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Potential Preharvest Application of γ-Aminobutyric Acid (GABA) on Improving Quality of 'Verna' Lemon at Harvest and during Storage

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Abstract: γ-aminobutyric acid (GABA) is a naturally occurring plant compound that acts as a signalling agent during stress conditions, mainly due to unstable events, although sometimes the endogenous content does not reach sufficient levels. Thus, the main aim of this study was to apply GABA preharvest treatments in lemon and to study its effects on quality attributes at harvest and during postharvest storage. GABA was applied as foliar spray at 10, 50, and 100 mM, and quality traits during 28 days of storage at two temperatures (at 2 and 10 °C) were determined. Results show that all GABA treatments had a positive effect on reducing the weight losses and fruit softening. In addition, crop yield in terms of kg tree^{-1} and fruit number tree^{-1} was improved for the first and second harvest as well as the total phenolics content and total antioxidant activity (TAA). In conclusion, GABA at 50 mM concentration was the most effective preharvest treatment, enhancing shelf life being enhanced for 14 and 7 days at 2 and 10 °C, respectively, with respect to control lemons.

Keywords: crop yield; elicitor; firmness; postharvest; total phenolics

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1. Introduction

Citrus limon (L) is a yellow fruit originated from an important evergreen fruit crop belonging to the *Rutaceae* family, mainly cultivated in tropical and subtropical regions. World lemon production significantly increased in the last decade, with countries such as Mexico, Turkey, and Spain leading crop production [1]. Spain is one of the main exporters and producers worldwide and Europe accounts for about 60% of its cultivation area [2]. The Valencian Community, Andalucía, and Murcia lead the production, with the 'Fino' and 'Verna' autochthonous cultivars being the most widespread.

Lemon is a fruit highly appreciated for its composition and is rich in bioactive compounds, such as ascorbic acid and phenolic compounds (flavonoids and hydroxycinnamic acids), which contribute to its total antioxidant activity and has beneficial health effects [3]. However, this fruit is susceptible to physiological, biochemical, and pathological disorders.

The effects of the climate change are a real challenge to produce plant-based food commodities, negatively affecting both plant growth and crop physiology and productivity. It was predicted that citrus cultivation will be affected in the near future [4]. Although climate change will be differentially affected depending on the region, the main problem of the citrus growing area of the Mediterranean basin will be related to drought problems attributed to the increase in average temperatures [5].

During fruit growth and development, both cell division and expansion occur rapidly, and the fruit is more sensitive to external elicitors. Therefore, the preharvest strategy based on the application of elicitors induces a more active metabolism compared to the recently non-treated harvested fruit, as well as prolonged storage at low temperatures [6]. Accordingly, the application of preharvest elicitors could improve the fruit resistance better than any postharvest tool, which is usually subjected to regulations.

In the last few years, γ-aminobutyric acid (GABA) was recognised as an elicitor for its application in crops affected by different abiotic stresses: low temperatures (chilling or freezing), high and fluctuated temperatures, solar radiation, flooding, and drought, among others [7,8]. GABA is a non-protein aminoaceus of four carbon atoms with a wide range of roles and modulates several physiological processes, including the cytosolic pH, redox status, osmotic moiety, plant growth, and senescence processes, among others [9], the latter being attributed to GABA shunt, which is the conversion of glutamate into GABA by glutamate decarboxylase enzyme [10]. At molecular level, GABA shunt acts as a signalling moiety, which is accumulated in the cytosol, playing a key role as a scavenger of reactive oxygen species (ROS), and thus, protecting the plant against the oxidative damage [11].

The association between fruit ripening and GABA was also proven in tomato. During fruit growth, GABA shows the higher concentration at the green mature stage and then sharply diminishes when the ripening processes advances [8]. Furthermore, the crosstalk between GABA and other plant hormones shows that the preharvest application of salicylates or jasmonates enhanced the endogenous concentration of GABA, which could be responsible for the observed increase in the yield and total antioxidant capacity [12]. Recently, GABA was postulated as a novel crop tool to increase quality and yield of lemon fruit (cv. Fino), even under undesired conditions of stress due to flash flooding as a consequence of torrential rain [13]. In 'Valencia' sweet orange [*Citrus sinensis* (L.) Osbeck] trees, the application of GABA increased the endogenous GABA level, some amino acids, and several phytohormones, including auxins, salicylates, jasmonic acid, and abscisic acid. This fact suggests that these plant hormones are upregulated in GABA-treated plants and could improve the plant response to abiotic stress conditions [14].

The exogenous application of GABA leads to increasing its endogenous level and promotes the GABA shunt, which increases the carbon flux to the respiration pathway with the enhancement of NADH, NADPH, and ATP, which is being postulated as a good strategy to prolong storability in fruits [15]. Accordingly, GABA treatment was effective in maintaining quality traits during postharvest storage of oranges by reducing both the consumption of the respiratory substrates, mainly citrate and amino acids, and reducing the decay incidence rots and by increasing the antioxidant enzyme activities [16].

As far as we know, the literature about the use of GABA preharvest treatment and its effect during postharvest storage of fruit is limited; thus, to our better knowledge, this is the first evidence in lemon fruit. Therefore, the main aim of this study was to apply different concentrations of GABA (10, 50, and 100 mM) throughout lemon growth on yield, and to observe its influence on quality and functional traits (weight loss, fruit firmness, total phenolics content, and total antioxidant activity) as well as the evolution of the total phenolics in 'Verna' lemon stored at 2 and 10 °C for 28 days of postharvest storage.

