1 Effects of industrial pre-freezing processing and freezing
2 handling on glucosinolates and antioxidant attributes in
3 broccoli florets

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ABSTRACT

The effects of industrial pre-freezing processing and freezing handling on the contents of glucosinolates and antioxidants (vitamin C, polyphenols, carotenoid and chlorophyll), as well as the antioxidant capacity in broccoli (*Brassica oleracea* L. var. *italica*) florets were investigated in the present study. Our results showed that the glucosinolate accumulations were significantly decreased after pre-freezing processing, whereas elevated levels of phenols, carotenoids, chlorophyll, and also antioxidant capacity were observed in frozen broccoli florets. The contents of vitamin C remained constant during above mentioned processing. In conclusion, the current industrial freezing processing method is a good practice for the preservation of main antioxidant nutrients in broccoli florets, although some improvements in pre-freezing processing, such as steam blanching and ice-water cooling, are needed to attenuate the decrease in glucosinolate content.

Keywords: Broccoli; Freezing; Glucosinolates; Antioxidant capacity

1. Introduction

Broccoli (*Brassica oleracea* L. var. *italica*) has been described as one of the most important vegetables worldwide, and it is also a rich source of anti-carcinogenic compounds and antioxidants, such as glucosinolates, phenolic compounds, vitamin C, and carotenoids. As a climacteric and perishable vegetable, broccoli usually needs suitable post-harvest techniques to extend its shelf life and marketability, among which freezing is one of the most popular and effective processing approaches to maintaining post-harvest quality and nutritional properties of broccoli products for extensive periods

of time. Broccoli is subjected to processing including cutting, washing, blanching and cooling prior to freezing. Although several studies have demonstrated that contents of glucosinolates and main antioxidants in broccoli were affected by post-harvest processing such as cooking (Yuan, Sun, Yuan, & Wang, 2009), blanching (Cieřlik, Leszczyńska, Filipiak-Florkiewicz, Sikora, & Pisulewski, 2007; Nugrahedi, Verkerk, Widianarko, & Dekker, 2015), and freezing (Alanis-Garza, Becerra-Moreno, Mora-Nieves, Mora-Mora, & Jacobo-Velazquez, 2015; Cieřlik, Leszczyńska, Filipiak-Florkiewicz, Sikora, & Pisulewski, 2007), little is known about the dynamics of main bioactive compounds in broccoli during pre-freezing processing, namely cutting, washing, blanching, cooling, and freezing handling period.

Several epidemiological studies have shown that broccoli has great potential to prevent a variety of diseases such as cancer and cardiovascular diseases (Dinkova-Kostova & Kostov, 2012; Wattenberg, 1992), and accumulating evidence indicates that its remarkable healthy effects are mainly attributed to its relatively high content of glucosinolates (Guo, Yuan, & Wang, 2013). Glucosinolates are a diverse group of sulfur- and nitrogen-containing secondary metabolites which are found mainly in *Brassicaceae* including broccoli, and their content can be regulated by many biotic and abiotic factors (Gigolashvili, Berger, & Flügge, 2008; Redovnikovic, Glivetic, Delonga, & Vorkapic-Furac, 2008; Yan & Chen, 2007). These phytochemicals have gained increased interest in recent years due to the chemoprotective properties of their major hydrolysis products, isothiocyanates, which exert health promoting effect of cancer protection in several epidemiological studies (Cartea & Velasco, 2008). Broccoli contains high levels of glucoraphanin and glucobrassicin, the hydrolysis of which produce sulforaphane and

indole-3-carbinol under the action of myrosinase. Both sulforaphane and indole-3-carbinol are major anticancer isothiocyanates responsible for the protective effects against cancer by inducing the detoxification phase 2 enzymes in anticarcinogenic metabolism (Hayes, Kelleher, & Eggleston, 2008; Holst & Williamson, 2004; Zhang & Tang, 2007). In addition to glucosinolates, broccoli is also particularly rich in antioxidants. It has been reported that phenolic compounds, vitamin C and carotenoids can contribute to antioxidant activity and are known as health-promoting compounds in *Brassica* vegetables including broccoli (Bhandari & Kwak, 2015; Byers & Perry, 1992; Velioglu, Mazza, Gao, & Oomah, 1998).

