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Glucosinolates in Mixed-Packaged Mini Broccoli and Mini Cauliflower under Modified Atmosphere

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Contents of total and individual glucosinolates of mini broccoli cv. Milady and mini cauliflower cv. Clarke were assessed to determine the effect of modified atmosphere packaging on postharvest glucosinolate dynamics of mixed mini *Brassica* vegetables. Therefore, mixed-packaged mini broccoli and mini cauliflower stored in food trays sealed with two different microperforated biaxial-oriented polypropylene films for up to 7 days at 8 °C were analyzed. The results indicate that modified atmosphere at 8% O_2 + 14% CO_2 was a suitable gaseous combination to maintain aliphatic and indole glucosinolates in mini broccoli for 7 days after an initial decrease at 4 days. In contrast, modified atmosphere at 1% O_2 + 21% CO_2 resulted in the best retention of indole glucosinolates of mini cauliflower for 7 days and also of aliphatic glucosinolates after an initial decrease at 4 days. Thus, to maintain glucosinolates and external appearance and to prevent off-odor, mini broccoli and mini cauliflower should be packed separately in suitable altered gas composition.

KEYWORDS: Glucosinolates; *Brassica oleracea* var. *italica* Plenck; *Brassica oleracea* var. *botrytis* L.; postharvest

INTRODUCTION

The consumption of mini vegetables including mixed-packaged mini vegetables, for example, mini broccoli and mini cauliflower, is on the increase due to a higher demand for healthy, single-serving snacks and convenience foods (*1*−*3*).

Numerous studies have already shown the health-promoting effects of *Brassica* vegetables (see, e.g., refs *4* and *5*). Due to their anticarcinogenic properties, glucosinolates and their hydrolyzed products have generated considerable interest, especially in regard to the pharmaceutical industry (*6*).

Glucosinolates are a group of phytochemicals found in plants of the order Capparales, including agricultural important crop plants of the family Brassicaceae (*7*). They consist of a β-D-thioglucose reduced group, a sulfonated oxime moiety, and a variable side chain derived from amino acids (*8*). On the basis of the chemical structures of their side chains, glucosinolates can be subdivided in different classes such as aliphatic, aromatic, and indole (*9*, *10*).

At present, only limited information is available on the postharvest glucosinolate dynamics of two highly consumed *Brassica* vegetables, namely, broccoli and cauliflower. Furthermore, to date, there are no reports on the effect of modified atmosphere on glucosinolate content in mixed-packaged mini broccoli and mini cauliflower.

In regard to external and sensory quality, broccoli and cauliflower are known to benefit from storage in controlled and modified atmosphere, respectively (*11*, *12*). Hansen et al. (*13*) reported that compared to freshly harvested broccoli, the total glucosinolate content increased by 21% with storage over 7 days at 10 °C under 0.5% O_2 + 20% CO_2, whereas controlled atmosphere (CA) storage with 20% CO_2 in the absence of O_2 reduced the content of the main indole glucosinolate glucobrassicin by 58% and resulted in visible CO_2 injuries. In contrast, Rangkadilok et al. (*14*) found in broccoli stored at 4 °C under CA conditions (1.5% O_2 + 6% CO_2) for up to 25 days or stored in modified-atmosphere packaging (MAP; 0.2% O_2 + 15% CO_2) for up to 10 days no significant changes in the main aliphatic glucosinolate glucoraphanin. Moreover, Vallejo et al. (*15*) demonstrated a distinct decrease of aliphatic and indole glucosinolates by 71% in low-density polyethylene film-wrapped broccoli (17% O_2 + 3% CO_2) within 7 days at 1 °C.

With regard to cauliflower stored under altered O_2 and CO_2 compositions, the external appearance and vitamin or other nutritionally valuable compound contents have been investigated (see, e.g., refs *16*−*18*); however, glucosinolate contents have not.

Due to the limited and partly contradictory literature relating to postharvest glucosinolate dynamics of broccoli and cauliflower under controlled and modified atmosphere, respectively, coupled with the knowledge of increased consumption as healthy, single-serving snacks, the objective of the present study was to determine the effect of MAP with very low and moderate O_2 concentrations combined with high and very high CO_2 concentrations on total and individual glucosinolates in mixed-packaged mini broccoli and mini cauliflower.