2. Materials and Methods

2.1. Experimental Design and Plant Material

Lemon trees of 15 years old and grafted on *Citrus macrophylla* rootstock (*Citrus limon* (L.) Burm. F. cv. Verna) were used for the experiment in the growing cycle of 2020–2021 from a commercial plot located in La Matanza (Alicante, Spain). The 'Verna' lemon tree usually blooms twice a year in the spring and summer season. The crop was under organic certification, and the climatic conditions were typical of Southern Spain: semi-arid climate with mean temperature and rainfall of 19 °C and 230 mm, respectively. Within the orchard, the experiment was randomly designed by using 3 blocks of 3 trees and planted at 4×5 m for GABA treatments (GABA was acquired from Sigma™ company, Madrid, Spain) and was applied at concentrations of 10, 50, and 100 mM, while control trees were only sprayed with distilled water. GABA (containing 0.5% Tween-20 as surfactant) solutions were freshly prepared and applied by foliar spray, using 5 L solution per tree. Along the growing cycle, treatments were repeated three times (T = 3): the first, being applied once, occurred at the normal physiological fruit drop (T1), the second application coinciding with the onset of colour changes (T2), while the third one was applied 4 days before harvest (T3). 'Verna' lemon was harvested at 3 dates (from May to June), and fruits from the 1st and 2nd harvests were used for the storage experiments. For each harvest date, 360 (10 lemons for each block of trees and treatment) lemons, that were uniformly mature and with colouration (full yellow), were manually harvested. At laboratory, lots of 30 fruits (grouped into 3 replicates of 10 fruits for each treatment; n = 3) were randomly selected at random for storage at 2 temperatures conditions (2 and 10 °C), and samples were transferred to a chamber at 20 °C for shelf life, in which the analytical measurements were carried out.

2.2. Lemon Fruit Yield and Quality Characteristics

Two measurements were carried out to evaluate the crop yield in terms of total yield (in kg tree^{-1}) and the number of lemons per tree (data are the mean ± SE). Quality parameters (weight loss, fruit firmness, total soluble solids, and titratable acidity content) were individually measured in each lemon fruit (data are the mean ± SE, n = 3). The weight loss was determined by weighing the recently harvested fruit and the weight obtained at each sampling date and results are expressed in percentage (data are the mean ± SE, n = 3). Regarding fruit firmness, a texturometer (TA-XT2i Texture Analyzer, Stable Microsystems^{TM}, Godalming, UK) was used with a flat probe of 10 cm. A force deformation was recorded by applying a 3% of the fruit deformation and results are expressed as N mm^{-1} (data are the mean ± SE, n = 3).

2.3. Extraction and Quantification of Total Phenolics and Total Antioxidant Activity (TAA)

The method of Folin-Ciocalteu was used following the protocol previously described by García-Pastor et al. (2020) [17]. A stainless steel lemon peeler was used to obtain the flavedo tissue by homogeneously splitting several 0.5 mm-thick slices of lemon peel. To obtain the lemon juice, a domestic squeezer ('Citromatic', Braun Española S.A., Barcelona, Spain) was used for the preparation of the juice by carefully squeezing the fruits manually in order to avoid contamination by components in the albedo tissue. Thus, 2 g of flavedo or 2 mL of juice were added to water:methanol (2:8) solution with 2 mM NaF (which inhibits the degradation of phenolics by the enzyme polyphenoloxidase). Then, the homogenized was obtained by using an Ultraturrax homogenizer (T18 basic, IKA, Berlin, Germany) at maximum speed for 1 min. The extract was centrifuged at 4 °C and 10,000× g for 10 min. An aliquot of 200 µL was mixed with the Folin reagent and the absorbance was quantified at 760 nm in a UV-Vis spectrophotometer (UV-1900i, Shimadzu, Duisburg, Germany). Total antioxidant activity (TAA) was measured in duplicate in each extract sample by using the ABTS peroxidase system, previously described in Martínez-Esplá et al. (2018) [18]. Results (mean ± SE) are expressed as mg equivalents of Trolox 100 g^{-1} of fresh weight.

2.4. Statistical Analysis

The software IBM SPSS Statistics 22.0 (IBM Corp., Armong, NY, USA) was used for the statistical analyses. Data for crop, quality, and functional parameters were subjected to analysis of variance (ANOVA) using the treatment studied and storage time as the factors. Thus, one-way ANOVA was used to determine the significance of mean differences among the four treatments: control and GABA at 10, 50, and 100 mM in 'Verna' lemon. Mean comparisons of analytical determinations were performed using a multiple range test (Tukey's test) to examine if differences among the treatments were significant at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$.

3. Results

3.1. Crop Yield

Crop yield was expressed in terms of kg tree^{-1} (Figure 1) and the total number of lemon trees^{-1} (Figure 2). Three harvest dates is the normal harvest schedule for this lemon cultivar (between May and June). For each harvest date, two lemon categories were established: commercial (yellow colour) and waste (non-commercial) fruits, according to the marketing procedure's date (harvest 3). Results show that the lower yield was obtained in the June harvest compared to the yield for harvest 1 and 2. However, the preharvest application of GABA significantly increased the yield for harvest 1 and 2 ($p < 0.01$ and $p < 0.001$, respectively; Supplementary Table S1), while remaining unaffected for harvest 3. The treatment of GABA at 50 mM significantly showed the highest yield of commercial lemons (105.2 ± 5.05 and 108.63 ± 6.1 kg tree^{-1} for harvest 1 and 2, respectively) compared to control (78.6 ± 6.2 and 63.56 ± 5.1 kg tree^{-1} for harvest 1 and 2, respectively) ($p < 0.001$; Supplementary Table S1). Generally, the yield of waste (non-commercial lemons) did not show significant differences among all treatments ($p \geq 0.05$; Supplementary Table S1).