Industrial freezing has become one of the most popular processing methods for broccoli in China, however, it is still an open question if it could also maintain the main phytochemicals and antioxidant activity in broccoli. The present study was therefore undertaken to investigate the effects of industrial pre-freezing processing and also freezing handling on glucosinolates and antioxidant attributes in broccoli. For that reason, broccoli florets were sampled along the corresponding industrial chain at different steps of the pre-freezing processing and freezing handling, and analyzed for the contents of glucosinolates and antioxidants as well as the antioxidant capacity.

2. Material and methods

2.1. Sample collection and preparation

Broccoli (*Brassica oleracea* var. *italica* cv. ‘Youxiu’) sampling along an industrial freezing chain was carried out in December 25, 2013. Seeds of broccoli ‘Youxiu’ were ordered from Sakata Seed Corporation (Japan). The broccoli heads of prime quality were

harvested in the early morning from the greenhouse of Zhejiang University (Hangzhou, China), top-iced and then transported to the laboratory of Vegetable Institute, Zhejiang University within 10 min. Five broccoli heads without visual defects and with a diameter ranging from 13 to 14 cm were chosen. Pre-freezing processing and freezing handling protocol was according to the industrial freezing chain conditions of Haitong Food Group Co. in Cixi, China. The inner branches (with florets having stalks of approximately 2 cm) were then cut from these heads, and immediately sampled as the “Control” group. The Cut florets were placed in room temperature for 1 h, and then sampled as “Cut” group. The florets were then immersed in 1% sodium chloride solution for 15 min, followed by washing in clean water, and sampled as “Washed” group afterwards. The washed florets were put into boiling waters for 90 s, and sampled as “Blanched” group. Cooling was then carried out by using a hydro-cooling shower operating at 3 °C for 8 min, and the florets was sampled as “Cooled” group. Finally, after all above pre-freezing processing, florets were frozen by subjecting the samples to -26 °C for 8 min in an Individual Quick Freezing (IQF) fluidized tunnel system, obtaining a final temperature of -18 °C in the center of broccoli florets, and sampled as “Frozen” group. At each sampling point, four florets were randomly chosen, collected and directly stabilized in liquid nitrogen and then lyophilized in an ultralow -80 °C freezer for further analysis of glucosinolates, antioxidants, and antioxidant capacity. The experiment was repeated three times.

2.2. *Glucosinolate assay*

Glucosinolates were extracted and analyzed as previously described with slight

modifications (Qian, Sun, Miao, Cai, Xu, & Wang, 2015). Freeze-dried samples (200 mg) were boiled in 4 ml water for 20 min. After centrifugation at $7500 \times g$ for 5 min, the supernatant was decanted and the residues were re-extracted with 4 ml water. The combined aqueous extract was applied to a DEAE-Sephadex A-25 (30 mg) column (pyridine acetate form). The column was washed three times with 0.02 M pyridine acetate and twice with water. The glucosinolates were converted into their desulpho analogues by overnight treatment with 100 μ l of 0.1% (1.4 units) aryl sulphatase (Sigma), and the desulphoglucosinolates were eluted with 2×0.5 ml water. The high-performance liquid chromatography (HPLC) analysis of desulphoglucosinolates was carried out using a Shimadzu HPLC instrument (Shimadzu, Kyoto, Japan) equipped with a SPD-M20A diode array detector. Samples (20 μ l) were separated at 30 °C on a Spherisorb C18 column (5 μ m particle size, 4.6 mm $\times$ 250 mm; Elite Analytical Instruments Co. Ltd, Dalian, China) using acetonitrile and water at a flow rate of 1.0 ml min⁻¹. The procedure employed isocratic elution with 1.5% acetonitrile for the first 5 min; a linear gradient to 20% acetonitrile over the next 15 min followed by isocratic elution with 20% acetonitrile for the final 13 min. Absorbance was detected at 226 nm. Ortho-nitrophenyl- β -D-galactopyranoside (Sigma) was used as an internal standard for HPLC analysis. The glucosinolate concentration was expressed as μ mol g⁻¹ dry weight (DW) of broccoli. Response factors for glucosinolate profiles in broccoli by using ortho-nitrophenyl- β -D-galactopyranoside as the standard were listed in supplementary Table S1.

