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Effect of light conditions on sulfur-containing phytochemicals from radish and mustard sprouts

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Abstract

In the recent years the experts dealing with the healthy nutrition turned their attention towards the determination of the biological value of sprouts. Nowdays, the consumption of germinated seeds became popular also in Romania. This study aim was to determine the effect of light conditions on the content of sulfur-containing phytochemicals of different varieties of radish and mustard. For that, red radish, white radish, black radish, white mustard and black mustard seeds were germinated under 24 h light and 24 h dark conditions, at 23 °C, for 7 days and glucosinolates along with S-methyl cysteine sulfoxide levels were determined and compared. These compounds are derived from amino acid biosynthesis and they appear to possess anticarcinogenic properties. Results showed that S-methyl cysteine sulfoxide is synthesised better under light condition meanwhile sprouts germinated in the dark, were characterized by a higher total glucosinolates content.

Keywords: glucosinolates, S-methyl cysteine sulfoxide, radish, mustard, sprouts

1. Introduction

Currently, the idea of anti-cancer protection by the means of foods it is very apealing, especially considering that in many tipes of cancer (eg. lung cancer) little progress was achieved by medicine [7]. Hence the consumers growing interest in a higher consumption of functional foodstuffs that contain health-promoting bioactive compounds.

It is communly accepted that sprouts have a higher nutritional value and they have long been consumed worldwide. During the germination process, the amount of the antinutritive compounds (trypsin inhibitor, phytic acid, pentosan, tannin) decreases and compounds with health-promoting effects and phytochemical properties are synthesized. Nowadays, the nutritional sprouts are new foodstuffs that can be produced and consumed without special product development, new appliances or expensive marketing [1].

Over the last decades, crops of the Brassicaceae family have been the focus of intense research based on their human health benefits [2] and studies point out sulfur-containing phytochemicals as being responsible [3,4]. One group of this sulphur compounds are known as glucosinolates and they have been the primerly focus of research in this area. However, another sulphur compound, S-methyl-

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cysteine sulphoxide it is known to have strong antimicrobial activity and appears to have been overlooked [5].

In the Brassicaceae family, mustards have been highly prized as medicinal plants as well as culinary spice being used since earlier times while radish is one of the most common vegetable consumed during spring in Romania.

Many studies have been conducted in order to investigate the factors that affect the distribution and content variation of both glucosinolates and their hydrolyzed products. Such factors include plant species, genotype, growth environment and post-harvest processing. Among this factors, the genotype and plant growth environment seem to have the most significant effects on the contents of individual glucosinolates and their hydrolyzed products. Furthermore, as plant growth environment being a vital factor, a lot of researches have been conducted to manipulate plant glucosinolates contents in order to make Brassica vegetables more beneficial to human health [6].

Most of the investigations concerning glucosinolates in developing plants are focused on the plant physiology and metabolic function of glucosinolates, primarily in the later stages of morphogenesis. Thus the studies investigating the obtaining of sprouts as food products with health-promoting phytochemicals are rare and comprise only a few species.

Also there are only a few investigations that have explored the S-methyl-cysteine sulphoxide (SMCSO). Some of the studies show that high concentrations of SMCSO in Brassica fodder can reduce palatability to livestock and cause toxicity [8] and other reports show anti-carcinogenic, anti-diabetic and cardiovascular effects in experimental animals. Recently, S-methyl-cysteine sulphoxide, has been identified as a biomarker of cruciferous vegetable intake in a human dietary intervention study and a potentially significant constituent of the human metabolome [5].

The main objective of this study was to investigate the effect of light conditions on the two important secondary metabolites (glucosinolates-GLs and S-methyl-cysteine sulphoxide – SMCSO) in different varieties of radish and mustard sprouts .

2. Materials and Methods

Plant Material: Seeds of white mustard (*Sinapsis Alba*), black mustard (*Brassica Nigra*), white radish and black radish (*Raphanus Sativus*) were purchased from different trade companies and a red type of Romanian radish – Red of Iernut (*Raphanus Sativus*), was kindly provided by the manager of the Vegetable Growing Research and Development Station of Iernut, Mureș county.

