

Effect of Postharvest and Industrial Processing on Glucosinolate from Broccoli: A Review

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Abstract: Glucosinolate, is a kind of bioactive sulfur-containing secondary metabolites, which are mainly distributed in cruciferous vegetables such as broccoli, cabbage, cauliflower turnip, and radish. In recent years, Glucosinolate has been widely studied for its anticancer and cardiovascular activities. The types and contents of Glucosinolate in broccoli are related to many factors, such as the cultivars, growth environment and stages, postharvest practices, and processing treatments. To make full use of the potential activities of Glucosinolate, the effects of postharvest practices and food processing treatments on Glucosinolate have been carried out in recent years. The authors have shown that postharvest processing conditions, for example, temperature, relative humidity, storage under a controlled atmosphere or modified atmosphere packaging, and food processing treatments can significantly affect the contents of Glucosinolate in broccoli. Therefore, this review updates the scientific literature on postharvest and food processing treatments on Glucosinolate in broccoli. In addition, the effect of cooking practice on the content of Glucosinolate is also highlighted.

Keywords: Glucosinolate, Broccoli, Postharvest Treatment, Processing, Cooking

Introduction

Broccoli, one of the most important crops, has been consumed throughout the world (Miao *et al.*, 2017; Sun *et al.*, 2021). China is the largest producer of broccoli, which is followed by India, the United States, Spain, Mexico, and Italy. In the last decade, the consumption of broccoli has increased by several times due to the awareness of the active ingredients including polyphenols, flavonoids, vitamins (V_A , V_{B6} , V_{B12} , V_C , etc.), minerals (calcium, potassium, sodium, phosphorous, etc.), carotenoids, Glucosinolate (GLS), etc., (Alexandre *et al.*, 2020; Soares *et al.*, 2017). Epidemiological studies have demonstrated that broccoli can reduce the occurrence risk for many types of cancers due to GLS and their hydrolysates (Nugrahedhi *et al.*, 2016; Sun *et al.*, 2021). GLS, a group of secondary metabolites containing elements sulfur and nitrogen, are primarily active substances in broccoli (Raiola *et al.*, 2017; Vig *et al.*, 2009). In general, GLS is composed of a β -D-thioglucose group, a sulfonated oxime group, and a side chain derived from amino acids (Barba *et al.*, 2016; Seo and Kim, 2017). More than 120 kinds of GLS have been found in the Brassicaceae family, whereas around 17 varieties of GLS in broccoli have been reported (Table 1, Lafarga *et al.*, 2018;

Van Etten and Tookey, 2018). Briefly speaking, according to the derived amino acid precursors, GLS can be classified into three types: Aliphatic type GLS mainly from methionine, isoleucine, leucine, or valine, indole type GLS originated from tryptophan, and aromatic type GLS generated from phenylalanine or tyrosine (Romeo *et al.*, 2018; Soares *et al.*, 2017).

The distribution of GLS in many broccoli cultivars has been studied. GLS contents in broccoli are highly dependent on various factors, for example, the cultivars, developmental stages, preharvest, and postharvest processing treatments (Baenas *et al.*, 2020; Miao *et al.*, 2017; Prieto *et al.*, 2019; Soares *et al.*, 2017). The most common GLS in broccoli, are glucoraphanin (GLA, aliphatic type GLS), sinigrin, progoitrin, gluconapin (alkenyl), the indole glucobrassicin and neoglucobrassicin (Table 1). In the report of Farnham *et al.* (2000), GLA contents in investigated broccoli cultivars varied from 0.04 to 2.94 $\mu\text{mol g}^{-1}$ fresh weight. Similarly, Wang *et al.* (2012) indicated that GLA contents varied between 1.57 and 5.95 $\mu\text{mol g}^{-1}$ dry weight in five Chinese broccoli cultivars.

Usually, GLS in broccoli are not directly bioactive, but their hydrolysates have the flavor and anticancer ability (Abdull Razis and Noor, 2013; Baenas *et al.*, 2020). From the health standpoint, the most extensively investigated

compounds in broccoli are the Isothiocyanates (ITCs), the hydrolysates of GLS, which are regarded as the main bioactive with anti-tumor activity (Miao *et al.*, 2017; Prieto *et al.*, 2019). GLS is distributed in the vacuole, while myrosinase is stored in the cytoplasm. After broccoli cell is damaged by external factors, for example chewing, insects attacking, or cooking processing, the hydrolysis of GLS begins once the contact of GLS and myrosinase occurs (Wu *et al.*, 2021). The hydrolysates like thioglucose, sulfate, and unstable intermediates, are formed through the GLS-myrosinase system. Among the bioactive hydrolysates, the most popular compounds like nitriles, ITCs, thiocyanates, epithionitriles, and vinyl oxazolidinethiones, have been reported (Prieto *et al.*, 2019). The factors, for instance, pH, the availability of ferrous ions, and the presence of myrosinase-interacting proteins can significantly influence the composition of the hydrolysates (Baenas *et al.*, 2020; Nugrahi *et al.*, 2016; Prieto *et al.*, 2019). However, because GLS are thermally sensible compounds, it is necessary to employ some methods to enhance their storage stability. Generally, all postharvest and processing treatments can cause a decrease in broccoli quality and a change in GLS contents. Many strategies have been employed to reduce GLS loss by radiation processing, heat, microwave, High-Pressure Processing (HPP), Modified Atmosphere Packaging (MAP), pre-freezing processing, freeze-drying, etc., (Aguilar-Camacho *et al.*, 2019; Cai *et al.*, 2016; Deng *et al.*, 2017; Ferreira *et al.*, 2018; Lu *et al.*, 2020b, c; Paulsen *et al.*, 2018; Torres-Contreras *et al.*, 2017, 2018; Villarreal-García *et al.*, 2016; Wang *et al.*, 2016, 2018). Cooking is not often considered a critical postharvest process. However, at present, numerous studies have shown that cooking can significantly affect GLS content in broccoli (Baenas *et al.*, 2019, 2020; Miao *et al.*, 2017; Nugrahi *et al.*, 2016; Soares *et al.*, 2017). Therefore, this review updates the latest development on the effects of postharvest treatments and food processing on GLS content in broccoli.

