Glucosinolate and Isothiocyanate Contents of Frozen Broccoli, Brussels Sprouts and a Whole-food Cruciferous Supplement Over Time

Stephanie Mondloch[1], Chris D. Scholl[2], Sara A. Arscott[2], Sherry A. Tanumihardjo[1,*]

[1]University of Wisconsin-Madison, Interdepartmental Graduate Program in Nutritional Sciences, 1415 Linden Dr.; Madison, Wisconsin, 53706; United States of America (USA).

[2]Standard Process, Inc., 1200 W. Royal Lee Drive, Palmyra, Wisconsin, 53156; USA.

RESEARCH

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***Corresponding Author:**

Prof. Sherry A. Tanumihardjo

Department of Nutritional Sciences, University of Wisconsin-Madison, 1415 Linden Dr., Madison, WI 53706, USA, Tel: 608-265-0792; Fax: 608-262-5860; E-mail: sherry@nutrisci.wisc.edu

ABSTRACT

Cruciferous vegetables have gained status as a functional food. Putative chemoprotective effects of cruciferous vegetables have been attributed to glucosinolates, which can be hydrolyzed to isothiocyanates (ITCs). Indole-3-carbinol (I3C), a product of glucobrassicin hydrolysis, is associated with in vitro and in vivo chemoprotective effects. The total glucosinolate and ITC contents and selected glucosinolate profiles of frozen broccoli and Brussels sprouts and a whole-food cruciferous supplement were determined. Brussels sprouts contained more total glucosinolates and glucobrassicin, whereas the supplement contained more ITC than either vegetable. Steaming the vegetables for 3-4 min did not affect glucosinolate or ITC content, except for decreases in glucobrassicin and gluconasturttin and an increase in ITC content in broccoli. Total glucosinolate, glucobrassicin, and ITC content declined over time in frozen broccoli and the supplement, but not in Brussels sprouts; however, substantial variability was observed. Interventions using whole-food cruciferous vegetables, should consider the cultivar, myrosinase content, and phytochemical degradation during storage.

Key Words: Cancer prevention; Myrosinase; Phytochemicals; Vegetables.

Abbreviations: DIM, 3, 3'-diindolylmethane; I3C, indole-3-carbinol; ITC, isothiocyanate.

Introduction

Case-control and cohort studies suggest a protective effect of cruciferous vegetable consumption against various cancers [1, 2]. While Brussels sprout utilization has remained relatively stable and low, i.e., <1 lb/capita/year, over the past few decades, broccoli utilization has steadily increased since 1970 with ten-times higher utilization than Brussels sprouts. This is likely due in part to the purported health benefits associated with broccoli. The potential chemoprotective effect of cruciferous vegetables is largely attributed to their glucosinolate content.

Glucosinolates are sulfur-containing phytochemicals found in plants (Figure 1). Upon damage to plant tissues and depending on pH and other factors [3], aliphatic or aromatic glucosinolates can be hydrolyzed by the endogenous plant or microbial myrosinase to form glucose and an unstable intermediate, which can rearrange to form isothiocyanates (ITCs), thiocyanates or nitriles depending on the structure of the original glucosinolate [4]. Indoles, including indole-3-carbinol (I3C), and hydrolysis product of glucobrassicin, are formed when the unstable ITC from myrosinase hydrolysis of indolyl glucosinolates rearranges to form an alcohol [4].

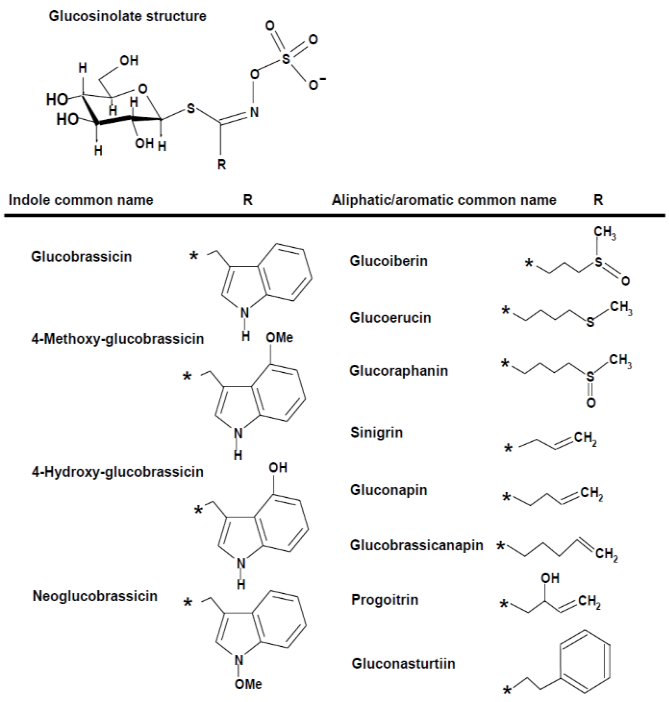


Figure 1. The basic structure of glucosinolates and the various chemical groups found in glucosinolates quantified in broccoli and Brussels sprouts.