The seeds were germinated following Ciska et al`s (2008) [9] protocol with some modifications: 5 g of each seeds samples were soaked in 50 mL of distilled water at room temperature and shaken every 30 min. After 4 h, the water was drained off, and the seeds were lined (at 10 mm distance between them) on the germination bed prepared in a Linhard dish with water and filter paper. The seeds were germinated under 24h light and 24h dark conditions, at 23 °C, for 7 days. The 7 days old sprouts were gently and rapidly collected and stored at -20 °C until lyophylization. The samples were freeze-dried using a freeze dryer Alpha 1–2 Christ (Martin Christ, Osterode am Harz, Germany) then grinded in a fine powder and stored at at −4 °C until analysis.

Germination rate and humidity were recorded. The germination percentage was determined using the method described by the Romanian standard SR 1634:1999 and the humidity was determined with the lyophilization method.

Glucosinolates Analysis: The glucosinolates extraction method was conducted according to the EEC Regulation N1864/90 following Vicaș et al`s (2013) [10] protocol with minor modifications . Briefly, 200 mg of the liophylized samples were extracted with 5 ml hot methanol 70 % for 5 min at 80 °C, then homogenized using a high-speed PRO 400 tissue homogenizer (Pro Scientific Inc., Oxford, CT, USA) and centrifuged at 5,000 rpm for 20 min using a Hettich centrifuge (EBA20, Tuttlingen Germany). Aliquots of 1 ml extract were loaded twice on a mini-column filled with 0.6 ml DEAE-Sephadex A25 anion-exchange resin, conditioned with 25 mM acetate buffer (pH 5.6). After washing with 3 ml buffer, volumes of 200 µl purified sulphatase were loaded on each mini-column, left

overnight at room temperature. The desulfoglucosinolates were eluted with 3 ml of ultra pure water and then analyzed by HPLC. A known amount of glucotropaeolin (200 µl of 1 mg/ml) was added to each sample, as internal standard, before the first extraction, for quantitative determination of glucosinolates.

The HPLC quantification of the glucosinoles was done by an HPLC-PDA system (Shimadzu Corporation,Scientific Instruments, Kyoto, Japan) equipped with a CBM20A controller, LC-20 AD pump, a DGU-20A degaser, a SIL-20 AC autosampler, CTO-20 AC column oven and a SPD-M20A photodiode array detector. Desulfoglucosinolates were separated on a Platinum (C 18) 100 A column (250×4.6 mm,5 µm), at 30 °C, using a flow rate of 0.5 ml/min and an injection volume of 20 µl. The mobile phases consisted of water (eluent A) and acetonitrile (eluent B), using a gradient program as follows: 1 min 1 % B; 22 min linear gradient up to 22 % B; 10 min linear gradient down to 1 % B. Elution of desulfoglucosinolates was monitored at 229 nm.

Data were processed using a Labsolution version 5.10.153 (Shimadzu) software and desulfoglucosinolates were identified by retention time using standards, and the UV–vis spectra. The content of each GLs, expressed in µmol/g dry weight (DW) was calculated using glucotropaeolin as internal standard and considering the response factors of the other desulfoglucosinolates relative to the desulfo-glucotropaeolin.

S-methyl-cysteine sulphoxide Analysis: SMCSO was determined following Traka et al`s protocol (2013) [12] with some modifications. For the extraction of the SMCSO, 200 g of freeze- dryed powder was left to macerate overnight in 30 ml acidified cold. After that the plant material was thoroughly homogenized by using a high-speed PRO 400 tissue homogenizer (Pro Scientific Inc., Oxford, CT, USA) and incubated at 70°C for 10 min vortexing every 2–3 min. After centrifugation, the methanolic fraction was decanted into a separate tube and the remaining pellet was reextracted twice with 30 ml of boiling acidified methanol (each extraction was followed by 10 min incubation). The combined methanolic extracts were concentrated to 2–3 ml under reduced pressure (40°C) and adjusted to 5 ml with 20 mM borate buffer (pH 9.2). The extract was stored at -20°C until derivatization.