Effect of Postharvest on GLS in Broccoli

Broccoli is one kind of highly perishable vegetable. Storage conditions, for instance, temperature, RH, atmosphere composition, Controlled Atmosphere (CA), MAP, etc., can significantly affect its quality (Baenas *et al.*, 2020; Jones *et al.*, 2006; Miao *et al.*, 2017). Additionally, the treatment of broccoli with sucrose, 1-Methylcyclopropene (1-MCP), or melatonin, is also an effective method to increase the GLS retention (Miao *et al.*, 2017). The effects of postharvest practices on GLS in broccoli were mainly discussed as follows. Some results on the effects of postharvest on GLS were presented in Table 2.

Storage Treatment

The level of GLS in broccoli is strongly affected by storage conditions (Baenas *et al.*, 2020; Banerjee *et al.*,

2014). In general, the stability of GLS in broccoli depends on many factors such as their chemical formula, storage temperature and time, packaging atmosphere, etc., and all these parameters need to be taken into consideration before the broccoli is stored (Jones *et al.*, 2006; Miao *et al.*, 2017).

Storage Temperature and Time

During the storage, the quality of broccoli usually decreases, which is accompanied by a decrease in GLS content (Miao *et al.*, 2017). Among the factors affecting GLS content during broccoli transport and storage, storage time and temperature may be the most important ones. Lowering the temperature (<4°C) can maintain higher levels of GLS in broccoli. In one early study, GLA content in broccoli decreased by 82% after it was stored at 20°C for 5 days, while it declined by around 31% when broccoli was at the storage of 4°C (Rodrigues and Rosa, 1999). Similarly, Rangkadilok *et al.* (2002) found that GLA level in broccoli cultivar 'Marathon' decreased by 55% after it was kept at 20°C for 7 days, while there was not any loss after broccoli was stored at 4°C for the same time. The reasonable explanation was that high temperature could disrupt the cellular integrity of broccoli, and so the improved contact between myrosinase and GLS led to the fast hydrolysis of GLS (Prieto *et al.*, 2019; Zinoviadou and Galanakis, 2017). Recently, Oliviero *et al.* (2018) reported that cold storage of broccoli at 4~8°C for 7 days caused the GLS loss (27%).

Pre-cooling treatment is a popular method to increase the quality of broccoli. Wang *et al.* (2020) employed three methods (0°C cold storage pre-cooling (control), ice pre-cooling, cold water pre-cooling) to improve the quality of broccoli. The results showed that ice pre-cooling and cold water pre-cooling treatments could reduce the GLS loss. Slurry ice precooling was also used to treat broccoli (Liu *et al.*, 2019). The results also demonstrated that it could reduce the GLS loss. Recently, Xie *et al.* (2021) investigated the effect of the combination of pre-cooling treatment and low-temperature storage (0±1°C) on the storage quality of broccoli. The authors indicated that pre-cooling treatment and low-temperature storage of broccoli could delay yellowing more effectively and maintain higher GLS content in broccoli.

Barba *et al.* (2016) studied the contents of the total indolyl and aliphatic types GLS in pre-stored broccoli at 0~4°C for 4~7 days and after storage at 10 and 18°C. The authors found that the total GLS and total aliphatic and indolyl types GLS increased after broccoli was stored at 10°C. Storage at 18°C increased the content of 4-hydroxyglucobrassicin. Similarly, Yuan *et al.* (2010) reported that the level of 4-methoxy glucobrassicin was improved when broccoli was preserved at 20°C. The results indicated that the contents of some indole types of GLS could be increased by postharvest treatment, and thus counteracted the breakdown of GLS induced by myrosinase. Actually, in these studies, the content of total GLS did not change significantly. The authors concluded that the increased level

of indole type GLS could counteract the decrease of aliphatic type GLS (such as GLA) content.

Relative Humidity (RH)

It is necessary to remember that RH is the crucial factor to maintain a high quality of broccoli. A high RH of 98~100% is highly recommended to keep the harvested quality of broccoli (Miao *et al.*, 2017). Jones *et al.* (2006) reported that the most important storage factor to keep the high quality of broccoli was high RH, next to low temperature. GLA in broccoli decreased by more than 80 % after it was stored under low RH, 20°C for 5 days. Similarly, GLA level decreased by 50% when broccoli was preserved in open boxes (low RH) at 20°C within the first 3 days, while the GLA loss was not obvious when broccoli was preserved in plastic bags under high RH (>90%) and the same temperature (Rangkadilok *et al.*, 2002). The decrease of GLA in broccoli was generally accompanied by a significant deterioration of the quality, which implied that the hydrolysis generated by myrosinase, might occur. However, the difference in the change of GLA level was not found when broccoli was stored under low temperature (4°C) for 7 days whether in either open boxes with about 60% RH or plastic bags with around 100% RH (Rangkadilok *et al.*, 2002). Shakeel *et al.* (2019) investigated the quality of broccoli under ambient conditions and proposed that the harvested broccoli had better be stored under lower temperatures and higher RH (>60%) to keep the good visual quality. Therefore, it seems that it is not too necessary to use 100% RH if broccoli is stored at a cooling temperature (below 4°C), whereas high RH combined with packaging is necessary to keep the high quality of broccoli when broccoli is preserved at 20°C (Shakeel *et al.*, 2019).

