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Hydrolysis before Stir-Frying Increases the Isothiocyanate Content of Broccoli

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ABSTRACT: Broccoli is found to be a good source of glucosinolates, which can be hydrolyzed by endogenous myrosinase to obtain chemopreventive isothiocyanates (ITCs); among them, sulforaphane (SF) is the most important agent. Studies have shown that cooking greatly affects the levels of SF and total ITCs in broccoli. However, the stability of these compounds during cooking has been infrequently examined. In this study, we proved that the half-lives of SF and total ITCs during stir-frying were 7.7 and 5.9 min, respectively, while the myrosinase activity decreased by 80% after stir-frying for 3 min; SF and total ITCs were more stable than myrosinase. Thus, the contents of SF and total ITCs decreased during stir-frying largely because myrosinase was destroyed. Subsequently, it was confirmed that compared to direct stir-frying, hydrolysis of glucosinolates in broccoli for 90 min followed by stir-frying increased the SF and total ITC concentration by 2.8 and 2.6 times, respectively. This method provides large quantities of beneficial ITCs even after cooking.

KEYWORDS: *broccoli, stir-frying, sulforaphane, isothiocyanate, myrosinase*

INTRODUCTION

Broccoli has been widely approved for its beneficial effects on human health because it contains high concentrations of vitamins, minerals, polyphenols, and in particular a group of phytochemicals named glucosinolates.[1] Epidemiological studies have shown that broccoli has potential beneficial effects, including the prevention or reversal of cancer,[2] hepatic steatosis,[3] and cardiovascular disease.[4] The health effects of broccoli are related to its high content of glucosinolates.[5] When fresh broccoli florets or broccoli sprouts are crushed, chopped or chewed, glucosinolates are hydrolyzed to equimolar amounts of glucose and unstable thiono compounds. The latter are spontaneously converted into bioactive isothiocyanates (ITCs) by the action of an endogenous enzyme called myrosinase, or non-bioactive nitriles by the action of epithiospecifier protein (ESP).[5,6] The most abundant glucosinolate found in broccoli is glucoraphanin, followed by glucobrassicin, which are hydrolyzed to sulforaphane (SF) and indole-3-carbinol by the action of myrosinase.[7]

Before being consumed, broccoli is commonly cooked. Cooking methods such as steaming and microwaving are common in Western society, whereas stir-frying is the most popular method in China.[8] SF has many bioactivities, and methods for retaining or improving its content in cooked broccoli are vital. Some studies have shown that cooking methods greatly alter the content of total glucosinolates and their hydrolysates, as well as the myrosinase activity in brassica vegetables.[9] For example, steaming broccoli for 2 min has a significant inhibitory effect on SF production.[10] A study by Rungapamestry et al.[11] showed that hydrolysis of glucoraphanin to SF and absorption of SF in humans after consumption of lightly cooked broccoli (microwaved for 2 min) were ∼3 times those after consumption of fully cooked broccoli (microwaved for 5.5 min), indicating that a longer cooking time inhibits SF production. Jones et al.[9] reported that the rate of SF production decreased from 51.9 to <1.1 mg/kg of dry weight (DW) after microwaving or boiling for 2−5 min, and steaming for 2 min resulted in an ∼50% SF yield loss. Therefore, the authors suggested that for optimum SF intake, broccoli should be eaten raw or lightly steamed. Vermeulen et al.[12] found that, compared with that of microwaved broccoli, consumption of raw broccoli led to much higher SF levels in urine and blood. SF was not detectable in broccoli florets after boiling for 15 min or steaming for 23 min, whereas pressure/microwave cooking for 2 min caused an only 17% loss of SF.[13] Generally, compared with the consumption of raw broccoli hydrolyzed by endogenous myrosinase during digestion, the intake of cooked broccoli usually provides approximately one-tenth of the amount of SF.[14−16] These results show that only raw or lightly processed broccoli provides high levels of beneficial SF.

