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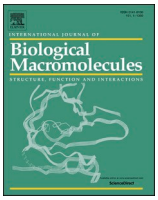

The combined application of Arabic gum coating and γ-aminobutyric acid mitigates chilling injury and maintains eating quality of 'Kinnow' mandarin fruits

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ABSTRACT

Low temperature storage of citrus generally extends the storage potential but leads to chilling injury appearance on the rind of fruits. The said physiological disorder has been found associated with changes in the metabolism of cell walls and other attributes. In this work, the influence of Arabic gum [AG (10 %)] and γ-amminobutyric acid [GABA (10 mmol L^{-1})] either alone or in combined form was studied on fruits of 'Kinnow' mandarin during storage at 5 ± 1 °C for 60 days. The results exhibited that the combined AG + GABA treatment significantly suppressed weight loss (5.13 %), chilling injury (CI) symptoms (2.41 score), incidence of disease (13.33 %), respiration rate [(4.81 μmol kg^{-1} h^{-1}) RPR] and ethylene production [(0.86 nmol kg^{-1} h^{-1}) EPR]. In addition, AG + GABA application reduced relative electrolyte (37.89 %) leakage (REL), malondialdehyde [(25.99 nmol kg^{-1}) MDA], superoxide anion [(15.23 nmol min^{-1} kg^{-1}) $O_2^{\bullet-}$] and hydrogen peroxide [(27.08 nmol kg^{-1}) H_2O_2] along with lower lipoxygenase [(23.81 U mg^{-1} protein) LOX] and phospholipase D [(14.07 U mg^{-1} protein) PLD] enzyme activities compared with control. The AG + GABA treated 'Kinnow' group showed higher glutamate decarboxylase [(43.18 U mg^{-1} protein) GAD] and lower GABA transaminase [(15.93 U mg^{-1} protein) GABA-T] activity having higher endogenous GABA (42.02 mg kg^{-1}) content. The fruits treated with AG + GABA exhibited higher cell walls (CW) components such as Na_2CO_3-soluble pectin [(6.55 g kg^{-1}) NCSP], chelate-soluble pectin [(7.13 g kg^{-1}) CSP] and protopectin [(11.03 g kg^{-1}) PRP] concentrations along with lower water-soluble pectin [(10.64 g kg^{-1}) WSP] compared to control. In addition, 'Kinnow' fruits treated with AG + GABA showed higher firmness (8.63 N) and lower activities of CW degrading such as cellulase [(11.23 U mg^{-1} protein) CX], poly-galacturonase [(22.59 U mg^{-1} protein) PG], pectin methylesterase [(15.61 U mg^{-1} protein) PME] and β-galactosidase [(20.64 U mg^{-1} protein) β-Gal] enzymes. The activity of catalase [(41.56 U mg^{-1} protein) CAT], ascorbate peroxidase [(55.57 U mg^{-1} protein) APX], superoxide dismutase [(52.93 U mg^{-1} protein) SOD] and peroxidase [(31.02 U mg^{-1} protein) POD] was also higher in combined treatment. In addition, AG + GABA treated fruits showed better biochemical and sensory attributes than the control. So, combined AG + GABA could be used for CI mitigation and storage life prolongation of 'Kinnow' fruits.

1. Introduction

Citrus nobilis L. × *Citrus deliciosa* T., commonly known as 'Kinnow' mandarin is an important fruit in citrus group. It is a rich source of various nutrients including phenols, antioxidants, flavonoids, minerals and carotenoids. These nutrients are considered important for the consumer's health [1,2]. Due to its rich nutrient availability, citrus fruit is widely consumed and traded worldwide. It is exported to various countries of the world from the producing areas and washing of the fruit is a routine practice in the pack-house operations. However, washing treatment has been found responsible for the removal (to certain extent) of natural wax from the surface of fruits [3]. Therefore, fruits lose their

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natural layer of protection and the produce thus shows excessive transpiration-induced water loss. Excessive water loss eventually results in mass loss and poor quality of the fruits [4].

