JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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Physiological and Biochemical Metabolism of Germinating Broccoli Seeds and Sprouts

Yingjuan Gu, Qianghui Guo,[‖] Liang Zhang, Zhigang Chen, Yongbin Han, and Zhenxin Gu*

College of Food Science and Technology, Nanjing Agricultural University, Nanjing, Jiangsu 210095, People's Republic of China

ABSTRACT: Changes in physiological and biochemical metabolism as well as glucoraphanin and sulforaphane contents of germinating broccoli seeds and sprouts were investigated in this study. Sprout length, root length, and fresh weight increased with germination time. Dry weight varied from 2.5 to 3.0 mg per sprout. A rapid increase in respiratory rate of sprouts occurred between 24 and 36 h of germination and then stayed at a high level. HPLC analysis found that glucoraphanin content increased at the early stage (0−12 h) of germination, decreased to a low value of 3.02 mg/g at 48 h, and then reached the highest value of 6.30 mg/g at 72 h of germination. Sulforaphane content decreased dramatically during the first day of germination, then increased slowly, and reached a high value of 3.38 mg/g at 48 h before declining again.

KEYWORDS: *broccoli seed germination, sprout, physiological and biochemical metabolism, glucoraphanin, sulforaphane*

INTRODUCTION

Broccoli (*Brassica oleracea* L. var. *italica* Plenck) belongs to the genus of *Cruciferous Brassica*. Large amounts of anticarcinogenic compounds,[1,2] antioxidants, vitamin C, and health-promoting compounds such as glucosinolates and phenolic compounds[3−5] exist in its seeds and sprouts. Glucosinolates are an important and unique class of thioglucosides, which are the key components of an active chemical defense found in Brassicales. When plants are mechanically damaged, infected, or attacked by pests, and metabolically induced, glucosinolates and myrosinase directly bond together.[6] Then glucosinolates are broken down and converted into a variety of degradation compounds (glucose, sulfate, isothiocyanates, thiocyanates, and nitriles).[7] Isothiocyanates have a preventive effect against cancers, particularly bladder, colon, and lung cancers.[8]

Sulforaphane (4-methylsulfinylbutyl isothiocyanate) is a type of sulfur-containing isothiocyanate receiving increasing attention due to its anticarcinogenic function.[9] Sulforaphane is a naturally occurring inducer of phase II enzymes in human and animal bodies to detoxify cancer-causing chemicals. Thus, it can decrease the occurrence of cancer.[10,11] Sulforaphane also has a cytoprotective effect against tissue damage associated with oxidative stress.[12] Glucoraphanin, the precursor of sulforaphane, is the predominant glucosinolate in broccoli, which is a rich source of sulforaphane.[13,14] It is necessary to investigate the changing pattern of glucoraphanin content during broccoli germination.

Seed germination begins with water absorption. Significant changes occur during germination, including the interconversion and synthesis of new compounds.[15] Seed germination also leads to radicle and hypocotyl growth and activation of stored substances. The content of alkyl glucosinolates decreased, whereas that of indol-3-ylmethylglucosinolates increased in germinating broccoli seeds.[16] Williams et al.[17] investigated changes of myrosinase and activities of epithiospecifier proteins (ESP) during broccoli seed germination and found that ESP activity increased up to day 2 after germination and then decreased to seed activity level at day 5. These changes varied with myrosinase activity and final alkenyl glucosinolate content.

There have been a number of studies on sulforaphane distribution and content in mature broccoli plants.[18−20] Previous studies mainly dealt with the effects of exogenous treatments such as light conditions,[21] air pressure and temperature,[22] nitrogen and sulfur fertilization,[23] and UV-C radiation.[24] Little literature is available on the changing patterns of sulforaphane and physiological and biochemical metabolism of germinating broccoli seeds without exogenous treatments.

Sprouts are a popular healthy product not only in China but also around the world. Broccoli sprouts are rich in health-beneficial compounds. It is important and necessary to improve our knowledge on the changing patterns of physiological and biochemical metabolism as well as glucoraphanin and sulforaphane contents during broccoli seed germination. The purpose of the present study is to investigate these important indices including sprout length, root length, dry substances, crude fat, respiratory rate, and glucoraphanin and sulforaphane contents to provide scientific evidence for future development of functional edible sprouts with high sulforaphane accumulation.