The stability of SF under different conditions and in different systems has been reported in a number of studies.[17,18] As an inherently reactive small molecule, SF is reported to be thermally sensitive and readily degradable.[17,18] Surprisingly, few methods have reported the SF concentrations in stir-fried broccoli, and to the best of our knowledge, no report has focused on SF stability during the stir-frying process. In a

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previous study, we showed that the half-life ($t_{1/2}$) of SF at 90 °C and pH 6.0 was 1.5 h.[19] Considering that the stir-frying time is usually 4 min, SF and total ITCs may not be as labile as we thought. Nevertheless, myrosinase is very labile, which in broccoli tissue and juice was stable up to 45 and 40 °C, respectively.[20,21] Consequently, we expect that because myrosinase is much more labile than ITCs, myrosinase will be destroyed during cooking, and the levels of formation of SF and total ITCs will greatly decrease. Thus, allowing for hydrolysis before stir-frying should increase the SF and total ITC contents of broccoli.

In this study, we evaluated the stabilities of SF, total ITCs, and myrosinase during the stir-frying process. A method for modifying the broccoli stir-frying process to increase the level of SF and total ITC formation is proposed.

MATERIALS AND METHODS

Materials. Broccoli seeds, namely LS-1, were preserved at Zhejiang Provincial Key Lab for Chem & Bio Processing Technology of Farm Products. Broccoli and carrots were purchased from a local market. One single cultivar of broccoli purchased at one time was used to study myrosinase activity, and another single cultivar of broccoli purchased at another time was used to study ITC contents before and after stir-frying.

Sulforaphane (98%) and sinigrin hydrate (99%) were purchased from Sigma Chemical Co. (St. Louis, MO). 1,2-Benzenedithiol was purchased from TCI Co. (Tokyo, Japan). The glucose GOD/PAP kit was purchased from Nanjing Jiancheng Biotech Inc. (Jiangsu, China). The protein quantitation kit was purchased from Sangon Biotech (Shanghai, China). Methanol (Merck) was HPLC grade, and all other chemicals used were of analytical grade and purchased from either Aladdin (Shanghai, China) or Sangon Biotech.

Total ITC Extraction Methods. Total ITCs were extracted by the method previously reported.[19] Briefly, 50 g of broccoli seed meal was ground using a Chinese herbal medicine grinder for 15 s; 400 mL of hexane was added to the seed meal and the mixture stirred for 3 h. The seed meal was then transferred and dried in a fume hood overnight to obtain defatted seed meal. In the hydrolysis and extraction step, 200 mL of ethyl acetate and 100 mL of potassium phosphate buffer (0.05 M, pH 5.8) were added simultaneously to the seed meal and agitated for 4 h, and then 20 g of sodium chloride and 10 g of anhydrous sodium sulfate were added to the solution and agitated. The ethyl acetate layer was transferred, and the residual seed meal was extracted twice with an equal volume of ethyl acetate; the resulting extracts were combined and dried in a rotary evaporator (Yarong Instrument, Shanghai, China). The residue was diluted with water and stored in a refrigerator at −20 °C.

Stability of SF and Total ITCs during Stir-Frying. Carrots (100 g) were cut into a 0.3 cm dice, and 10 mL of broccoli extracts with a SF concentration of 4.17 mg/mL was added and mixed. An electromagnetic oven with an output power of 1200 W was preheated for 1 min, and then the carrot/broccoli extract mixture was stir-fried in a wok; at 0, 1, 2, 3, 4, 6, 8, and 10 min, a 5 g sample was taken and rapidly cooled by being placed in an ice/water bath. Each sample was extracted three times with 15 mL of ethyl acetate, and the resulting ethyl acetate layers were combined and evaporated at 30 °C. The residue was dissolved with methanol and constant-volumed to 10 mL. The SF and total ITC concentrations of each sample were measured by HPLC, as described below. Three independent replicates were performed for each experiment.