2.3. Vitamin C content assay

Vitamin C content was analyzed as previously described with minor modifications

(Qian, Sun, Miao, Cai, Xu, & Wang, 2015). Briefly, freeze-dried samples (0.1 g) were homogenized twice with 2 ml of oxalic acid solution (1%, w/v). The mixture was diluted to 6 mL with water and centrifuged at $7000 \times g$ for 5 min. The supernatant was filtered through a 0.45 μm cellulose acetate filter. HPLC analysis of vitamin C was carried out using a Shimadzu HPLC instrument (Shimadzu, Kyoto, Japan) equipped with a SPD-M20A diode array detector. Samples (20 μl) were separated at 30 $^{\circ}\text{C}$ on a spherisorb C18 column (5 μm particle size, 4.6 mm $\times$ 250 mm; Elite Analytical Instruments Co. Ltd, Dalian, China) using a solvent of 0.1 % oxalic acid at a flow rate of 1.0 ml min⁻¹. Absorbance was detected at 243 nm. Authentic ascorbic acid was used as a standard for calculation of the amount of vitamin C. Result was presented as mg 100 g⁻¹ dry weight (DW).

2.4. Total phenolic content assay

Total phenolic content was evaluated based on previous method with modifications (Volden, Bengtsson, & Wicklund, 2009). Freeze-dried samples (0.2 g) were ground twice with 2 ml of 30% ethanol. The obtained homogenates were diluted to 6 mL with water, followed by incubating at 80 $^{\circ}\text{C}$ for one hour in the dark and centrifuging at $7000 \times g$ for 10 min. Then, 0.3 ml of supernatant was mixed with 1.5 ml of 0.2 M Folin-Ciocalteu reagent, and incubated at room temperature for 3 min prior to addition of 1.2 ml of 7.5% (w/v) sodium bicarbonate solution. After incubating the reaction mixture at room temperature for one hour, absorbance was detected at 760 nm with the spectrophotometer. Total phenolic content was determined according to a calibration curve of gallic acid. Results were expressed as mg gallic acid equivalent (GAE) 100 g⁻¹ DW.

2.5. Carotenoid and chlorophyll content assay

The contents of carotenoid and chlorophyll were determined according to the HPLC method of previous reports (Lefsrud, Kopsell, & Kopsell, 2007). Briefly, 0.1 g sample was hydrated with 0.8 ml of deionized water in a 40 °C water bath for 20 min. After hydration, 0.8 ml of the internal standard, ethyl- β -apo-8'-carotenoate and 2.5 ml of tetrahydrofuran (THF) were added and the mixture was homogenized before being placed into a clinical centrifuge for 3 min at 500 $\times$ g. The supernatant was placed into a test tube, capped and held on ice. The sediment was re-suspended in 2.0 ml THF and homogenized for the second time. The sample was then centrifuged for 3 min at 500 $\times$ g per min and the supernatant was collected and combined with the first extracted supernatant. The combined supernatants were reduced under a stream of nitrogen gas, and brought up to a final volume of 5 ml with methanol. A 2 ml aliquot was filtered prior to HPLC analysis. Samples (20 μ l) were separated at 16 °C on a RP C18 column (250 $\times$ 4.6 mm i.d.; 5 μ m particle size) using gradient elution at a flow rate of 0.7 ml min⁻¹. Eluted carotenoids and chlorophyll compounds were separately detected at 452 nm (carotenoids and internal standard), 652 nm (chlorophyll b), and 665 nm (chlorophyll a). Peak assignment for individual pigments was performed by comparing retention times and line spectra obtained from photodiode array detection using external standards.