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MATERIALS AND METHODS

Experimental Setup. Mini broccoli cv. Milady and mini cauliflower cv. Clarke were grown at the experimental station of the Institute of Vegetable and Ornamental Crops Grossbeeren/Erfurt e.V. (Golzow, Germany) in the spring growing cycle (planting in April) and were harvested randomly at the end of June. Fertilization, irrigation, and plant protection corresponded to the guidelines of the integrated cultivation for broccoli and cauliflower (*19*). After harvest, mini broccoli and mini cauliflower were transported to the Institute of Vegetable and Ornamental Crops Grossbeeren/Erfurt e.V. (Grossbeeren, Germany) in an air-conditioned (8 °C) vehicle. Broccoli and cauliflower heads were sorted on the same day to eliminate those that were damaged or misshaped, and heads were then selected for uniform weight and size (mini broccoli, 70 ± 5 g; mini cauliflower, 100 ± 5 g; length of head arc, 10−12 cm).

Postharvest Treatments. Three broccoli and two cauliflower heads were mixed-packed in polypropylene food trays (ES Plastic GmbH Co. KG, Hutthurm, Germany; 275 mm × 175 mm × 75 mm in size; weight, 33.4 g; volume, 2.2 L) sealed with a biaxial-oriented polypropylene (BOPP) film (NNZ GmbH, Lüneburg, Germany; 30 μm thick) with two different microperforations: two or eight microholes (diameter = 0.37 mm). Due to the different microperforations of the film, two modified atmospheres were created after 24 h of equilibration in the food tray packaging: 1% O_2 + 21% CO_2 (two microholes) and 8% O_2 + 14% CO_2 (eight microholes). O_2 and CO_2 concentrations were verified daily by analyzing 5 mL gas samples by an O_2/CO_2 headspace gas sampler (type Checkmate 9900; PBI Dansensor, Ringsted, Denmark). The O_2 and CO_2 concentrations were maintained within 10% of the required concentration. The sealed food trays were stored in an environmental chamber (14 m^3 air volume; York International, Mannheim, Germany) at 8 °C combined with >80% relative humidity for up to 7 days, simulating marketing in cooled, mist-equipped display cases or short-term storage in cold rooms.

The entire experiment comprised three replications conducted in the spring growing cycle (April−June) in the years 2003, 2004, and 2005.

Sample Preparation and Glucosinolate Analysis. At harvest and after 4 and 7 days of storage, three samples per treatment were removed for glucosinolate analysis. For the determination of glucosinolates, broccoli and cauliflower heads were frozen (−28 °C), freeze-dried, and then ground to a fine powder.

The HPLC method reported by Krumbein et al. (*20*) was used for glucosinolate determination. Duplicates of freeze-dried sample material (0.5 g) were heated to and incubated at 75 °C for 1 min, extracted with 4 mL of a methanol/water mixture (v/v = 7:3, T = 70 °C), and then, after the addition of 1 mL of 0.4 M barium acetate, centrifuged at 4000 rpm for 10 min. Two hundred microliters of a 5 mM stock solution of sinigrin in methanol was added to one of the duplicates just before the first extraction as internal standard. The residue was extracted twice more with 3 mL of the methanol/water mixture (v/v = 7:3, T = 70 °C). The supernatants were pooled and made up to 10 mL with methanol/water mixture (v/v = 7:3, T = 70 °C). From this, 5 mL of the extract was applied to a 250 μL DEA-Sephadex A-25 ion exchanger (acetic acid-activated, Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) and rinsed with 10 mL of bidistilled water. Next, 250 μL of a purified solution of aryl sulfatase (Boehringer-Mannheim GmbH, Mannheim, Germany) was applied and left for 12 h before the desulfo compounds were flushed with 5 mL of bidistilled water.

Desulfoglucosinolate analysis was conducted by HPLC (Merck HPLC pump L-7100, DAD detector L-7455, automatic sampler AS-7200, and HPLC Manager software D-7000) using a Spherisorb ODS2 column (5 μm, 250 × 4 mm). A gradient of 0−20% acetonitrile in water was selected from 2 to 34 min, followed by 20% acetonitrile in water until 40 min, and then 100% acetonitrile for 10 min until 50 min. Determination was conducted at a flow of 1.3 mL min^{-1} and a wavelength of 229 nm. Sinigrin (Sigma-Aldrich Chemie GmbH) and glucotropaeolin (AppliChem GmbH, Darmstadt, Germany) were used as standards. Individual glucosinolates were identified by comparison of their retention times with individual glucosinolates in standard reference materials of oilseed rape (BCR-190R and BCR-367 R) (*21*).

