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# γ-Aminobutyric Acid and Antioxidant Changes in Fresh-cut Cantaloupe, Pineapple, and Cauliflower during Storage

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***Abstract.* γ-Aminobutyric acid (GABA), a nonprotein amino acid, can accumulate in plants in response to abiotic stresses. The effects of postharvest treatments on endogenous GABA concentrations and exogenous GABA on whole horticulture product quality has recently received attention. However, knowledge of the effects of mechanical damage events such as peeling and cutting on GABA concentrations of fresh-cut products is limited. In this study, concentrations of GABA and antioxidants including total phenolics and ascorbic acid in fresh-cut cantaloupe, pineapple, and cauliflower during storage at 5 °C for 9 days were investigated. We found that GABA accumulated in fresh-cut pineapple and cauliflower during storage, but that the increase in cantaloupe was not significant. Total phenolics, total ascorbic acid, and dehydroascorbic acid (DHA) remained stable in fresh-cut cantaloupe and cauliflower. In pineapple, however, total phenolics and total ascorbic acid concentrations decreased, whereas the DHA concentration increased. No correlation was found between GABA and antioxidants in fresh-cut cauliflower and cantaloupe; however, GABA was negatively related to antioxidants, including total phenolics and total ascorbic acid, in fresh-cut pineapple. The results show that GABA accumulation may represent a stress response to damage that occurs during the preparation of fresh cut products, but that the degree of response is affected by the specific product. Further research of GABA metabolism in response to minimal processing, including GABA biosynthesis, in a wider range of horticultural products and relationships with antioxidants is warranted.**

γ-Aminobutyric acid (GABA) is a ubiquitous four-carbon nonprotein amino acid that is widely distributed in plants, animals, and microorganisms (Shelp et al. 2021). In humans and other animals, GABA functions as an inhibitory neurotransmitter in the brain and central nervous system (Siucinska 2019), and it is believed to have a range of health benefits (Diana et al. 2014; Hou et al. 2023). These effects have resulted in GABA being considered a bioactive compound in foods and manufactured GABA-rich foods, including tea, brown rice, soybean, eggs, sourdough bread, and yogurt (Liao et al. 2017).

GABA has important roles in the physiological responses of plants to environmental stresses, such as cold temperature, heat, salinity, drought, water logging, increased carbon dioxide levels, and decreased oxygen levels (Aghdam et al. 2022; Bouché and Fromm 2004; Shelp et al. 2017; Yuan et al. 2023). The involvement of GABA in the responses of horticultural products to postharvest treatments has received increased attention; this has included the effects of gas composition and storage temperature on endogenous GABA concentrations, as well as the effects of exogenous GABA on the resistance of treated products to chilling injuries, decay, and senescence (Aghdam et al. 2022).

Interest in the effects of minimal processing on GABA accumulation has been extended to fresh-cut fruit and vegetables. Although fresh-cut products generally spoil before significant nutrient loss occurs (Gil et al. 2006), their preparation can result in the loss of dietary antioxidants, such as ascorbic acid, and the triggering or speeding of senescence and associated processes that lead to the accumulation of reactive oxygen species that are closely related to stress responses in plants (Hodges and Toivonen 2008; Iturralde-García et al. 2022). In a study of GABA concentrations and their relation to ascorbic acid, total phenolics and total antioxidant activity in seven fresh-cut fruits and five fresh-cut vegetables, the concentrations ranged from 90 $\mu mol \cdot kg^{-1}$ in cauliflower to 2480 $\mu mol \cdot kg^{-1}$ in cantaloupe based on fresh weight (Zhang et al. 2021). Total phenolics and total ascorbic acid (TAA) concentrations, but not those of GABA, were positively related to the total antioxidant activity in these products (Zhang et al. 2021).

