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Impact of Thermal Processing on Sulforaphane Yield from Broccoli (*Brassica oleracea* L. ssp. *italica*)

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ABSTRACT: In broccoli, sulforaphane forms when the glucosinolate glucoraphanin is hydrolyzed by the endogenous plant thiohydrolase myrosinase. A myrosinase cofactor directs hydrolysis away from the formation of bioactive sulforaphane and toward an inactive product, sulforaphane nitrile. The cofactor is more heat sensitive than myrosinase, presenting an opportunity to preferentially direct hydrolysis toward sulforaphane formation through regulation of thermal processing. Four broccoli cultivars were microwave heated, boiled, or steamed for various lengths of time. Production of nitrile during hydrolysis of unheated broccoli varied among cultivars from 91 to 52% of hydrolysis products (Pinnacle > Marathon > Patriot > Brigadier). Boiling and microwave heating caused an initial loss of nitrile, with a concomitant increase in sulforaphane, followed by loss of sulforaphane, all within 1 min. In contrast, steaming enhanced sulforaphane yield between 1.0 and 3.0 min in all but Brigadier. These data are proof of concept that steaming for 1.0−3.0 min provides less nitrile and more sulforaphane yield from a broccoli meal.

KEYWORDS: *broccoli, sulforaphane, nitrile, steam*

INTRODUCTION

Epidemiological studies have shown that the consumption of cruciferous vegetables, such as broccoli and Brussels sprouts, is linked to reduced cancer risk.[1,2] Broccoli is considered to have relatively high anticancer potential due to its high levels of glucoraphanin (GR; 4-methylsulfinylbutyl glucosinolate). When broccoli is crushed, GR and the endogenous thiohydrolase myrosinase (EC 3.2.3.147) are brought into contact, and GR hydrolysis releases an unstable product (a thiohydroximate *O*-sulfinate) that breaks down to form the cancer-preventive agent sulforaphane (SF; 4-methylsulfinylbutyl isothiocyanate) or the corresponding nitrile (5-methylsulfinylpentane nitrile; Figure 1).[3] Nitrile formation, which predominates over SF in many cultivars, is not associated with health benefits.[3,4]

A myrosinase cofactor, the epithiospecifier protein (ESP), affects the ratio of SF to nitrile by directing breakdown of the thiohydroximate *O*-sulfinate intermediate toward nitrile formation, at the expense of SF.[5,6] Importantly, ESP is more heat sensitive than myrosinase, suggesting that heating/cooking may be a practical way to inactivate ESP while preserving myrosinase and, thus, optimizing SF formation.[5]

There is a growing literature describing the lack of SF bioavailability from cooked broccoli and, thus, an expected lack of health benefits. However, most studies do not evaluate short cooking periods or nitrile formation.[7−10] One study showed full myrosinase activity (using exogenous sinigrin as substrate) following 2.5 min of steaming and loss of myrosinase activity at 5 min, but did not measure formation of GR hydrolysis products.[11] Another study used short cooking times (0, 2, or 5 min) and measured SF and nitrile. However, they saw no differential heat sensitivity for nitrile formation: formation of both SF and nitrile persisted until myrosinase was destroyed.[12] Reviewing their methods, we found that hydrolysis was carried out in 0.1 M HCl. A low pH supports myrosinase-dependent nitrile formation in the absence of any cofactor, and thus nitrile formation was not ESP-dependent and the temperature sensitivity of ESP was not evaluated.[5]

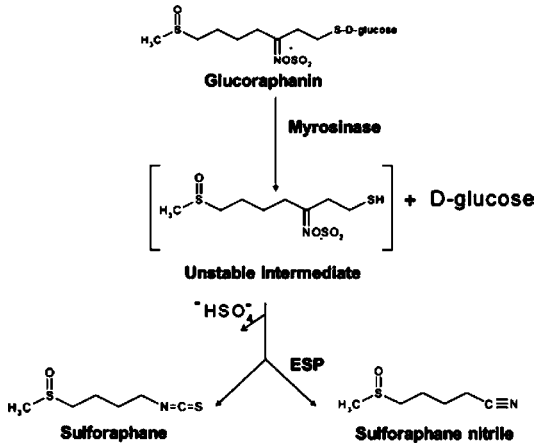

Figure 1. Hydrolysis of glucoraphanin to sulforaphane and sulforaphane nitrile.