Determination of Myrosinase Activity during Stir-Frying. Myrosinase activity was measured by the method of Guo et al.[22] with some modifications. Briefly, an electromagnetic oven with an output power of 1200 W was preheated for 1 min, and then a total of 100 g of broccoli was stir-fried in a wok, as described above. At 0, 1, 2, 3, 4, 6, and 8 min, a 5 g sample was taken and was cooled by being placed in an ice/water bath. Each sample was divided into two equal parts; one part was heated at 80 °C overnight to obtain the dry weight. Another part was homogenized with 15 mL of 0.1 M phosphate buffer (pH 6.5) in an ice bath for 3 min. The homogenate was centrifuged at 10000*g* and 4 °C for 15 min; 500 μL of the supernatant was mixed with 500 μL of distilled water and boiled for 5 min, and then the amount of glucose in the mixture (A1) was quantified using the glucose GOD/PAP kit (Nanjing Jiancheng Biotech Inc.). An additional 500 μL of supernatant was mixed with 500 μL of a 0.25 mM sinigrin solution and incubated at 37 °C for 15 min. The reaction was stopped by boiling the mixture for 5 min. The amount of glucose in the mixture (A2) was analyzed using the glucose GOD/PAP kit. The amount of glucose formed in the enzymatic hydrolysis was calculated by subtracting A1 from A2. One myrosinase unit was expressed as 1 nmol of glucose formed/min. The specific activity was defined as units per gram of broccoli (dry weight). Three independent replicates were performed for each experiment.

Hydrolysis Rate of Myrosinase. Two and one-half grams of broccoli was homogenized with 15 mL of 0.1 M phosphate buffer (pH 6.5) in an ice bath for 3 min. The homogenate was centrifuged at 10000*g* and 4 °C for 15 min to obtain the supernatant; 0.25 mL of 2.5 mM sinigrin was mixed with 0.25 mL of supernatant, well capped, and incubated at 37 °C. At 0, 10, 15, 30, 45, 60, 90, 120, 150, and 210 min, one tube was randomly taken from the water bath, and its contents were boiled for 5 min to quench the reaction. The amount of glucose was measured using the glucose GOD/PAP kit. Three independent replicates were performed for each experiment.

Effect of Hydrolysis on ITC Content after Stir-Frying. Fresh broccoli florets were cut into small pieces (size of approximately 0.2 cm × 0.2 cm × 0.2 cm) and divided into three groups, namely, the hydrolyzed and stir-fried (HS) group, the directly stir-fried (DS) group, and the raw broccoli (RB) group. For the HS group, 100 g of chopped fresh broccoli was mixed with 3 mL of water and incubated in a 37 °C water bath for 90 min. An electromagnetic oven with an output power of 1200 W was preheated for 1 min, and the broccoli was stir-fried in a wok for 4 min. Then, the broccoli meal was mixed with 200 mL of ethyl acetate and 100 mL of 0.05 M potassium phosphate buffer (pH 7.0) and agitated for 30 min; 25 g of NaCl were then added, and the mixture was shaken and filtered with filter paper. The ethyl acetate layer was transferred, and the water layer was extracted with 200 mL of ethyl acetate two additional times. The ethyl acetate layers were combined and evaporated. The residue was dissolved using 25 mL of methanol. For the DS group, the electromagnetic oven with an output power of 1200 W was preheated for 1 min, and 100 g of chopped broccoli florets was stir-fried for 4 min; then the broccoli florets were rapidly cooled by being placed in an ice/water bath. The florets were mixed with 200 mL of ethyl acetate and 100 mL of potassium phosphate buffer, and the following steps were the same as those for the HS group. For the RB group, 100 g of chopped fresh broccoli was mixed with 200 mL of ethyl acetate and 100 mL of 0.05 M potassium phosphate buffer (pH 7.0), and the following extraction steps were the same as those for the HS group. Three independent replicates were performed for each condition. The total ITC concentration in the methanol solution was measured by HPLC, and concentrations of individual ITCs, nitriles, and degradation products were determined by GC-MS, as described below.

Quantification of Total ITCs. Quantification of total ITCs was performed using the cyclocondensation method.[23] Briefly, the ITC sample was mixed with 10 mM 1,2-benzenedithiol and 25 mM potassium phosphate buffer. After being incubated for 2 h at 65 °C, the mixture was cooled to room temperature and then centrifuged at 16000*g* for 10 min. The supernatant was collected and analyzed using a Waters (Milford, MA) e2695 HPLC system equipped with a 4.6 mm × 250 mm inside diameter, 5 μm WondaCract ODS-2 column (Shimadzu). The mobile phase consisted of 80% methanol and 20% water at a flow rate of 1.0 mL/min, and a 10 μL sample was injected onto the column. The cyclocondensation product, 1,3-benzodithiole-2-thione, was detected by absorption at a wavelength of 365 nm using a Waters 2489 detector. The standard curve was prepared using varying amounts of pure SF to estimate the ITC concentration in each sample.