Early physiological studies using systems such as soybean leaf responses to insect larvae found that GABA accumulated in response to mechanical damage (Ramputh and Bown, 1996; Wallace et al. 1984). It can be hypothesized that the accumulation of GABA in fresh-cut products would occur as a result of processing; however, until recently, little information was available. GABA accumulates in shredded carrots (Hou et al. 2023; Zhang et al. 2023). GABA treatment of fresh-cut products with GABA also showed effects such as slowed tissue browning and bacterial growth of apple (Gao et al. 2018a; Zhao et al. 2021), browning of potato (Gao et al. 2018b; Zhou et al. 2023), and alleviated oxidative damage and programmed cell death of pumpkins (Liang et al. 2022). Treatment of fresh-cut pear and cantaloupe with calcium chloride (Chi et al. 2021; You et al. 2024) and fresh-cut peaches with hydrogen sulfide (Wang et al. 2023) resulted in GABA accumulation, with the latter being associated with decreased tissue browning and quality maintenance.

Increased knowledge of the effects of GABA on individual fresh-cut products is an important step toward understanding the role of using GABA as a possible stress metabolite, as well as for the potential of fresh-cut fruits and vegetables as sources of health-promoting compounds. The objective of this study was to investigate GABA concentrations in selected fresh-cut fruit and vegetables in relation to the change in antioxidants, including vitamin C and total phenolics, during storage.

## Materials and Methods

*Plant material and sample preparation.* Whole cantaloupes, pineapples, and cauliflowers were obtained from a local supermarket in Ithaca, NY, USA. Replication was based on three individual fruits or vegetables. Cantaloupes and pineapples were prepared by slicing the fruit perpendicular to the blossom end-stem scar axis with a sharp stainless-steel knife. Then, 4-cm-thick slices were cut into uniform 2-cm-long cylinders with a cork-corer and stainless-steel knife. Cauliflowers were also cut with a sharp stainless-steel

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knife, and leaves were pulled away from the base of the head and sliced through the core into four quarters; the thicker stems were discarded. Similar bite-sized florets were made by cutting the stem. All samples were dipped in chlorinated (1.3 mM sodium hypochlorite) cold tap water for 2 min. Fresh-cut tissues were sampled at the time of preparation and after 3, 6, and 9 d of storage at 5 °C in the darkness. After measuring the quality of each product, the tissues were frozen in liquid nitrogen and maintained at −80 °C until analysis. Samples were ground to a fine powder using an IKA® A11 basic grinder (IKA Works, Inc., Wilmington, NC, USA) in liquid nitrogen. Powders were used for the extraction of GABA, total phenolic compounds, and vitamin C.

*Quality evaluation.* Color measurements of cantaloupes and pineapples were assessed using a Minolta Chroma Meter (model CR-300; Minolta, Osaka, Japan) calibrated with a white plate. The color was expressed as L*, a*, and b*, which define luminosity, red-greenness, and blue-yellowness, respectively, and a* and b* values were used to derive chroma and hue. The firmness of cantaloupes and pineapples was determined with a texture analyzer (model FDV-30; FORCE FIVE, Greenwich, CT, USA) as the force required for a 3-mm-diameter tip to penetrate to a depth of 5 mm.

*GABA concentrations.* The GABA concentrations were measured according to Zhang and Bown (1997). Frozen powder (0.4 g) was mixed with 400 μL methanol to extract GABA and inactivate glutamate decarboxylase activity. After 10 min at 25 °C, the sample was vacuum-dried, and 1 mL of 70 mM lanthanum chloride was added to remove the water-soluble phenolic pigments. After 15 min of shaking, the samples were centrifuged at 13,000 $g_n$ for 5 min, and 0.8 mL of the supernatant fluid was transferred to a new tube in which 160 μL 1 M KOH was added to precipitate lanthanum chloride. After 15 min of shaking and centrifugation at 13,000 $g_n$ for 10 min, the supernatant fluid was used for GABA determination. The 1 mL assay system contained 550 μL of a sample extract, 150 μL 4 mM $NADP^+$, 200 μL 0.5 M potassium pyrophosphate buffer (pH 8.6), 50 μL of 2 units of GABASE per mL and 50 μL of 20 mM α-ketoglutarate. The initial absorbance was read at 340 nm before adding 20 mM α-ketoglutarate, and the final absorbance was read at the same wavelength after 60 min of incubation at 25 °C. The GABA concentration was calculated as $\mu mol \cdot kg^{-1}$ based on fresh weight.