I3C has received substantial research attention because it decreased tumor formation in mouse models of mammary [5] and uterine [6] tumors, although it has been observed to increase tumor formation in some models [7, 8]. 3,3'-Diindolylmethane (DIM), an acid condensation product of I3C, may act as a chemo preventive compound through a variety of potential mechanisms [9 10], including effects on Nrf2 signaling pathways; chemokine receptors; transcription factors involved in cell-cycle regulation, inflammation, and apoptosis; and inhibition of aromatase. Additionally, DIM weakly associates with the aryl hydrocarbon receptor (Figure 2) [11], inducing cytochrome P450 1A1 (CYP1A1) and CYP1A2 activity in human colon cell lines [12] and rats [13]. Supplemental I3C (400-800 mg) given for eight weeks [14] to humans induced CYP1A2 activity, which is associated with 2-hydroxylation of estradiol and estrone [15]. Inducing CYP1A2 activity may increase 2-hydroxylation of estrogens, the constituents of which are thought to be less estrogenic [16] and theoretically protective against breast cancer [5]. Large prospective cohort studies suggest 2-hydroxylation of parent estrogens may provide breast cancer protection in postmenopausal women [17].

More research is needed on whole-food dietary supplements compared with the whole vegetables from which they are derived. The interactions among phytochemicals likely exert synergistic effects on health through multiple mechanisms. Cruciferous vegetables are a fast-growing source of phytochemicals often considered a functional food [18]. Studies examining the effect of cruciferous vegetables or supplemental I3C on biomarkers associated with health outcomes need to assess the profiles of the supplements or the whole foods and their stability over time during typical storage conditions. In this study, frozen broccoli and Brussels sprouts and a whole-food cruciferous supplement to be used in a clinical study were analyzed for 12 glucosinolates (Figure 1) and total ITCs (sulforaphane equivalents) over time. Consumers often store frozen vegetables and keep supplements for a long time. This study uniquely analyzed glucosinolate content in frozen cruciferous vegetables and supplements for 1.5 year.

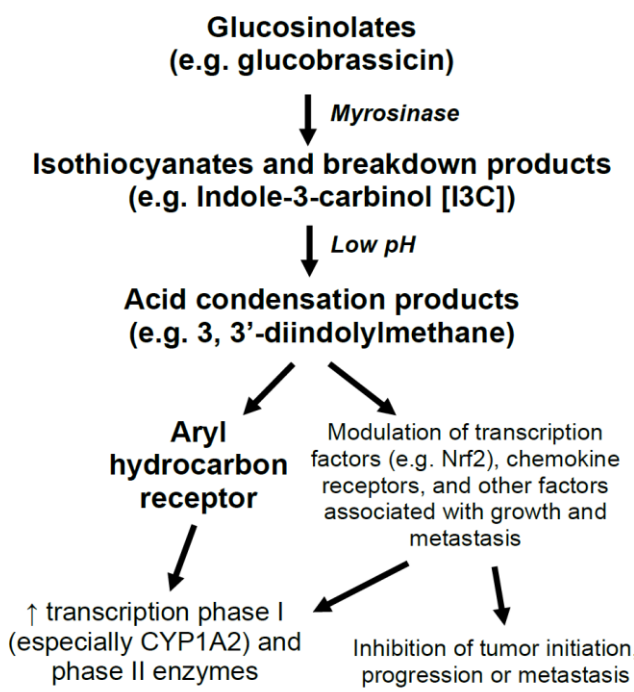


Figure 2. Schematic of current hypotheses of the chemoprotective effect of indole-3-carbinol, an hydrolysis product of the indole glucosinolate glucobrassicin. The cytochrome P450 system is likely induced.

Materials and Methods

Procurement and sampling of vegetables and capsules

Commercial frozen broccoli and Brussels sprouts (Nature Blessed™) were purchased from the University of Wisconsin (UW) Meat Market (Madison, WI) and stored in a household freezer at -20°C, which did not go through defrosting cycles. Both were sampled every 6-8 wk for about 1.5 y for analysis of glucosinolate and ITC contents using well-established HPLC methods confirmed by mass spectrometer analysis [19]. Approximately 20 g frozen broccoli and Brussels sprouts were portioned into pre-weighed sample cups, and three replicates were performed per vegetable. Vegetables were steamed from frozen for 3-4 min and subsequently flash-frozen in liquid nitrogen. All samples were stored at -80°C until transferred on dry ice to the laboratory for freeze-drying and glucosinolate profile and ITCs analysis. The cruciferous supplement (Standard Process, Inc.) consisted of 550 mg dried Brussels sprouts and kale, water, and calcium stearate as a flow agent in a gelatin capsule. The suggested use is 1 capsule/d or as directed, and the dosage used in the associated human study was 6 capsules/d (registered at Clinicaltrials.gov: NCT01726127).