2.6. Antioxidant capacity assay

Ferric reducing ability of plasma (FRAP) assay proposed by Benzie and Strain (1996) was adopted to evaluate the antioxidant capacity of sprouts. The sample extracts were prepared in the same way as described in total phenolic content assay above. Then, 0.3 ml

of the extracts were added to 2.7 ml of FRAP working solution composed of 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-S-triazine in 40 mM HCl and 20 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ with the ratio of 25:1:1. The mixture was vortexed and incubated at 37 °C for 10 min. The absorbance was recorded at 593 nm. FRAP values of sprouts were calculated against $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ standard curves and expressed as $\mu\text{mol g}^{-1}$ DW.

2.7. RNA extraction and real-time PCR analysis

Total RNA was isolated from ~100 mg broccoli florets using Trizol reagent according to manufacturer's instruction (Takara, Japan). RNA samples were reversed transcribed into cDNAs using Prime Script RT Master Mix (Takara, Japan).

Real-time quantitative PCR (qPCR) was performed in a total volume of 20 μl , including 2 μl of diluted cDNA, 1 μl of each primer (5 μM), and 10 μl of 2 $\times$ SYBR Green PCR Master Mix (Takara, Japan) on an Applied Biosystems StepOne Real-Time PCR Systems (Applied Biosystems, USA) according to the kit manual. The qPCR program was conducted at 95 °C for 10 s first, followed by 40 cycles of 95 °C for 5 s, and 60 °C for 30 s. The expression level of *BoActin7* (Accession number: Bol024627) was used as an internal control and the expression of other genes was computed with the $2^{-\Delta\Delta\text{CT}}$ method. The primers used in this work are listed in supplementary Table S2.

2.8. Statistical analysis

Statistical analysis of data was performed using the SPSS package program version 11.5 (SPSS Inc. Chicago, IL, USA). Data were analyzed by one-way ANOVA, in

combination with Tukey's HSD multiple comparison test to evaluate the effects of pre-freezing processing and freezing handling. Different letters within the same group indicate significant difference at 95% level.

3. Results

3.1 Glucosinolates

The glucosinolate composition and contents were quantitatively determined in broccoli florets. Nine glucosinolate profiles were identified, belonging to two chemical classes: five aliphatic (glucoiberin, glucoraphanin, glucoerucin, sinigrin, gluconapin and progoitrin) and four indolic (glucobrassicin, 4-hydroxy glucobrassicin, 4-methoxy glucobrassicin and neoglucobrassicin) glucosinolates (Table 1).

The contents of aliphatic glucosinolates and total glucosinolates were decreased by 44.76% and 35.16% respectively in frozen broccoli florets, while the content of indolic glucosinolates was not significantly affected. Glucosinolate content remained constant following cutting and washing, and was reduced by 37.59% in blanched florets and 64.93% in cooled florets. Final freezing treatment significantly increased both aliphatic and indolic glucosinolate content in pre-freezing processed broccoli florets. Our result showed that gluconapin and glucoraphanin, suffering a 49% and 50% loss respectively, were most influenced types of glucosinolates in frozen broccoli florets, and the amount of glucoraphanin, the most predominant glucosinolate in broccoli florets, was reduced by 61.21% after pre-freezing processing, and notably, its content was decreased by 29.5% and 61.2%, respectively upon blanching and cooling (Table 2).

3.2 Vitamin C content

Generally, the content of vitamin C in frozen broccoli florets remained at the same level as that in the control. No change was observed after cutting. However, washing treatment led to a significant decrease of vitamin C content. There was also no significant difference during blanching, cooling and freezing (Figure 1).

3.3 Total phenolic content

Broccoli subjected to pre-freezing processing and freezing handling showed a 29.34% increase in total phenolic content as compared with the fresh broccoli. No change was observed after cutting and washing, but total phenolic content was increased by 25.92% after blanching. Cooling treatment caused a 22.04% reduction in total phenolic content of blanched broccoli florets, while final freezing handling led to a 31.92% elevation in total phenolic content of cooled broccoli florets (Figure 2).