*Total and reduced ascorbic acid concentrations.* For measurements of TAA and dehydroascorbic acid (DHA) (Shin et al. 2008), frozen powder (0.3 g) was added to prechilled centrifuge tubes containing 1.5 mL extraction buffer (2% metaphosphoric acid and 2 mM EDTA). After centrifugation at 12,000 $g_n$ at 4 °C for 15 min, 900 μL supernatant was neutralized with 600 μL 10% sodium citrate. The DHA was determined by measuring the decrease in absorbance at 265 nm upon the addition of 10 μL 0.4 unit $\mu L^{-1}$ ascorbate oxidase to a mixture containing 500 μL 0.2 M phosphate buffer (pH 5.6), 390 μL distilled water, and 100 μL sample. The TAA was determined by measuring the decrease in absorbance at 265 nm upon the addition of 10 μL 0.4 unit $\mu L^{-1}$ ascorbate oxidase to a mixture containing 500 μL 0.2 M phosphate buffer (pH 5.6), 350 μL distilled water, 20 μL 20 mM dithiothreitol that was prepared with ethanol, 20 μL 0.5 M HEPES-KOH buffer (pH 7.5), and 100 μL neutralized sample. The amount of DHA was determined by subtracting the reduced ascorbic acid from the TAA. The concentrations of TAA and DHA were expressed as $mg \cdot kg^{-1}$ based on fresh weight.

*Total phenolic concentrations.* The total phenolics concentrations of the flesh extracts were measured using a modified colorimetric assay (Meyers et al. 2003). Frozen powder (1 g) was added to 5 mL 80% acetone containing 0.02% formic acid. After the mixture was vortexed and centrifuged at 12,000 $g_n$ for 20 min, the supernatant was used to measure total phenolics. For phenolics, 0.5 mL of the supernatant was added to 3 mL distilled water and 0.25 mL Folin-Ciocalteu reagent. After shaking for 5 min, 3 mL 7% $Na_2CO_3$ was added. The sample was mixed and the absorbance was measured at 750 nm and compared to a blank after 90 min at 25 °C. The results were expressed as gallic acid equivalents ($mg \cdot kg^{-1}$) based on fresh weight.

*Statistical analysis.* All data collected for each product type are reported as the means ± *SE* of three replications. An analysis of variance of all data was performed. Means were separated at the 5% significance level by the least significant difference test. All statistical analyses were performed using R Statistical Software (Foundation for Statistical Computing, Vienna, Austria).

## Results and Discussion

*Quality indices.* The firmness and color coordinates of the fresh-cut cantaloupes and pineapple during storage are shown in Table 1. The fresh-cut cantaloupes softened rapidly during the first 3 d, and then more slowly, declining to 48% of the original firmness on day 9. Softening has also been observed in fresh-cut cantaloupe in storage (Amaro et al. 2013; Bai et al. 2001; Syahidah et al. 2015), although not during all studies (Gil et al. 2006). However, with the exception of a lower C* value on day 9, color coordinates of the cantaloupe were unaffected by storage time. Stability of color coordinates has been observed previously (Machado et al. 2008). Browning or water soaking of the tissues, which can be indicated by changes in L* and C* (Bai et al. 2001; Syahidah et al. 2015), were not observed.

In contrast with the cantaloupe, fresh-cut pineapples did not soften during storage (Table 1). However, the L*, a*, and b* values increased, C* decreased, and h* remained unchanged. Similar findings were obtained by Azarakhsh et al. (2014), who found that the firmness of fresh-cut pineapple remained constant during 8 d of storage and decreased only by day 12. Firmness of fresh-cut pineapples during storage can be affected, depending on the cultivar (Xing et al. 2022). Decreasing b* values of fresh-cut pineapple during storage have been reported by others (Gil et al. 2006; González-Aguilar et al. 2005; Mantilla et al. 2013). No visual signs of tissue browning were detected; these would also have been indicated by decreasing L* values over time. No changes in quality characteristics of cauliflower were detected (data not shown), consistent with its high stability as a fresh-cut product (Róth et al. 2010).

