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# BIOLOGICALLY ACTIVE COMPOUNDS IN *CRUCIFERAE* SPROUTS AND THEIR CHANGES AFTER THERMAL TREATMENT

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Key words: cruciferous sprouts, pasteurization, sterilization, total phenolic compounds, tocopherols, reduced glutathione, glucosinolates, trolox equivalent antioxidant capacity

Since thermal treatment of ready-to-eat sprouts is suggested in order to obtain microbiologically safe sprouts, changes in bioactive compound levels in rapeseed and radish sprouts, after pasteurization and sterilization, were addressed in this study.

The seeds of double low rapeseed (*Brassica napus* L. *oleifera*.) and radish (*Raphanus sativus* L. *major*) after four days of germination were collected and subjected to thermal treatment, including pasteurization (95°C/30 min) and sterilization (1.5 atm/30 min). The following bioactive compounds were analysed before and after the thermal treatment of sprouts: tocopherols ($\alpha$-T, $\beta$-T, $\gamma$-T, $\delta$-T), glucosinolates (GLS), total phenolic compounds (TPC), and reduced glutathione (GSH). The trolox equivalent antioxidant capacity (TEAC) of thermally-treated sprouts was compared to that of the ready-to-eat ones.

The results showed that after the thermal treatment the content of all tocopherols decreased drastically in the investigated rapeseed and radish sprouts. The pasteurization of rapeseed and radish sprouts caused a decrease in the content of all aliphatic, indolic and aryl GLS (present only in rapeseed). The heat treatment *via* sterilization caused greater decreases in the above-mentioned groups of GLS when compared to the pasteurization. Both thermal treatment processes were found to determine the content of TPC. In the case of rapeseed sprouts only, a small decrease was noted, however an increase was found in respect to radish sprouts. A decrease in TEAC of thermally-treated rapeseed and radish sprouts was observed. Among the compounds investigated in this study, only the amount of GSH found in the heat treated sprouts was at least two- or threefold higher when compared to the untreated sprouts.

Thermal processes used in this study were observed to negatively interact with some biologically active compounds present in the investigated sprouts, hence a need of the search for another method for fresh sprouts preservation was postulated.

## INTRODUCTION

A plant-based diet, composed mainly of vegetables, fruit and whole grains, has become one of the most important guidelines for lowering the risk of human diseases, in which the increased level of free radicals is implicated [Lawrence & Machlin, 1995]. Moreover, a number of the natural biologically active compounds present in the plant-based diet, despite of their antioxidant functions, exhibit a wide range of biological effects, including antibacterial, antiviral, anti-inflammatory, antiallergic, antithrombotic, and vasodilatory actions [Cook & Samman, 1996].

One of more valuable still underappreciated dietary supplements are sprouts which may be considered as functional food and meet consumer's demands as well. Up-to--day studies into the chemical composition of sprouts obtained from different seeds and cereals have pointed to their high nutritive value and indicated that they contain biologically-active, positive components which can improve health and well being [King & Perwastien, 1987; Price, 1988]. It has been proved that sprouts demonstrate a higher nutritive value than seeds and that the process of their production is inexpensive and simple [Finley, 1978; Vidal--Valverde *et al.*, 2002]. Hence, as early as in 1988 Kuo and Van Middlesworth [1988] suggested popularisation of their consumption. So far, the chemical composition of sprouts from legume seeds has been best recognised [Finley, 1978; Frias *et al.*, 2000]. While searching for new sources of sprouts, special attention has been paid to sprouts obtained from *Cruciferae* family seeds that have been increasingly used in human diets. It has been reported that these sprouts contain glucosinolates [Kozłowska *et al.*, 2002], tocopherols [Zieliński & Kozłowska, 2003], reduced glutathione [Zieliński *et al.*, 2002], and have higher total antioxidant status [Zieliński *et al.*, 2003] when compared with raw seeds.

Data compiled so far on bioactive compounds in sprouts obtained from *Cruciferae* seeds lacks information on the effect of thermal treatments on these compounds. Since the

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thermal treatment of ready-to-eat sprouts is suggested in order to obtain microbiologically safe sprouts, changes in the levels of selected bioactive compound after thermal treatment were addressed in this study.