It has been reported that low temperature conditions protect the fruits from desiccation-induced mass loss and extend the storage life in contrast with the ambient storage environment [5,6]. At the same time, low temperature storage of 'Kinnow' fruits generally leads to the occurrence of chilling injury (CI) which is a physiological disorder that not only negatively affects the visual appearance but also reduces the eating quality as reported previously [1]. In addition, excessive decay, firmness loss, the decline of bioactive compounds and cell wall components degradation have also been found associated with long-term stored fruits of 'Kinnow' mandarin [7]. The severity of chilling stress substantially reduces the membrane integrity probably owing to the degradation of cell walls polysaccharides in fresh horticultural produce [8,9]. It is evident from the literature that some suitable treatments are required to reduce the desiccation-induced mass and associated quality losses during ambient storage of 'Kinnow' fruits. On the other side, some protective agents (anti-chilling treatments) are also indispensable to apply before long-term cold storage to mitigate CI occurrence and chilling-induced disassembly of cell walls polysaccharides (WSP, PRP, NCSP and CSP) to prolong the storage life of 'Kinnow' fruits with conserved quality.

It is a common and commercial practice to apply coatings on citrus fruits to replace natural waxes (lost or reduced during washing) in the packhouses. The edible coating treatment forms a protective barrier to protect from the loss of water and gases exchange [3]. The plant based natural and biodegradable edible coatings do not cause any potential negative effects on the produce quality and are also considered eco-friendly in nature [1,10]. Arabic gum (AG) is a natural plant based polysaccharide. It is derived from *Acacia senegal* exudates and consists of polysaccharides (galactose, arabinose, rhamnose and glucuronic acid) and a small concentration of proteins [10]. It has good film-forming, solubility, emulsification and antioxidant activity. Previous studies have proven that AG edible coating efficiently reduced postharvest decay and conserved the general eating quality of various horticultural crops. The AG coating application has been reported on banana [11], guava [12], papaya [13], mango [14], persimmon [15], tomato [16], blueberry [17], apricot [18] and mandarin [10]. Based on the literature, it is evident that AG coating has a promising potential for conserving the quality of harvested horticultural crops but its use in combination with GABA has not been reported yet.

The γ-aminobutyric acid (GABA) is a non-protein based four-carbon amino acid. It plays multiple functions in the metabolism of plants such as cellular signaling, conservation of tricarboxylic acid and carbon-nitrogen activities, growth as well as development modulation and induction of certain biotic and abiotic stresses tolerance [19]. In fresh horticultural produce, GABA has been found to delay senescence and induce resistance against CI under cold stored conditions. The suppressed CI ultimately helps to prolong the storage potential of susceptible fruits and vegetables with maintained eating quality [20]. The exogenous GABA applications impart chilling tolerance by regulating GABA shunt mediated by GAD and GABA-T ultimately helping in the reactive oxygen species (ROS) detoxification by stimulating the higher activities of antioxidative enzymes system [21]. The GABA application has been reported for the alleviation of CI in bananas [22], cut flowers of anthurium [23], peaches [24], zucchini [25], cherries [26], pomegranates [27], persimmons [28], Chinese olive [29] and aonla [21]. On the other side, its combined use with AG coating has not been investigated yet. So, objective of the current work was to investigate the effect of AG coating in combination with GABA on chilling inury, physiological weight loss, GABA shunt attributes, metabolic activities, oxidative stress indicators, membrane stability degrading enzymes, components of cell walls, cell walls degrading enzymes, antioxidant activities, biochemical quality and sensory attributes of cold stored 'Kinnow' fruits.

2. Materials and methods

2.1. Experimental fruits

The fruits of 'Kinnow' cultivar were obtained from a local orchard. These were harvested at a commercial maturity and brought to the laboratory within 1 h of the harvest. In the laboratory, the fruits were first washed with fresh tap water and then surface disinfected for 3 min with 0.01 % NaOCl and dried for ~60 min at room temperature (20 ± 1 °C).