*GABA concentrations.* The GABA concentrations of fresh-cut cantaloupe were much higher than those of pineapple and cauliflower (1100, 62, and 55 $\mu mol \cdot kg^{-1}$, respectively) at the time of preparation (Fig. 1). Visually, GABA increased in all products over time, but not necessarily significantly. The GABA concentrations increased by 132% and 120%, respectively, in fresh-cut pineapple and cauliflower during storage. In pineapple, the GABA concentration increased after 3 d, whereas the increase occurred after 6 d in cauliflower, indicating that storage times resulting in GABA accumulation depend on the product type.

No statistical increase of GABA was detected in cantaloupe. Although GABA concentrations increased by 117 $\mu mol \cdot kg^{-1}$ from the day of preparation to day 9 (Fig. 1), the increase was only 10%. It is possible that the failure to detect a significant increase was attributable to the small differences above the high baseline GABA concentration.

We compared the changes in GABA in the published literature; however, experimental timelines vary greatly for different fresh-cut products. Changes cited here are approximate because the calculations were based on published figures. In the studies by Hou et al. (2022) and Zhang et al. (2023), GABA in shredded carrots increased by 22% and 13%, respectively, above the initial concentrations (time zero) by 3 h; thereafter, they declined slightly over time. Using untreated cut products in several studies in which treatments were applied, the GABA concentration increased by 28% by 12 h before declining slightly in cantaloupe (You et al. 2024), by 54% after 12 h in peaches, by 12% after 6 d in pear fruits (Chi et al. 2021), and 109% after 6 d in Asian pear fruits (Wang et al. 2021). Only minor changes (4%) in fresh-cut pumpkins were found (Liang et al. 2022). Collectively, these results suggest that the effects of mechanical damage on GABA concentrations of fresh-cut products can vary greatly. Also, interestingly these increases from the published literature reported here and from our own research are small compared with the increases of 1800% to 2000% within 1 to 5 min in mechanically damaged soybean leaves (Ramputh and Bown 1996; Wallace et al. 1984). The tissue damage that occurs during preparation during minimal processing is much milder than that associated with manipulation or damage to leaves.

*Vitamin C concentrations and total phenolics concentrations.* The TAA and DHA concentrations in fresh-cut cantaloupe and

Table 1. Flesh firmness and color coordinates of fresh-cut cantaloupe and pineapple during storage at 5 °C.

| Storage time (d) | Firmness (N) | L* | a* values | b* values | Chroma (C*) | Hue (h*) |
|---|---|---|---|---|---|---|
| | | | Cantaloupe | | | |
| 0 | 12.4 a | 64.66 a | 11.36 a | 31.86 a | 33.83 a | 1.23 a |
| 3 | 7.4 b | 62.91 a | 10.90 a | 31.16 a | 33.01 a | 1.23 a |
| 6 | 7.1 b | 65.09 a | 10.73 a | 32.50 a | 34.23 a | 1.25 a |
| 9 | 6.4 b | 63.05 a | 10.46 a | 30.63 a | 32.37 b | 1.24 a |
| | | | Pineapple | | | |
| 0 | 4.9 a | 78.03 a | −4.34 b | 38.35 a | 38.60 a | −1.46 a |
| 3 | 4.5 a | 74.32 c | −4.25 b | 36.41 a | 36.66 a | −1.46 a |
| 6 | 4.9 a | 76.29 b | −3.38 a | 32.69 b | 32.86 b | −1.47 a |
| 9 | 4.3 a | 76.41 b | −3.14 a | 31.19 b | 31.35 b | −1.47 a |

Means with different lowercase letters were significantly different at $P = 0.05$.

cauliflower did not change significantly over time, but the TAA decreased and DHA increased in pineapple (Table 2). A decrease in TAA was also observed in fresh-cut pineapple during 5 d of cold storage (Barberis et al. 2012). However, Gil et al. (2006) found that TAA decreased in both cantaloupe and pineapple, although the losses in pineapples were highest. In contrast, no significant changes in ascorbic acid levels were observed in pineapple slices during storage (González-Aguilar et al. 2005). Cauliflower is very stable as a fresh-cut product (Róth et al. 2010), and high ascorbic acid retention may be related to their high sulfur-containing compounds (Albrecht et al. 1990).