## MATERIALS AND METHODS

**Reagents.** Glutathione ($\gamma$-glutamyl-cysteinyl-glycine; GSH), oxidized glutathione (GSSG), myoglobin from horse heart, (±) catechin, 2,2'-Azinobis(3-ethylbenzothiazoline-6--sulfonic acid) diammonium salt (ABTS) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma (Sigma Chemical Co., St. Louis, MO, U.S.A.). Tocopherol standards ($\alpha$-T, $\beta$-T, $\gamma$-T, $\delta$-T) were obtained from Merck and Sigma. All other reagents of reagent-grade quality were from POCh, Gliwice, Poland.

**Samples.** Seeds of double low oilseed rape of Mango variety (*Brassica napus var. oleifera*) and radish (*Raphanus sativus* L.) were obtained from a local plant breeding station in the North-East Poland. The seeds were stored at room temperature in polyethylene bags until germination.

**Seed germination.** Cruciferous seeds (25 g) were soaked in 125 mL of cooled at room temperature boiled water and shaken every 30 min. After 4 h, the water was drained off and the seeds were transferred to an incubator (Cliambic Cabinet, Economic Deluxe EC00-065 model, Snijders Scientific b.v, Netherlands). The seeds were germinated in dark at 25°C in humidity of 95%, for 4 days. The seeds were layered over a moist filter paper (qualitative medium-speed filter paper). Sprouts from each species were removed from the incubator every 24 h, frozen in liquid nitrogen and lyophilized. The germination was carried out in triplicate.

**Thermal treatment.** Fresh 4-day germinated under dark conditions rapeseeds (*Brassica napus var. oleifera*) and radish seeds (*Raphanus sativus* L.) were canned as follows: 100 g of sprouts were put into 150 mL jars and covered with water containing 2 g of citric acid, 24 g of sugar and 6 g of salt per liter. Jars were submitted to preserving procedures such as pasteurization (95°C for 30 min) and sterilization into a vertical steam autoclave (ASV, SMS, Poland) to 1.5 atm for 30 min. After treatments, the content was frozen in liquid nitrogen and lyophilized for further analysis. The canning process was carried out in triplicate.

**Analytical methods.** Tocopherols ($\alpha$-T, $\beta$-T, $\gamma$-T, $\delta$-T) were extracted from the material by methanol and after solvent evaporation the residue was redissolved in n-hexane. The tocopherols were separated by HPLC on Lichrospher Si 60 5-$\mu$m particle size, 4 × 250-mm column, according to the method described by Peterson and Qureshi [1993]. The HPLC systems consisted of a Shimadzu model LC pump series 10 AD, and a Shimadzu RF-535 fluorescence spectrometer. The tocopherol contents were calculated from the peak areas using standard curves of particular tocopherols.

GLS were extracted from the samples with boiling 70% methanol. The isolation, desulphatation and HPLC determination on Spherisorb ODS-2 column were carried out according to the methods reported by Heaney *et al.* [1986]. Individual GLS were identified by comparing the retention times with those for standards or on the basis of available literature data for 4-methylthiobut-3-enyl GLS [Carlson *et al.*, 1985] and glucoraphenin [Carlson *et al.*, 1987].

Phenolics from the samples were extracted at room temperature using 80% methanol. After centrifugation, TPC were assayed according to the method of Shahidi and Naczk [1995] using Folin-Ciocalteu reagent. A spectrophotometer UV-160 1PC (Shimadzu, Japan) was employed and the results were expressed as (±) catechin equivalents.

Reduced glutathione (GSH) was extracted from the samples with 1% methaphosphoric acid. GSH was determined by the enzyme recycling method [Tietze, 1969] modified for use in a microplate reader EF 340 (Biotek Instruments Inc, USA). The detailed protocol of the assay was described previously [Zieliński *et al.*, 1999].