Analysis of glucosinolates in the supplement and vegetables

Glucosinolate concentrations were measured using previously described methods [20, 21]. Dry samples (200 mg) were extracted two times with 2 mL boiling 70% methanol with 500 µL benzylglucosinolate (1 mM; Sigma-Aldrich, St. Louis, MO) added as an internal standard. During extraction, samples were incubated at 95°C and mixed with a vortex each min. Samples were centrifuged at 1200 x g at 10°C for 15 min and the supernatant was transferred to a new tube. One mL of the pooled supernatants was transferred to a new tube and 150 µL 0.5 M barium acetate/0.5 M lead acetate was added. The sample was mixed and applied to a column with 100 mg A-25 Sephadex pre-conditioned with 5 mL 0.5 M NaOH, 5 mL 0.5 M pyridine acetate, and 5 mL water. After the sample had passed through the column, the column was washed with 3 mL 0.02 M pyridine acetate followed by 3 mL water. After washing, 500 µL sulfatase solution (20 U/mL) was allowed to penetrate the column and the column was incubated overnight at room temperature. The next day, glucosinolates were eluted with 2 mL water followed by an additional 1 mL water. Samples were syringe-filtered (0.2 µm nylon) before 10 µL was injected onto a 3-µm, 3.0 x 100 mm Inertsil® ODS-3 column (GL Sciences, Inc.; Torrance, CA) in line with an Agilent 1260 HPLC system (Agilent Technologies; Santa Clara, CA). Desulfoglucosinolates were eluted from the column at a flow rate of 0.4 mL/min with a mobile phase consisting of water and acetonitrile according to the following gradient: 1) 2% acetonitrile held for 4 min, 2) increased to 7.5% over 6 min, 3) increased to 35% over 19 min, 4) increased to 60% over 1 min, and 5) held for 10 min. The column was re-equilibrated at 2% acetonitrile for 6 min. Chromatograms were generated and glucosinolates were detected at 229 nm and determined using relative response factors [19, 22].

Total isothiocyanates in the supplement and vegetables

Total ITCs were measured as previously described [23]. To 250 mg dry sample, 5 mL distilled water was added and the sample was mixed with a vortex and incubated at room temperature for 1 h. Samples were centrifuged at 4,500 x g for 10 min and the supernatant was syringe-filtered (0.2 µm nylon) before 100 µL extract was combined with 400 µL (100 mM, pH 8.5) potassium phosphate buffer and 500 µL (8 mM) 1, 2-benzenedithiol in an HPLC vial. Vials were capped, flushed with nitrogen, and incubated at 65°C for 2 h. Samples (40 µL) were injected onto a 3.0 x 100 mm, 2.7 µm Agilent Poroshell 120 EC-C8 column on the same HPLC system noted above. The mobile phase at a flow rate of 2 mL/min, consisted of water and acetonitrile, where 5%

acetonitrile was held for 2 min, and increased to 90% in 15 min, held for 5 min, and re-equilibrated at 5% for 12 min. Condensation products were detected at 365 nm. The cyclo-condensation product 1,3-benzodithiole-2-thione eluted at approximately 14 min. Serial dilutions of a 10 µM d, l-sulforaphane (Sigma Aldrich; St. Louis, MO) stock solution were used to create a linear standard curve. The resulting concentration of the condensation products was used to estimate ITC concentrations by converting to sulforaphane equivalents (µmol sulforaphane equivalents/g sample) and reported as "total ITC" estimates.

Comparisons of glucosinolate and isothiocyanate content of vegetables and the supplement

In-text values reflect mean ± SD unless otherwise noted. Statistical comparisons were carried out in SAS 9.4 (Cary, NC). Content of individual and total glucosinolates (defined as the sum of the 12 analyzed glucosinolates) and ITCs were compared among vegetables and the supplement using ANOVA or RM-ANOVA (mixed procedure, with treatment as a fixed effect and time as a random effect) with time slices for comparisons over time. Bonferroni correction of least squares mean differences was used for multiple comparisons if the overall *F*-test was significant.

Results

Baseline total glucosinolate, glucobrassicin, and total ITC content of broccoli, Brussels sprouts, and the cruciferous supplement were quantified on a per-dose or 40-g serving (equivalent to 1/4 cup vegetables). Broccoli contained 65.9, 9.48, and 1.0 µmol of total glucosinolate, glucobrassicin, and total ITC, respectively; Brussels sprouts contained 146, 33.8, and 1.95 µmol of total glucosinolate, glucobrassicin, and total ITC, respectively; and the supplement (6 capsules) contained 33.6, 3.61, and 4.47 µmol of total glucosinolate, glucobrassicin, and total ITC, respectively. In a small serving of vegetables or the dose amount analyzed, the total glucosinolate and glucobrassicin content was higher in the vegetables than the supplement (<0.004 for all comparisons).