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SF Quantification. SF was quantified using a Waters e2695 HPLC system using the method previously reported.[19] Ten microliters of sample was injected onto a 4.6 mm × 250 mm inside diameter, 5 μm WondaCract ODS-2 column (Shimadzu). The mobile phase consisted of methanol and water; the flow rate was 1 mL/min, and the column oven temperature was 25 °C. The gradient conditions were as follows. The level of methanol was increased from 20% in water to 60% over 10 min, then increased to 100% over 2 min, and maintained for an additional 2 min. SF was detected by the absorbance at 241 nm using a Waters 2489 detector.

GC-MS Analysis. Glucosinolate hydrolysis products were assessed by a model 7890A GC system (Agilent Technologies Inc., Palo Alto, CA) by the method previously reported.[24] Mass spectra were recorded with a model 5975C inert XL/CI MSD with a triple-axis detector enabling recording of ions from m/z 35 to 500. The capillary column contained HP-5MS UI capillaries (0.25 μm film thickness, 30 m × 0.25 mm inside diameter). The injection volume was 1.0 μL. The injector and detector temperatures were set to 270 and 280 °C, respectively. The column oven temperature started at 50 °C for 2 min, increased at a rate of 10 °C/min to 190 °C, then increased at a rate of 20 °C/min to 300 °C, and was maintained for 5 min. The carrier gas was ultra-high-purity helium at a flow rate of 1 mL/min, and the split ratio was 10:1. Cyclohexanone was used as the internal standard.

Statistical Analysis. All experiments were performed in triplicate. Treatment effects were determined by analysis of variance (one-way ANOVA) using SPSS version 22.0 (IBM Corp.). Differences were considered significant at p values of <0.05.

RESULTS AND DISCUSSION

Degradation of SF and Total ITCs during Stir-Frying. Studying the fate of bioactive compounds during cooking is important for optimizing the processing conditions for maintaining or even improving the health benefits of these compounds. In this study, the degradation of SF and total ITCs during stir-frying was investigated. Broccoli contains glucoraphanin, which can undergo hydrolysis by endogenous myrosinase to produce SF and other ITCs. A study of the stability of SF during stir-frying showed that SF degradation and formation occurred simultaneously in broccoli florets.[25] Consequently, broccoli seed extract, which has a high content of SF and total ITCs, was mixed with diced carrot, which does not contain any ITCs. There has been no report of the effects of components in carrots on ITC stability, and in this study, we mixed broccoli seed extracts with only carrots and immediately stir-fried them. Therefore, the effect the carrots might have on ITC stability could be ignored. After the samples had been stir-fried for 10 min, the concentrations of SF and total ITCs were determined (Figure 1). The results showed that SF and total ITCs were more stable than we expected. After the samples had been stir-fried for 2 min, only 15% of the total ITCs were degraded, but almost no obvious SF degradation was observed. During a 4 min stir-fry, ITCs degraded faster than SF, but after 4 min, the degradation rates of SF and total ITCs were almost the same. This could be attributed to the hydrolysis products of the indolyl glucosinolates, such as indole-3-carbinol, being more heat labile than SF.[26] The predominant glucosinolate in broccoli is glucoraphanin, regularly contributing >50% of the total glucosinolates, with the other 50% being aliphatic and indolyl glucosinolates.[27] After the samples had been stir-fried for 10 min, even though some carrots were charred, >40% of SF and total ITCs remained. This is interesting because many studies have reported that the ITC contents decrease significantly after cooking,[9−11] which does not agree with our results.