The trolox equivalent antioxidant capacity (TEAC) of the 80% methanol extract of the samples was measured by a spectrophotometric technique according to Miller and Rice-Evans [1996]. The standard curve was constructed with different Trolox solution. The temperature-controlled recording spectrophotometer (UV-160 1PC with CPS-Controller, Shimadzu, Japan) was used for the respective determinations.

All analysis of the selected bioactive compounds and antioxidant capacity were carried out in triplicate.

**Statistical analysis.** Each extract was considered as a "treatment". All measurements were replicated three times for each treatment and their means are reported.

## RESULTS AND DISCUSSION

Our earlier works, in general regarding the phenomena of germination with the outcome of biologically active component contents showed that in respect to rapeseed and radish sprouts, they had sufficient sensory quality in terms of consumers' acceptance [Troszyńska *et al.*, 2002]. Since thermal treatment of ready-to-eat sprouts is suggested in order to obtain microbiologically-safe sprouts [Bielecka *et al.*, 2002], changes in the levels of bioactive compound after thermal treatment were addressed in this study. The following biologically active compounds were identified and determined in ready-to-eat and thermally-treated sprouts: $\alpha$-T, $\beta$-T, $\gamma$-T, $\delta$-T, GLS, TPC and GSH. In addition, the trolox equivalent antioxidant capacity (TEAC) of the sprouts was assayed before and after the thermal treatment.

The first group of bioactive compounds investigated in this study were tocopherols, including $\alpha$-T, $\beta$-T, $\gamma$-T, $\delta$-T. In determining tocopherol content of radish sprouts it was shown that the amount of total tocopherols was higher (about 40%) than in rapeseed sprouts (Table 1). Of individual tocopherols, in both kinds of sprouts, the highest amounts were reported for $\gamma$-T and $\alpha$-T, whereas low levels for $\beta$-T and $\delta$-T. The results showed that after the thermal treatment the content of all tocopherols ($\alpha$-T, $\beta$-T, $\gamma$-T, $\delta$-T) decreased by 90–99% in rapeseed and radish sprouts. There was no evidence for the effect of pasteurization (95°C for 30 min) and sterilization (1.5 atm for 30 min) on the

TABLE 1. The content of tocopherols ($\alpha$-T, $\beta$-T, $\gamma$-T, $\delta$-T) due to the thermal treatment of rapeseed and radish sprouts ($\mu$g/ g d.m.).

| Cruciferous sprouts | $\alpha$-T | $\beta$-T | $\gamma$-T | $\delta$-T | Total tocopherols |
|---|---|---|---|---|---|
| Rapeseed sprouts | | | | | |
| fresh | 104.37 | 43.24 | 148.93 | 14.98 | 311.52 |
| pasteurized | 6.09 | 2.40 | 6.37 | 0.50 | 15.36 |
| sterilized | 3.64 | 2.31 | 4.05 | 0.29 | 10.29 |
| Radish sprouts | | | | | |
| fresh | 76.72 | 24.87 | 334.01 | 70.92 | 506.52 |
| pasteurized | 1.44 | 0.69 | 9.42 | 1.76 | 13.31 |
| sterilized | 0.69 | 0.41 | 4.10 | 0.79 | 5.99 |

remaining tocopherols in both kinds of sprouts. A similar phenomenon was observed in other reports on the impact of thermal treatment of plant-originated food [Kamal-Edin & Appelqvist, 1996].