For the derivatization 100 µl of the sample extracts were mixt with 250 µl of Dansyl-Chloride reagent (10 mM dansyl chloride in acetonitrile) and 650 µl of 20 mM borate buffer (pH 9.2). The mixture was briefly shaken, allowed to stand at room temperature for 30 min, centrifuged at 16 200 g for 10 min and analysed by HPLC-DAD/MS using the positive polarity mode.

Dansyl derivatives were analysed using a Spherisorb ODS2 (250 x 4.6 mm 5 µm) column (Waters, Milford, MA, USA) connected to an 1100 HPLC system (Agilent Technologies, Waldbronn, Germany) comprising a binary pump, degasser, cooled autosampler, column oven, diode array and mass spectrometer detectors. Samples were eluted at 0.9 ml/min with a gradient of increasing methanol using 50 mM ammonium acetate buffer (pH 5) as solvent A and methanol as solvent B. The gradient started at 30% solution B increasing to 40% after 35 min and to 75% B after 60 min were it was maintained for 5 and finally re-equilibrated to 30% B for 5 min.

Dansyl derivatives were monitored at 250 nm, full scan and selecting ion monitoring mode. Results were expressed as µmols/g DW.

Statistical Analisys: All of the experiments were conducted in duplicate and all of rge statistical analysis were performed by Analysis of Variance (ANOVA) test using the general linear model. Tukey's honest significance test was carried out at a 95% confidence level ($p< 0.05$). The contribution percentage of each factor and interaction was calculated using eta-square. The Pearson's correlation ($\alpha=0.05$) with two-tailed probability values was used to check the correlation between the two sulphur containg phytochemicals. Statistical analysis of the data was performed by Minitab Statistical software version 16.1.0 (LEAD Technologies, Inc.).

3. Results and discussion

In the matter of sprouting seeds, the germination rate and humidity are important data, especially from the economic point of view. Such data, for the studied samples, are presented in table 1.

The light conditions used during seeds germination had no significant effect on the germination rate, humidity or the dry weight of the samples, however the type and variety of samples had significant influence on these parameters. The highest germination rates were registered by the white radish and white mustard samples (~98%) and the lowest germination rate by the black mustard samples (~20%). This low germination rate may be given because of a poor quality of the seeds. White and black radish had around 91% humidity and red radish, white mustard and black mustard around 90% humidity. A good and profitable germination rate it is considered above 90%. Similar results regarding the germination rates and dry weight were foynd by Ciska E. et al., 2008 [9] and Gaofeng Y. et al., 2010 [13].

Seven GLs were identified in the radish sprouts: four aliphatic, glucoraphenin (GRE), progoitrin (PRO), glucoiberin (GIB) and glucoraphanin (GRA), representing between 83.11% and 94.15%, and three indolic, namely 4-hydroxyglucobrassicin (4OHGBS), glucobrassicin (GBS) and 4-methoxyglucobrassicin (4OMeGBS), accounting for the remaining 5.85% and 16.89% depending on the variety and light conditions. The individual GLs of radish sprouts are graficaly represented in figure 1, and as it can bee seen, all radish varieties have the same GLs profile with the major components being glucoraphenin and glucoiberin.

In mustard sprouts we have identified 8 GLs: two aliphatic – sinigrin (SIN) and glucoraphanin(GRA), one aromatic – sinalbin (SNB) and four indolic - 4-hydroxyglucobrassicin (4OHGBS), glucobrassicin (GBS), 4-methoxyglucobrassicin (4OMeGBS) and neoglucobrassicin (NeoGBS). Aromatic glucosinolates include indolic glucosinolates. As showed in figure 2, the main GLs of white mustard sprouts is sinalbin, meanwhile in the black mustard sprouts, sinigrin is the main component.

Therefore the percentage of aliphatic GLs and indolic GLs differes greatly between the two varieties of mustard.

The statistical analysis regarding the percentage of aliphatic and indolic GLs, of all samples it is summarized in table 2. As it can be observed variety, light conditions and their firt degree interaction have a significant influence on the aliphatic and indolic GLs. The content of aliphatic glucosinolates increases in the case of the sprouts germinated under dark conditions, meanwhile the indolic glucosinolates register a decrease.