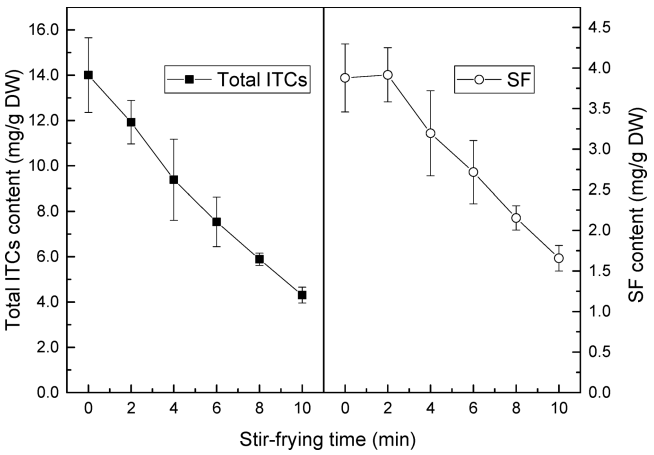

Figure 1. Degradation of SF (○) and total ITCs (■) during the stir-frying process. The errors in experimental data from the mean values are expressed as the standard deviation (SD) and illustrated as error bars (n = 3).

The rates of degradation of SF and total ITCs during heat processing can be modeled by the following equations:[28]

$$\ln r = \ln(C_t/C_0) = -kt \quad (1)$$

$$t_{1/2} = \ln(0.5)k^{-1} \quad (2)$$

where r is the retention value at time t, C_0 is the initial SF or total ITC concentration (milligrams per gram of DW), C_t is the SF or total ITC concentration (milligrams per gram of DW) after stir-frying for time t, t is the stir-frying time (minutes), k is the constant of the degradation rate (inverse hours), and $t_{1/2}$ is the half-life (minutes), which is the time required for 50% degradation of SF or total ITCs. $\mathrm{Ln}(-C_t/C_0)$ was plotted versus t for the degradation of SF and total ITCs during stir-frying (Figure 2). The correlation coefficient was >0.9, showing

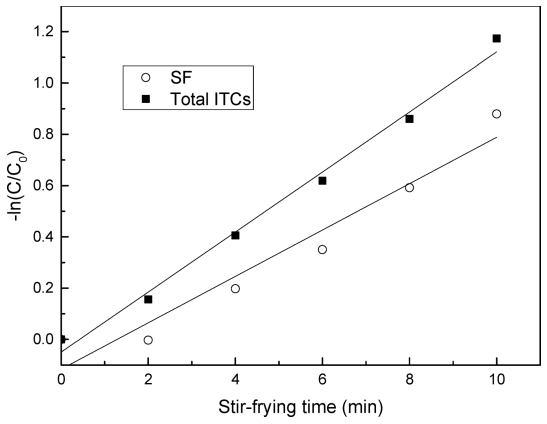

Figure 2. Plots of $-\ln(C_t/C_0)$ of SF (○) and total ITCs (■) vs stir-frying time.

that the degradation of SF and total ITCs during stir-frying followed first-order reaction kinetics. The $t_{1/2}$ values of SF and total ITCs were calculated by linear regression of the observed data in Figure 2 and were 7.7 and 5.9 min, respectively. In our previous study, the $t_{1/2}$ of SF in a pH 6.0 solution at 90 °C was 1.5 h,[19] much longer than that observed in this study. This is reasonable because SF was not in solution in this study and the temperature was >90 °C. Considering that the stir-frying time

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is always much shorter than 10 min, our results indicate that SF and total ITCs are somewhat stable during stir-frying.

Myrosinase Stability and Activity during Stir-Frying. The stability of myrosinase during stir-frying was studied. Fresh broccoli was chopped and stir-fried for 8 min, and the myrosinase activity was measured at 0, 1, 2, 4, 6, and 8 min (Figure 3). On the basis of sinigrin hydrolysis measured with a glucose assay kit, the myrosinase activity of raw broccoli was 128 units/g of DW of broccoli. Guo et al.[22] reported a myrosinase activity of 75.2 units/mg of protein. Okunade et al.[29] reported that the myrosinase activity of brown mustard was the highest (2.04 units/mg) while that of yellow mustard was the lowest (0.48 unit/mg). The variations in myrosinase activity within and between brassica species have been attributed to genetic and/or environmental factors (agronomic and climatic conditions)[30] as well as different enzyme activity definitions.

