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DOI: 10.1007/s00217-016-2764-3

Li, R., Song, D, Vriesekoop, F., Cheng, L.,Yuan, Q. and Liang, H. 2016. Glucoraphenin, sulfuraphene and antiproliferative capacity of radish sprouts in germinating and thermal processes. *European Food Research and Technology*, 243(4), pp.547-554.

Glucoraphenin, sulforaphene, and antiproliferative capacity of radish sprouts in germinating and thermal processes

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Abstract

Glucoraphenin, the predominant glucosinolate in radish sprouts, is hydrolyzed by myrosinase to sulforaphene that is implicated to exert anti-cancerogenic effects. The effects of germination and subsequent cooking processes on the levels of glucoraphenin and its hydrolysis products were investigated in this research. HPLC analysis revealed that the levels of glucoraphenin and sulforaphene decreased with germination time. In agreement with the above results, the antiproliferation activity of radish sprouts extracts on human lung cancer cells was also found to decline gradually in line with the germination process. Furthermore, when we applied three traditional cooking treatments to radish sprouts, the glucoraphenin and sulforaphene were markedly decreased; while the antiproliferation activity of cooked radish sprouts was considerably decreased. This research showed that 3-day-old radish sprouts are an excellent source of bioactive compounds that could potentially benefit human health; while any cooking process appears to cause the devastation of beneficial attributes in radish sprouts.

Keywords Radish sprouts · Glucoraphenin · Sulforaphene · Degradation · Antiproliferation.

Introduction

A large body of epidemiological studies have indicated that a diet rich in cruciferous vegetables has been associated to a reduction in cancer risk [1-4]. Several studies have revealed that various bioactive functions appear to be related to high amounts of glucosinolates and myrosinase in cruciferous vegetables [5-6]. When plant tissue is mechanically damaged or attacked by pests, glucosinolates and myrosinase are brought into contact, resulting in the glucosinolates being hydrolyzed by myrosinase and they produce a variety of degradation products such as isothiocyanates, thiocyanates, nitriles, epithionitriles and oxazolidine-2-thiones [6-7]. Importantly, the anticancer effects of cruciferous vegetables is thought to arise from isothiocyanates which can increase the activity of phase II enzymes and inhibit the proliferation of cancer cells [8-10].

Sprouts are a popular healthy product in many countries. In recent years, radish sprouts have gained people's attention due to their abundance in bioactive compounds and their perceived health benefits [11-15]. Among bioactive compounds in radish sprouts, sulforaphene (SFE: 4-methylsulfinyl-3-butenyl isothiocyanate), an important isothiocyanate derived from glucoraphenin (GRE: 4-methylsulfinyl-3-butenyl glucosinolate), is strongly associated with anticancer activity [16-17]. In our previous study, we showed that SFE was unstable and easily degraded in the hydrolytic process of GRE and then converted to a cyclic degradation product (MSMTT: 6-[(methylsulfinyl)-methyl]-1, 3-thiazinan-2-thione) [17]. MSMTT was early found and identified by Zhang et al. in radish seed and then they discovered the compound showed no cytotoxicity on human tumor cell lines [18]. Therefore, the degredation process could reduce the bioactivity of SFE (Fig. 1). Moreover, others have reported that the quantity of some bioactive compounds in radish sprouts change during the germination period and that sulfur fertilization could also affect the accumulation of health-promoting phytochemicals in

radish sprouts [13,15]. However, the changes of GRE and its hydrolytic products during the germination process of radish seed have not yet been studied.

Cruciferous vegetables and their sprouts are usually subjected to some form of heat treatment to make them suitable for human consumption. However, the choice of cooking method is likely to influence the quantity of glucosinolates and their hydrolysis products. Many of the previous studies investigating the effects of heat treatments on cruciferous vegetables focused on the quantity of glucosinolates in general, but only very few papers studied the change of the quantity of isothiocyanates, which are the actual compounds with biological activity in humans [18,19]. Furthermore, effects of cooking have been widely investigated in abundantly available cruciferous vegetables such as broccoli and cabbage [18-22], however there are no studies that investigated the thermal effects on radish sprouts.

In the present study we investigated radish sprouts with the aim of examining the changes of levels of GRE, SFE and MSMTT during the germination process to provide an optimum yield of health-promoting compounds for human consumption. In addition, this study tested how GRE and its hydrolysis products vary following a variety of traditional cooking methods, including boiling, steaming and microwaving. Lastly, in order to determine whether the germination and cooking processes affect the bioactivity of radish sprouts: the antiproliferation activity of aqueous extracts from radish sprouts with different germination times and heat treatments on human non-small cell lung cancer cell lines (H1299 and HCC827) were evaluated.

Materials and methods

Materials

Radish seeds (*Raphanus sativus* L. Mantanghong) were kindly provided by Vegetables and Flowers

Institute, China Academy of Agriculture Science. The standards (purity > 98%) of SFE, GRE and MSMTT were separated and purified from radish seeds in our laboratory and its purity and chemical structure were identified by analytical HPLC, ESI-MS and NMR [17, 23]. Sinigrin (purity > 98%) was purchased from Sigma (St. Louis, MO). Methanol and trifluoracetic acid (TFA) used for HPLC were of HPLC grade and purchased from Fisher Scientific Co., LTD (Tustin, CA). Ultra pure water was obtained by Q Millipore System (Millipore, USA). RPMI-1640 media, fetal bovine serum, penicillin, streptomycin were purchased from Hyclone (Thermo Scientific, USA). KH_2PO_4, K_2HPO_4, DMSO (dimethylsulfoxide) and MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] were analytical grade and purchased from Beijing Chemical Works (Beijing, China).

The analytical HPLC equipment used in our experiment was a Shimadzu LC-20AT system (Kyoto, Japan) with two LC-20AT solvent delivery units, a SPD-M20A DAD detector, a SIL-20A auto sampler, a CTO-10ASVP column oven, a LC solution workstation and an analytical reverse phase C_{18} column (4.6 × 250 mm, 5 μm; Shimadzu, Japan).