Individual glucosinolate content at baseline on a per-gram dry weight basis in the vegetables (frozen and steamed) and cruciferous supplement are listed in Table 1. The glucosinolate profiles of broccoli and Brussels sprouts were different from each other and that of the supplement. Steamed Brussels sprouts had significantly more glucobrassicin than steamed broccoli or the supplement, but also exhibited substantial variation for this glucosinolate. While the vegetables had higher glucosinolate concentrations than the supplement for most compounds, the supplement contained more glucoiberin, gluconapin, and glucoerucin. For most individual glucosinolate compounds, steaming the vegetables preserved glucosinolate content, with the exceptions of 30% and 4% decreases in gluconasturtiin and neoglucobrassicin in broccoli, respectively. Steaming tended to concentrate gluconapin and total glucosinolates in Brussels sprouts, although it did not reach significance (Table 1).

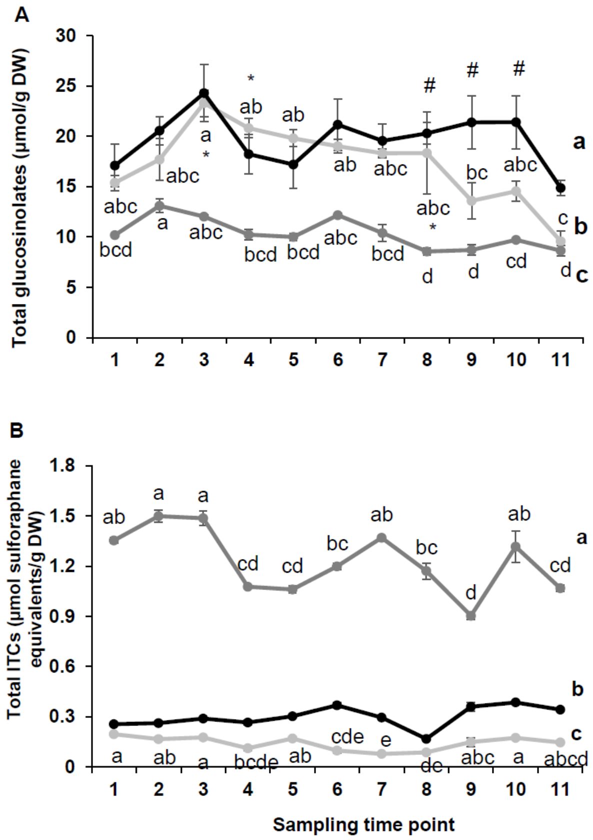


Figure 3. Total glucosinolates (A; µmol/g dry weight) were different by vegetable or supplement (P < 0.0001; large letters) and over time (P < 0.0001; small letters), and the interaction was significant (P = 0.04). Total isothiocyanate (B; µmol sulforaphane equivalents/g dry weight) content also varied by vegetable or

supplement, over time, and an interaction existed (P < 0.0001 for all). Frozen broccoli is the light grey line, frozen Brussels sprouts is the black line, and the supplement is the darker grey line. Vegetables were tested every 6-8 weeks, beginning in July 2014. Error bars represent ± SEM. Large and small letters represent Bonferroni-corrected least squares differences for treatment and time point comparisons, respectively, after overall treatment by time comparison with RM-ANOVA (mixed procedure, SAS 9.4). Symbols indicate a significant difference at a time point between frozen broccoli (*) or frozen Brussels sprouts (#) and the supplement, respectively.

Total glucosinolate concentration varied by time and treatment (P = 0.04; Figure 3A), with substantial variation in broccoli and Brussels sprouts. Both frozen broccoli and the cruciferous supplement lost total glucosinolates over time (P < 0.0006 for frozen broccoli and P < 0.0001 for the supplement, Figure 3A). Total glucosinolate content was not different across time points in frozen Brussels sprouts (P = 0.2; Figure 3A).

On a dry weight basis, the majority of the individual glucosinolates declined over time in frozen broccoli, with the exception of glucoraphanin and neoglucobrassicin, whereas several glucosinolates did not change over time in frozen Brussels sprouts (Figure 4 and 5), except for 4-OH-glucobrassicin, 4-methoxy-glucobrassicin, and gluconasturtiin. Glucobrassicanapin was only detected in the supplement (Table 1) and remained relatively stable over time, whereas glucoerucin was only detected in the supplement and frozen broccoli (Table 1). Glucoerucin decreased in the supplement (P < 0.0001) and increased in the broccoli (P = 0.02) with time, but with appreciable variability. Importantly, glucobrassicin content was different among the vegetables and supplement (P < 0.0001). Glucobrassicin was not different over time in Brussels sprouts (P = 0.9), but decreased over time in frozen broccoli and the supplement (Figure 4A). Substantial variation was observed for the majority of glucosinolates in Brussels sprouts. Variability was lower for the cruciferous supplement.

The estimated total ITC content of vegetables (frozen and cooked) was lower than the supplement on a dry-weight basis (Figure 3B), with less variation than that observed for glucosinolates. Steaming did not substantially affect the ITC content of the vegetables; however, steaming broccoli increased ITC content on a dry weight basis and not Brussels sprouts at time point 1 (Table 1). The supplement and frozen broccoli lost total ITCs over time (Figure 3B). However, there was substantial variation in ITC content with time. There was no difference in ITC content over time for frozen Brussels sprouts (Figure 3B).