CA Storage

CA storage is an effective means to keep the quality of broccoli and has been extensively applied to improve the shelf life of broccoli (Caleb *et al.*, 2016; Fernandez-Leon *et al.*, 2013a, b, c; Singh *et al.*, 2018; Wang *et al.*, 2017). CA conditions should be carefully investigated. Lower concentration of gases in CA (0.5~1.0% O₂; 0.5% CO₂; 0.5~1.0% O₂; 1% CO₂; 0.50~1.0% O₂; 2% CO₂; 1.0% O₂; 1.0% CO₂) or controlled dynamic atmosphere (0.5%; 1.0%; 2.0% CO₂)

could cause greater loss of indole-3-carbinol GLS in broccoli compared to the control, after a 3-month storage. The best atmosphere conditions to preserve the quality of broccoli were 1~2% O₂, and 5~10% CO₂ when it was stored at a temperature from 0 to 5°C (Jones *et al.*, 2006). In the report of Fernández-León *et al.* (2013a), when broccoli was stored under cooling or room temperature (20°C), the decrease of GLS in broccoli was significantly reduced after it was treated by CA storage (10% O₂, 5% CO₂). Similarly, the content of GLA in broccoli stored at 4°C and CA (1.5% O₂ + 6% CO₂) was higher than that under air conditions (Rangkadilok *et al.*, 2002). The authors indicated that higher CO₂ levels might induce GLA biosynthesis and/or the decrease of its degradation pathway. However, after being stored for 20 days at 1% O₂ or 1% O₂ +10% CO₂, GLA levels were significantly lower than those under 5°C, air condition, and the total GLS level in broccoli cultivar ‘Marathon’ was reduced by 15% under the storage of 10°C for 7 days after it was treated by 20% CO₂ (Jones *et al.*, 2006). Therefore, CA conditions such as normal content of O₂ and higher content of CO₂ are highly suggested for the storage of broccoli.

Table 1: GLS distributed in broccoli*

Commonly known name	Chemical name
Aliphatic type GLS	
Glucoraphanin	4-methylsulfinylbutyl-GLS
Progoitrin	(2R)-2-hydroxybut-3-enyl-GLS
Glucorucin	4-methylthiobutyl-GLS
Gluconapin	3-butenyl-GLS
Epiprogoitrin	(2S)-2-hydroxy-3-butenyl-GLS
Glucoiberin	3-methylsulfinylpropyl-GLS
Glucoiberverin	3-methylthiopropyl-GLS
Glucobrassicinapin	4-pentenyl-GLS
Sinigrin	2-propenyl-GLS
Glucoalysin	5-methylsulfinylpentyl-GLS
Gluconapoleiferin	2-Hydroxy-4-pentenyl-GLS
Indole type GLS	
Glucobrassicin	Indol-3-ylmethyl-GLS
4-Hydroxy-glucobrassicin	4-Hydroxy-indol-3-ylmethyl-GLS
Neo-glucobrassicin	N-methoxyindol-ylmethyl-GLS
4-Methoxy-glucobrassicin	4-Methoxy-indol-3-ylmethyl-GLS
Aromatic type GLS	
Gluconastrutiin	2-phenylethyl-GLS

*Miao *et al.* (2017); Nugraedi *et al.* (2016); Soares *et al.* (2017)

Table 2: Effects of postharvest treatments on GLS in broccoli

Methods	Results	References
Ethylene treatment	Both aliphatic and indole type GLS (up to two times) levels were improved by ethylene treatment (1000 ppm, 24 h, 20°C).	Villarreal-García <i>et al.</i> (2016)
1-MCP treatment	The total GLS content was significantly enhanced by the treatment of 1-MCP (25 µL ⁻¹).	Fernández-León <i>et al.</i> (2013a)
Melatonin treatment	The degradation of GLS was significantly inhibited after broccoli was treated with melatonin treatment.	Miao <i>et al.</i> (2020)
LED treatment	The decrease rate of total GLS was prevented after broccoli was treated by LED.	Jin <i>et al.</i> (2015)
Stored at 10% O ₂ , 5% CO ₂	CA storage could effectively low the decrease of GLS in broccoli florets when broccoli was stored at cooling and 20°C.	Fernández-León <i>et al.</i> (2013a)
Stored in MAP using Micro-perforated polypropylene plastic	The loss of the GLS content in the MAP sample was about 23%, while it was about 57% in the control.	Fernandez-Leon <i>et al.</i> (2013b)

Ultraviolet (UV)

Some studies on the effect of UV-B irradiation on the quality of broccoli and the contents of phytochemicals in harvested broccoli have been carried out (Aiama-or *et al.*, 2012; Duarte-Sierra *et al.*, 2019, 2020). UV-B conditions could significantly affect the accumulation of GLS in broccoli (Aiama-or *et al.*, 2019; Rybarczyk-Plonska *et al.*, 2014, 2016). Darre *et al.* (2017) evaluated the effect of UV-B radiation conditions on GLS in broccoli after it was stored at 4°C for 17 days. The results showed that the levels of aliphatic type GLS were improved when broccoli was treated by UV-B irradiation for 18 h. In addition, the authors also found that aliphatic type GLS was the most easily accumulated when broccoli was treated by UV-B irradiation. Recently, Duarte-Sierra *et al.* (2020) also examined the effects of UV-B radiation on the quality as well as GLS levels in broccoli during the storage periods. The results indicated the contents of indole type GLS in broccoli treated with both doses of UV-B irradiation, were significantly higher than those in untreated broccoli. The total GLS contents were increased by 18 and 22%, respectively after broccoli was accordingly treated by hormetic and higher doses of UV-B irradiation. In addition, the contents of aliphatic type GLS in broccoli treated with both doses of UV-B irradiation, increased by 15% compared with those in the untreated broccoli. But, the aliphatic type GLS contents increased less than that of indole type GLS.

Packaging Treatment

One of the purposes of packaging coating for fresh broccoli is to reduce the exposure GLS to myrosinase, and GLS breakdown (Prieto *et al.*, 2019; Singh *et al.*, 2018). Generally, the investigations are focused on the studies on how packaging materials (holes or without holes, different polymers, edible coating, etc.) can preserve GLS in broccoli.