Seeds germination and sprouts cultivation

One hundred grams of radish seeds were cleaned by rinsing with deionized water then they were immersed in a 0.7% sodium hypochlorite solution for 30 min. Seeds were drained and washed with deionized water until they reached a neutral pH. Afterwards, seeds were soaked in 500 mL deionized water overnight. The imbibed seeds were germinated on four layers of moist sterile gauzes in culture trays. The trays were placed in incubators and in order to maintain an even water content the seeds were watered every 8 h. During first two days, seeds were germinated in darkness at 25 ^{o}C, after that they were cultivated under photoperiod conditions (16 h light and 8 h darkness) at 25 ^{o}C. Sprouts were harvested daily during germination for up to seven days [13, 15]. One half of the harvested sprouts

were stored at -20 °C and the others were freeze-dried.

Quantitation of GRE by HPLC

The extraction of GRE was conducted according to a previously published method [19, 24], with modifications. Two grams of freeze-dried radish sprouts were ground into powder with an analytical grinder and then added to 20 mL boiling water. The mixture was heated in a water bath set at 100 °C for 15 min before being centrifuged at 10625 g for 5 min. Subsequently, the supernatant was decanted, and the residue was extracted twice with 20 mL of boiling water. Then the combined supernatants were concentrated to 20 mL under vacuum. The extracted concentrate was filtered with 0.22 μm nylon membrane and GRE in it was separated using the HPLC system described above with 1 mL/min flow rate at 30 °C by eluting with a isocratic elution of methanol (mobile phase A) and 0.02% (v/v) TFA aqueous solution (mobile phase B) as follows: isocratically 5% A kept for 10 min, 100% A for 2 min, then 5% A for 8 min. The injection volume was 20 μL per sample and the detection wavelength was set at 235 nm. The concentration of GRE was expressed as μmol/g dry weight (DW).

Analysis of the formation of SFE and MSMTT by HPLC

Ten grams of treated or untreated sprouts and 20 mL deionized water were juiced for 3 min using a juice centrifuge (Joyoung model JYL-C18D, China). The obtained slurry was allowed to hydrolyze *in situ* at room temperature for 20 min to facilitate product formation. Following hydrolysis the slurry was centrifuged at 10625 g for 5 min and filtered with 0.22 μm nylon membrane. SFE and MSMTT formed in the slurry were separated by HPLC with 1 mL/min flow rate at 30 °C by eluting with a gradient elution of methanol (mobile phase A) and 0.02% (v/v) TFA aqueous solution (mobile phase B) as follows: linear gradient from 5% A to 80% A for 30 min, 100% A kept for 2 min, then 5% A kept for 8 min. The injection volume was 20 μL and the detection wavelength was set at 254 and 281 nm for SFE

and MSMTT respectively [17]. The formation of SFE and MSMTT were expressed as μmol/g dry weight (DW).

Cooking treatments

Radish sprouts germinated for three days were collected and processed within 24 h. A control and three cooking treatments including boiling, steaming and microwaving were conducted in this experiment. Different cooking times were executed aimed to evaluate the influence of different durations of cooking on the levels of GRE, SFE and MSMTT and the potential anticancer activity of radish sprouts. For steam treatment, ten grams of radish sprouts were steamed using an electric steamer (Supor model Z09YA4-G2, China) for 0.5, 1, 1.5, 2, 3, 5 min. For boiling, ten grams of radish sprouts were placed into a pot of boiling water around 500 g for 0.5, 1, 1.5, 2, 3, 5 min to conduct boiling treatment. Microwaving was conducted in a microwave oven (Galanz model MC-83105FB, China) at 800 W. Ten grams of radish sprouts were put in a heat-resistant dish containing ten grams of water and then microwave heating for 0.5, 1, 1.5, 2 min [21-22]. Samples of germinating radish sprouts were withdrawn at appropriate times and the thermal treatment halted by immediate immersion in iced-water. Subsequently, one half of the sprouts were subjected to analysis of SFE and MSMTT by HPLC and the others was freeze-dried then analyzed GRE as described above.

The extraction of myrosinase was conducted according to previously published method [25], with modifications. Ten grams of raw or heat treated radish sprouts (boiling, steaming or microwaving for 0.5 min) were homogenized with 20 mL buffer (sodium phosphate 10 M, pH 7.0, containing ethylene diamine tetraacetic acid 1 mM, dithiothreitol 3 mM, and 5% glycerol) for 3 min in an ice bath and centrifuged at 10625 g for 5 min at 4 °C. The supernatants were collected and ammonium sulfate was added until the final concentration of ammonium sulfate reached saturation (~55%). The mixture was

kept at 4 °C for 2 h to precipitate the protein fraction. This was then centrifuged at 10625 g for 15 min at 4 °C. The pellet was dissolved in 2 mL buffer (sodium phosphate 10M, pH 7.0) and dailysed against the same buffer at 4 °C for 24 h. Then the mixture was used as a crude myrosinase preparation. Myrosinase activity was determined by evaluating the rate of hydrolysis of sinigrin. Briefly, 200 µL of myrosinase and 10 µL sinigrin (2 mg/mL) were reacted at 37 °C for 15 min, then it was boiled for 5 min to stop the reaction. The residual sinigrin in the reaction system was measured by HPLC with 1 mL/min flow rate at 30 °C by eluting with a gradient elution of methanol (mobile phase A) and 0.02% (v/v) TFA aqueous solution (mobile phase B) as follows: linear gradient from 1% A to 70% A for 20 min, 100% A kept for 2 min, then 1% A kept for 8 min. The injection volume was 20 µL and the detection wavelength was set at 235 nm. The protein content of the myrosinase preparation was determined by the commassie brilliant blue method (REF) [26]. Myrosinase activity was expressed as units per milligram of protein.