The second group of bioactive compounds investigated in this study were GLS which comprise a group of thioglucosides naturally occurring in cruciferous vegetables which are the main source of GLS in a human diet [Carlson *et al.*, 1987]. It is known that particular species, varieties and cultivars differ in regard to the type and amount of the GLS present. A variety of factors influencing the ultimate GLS content of vegetables causes that the values reported by different authors are within a broad range for the vegetables of the same variety [Ciska *et al.*, 2000]. For example, average GLS content ranges from about 160 to over 250 mg/100 g for Brussels sprouts and from 10 to 70 mg/100 g for radish [Fenwick *et al.*, 1983; Carlson *et al.*, 1987]. An HPLC analysis indicated that the total content of dominating aliphatic GLS in fresh radish sprouts, including glucoraphenin, glucoraphanin, napoleiferin and 4-methylthiobut-3-enyl, was about 36 times higher when compared to the total aliphatic GLS found in rapeseed sprouts, including napoleiferin, progoitrin and gluconapin (Table 2). Moreover, an about threefold higher level was noted in total indolic GLS content in radish sprouts when compared to the total indolic GLS content in rapeseed sprouts. The profile of indolic GLS was similar in both kinds of sprouts, and included mainly 4-hydroxy-glucobrassicin, glucobrassicin and 4-metoxy-glucobrassicin. In addition, a sole aryl GLS – gluconasturtin was found in trace amount only in rapeseed sprouts. An analysis of thermally-treated sprouts indicated the influence of the thermal treatment on the loss of glucosinolates (Table 2). Pasteurisation of rapeseed sprouts caused a decrease in the content of all aliphatic, indolic and aryl GLS by 49%, 59% and 100% respectively. The respective loss of all aliphatic and indolic GLS by 36% and 73% was also noted for the treated radish sprouts. The heat treatment by sterilization caused greater decreases in the above-mentioned groups of GLS when compared to the pasteurisation. It cannot be excluded that during the initial stages of thermal treatment, the native mirosinase gets into contact with GLS, which results in GLS breakdown. The GLS bioactive breakdown products, particularly the aliphatic and indolyl ones, have been shown to act as anticarcinogens by inhibiting the phase I enzymes and inducing the phase II enzymes which affect xenobiotic transformations [Bailey & Williams, 1993]. Similar effects have also been described for some degradation products of the aliphatic GLS [Verhoeven *et al.*, 1997]. Negative effects of degradation products, especially of the aliphatic GLS, have been related to their harmful influence on the thyroid gland, particularly at iodine deficiency in the organism [Michajlovski , 1986].

Both thermal treatment processes were found to have an influence on the content of TPC. In the case of rapeseed sprouts (8.11 mg/g d.m.), a small decrease by 8–15% was noted however an increase by 16-50% was found in respect to ready-to eat radish sprouts (8.90 mg/g d.m.). This finding indicates that not only heat treatment conditions but also the kind of phenolic compounds present in the processed material should be taken for independent consideration (Figure 1A). Further detailed investigations of the quality of phenolic compounds are necessary since it was reported that phenolic acid esters and free phenolic acids constituted as much as 80% and 16% of total phenolic compounds of rapeseeds meals, respectively [Krygier *et al.*, 1982]. Moreover, Bjergegaard and coworkers [Bjergegaard *et al.*, 1994]

TABLE 2. The content of GLS due to the thermal treatment of radish and rapeseed sprouts ($\mu$mol/ g d.m.).

| GLS | Fresh | Pasteurized (95°C/30 min.) | Sterilized (1.5 atm/ 30 min.) |
|---|---|---|---|
| Radish sprouts | | | |
| • glucoraphanin | 1.85 | 1.37 | tr. |
| • glucoraphenin | 74.37 | 60.19 | 14.00 |
| • napoleiferin | 11.37 | 3.93 | 2.23 |
| • 4-methylthiobut-3-enyl | 46.62 | 19.82 | 2.42 |
| Total aliphatic GLS | 134.21 | 85.31 | 18.65 |
| • 4-hydroxy-glucobrassicin | 5.28 | 1.20 | 0.06 |
| •glucobrassicin | 0.07 | 0.21 | 0.05 |
| • 4-metoxy-glucobrassicin | 0.49 | 0.12 | tr. |
| Total indole GLS | 5.84 | 1.53 | 0.11 |
| Rapeseed sprouts | | | |
| • progoitrin | 2.99 | 1.46 | 0.75 |
| • napoleiferin | 0.11 | 0.23 | 0.24 |
| • glucoalyssin | 0.12 | 0.05 | tr. |
| • gluconapin | 0.52 | 0.17 | 0.06 |
| Total aliphatic GLS | 3.74 | 1.91 | 1.05 |
| • 4-hydroxy-glucobrassicin | 0.67 | 0.13 | tr. |
| • glucobrassicin | 0.28 | 0,13 | tr. |
| • 4-metoxy-glucobrassicin | 0.36 | 0.08 | tr. |
| • neoglucobrassicin | 0.68 | 0.47 | 0.05 |
| Total indole GLS | 1.99 | 0.81 | 0.05 |
| Aryl GLS (gluconasturtin) | 0.09 | tr. | tr. |

tr. (trace) < 0.05 $\mu$mol/g d.m.