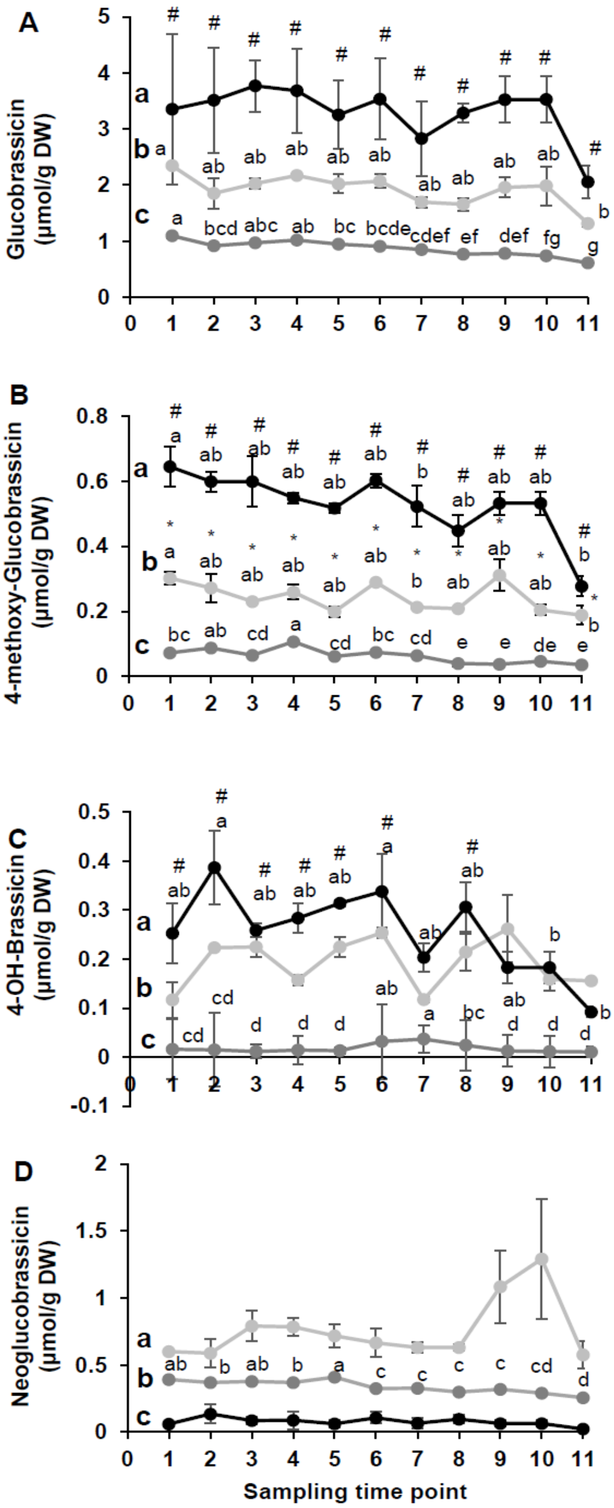
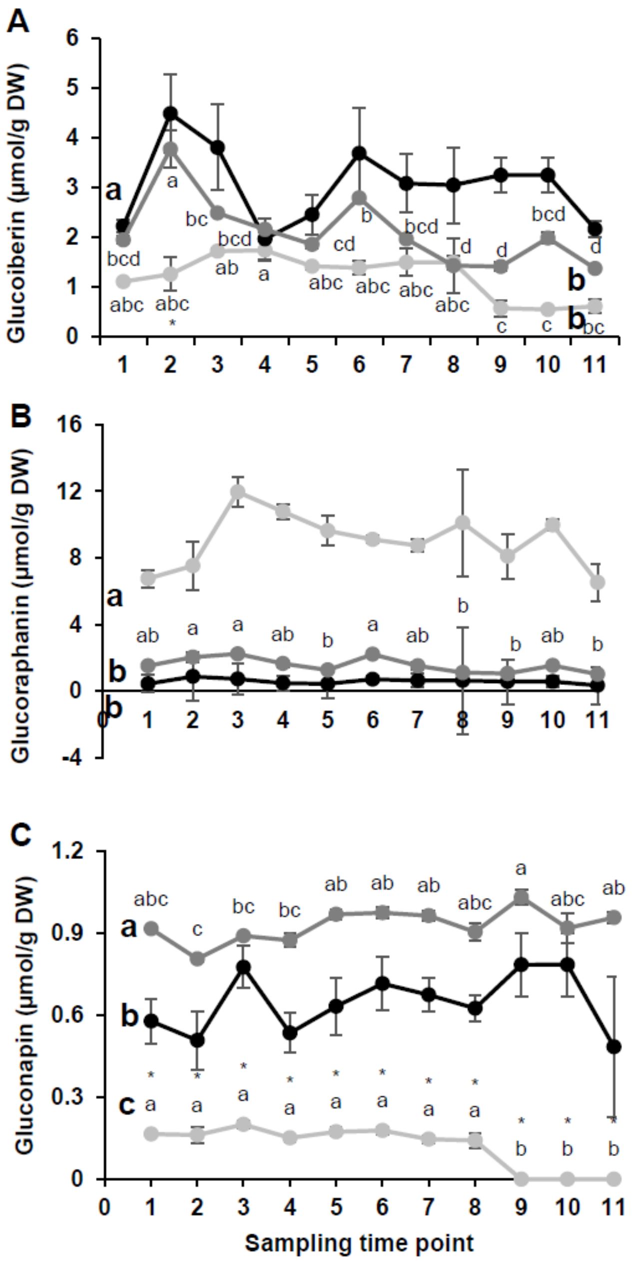