Non-small cell lung cancer culture

Human non-small cell lung cancer H1299 cells and HCC827 cells obtained from the American Type Culture Collection (Manassas, VA), were cultured in RPMI-1640 media supplemented with 10% fetal bovine serum, 100 units/mL penicillin, and 100 µg/mL streptomycin. Cultured cells maintained at 37 °C in a humidified 5% CO_2 atmosphere.

Preparation of radish sprouts extracts for cell experiment

Approximately ten grams of raw and heat treated radish sprouts were juiced with 20 mL deionized water for 3 min using a commercial juice centrifuge (Joyoung model JYL-C18D, China). The obtained slurry was allowed to hydrolyze *in situ* at room temperature for 20 min to facilitate product formation. The slurry was centrifuged at 10625 g for 5 min and filtered with 0.45 µm nylon membrane, following

which the supernatants were freeze-dried [27].

Antiproliferative effects

The antiproliferative effects of radish sprouts extracts was evaluated by tetrazolium reduction assay, based on the reduction of metabolically active cells. Briefly, 5000 cells/100 μL were seeded into each well of 96-well plates and cultured for 12 h. Then cells were treated with 100 μL of media containing 10 μg raw or heat treated radish sprouts extracts. Five replicate wells received each treatment, and they were cultivated for 24, 48 and 72 h. Afterwards, 20 μL of MTT at a concentrate 5 mg/mL in PBS was added to each well and the cells were cultured at 37 °C for 4 h. Treatment media was removed and then 150 μL of DMSO was added, and then the absorbance of each well was measured in a model 680 microplate reader (BIO-RAD, USA) at 490 nm [27-29]. The results were expressed as percentage of the controls.

Statistical analysis

All experiments were carried out in three independent replicates and data were expressed as mean ± standard deviation (n=3). Origin 7.5 (OriginLab, USA) was used to prepare figures. SPSS 18.0 (SPSS Inc., Chicago, IL) were used to analyse significant difference followed by Duncan's multiple range tests ($P<0.05$).

Results and discussion

Effects of germination on the quantity of GRE in radish sprouts.

Like other cruciferous plants, radish sprouts contain several different glucosinolates. However, GRE is the predominant glucosinolate in raw radish seeds and sprouts [13-15]. Fig. 2 shows the influence of germination time on the quantity of GRE in radish sprouts. The concentration of GRE was depleted during germination, it decreased by 80.2% after 7 days germination under our conditions. Similarly,

Zhou et al. reported a reduction of 89.2% in the GRE when radish sprouts were cultivated for 7 days [15]. This decrease of the GRE in germination process was from tissue expansion and the growth of sprouts [30].

Effects of germination on the formation of SFE and MSMTT in radish sprouts.

SFE is produced from GRE by myrosinase when radish sprouts are damaged or otherwise attacked by herbivores. In our previous study, we found that SFE was unstable and readily degraded in the hydrolytic process of GRE and then converted to a cyclic degradation product (MSMTT) [17], which could reduce the bioactivity of SFE (Fig. 1). In our current study, along with the depletion of the GRE content, SFE formation decreased (Fig. 1 and Fig. 2) in a time-dependent manner during germination process. A high level of SFE (96.9 μmol/g DW) was detected in radish seeds. After 3 days of germination, it decreased to 85.2 μmol/g DW, and then followed by a further decrease from 3 to 7 days, reaching 13.5 μmol/g DW at 7 days. However, the formation of MSMTT increased and reached its highest value (4.5 μmol/g DW) at 5 days and then stayed at this level (Fig. 2). It is well known that SFE mainly evolve from the hydrolysis of GRE facilitated by myrosinase [31]. Our results suggest that the activities or contents of myrosinase in radish sprouts increase at 3 days into the germination process, and with a marked decrease in SFE levels after 3 days of germination. Therfore, based on the results, 3-day-old radish sprouts appear to represent the most optimized condition for the synthesis of functional compounds.

Antiproliferative effects of aqueous extracts from raw radish sprouts on human non-small cell lung cancer.

In the present study, the antiproliferative effects of aqueous extracts from radish sprouts germinated for different times were evaluated on human non-small cell lung cancer H1299 cells and HCC817 cells.

When cells were treated with 0.1 mg of radish sprouts extracts/mL of media, the maximum concentration of SFE received by cells was 35.2 μM from the extracts of radish seeds (cultivated 0 day) and the minimum was 5.3 μM from the extracts of 7-day-old sprouts. Fig. 3 shows the antiproliferative effects on two lung cancer cell cultures (H1299 and HCC299) exposed to extracts from radish sprouts. Radish sprouts germinated less than 3 days could significantly ($p < 0.05$) inhibit the proliferation of the cells. However, 7-day-old sprouts had a negligible inhibitory influence ($P>0.05$) compared to the control. The decrease of SFE formation in the germination process of radish sprouts contributed much to these results. These findings indicate that germination time of radish sprouts could be an important impact on their antiproliferative activity with regards to lung cancer cell cultures. Our results are in contrast with those of Martínez-Villaluenga who reported no noticeable toxic effects of fresh radish sprouts on the proliferation and viability of the human promyelocytic leukemia cells: HL-60 [32]. The differences in our observations may be due to the differences in species of radish sprouts and/or cell lines used. Especially, the drug sensitivity of different cell lines can be distinctively different [33].

In this work, 3-day-old radish sprouts had noticeable antiproliferative effects on human lung cancer H1299 and HCC817 cells. When cells treated with 0.1 mg of 3-day-old sprouts extracts/mL of growth medium, the concentration of SFE was 20.4 μM. We compared these crude extracts with 20.4 μM of purified SFE with regards to their antiproliferative effects. Interestingly, Fig. 4 shows that the extracts provide some advantage ($P<0.05$) compared to purified SFE on HCC817 cells. This result could be due to the other isothiocyanates in radish sprouts which had antiproliferative effects as well.