noted the presence of flavonoids and aromatic choline esters in cruciferous plant extracts of *Brasica napus* (leaves) and *Sinapis alba* (seeds). A distinct increase in the total phenolic compounds measured in radish sprouts after pasteurization points to better accessibility of extracting solvent into thermally-treated plant matrix or to the effect of the process applied on their release. In the case of sterilization of rapeseed and radish sprouts, this effect was not as noticeable as after pasteurization.

Different levels of GSH found in the fresh rapeseed sprouts (1.18 $\mu$mol/ g d.m.) and fresh radish sprouts

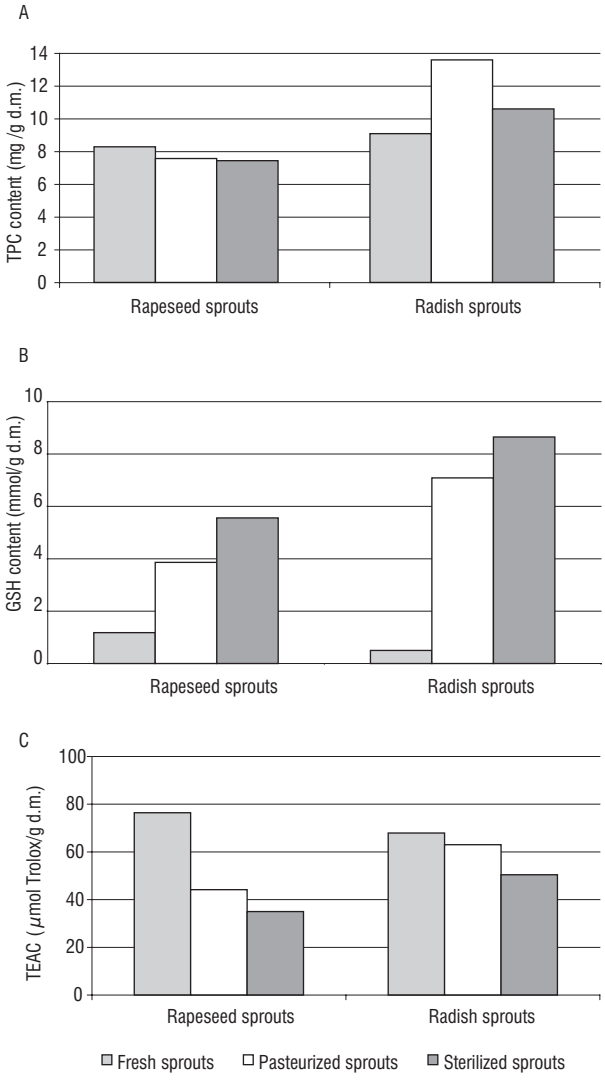


FIGURE 1. Changes in TPC (A), GSH (B) and TEAC (C) upon the thermal treatment of rapeseed and radish sprouts.

(0.50 $\mu$mol/ g d.m.) confirmed, in part, the previously observed differences in levels of bioactive compounds investigated in this study. It is worth emphasizing that among the compounds investigated in this study, only the amount of GSH found in thermally-treated sprouts was at least two- or threefold higher when compared to these of the untreated sprouts (Figure 1B). The results obtained indicate that sprouts originated from rapeseed and radish seeds, especially those treated thermally, represent a better source of reduced glutathione with concentrations comparable with these of selected fresh and cooked vegetables and fresh fruit [Valencia *et al.*, 2001]. They are also richer in GSH than the hydrothermally-processed cereal grains like wheat, barley rye and oat [Zieliński & Rzedzicki, 2001].

