLIAMS 2000).

Vegetables are a major source of flavonoid compounds in the diet. For example onions and lettuce contain significant amounts of quercetin, while broccoli is the major source of kaempferol (MANACH *et al.* 2004; LUGASI & HOVARI 2000; US DEPARMENT OF AGRICULTURE 2003). Flavonoids also occur in seeds and sprouts of many plants (TAKAYA 2003).

The aim of this study was to determine the content of four flavonols: myricetin, morin, quercetin and kaempferol, in alfalfa and radish seeds and sprouts germinated under variable conditions. Seeds were germinated in total darkness, temperature 20°C with and without an UV and IR light period, and at a temperature of 4 and 30°C without light.

Material and Methods

C h e m i c a l s. Methanol was obtained from Przedsiębiorstwo Chemiczne (Poland), hydrochloric acid from Przedsiębiorstwo Przemysłowo-Handlowe "Standard" (Poland), acetonitrile of HPLC-grade, Super-grade, PoCh (Poland), trifluoroacetic acid (TFA) from Sigma-Chemical (USA), myricetin and kaempferol from Fluka BioChemica, morin from Sigma-Aldrich (U.K.) and quercetin from Merck (Germany). All chemicals were analytical grade.

S e e d s. Research material included radish – *Raphanus sativus* and alfalfa – *Medicago sativa* seeds suitable for producing sprouts (DIET-FOOD, Warsaw, Poland) purchased in a local market with "healthy food".

T r e a t m e n t s. Five different treatments with various germination conditions were performed (Table 1). Each sample of seeds in our experiment weighed 5 g and accumulated to 500±10 and 3750±50 seeds for radish and alfalfa, respectively. In the experiment with UV and IR light period, EAM 40-1 and EMTA-VT-410 (FAMED Łódź, Poland) lamps were used, which emitted electromagnetic light within the range of 200-400 nm and 780-1500 nm, respectively.

Table 1

Sprouting conditions of alfalfa and radish seeds

Number of sample	Conditions
1	total darkness (temp. +20°C)
2	total darkness (temp. +20°C) + UV (20 min/24 h)
3	total darkness (temp. +20°C) + IR (20 min/24 h)
4	total darkness, temp. +30°C (24h)
5	total darkness, temp. +4°C (24h)

S a m p l e p r e p a r a t i o n. Flavonoid content was determined in alfalfa and radish seeds (sample nr 0) and their sprouts, which were germinated in a plastic "sprouter". During growth, samples were washed with distilled water (20°C) once a day. Harvest was set in after four days of growth. Then plant sprouts were lyophilised and ground. Samples were stored in – 20°C until extraction and hydrolysis.

Freeze-dried material was extracted and hydrolysed. Approximately 0.2 g of lyophilised material was accurately weighed into a screw cap test tube. 2.5 ml 80% (v/v) methanol was added to the sample, shaken for 1 min on a vortex and placed for 10 min into an ultrasonic bath to improve extraction. Then 0.5 ml 4 mol·l^{-1} hydrochloric acid was added and placed in a water bath (98-100°C) for 1 h. After hydrolysis the samples were centrifuged for 10 min at 2000 g.

100 μl of supernatant was injected onto the chromatographic column. Alfalfa and radish seed samples were prepared in a similar manner. All treatments were carried out using two replicates and each sample was analyzed in triplicate.

C h r o m a t o g r a p h i c c o n d i t i o n s. HPLC with column Sephasil C_8 (250 * 4.6 mm, 5 μm) (Amersham Pharmacia Biotech., Sveden) was applied to the samples. Flavonols were separated at a flow rate of 1 ml·min^{-1} and a mixture of acetonitrile (ACN), water and trifluoroacetic acid (TFA) 25.4:73.6:1 (v/v) as the mobile phase. A UV-VIS detector was used, wavelength was set at 370 nm, 365 nm and 360 nm. Flavonol concentrations in samples were calculated using the corresponding standard calibration curve. In Table 2 the detection and quantitation limits of this method are shown.

Table 2

Detection and quantitation limits of flavonols by HPLC method

Parameter	Unit	Myricetin	Morin	Quercetin	Kaempferol
Detection limit (DL)	μg/ml	0.048	0.066	0.040	0.034
Quantitation limit (QL)	μg/ml	0.146	0.200	0.120	0.104

S t a t i s t i c a l e v a l u a t i o n. All determinations were carried out in three replications. Results were analysed by one-way analysis of variance (ANOVA) for each plant. Normality of a variable distribution and homogeneity of variances were tested with the Shapiro-Wilk and Levene Test, respectively.