MATERIALS AND METHODS

Materials. Seven cultivars of broccoli seeds (ZaoSheng, ZS; XueBai, XB; RuiFan N732, RF; YinXing 100, YX; TaiYou, TY; LuLingxiang, LLX; and XiaMenYinhua, XMYH) were purchased from Nanjing Jinshengda Seed Co. (Jiangsu, China). Standard samples of glucoraphanin and bovine serum albumin (BSA) were purchased from Chrom-Matrix Co. (Richland, WA) and Lanji Science and Technology Development Co. (Shanghai, China), respectively. Acetonitrile was of high-performance liquid chromatography (HPLC) grade. Other chemicals and reagents were of analytical grade and purchased from Shanghai Institute of Biochemistry (Shanghai, China).

Seed Germination. One gram of broccoli seeds was surface-sterilized by soaking for 15 min in 1.5% sodium hypochlorite and then

Received: September 6, 2011
Revised: December 5, 2011
Accepted: December 5, 2011
Published: December 5, 2011

dx.doi.org/10.1021/jf203599v | J. Agric.Food Chem. 2012, 60, 209−213

rinsed three times with distilled water. Sterilized seeds were then germinated on a filter paper in Petri dishes filled with sterilized quartz sand (15 cm in diameter) and grown in incubators at 28 °C in darkness for 3 days. Seeds were supplied with distilled water every 6 h. The germinating broccoli seeds at the times of 0, 4, 8, 12, 18, 24, 36, 48, 60, and 72 h, respectively, after the above treatments were carefully washed with distilled water, dried on a filter paper, and then flash-frozen in liquid nitrogen for further experiments.

Determination of Biochemical Components in Broccoli Seeds and Sprouts. Water, crude fat (solvent extraction), crude protein (Kjeldahl, multiplying factor of 6.25), and ash contents were determined according to standard AOAC methods.[25] Free amino acid content was estimated using a ninhydrin colorimetric method. Reducing sugars were estimated by Miller's method.[26] Quantitative determination of total soluble sugars was carried out according to the colorimetric method.[27]

Measurement of Sprout Length and Root Length. Sprout length and root length were measured directly using a caliper on different sampling schedules during broccoli seed germination. Twenty germinated sprouts were set as a sampling group for each measurement.

Measurement of Respiratory Rate. One gram of fresh broccoli seeds or sprouts was put into a sealed container at 28 °C for 1 h. An infrared gas analyzer was used to determine its CO_2 concentration. Another sample was measured for its dry weight. The respiratory rate was expressed as micromoles per gram per minute.

Determination of Sulforaphane Content. Freeze-dried germinating broccoli seeds and sprouts were ground into powder. One gram of sample was defatted three times with excess hexane and dried in a fume hood. Following this, defatted seeds were hydrolyzed by adding distilled water in a ratio of 4:1 both in weight of water and in defatted seed. The mixture was hydrolyzed at 35 °C for 2 h in a shaking water bath. After hydrolyzing, it was extracted three times with 10 mL of ethyl acetate, which was combined and salted with 1 g of sodium chloride. The ethyl acetate fraction was dried at 35 °C under vacuum on a rotary evaporator. The residue was dissolved in 1 mL of 10% acetonitrile and then filtered through a 0.45 μm membrane filter before injection into the HPLC.

Determination of Glucoraphanin Content. The glucoraphanin content was determined using the procedure of Rochfort et al.[28] with slight modifications as below.

Crude Sample Preparation. Five milliliters of boiling water was added to approximately 0.5 g of freeze-dried germinating broccoli seeds. The mixture was boiled for 5 min. The bulk of the water was decanted, and the seeds were transferred to a mortar with 5 mL of water. The mixture was extracted with 5 mL of boiling water twice, then combined the extractions, and concentrated to 3 mL under vacuum.

C18 Solid Phase Extraction (SPE). A C18 SPE cartridge was activated with 3 mL of methanol and washed with 3 mL of water. A crude glucosinolate concentrate was obtained from the above crude sample by passing the solution through the cartridge and washed with 3 mL of 10% methanol.