Data regarding the total GLs and SMCSO content are presentented in table 3. Total GLs contents were obtained by summarizing the contents of the individual GLs.

Mustard samples registered higher levels of GLs than radish samples. Significant differences were obtained between the different radish varieties, red radish sprouts registering the highest levels (74.2 µmol/g DW) and white radish the lowest (46.5 µmol/g DW). No significant differences were noticed in the case of the mustard varieties (214.2 µmol/g DW in black mustard sprouts and 207.9 µmol/g DW in white mustard sprouts).

The light conditions used during germination had a significant effect on the content of total GLs wich was up to 27% higher in the case of the sprouts germinated under dark conditions.

The highest GLs content was observed in the case of black mustard sprouts germinated under dark conditions (301.69 µmol/g DW) and the lowest in the case of white radish sprouts germinated under light conditions (39.07 µmol/g DW).

The results regarding the GLs are in agreeament with the data available in the literature. Ciska E. et al., 2008 [9], Kubec R. et.al., 2009 [11], De Nicola G. et al., 2013 [14] and Gaofeng Y. et al., 2010 [13] found similar results.

Aldough SMCSO is also a sulphur containing phytochemical, no correlation was determined between the total GLs and SMCSO content by Pearson correlation (p=0.098).

Table 1. Germination rate [%], moisture [%] and dry weight [%] of the studied samples

Crt. Nr.	Sample Type	Sample Variety	Light Conditions	Germination rate	Humidity	Dry Weight
1	Radish	Red	Light	88.75±1.77 ^{d}	89.26±0.51 ^{d}	10.74±0.51 ^{a}
2		White		98.5±0.71 ^{ab}	90.34±0.37 ^{bcd}	9.66±0.37 ^{abc}
3		Black		86±2.83 ^{d}	90.77±0.36 ^{abc}	9.24±0.36 ^{bcd}
4		Red	Dark	91.5±2.12 ^{cd}	90.29±0.42 ^{bcd}	9.72±0.42 ^{abc}
5		White		98.75±0.35 ^{a}	91.07±0.06 ^{ab}	8.94±0.06 ^{cd}
6		Black		92.25±1.06 ^{bcd}	91.5±0.13 ^{a}	8.51±0.13 ^{d}
7	Mustard	White	Light	96.75±0.35 ^{abc}	90.22±0.08 ^{bcd}	9.79±0.08 ^{abc}
8		Black		22.5±1.41 ^{e}	90.3±0.13 ^{bcd}	9.7±0.13 ^{abc}
9		White	Dark	98.25±0.35 ^{ab}	89.59±0.05 ^{d}	10.42±0.05 ^{a}
10		Black		18.25±2.47 ^{e}	89.73±0.09 ^{cd}	10.28±0.09 ^{ab}

Note: Values are expressed as mean± standard deviation of two replicates. Different letters in the same column indicate statistically significant differences (Tukey's test $p< 0.05$).

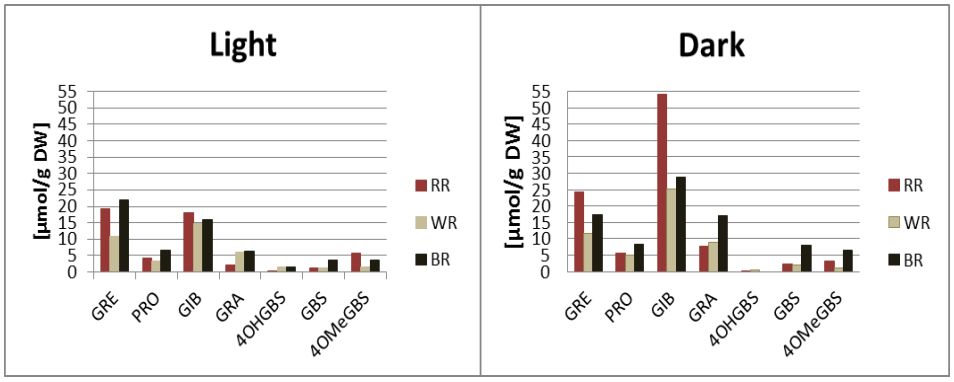

Figure 1. Individual glucusinolates identified in 7 days old radish sprouts germinated under 24h light and 24h dark conditions (RR – Red Radish, WR – White radish, BR – Black Radish, GRE - glucoraphenin, PRO - progoitrin, GIB - glucoiberin, GRA - glucoraphanin, 4OHGBS – 4-hydroxyglucobrassicin, GBS – glucobrassicin, 4OMeGBS – 4-methoxyglucobrassicin)