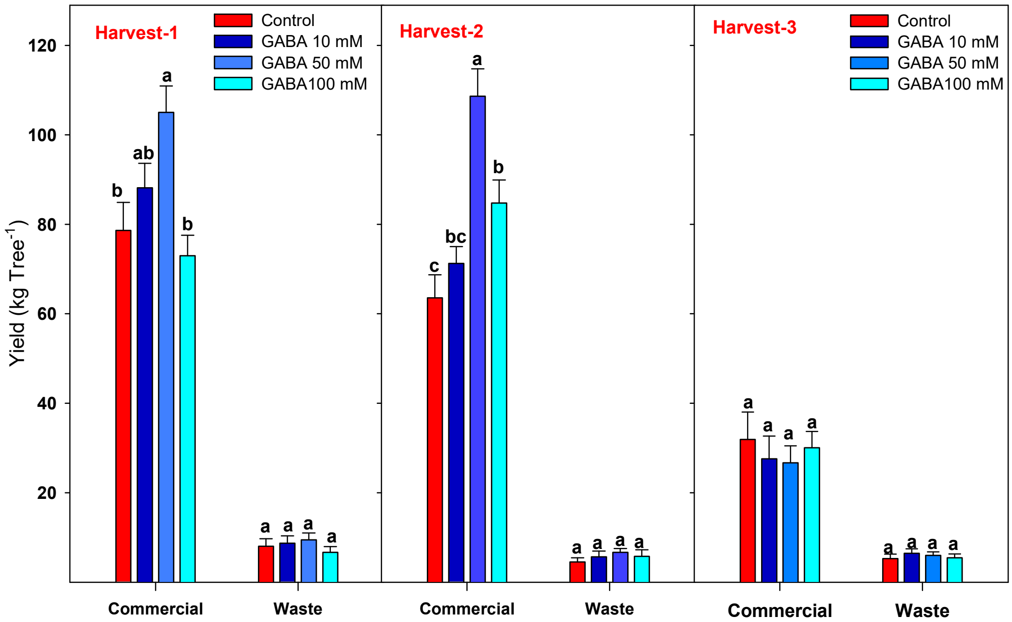

Figure 1. Influence of GABA preharvest treatment on yield (kg tree^{-1}) in three harvest dates as according to two categories: commercial and waste. Data are the mean ± SE. Different letters are significantly different at $p < 0.05$ according to Tukey's test.

The same behaviour was shown in relation to the number of fruit trees^{-1}, where the preharvest treatment of 50 mM GABA significantly produced the highest number of commercial lemons (550.67 ± 25.4 and 642 ± 30.8 number of fruits for harvests 1 and 2, respectively) compared to control fruits (367.7 ± 12.9 and 407.2 ± 34.2 kg tree^{-1}, respectively) ($p < 0.001$; Supplementary Table S1).

3.2. Postharvest Storage

Lemon fruits from each preharvest treatment (control and GABA at 10, 50, and 100 mM), which were harvested in May and June (harvest 1 and 2), were stored during 28 days at two temperatures (2 and 10 °C) and samples were weekly transferred to shelf life conditions for 2 days at 20 °C. During postharvest storage, the percentage of weight loss was higher at 10 than 2 °C for both harvest dates. Control fruits showed the highest weight loss percentage (≈6 and 7%) at both temperatures compared to GABA-treated fruits, which showed (Figure 3) the lowest weight losses ($p < 0.001$; Supplementary Table S2). Among GABA treatments, the concentration of 50 mM significantly showed the lowest percentage of weight loss (3–4 and 5% for 2 and 10 °C, respectively) compared to that obtained for 10 mM GABA-treated ($p < 0.001$; Supplementary Table S2) and control lemons.

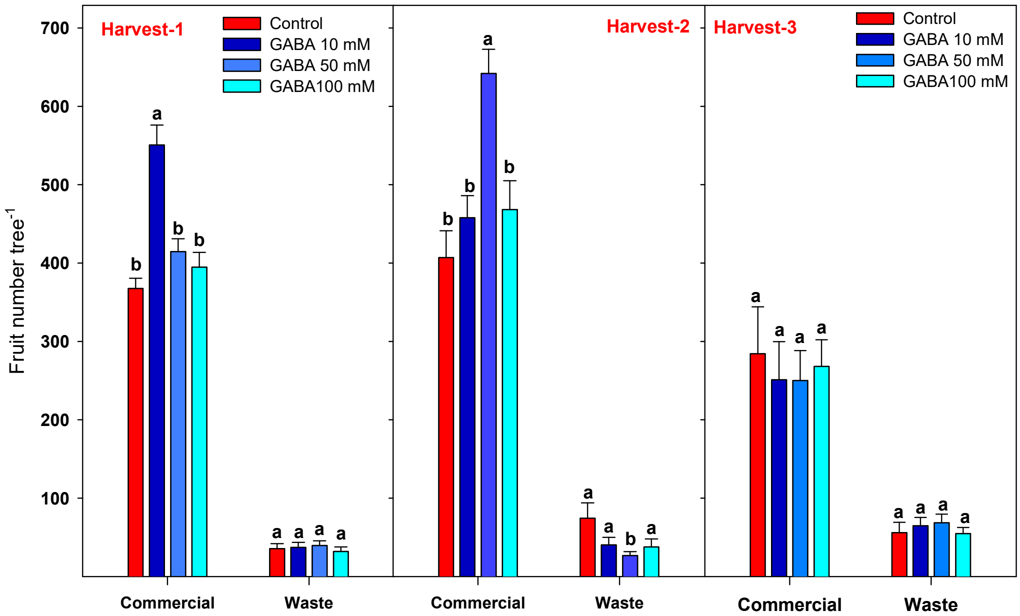

Figure 2. Influence of GABA preharvest treatment on fruits number tree^{-1} in three harvest dates as according to two categories: commercial and waste. Data are the mean ± SE. Different letters are significantly different at $p < 0.05$ according to Tukey's test.

Firmness values were higher in lemons harvested in May (harvest 1) than those picked in June (harvest 2). Firmness was expressed as fruit deformation (N mm^{-1}), and as expected, this parameter diminished during postharvest storage, for both harvest dates and storage temperatures (Figure 4). Control lemons showed the highest softening process compared to GABA-treated fruits. The most effective GABA concentration on maintaining lemon firmness was 50 mM.

Regarding total soluble solids (TSS). Control fruits showed a content of 6.33 ± 0.03 and 6.12 ± 0.05 g 100 g^{-1} at harvest for 10 and 2 °C, respectively, in the first harvest date, and no significant increment occurred during postharvest. During the storage, TSS slightly decreased for both temperatures and harvest dates. GABA preharvest treatments showed similar results to control lemons without significant differences among them for both harvest dates and temperatures. Concerning the TA content, in general, there were no significant differences during the storage between the control and treated lemons at the two temperatures and in both harvest dates.

Total phenolic content in the flavedo of lemon fruit ranged from 180 to 196 mg and 100 g^{-1} at the first sampling date and increased during storage, reaching final values of 182 and 197 mg 100 g^{-1} in the control and GABA 50 mM-treated fruit, respectively, in harvest 1 at 10 °C (Figure 5). During the second harvest (at 10 °C) phenol content showed an overall increase compared to the first harvest, while in GABA treatments this bioactive compound increased significantly compared to the control ($p < 0.001$; Supplementary Table S2), being that this effect was also observed at 2 °C.