Because most consumers eat broccoli after it has been cooked, the objective of this study was to test how SF and nitrile formation vary following three common cooking

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methods, using four commercially available broccoli cultivars. Our aim was to determine if there is a duration of heating that halts nitrile formation but does not destroy myrosinase activity and thus optimizes SF formation.

MATERIALS AND METHODS

Pinnacle, Marathon, and Patriot broccoli (*Brassica oleracea* L. ssp. *italica*) were grown to commercial maturity at the U.S. Vegetable Laboratory (Charleston, SC, USA) during fall 2003 and spring 2004 using standard practices for this location.[13] Plants were grown on raised beds, spacing between rows was 102 cm, and spacing between plants in a row was 15 cm. All cultural practices (e.g., cultivation, fertilization, irrigation, pest management) used were standard for local conditions. As plants approached maturity they were observed every 2 days, and those heads that reached 10−12 cm in diameter were cut from the main stem with subtending stalks were cut to a 16 cm length. For both seasons, heads were harvested, immediately packed on ice, and shipped overnight to the University of Illinois, where they were quickly processed. The cultivar Brigadier was grown to commercial maturity at the University of Illinois South Farms (Champaign, IL, USA) in the fall of 2004, harvested, and transported on ice to the laboratory. Ten to twelve heads of each cultivar were harvested, stored in a refrigerator at 4 °C, and used within 48 h.

Methylene chloride (Optima grade) was purchased from Fisher Scientific (Fair Lawn, NJ, USA) and benzyl isothiocyanate from LKT Laboratories (St. Paul, MN, USA). Sulforaphane and sulforaphane nitrile were purified from broccoli seed as previously described.[4] All other solvents and chemicals were of reagent grade.

Sample Preparation. Broccoli florets were sampled randomly from 10−12 heads and cut 2 in. below the floret crown. A control and three cooking treatments, including microwave heating, boiling in water, and steaming, were evaluated. The time periods chosen for evaluating cooking differed among the primary methods and were chosen to represent a range of possible cooking times, keeping in mind consumer acceptability. For example, microwave heating for >1 min is undesirable due to scorching of the samples. Similarly, excessive boiling in water causes the cooked broccoli to lose physical integrity, becoming soft, as well as causing significant leaching of glucosinolates.[12]

For the microwave treatment, florets (35 g composite from three heads) were placed in a shallow dish with approximately 30 g of water. The microwave oven (Panasonic 900 W with rotating dish) was used on high power for 0.25, 0.50, 0.75, or 1.0 min. For the boiling water treatment, florets (35 g) were placed into a pot of boiling water (∼1.5 L) for 0.5, 1.0, or 3.0 min. For the steam treatment, florets (35 g) were steamed using a benchtop steamer (Black and Decker Flavor Scenter Handy Steamer HS800) for 1.0, 3.0, or 5.0 min. In all cases, unheated broccoli florets were used as controls. Immediately following cooking treatment, all samples (including controls) were drained, cooking water was discarded, and the florets were placed in an iced-water bath for 1.5 min and then immediately hydrolyzed.

Postprocessing Hydrolysis. Samples were chopped roughly using a food processor (Black and Decker One-Touch Chopper HC21K). Chopped broccoli was then measured into 50 mL tubes, in triplicate (10 g each). Ten milliliters of ddH_2O was added, and the mixture was homogenized for approximately 20 s using a tissuemizer (Tekmar, Cincinnati, OH, USA). The slurry was allowed to hydrolyze in the dark for 8 h at room temperature for maximum hydrolysis product formation.[4]

Extraction and Analysis. The extraction and analysis of SF and the nitrile were carried out according to previously published methods, with modifications.[5] In brief, the broccoli slurry was filtered through two layers of cheesecloth, and the extracts were centrifuged for 20 min at 12000 rpm, using an Eppendorf 5415C microcentrifuge. An aliquot (500 μL) of the supernatant was removed into a 2 mL Teflon tube, 20 μL of benzyl isothiocyanate (0.5 mg/mL) was added as the internal standard, with 1 mL of methylene chloride, and the samples were shaken vigorously before being centrifuged for 2 min at 12000 rpm. The methylene chloride layer was analyzed by GC.