Cooking treatments

Radish sprouts are usually subjected to some form of heat treatment to make them more suitable for human consumption. However, any cooking process could influence the glucosinolate-myrosinase

system in radish sprouts. Thus, we also investigated the effects of various cooking processes on the level of GRE and its hydrolysis products in radish sprouts. GRE and its hydrolysis products were affected by cooking treatments and cooking time (Fig. 5). Earlier work had indicated that the glucosinolate-myrosinase system was modified during the heating process of cruciferous vegetables due to partial or total inactivation of myrosinase or leaching of glucosinolates [21]. In order to evaluate the influence of a variety of household-like thermal treatments on the levels of GRE, SFE in radish sprouts, we used boiling, steaming and microwave treatments. As shown in Fig. 5A, boiling significantly ($P<0.05$) depleted the GRE content and SFE formation. Our analysis shows that the content of GRE was decreased to 21.1% and 15.1% after boiling sprouts for 3 and 5 min respectively. Boiling is a conventional way of cooking with a relatively large amount of water involved in the preparation of vegetables where a substantial surface area of vegetables is contacted with boiling water. We measured GRE in the cooking water and found that 75.8% and 80.4% of the total GRE was leached into cooking water after sprouts were boiled for 3 and 5 min. Therefore, leaching of GRE into the cooking water can be considered to be the main route by which GRE is lost from sprout tissue [34]. Furthermore, the level of SFE experienced an even greater loss during boiling. Even boiling for 0.5 min prior to hydrolysis resulted in a loss of SFE production of 97.2%; while MSMTT gradually decreased during the boiling process.

Steaming of the radish sprouts resulted in the greatest retention of GRE, with no significant ($P>0.05$) loss compared with raw sprouts (Fig. 5B). However, the formation of SFE dropped markedly after steaming even for a short time, however more SFE was retained compared to boiling even after extended steaming.

The content of GRE decreased somewhat while microwaving at 800W for 0.5 or 1 min. However, it

declined dramatically when microwaving continued beyond 1 min. Microwaving for 1.5 or 2 min contributed to almost complete loss of GRE and its hydrolysis products (Fig. 5C). Thus, microwave heating for more than 1 min is undesirable due to the serious loss of the predominate glucosinolate. However, even if a short microwaving time (0.5 min) was utilized, sprouts showed significantly ($P<0.05$) decreased of SFE formation compared to control sample.

Myrosinase activity was significantly decreased ($P<0.05$) even after a short (0.5 min) cooking treatment, including boiling, steaming and microwaving (Table 1). The myrosinase activity in raw radish sprouts was 1.81±0.006 U/mg, which decreased by 95% and 95.6% after microwaving and boiling for 0.5 min respectively. Steaming provided the highest retention of myrosinase activity, however, it still caused a marked decrease of 88.4% after 0.5 min. These data suggest that myrosinase in radish sprouts is heat sensitive, and is readily inactivated during any cooking process and duration.

Effects of cooking have been widely investigated in broccoli. Early work has reported that microwaving and boiling could decrease the content of glucosinolates in broccoli. Steaming, on the other hand, appeared to minimize the loss of glucosinolates [19]. Our present study is in agreement with previous results. Furthermore, it has been known that short steaming periods yield less nitrile and more sulforaphane yield from broccoli [20]. Unlike broccoli, radish has been shown to contain no ESP and therefore heating to destroy ESP does not switch formation of nitrile to formation of isothiocyanate, providing no improvement in isothiocyanate formation from low heat [35-36]. This study demonstrated that all cooking methods significantly ($P<0.05$) diminished SFE formation. Even following a brief heating period, sprouts were no longer able to yield large amounts of SFE. We found that the leaching of GRE and denaturing myrosinases during cooking treatments are the main factors that contributed the significant loss of SFE. Our results can be generalized to suggest that the consumption of raw radish

sprouts will provide the greatest SFE availability.

MSMTT is reported to be generated due to the presence and availability of the hydrogen sulfide in cruciferous vegetables [17, 37-38]. From Fig. 5, we found that the content of MSMTT decreased after three cooking treatments. We inferred that heating could drive away the hydrogen sulfide in the radish sprouts and slightly inhibit the degradation of SFE.

Antiproliferative effects of aqueous extracts from heat treated radish sprouts on human non-small cell lung cancer.

As stated previously, all cooking methods depleted the SFE formation. In order to determine whether the cooking treatment could influence the anticancer activity in spite of a marked drop in SFE, as such we evaluated the effects of short time cooking on the antiproliferative activity of radish sprouts on human non-small lung cancer H1299 and HCC827 cells. Three-day-old radish sprouts were exposed to a a brief heat treatment (boiled or steamed or microwaved for 0.5 min) and then prepared their aqueous extracts. The addition of freeze-dried aqueous extracts from any of the cooking methods did not impose strong antiproliferative activity of radish sprouts on human lung cancer H1299 and HCC827 cells compared to a control culture with no additions (Fig. 6). Contrary, the raw sprouts showed obvious inhibitory effects ($P<0.05$). Our results indicate that radish sprouts essentially lose their antiproliferative activity on lung cancer cells even after a very short cooking time. Moreover, the decrease of SFE formation during the cooking processes contributed much to these results.

Conclusions

The effects of germination and cooking processes on levels of GRE, SFE and MSMTT in radish sprouts were studied in this research. Our findings suggest that 3-day-old radish sprouts are an excellent source of GRE and SFE which could potentially benefit human health. However, even modest

cooking processes could deplete the GRE content and SFE formation, resulting the loss of the antiproliferative activity of radish sprouts. Thus eating radish sprouts in raw may be desirable from health perspective.

Acknowledgments

This study was supported by the Natural Science Foundation of China (Grant No.20806005, 21176018) and the National High Technology Research and Development Program of China (863 Program, Grant No. 2012AA021403).