On the other hand, the total phenolic content analyzed in the juice of lemon fruits showed a higher concentration in the harvest 1 compared to harvest 2 (Figure 6). During storage, total phenolics showed a decrease at 10 °C and an increase at 2 °C. All GABA-treated lemons showed a significantly higher content of total phenolics compared to control fruits ($p < 0.001$; Supplementary Table S2), and especially for the 50 mM concentration, in both harvest dates and storage temperatures (2 and 10 °C).

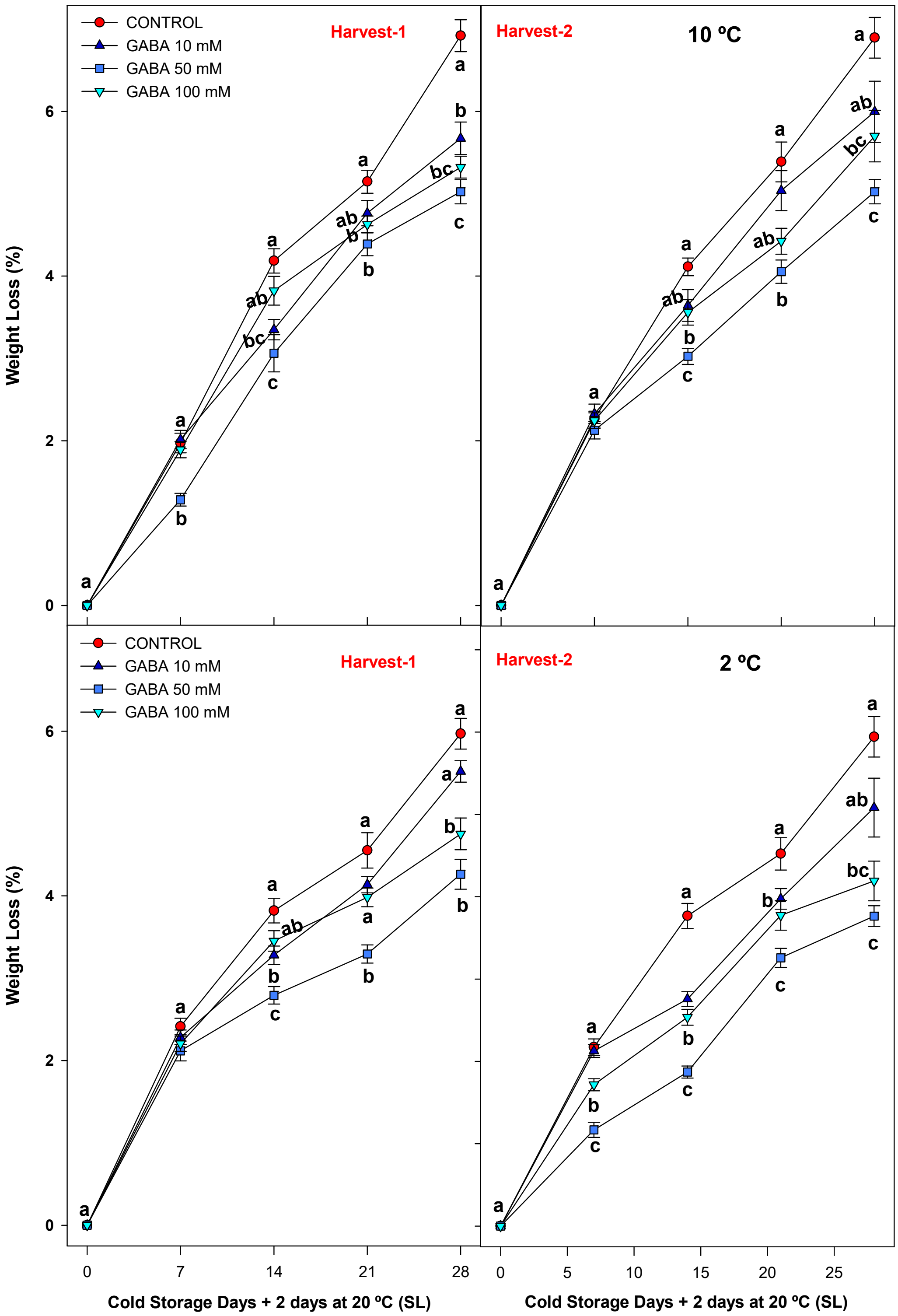

Figure 3. Weight loss (%) during 28 days of storage + shelf life (SL) at two temperatures (2 and 10 °C) from two harvest dates in lemon fruit. Data are the mean ± SE (n = 3). Different letters are significantly different at $p < 0.05$ according to Tukey's test.