The total phenolics concentrations in cantaloupe, pineapple, and cauliflower are shown in Table 3. The total phenolics concentrations in pineapple decreased over time whereas no significant changes in the total phenolics concentration were detected in cantaloupe and cauliflower. Similar results were found during storage of fresh-cut cantaloupe (Amaro et al. 2013). Phenolic biosynthesis is induced by wounding stress in various fresh-cut products, but antioxidant responses to wounding vary greatly by product (Gil et al. 2006; Reyes et al. 2007).

*Correlations among GABA, total phenolics, and TAA concentrations.* No significant differences in total phenolics were detected in the fresh-cut cauliflower or cantaloupe during storage, and no correlation with the GABA accumulation was detected. However, in pineapple, both phenolics and TAA were negatively related to GABA (Fig. 2). An explanation of the relationships for pineapple is unavailable and warrants further research.

## Conclusion

Our results indicate that GABA accumulated in fresh-cut pineapple and cauliflower, but not statistically significantly in cantaloupe, during storage for up to 9 d at 5 °C. During storage, total phenolics, TAA, and DHA remained stable in fresh-cut cantaloupe and cauliflower. However, in pineapple, total phenolics and TAA concentrations decreased while those of DHA increased. The GABA accumulation in cauliflower has no relation with antioxidants, including total phenolics and TAA; in contrast, in fresh-cut pineapple, GABA accumulation is negatively related to total phenolics and TAA. Considering other data in the literature, it is clear that GABA accumulations in different fresh-cut products after preparation vary greatly. These accumulations are small compared with those found in mechanically damaged leaves, suggesting that the specific fresh-cut process used may affect the extent of accumulation. Overall, we cannot identify any unifying factor, especially because of the varying experimental approaches that have been taken by different

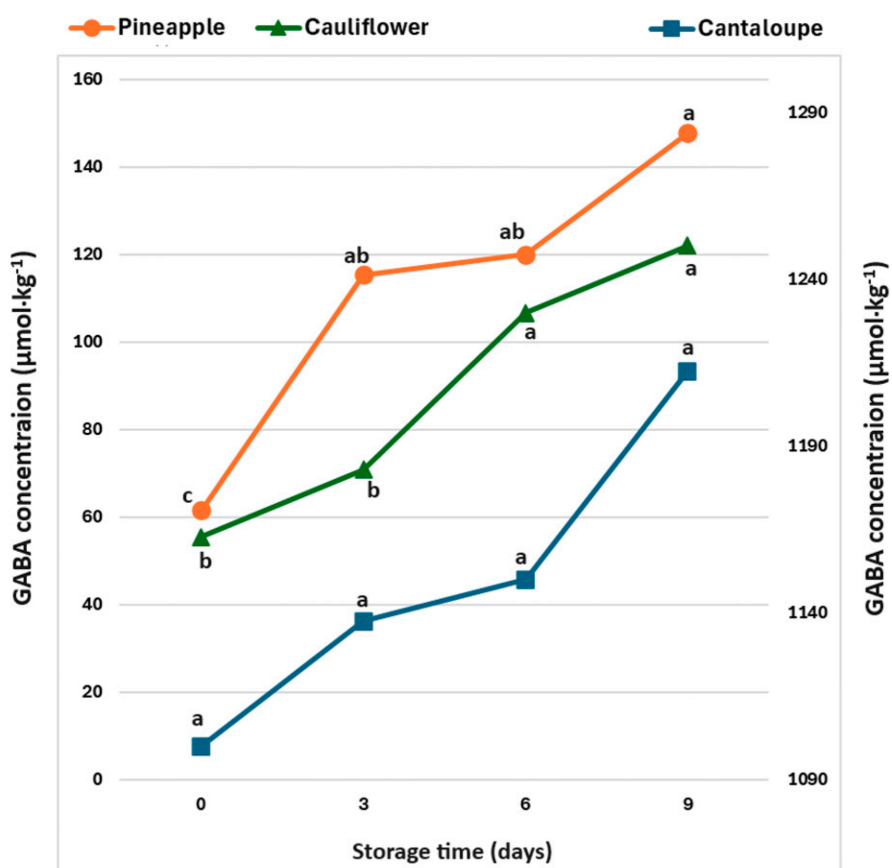


Fig. 1. γ-Aminobutyric acid (GABA) concentrations of fresh-cut cantaloupe, cauliflower, and pineapple during storage at 5 °C. Within each product type, means with different lowercase letters were significantly different at $P = 0.05$. The GABA concentrations for pineapple and cauliflower are on the left-hand $y$ axis, while that for cantaloupe is on the right-hand $y$ axis.