Results and Discussion

The four flavonol aglycons were separated and determined simultaneously. The retention time of myricetin, morin, quercetin and kaempferol were: 6.87 min, 10.58 min, 15.17 min and 21.62 min respectively (Fig. 1A). Figure 1B depicts the chromatogram of alfalfa sprout extract after acid hydrolysis.

In Table 3 the results for each of the four flavonols and total flavonol content from the experiment with different germination conditions are shown. Alfalfa seeds contained all four flavonols, while in radish seeds only morin and kaempferol were determined (US DEPARTMENT OF AGRICULTURE 2003; SEGUIN *at al.* 2004). Total flavonol content in alfalfa seeds was 459.63 μg·g^{-1} dry weight and was almost 4-times greater than in radish seeds (125.59 μg·g^{-1} d.w.).

As shown in Table 3, the presence of three flavonols: quercetin, morin and myricetin depended on germination conditions, however kaempferol occurred in both plant seeds and sprouts regardless of experiment number.

Germination in the dark and 20°C caused a statistically significant increase (about 1.5-2-times) in flavonol content in sprouts in comparison to the seeds (sample 1). Raw radishes contained only

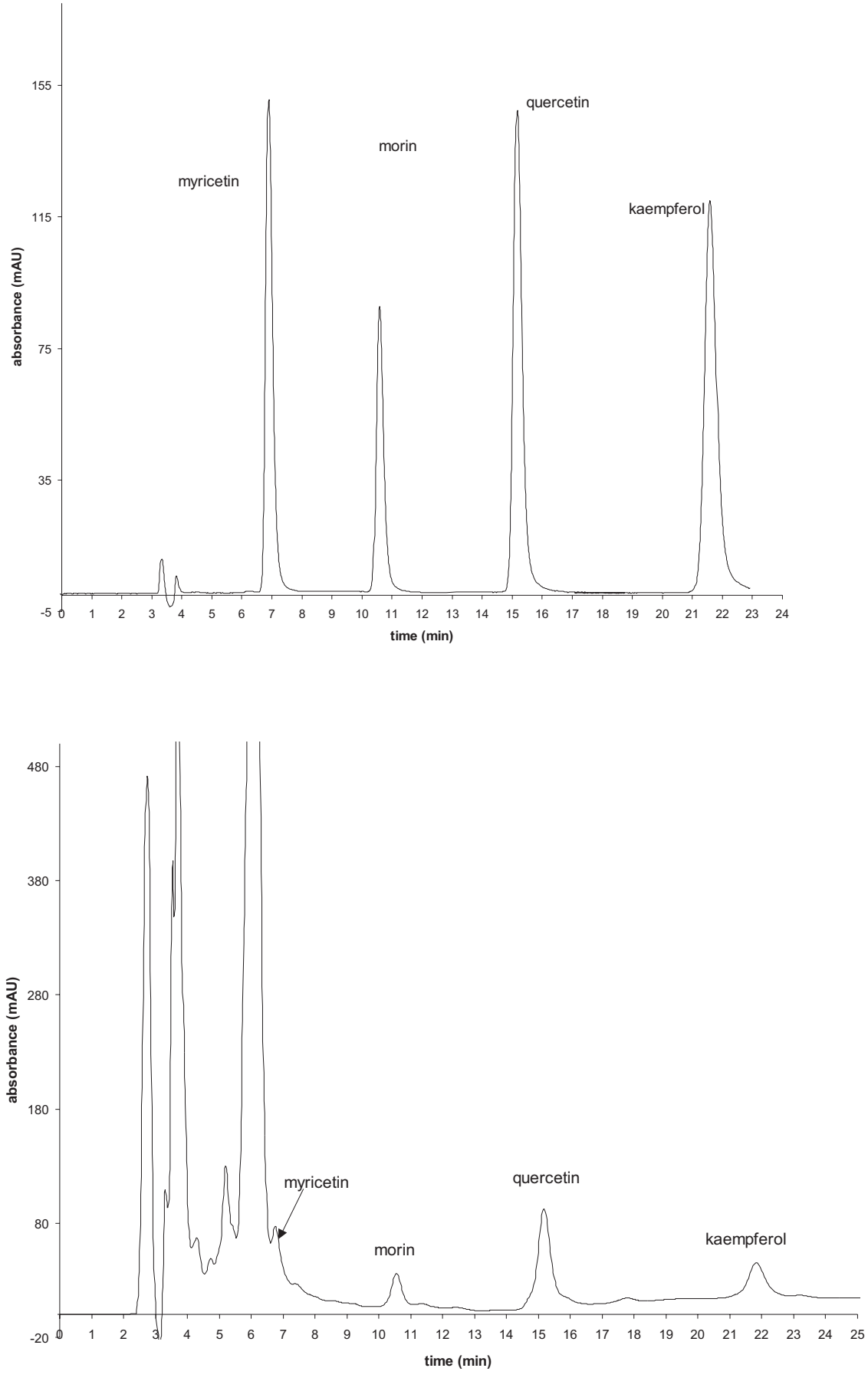

Fig. 1. A HPLC chromatogram of a standard flavonoid mixture (A) and alfalfa sprout extract (B)

Table 3

The effect of different germination conditions on the flavonols concentration in radish and alfalfa seeds and sprouts

(a) radish

Sample number	Content (μg/g d.w. ±SD)				
	Myricetin	Morin	Quercetin	Kaempferol	Total
0	ND	15.71±0.13	ND	109.88±26.61	125.59±26.73^{A,a}
1	ND	ND	ND	216.20±1.45	216.20±1.45^{A,b}
2	8.79±0.70	ND	ND	130.48±26.86	139.27±26.31^{A,a}
3	3.24±1.15	2.08±2.47	ND	126.13±30.68	131.39±28.58^{A,a}
4	ND	88.34±21.27	14.41±11.39	83.29±27.33	106.96±21.02^{A,a}
5	3.22±0.90	9.37±1.89	ND	42.84±4.74	55.39±4.93^{B,a}

(b) alfalfa

Sample Number	Content (μg/g d.w. ±SD)				
	Myricetin	Morin	Quercetin	Kaempferol	Total
0	33.70±3.35	45.87±6.97	7.65±12.71	372.40±49.77	459.63±42.82^{A}
1	116.02±5.48	8.10±1.33	15.97±7.91	580.96±126.72	721.05±136.57^{B,b}