Figure 4. Indole glucosinolates in frozen broccoli (light grey) and Brussels sprouts (black) and a cruciferous supplement (dark grey) over time. Glucobrassicin (A) was affected by time in broccoli (P = 0.02) and the supplement (P < 0.0001). 4-Methoxy-glucobrassicin (B) was affected by time in all products (P < 0.007) as was 4-hydroxy-glucobrassicin (C) (P < 0.01). Neoglucobrassicin (D) only varied by time in the supplement (P < 0.0001). Error bars are ± SEM. Large lower case letters reflect overall effect of storage on the indole glucosinolate. Small letters reflect Bonferroni-corrected least squares means if the overall F-test was significant. Symbols indicate a significant difference at a time point between frozen broccoli (*) or frozen Brussels sprouts (#) and the supplement, respectively. [Note: the scales are different for each glucosinolate.].



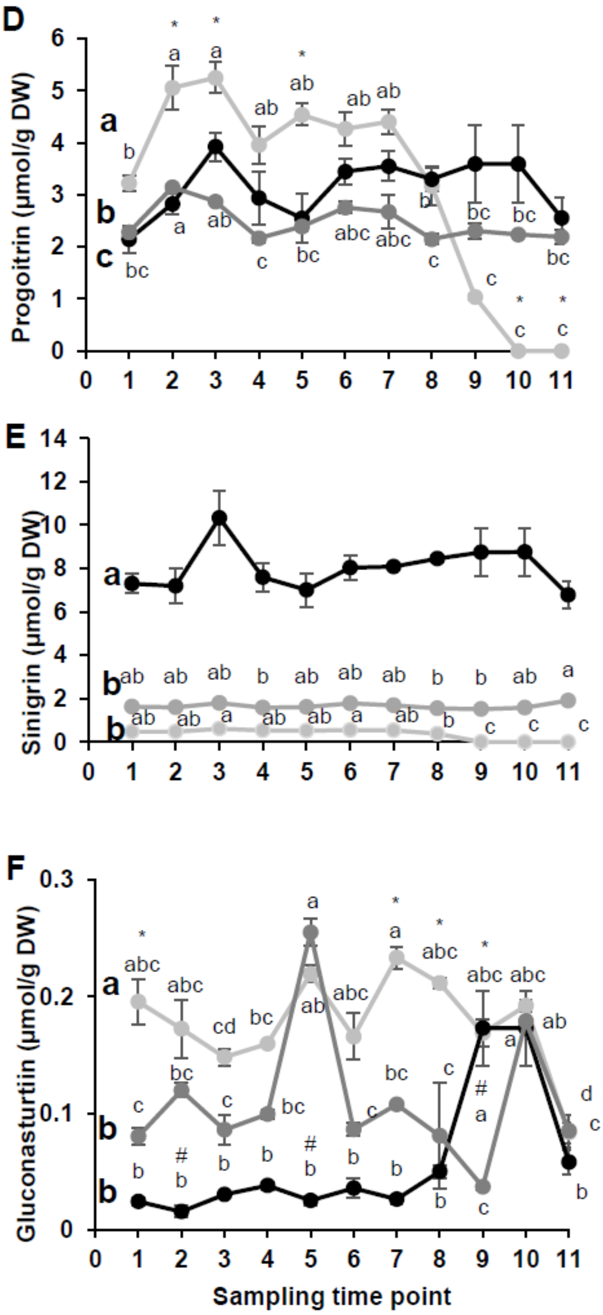


Figure 5. Aliphatic and aromatic glucosinolates in frozen broccoli (light grey) and Brussels sprouts (black) and a cruciferous supplement (dark grey). Glucoiberin (A) was affected by time in the broccoli (P = 0.001) and supplement (P < 0.0001), but not Brussels sprouts. Glucoraphanin only fluctuated in the supplement (P < 0.0001). Gluconapin (C), progoitrin (D) and sinigrin (E) were affected by time in the broccoli (P < 0.0001 for all) and the supplement (P < 0.003), but not Brussels sprouts. Gluconasturtiin (F) was the lowest glucosinolate and was universally affected by time (P < 0.0001 for all). Error bars are ± SEM. Large letters reflect overall treatment effect. Small letters reflect Bonferroni-corrected least squares means if the overall F-test was significant. Symbols indicate a significant difference at a time point between frozen broccoli (*) or frozen Brussels sprouts (#) and the supplement, respectively.