The storage of cooling and CA are effective methods to remain the quality of broccoli (Singh *et al.*, 2018). But, these methods are not popular in developing countries, where broccoli is challenged by high temperatures during the post-harvest processing (Jones *et al.*, 2006). MAP, as one of the storage methods, possesses simple and economical properties and has great potential in maintaining the quality of broccoli (Fernández-León *et al.*, 2013b, c; Singh *et al.*, 2018; Wang *et al.*, 2017). Meanwhile, MAP also is an effective method to preserve GLS in broccoli whether at low or high temperatures. Certainly, many factors, for example, the types of packaging, broccoli cultivars, etc., can significantly affect the content of GLS. Fernandez-Leon *et al.* (2013c) investigated the quality of broccoli when it was stored at 5°C in MAP using microperforated polypropylene plastic. The GLS content in broccoli stored in MAP decreased by about 23%, whereas the GLS level in the control was reduced by around 57% at the same time. Barba *et al.*

(2016) observed the GLA level declined by 55% when broccoli was under the storage of open-air boxes for 3 days, and the level of GLA decreased by 56% when broccoli was stored in plastic bags for 7 days. The GLS content decreased more obviously when broccoli cultivar 'Parthenon' was under the storage of air conditions than that was stored under modified atmospheres using micro-perforated polypropylene plastic at 5°C for 12 days (Fernández-León *et al.*, 2013b).

The GLS content is also significantly affected by storage temperature when broccoli is under the storage of MAP. No significant difference in GLA content was observed when broccoli was under the storage of air or MAP at 4°C for 10 days (Rangkadilok *et al.*, 2002), whereas, the content of GLA decreased by 50% when broccoli was under the storage of air condition and room temperature for 7 days. Under the same storage temperature, GLA level was not significantly reduced when broccoli was stored under MAP for 10 days (Rangkadilok *et al.*, 2002). Jia *et al.* (2009) also investigated the effect of MAP processing on the GLS content in broccoli cultivar 'Youxiu'. In their study, polyethylene bags (40 µm thick) with no holes (M₀), two Micromoles (M₁), and four macro holes (M₂) were used to package broccoli samples, respectively, and then they were under the storage of 4 or 20°C. As for the control, the total GLS level was significantly reduced after it was stored at 4°C over 23 days. In addition, the total aliphatic and indole type GLS contents decreased by 56, and 42%, respectively, under the same storage conditions. However, for broccoli coated with polyethylene bags, the contents of the total aliphatic and indole type GLS were reduced by 26 and 15%, respectively, when it was preserved at 4°C for 23 days. The authors suggested that polyethylene bags (40 µm thick) without hole (M₀) was one of the effective packaging materials to keep the quality of broccoli whether at low or high temperatures. Recently, Zinoviadou and Galanakis (2017) presented the results of broccoli stored at 4 or 20°C in MAP and found the losses of the total aliphatic and indole types GLS decreased. The positive effect generated by MAP during postharvest storage was probably due to the change of amino acid contents since they were the precursors of some types of GLS (Bonte *et al.*, 2017).

The use of Exogenous Metabolic Regulators

Methylcyclopropene (1-MCP)

1-MCP has been extensively applied to the preservation of fruit and vegetables (Miao *et al.*, 2017). Yuan *et al.* (2010) showed that the employment of 1-MCP at the content of 2.5 µL⁻¹ could reduce the degradation rate of GLS when broccoli was stored at 20°C. Similarly, in the work of Fernández-León *et al.* (2013a), after broccoli was treated with 1-MCP at the content of 0.6 µL⁻¹, the decrease rate of GLS was reduced. Additionally, the use of 1-MCP at the content of 25 µL⁻¹ could improve the total GLS level when broccoli was preserved at 15°C for 5 days

(Xu *et al.*, 2013). In short, 1-MCP shows a great potential to be used as a chemical preservative to reduce the degradation of GLS in broccoli.

Melatonin

Melatonin is regarded as one kind of bio-preservatives and has been widely used for the preservation of fruits and vegetables (Arnao and Hernández-Ruiz, 2019; Luo *et al.*, 2018; Miao *et al.*, 2020; Zheng *et al.*, 2019). Miao *et al.* (2020) studied the effect of melatonin on GLS degradation when broccoli was stored at room temperature. In their work, a higher GLS retention rate and GLA accumulation were observed after broccoli was treated with melatonin at the content of 1.0 μL^{-1} . However, the level of GLS in the control was significantly reduced during the storage. As for the total contents of aliphatic and indolic types GLS, they decreased by 50 and 52%, respectively, after the control was stored for 3 days. Whereas, the total levels of GLS in broccoli handled by melatonin, were reduced by 17 and 35%, respectively, under the same storage time. Wei *et al.* (2020) also investigated the effect of melatonin treatment on GLS levels in fresh-cut broccoli when broccoli was stored at 4°C. The results showed that the total GLS level in broccoli treated with 100 μm melatonin was 16.08 mmol kg^{-1} , which was almost two times higher than that in untreated broccoli after all broccoli samples were stored for 20 days. Furthermore, the total level of GLS in melatonin-treated broccoli was still higher than that in untreated broccoli.

Sucrose Treatment

Sucrose also has been employed to delay the senescence, and improve the storage quality of broccoli (Miao *et al.*, 2017). Xu *et al.* (2016) investigated the effect of sucrose treatment on the levels of GLS in broccoli. The results showed the degradation rate of GLS in sucrose-treated broccoli, was significantly reduced compared to that in the control. Therefore, the authors indicated that sucrose exhibited great potential to be applied in maintaining the quality of broccoli. Generally, GLS contents in broccoli are highly related to two opposing mechanisms (Nugrahedhi *et al.*, 2015; 2016; Yuan *et al.*, 2010). One is that GLS can be hydrolyzed by myrosinase, and the other is that the accumulation of GLS can be controlled by an unknown mechanism. According to this hypothesis, the authors inferred that the higher GLS content in sucrose-treated broccoli might be attributed to the regulation of myrosinase activity (Xu *et al.*, 2016).