2.2. AG coating preparation and concentrations selection

The edible coating based on AG was prepared by following the earlier method of Khaliq et al. [14]. AG powder was sourced from Sigma Aldrich (St Louis, USA). The powder of AG was heated at 40 °C on a magnetic stirrer for about 3 h after dissolving in deionized water. After the complete dissolution of AG, 1 % glycerol was incorporated as a plasticizer and 0.25 % Tween-20 was added as a surfactant. The AG coating pH was adjusted to 5.6 by using NaOH (1 mol L^{-1}). The AG coating solution was then sonicated to exclude any possible air bubbles for about 30 min and left at room temperature for another 60 min before use. The used concentration i.e. 10 % AG was optimized during a preliminary work in which 2.5 %, 5 %, 10 %, 15 % and 20 % treatments were used (Supplementary Figs. 1–9). The preliminary work was conducted twice for confirmation. The thickness of 10 % AG coating used in the main trial ranged from 0.16 ± 0.009–0.18 ± 0.006 mm.

2.3. GABA solution preparation and doses selection

The analytical pure GABA was purchased from Sigma Aldrich (St Louis, USA). The solution of GABA treatment was prepared by dissolving it in double distilled water. Various concentrations of GABA were used during a preliminary work in which 0, 2, 4, 6, 8 and 10 mmol L^{-1} doses were applied (Supplementary Figs. 1–9). Based on the results obtained, the effect of 10 mmol L^{-1} GABA was the most significant, so, this concentration was selected to use in the main experiment of the present work. The preliminary trial was carried out twice for the confirmation of the results.

2.4. Treatments application and fruits storage

The fruits were divided into 4 groups of 225 (15 fruits per replicate and 45 per treatment at the period of each sampling) and subjected to treatment application. A total of 945 'Kinnow' fruits were used in the trial including 45 fruits for sampling on the day of harvest. Total 4 treatments were used i.e. T_1 = control (dipped in double distilled water only), T_2 = 10 mmol L^{-1} GABA, T_3 = 10 % AG coating and T_4 = GABA (10 mmol L^{-1}) + GA [10 % (*w/v*)] coating. In treatment T_1, fruits were dipped (for 5 min) only in double distilled water. Fruits of T_2 and T_3 were dipped in GABA and AG coating solutions, respectively for 5 min. On the other hand, the fruits of T_4 were first dipped in GABA solution for 5 min, dried at 20 °C and then dipped in AG coating solution for 5 min. After dipping in the coating solutions, the fruits were dried at room temperature for ~60 and packed in corrugated cardboard boxes which were stored at 5 °C with 90 ± 2 % relative humidity for 60 days. The fruit sampling was carried out at 12 days interval after 0 (before application of the treatments), 12, 24, 36, 48 and 60 days plus 2 days at 20 °C.

2.5. Weight loss and disease incidence

It was assessed by measuring the final and initial weight of the stored 'Kinnow' fruits at a specific sampling period. Briefly, a total of 15 'Kinnow' fruits per replicate and 45 per treatment were used for weight loss assessment at every period of sampling. The loss in weight was

determined and derived as a percent after calculation as appended below.

$$\text{Weight loss}\,(\%) = \frac{\text{Initial weight on storage day} - \text{Weight on sampling day}}{\text{Initial weight on storage day}} \times 100$$

Fruits of 'Kinnow' mandarin were evaluated for the determination of disease incidence. The disease incidence was calculated by counting the disease-infected 'Kinnows' from the total fruits per replicate. A total 45 'Kinnows' per treatment were assessed for disease incidence and expressed as a percent.