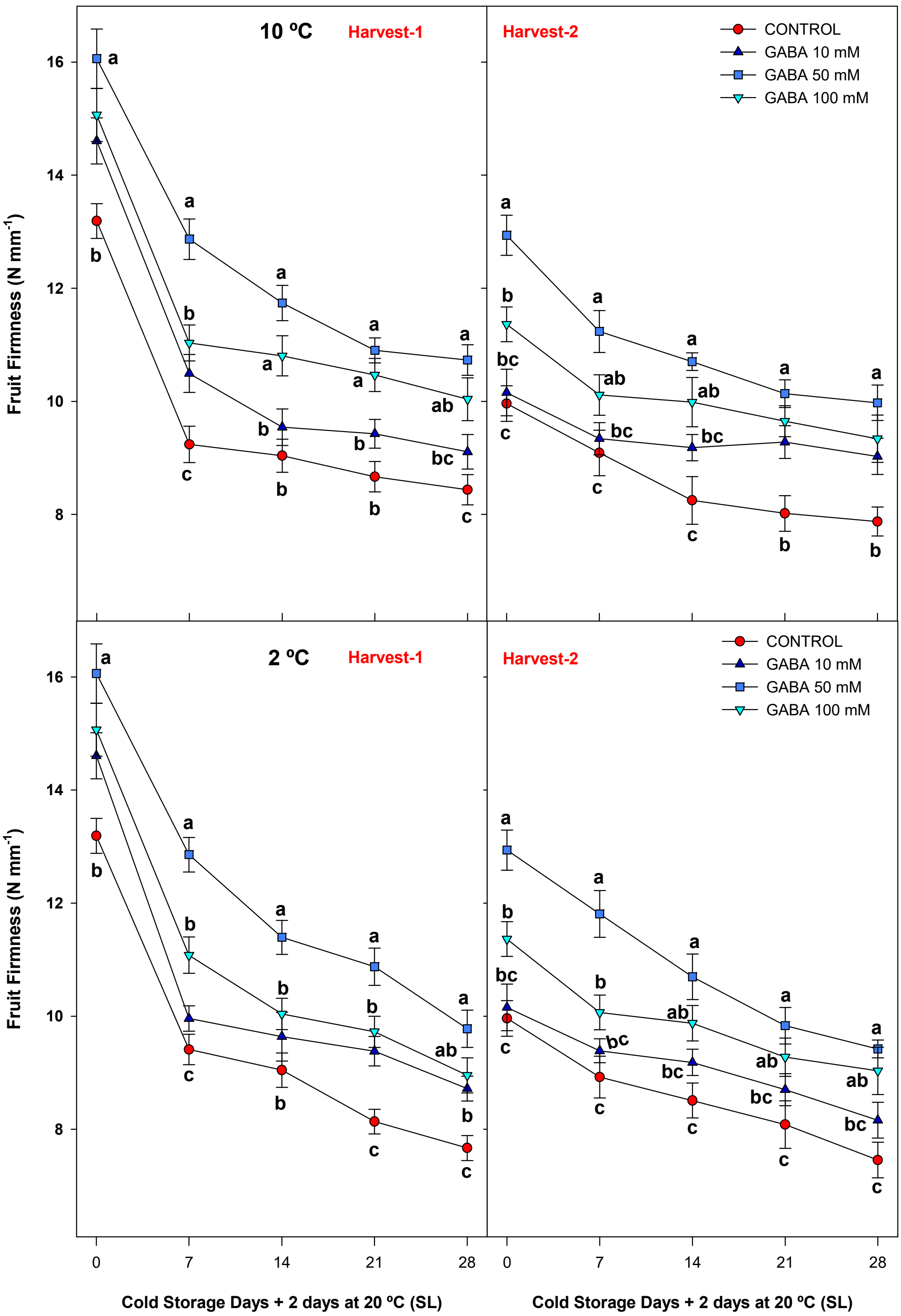

Figure 4. Firmness (N mm^{-1}) during 28 days of storage + SL at two temperatures (2 and 10 °C) from two harvest dates. Data are the mean ± SE (n = 3). Different letters are significantly different at $p < 0.05$ according to Tukey's test.

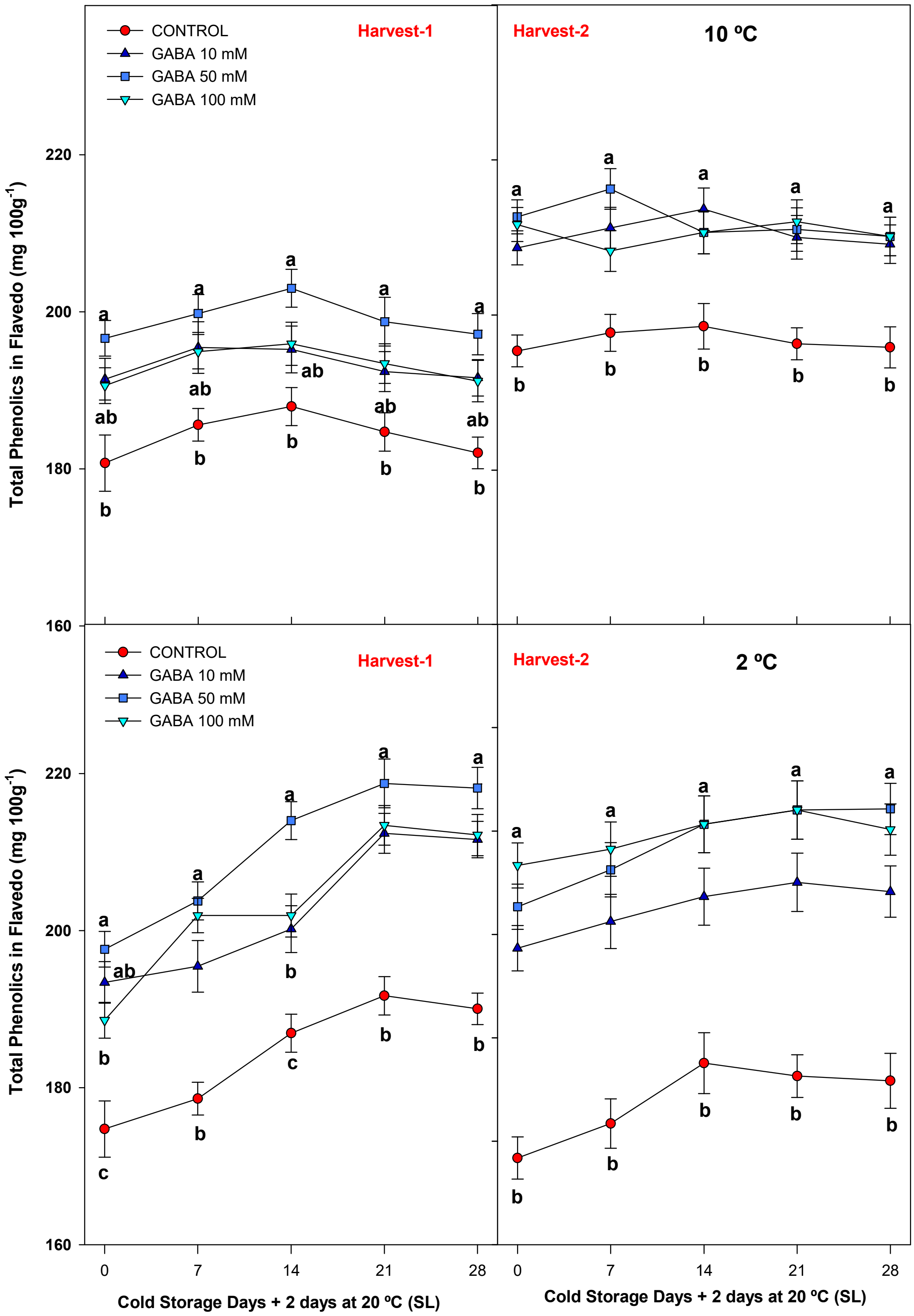

Figure 5. Total phenolics in the flavedo of lemon (mg 100 g^{-1}) during 28 days of storage + SL at two temperatures (2 and 10 °C) from two harvest dates. Data are the mean ± SE (n = 3). Different letters are significantly different at $p < 0.05$ according to Tukey's test.