Table 1. Contents of Aliphatic Glucosinolates of Mini Broccoli Cv. Milady Stored in Modified Atmosphere for up to 7 Days at 8 °C

	aliphatic glucosinolates[a] (μmol/g of dw)		
MAP	glucoraphanin	glucoiberin	total aliphatic glucosinolates
1% O_2 + 21% CO_2			
harvest	4.34 a	0.65 a	5.00 a
4 days of storage	3.12 b	0.52 b	3.67 b
7 days of storage	3.42 b	0.53 b	4.00 b
HSD[b]	0.68	0.11	0.78
8% O_2 + 14% CO_2			
harvest	4.34 a	0.65 a	5.00 a
4 days of storage	3.07 b	0.47 b	3.57 b
7 days of storage	3.71 ab	0.58 ab	4.35 ab
HSD	0.71	0.12	0.84

[a] Values represent the mean of nine samples. Values followed by the same letter are not significantly different. The differences are compared for each MAP and each individual glucosinolate, particularly at harvest and after 4 and 7 days after harvest. [b] HSD, least honestly significant difference.

Glucosinolate content was calculated using sinigrin as internal standard and the response factor of each compound relative to sinigrin. Determination of glucosinolates was performed in duplicate.

Statistical Analysis. The contents of total and individual glucosinolate in broccoli and cauliflower heads were analyzed using multifactorial analysis of variance, and least-significant differences were calculated with Tukey's honestly significant difference test (significance level, $P < 0.05$). All statistical analyses were performed with Statistica for Windows (version 6.1, Statsoft Inc.).

RESULTS AND DISCUSSION

The total glucosinolate content as well as the individual aliphatic (methylsulfinylalkyl glucosinolates: glucoraphanin and glucoiberin) and indole (glucobrassicin, neoglucobrassicin, 4-hydroxyglucobrassicin, and 4-methoxyglucobrassicin) glucosinolate contents were quantitatively determined in mini broccoli heads at harvest and after 4 and 7 days of storage (**Tables 1** and **3**). The predominant glucosinolate was glucoraphanin, followed by glucobrassicin. In addition to the aliphatic glucosinolates glucoraphanin and glucoiberin, relatively high levels of sinigrin and low levels of progoitrin were found in cauliflower. The group of indole glucosinolates in cauliflower consists of glucobrassicin, 4-methoxyglucobrassicin, and neoglucobrassicin; 4-hydroxyglucobrassicin was also detected, but at very low levels (**Tables 2** and **4**). Similar glucosinolate profiles in broccoli and cauliflower were reported by Hansen et al. (*13*), Branca et al. (*22*), and Schonhof et al. (*23*).

Aliphatic Glucosinolates. Contents of both methylsulfinylalkyl glucosinolates glucoraphanin and glucoiberin decreased in mini broccoli heads at very low O_2 concentration (1%) combined with very high CO_2 concentration (21%), whereas moderate O_2 concentration (8%) with high CO_2 concentration (14%) led to decreased contents of glucoraphanin and glucoiberin until 4 days of storage followed by a tendentious accumulation of these methylsulfinylalkyl glucosinolates at the end of the storage after 7 days (**Table 1**).

Radishes packed in modified atmosphere (8% O_2 + 5% CO_2) also showed after an initial decrease an accumulation of aliphatic glucosinolates after 5 days of storage (*24*). Moreover, the glucoraphanin and glucoiberin contents of mature broccoli heads stored in a controlled atmosphere (0.5% O_2 + 20% CO_2) were reported to increase tendentiously during 7 days of storage (*13*). Hansen et al. (*13*) proposed that this increase of 8% could be associated with enhanced levels of metabolites (e.g., amino acids) being available for a de novo glucosinolate biosynthesis