Gas Chromatography. A splitless HP 5890 GC system and 7363A autosampler were used. A deactivated cyclo-double gooseneck liner (Restek Inc., Bellefonte, PA, USA) was followed by a 3 m J&W DB-5 guard column and a 30 m J&W DB-5 capillary column (0.25 mm i.d., 0.25 μm film) with flame ionization detection. The injector and detector temperatures were 200 and 280 °C, respectively. The temperature cycle began at 40 °C for 2 min, increased to 260 at 10 °C/min, and was held at 260 °C for 10 min. The carrier gas was helium, at 25 psi. Standard curves (0−25 μg/mL) were constructed using purified SF, SF nitrile, and benzyl isothiocyanate in methylene chloride.

RESULTS AND DISCUSSION

Variation in Hydrolysis Product Formation among Cultivars. Health benefits associated with SF can be expected to vary considerably with cultivar, not only because of variation in GR levels across genotypes but also because of variation in fractional SF formation across genotypes. When raw florets were hydrolyzed, Brigadier was found to have a greater SF content than the other three cultivars (Figure 2; raw, $p < 0.05$). Comparison of the levels of the nitrile formed when raw broccoli was hydrolyzed showed a reverse pattern, by which Brigadier and Patriot had the lowest nitrile values. In unheated tissue, both the amount of SF formed (0.16 ± 0.02 μmol/g FW) and the percentage hydrolysis products as SF (48%) were greatest for Brigadier. However, total hydrolysis products in raw broccoli (SF + nitrile) showed Pinnacle (0.59 ± 0.01 μmol/g FW) with almost double that of Brigadier (0.34 ± 0.03 μmol/g FW). These data point to a potential for enhanced formation of SF levels if hydrolysis can be directed away from the formation of the inactive nitrile and toward SF formation.

Most U.S. consumers cook broccoli prior to eating it. Therefore, our ultimate goal in conducting this work was to harness differential heat sensitivity of SF and nitrile formation to positively affect the SF yield in cooked broccoli. We postulated that nitrile formation would be decreased due to ESP heat sensitivity following a shorter heating period than that required to decreased myrosinase activity. In general, heating for a brief period before hydrolysis did cause loss of nitrile formation and a concomitant increase in SF formation, confirming our hypothesis (Figure 2). However, the time window for enhanced SF levels with heating varied both among cultivars and among heating methods employed. Even following a brief heating period, Brigadier no longer provided the greatest amount of SF. Comparison of the maximum levels of SF formed after 3 min of steaming (Figure 2c) showed that Pinnacle (0.78 ± 0.21 μmol/g FW) provided the greatest level, greater than steamed Marathon (0.19 ± 0.02 μmol/g FW) or steamed Patriot (0.07 ± 0.01 μmol/g FW). When Brigadier was steamed for 3 min, no measurable SF was produced upon hydrolysis. When Brigadier was examined in more detail, using shorter steaming intervals, increased SF production (0.36 ± 0.02 μmol/g FW) was observed following 1.5 min of steaming, with SF levels decreasing with longer steaming times (data not shown).

There was also an absolute increase in hydrolysis products with heating. The total hydrolysis products (SF + nitrile) formed following brief heating rose from 0.34 ± 0.03 μmol/g FW in uncooked Brigadier to 0.42 ± 0.02 and 0.53 ± 0.02 μmol/g FW following 0.5 and 0.75 min of microwave heating (Figure 2b), 24 and 56% increases, respectively. Whereas increases in totals were seen in all cultivars following 0.25 min of microwave heating, any increase was lost in Marathon by 0.5 min (Figure 2b). This is in contrast to Patriot and Pinnacle,