3.4 Carotenoid and chlorophyll content

As shown in Table 3, significant increases in the contents of carotenoids and chlorophylls were observed in frozen broccoli florets when compared with the control. The content of carotenoids and chlorophylls in frozen broccoli florets was increased by 21.8% and 10.42% respectively. Pre-freezing processing increased the content of chlorophyll but did not affect the content of carotenoids, while freezing handling elevated carotenoid content but did not cause a significant change in chlorophyll content.

243

244 3.5 Antioxidant capacity

245 The antioxidant capacity of broccoli florets was measured as the value of FRAP. As
246 shown in Figure 3, FRAP value was increased by 21.26% in frozen broccoli florets.
247 There was no significant change after cutting and washing treatment, and it was
248 decreased by 17.03% after cooling, while elevated by 23.36% and 22.88% respectively,
249 after blanching and freezing.

250

251 4. Discussion

252 We investigated the effects of individual industrial pre-freezing processing and
253 freezing handling on glucosinolate content of broccoli florets in current survey, and our
254 results showed that glucosinolate content was decreased in frozen broccoli florets
255 compared with that in fresh broccoli. Cutting and washing treatments did not have an
256 influence on glucosinolate content, while remarkable reductions in glucosinolate levels
257 were observed after blanching and cooling treatments, which might be attributed to the
258 dissolution of glucosinolates into water as cell lysis occurred during hydrothermal
259 treatment. Studies on other *Brassica* vegetables such as Brussel sprouts, white
260 cauliflower, green cauliflower, and curly kale showed a similar result (Cieřlik,
261 Leszczyńska, Filipiak-Florkiewicz, Sikora, & Pisulewski, 2007). In the present study, the
262 final freezing handling enhanced glucosinolate content. As the glucosinolate level in
263 broccoli is a reflection of two opposing physiological processes, induction of
264 glucosinolate biosynthesis in an unknown way and hydrolysis of glucosinolates by

myrosinase (Jia, Xu, Wei, Yuan, Yuan, Wang, *et al.*, 2009), two reasons may account for our finding of increased glucosinolates under freezing handling. On one hand, freezing stress may lead to induction of glucosinolates biosynthesis. On the other hand, according to previous studies (Dosz & Jeffery, 2013; Ludikhuyze, Ooms, Weemaes, & Hendrickx, 1999; Van Eylen, Oey, Hendrickx, & Van Loey, 2007), myrosinase is not thermal-stable and therefore prior blanching step will cause an almost complete loss of myrosinase activity, which subsequently resulted in the inhibition of glucosinolate hydrolysis.

Glucoraphanin is the most predominant glucosinolate in both fresh and frozen broccoli florets. It is more stable than sulforaphane in food, and also can be hydrolyzed to sulforaphane in human gastrointestinal system (Luang-In, Narbad, Nueno-Palop, Mithen, Bennett, & Rossiter, 2014). Therefore, it will be better to keep high level of glucoraphanin instead of sulforaphane in broccoli before consumption by the consumers for better health benefit. Our result showed that the amount of glucoraphanin in broccoli florets was substantially reduced after pre-freezing processing. Thus, the current industrial pre-freezing processing chain needs to be improved. With respect to blanching, it will be better to apply steam blanching by a steamer instead of hydrothermal treatment. Blanching with steam does not require immersion in water and, thus, leaching of water-soluble glucoraphanin and other glucosinolates was hindered. Our previous study also showed that the steam cooking led to the lowest loss of total glucosinolates when compared with stir-frying and boiling (Yuan, Sun, Yuan, & Wang, 2009). As for cooling, an ice-water cooling system is suggested to replace water cooling method used in this study. Application of ice-water cooling system will shorten the duration of broccoli florets in water and thus minimize the loss of glucosinolates.