Amino Propyl SPE. A protonated amino propyl anion exchange SPE cartridge was activated with 3 mL of methanol and equilibrated with 3 mL of water. The crude glucosinolate concentrate was placed into the cartridge and washed with 3 mL of methanol, and then the glucosinolates were removed by washing with 5 mL of 2% ammonia solution. The ammonia solvent was removed under vacuum, leaving the residue with glucoraphanin. The latter was dissolved in 1 mL of distilled water and filtered through a 0.45 μm membrane filter prior to HPLC.

Statistical Analysis. In this paper, the sulforaphane content in seeds was measured in seven broccoli cultivars. The rest was represented by the cultivar LLX only. Experimental data were expressed as the mean ± standard deviation (SD) with three replications ($n = 3$). SPSS 18.0 (SPSS Inc., Chicago, IL) was applied for the significant difference test. Excel 2003 (Microsoft Co., Redmond, WA) and Agilent1200 chemical station software (Agilent Co., Santa Clara, CA) were used to prepare the figures.

RESULTS AND DISCUSSION

Effect of Cultivars on Sulforaphane Content during Seed Germination. Figure 1 shows the differences in sulforaphane content of seven broccoli cultivar seeds. Sulforaphane content varied greatly among cultivars. A high sulforaphane content was detected in cultivars LLX, ZS, RF, and TY. LLX was as high as 3.46 mg/g, whereas XB was as low as 0.24 mg/g. This result was in agreement with that of Liang et al.[29] for sulforaphane content in 18 varieties of broccoli. They also found significant differences in sulforaphane content among different cultivars. Pérez-Balibrea et al.[14] observed that the glucoraphanin content of seeds and sprouts differed among three commercial broccoli cultivars, but they did not measure sulforaphane content hydrolyzed from the sprouts. Glucoraphanin is the precursor of sulforaphane, and its content varies with different plant genotypes/cultivars,[30] so sulforaphane formation will be consequently influenced by its heterogeneity, which is in accordance with our present experimental results.

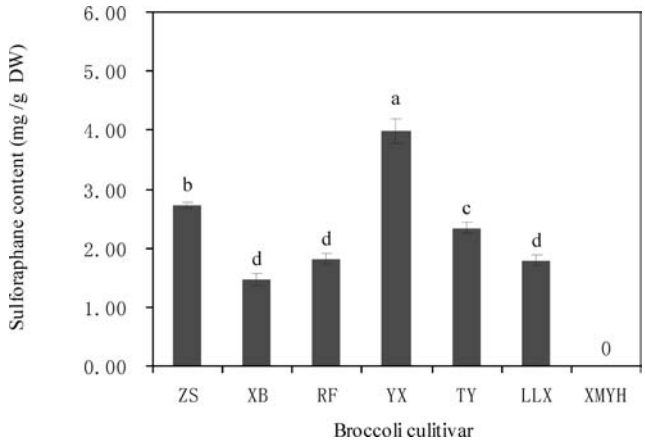

Figure 1. Sulforaphane content in seeds of different broccoli cultivars. Values are the means of triplicate analyses. Error bars show the standard deviation. Lower case letters reflect the significance of differences in sulforaphane content ($p < 0.05$).

Nutritional Contents in Broccoli Seeds and Sprouts. Nutritional contents in broccoli (cultivar LLX) seeds and sprouts (48 h after germination) are shown in Table 1.

Table 1. Nutritional Contents of Broccoli Cultivar LLX Seeds and Sprouts

	content[a] (g/100 g)	
index	seed	sprout
water content	4.16 ± 0.22 b	70.20 ± 8.22 a
crude fat	31.99 ± 2.06 b	40.31 ± 2.26 a
crude protein	21.01 ± 3.30 a	16.86 ± 2.44 a
total sugars	19.02 ± 2.02 a	20.22 ± 5.31 a
reducing sugar	3.24 ± 0.07 b	10.64 ± 1.44 a
free amino acid	0.25 ± 0.01 b	2.33 ± 0.56 a

[a]Data are mean ± SD with three replications. Those in the same row with different letters are significantly different ($p < 0.05$). Dry weight except water content for fresh weight.