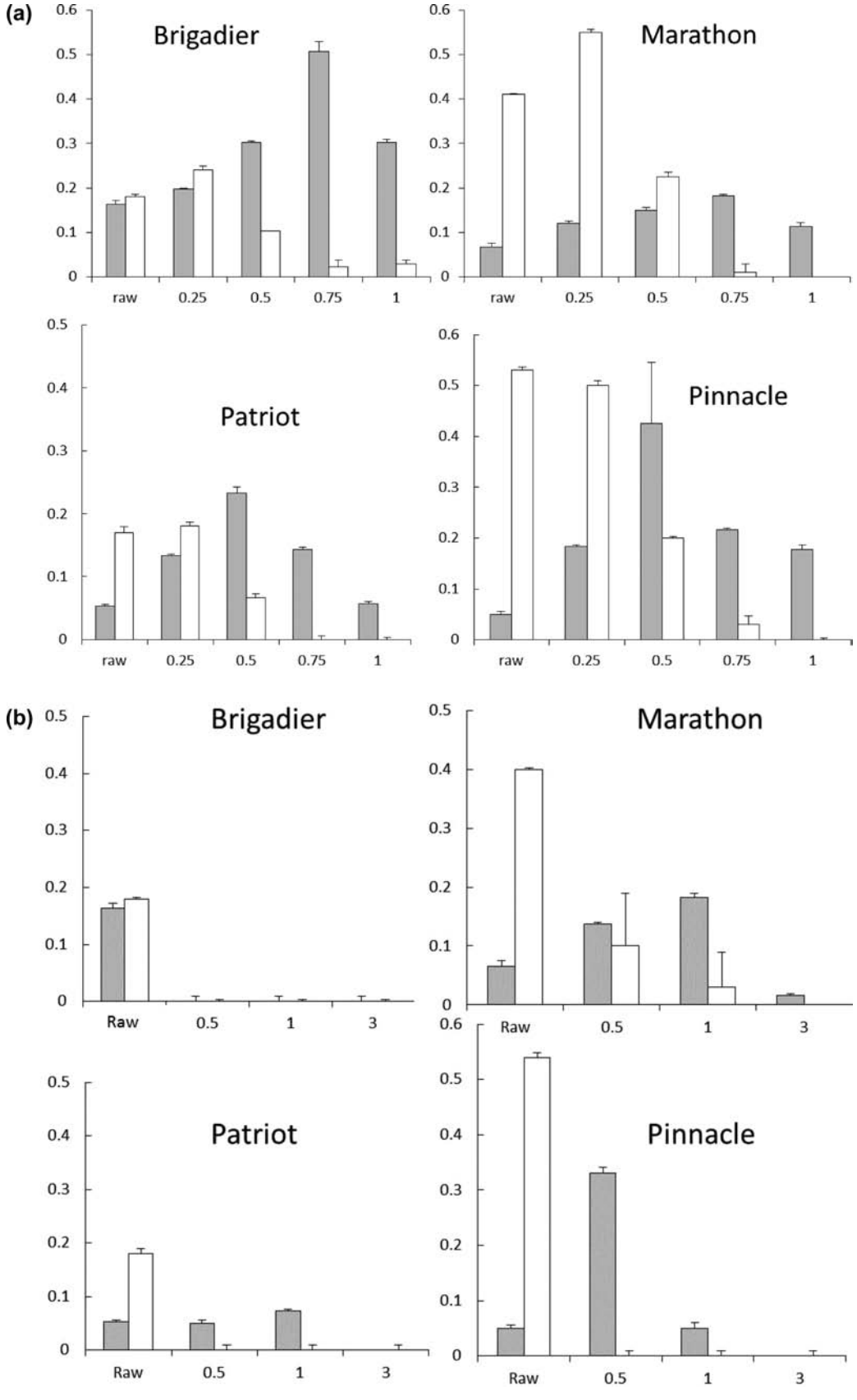

Figure 2. continued

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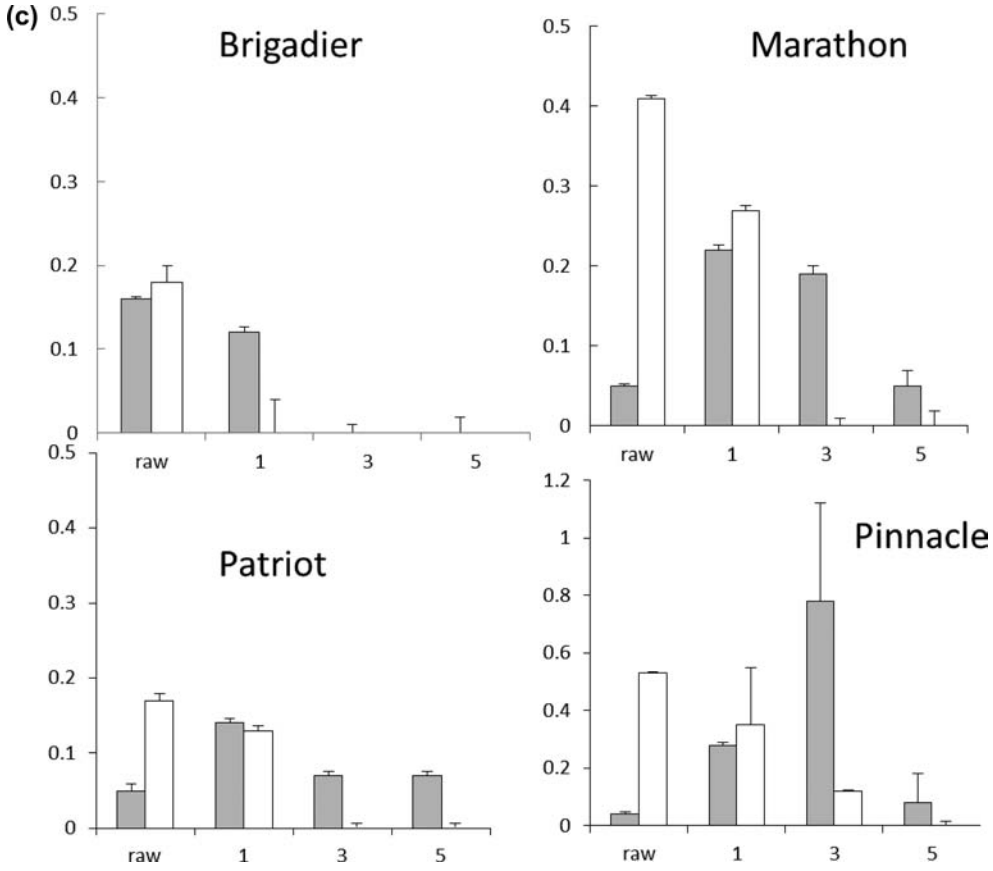

Figure 2. Impact of heating broccoli before hydrolysis on sulforaphane (shaded bars) and sulforaphane nitrile (open bars) formation. Four cultivars of broccoli were heated by (a) microwave heating, (b) boiling, or (c) steam heating for various time periods, as shown. All samples, including unheated controls, were immersed in an ice−water bath immediately following the heating, before undergoing homogenization and hydrolysis (see Materials and Methods). Data are reported as the mean ± SE (n = 3), μmol hydrolysis product/g FW broccoli (y-axis); period of heating is reported in minutes (x-axis).

with which microwave heating for 0.5 min, as in Brigadier, maintained an increase in total hydrolysis products (SF + nitrile) over control (unheated) values (Figure 2b). The reason for this increase in yield is not known. It is possible that heating released protein-bound glucosinolates or caused a more complete destruction of the effective compartmentalization of myrosinase and glucosinolates.[12,14,15]

Effect of Microwave Heating on SF Formation. Because microwave heating is based on intramolecular friction and not traditional heat transfer, this method effectively heats samples more rapidly compared to boiling or steaming. Thus, we evaluated the effects of microwave cooking for periods of <1.0 min. With all four cultivars, microwave heating for 0.5 and 0.75 min resulted in SF production that was significantly greater than that found in control, unheated samples (Figure 2a). However, by 1.0 min, SF production was decreased from the maximum. No period of microwave heating clearly reflected complete loss of nitrile formation without some loss of SF formation also. Microwave heating for the shortest time (0.25 min) caused no significant change from values in unheated tissue in nitrile formation in Patriot or Pinnacle and actually caused a small increase in nitrile formation in Brigadier and Marathon. After 0.5 min, all nitrile levels were less than those found in control florets, and nitrile formation continued to decrease as the cooking duration was extended to 1.0 min, a time when SF formation was also substantially compromised. The relatively slow temporal loss of nitrile formation following microwave heating indicates that ESP was not immediately destroyed. One cause for this inconsistent loss of nitrile formation could be a lack of homogeneity in microwave heating. Nitrile formation was affected similarly across all cultivars, suggesting that this did not reflect variation due to genotype. It would be interesting to determine if the striking difference in percent nitrile formation in raw broccoli among the cultivars studied here is under genetic control and if this reflects the presence of different isoforms of ESP or some other factor regulating the amount/activity of ESP present.