2.6. Assessment of CI symptoms

The CI index was evaluated on a visual basis by noting the discoloration/rind pitting on the peel of fruits as described earlier by Imahori et al. [30]. The CI scale ranged from 1 to 5 based on the severity that occurred on the rind of the fruits. The CI was observed as 1 = no CI, 2 ≤ 20 %, 3 = 21–40 %, 4 = 61–80 % and 5 = 81–100 % and expressed as a score. A total of 15 fruits per replicate and 45 in each treatment were assessed for CI at every period of sampling.

2.7. Respiration rate (RPR) and ethylene production (EPR)

RPR and EPR of 'Kinnow' fruits were determined at each sampling period. An F-950 digital gas analyzer was used (Felix Inst., USA) for this purpose [31]. Briefly, one 'Kinnow' fruit of known weight was put in a plastic jar (with known volume) at 25 °C. The jars were carefully made airtight and the 'Kinnow' containing jars were incubated for 60 min and gas readings of RPR and EPR were taken from the headspace (through the insertion of a probe). The concentrations of RPR and EPR were calculated by comparing them with the blank readings (without fruit jars) and expressed as μmol kg^{-1} h^{-1} and nmol kg^{-1} h^{-1}, respectively.

2.8. Determination of REL and MDA

Flesh REL was determined with the assay of Chen et al. [32]. The 'Kinnow' peel discs were added to the solution of deionized water (20 mL) and then placed at a temperature of 25 °C to take the initial REL reading. Thereafter, the discs solution was heated in a water bath 100 °C for 15 min and the final reading was noted. Finally, REL was derived as a percent.

The production of MDA was determined based on the assay of Sun et al. [33]. The sample of 'Kinnow' fruit flesh (1 g) was macerated in 100 g L^{-1} 5 mL of thiobarbituric acid (TBA) and subjected to centrifugation at 10,000 ×*g*. After that, 2 mL TBA was reacted with 2 mL of the collected supernatant and boiled for 20 min in a water bath. Finally, MDA concentration was expressed as nmol min^{-1} kg^{-1} FW.

2.9. Determination of ROS concentrations

2.9.1. Assay of H_2O_2 content

The fruit flesh of 'Kinnow' mandarin (1 g) was homogenized in the solution of 5 mL trichloroacetic acid and the upper supernatant layer was collected following centrifugation at 12000 ×*g* for 10 min. After the centrifugation, the obtained supernatant (1 mL) was mixed in phosphate buffer (10 mmol L^{-1}, 7.0 pH) which contained potassium iodide (1 M) and optical density was read at 390 nm. The concentration of H_2O_2 was expressed as μmol kg^{-1} FW [34].

2.9.2. Assay of $O_2^{\bullet-}$ content

The flesh tissue of 'Kinnow' fruits (1 g) sample was macerated in 50 mmol L^{-1} 7.8 pH based phosphate buffer. Thereafter, the sample's supernatant was taken and reacted with 10 mmol L^{-1} concentrated hydroxylamine at 25 °C. After that, sulfanilic acid (17 mmol L^{-1}) and α-naphthylamine (7 mmol L^{-1}) were added and left for incubation. Following the incubation, the assay mixture was centrifuged at 10,000 ×*g* for 20 min and absorbance was taken at 530 nm to express the $O_2^{\bullet-}$ content in terms of μmol min^{-1} kg^{-1} FW [35].

2.10. Determination of LOX and PLD activity

The sample tissues (5 g) were extracted in potassium phosphate buffer which was 0.1 mmol L^{-1} with pH of 6.8. After that, 0.25 mL Tween-20, 0.5 mL linoleic acid and 10 mL deionized H_2O were incorporated into the collected supernatant to obtain a substrate reaction. The mixture of the reaction consisted of 2.98 mL 5.5 pH based buffer (0.1 mmol L^{-1}), 0.1 mol L^{-1} 100 μL linoleic acid and 100 μL extract (supernatant) of the crude enzyme. The mixture of reaction was subjected to incubation for 15 min at 30 °C to note optical density at 234 nm as described previously by Liu et al. [36]. The activity of the enzyme was derived as U mg^{-1} protein. The one unit (U) of LOX was enzyme quantity that resulted in 0.001 change in absorbance under the conditions of the assay.