Besides the discussion above, Methyl Jasmonate (MeJA) and 6-Benzylaminopurine (6-BA) also have been used to maintain GLS content in broccoli (Chiu *et al.*, 2019, 2020; Miao *et al.*, 2017; Xu *et al.*, 2020). 6-BA could significantly improve the retention rate of GLS (Xu *et al.*, 2012). MeJA at the content of 250 μm also could increase the preservation of GLS in broccoli.

Even if broccoli was treated by boiling, steaming, or microwaving, the total GLS level in broccoli treated by MeJA was markedly higher than that in uncooked broccoli (Chiu *et al.*, 2019, 2020). Thus, the use of MeJA can improve the remained amount of GLS, and shows a great potential to be applied in the postharvest treatment of broccoli.

Light Treatment

Many authors have carried out the effects of radiation types on the biosynthesis of GLS in broccoli, and the results are different. Casajus *et al.* (2021) investigated the effect of continuous white light irradiation on the biosynthesis of GLS in broccoli during the storage. Visible radiation was found to reduce the decline of GLS content. The total GLS content in the control was reduced from 10.1 $\mu\text{mol/g}$ dry tissue to 1.4 $\mu\text{mol/g}$ dry tissue when it was stored for 5 days, whereas the total GLS content in treated broccoli was only reduced to 3.0 $\mu\text{mol/g}$ dry tissue. Continuous white light irradiation treatment, not only could keep GLS levels, but also maintain the visual quality of broccoli at the same time. While, the content of aliphatic type GLS was improved when the visible light of 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$ was used to treat broccoli preserved at 18°C, and the content of GLS also increased when the same radiation conditions were employed to treat broccoli stored at 10°C (Rybarczyk-Plonska *et al.*, 2016). Recently, Casajus *et al.* (2020) found that the senescence of broccoli during the storage was significantly affected by harvesting time. Harvesting time could affect the composition and level of GLS. During the day, with the extension of harvesting time, indolic type GLS content only decreased slightly. The level of aliphatics type GLS was reduced during the whole storage period. The possible reason was that darkness storage might give rise to the degradation of GLS since many studies indicated that light radiation could improve GLS accumulation (Rybarczyk-Plonska *et al.*, 2016). In addition, the reason for the different GLS levels in broccoli harvested at different time points must be not related to the light radiation, since all broccoli samples were stored in the darkness.

Some investigations also have shown that Light-Emitting Diode (LED) lights, including LED green light, red LED irradiation, and yellow LED light, are better than fluorescent lights for maintaining the quality stability of broccoli (Jiang *et al.*, 2019; Loi *et al.*, 2019; Wang *et al.*, 2021). Jin *et al.* (2015) investigated the effects of the treatments of fluorescent and LED green lights on GLS levels in broccoli. The results showed that the retention rate of the total GLS in broccoli treated with LED green light was significantly higher than that of the samples treated with fluorescent light. So, the authors suggested that LED green light was an effective way to reduce GLS loss, and improve the quality of broccoli.

In addition, emulsion technology and ethanol vapor treatments also have been applied to maintain GLS in broccoli. Wang *et al.* (2014) investigated the effect of ethanol vapor processing on bioactive substances, for example, polyphenols, total GLS, sulforaphane, etc., and the antioxidant activity of fresh-cut broccoli. The samples were pretreated with 2, 5, 10, or 20% ethanol vapor at 20°C for 6 h, then cut into small florets and stored at 10°C for 10 days. The results showed that the pretreatment with 10% ethanol significantly delayed the decrease of the total GLS content. During the investigated storage, the remained GLS in broccoli treated with 10% ethanol was 1.82 times higher than that of the control. Lu *et al.* (2020a) studied the effect of a double emulsion system (W/O/W) on the quality of broccoli and indicated that double emulsion technology could improve the quality of broccoli, and maintain higher GLS content.

Effect of Cooking Process on GLS

Before it is eaten, broccoli is usually treated by many kinds of cooking practices, for example, steaming, boiling, stir-frying, microwave, stir-frying followed by boiling, etc. The employed cooking process and cooking time can significantly affect the GLS level in broccoli and thus result in the cooked broccoli with different nutritional values (Nugrahedhi *et al.*, 2016). Until now, there have been reported on the mechanisms of the change of GLS content, including GLS leakage, GLS hydrolysis, heat-reduced myrosinase inactivation, and the degradation of GLS hydrolysates, etc., (Baenas *et al.*, 2019, 2020; Nugrahedhi *et al.*, 2016; Sun *et al.*, 2021). As for the mechanisms involved during the cooking practice for broccoli, it highly depends on the investigated cooking conditions. Generally, among the cooking practices, microwaving and boiling can lead to the greatest GLS drop (Baenas *et al.*, 2020; Wu *et al.*, 2021). However, the decrease in GLS level in steamed broccoli showed the lowest drop (Tabart *et al.*, 2018; Wu *et al.*, 2021).

Cutting Treatment

Cutting is a common handling method for broccoli pretreatment, which destroys the tissue of broccoli and thus promotes the formation of GLS hydrolysates (Jia *et al.*, 2009). Torres-Contreras *et al.* (2017) examined the effect of cutting treatment on GLS in broccoli, which was cut as whole florets, two pieces of florets, four pieces of florets, and shredded pieces of florets. The authors pointed out that the contents of glucoerucin and gluconasturtiin in cut four-piece florets were reduced by 62 and 50%, respectively, and thought that the hydrolysis reaction could occur. In another study, Jones *et al.* (2006) examined the levels of GLS in shredded pieces of broccoli after broccoli was stored for 48 h at room temperature. The authors found that the contents for most kinds of GLS were reduced, whereas the content of 4-methoxy-3-

indolylmethyl GLS increased 15 times (Jones *et al.*, 2006). Through analyzing the content of GLS in the finely shredded broccoli, it was also found that GLS content could be reduced by 75% after 6 h (Prieto *et al.*, 2019; Song and Thornalley, 2007).