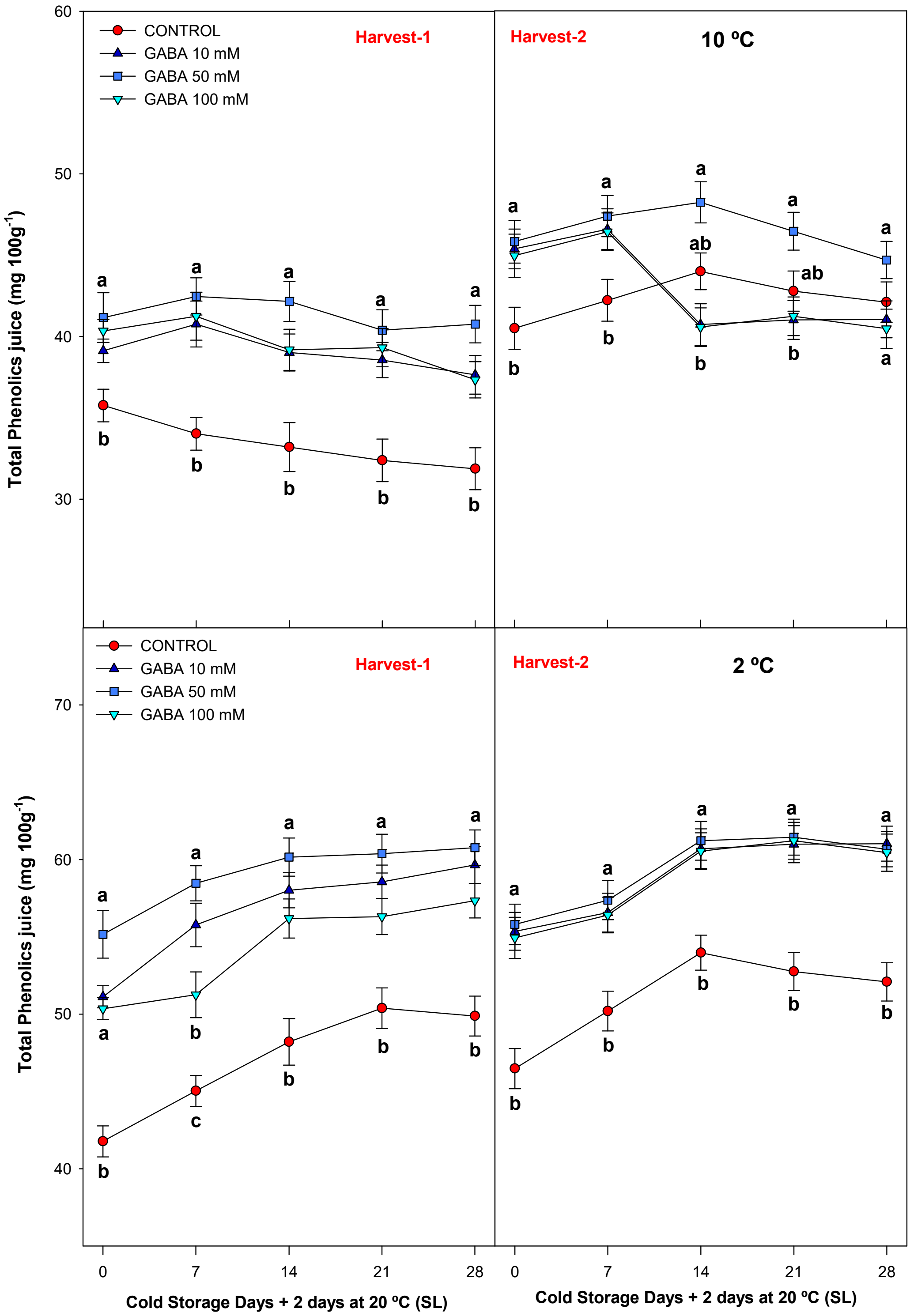

Figure 6. Total phenolics in the lemon juice (mg 100 g^{-1}) during 28 days of storage + SL at two temperatures (2 and 10 °C) from two harvest dates. Data are the mean ± SE (n = 3). Different letters are significantly different at $p < 0.05$ according to Tukey's test.

Total antioxidant activity (TAA) in the lemon juices was also significantly increased ($p < 0.001$; Supplementary Table S2) during storage at 2 °C from the first to fourth week at both harvest dates as a consequence of GABA treatments ($p < 0.001$; Supplementary Table S2). This increment was from 26.2 ± 1.7 to 35.7 ± 1.6 mg 100 g^{-1} in control and 50 mM in GABA-treated fruits, respectively, in the first harvest. For harvest 2, the TAA was from 31.7 ± 0.96

to 40.8 ± 1.7 mg 100 g^{-1} in control and 50 Mm GABA, respectively, also in the first harvest date. The values were significantly higher in the treated fruits than in the control, 50 mM being the most effective concentration of GABA treatment (Figure 7) ($p < 0.001$; Supplementary Table S2). Total antioxidant activity showed more variance in data during the storage at 10 °C in both harvest dates, although GABA at 50 and 100 mM exhibited significantly higher levels of TAA compared to control ($p < 0.001$; Supplementary Table S2) in the first harvest date.