Nongerminated seeds contain a low level of water content with high contents of crude fat and protein, but lower contents of free amino acids and reducing sugars. After germination for 48 h, the moisture content of sprouts increased over 15-fold.

The contents of crude fat, free amino acids, reducing sugars, and total sugars increased at different levels, respectively, whereas that of crude protein declined. These results agreed with the results of Oloyo,[31] who studied germinating seeds of pigeon pea and observed that crude protein and nitrogen free extractives (crude carbohydrate) decreased gradually, whereas fat, ash, and bioenergy increased during germination. In the present experiment, the contents of free amino acids and reducing sugars increased by 10- and 3-fold, respectively. They indicated that quantities of free amino acids and reducing sugars were formed during seed germination. This phenomenon was also observed by Yang et al.,[32] Rimsten et al.,[33] and Saman et al.[34] Crude fat, crude protein, and carbohydrates were major nutrient components in broccoli sprouts. They are important sources of bioenergy and stored nutrients for seed germination and sprout growth. These important nutritional components can subsequently provide both seed and sprout with essential nutrients and functional substances for growth and development during the germination process.

Seed germination is an efficient and low-cost approach to changing chemical compositions of broccoli sprouts without complicated treatments. It has a potential to increase the nutritive value of broccoli sprouts and improve its acceptance for human consumption.

Sprout Length, Root Length, and Respiratory Rate of Germinated Broccoli. Figure 2 indicates that broccoli seeds initiated their sprouts and roots after 12 h of germination. The lengths of sprouts and roots reached 3 and 4 mm at 36 h and 20 and 30 mm at 72 h, respectively. These facts evidenced that broccoli sprouts grew rapidly after 36 h of germination.

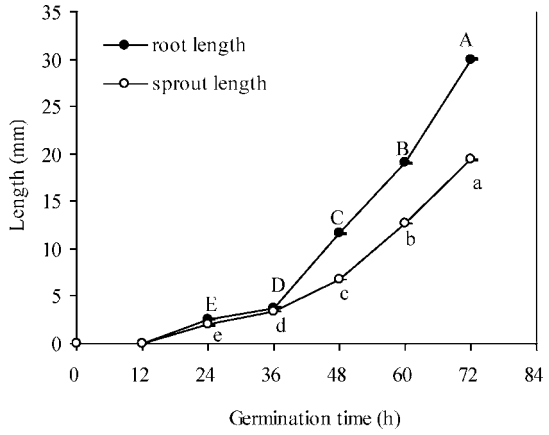

Figure 2. Changing patterns in sprout and root length of broccoli cultivar LLX during germination. Values are the means of triplicate analyses. Error bars show the standard deviation. Capital letters and lower case letters reflect the significance of differences in root length and sprout length ($p < 0.05$), respectively.

An actively germinating seed supplies biochemical nutrients and bioenergy through phytophysiological respiration metabolism and relies exclusively on the seed reserves for the respiration process as well as other anabolic reactions.[35] The respiration process is triggered by ascorbic acid and thus leads to a remarkable increase in the ascorbic acid content of broccoli sprouts, making the sprouts more nutritive for human comsumption.[14]

A higher respiratory rate promotes vigorous growth of sprouts. Results in Figure 3 show the changing pattern in respiratory rate of broccoli (cultivar LLX) seeds and sprouts during germination. In the first 24 h of germination, a low level of respiration

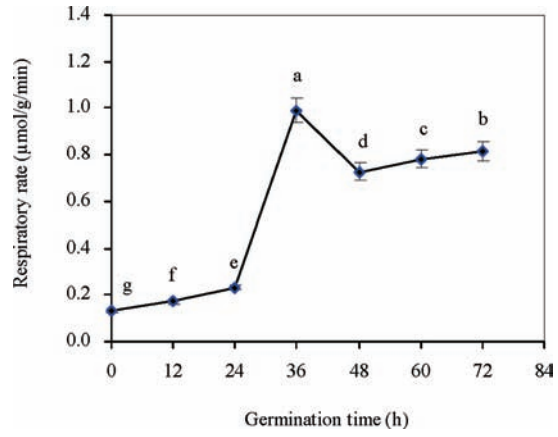

Figure 3. Changing pattern in respiratory rate of broccoli cultivar LLX seeds and sprouts during germination. Values are the means of triplicate analyses. Error bars show the standard deviation. Lower case letters reflect the significance of differences in respiratory rate ($p < 0.05$).