Discussion

The vegetables had higher total glucosinolate content on a dry weight or small serving basis than the supplement. The total glucosinolate content of the frozen vegetables (0.63 and 1.24 mg/g wet weight for broccoli and Brussels sprouts, respectively) was higher than previously reported literature values (0.27 and 0.61 mg/g wet weight for broccoli and Brussels sprouts) [24]. In a prior study, total glucosinolates did not change over 7 days in broccoli and Brussels sprouts when stored at room temperature and in the refrigerator [25]. Shredding and boiling, on the other hand, caused significant losses [25]. Total glucosinolate content decreased over time in frozen broccoli and in the supplement, but not in Brussels sprouts, which showed greater variation in content. Such variation could have obscured a decrease over time or be due to Brussels sprouts' more compact form, which may have protected glucosinolates during prolonged frozen storage compared with the more branched structure of broccoli.

Such observed differences in glucosinolates serve as a reminder about phytonutrient variability in nutritional interventions, which must be considered when assigning treatment groups and formulating supplements to ensure that treatments are comparable and that supplements contain the reported active ingredients. Glucosinolate content varies substantially by cultivar, growing season, location, and abiotic stressors [26, 27]. The supplement contained kale instead of broccoli, which resulted in a different glucosinolate profile. Broccoli is much more common in the US diet than Brussels sprouts and kale, which is why broccoli was chosen for analysis. Broccoli was included in the subsequent human intervention (reported elsewhere) because it was easier to find a supplier, manage in the frozen state, and is preferred by most consumers.

The supplement had higher ITC content than the vegetables. It is important to understand that ITCs measured in the vegetables and supplement are actually a reflection of the amount produced during the incubation period of the analysis and the results of this study are relative and only comparable under the specific conditions of this analysis. ITCs are unstable compounds that can quickly degrade or be bioconverted. Moreover, we did not determine percent recovery from the plant matrix and it is possible that not all of the ITCs were extracted in the watery phase, especially given that we did not add additional methanol to better solubilize ITCs. As such, the lower ITC content in the vegetables may be a result of incomplete extraction or be suggestive of destruction of intrinsic myrosinase, likely during blanching prior to frozen storage or vegetable preparation. However, steaming preserves myrosinase activity than more intensive cooking methods, such as boiling [4]. Steaming did not affect ITC content in Brussels sprouts in the current study. Total ITCs of the frozen vegetables (8 and 5 µg/g wet weight for broccoli and Brussels sprouts, respectively) were lower than that reported for raw broccoli and Brussels sprouts (12.2 and 17.0 µg/g wet weight, respectively) [28], which may be explained by the processing of the frozen vegetables prior to freezing, which typically involves blanching and flash freezing. While many glucosinolates decreased over time, the variability was much lower in the broccoli and therefore a difference was more apparent in comparison with the Brussels sprouts.

Cruciferous vegetables have been implicated in cancer prevention and should be part of an optimal diet, not only for their glucosinolate content, but also because they are rich in vitamin C, minerals, and fiber. This study uniquely analyzed important phytochemicals during long-term frozen storage and found little variation for over a year's time. The US Dietary Guidelines for Americans recommends that people consume 2.5 cups of vegetables per day (2000-Calorie diet) at minimum, with at least 1.5 cups of dark-green vegetables per week [29]. While few Americans meet the recommendations for vegetable intake, evidence suggests that Americans may be replacing white potatoes with other types of vegetables, because consumption of leafy greens, broccoli, and cauliflower has been rising [30]. Promoting frozen vegetables with nutrition education messages may be a way to circumvent some people's hesitance to buying fresh vegetables due to short shelf-life.

The US supplement industry is now estimated at approximately $40 billion, with herbs and botanicals accounting for 18% of the market [31]. In the National Health Interview Survey 2012, 17.7% of respondents reported taking a non-vitamin or non-mineral dietary supplement [32] and 29% of US supplement users reported taking herbals or botanicals in 2017 [33]. Reports that herbal and dietary supplements may not contain the claimed active ingredients and may be contaminated with pharmaceuticals [34] and that supplement manufacturers may fail to test for active ingredients [35] have raised questions of supplement safety and quality. Even when active ingredients are identified, there may be a large degree of variability in the concentration of the active component [36].

This study showed the importance of determining the unique glucosinolate patterns of cruciferous vegetables and supplements over time. In the steamed form, the Brussels sprouts had total glucosinolate content that was higher than that measured in broccoli or the supplement, but the broccoli and supplement did not differ. The strength of the results of epidemiological and intervention studies [1, 2] could be improved with analytical analyses of phytochemicals with purported health effects.