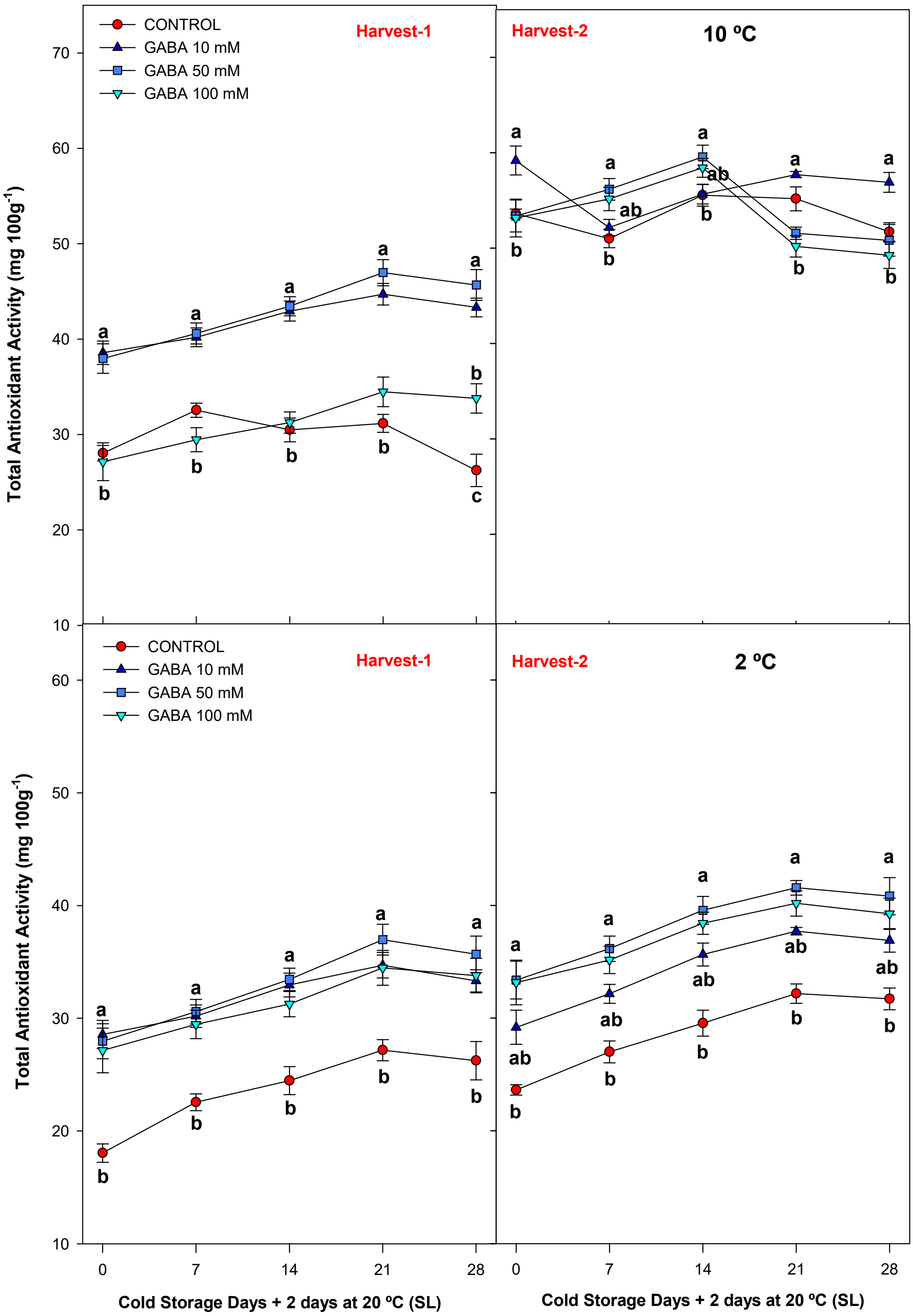

Figure 7. Total antioxidant activity in lemon juice (mg 100 g^{-1}) during 28 days of storage + SL at two temperatures (2 and 10 °C) from two harvest dates. Data are the mean ± SE (n = 3). Different letters are significantly different at $p < 0.05$ according to Tukey's test.

4. Discussion

It is clear that the consequence of climate changes associated with global warming is negatively affecting the fruit trees in general, and particularly in the citrus sector. In Southern parts of Spain, such as Alicante and Murcia, the scarcity of water is ruining crops and forcing trees to be uprooted. Apart from the episodes of extreme heat, the main problem for citrus is the mild winter temperatures, since the plant needs cold so that the skin of the fruit gains consistency, which directly affects the storability time once fruit is harvested. Therefore, it is necessary to deal with these stress situations in order to mitigate them, and among the different tools, we propose the use of preharvest elicitors, such as γ-aminobutyric acid (GABA), as this molecule is related to different growth environments [8].

Preharvest GABA application was effective in increasing crop yield as determined by the kg tree^{-1} and the number of lemons per tree, with the most effective GABA treatment being 50 mM. It is interesting to highlight that the improvement of crop yield was not dose dependent, since preharvest GABA at 10 mM showed better proficiency than 100 mM. The literature about the possible role of preharvest GABA applied in preharvest fruit productivity is scarce. The present results agree with the previous report on 'Fino' lemon, in which GABA at 100 mM GABA was the most effective dose in enhancing the yield during two consecutive seasons [13]. On the contrary, in 'Mollar de Elche' pomegranate trees, GABA preharvest treatments increased crop yield in a dose-dependent way [19]. The enhancement of lemon yield could be related to a higher content of photoassimilates through the developing fruits. Accordingly, the effects of GABA on enhancing the net photosynthesis rate were demonstrated in plants under stress conditions, the photosynthesis improvement was attributed to the exogenous application of GABA [8]. Furthermore, the relationship between kg tree^{-1} and number of fruit trees^{-1} in which similar results were observed, clearly shows that GABA treatments increased binding strength of the fruit branch, leading to reduction in normal fruit drop, which occurs throughout the fruit developmental cycle and is associated with environmental factors [19]. Current global warming predictions indicate that field temperature is expected to increase by 1.5–2.4 °C by 2050, and fruit crops will be subjected to more extreme weather conditions and increased abiotic stress, which in turn will reduce crop yield [20]. Therefore, the exogenous application of GABA could be considered an effective and sustainable strategy against different types of stress. An example of this effect was observed in creeping bentgrass, in which there was an alleviation of detrimental effects of heat stress, by reducing chlorophyll content, net photosynthesis, and water use efficiency among others, which was related to a GABA-induced increase in the activity of antioxidant enzymes (SOD, CAT, APX, POD, and DHAR) and with a reduction in ROS damage in the photosynthetic tissues [21].