Blanching/Boiling

Since GLS are sensitive to heat, heat treatments of broccoli will affect the GLS content, and generally leads to a decrease in GLS content (Lafarga *et al.*, 2018). The GLS loss highly depends on the chemical structure of GLS, for instance, the indole type GLS is more sensitive to heat than the aliphatic type GLS (Zinoviadou and Galanakis, 2017). During boiling, broccoli is dipped into water at 100°C, for at least 10 minutes, while blanching involves blanching broccoli in boiling water for up to 3 min, then removing it, and immersing it in cold water (Hanschen *et al.*, 2018; Lafarga *et al.*, 2018; Preciado-Iniga *et al.*, 2018). Among the investigated processes for cooking broccoli, boiling may produce the greatest impact on GLS content. The decrease of GLS content in broccoli treated by cooking practice is mainly due to GLS leakage and the degradation of GLS hydrolysates. The loss amount of GLS is highly related to boiling time (Hanschen *et al.*, 2018; Nugrahedhi *et al.*, 2015). Song and Thornalley (2007) compared four cooking practices, for instance steaming, boiling, microwaving, and stir-frying, and found that among these studied processes, only boiling could reduce GLS content significantly. Similarly, Cieslik *et al.* (2007) compared the effects of blanching and boiling treatments on GLS contents in the chosen cruciferous vegetables including broccoli, curly kale, Brussels sprouts, etc. The results also showed that the total content of GLS decreased significantly after the selected vegetables were treated by blanching and boiling. Recently, Hanschen *et al.* (2018) also found that boiling and blanching could affect the formation of the hydrolysates in cruciferous vegetables, thus resulting in the difference in GLS levels. Blanching was favorable for the formation of ITCs due to the heating denaturation of the epithio-specifier protein, while boiling could give rise to the leakage of GLS and the hydrolysates into the cooking water.

Mrkic *et al.* (2010) studied the effect of the combination of blanching and hot air drying (50~100°C) on GLS content in broccoli. The results indicated that GLS content decreased by appropriately 64% after broccoli was treated by water blanching, which was likely attributed to the leakage of GLS into cooking water (Mrkic *et al.*, 2010). Some studies indicated that steam blanching could achieve the same purposes as water blanching (Baenas *et al.*, 2020; Ndiaye *et al.*, 2009). Compared with water blanching, steam blanching has some advantages, for example, the less leakage of GLS, the higher preservation of myrosinase activity. Compared with boiling and branching processes, the fermentation process could reduce GLS. Sosinska and Obiedinski

(2011) investigated the effects of heating treatment, pickling, and fermentation on glucobrassicin levels and the hydrolysates in broccoli and cauliflower. Heating treatment, for example, boiling and steam cooking, could remain the highest glucobrassicin level in selected vegetables, however, pickling and fermentation could cause the greatest loss of glucobrassicin. Xu *et al.* (2021a, b) investigated the effect of fermentation using animal- and plant-sourced *Pediococcus pentosaceus* on the formations of bioactive compounds in broccoli juice. The results indicated that the total GLS content decreased significantly after broccoli juice was fermented by the investigated *Pediococcus pentosaceus*.

Steaming

Steaming, by reducing the direct contact of broccoli with the cooking water, maybe the most effective method for remaining GLS level in post-harvested broccoli. Many studies have demonstrated that steaming can cause a slight decrease, or even improve the total GLS content (Lafarga *et al.*, 2018; Sun *et al.*, 2021; Wu *et al.*, 2021; Xu *et al.*, 2016; Zinoviadou and Galanakis, 2017). Miglio *et al.* (2008) studied the effects of three common cooking practices, for instance, boiling, steaming, and frying, on GLS contents. The results indicated that the total level of GLS was improved by 30.8% after broccoli was treated by steaming, however, when broccoli was treated by boiling and frying, the total levels of GLS decreased by 59.0 and 84.0%, respectively (Miglio *et al.*, 2008). Interestingly, Jones *et al.* (2010) evaluated the effects of three cooking practices named boiling, microwaving, and steaming, on the content of GLS in broccoli. Regardless of treatment time, the higher retention of GLS was observed after broccoli was treated by steaming, whereas boiling and microwave processes could cause more losses of GLS in investigated broccoli. Similarly, Lu *et al.* (2020b) studied the effect of cooking time on GLA content in broccoli treated by four processes including steaming, boiling, stir-frying, and microwaving. The results showed that GLA content decreased with the extension of cooking time in each cooking method. Steamed broccoli retained a higher content of GLA. Among the investigated methods, the authors thought that the best methods to maintain the highest level of GLS (and/or) their derivatives were steaming (3~50 min) and microwaving (45~590 W). However, Bongoni *et al.* (2014) reported that the total GLS content increased by 17% after broccoli was treated by steaming. The reason was that the extractability of GLS was improved after broccoli was treated by heating.

Microwave

Many authors consider microwaving an effective method to preserve the GLS content (Table 3; Guo *et al.*,