In this study, we found that pre-freezing processing and freezing handling produced a significant increase in the amount of phenolic compounds and carotenoids along with antioxidant capacity, with the exception of the vitamin C. A former report also revealed that broccoli subjected to the industrial freezing process showed higher concentration of total phenolic compounds and carotenoids as compared with fresh broccoli florets (Alanis-Garza, Becerra-Moreno, Mora-Nieves, Mora-Mora, & Jacobo-Velazquez, 2015). Increases in the content of phenolic compounds are a common response to infection, damage or other stress-inducing factors in plants (Dixon & Paiva, 1995), which may explain its increased concentration observed in our study. It has also been previously reported that total phenolic in broccoli was enhanced by salt stress (Guo, Yang, Wang, Guo, & Gu, 2013) and thermal stress during steam cooking (Gliszczynska-Swiglo, Ciska, Pawlak-Lemańska, Chmielewski, Borkowski, & Tyrakowska, 2006). This situation may also be attributed to an increase in the extractability of these compounds triggered by pre-freezing processing, which was also reported by several former studies (Alanis-Garza, Becerra-Moreno, Mora-Nieves, Mora-Mora, & Jacobo-Velazquez, 2015; Pellegrini, Chiavaro, Gardana, Mazzeo, Contino, Gallo, *et al.*, 2010). However, further method validation analysis need to be performed to test whether analytical recoveries are the same for fresh, blanched, and frozen plant tissues.

Antioxidant capacity is the reflection of synergetic effect of multiple antioxidants including phenolic compounds, vitamin C and carotenoids. It has been previously reported that phenolic compounds contributed more to antioxidant capacity than vitamin C and other antioxidants in *vitro* study (Vinson, Dabbagh, Serry, & Jang, 1995), which is in accordance with our observation of high conformity between variation tendency of

total phenol content and that of antioxidant capacity in current survey.

Previous study reported that freezing slightly increased the chlorophyll content of plant food (Alanis-Garza, Becerra-Moreno, Mora-Nieves, Mora-Mora, & Jacobo-Velazquez, 2015). On the basis of our study, it can be pointed out that pre-freezing processing and freezing handling resulted in a statistically significant elevation of chlorophyll content, which was mainly caused by blanching process. This result was consistent with previous report by Dong *et al* (2004). The degradation of chlorophyll is caused by the chlorophyllase enzyme and chlorophyll decomposition is linked to senescence and peroxidation of the cell membranes. During blanching, the chlorophyllase enzyme activity was blocked and the cell membrane structure was disrupted, which might led to the delayed degradation of chlorophyll (Dong, Jiang, Wang, Liu, & Guan, 2004).

In conclusion, current industrial pre-freezing processing and freezing handling significantly reduced the content of glucosinolates, while enhanced the levels of phenols, carotenoids and chlorophyll, and thereby boosted the antioxidant capacity. The reduction of glucosinolate content was mainly caused by blanching and cooling treatment, and the elevation of total phenolic content and antioxidant capacity was due to blanching and freezing. Carotenoids content was substantially increased by freezing while chlorophyll content was dramatically enhanced by blanching. Although pre-freezing processing and freezing handling are good approach to maintaining antioxidant ability of broccoli florets, steam blanching and ice-water cooling are suggested to replace the current blanching and cooling processing in common industrial pre-freezing chain to minimize the reduction of glucosinolate contents.

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FIGURE CAPTIONS:

Fig. 1. Effects of pre-freezing and freezing treatments on vitamin C of broccoli florets.

Each data is the mean ($\pm$ standard error) of three replicates ($n = 3$). Values not sharing the same letter are significantly different at $p < 0.05$.

Fig. 2. Effects of pre-freezing and freezing treatments on total phenols of broccoli florets.

Each data is the mean ($\pm$ standard error) of three replicates ($n = 3$). Values not sharing the same letter are significantly different at $p < 0.05$.