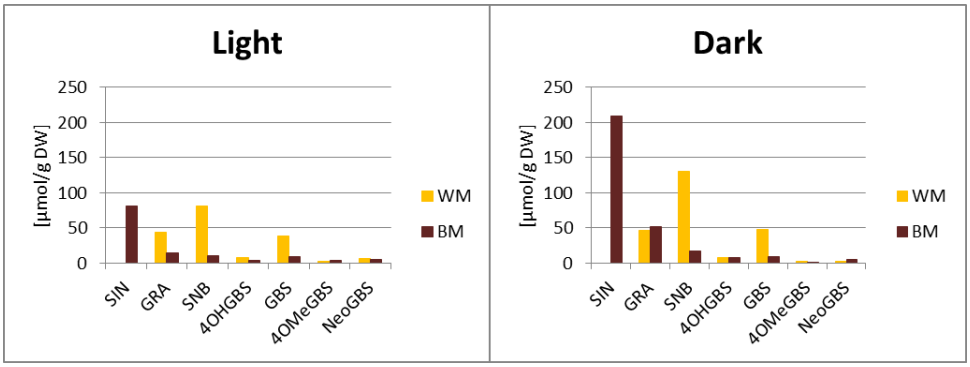

Figure 2. Individual glucusinolates identified in 7 days old musard sprouts germinated under 24h light and 24h dark conditions (WM – White mustard, BM – Black Mustard, SIN - sinigrin, GRA – glucoraphanin, SNB - sinalbin, 4OHGBS – 4-hydroxyglucobrassicin, GBS – glucobrassicin, 4OMeGBS – 4-methoxyglucobrassicin, NeoGBS - neoglucobrassicin)

Table 2. Effects of variety, light conditions and their first- degree interaction on the aliphatic and indolic GLs [%]

Sample Type	Factor	Aliphatic GLs	Indolic GLs
Radish	**Variety (V)**		
	Red	89.9 ^{b}	10.1 ^{b}
	White	91.4 ^{a}	8.6 ^{c}
	Black	84.1 ^{c}	15.9 ^{a}
	p/contribution (%)	*< 0.001***/53.3*	
	Light Conditions (LC)		
	Light	86.5 ^{b}	13.5 ^{a}
	Dark	90.5 ^{a}	9.5 ^{b}
	p/contribution (%)	*< 0.001***/21.3*	
	V x LC		
	Red x Light	85.74±0.21 ^{c}	14.26±0.21 ^{b}
	Red x Dark	94.15±0.29 ^{a}	5.85±0.29 ^{d}
	White x Light	88.61±0.32 ^{b}	11.39±0.32 ^{c}
	White x Dark	94.1±0.35 ^{a}	5.9±0.35 ^{d}
	Black x Light	85.01±0.1 ^{c}	14.99±0.1 ^{b}
	Black x Dark	83.11±0.05 ^{d}	16.89±0.05 ^{a}
	p/contribution (%)	*< 0.001***/25.2*	
Mustard	**Variety (V)**		
	White	21.9 ^{b}	78.1 ^{a}
	Black	81.1 ^{a}	18.9 ^{b}
	p/contribution (%)	*< 0.001***/37.2*	
	Light Conditions (LC)		
	Light	75.6 ^{b}	50.1 ^{a}
	Dark	86.5 ^{a}	46.9 ^{b}
	p/contribution (%)	*<0.01**/0.1*	
	V x LC		
	White x Light	24.15±1.04 ^{c}	75.85±1.04 ^{b}
	White x Dark	19.64±0.2 ^{d}	80.36±0.2 ^{a}
	Black x Light	75.63±0.93 ^{b}	24.37±0.2 ^{c}
	Black x Dark	86.52±0.23 ^{a}	13.48±0.23 ^{d}
	p/contribution (%)	*< 0.001***/62.7*	

Note: Values are expressed as mean± standard deviation of two replicates for each variety x vegetative stage. Different letters in the same column indicate statistically significant differences (Tukey's test $p< 0.05$). Significant differences are denoted by asterisks: *$p< 0.05$; **$p < 0.01$; ***$p< 0.001$; $p \geq 0.05$, non-significant.