Most of the above-studied compounds were shown to exhibit antioxidant properties [Halliwell *et al.*, 1995]. Therefore, to establish how far the thermal processes influence this ability, the antioxidant capacity of thermally-treated sprouts was determined in the study (Figure 1C). As a result of antioxidant contents, the TEAC of ready-to-eat rapeseed and radish sprouts was 76.4 $\mu$mol Trolox/ g d.m. and 67.9 $\mu$mol Trolox/ g d.m., respectively. The hydrothermal treatment not equally influenced TEAC of rapeseed and radish sprouts. The observed drop in TEAC reached 30–54% and 7–25%, respectively, and compiled all changes induced by the applied thermal processes.

## CONCLUSIONS

The applied thermal processes affected the investigated compounds in different ways. The content of tocopherols was observed to drop drastically. Also a significant drop was noted for glucosinolates. The content of the remaining compounds either did not change (TPC in rapeseed) or significantly increased (TPC in radish sprouts and GSH in both radish and rapeseed sprouts). Antioxidant capacity, which is formed by all sprout components displaying antioxidant activity, decreased after the hydrothermal treatment. For this reason, there is a need for elaborating another method for fresh sprout preservation that would deliver a safe, valuable for consumers product with minimized loss of beneficial bioactive components.

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# ZWIĄZKI BIOLOGICZNIE AKTYWNE W KIEŁKACH NASION ROŚLIN KRZYŻOWYCH I ICH ZMIANY PO ZASTOSOWANIU PROCESÓW TERMICZNYCH

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W pracy przedstawiono zmiany zawartości związków bioaktywnych zachodzące podczas termicznego traktowania kiełków nasion rzepaku i rzodkwi mającego na celu uzyskanie mikrobiologicznie bezpiecznego dla konsumenta produktu.

Nasiona rzepaku dwuzerowego oraz rzodkwi poddano procesowi kiełkowania w szafie klimatyzacyjnej w temperaturze 25°C i wilgotności 95% bez dostępu światła przez 4 dni. Wcześniejsza analiza kiełków wykazała, że właśnie 4-dniowe kiełki wyprodukowane w powyższych warunkach są najbardziej wartościowe i akceptowane przez konsumentów. Dlatego też tego rodzaju kiełki poddano pasteryzacji (95°C/30 min) oraz sterylizacji (1.5 atm/30 min). W badanym materiale przed i po zastosowaniu w/w procesów termicznych wyznaczono zawartość tokoferoli ($\alpha$-T, $\beta$-T, $\gamma$-T, $\delta$-T), glukozinolanów (GLS), związków fenolowych ogółem (TPC), zredukowanego glutationu (GSH) oraz wyznaczono pojemność przeciwutleniającą w jednostkach Troloksu (TEAC).

Po zastosowaniu pasteryzacji i sterylizacji zawartość tokoferoli w kiełkach uległa drastycznemu obniżeniu. Utrwalanie termiczne prowadziło także do spadku alifatycznych, indolowych i arylowych GLS (te ostatnie były tylko obecne w świeżych kiełkach rzepaku), przy czym sterylizacja prowadziła do większej destrukcji GLS niż pasteryzacja. Ponadto, po zastosowaniu obydwu rodzajów procesów termicznych zawartość TPC była niższa w utrwalonych kiełkach rzodkwi w przeciwieństwie do utrwalonych kiełków rzepaku, w których to zaobserwowano wzrost TPC. Obniżeniu uległa także pojemność przeciwutleniająca kiełków. Spośród badanych w pracy związków bioaktywnych, jedynie zawartość GSH była znacząco wyższa w utrwalonych termicznie kiełkach. W podsumowaniu należy stwierdzić, że zastosowane procesy termiczne negatywnie oddziaływały, z punktu widzenia żywienia człowieka, na większość badanych związków biologicznie aktywnych, co wymaga poszukiwania innych metod konserwacji kiełków.