metabolism was detected with little release of CO_2, followed by a dramatic increase in respiration rate from 24 to 36 h, reaching a peak value of 0.992 μmol/g/min at 36 h. Then a decline occurred, and a relatively high level of respiratory rate was retained until the end of germination. This change was in accordance with the developing trend of sprout and root length (Figure 2), suggesting that a large amount of bioenergy required for sprout growth and root elongation was associated with an increase in the respiration rate of the germinating sprouts.

Changing Patterns in Fresh Weight, Dry Weight, and Crude Fat Content. As shown in Figure 4, the fresh weight of sprouts increased with germination time, whereas the dry weight varied from 2.5 to 3.0 mg per sprout.

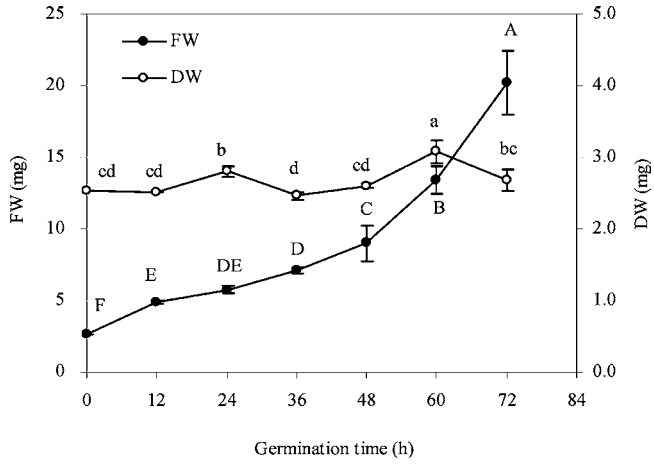

Figure 4. Changing patterns in fresh weight and dry weight of broccoli cultivar LLX seeds and sprouts during germination. Values are the means of triplicate analyses. Error bars show the standard deviation. Capital letters and lower case letters reflect the significance of differences in fresh weight and dry weight ($p < 0.05$), respectively.

The changing pattern in crude fat content is displayed in Figure 5. No obvious variation was found during the first 36 h of germination. It remained at a high level from 48 to 60 h, but significantly decreased after 60 h of germination. These facts indicate that crude fat was hydrolyzed into fatty acids at an early stage of germination, which led to an increase in crude fat

dx.doi.org/10.1021/jf203599v | J. Agric.Food Chem. 2012, 60, 209–213

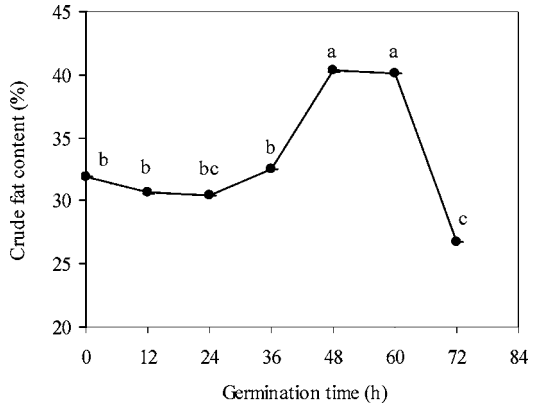

Figure 5. Changing pattern in crude fat content of broccoli cultivar LLX seeds and sprouts during germination. Values are the means of triplicate analyses. Error bars show the standard deviation. Lower case letters reflect the significance of differences in crude fat ($p < 0.05$).

content; but the latter declined after 60 h of germination due to sprout consumption on the crude fat. This result corresponded to that of Lorenz,[36] who found the increase resulted from an actual increase in the ratio of other nutrients due to a loss of dry matter, mainly in the form of carbohydrates, for respiration during sprouting. Parameswaran and Sadasivam[37] also observed an increase in the percentage of protein in germinated grains of proso millet as a consequence of dry matter loss during germination. However, the content of crude fat decreased drastically to a level lower than that of nongerminated seeds after 60 h of germination in the present study, which was caused by the hydrolysis of crude fat into carbohydrates at the late stage of germination.