Table 3. Effects of variety, light conditions and their first- degree interaction on the GLs and SMCSO content [µmol/g DW]

Sample Type	Factor	Total GLs content	SMCSO content
Radish	**Variety (V)**		
	Red	74.2 ^{a}	22.5 ^{a}
	White	46.5 ^{b}	21.3 ^{a}
	Black	72.9 ^{a}	25.3 ^{a}
	p/contribution (%)	*< 0.001***/39.0*	*>0.05 n.s*
	Light Conditions (LC)		
	Light	50.0 ^{b}	26.8 ^{a}
	Dark	79.1 ^{a}	19.2 ^{b}
	p/contribution (%)	*< 0.001***/50.6*	*< 0.01**/37.6*
	V x LC		
	Red x Light	51.13±1.56 ^{d}	32.1±1.58 ^{a}
	Red x Dark	97.21±1.21 ^{a}	12.85±1.25 ^{c}
	White x Light	39.07±2.5 ^{e}	23.61±2.37 ^{b}
	White x Dark	53.94±2.09 ^{cd}	18.93±1.46 ^{bc}
	Black x Light	59.78±1.56 ^{c}	24.55±2.53 ^{ab}
	Black x Dark	86.04±1.95 ^{b}	25.96±2.03 ^{ab}
	p/contribution (%)	*< 0.001***/10.0*	*< 0.01**/50.1*
Mustard	**Variety (V)**		
	White	207.9 ^{a}	13.4 ^{b}
	Black	214.2 ^{a}	26.2 ^{a}
	p/contribution (%)	*> 0. 05 n.s*	*< 0.001***/70.1*
	Light Conditions (LC)		
	Light	152.5 ^{b}	22.5 ^{a}
	Dark	269.5 ^{a}	17.1 ^{b}
	p/contribution (%)	*< 0.001***/79.4*	*< 0.05*/12.3*
	V x LC		
	White x Light	178.41±3.23 ^{c}	19.05±1.4 ^{b}
	White x Dark	237.33±0.14 ^{b}	7.74±0.86 ^{c}
	Black x Light	126.63±13.84 ^{d}	25.9±2.1 ^{ab}
	Black x Dark	301.69±8.41 ^{a}	26.49±2.12 ^{a}
	p/contribution (%)	*< 0.01**/19.6*	*< 0.01**/15.1*

Note: Values are expressed as mean± standard deviation of two replicates for each variety x vegetative stage. Different letters in the same column indicate statistically significant differences (Tukey's test $p< 0.05$). Significant differences are denoted by asterisks: *$p< 0.05$; **$p < 0.01$; ***$p< 0.001$; $p \geq 0.05$, non-significant.

4.Conclusions

Nowadays, people interested in maintaining their health are aware of the need for a vegetable-based diet in order to achieve an intake of phytochemicals that are related to the prevention of chronic disease.

The results of the present study show that radish and mustard sprouts, members of brassica vegetables, are a good source of sulphur compounds. Our findigs point out some general dependencies between various species, varieties and light conditions used in the germination process and the content of sulphur phytochemical such as GLs and SMCSO.

This may be useful in order to select those species and varieties of seeds and the application of those parameters for the germination process that would ensure a food product with the optimum composition of bioactive phytochemicals.

Moreover, given the lack of data regarding SMCSO contents, our paper can contribute to filling in this knowledge gap, especially since this compound is the precursor of a variety of sensory-active and health-beneficial compounds such as dimethyl thiosulfinate, and dimethyl sulphides.

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Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards.

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