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Figure captions:

Fig. 1 The formation and degradation of sulforaphene (SFE) and chromatograms of the analysis HPLC of SFE in radish sprouts germinated for 0, 3, 7 days

Fig. 2 Levels of glucoraphenin (GRE), sulforaphene (SFE) and its hydrolysis product (MSMTT) in radish sprouts germinated for 0-7 days. The data were expressed as mean ± standard deviation (n=3). Means with different letters reflect the significant difference in the levels of GRE, SFE and MSMTT respectively ($P<0.05$)

Fig. 3 Effects of aqueous extracts from radish sprouts on the proliferation of human lung cancer H1299 (A) and HCC817 cells (B). The cells were treated with the aqueous extracts from radish sprouts germinated for 0-7 days. The data were expressed as mean ± standard deviation (n=3). Means with different letters at each time point differed significantly ($P<0.05$)

Fig. 4 The proliferation curves of human lung cancer H1299 (A) and HCC817 cells (B) exposed to the aqueous extracts from 3-day-old radish sprouts or 20 μM sulforaphene (SFE). The data were expressed as mean ± standard deviation (n=3). Means with different letters at each time point differed significantly ($P<0.05$)

Fig. 5 Effects of three heat treatment methods on the levels of glucoraphenin (GRE), sulforaphene (SFE) and its hydrolysis product (MSMTT) in radish sprouts. Three-day-old radish sprouts radish sprouts were heated by boiling (A), steaming (B) and microwaving (C) for various time periods. The data were expressed as mean ± standard deviation (n=3). Means with different letters reflect the significant difference in the levels of GRE, SFE and MSMTT respectively ($P<0.05$)

Fig. 6 The proliferation curves of human lung cancer H1299 and HCC817 cells. (A) The proliferation curve of H1299 cells. (B) The proliferation curve of HCC827 cells. The cells were treated

with the aqueous extracts of 3-day-old radish sprouts which had been boiled or steamed or microwaved for 0.5 min before used. The data were expressed as mean ± standard deviation (n=3). Means with different letters at each time point differed significantly ($P<0.05$)

Table 1 Effects of cooking treatments on the myrosinase activity in radish sprouts

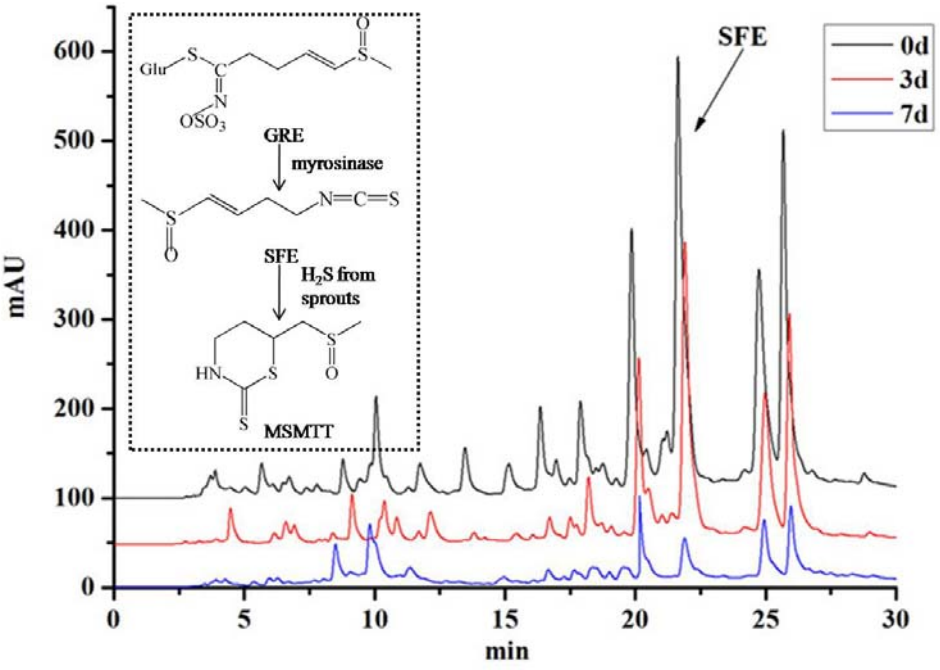


Fig. 1 The formation and degradation of sulforaphene (SFE) and chromatograms of the analysis HPLC of SFE in radish sprouts germinated for 0, 3, 7 days