Table 2. Total ascorbic acid (TAA) and dehydroascorbic acid (DHA) concentrations (mg·kg$^{-1}$) of fresh-cut cantaloupe, cauliflower, and pineapple during storage at 5 °C.

| Storage time (days) | Cantaloupe | Cauliflower | Pineapple |
|---|---|---|---|
| | TAA (mg·kg$^{-1}$) | | |
| 0 | 295 a | 576 a | 495 a |
| 3 | 274 a | 508 a | 428 b |
| 6 | 268 a | 573 a | 383 c |
| 9 | 257 a | 537 a | 372 c |
| | DHA (mg·kg$^{-1}$) | | |
| 0 | 11 a | 75 a | 10 b |
| 3 | 19 a | 22 a | 53 a |
| 6 | 19 a | 84 a | 80 a |
| 9 | 10 a | 24 a | 70 a |

Means with different lowercase letters were significantly different at $P = 0.05$.

Table 3. Total phenolics (TP) concentrations (mg·kg$^{-1}$) of fresh-cut cantaloupe, cauliflower, and pineapple during storage at 5 °C.

| Storage time (days) | Cantaloupe | Cauliflower | Pineapple |
|---|---|---|---|
| | TP (mg·kg$^{-1}$) | | |
| 0 | 386 a | 673 a | 750 a |
| 3 | 414 a | 632 a | 677 b |
| 6 | 427 a | 681 a | 645 b |
| 9 | 436 a | 730 a | 638 b |

Means with different lowercase letters were significantly different at $P = 0.05$.

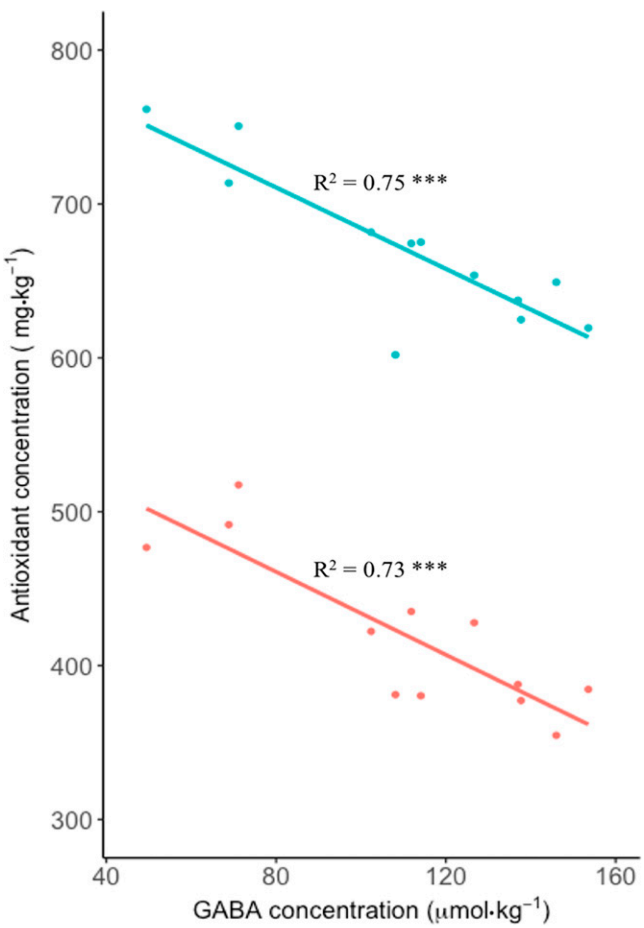


Fig. 2. Regressions of total ascorbic acid (TAA) and total phenolics (TP) with γ-aminobutyric acid (GABA) concentrations in fresh-cut pineapple during storage 5 °C. *** Significant at $P = 0.001$.

research groups. More research of GABA metabolism in a wider range of fresh-cut fruits and vegetables and the relationships of GABA with antioxidants is warranted.

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