2017; Soares *et al.*, 2017; Tabart *et al.*, 2018; Xu *et al.*, 2016). Some studies indicated that GLS loss was observed when microwaving was used to treat broccoli even under the optimized microwaving process (Vallejo *et al.*, 2002). So, microwave conditions such as microwave power and time can significantly affect the preservation of GLS (Sun *et al.*, 2021). Conflicting results regarding the effect of microwave processing on GLS content have been reported. Some studies indicated microwave treatment could cause a significant loss of GLS (Jones *et al.*, 2010; Vallejo *et al.*, 2002), whereas other investigations manifested it was a good way to preserve or even improve GLS content (Barakat and Rohn, 2014; Lu *et al.*, 2020c; Soares *et al.*, 2017; Wu *et al.*, 2019). The inconsistent results are ascribed to the employed conditions, such as microwave power, time, etc., (Baenas *et al.*, 2020; Tabart *et al.*, 2018). Vallejo *et al.* (2002) demonstrated that the total GLS content decreased by 74% after broccoli was cooked using microwave treatment at 1000 W for 5 min, which was mainly attributed to the leakage of GLS into the cooking water. GLA content in broccoli treated by microwave treatment was reduced by 62% (Vallejo *et al.*, 2002). Also, in the study of Jones *et al.* (2010), GLA content decreased by 15~17% after broccoli was treated by microwaving at 1,100 W for 5 min. However, Song and Thornalley (2007) found that the decrease in GLS level was not significant when broccoli was cooked by microwaving for up to 3 min. With the increase of microwave time to 19 min at 950 W, Tabart *et al.* (2018) manifested that the total GLS level in broccoli was still not significantly affected. The difference was mainly ascribed to the user conditions such as microwave time, microwave power, etc., Pellegrini *et al.* (2010) investigated the effects of cooking processes such as boiling, microwaving, and steaming on GLS content in broccoli. The results showed that microwaving was the best cooking practice for preserving GLS content among the investigated methods. The change in the total GLS content was not observed during the microwaving process, but only a slight variation of single-type GLS content was found. The high preservation of GLS was mainly ascribed to the loss of water during the microwaving process (Armesto *et al.*, 2019; Campos *et al.*, 2019; Guo *et al.*, 2017; Tabart *et al.*, 2018; Wu *et al.*, 2019; Zhao *et al.*, 2019). Wu *et al.* (2017) confirmed that microwave was an effective method to preserve GLS in broccoli. Lu *et al.* (2020c) investigated the effects of microwave and the low-temperature cooking process, on GLA levels in broccoli. The results showed that GLA content in broccoli cooked by both investigated methods was higher than that in the control. Compared to conventional heating, GLA content could be increased by around 80% after broccoli was cooked by microwaving at 60°C. Therefore, the authors suggested that GLA content could be improved when broccoli was

treated by microwaving treatment with a temperature below 60°C. Paulsen *et al.* (2021) carried out the effects of microwave bag cooking and conventional microwaving on GLS content in broccoli. A higher total GLS level (32.3±2.6 µmol/g) was observed when broccoli was cooked using a microwave bag, compared to that in broccoli (26.4±1.3 µmol/g) cooked by conventional microwaving. No significant change in total GLS level was observed when broccoli was cooked in a microwave bag for the first 3~5 min. So, the authors suggested that a microwave bag was a good manner to preserve GLS content in cooked broccoli.

Frying

Stir-frying is one of the most widely employed cooking practices in some Asian countries, which involves frying foods using a little number of hot oils (Baenas *et al.*, 2020; Nugrahedhi *et al.*, 2017; Tian *et al.*, 2018). Regarding the effect of stir-frying on the retention of GLS, some contradictory results have been shown. In some cases, stir-frying can cause little loss of GLS, while in other studies, it can result in a significant decrease of GLS. The differences in these reports are mainly due to the used stir-frying conditions (Bongoni *et al.*, 2014; Wu *et al.*, 2021). Nugrahedhi *et al.* (2017) reported that stir-frying was an excellent cooking practice to preserve GLS, due to the deactivation of myrosinase at the high temperatures during the process (160~250°C). Nugrahedhi *et al.* (2015) also reported that frying (as well as steaming and microwave cooking) was beneficial for the retention of GLS. Yuan *et al.* (2009) studied the effects of cooking practices, such as microwaving, steaming, stir-frying, boiling, and stir-frying followed by boiling, on GLS contents in broccoli. The authors showed that the contents of total aliphatic type GLS were markedly reduced by 55, 54, 60, and 41%, respectively, after broccoli was accordingly cooked by stir-frying, stir-frying/boiling, microwaving, and boiling. In contrast, the content of total aliphatic type GLS did not almost change after broccoli was cooked by steaming. All investigated cooking methods could significantly decrease the total contents of the indole type GLS. The loss of total indole type GLS could reach up to 67 and 64%, respectively, when broccoli was accordingly cooked by stir-frying and stir-frying/boiling. Besides, the effect of stir-frying using different edible oils on the GLS level was also studied (Moreno *et al.*, 2007). The results showed that stir-frying with different edible oils could significantly affect GLS content. Stir-frying with sunflower oil and refined olive oil could significantly cause the loss of total GLS of 49 and 37%, respectively, when broccoli was accordingly cooked by stir-frying with refined oil and sunflower oil. However, no significant change in total GLS was observed when broccoli was cooked using the rest investigated oils (Moreno *et al.*, 2007). The thermal degradation of GLS resulted in a significant decrease in GLS content during stir-frying. So, the degradation of

GLS was highly dependent on the used cooking edible oils. However, the relationship between stir-frying temperature and the retention or decrease of GLS content was not found. In the study of Song and Thornalley (2007), no significant change in GLS content was observed when broccoli was cooked at stir-frying temperature up to 200°C for 3~5 min. However, in their study, a high decrease in GLS content was observed when broccoli was cooked by stir-frying and stir-frying/boiling. The differences in the results were due to the used cooking temperature. The myrosinase was denatured rapidly at high cooking oil temperature (200°C), and thus the hydrolysis of GLS did not occur. However, when the cooking oil temperature was around 130~140°C, the hydrolysis of GLS induced by myrosinase was produced. According to these results, the authors indicated that cooking oil temperature had better reach up to 200°C to ensure the preservation of the total GLS.

Effect of Food Processing on GLS

Freezing

Freezing is one of the most widely employed methods to keep the quality of broccoli (Baenas *et al.*, 2020; Storey and Anderson, 2018). Broccoli usually goes through several treatments, for example, cutting, blanching, washing, and cooling before it is frozen. Indeed, for blanched broccoli, the content of GLS did not change after 3 months of storage (at 20°C), but for the non-blanched broccoli, a decrease of 33% of the total GLS content was observed at 85°C for seven-day storage (Oliviero *et al.*, 2018). Rungapamestry *et al.* (2008) investigated the effects of blanching and freezing processing on GLS content in broccoli. GLS content in the control could be retained for up to 90 days at 20°C when it was treated by the blanching/freezing process. In addition, the highest retained GLS content was observed after the blanching-frozen broccoli was cooked by stir-frying. Furthermore, Alanis-Garza *et al.* (2015) evaluated the effect of the freezing process on the retention rate of GLS in seven broccoli cultivars. The results showed that the extractability of total GLS was improved when all studied broccoli cultivars, except cultivar Florapack®, were treated by the freezing process. Similarly, Cai *et al.* (2016) studied the effects of pre-freezing processing and freezing processing on GLS content in broccoli. The results indicated that pre-freezing processing could significantly reduce the biosynthesis of GLS. The freezing process could cause the decrease of aliphatic type GLS level (44.76%) and total GLS level (35.16%) but did not significantly affect indolic type GLS level (Table 3). In addition, the GLS level also could be improved by the freezing process. As mentioned above, two main mechanisms for the formation of GLS were proposed. One was the accumulation of GLS induced by an unknown pathway, the other was the breakdown of GLS by myrosinase.