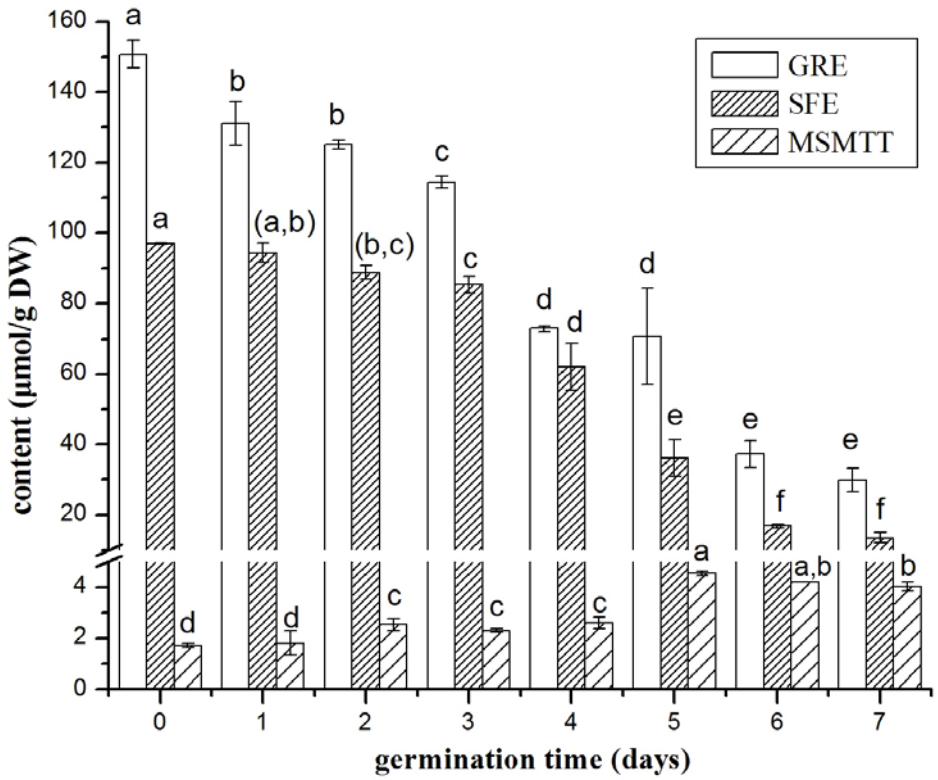
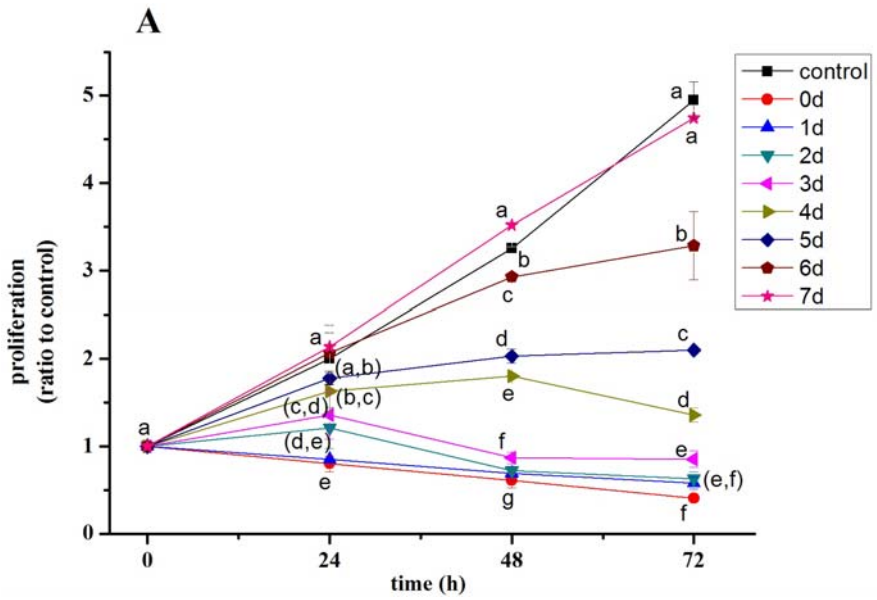


Fig. 2 Levels of glucoraphenin (GRE), sulforaphene (SFE) and its hydrolysis product (MSMTT) in radish sprouts germinated for 0-7 days. The data were expressed as mean ± standard deviation (n=3). Means with different letters reflect the significant difference in the levels of GRE, SFE and MSMTT respectively ($P<0.05$)



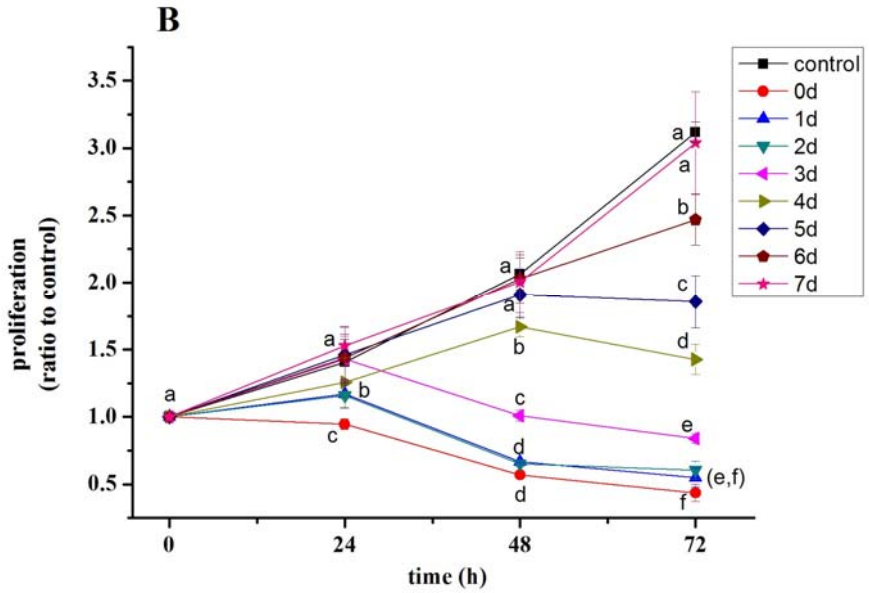


Fig. 3 Effects of aqueous extracts from radish sprouts on the proliferation of human lung cancer H1299 (A) and HCC817 cells (B). The cells were treated with the aqueous extracts from radish sprouts germinated for 0-7 days. The data were expressed as mean ± standard deviation (n=3). Means with different letters at each time point differed significantly ($P<0.05$)