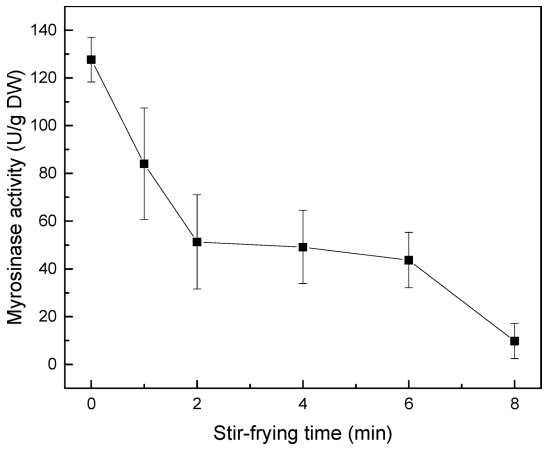

Figure 3. Myrosinase activities in broccoli after stir-frying for a few minutes. The errors in experimental data from the mean values are expressed as the standard deviation (SD) and illustrated as error bars (n = 3).

Myrosinase activity decreased by 80% after samples had been stir-fried for 3 min (Figure 3). This result is not unexpected because myrosinase has been reported to be easily inactivated at normal cooking temperatures, regardless of the cooking method used.[31] Myrosinase in Brassicaceae lost approximately 90% of its activity after samples had been heated at 60 °C for only 3 min.[32] Microwave cooking of red cabbage for 4.8 min at 900 W caused almost complete loss of myrosinase activity.[33] Gliszczyńska-Świgło et al.[34] reported that the black mustard enzyme activity in cabbage was lost after microwave cooking for 2 min or steaming for 7 min. Compared to other myrosinase sources, broccoli myrosinase has a low thermal stability,[35] and heating broccoli florets to ≥70 °C for 5 min inactivates the myrosinase.[36] Generally, myrosinase is sensitive to temperature, and cooking methods such as steaming or microwaving are known to decrease or completely eliminate the hydrolytic activity.

Furthermore, the enzyme hydrolysis rate of glucosinolates was studied. The result showed that the hydrolysis rate was very slow. In the first 60 min, the glucose content increased very slowly. After 90 min, the glucose content increased to a certain extent (0.34 μM) and then leveled off with further increases (Figure 4). This result was in line with earlier studies. Tian et al.[37] studied the effect of the hydrolysis time on the production of SF from broccoli sprouts and found that the best hydrolysis time for SF production was 1.5 h. Vastenhout et al.[38] evaluated the kinetics of glucosinolate hydrolysis by *Sinapis alba* myrosinase and found that production of ITCs increased gradually over 80 min. Similar results were reported by Klingaman et al.[39] using another glucosinolate. The results for these reports were different, because the hydrolysis rate is dependent on the structure of the ITC.[25] However, from these results, we could infer that the hydrolysis rate is very slow.

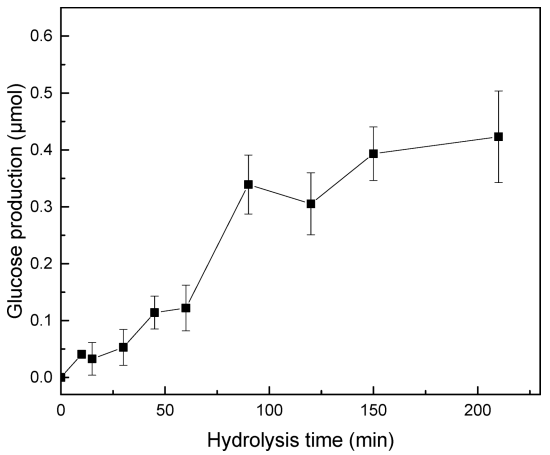

Figure 4. Glucose production after hydrolysis for 210 min. The concentration of glucose produced was calculated by subtracting the original glucose concentration from the formed glucose concentration. The errors in experimental data from the mean values are expressed as the standard deviation (SD) and illustrated as error bars (n = 3).

In summary, the enzymatic activity study showed that myrosinase was labile and that the cooking procedure inactivated myrosinase. The hydrolysis rate is very slow; therefore, during the cutting or mincing of broccoli, only a small quantity of the glucosinolates is hydrolyzed to ITCs.