Table 2. Contents of Aliphatic Glucosinolates of Mini Cauliflower Cv. Clarke Stored in Modified Atmosphere for up to 7 Days at 8 °C

	aliphatic glucosinolates[a] (μmol/g of dw)				
MAP	sinigrin	progoitrin	glucoraphanin	glucoiberin	total aliphatic glucosinolates
1% O_2 + 21% CO_2					
harvest	1.86 a	0.18 a	0.21 a	1.64	3.90 a
4 days of storage	1.50 b	0.13 ab	0.13 b	1.24	3.00 b
7 days of storage	1.49 b	0.14 b	0.16 ab	1.34	3.17 ab
HSD[b]	0.36	0.05	0.06	0.46 ns	0.87
8% O_2 + 14% CO_2					
harvest	1.86 a	0.18 a	0.21 a	1.64	3.90 a
4 days of storage	1.40 b	0.12 b	0.14 b	1.29	2.94 b
7 days of storage	1.27 b	0.11 b	0.14 b	1.23	2.75 b
HSD	0.26	0.04	0.06	0.42 ns	0.72

[a] Values represent the mean of nine samples. Values followed by the same letter are not significantly different (ns, not significant). The differences are compared for each MAP and each individual glucosinolate, particularly at harvest and after 4 and 7 days after harvest. [b] HSD, least honestly significant difference.

Table 3. Contents of Indole Glucosinolates of Mini Broccoli Cv. Milady Stored in Modified Atmosphere for up to 7 Days at 8 °C

	indole glucosinolates[a] (μmol/g of dw)				
MAP	glucobrassicin	4-hydroxyglucobrassicin	4-methoxyglucobrassicin	neoglucobrassicin	total indole glucosinolates
1% O_2 + 21% CO_2					
harvest	1.52	0.15 a	0.21	0.81 a	2.69 a
4 days of storage	1.27	0.10 b	0.21	0.59 b	2.16 b
7 days of storage	1.27	0.09 b	0.24	0.56 b	2.15 b
HSD[b]	0.27 ns	0.03	0.04 ns	0.23	0.50
8% O_2 + 14% CO_2					
harvest	1.52	0.15 a	0.21 b	0.81 a	2.69 a
4 days of storage	1.25	0.09 b	0.21 b	0.57 b	2.12 b
7 days of storage	1.35	0.12 b	0.27 a	0.52 b	2.56 ab
HSD	0.29 ns	0.03	0.04	0.24	0.52

[a] Values represent the mean of nine samples. Values followed by the same letter are not significantly different (ns, not significant). The differences are compared for each MAP and each individual glucosinolate, particularly at harvest and after 4 and 7 days after harvest. [b] HSD, least honestly significant difference.

Table 4. Contents of Indole Glucosinolates of Mini Cauliflower Cv. Clarke Stored in Modified Atmosphere for up to 7 Days at 8 °C

	indole glucosinolates[a] (μmol/g of dw)			
MAP	glucobrassicin	4-methoxyglucobrassicin	neoglucobrasscin	total indole glucosinolates
1% O_2 + 21% CO_2				
harvest	2.19	0.051	0.108	2.35
4 days of storage	1.58	0.055	0.091	1.72
7 days of storage	1.64	0.062	0.087	1.79
HSD[b]	0.69 ns	0.012 ns	0.069 ns	0.76 ns
8% O_2 + 14% CO_2				
harvest	2.19 a	0.051	0.108	2.35 a
4 days of storage	1.53 b	0.054	0.093	1.68 b
7 days of storage	1.21 b	0.047	0.067	1.33 b
HSD	0.56	0.012 ns	0.068 ns	0.61

[a] Values represent the mean of nine samples. Values followed by the same letter are not significantly different (ns, no significant). The differences are compared for each MAP and each individual glucosinolate, particularly at harvest and after 4 and 7 days after harvest. [b] HSD, least honestly significant difference.

that originated from the decomposition of other compounds. This process also seems to take place in mini broccoli packed in a modified atmosphere. It is assumed that the increase in glucosinolate content by a de novo biosynthesis in controlled and modified atmospheres is a stress response due to the increased CO_2 and decreased O_2 concentrations. The hypothesis of stress-induced accumulation of glucosinolates is supported by Bennett and Wallsgrove (*25*). They detected increased levels of glucosinolates due to environmental impact.