Nowadays, the food market trends are based on fruits and vegetables with high-quality attributes that must be maintained for a long time during storage and commercialization. Fruits are often exposed to several stresses during postharvest storage, such as low temperature, affecting the ripening process, the onset of senescence, the loss of nutritive and bioactive compounds, and the reduction in shelf life. In this experiment, we stored the lemon fruits at two temperatures (2 and 10 °C), the 10 °C being considered as optimal and the 2 °C as the non-suitable temperature for lemon storage because it could lead to produce slightly retaining chilling injury damages. However, for this experiment, we observed that both temperatures maintained good results for lemon fruits after being treated with GABA. Two of the main parameters determining fruit deterioration during postharvest are weight loss and fruit firmness [22]. Even so, the weight loss was significantly reduced in GABA-treated than control lemons for both storage temperatures. Weight loss was even greater at 10 °C than at the 2 °C temperature. Thus, the fruit treated with GABA at 2 °C increased the quality of Verna lemon, preserving the weight for almost 14 days more than the control, which could reach a total of 42 days of shelf life. The visual quality of lemons is compromised (dehydration, shriveling, and decay) when the percentage of weight loss is higher than 6–7%. Similarly, fruit firmness was maintained by the GABA preharvest application when lemons were stored at 2 or 10 °C. One of the most important factors that determine the commercial acceptance of lemons is fruit firmness, which is related to cell turgidity and thickness of skin [23]. For instance, GABA treatment can induce chilling tolerance in postharvest kiwifruit by regulating the ascorbic acid metabolism, keeping the firmness longer [24]. For both parameters, the best concentration of GABA was 50 mM with a non-dose dependence effect. In a highly sensitive zucchini fruit cultivar (Sinatra), the postharvest application of GABA improved zucchini quality during storage at 4 °C determined by a lower chilling injury index and weight loss [25]. In a similar way, GABA alleviated the chilling injury symptoms in peach fruit during cold storage due to the higher accumulation of ascorbic acid and glutathione [26]. In GABA-treated grapes, the percentage of weight loss was reduced, as well as stem browning and decay [27], while in tomato, GABA was able to obtain fruits with higher firmness [28]. TSS and TA are other important quality parameters in lemon fruit. In mature lemons, sugars account for 80–90% of TSS depending on cultivars, the rest being corresponded to TA and the major one, which was citric acid, which is the main organic acid present in citrus fruits [29]. TSS and TA content were not affected by GABA treatments and the slight decrease shown in both parameters during storage is to be expected, as this may occur due to increased fruit ripening [30].

The peel and the juice of lemon fruit have a wide range of natural phytochemical compounds, the most important being phenolic compounds (mainly flavonoids) that contribute to the total antioxidant activity. Carotenoids, vitamins, and essential oils are some of them and the content of these bioactive compounds is related to health properties attributed to their antioxidant nature [31]. In the present study, preharvest GABA treatments induced a higher concentration of total phenolics both in peel and juice. However, in both harvest dates and temperatures, GABA treatment significantly increased total phenolic concentration in flavedo and juice, with 50 mM being the most effective concentration. Furthermore, results of the present study show that the phenolic concentration was higher in the flavedo than in the juice, which is typical in lemon fruits [32]. The main factor responsible for the antioxidant properties attributed to lemon fruit is the concentration of phenolic compounds together with ascorbic acid. Thus, the effect of the treatment on the increase in the phenolic concentration in the juice and flavedo tissue of the lemon fruit would result in an increase in its antioxidant activity [33]. Therefore, GABA preharvest treatment of lemon trees increased total antioxidant activity at both harvest dates and was maintained at higher levels during storage at both temperatures, delaying the postharvest ripening process. This higher content of total phenolics could be attributed to a stimulation of activity of phenylalanine ammonia-lyase (PAL), which is the key enzyme in the phenolic biosynthesis pathway and a lower activity of polyphenol oxidase (PPO) as was reported for strawberries [34] and pomegranates [19]. In blood oranges stored at low temperatures (2 °C), GABA treatment increased PAL activity and enhanced or maintained total phenolic compound content [35]. In some GABA-treated fruits, some of the mechanisms that could explain the increased antioxidant capacity could also be that exogenous GABA alleviates cold damage through the maintenance of energy status by supplying nicotinamide adenine dinucleotide (NAD)+H+(NADH), adenosine triphosphate (ATP), and inhibition of cytoplasmic acidification, as well as by the endogenous accumulation of GABA during cold stress in the fruit. On the other hand, the reduction in cold damage is accompanied by the stimulation of the activity of antioxidant enzymes under cold storage conditions to scavange ROS, which could be another mechanism of action [35].

5. Conclusions

This study investigated the role of GABA in preharvest and postharvest, and overall, results indicate an improvement in the marketability of lemon fruits as a consequence of GABA preharvest treatments, the most effective concentration being 50 mM. Thus, GABA increases crop yield and quality and functional parameters in both temperatures and harvest dates. On average, shelf life could be prolonged 2 weeks more than that obtained for control lemons, leading to an increase in its economic value as well as its health beneficial effects for consumers.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture13071397/s1, Table S1: Analyses of variance (ANOVA) of crop parameters (kg tree -1 and fruit number tree^{-1}) for different harvest dates (1st, 2nd and 3rd harvest) as according to 2 categories: commercial and waste in 'Verna' lemon using the treatment studied as the factor. Table S2: Analyses of variance (ANOVA) of quality and functional parameters [weight loss, firmness, total phenolics in flavedo and juice and total antioxidant activity (TAA)] for different harvest dates (1st and 2nd harvest) in'Verna' lemon during 28 days of storage + shelf-life at 2 temperatures (2 and 10 °C) using the treatment studied and storage time as the factors.

Author Contributions: M.S. and D.V. conceived and designed the work; S.C. and J.M.V. performed the field treatments; F.B.-E.H., H.D.-M. and J.M.V. performed most of the analytical determination, in collaboration with the other authors; M.S. and D.V. analyzed the data and wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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