2	33.50±29.08	13.90±3.24	4.25±2.11	330.51±49.48	382.16±57.66^{A,a}
3	68.06±13.37	ND	ND	518.77±156.83	586.83±143.57^{A,B}
4	51.14±18.05	2.48±0.86	ND	457.34±114.94	510.96±113.52^{A}
5	67.41±6.39	ND	32.84±8.35	450.19±97.67	550.44±88.79^{A,B}

0 – seeds; SD – standard deviation; ND – not detected; A,B – significant difference between experiments at $P<0.05$; a,b,c – significant difference between experiments at $P<0.01$.

kaempferol – 0.86 mg in 100g of edible product (US DEPARTMENT OF AGRICULTURE 2003), similarly to radish sprouts – 0.22 mg·g^{-1}/g dry weight (Table 3); however a better source of kaempferol are the sprouts. Alfalfa sprouts were a rich source of all four flavonols, particularly kaempferol (0.58 mg in 1g lyophilised material). This is comparable to the content of kaempferol in 10 g raw broccoli or 100 g raw grapefruit (US DEPARTMENT OF AGRICULTURE 2003).

In sample 2 and 3 irradiation (20 min/24h) of UV and IR light was used. No significant differences in total flavonols in radish sprouts were observed depending on wavelengths of light. UV light caused a significant ($P<0.05$) decrease of total flavonol content in alfalfa sprouts compared to IR light (experiments 2 and 3). In both samples 2 and 3 total flavonol content in radish sprouts was lower than in experiment 1.

In sample nr 4 sprouts in total darkness and elevated (30°C) temperature were grown, then sample 5 in temperature 4°C was carried out. The total flavonol content of alfalfa sprouts obtained from samples 4 and 5 is similar to the seeds and significantly lower than in sprouts growing in a temperature of 20°C.

Conclusions

The results presented here demonstrate that different conditions of seed germination can influence the flavonol content. The highest contents of these compounds were observed in sprouts growing in total darkness at 20°C. Both increasing and decreasing of germination temperature doesn't favourably affect the efficiency of flavonol synthesis. Similarly UV and IR light don't induce a significant increase of flavonol content in sprouts compared to seeds.

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