Effect of Boiling on SF Formation. When florets were boiled for 5.0 min prior to hydrolysis, no hydrolysis products were formed (data not shown), suggesting that myrosinase was destroyed (data not shown). When a much shorter boiling time (0.5 min) was utilized, Marathon and Pinnacle showed increased SF production compared to unheated control samples (Figure 2b). However, no increases were seen in Brigadier (all SF was lost, even by 0.5 min of boiling) and Patriot after 1.0 min of boiling. A boiling period of 3.0 min resulted in the complete loss of SF production in all but Marathon, and in this case there was only 15% of maximum levels remaining (less than half of that in unheated florets). Even 1 min of boiling caused loss of SF formation in all cultivars except Marathon (Figure 2b). However, almost no nitrile was formed after 0.5 min of boiling, suggesting that boiling rapidly inactivated ESP, resulting in loss of nitrile formation at a time point that myrosinase remained active.

Because consumers would not in all cases immerse boiled broccoli in ice−water to rapidly stop the heating process, it is unlikely that consumers would be able to utilize boiling (for 0.5 min) to enhance SF levels over those present in the uncooked vegetable. In addition to myrosinase inactivation, product leaching into the boiling water may be expected to occur at least by 2 min.[12]

Effect of Steaming on SF Formation. When broccoli was steamed for various time periods, the four cultivars responded in different ways (Figure 2). Nitrile levels decreased with steaming, essentially reaching zero by 3 min in all cultivars. Brigadier showed no increase in SF production even following only 1 min of steaming, whereas the other cultivars all showed increases in SF by 1 min (Figure 2c). Apparently, Brigadier exhibits a more contracted temperature response than other cultivars (true also following boiling). This is unlikely to indicate that Brigadier myrosinase is more heat sensitive, because SF production remained even with 1 min of microwave heating. Possibly Brigadier contains less myrosinase or the structure of the Brigadier head is less dense, permitting the tissue to rise in temperature more rapidly than in other cultivars. Further work is needed to determine the cause of this difference.

The other three cultivars showed increases in SF formation after steaming for 1.0 min (Figure 2c), and values remained greater than those of unheated broccoli even after 3.0 min of steaming. By 5.0 min, all SF levels were the same as or less than levels seen in unheated tissue. Nitrile levels decreased with steaming, with small losses at 1 min but complete loss in all but one cultivar (Pinnacle) by 3 min. These data suggest that steaming for 1−3 min (then chilling) may improve SF content in many broccoli cultivars, compared to broccoli served raw or steamed for 5 min.

Comparing Heating Methods. It is clear that nitrile production was lost following less severe heat treatments than was necessary for loss of SF formation. Maximum SF formation was reached following a much shorter heating period for microwave heating or boiling than for steaming. It can also be seen that much, but not all, of the increase in SF formation following heating occurred at the expense of nitrile formation regardless of the heating method used: some of the increase was associated with an absolute increase in hydrolysis products, indicative of an absolute increase in GR hydrolysis This pattern of replacing nitrile with SF formation early in the heating process, followed by loss of SF formation as the heating period was extended. is consistent with our previous report that nitrile levels decreased and SF levels increased when broccoli samples were heated for 5 min at 50 °C prior to hydrolysis.[5] In that study, SF formation reached a maximum when the broccoli was preheated for 5 min at 60 °C prior to hydrolysis and dropped below detectable levels when the preheating temperature was raised above 70 °C. Duration of heating was not varied in that study, making difficult translation of those findings to the normal cooking process. In the present study, duration of heating was varied to better reflect kitchen preparation. We found a similar trend across all cultivars studied, although the duration of cooking required to effect this change varied somewhat among cultivars. Thus, boiling and microwave heating were able to enhance SF levels only when a very short heating period was utilized, making critical our use of an ice−water bath to rapidly stop the heating process. In contrast to microwave heating and boiling, steaming for 1.0−3.0 min enhanced SF levels in all but Brigadier and therefore can be expected to enhance the health benefit of a broccoli meal.

Whereas there are differences among cultivars in the amounts of hydrolysis product formed, results of this work indicate that such differences may result not only from differences in glucosinolate content but also from differences in myrosinase and ESP. Future studies should quantify these across genotypes. Boiling resulted in the most rapid loss of SF and is not recommended. Whereas microwave heating and steaming both enhanced levels of available SF compared to uncooked broccoli, it may be easier to maximize levels of SF by steaming because the window for microwave-induced increases is very short (<1.0 min) compared to steaming (1.0−3.0 min). Thus, the likelihood of overcooking and denaturing myrosinase during microwave cooking is greater than during steaming. Although broccoli sold to consumers is not labeled with a cultivar name, our results can be generalized to suggest that steaming for 1−3 min will provide the greatest SF availability.