Effect of Hydrolysis on ITC Content after Stir-Frying. On the basis of the results mentioned above, we hypothesized that after hydrolysis of glucosinolates in broccoli by endogenous myrosinase for a certain length of time (e.g., 90 min) before stir-frying, the nutritional value would then increase because ITCs are more stable than myrosinase. To verify this, the total and individual ITC contents in the HS group, the DS group, and the RB group were analyzed. The total ITC concentration in broccoli was quantified by the cyclocondensation method, which is both accurate and rapid,[23] and individual levels of ITCs and nitriles were determined by GC-MS. The cutting style greatly affected the ITC content of broccoli, and the total ITC concentration increased by 133% in florets processed into chops compared to other cutting styles.[40] Thus, we processed broccoli florets into chops and waited for 90 min before stir-frying to allow for production of maximum possible ITCs. During stir-frying, broccoli is usually cooked for ~4 min; therefore, stir-frying for 4 min was used in this study. The results verified our hypothesis; the amounts of ITCs formed in the RB, HS, and DS groups were 37.92 ± 13.35, 30.90 ± 10.88, and 11.89 ± 2.52 mg/100 g of DW of broccoli, respectively (Figure 5). The amounts of ITCs produced in RB and HS groups were larger than in the DS group; compared to that of the DS group, the ITC content of the HS group increased by 2.6-fold, while there was no significant difference between the RB and HS groups.

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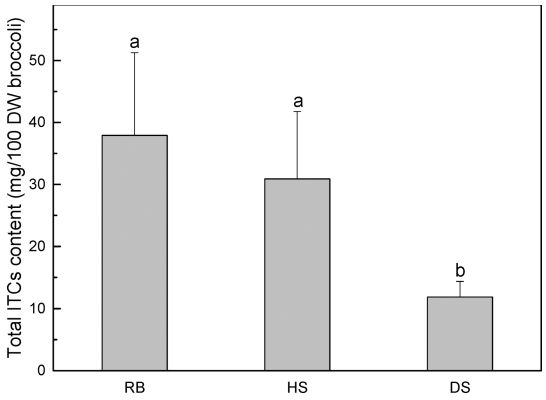

Figure 5. Total ITC content in the directly stir-fried (DS) group, the hydrolyzed and stir-fried (HS) group, and the raw broccoli (RB) group. Different letters indicate significant differences in the ITC concentration among the three groups ($p < 0.05$). The errors in experimental data from the mean values are expressed as the standard deviation (SD) and illustrated as error bars ($n = 3$).

Volatile compounds from the methylene chloride extracts of the RB, HS, and DS groups, such as ITCs, nitriles, and their degradation products, were identified and quantified by GC-MS (Table 1). SF was the most abundant ITC, followed by 1-butene 4-isothiocyanate and erucin; only trace amounts of PEITC were detected in the RB group. After stir-frying, there were no significant differences in SF and erucin content between the RB and HS groups, while in the DS group, the SF content decreased significantly and only trace amounts of erucin were found.

Some degradation products of SF were also found. Among them, dimethyl sulfone, *S*-methyl methylthiosulfinate, and *S*-methyl methylthiosulfonate have been reported as thermal degradation products of SF, and the mechanisms for the formation of these compounds from SF have been proposed by Jin et al.[18] The concentration of these degradation products was higher in the RB group but much lower in the HS and DS groups. We supposed that these compounds are volatile and volatilized in the stir-frying procedure.

Sulforaphane nitrile and 3-methyl-2-butenenitrile, produced in the action of epithiospecifier proteins (ESP), were also found. ESP is the cofactor of myrosinase, which can direct the hydrolysis reaction toward nitriles in the presence of Fe^{2+} ions.[36] Unlike those of ITCs, levels of nitriles are much lower in the DS and HS groups. For example, 3-methyl-2-butenenitrile was not present in the DS and HS groups, but in the RB group, its concentration was 5.43 ± 1.59 mg/100 g of DW of broccoli. There were no significant differences between the concentrations of sulforaphane nitrile in the HS and DS groups, but in the RB group, the concentration was higher. Nitriles are much more stable than ITCs are, which can present even during heat treatments above 100 °C and hardly degrade any further.[26] Therefore, their losses during stir-frying predominantly because of their volatility.