The oxygen dependence of the cytochrome P450-dependent monooxygenases of the CYP 79 family catalyzing the formation of aliphatic aldoxime—a key regulatory step in aliphatic glucosinolate biosynthesis (*26*, *27*)—seems not to be a limiting factor in broccoli for the de novo biosynthesis of glucoraphanin and glucoiberin at moderate O_2 concentrations in postharvest, because an O_2 level of 8% enables a significant increase of aliphatic glucosinolates in mini broccoli. However, at strongly reduced O_2 concentrations of 1%, decreased aliphatic glucosinolate contents were found in mini broccoli (**Table 1**), whereas tendentiously increasing methylsulfinylalkyl glucosinolate contents or unchanged glucoraphanin contents were obtained for mature broccoli in CA storage or in modified atmosphere packaging at very low O_2 concentrations (0.5 and 0.2%, respectively) (*13*, *14*). Regarding the decreasing contents of glucoraphanin and glucoiberin of mini broccoli at very low O_2 concentration of 1%, it could be assumed that younger broccoli heads have a more pronounced O_2 sensibility than mature broccoli heads. Kays (*28*) also stated that susceptibility to low-O_2 conditions is related to the product's nature, such as the stage of development.

Moreover, the CO_2 level also seems to be important for maintaining, decreasing, or increasing aliphatic glucosinolate

content after harvest in broccoli heads. In our experiments with mini broccoli at high CO_2 concentrations (14%) as well as in the investigations of Rangkadilok et al. (*14*) and Hansen et al. (*13*) with mature broccoli at higher CO_2 concentrations (15 and 20%, respectively) at low to moderate O_2 levels (0.5−8%), unchanged or rising contents of aliphatic glucosinolates after 7 and 10 days of storage, respectively, were detected. In contrast, Vallejo et al. (*15*) integrated a low CO_2 level (3%) at moderate O_2 concentration in the MAP of mature broccoli, resulting in a strong decomposition of aliphatic as well as indole glucosinolates. Taken together, these results indicate that enhanced CO_2 concentrations ≥14% are necessary for preventing loss in glucosinolates, even when the storage temperature is very low at 1 °C, as was demonstrated in mini broccoli and mature broccoli heads (*13*, *14*). However, with regard to the decreased content of aliphatic glucosinolates in mini broccoli at 1% O_2 + 21% CO_2, strongly enhanced CO_2 concentrations (21%) should be precautionarily avoided to prevent degradation of aliphatic glucosinolates in mini broccoli.

Total aliphatic glucosinolate content decreased in mini cauliflower heads at moderate O_2 concentration (8%) combined with high CO_2 concentration (14%) due to the degradation of sinigrin, progoitrin, and glucoraphanin (**Table 2**), whereas very low O_2 concentration (1%) with very high CO_2 concentration (21%) led to a reduced total aliphatic glucosinolate content until 4 days of storage followed by a tendentious final increase at the end of the storage after 7 days (**Table 2**). Thus, in mini cauliflower, the combination of reduced O_2 and enhanced CO_2 concentrations, especially at 8% O_2 + 14% CO_2, promoted the decomposition of aliphatic glucosinolates.

Indole Glucosinolates. In mini broccoli, the total indole glucosinolate content decreased at 1% O_2 + 21% CO_2, and this was predominantly due to the degradation of neoglucobrassicin and 4-hydroxyglucobrassicin. In contrast, moderate O_2 concentration (8%) with high CO_2 concentration (14%) resulted in, after a drop at 4 days of storage, an increased level of indole glucosinolates at the end of storage (**Table 3**). However, in mature broccoli heads, Hansen et al. (*13*) found a tendentiously increasing content of indole glucosinolates in CA storage (0.5% O_2 + 20% CO_2), whereas mature broccoli in MAP with low CO_2 levels (3%) showed a decrease in contents for all individual indole glucosinolates, particularly for neoglucobrasscin (*15*).