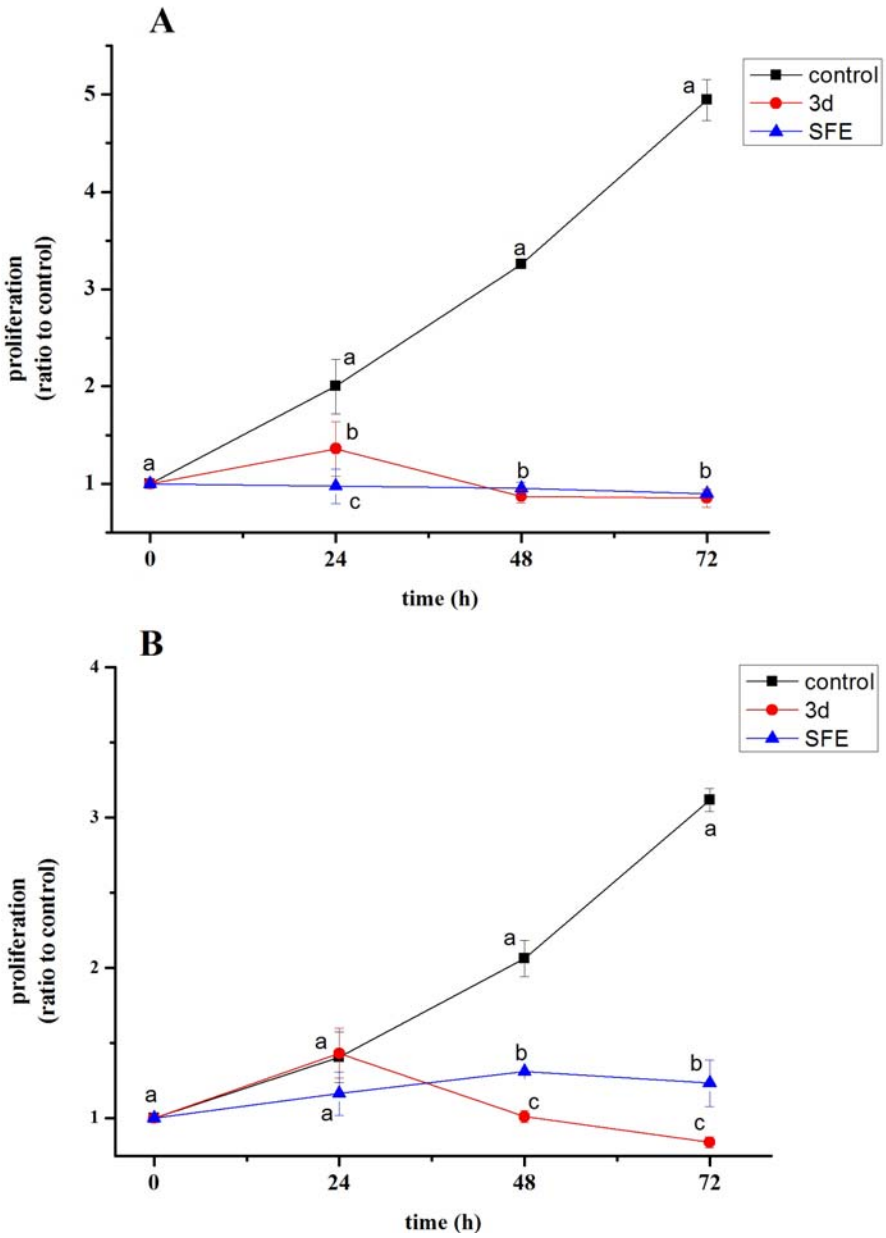
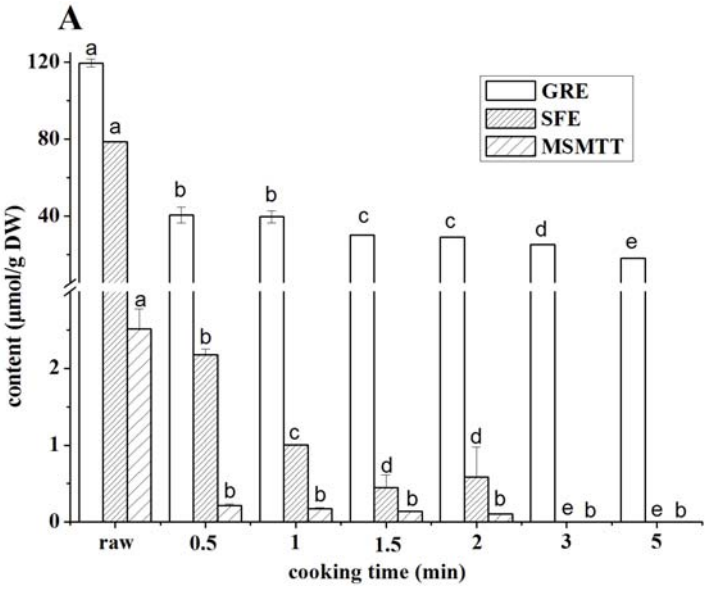
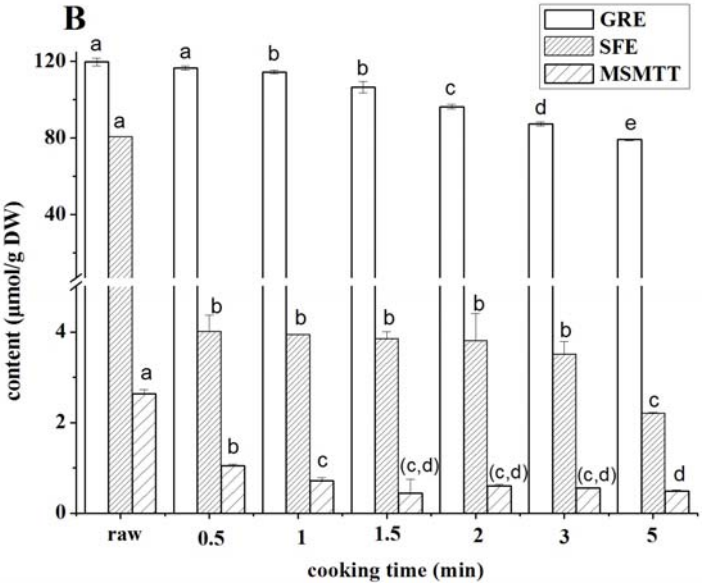