Table 3: Effects of processing methods on GLS levels in broccoli

Processing methods	Conditions	The retention of total GLS	References
Microwave	1000 W, 5 min	↓ 74%.	Vallejo <i>et al.</i> (2002)
	1000 W, 1~10 min	GLS were reduced by 0.95, 13.5 and 64.6% for 1 min, 5 min, 10 min, respectively.	Hwang and Kim (2013)
	590 W, 5 min	↑ 0.4%.	Barakat and Rohn (2014)
	950 W, 40~60°C	Sulforaphane was increased by 99.5% (40°C), 46.6 (50°C), 27.4% (60°C), respectively.	Lu <i>et al.</i> (2020b)
	300 W, 30 min	102.2%.	Pellegrini <i>et al.</i> (2010)
	1100 W, 2 or 5 min	83.9 ~104.1%.	Jones <i>et al.</i> (2010)
	1000 W, 5 min	25.5%.	Vallejo <i>et al.</i> (2002)
	1000 W, 5 min	41.8%.	Yuan <i>et al.</i> (2009)
	900 W, 0.5~3 min	~100%	Song and Thornalley (2007)
	900 W, 2.5 min	60%	Martinez-Hernandez <i>et al.</i> (2013)
Pre-freezing and freezing	3°C, 8 min; -26°C, 8 min	GLS were reduced by 64.9% at 3°C for 8 min, and reduced by 35.2% at -26°C for 8 min.	Cai <i>et al.</i> (2016)
HPP	100~500 MPa, 15 min, 20°C	No degradation of GLS was observed.	Van Eylen <i>et al.</i> (2009)
300 MPa, 35 min, 20°C or 300 MPa, 15 min, 40°C.	The degradation of 80% of total GLS was found.		

The remained GLS content was generally attributed to the balance of both mechanisms during freezing processing. Regarding the freezing process, it not only caused the accumulation of GLS, but also might give rise to the denature of myrosinase, and subsequently lead to the decrease in the formation of GLS hydrolysates. Qiu *et al.* (2020) studied the effects of microwave thawing, steam thawing, natural thawing, and still water thawing on GLS. No significant differences in GLS level were observed when broccoli was processed by microwave thawing and steam thawing. However, the decrease of ascorbic acid content induced by steam thawing was around 23.7% compared to that treated by microwave thawing. After the treatment of microwave thawing, the contents of total phenolics, GLS, ascorbic acid, and carotenoids were 1.15, 1.20, 1.93, and 1.39 times those of the natural thawing treatment, respectively.

HPP

HPP is one kind of extensively applied strategy for preserving and sterilizing foods. When it is applied to foods, it causes the inactivation of enzymes and pathogenic microorganisms and helps to maintain the compounds that can promote our health (Baenas *et al.*, 2020; Lafarga *et al.*, 2018; Zinoviadou and Galanakis, 2017). Experiments have been conducted on the effect of HPP on GLS content. The results showed that the inactivation of myrosinase was at the pressure of 300 or 500 MPa (Zinoviadou and Galanakis, 2017). Van Eylen *et al.* (2009) investigated the effect of the combination of temperature (20~40°C) and pressure (100~500 MPa) on GLS content. The results manifested that HPP could induce the hydrolysis of GLS, and thus led to the formation of ITCs (Table 3). Therefore, the authors indicated that HPP could be used as an effective method to avoid the decrease in GLS content.

Conclusion

Broccoli is one of the most popular vegetables due to the high content of GLS. GLS can be applied in many fields, for example, food additives, flavor enhancers, as well as anticancer agents. However, they are highly unstable compounds that can be easily hydrolyzed into some breakdown derivatives. Therefore, the preservation of GLS is critical work and should be considered as a part of the broccoli processing workflow for both food producers and the restoration sector. The high retention of GLS in broccoli can be obtained if correct post-harvest storage conditions, cooking handling, and food processing treatments are chosen. Therefore, the studies on optimizing postharvest conditions, food processing, and cooking handling for broccoli are very important. Postharvest treatments such as CA, cooling, MAP, freezing processing, and as well as 1-MCP and melatonin treatments, are effective methods to reduce GLS loss. Low temperature (<4°C) and high RH may be the most important storage factors to keep the high quality of broccoli, which can keep the integrity of broccoli cells, and reduce the contact of GLS and myrosinase. UV irradiation can effectively reduce microbial contamination in broccoli and improve the preservation of bioactive compounds including GLS. As for cooking practices, microwaving can retain or even increase GLS content in some cases. Boiling can reduce the GLS content significantly. The highly recommended method to cook broccoli is short-time steaming, which may be the best effective method to preserve GLS during cooking. Among thermal treatments, blanch-freezing treatment does not cause a change in GLS content significantly. HPP, one of the nonthermal treatments, has been regarded as a promising way to maintain high GLS content.

Although many studies on the effects of postharvest and processing on GLS have been investigated, however, many works on this topic are needed to be carried out. The effect of the combination of the heating with other non-thermal methods like HPP on GLS is critical to be investigated in the future and, the underlying mechanisms of how post-harvest processing regulates GLS metabolism are still needed to be further explained. More importantly, the relationship between the changes of individual type GLS and postharvest processing needs to be further investigated.

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Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and that no ethical issues are involved.

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