In contrast to mini broccoli, in mini cauliflower stored at 1% O_2 + 21% CO_2 the indole glucosinolate contents remained unchanged, whereas in a modified atmosphere with increasing O_2 and lower CO_2 levels (8% O_2 + 14% CO_2), a continuous reduction of total indole glucosinolates mainly due to the decomposition of glucobrassicin was observed (**Table 4**). Thus, mini cauliflower seems to need a very low O_2 concentration (1%) combined with a very high CO_2 concentration (21%) to avoid decomposition of indole glucosinolates (**Table 4**) and to induce a tendentious final increase of the total aliphatic glucosinolate content after 7 days of storage (**Table 2**), although for mature cauliflower with respect to color retention of the inflorescences and leaves, an even weaker gas composition of only 2−3% O_2 + 3−4% CO_2 has been recommended (*11*, *29*). In contrast, Cantwell and Suslow (*30*) recommended for cauliflower florets higher O_2 concentration (5−10%) to avoid discoloration and off-odors. However, with respect to glucosinolates lower O_2 levels should be applied, because no off-odors or color degradation occurred in our investigations.

MAP for Mini Broccoli and Mini Cauliflower. Degradation of glucosinolates is due to glucosinolate hydrolysis catalyzed by endogenous thiogluosidases, the myrosinases (see, e.g., ref *8*). The myrosinase−glucosinolate system is a preferred two-component system—myrosinase is located in myrosin cells and glucosinolates in the vacuoles (*10*, *31*)—which is activated by tissue damage or loss of cell integrity during product senescence. Chong and Berard (*32*) have already reported that cold-stored cabbage showed a rapid decline of glucosinolates at the beginning of product senescence. Additionally, myrosinase can also be inactivated by enhanced CO_2 concentrations (*33*). Thus, myrosinase hydrolysis in mature broccoli marked by progressed development could explain the glucosinolate decomposition in mature broccoli heads stored at low CO_2 levels, whereas mature broccoli heads (*13*, *14*) stored at enhanced CO_2 levels ≥6% combined with low (0.5 and 1.5%, respectively) or moderate O_2 levels (8%) showed no glucosinolate degradation. In contrast, in mini broccoli and mini cauliflower, modified atmospheres with high or very high CO_2 concentrations (8 or 21%) led to decreasing contents of aliphatic and indole glucosinolates, respectively. Moreover, no senescence symptoms, for example, color changes, were visible. Thus, it could be assumed that the decreasing glucosinolate contents should not related to myrosinase activity but to glucosinolate transport processes. As shown by *Arabidopsis thaliana*, glucosinolates could be transported by phloem, enabling a glucosinolate exchange between the individual plant organs (*34*, *35*). It is assumed that during 1 week of packaging glucosinolates were transported from the florets to the stalks due to the changing source-sink relationship induced by enhanced transpiration at the cut stalk edges. To confirm this hypothesis, in further experiments the glucosinolates have to be determined in both stalks and florets.

Moreover, the differences in postharvest glucosinolate metabolism of mini broccoli and mini cauliflower as well as the different responses to altered gas composition of broccoli are assumed to be due to the vegetables' precondition being influenced by the seasonal preharvest conditions and the development stage at harvest as was found for radish (*36*). The various products' precondition can be demonstrated by variable glucosinolate content at harvest. For example, the glucoraphanin content in broccoli ranged from 6 to 15 μmol/g of dry matter due to changing climate conditions (*37*).

In this study, the results indicate that modified atmosphere at 8% O_2 + 14% CO_2 was a suitable gaseous combination to maintain aliphatic and indole glucosinolates in mini broccoli for 7 days at 8 °C after an initial decrease at 4 days. In contrast, modified atmosphere at 1% O_2 + 21% CO_2 resulted in the best retention of indole glucosinolates of mini cauliflower for 7 days and also of aliphatic glucosinolates after an initial decrease at 4 days. Hence, mixed packaging of mini broccoli and mini cauliflower at either of the applied altered gas compositions could maintain the glucosinolate content of only one mini *Brassica* vegetable and simultaneously reduce the glucosinolate contents in the other.

No color loss in any food tray packaging was detected during the entire storage period. Additionally, the fresh weight loss at the end of any MAP was low and varied between 0.8 and 1.3%. Thus, to maintain glucosinolates and also external appearance, mini broccoli and mini cauliflower could be packed separately in suitable altered gas composition. To offer a combined packaging of mini broccoli and mini cauliflower, food trays with two compartments sealed with BOPP film having different microperforations to allow the buildup of the corresponding favorable modified atmospheres would be ideal for maintaining glucosinolate contents of both mini broccoli and mini cauliflower within 7 days. This would ultimately result in the

consumer benefiting from the high health-promoting quality of mini broccoli and mini cauliflower.

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