A significant increase ($p < 0.05$) in the level of indole-3-acetonitrile (I3ACN) in the HS and DS groups was recorded compared to that of the RB group. Sosińska and Obiedziński[41] also found that the I3ACN concentration increases along with cooking time. The authors suggested that while the myrosinase and ESP in vegetables were denatured during boiling, the I3ACN could have been formed through nonenzymatic decomposition of glucobrassicin, a precursor of indole-3-carbinol.

In this study, after stir-frying, a hydrolysis step was added for the DS and HS groups, as described in Materials and Methods, but the results obtained without this hydrolysis step were not very different from those obtained after hydrolysis (data not shown). We speculated that this was because the myrosinase was destroyed during cooking; thus, no further ITCs were produced even after the hydrolysis step.

Because cooking reduces the level of formation of isothiocyanates and allows for consumption of glucosinolates in unchanged form,[42] some methods for increasing the level of production of ITCs by adding an exogenous source of myrosinase to processed broccoli have been reported. According to Cramer et al.,[43] SF production was enhanced by adding a powder rich in glucoraphanin but lacking myrosinase to air-dried broccoli sprout powder. In another paper, they reported that upon co-consumption of glucoraphanin-rich powder with fresh broccoli sprouts, the level of SF formation increased,[44] because both air-dried broccoli sprout powder and fresh broccoli sprouts contain myrosinase, which can hydrolyze glucoraphanin to SF. Adding mustard seed powder to heat-treated broccoli also greatly increased the level of SF formation.[45] Another way to overcome the loss of hydrolyzing activity is addition of radish root.[46] However, addition of mustard seeds or radish root powder may affect the taste and flavor of broccoli.

Table 1. ITCs, Nitriles, and Their Degradation Product Contents in the Raw Broccoli (RB) Group, the Hydrolyzed and Stir-Fried (HS) Group, and the Directly Stir-Fried (DS) Group

		content* (mg/100 g of dry weight broccoli)		
category	compound	RB	HS	DS
ITCs	sulforaphane	15.20 ± 5.05 a	12.73 ± 4.16 a	4.50 ± 0.90 b
	erucin	0.61 ± 0.01 a	0.74 ± 0.19 a	trace
	1-butene 4-isothiocyanate	9.33 ± 3.25 a	6.67 ± 1.90 a	2.58 ± 0.45 b
	PEITC	0.58 ± 0.00	ND**	ND**
nitriles	sulforaphane nitrile	18.90 ± 5.90 a	6.60 ± 2.29 b	6.16 ± 1.55 b
	3-methyl-2-butenenitrile	5.43 ± 1.59	ND**	ND**
	1*H*-indole-3-acetonitrile	20.05 ± 3.74 c	62.75 ± 5.26 a	38.32 ± 9.86 b
degradation products	dimethyl sulfone	1.36 ± 0.44 a	0.70 ± 0.14 ab	0.46 ± 0.15 b
	S-methyl methanethiosulfinate	1.88 ± 0.15	trace	ND**
	S-methyl methanethiosulfonate	13.67 ± 0.39 a	0.77 ± 0.05 b	trace

*Different letters indicate significant differences in the concentration of ITCs among the three groups ($p < 0.05$). **Not detected.

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Our results suggest that after broccoli florets are cut into small pieces, they should be left for ~90 min before being cooked. Although we did not conduct experiments to investigate a shorter hydrolysis time, we believe that hydrolysis for 30 min would also be helpful. This procedure is simple and feasible and will significantly increase the total ITC content ($p < 0.05$) and, thus, increases the nutritional value of broccoli. However, to achieve thorough hydrolysis, we had to chop the broccoli florets into 2 mm pieces, which is a drawback of this method. Therefore, our future work will investigate how to achieve complete hydrolysis without cutting the broccoli into chops.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

ITCs, isothiocyanates; SF, sulforaphane; PEITC, phenethyl isothiocyanate; I3ACN, indole-3-acetonitrile; ESP, epithiospecifier protein; HS, hydrolyzed and stir-fried; DS, directly stir-fried; RB, raw broccoli; DW, dry weight

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