Conclusions

Given the chemopreventive potential of cruciferous vegetables, current dietary recommendations, and trends toward increased cruciferous vegetable consumption, research on the phytochemical content and their stability in food and food-based products over time is needed. Characterization of the variability in glucosinolate and ITC content is needed to better estimate glucosinolate and ITC intake for epidemiological studies, which may improve the strength of associations. Herein, relative contents of individual and total glucosinolates and total ITCs were determined in frozen broccoli and Brussels sprouts and a commercial supplement. Total glucosinolates and glucobrassicin were not different over time in frozen Brussels sprouts, but substantial variation was observed. Glucosinolates decreased over time in frozen broccoli and the supplement. Future studies seeking to use frozen cruciferous vegetables or a whole-food supplement should consider time of storage and variability when determining treatment amounts. While whole-food supplements will vary in active components according to the variability in the food sources themselves, this variation may make it difficult to estimate a supplement dose to demonstrate an effect in human studies. Quantification of bioactive compounds in supplements and determining stability over time are important for manufacturers and consumers. Consumers often keep supplements in their homes for more than a year. Bulk purchases often lead to savings. Therefore, determining the potency over time is important.

Conflicts of Interest

Stephanie Mondloch and Sherry Tanumihardjo have no conflicts of interest to declare. Standard Process, Inc., employed Chris Scholl and Sara Arscott at the time this study was done, but they were not involved in statistical analysis and their salaries were not contingent on the findings of this study.

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PEER REVIEW

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Tables

Table 1. Glucosinolates and total isothiocyanates in broccoli (frozen and cooked), Brussels sprouts (frozen and steamed), and a cruciferous supplement.[1]

µmol/g dry weight	Frozen broccoli	Steamed broccoli	*P*-value for steaming	Frozen Brussels sprouts	Steamed Brussels sprouts	*P*-value for steaming	Supplement	*P*-value for overall comparison
glucosinolates								
glucoiberin[2]	1.11 ± 0.14^{b}	1.02 ± 0.35^{b}	0.7	2.23 ± 0.22^{a}	1.95 ± 0.47^{a}	0.4	1.95 ± 0.01^{a}	0.001
progoitrin	3.22 ± 0.24	2.86 ± 0.57	0.4	2.15 ± 0.45	2.84 ± 0.66	0.2	2.28 ± 0.13	0.08
glucoraphanin	6.74 ± 0.89^{a}	6.22 ± 2.19^{a}	0.7	0.45 ± 0.06^{b}	0.40 ± 0.09^{b}	0.4	1.52 ± 0.07^{b}	<0.0001
sinigrin	0.48 ± 0.03^{b}	0.41 ± 0.06^{b}	0.1	7.30 ± 0.77^{a}	8.23 ± 1.83^{a}	0.5	1.63 ± 0.03^{b}	<0.0001
gluconapin	0.17 ± 0.01^{c}	0.16 ± 0.03^{c}	0.7	0.58 ± 0.14^{b}	0.73 ± 0.06^{ab}	0.2	0.91 ± 0.00^{a}	<0.0001
4-OH-glucobrassicin	0.12 ± 0.06^{ab}	0.10 ± 0.03^{ab}	0.7	0.25 ± 0.10^{a}	0.20 ± 0.03^{a}	0.4	0.02 ± 0.00^{b}	0.005
glucobrassicanapin[3]	ND	ND	-	ND	ND	-	0.08 ± 0.02	-
glucoerucin	0.03 ± 0.03^{b}	0.03 ± 0.01^{b}	0.9	ND	ND	-	0.15 ± 0.01^{a}	<0.0001
glucobrassicin	2.34 ± 0.07^{a}	1.98 ± 0.02^{a}	0.001	3.35 ± 2.33^{a}	4.56 ± 1.33^{a}	0.5	1.10 ± 0.07^{b}	0.04
gluconasturtiin	0.20 ± 0.03^{a}	0.14 ± 0.02^{b}	0.05	0.02 ± 0.01^{d}	0.02 ± 0.01^{d}	0.9	0.08 ± 0.01^{c}	<0.0001
4-methoxy-glucobrassicin	0.35 ± 0.02^{b}	0.30 ± 0.04^{b}	0.09	0.71 ± 0.07^{a}	0.64 ± 0.11^{a}	0.4	0.07 ± 0.01^{c}	<0.0001
neoglucobrassicin	0.60 ± 0.02^{a}	0.58 ± 0.13^{ab}	0.8	0.06 ± 0.01^{c}	0.13 ± 0.07^{c}	0.1	0.39 ± 0.02^{b}	<0.0001
total glucosinolates	15.4 ± 1.31^{ab}	13.8 ± 3.26^{ab}	0.5	17.1 ± 3.68^{ab}	19.7 ± 3.17^{a}	0.4	10.2 ± 0.06^{b}	0.02
isothiocyanates				µmol sulforaphane equivalents/g dry weight				
total isothiocyanates	0.20 ± 0.005^{c}	0.21 ± 0.005^{c}	0.02	0.26 ± 0.01^{b}	0.26 ± 0.01^{b}	0.4	1.35 ± 0.03^{a}	<0.0001

[1]Glucosinolates and isothiocyanates were analyzed in triplicate.
[2]Data are mean ± SD. P-value for the overall comparison reflects the F-test comparing frozen and cooked vegetables and the supplement. If the overall F-test was significant, group means (among broccoli [steamed, frozen] and Brussels sprouts [steamed, frozen]) were compared with Bonferroni correction of least squares means (letters: a > b > c > d).
[3]ND indicates that the compound was "not detected".