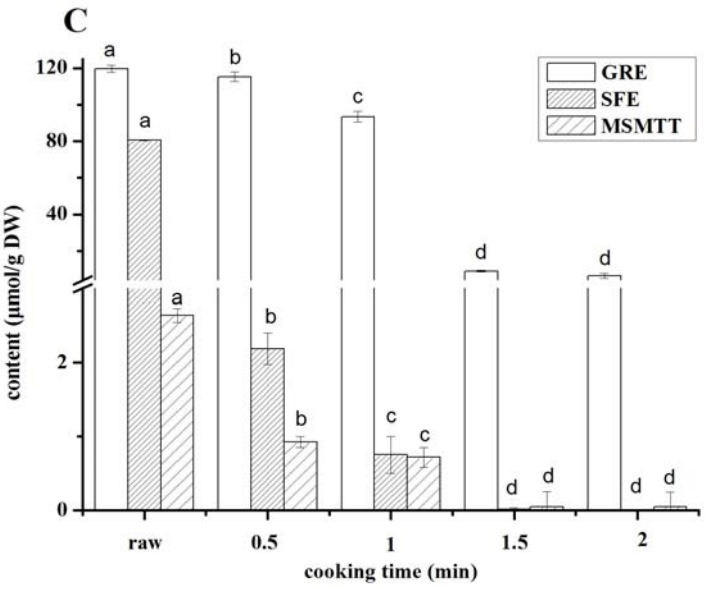
Fig. 4 The proliferation curves of human lung cancer H1299 (A) and HCC817 cells (B) exposed to the aqueous extracts from 3-day-old radish sprouts or 20 μM sulforaphene (SFE). The data were expressed as mean ± standard deviation (n=3). Means with different letters at each time point differed significantly ($P<0.05$)


A
GRE
SFE
MSMTT
content (μmol/g DW)
cooking time (min)
raw
0.5
1
1.5
2
3
5

1


B
GRE
SFE
MSMTT
content (μmol/g DW)
cooking time (min)
raw
0.5
1
1.5
2
3
5
(c,d)

2


C
GRE
SFE
MSMTT
content (μmol/g DW)
cooking time (min)
raw
0.5
1
1.5
2

3

Fig. 5 Effects of three heat treatment methods on the levels of glucoraphenin (GRE), sulforaphene (SFE) and its hydrolysis product (MSMTT) in radish sprouts. Three-day-old radish sprouts radish sprouts were heated by boiling (A), steaming (B) and microwaving (C) for various time periods. The data were expressed as mean ± standard deviation (n=3). Means with different letters reflect the significant difference in the levels of GRE, SFE and MSMTT respectively ($P<0.05$)

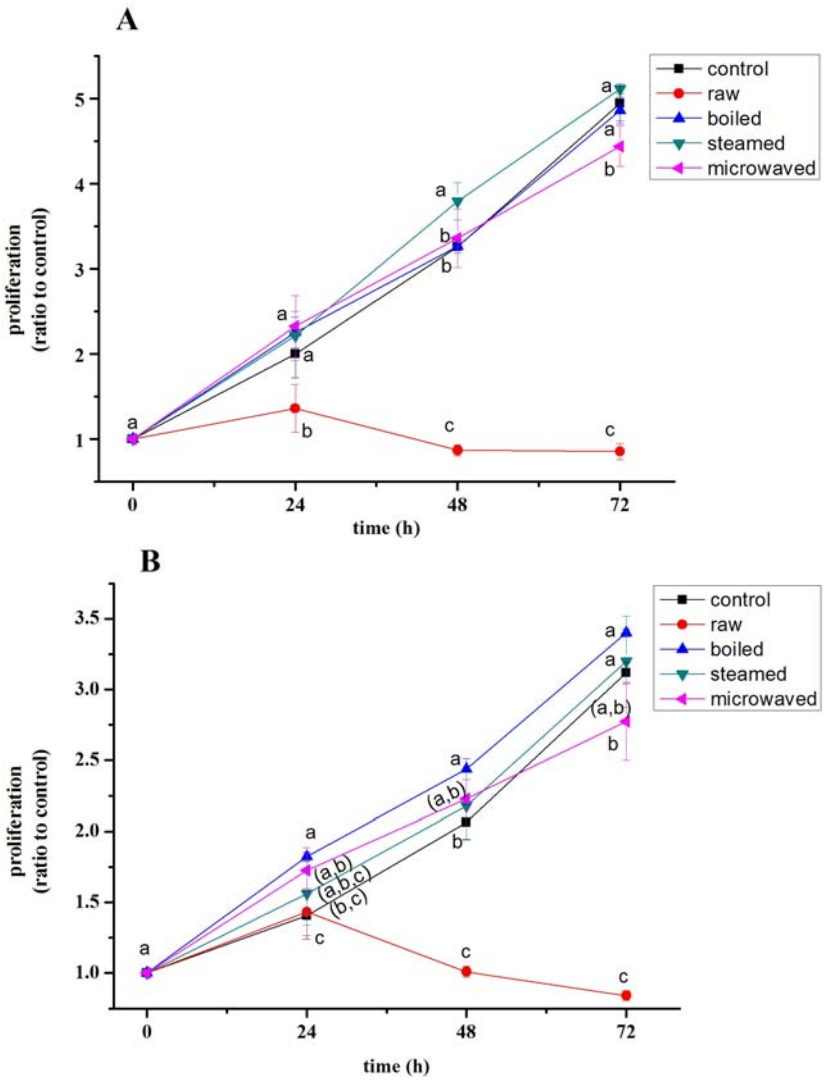


Fig. 6 The proliferation curves of human lung cancer H1299 and HCC817 cells. (A) The proliferation curve of H1299 cells. (B) The proliferation curve of HCC827 cells. The cells were treated with the aqueous extracts of 3-day-old radish sprouts which had been boiled or steamed or microwaved for 0.5 min before used. The data were expressed as mean ± standard deviation (n=3). Means with different letters at each time point differed significantly ($P<0.05$)

Table 1 Effects of cooking treatments on the myrosinase activity in radish sprouts

Cooking treatment	Myrosinase activity[a] (units[#]/mg protien)
Raw	1.81±0.006a
steaming	0.21±0.02b
microwaving	0.09±0.005c
boiling	0.08±0.003c

[a] The data were expressed as mean ± standard deviation (n=3). [#] One unit of enzyme activity is defined as the amount of enzyme which catalyzes 1 μmol sinigrin per minute at 37 ºC. Means with different letters in the same column differed significantly ($P<0.05$)