Fig. 3. Effects of pre-freezing and freezing treatments on antioxidant capacity of broccoli florets. Each data is the mean ($\pm$ standard error) of three replicates ($n = 3$). Values not sharing the same letter are significantly different at $p < 0.05$.

Table 1
Information of 9 identified desulfo-glucosinolates in broccoli florets

Glucosinolate group	Abbreviation	Trivial name	Chemical name
Aliphatic GS			
	GIB	Glucoiberin	3-Methylsulphinylpropyl
	GRA	Glucoraphanin	4-Methylsulphinylbutyl
	SIN	Sinigrin	2-Propenyl
	GNA	Gluconapin	3-Butenyl
	PRO	Progoitrin	2-(R)-Hydroxy-3-butenyl
Indolic GS			
	GBS	Glucobrassicin	3-Indolymethyl
	4-OH GBS	4-Hydroxy glucobrassicin	4-Hydroxy-3-indolymethyl
	4-OM GBS	4-Methoxy glucobrassicin	4-Methoxy-3-indolymethyl
	NGBS	Neoglucobrassicin	1-Methoxy-3-indolymethyl

Table 2

Effect of pre-freezing and freezing treatments on glucosinolate composition and content (μmol g⁻¹ DW) in broccoli florets

Treatment	Aliphatic						Indolic					Total glucosinolate
	GIB	GRA	SIN	GNA	PRO	Total AGS	GBS	4-OH GBS	4-OM GBS	NGBS	Total IGS	
Control	1.28 a	9.10 a	0.81 a	0.42 a	3.55 a	15.17a	3.18 a	0.42 a	0.68 a	3.15 a	7.44 a	22.61a
Cut	1.18 a	7.90 a	0.72 ab	0.49a	3.23 a	13.52 a	3.13 a	0.40 a	0.61 ab	3.60 a	7.73 a	21.25 a
Sanitized	1.13 ab	8.23 a	0.62 b	0.45 a	3.12 a	13.55 a	2.61 b	0.33 b	0.53 bc	3.48 a	6.96 a	20.51 a
Blanched	0.92 b	6.42 b	0.32 d	0.25 b	1.92 b	9.83 b	1.65 c	0.30 b	0.44 c	1.89 b	4.28 b	14.11 b
Cooled	0.50 d	3.53 d	0.15 e	0.11 c	0.99 c	5.27 c	0.93 d	0.17 c	0.26 d	1.30 c	2.66 c	7.93 c
Frozen	0.65 c	4.53 c	0.49 c	0.26 b	2.44 b	8.38 b	3.43 a	0.28 b	0.56 bc	2.01 b	6.28 a	14.66 b

Each value represents the mean (n=3). Values in the same column not share the same letter are significantly different at *p*<0.05. GIB: glucoiberin; GRA: glucoraphanin; SIN: sinigrin; GNA: gluconapin; PRO: progoitrin; GBS: glucobrassicin; 4-OH GBS: 4-hydroxy glucobrassicin; 4-OM GBS: 4-methoxy glucobrassicin; NGBS: neoglucobrassicin.

Table 3
Effect of pre-freezing processings and freezing handling on carotenoids and chlorophyll content ($\mu\text{g g}^{-1}$ DW) in broccoli florets

	Lutein	β -carotene	Carotenoids	Chlorophyll <i>a</i>	Chlorophyll <i>b</i>	Total Chlorophyll
Control	264.4b	91.2b	355.6b	1147.8b	403.3b	1551.1 b
Cut	261.7b	93.5b	355.2b	1192.4b	398.7b	1591.1b
Sanitized	258.6b	88.3b	346.9b	1205.3b	412.1b	1617.4b
Blanched	273.5b	94.3b	367.8b	1333.4a	475.9a	1809.3a
Cooled	268.4b	94.8b	363.2b	1298.5a	463.1a	1761.6a
Frozen	322.6a	110.6a	433.2a	1267.4a	445.3a	1712.7a

Each value represents the mean (n=3). Values in the same column not share the same letter are significantly different at $p<0.05$.

Figure 1

Figure 2

Figure 3