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Notes

The authors declare no competing financial interest.

REFERENCES

(1) Verhoeven, D.; Goldbohm, R.; vanPoppel, G.; Verhagen, H.; vandenBrandt, P. Epidemiological studies on brassica vegetables and cancer risk. *Cancer Epidemiol. Biomarkers Prevention* **1996**, *5*, 733−748.

(2) Jeffery, E. H.; Araya, M. Physiological effects of broccoli consumption. *Phytochem. Rev.* **2009**, *8*, 283−298.

(3) Matusheski, N. V.; Jeffery, E. H. Comparison of the bioactivity of two glucoraphanin hydrolysis products found in broccoli, sulforaphane and sulforaphane nitrile. *J. Agric. Food Chem.* **2001**, *49*, 5743−5749.

(4) Matusheski, N. V.; Wallig, M. A.; Juvik, J. A.; Klein, B. P.; Kushad, M. M.; Jeffery, E. H. Preparative HPLC method for the purification of sulforaphane and sulforaphane nitrile from *Brassica oleracea*. *J. Agric. Food Chem.* **2001**, *49*, 1867−1872.

(5) Matusheski, N. V.; Juvik, J.; Jeffery, E. H. Heating decreases epithiospecifier protein activity and increases sulforaphane formation in broccoli. *Phytochemistry* **2004**, *65*, 1273−1281.

(6) Matusheski, N. V.; Swarup, R.; Juvik, J. A.; Mithen, R.; Bennett, M.; Jeffery, E. H. Epithiospecifier protein from broccoli (*Brassica oleracea* L. ssp. *italica*) inhibits formation of the anticancer agent sulforaphane. *J. Agric. Food Chem.* **2006**, *54*, 2069−2076.

(7) Conaway, C. C.; Getahun, S. M.; Liebes, L. L.; Pusateri, D. J.; Topham, D. K. W.; Botero-Omary, M.; Chung, F.-L. Disposition of glucosinolates and sulforaphane in humans after ingestion of steamed and fresh broccoli. *Nutr. Cancer* **2000**, *38*, 168−178.

(8) Moreno, D. A.; López-Berenguer, C.; García-Viguera, C. Effects of stir-fry cooking with different edible oils on the phytochemical composition of broccoli. *J. Food Sci.* **2007**, *72*, S064−068.

(9) Song, L.; Thornalley, P. J. Effect of storage, processing and cooking on glucosinolate content of *Brassica* vegetables. *Food Chem. Toxicol.* **2007**, *45*, 216−224.

(10) Vermeulen, M.; Klöpping-Ketelaars, I. W. A. A.; Van Den Berg, R.; Vaes, W. H. J. Bioavailability and kinetics of sulforaphane in humans after consumption of cooked versus raw broccoli. *J. Agric. Food Chem.* **2008**, *56*, 10505−10509.

(11) Verkerk, R.; Knol, J. J.; Dekker, M. The effect of steaming on the glucosinolate content in broccoli. *Acta Hortic.* **2010**, *867*, 37−45.
(12) Jones, R. B.; Frisina, C. L.; Winkler, S.; Imsic, M.; Tomkins, R. B. Cooking method significantly effects glucosinolate content and sulforaphane production in broccoli florets. *Food Chem.* **2010**, *123*, 237−242.
(13) Farnham, M. W.; Wilson, P. E.; Stephenson, K. K.; Fahey, J. W. Genetic and environmental effects on glucosinolate content and chemoprotective potency of broccoli. *Plant Breed.* **2004**, *123*, 60−65.
(14) Vallejo, F.; Tomás-Barberán, F. A.; Garcia-Viguera, C. Glucosinolates and vitamin C content in edible parts of broccoli florets after domestic cooking. *Eur. Food Res. Technol.* **2002**, *215*, 310−316.
(15) Ciska, E.; Kozlowska, H. The effect of cooking on the glucosinolates content in white cabbage. *Eur. Food Res. Technol.* **2001**, *212*, 582−587.

NOTE ADDED AFTER ASAP PUBLICATION

The citations for Figures 2a and 2b were reversed in the version of this paper published May 15, 2012. The correct version published May 17, 2012.