Changing Patterns in Glucoraphanin and Sulforaphane Contents. Glucoraphanin content (Figure 6) experienced

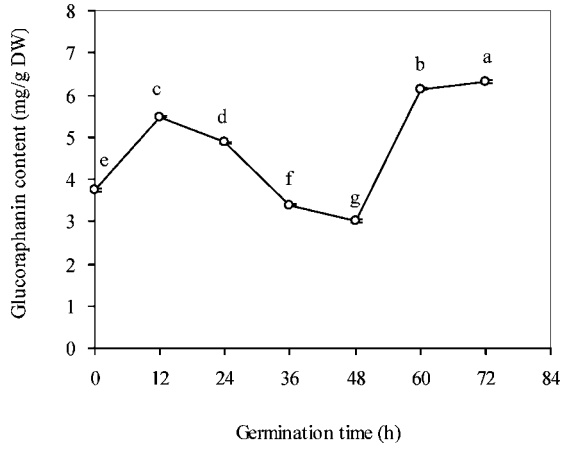

Figure 6. Changing pattern in glucoraphanin content of broccoli cultivar LLX seeds and sprouts during germination. Values are the means of triplicate analyses. Error bars show the standard deviation. Lower case letters reflect the significance of differences in glucoraphanin content ($p < 0.05$).

an increasing trend over the first 12 h of germination, but decreased to the lowest value of 3.02 mg/g DW at 48 h and increased again to 6.14 mg/g DW at 60 h until staying at this level. The glucoraphanin content of 3.74 mg/g DW in nongerminated broccoli seeds in this study was different from that of 5.87 mg/g DW purified from 3 g of broccoli seeds by Rochfort et al.[28] A similar variation in glucoraphanin content among different broccoli cultivars was also observed by West et al.[30] and Pereira et al.[38]

Sulforaphane content dropped markedly over the first 18 h of germination and then increased gradually to the highest level of 3.38 mg/g DW at 48 h before declining again to a low level of 0.93 mg/g DW at 60 h (Figure 7). A similar result was obtained

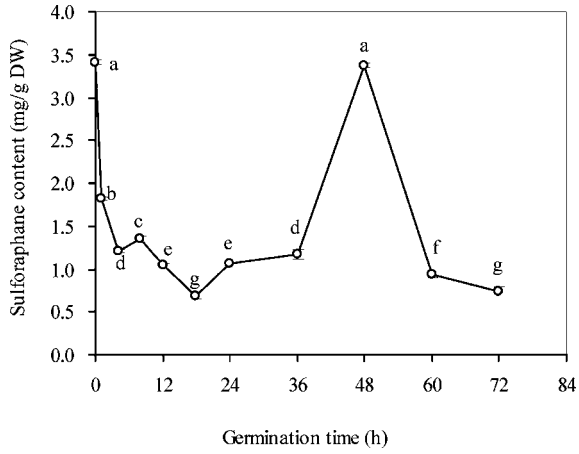

Figure 7. Changing pattern in sulforaphane content of broccoli cultivar LLX seeds and sprouts during germination. Values are the means of triplicate analyses. Error bars show the standard deviation. Lower case letters reflect the significance of differences in sulforaphane content ($p < 0.05$).

by Williams et al.[17] with a slight decrease in sulforaphane content on day 2 of germination, then increasing slightly while dropping dramatically again after day 4, and then remaining stable at a low value.

In this study, the authors have found that broccoli sprout and root length increase significantly with time of seed germination. There is a continuous increase in its respiratory rate during the first 36 h of germination, whereas a decreasing trend occurs in its dry weight, and glucoraphanin content significantly increases at 48 h of germination, with a simultaneous decrease in sulforaphane content. On the basis of the present experimental evidence, it is suggested that 2-day-old sprouts of broccoli cultivar LLX are an excellent source of bioactively functional compounds benefiting human health.

AUTHOR INFORMATION

Corresponding Author

*Phone/fax: 86-25-84396293. E-mail: guzx@njau.edu.cn.

Author Contributions

‖Shares an equal contribution as the first author.

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