The activity of PLD enzyme was determined with the detailed procedure of Liu et al. [36]. The sample tissues (5 g) were macerated in polyvinylpyrrolidone containing 20 mL sodium acetate (5.6 pH, 0.1 mol L^{-1}) buffer. The assay mixture for PLD contained 27.4 mmol L^{-1} 1,3-phosphatidyl choline, 900 μL acetate buffer (50 mmol L^{-1}, 5.6 pH) and 300 μL extract of the enzyme. In the end, 5.6 pH based acetate buffer (50 mmol L^{-1}) having 100 μL acid phosphatase (0.4 mmol L^{-1}) was incorporated for the reaction completion following incubation for 1 h at 37 °C. The absorbance was noted at 520 nm and one PLD unit (U) was defined as an absorbance change of 0.001 under specific conditions of the assay.

2.11. GAD and GABA-T activity assays and endogenous GABA content determination

The activity of GAD and GABA-T enzymes was assessed with the method of Deewatthanawong et al. [37]. The flesh samples of 'Kinnow' fruits were extracted with 9.1 pH based Tris-HCl buffer. The absorbance was noted at 340 nm and the activities of both enzymes were expressed as U mg^{-1} protein. One GAD activity unit (U) was defined as the production of 1 μg of GABA per h under specific conditions of the assay. On the other side, one GABA-T activity unit (U) was the quantity of enzyme that caused the production of 1 μg of alanine per h under assay conditions.

The endogenous content of GABA was assayed by following the assay of Hu et al. [38]. The flesh sample (1 g) was extracted with 3 mL of 0.05 mol L^{-1} lanthanum chloride and centrifuged at 13,000 ×*g* for 5 min. The supernatant was re-centrifuged after mixing 0.2 mL of 2 mol L^{-1} potassium hydroxide. Following centrifugation, the mixture was reacted with 0.6 mL phosphate buffer (0.05 mol L^{-1}, 10.0 pH), 0.2 mL phenol (6 %) and 0.4 mL supernatant along with NaOCl (5 %). The absorbance was read at 645 nm and GABA concentration was derived as mg kg^{-1} FW.

2.12. Determination of cell walls polysaccharides

The cell walls components such as WSP, PRP, NCSP and CSP were extracted and determined with the comprehensive protocols of Wang et al. [39] and Chen et al. [40]. Briefly, 'Kinnow' fruits flesh tissue samples were boiled in 95 % ethanol for 30 min and centrifuged at 8000 ×*g* for 15 min. The sediment was dissolved in double distilled water and the supernatant (as WSP) was collected and the lower sediment (for PRP) was incorporated into 25 mL sulfuric acid (0.5 mol L^{-1}) and boiled for 1 h. For NCSP and CSP fractions, the cell walls material (300 mg) was homogenized in acetic acid buffer (6.5 pH) and continuously stirred for 6 h. The sediment was collected following centrifugation and poured into 50 mmol L^{-1} Na_2CO_3 (50 mmol L^{-1}) which contained CDTA and the assay solutions were re-stirred for 6 h. The subsequent residues were collected as NCSP and CSP fractions of the cell walls. Finally, the

concentration of WSP, PRP, NCSP and CSP were expressed as mg kg^{-1} FW.

2.13. Flesh firmness measurement and softening enzyme activity assays

2.13.1. Flesh firmness

The FR-5120 penetrometer was used for 'Kinnow' fruits firmness determination. A tip of 8 mm was fitted with the penetrometer and firmness was determined from 15 fruits of 'Kinnow' mandarin per replicate and 45 fruits in each treatment. The results were expressed in newtons (N).

2.13.2. Assay of PG enzyme activity

The procedure of Ge et al. [41] was used for PG enzyme activity determination in 'Kinnow' fruits flesh. The assay of the reaction consisted of 0.1 mL extract of enzyme, 0.4 mL 1 % pectin and acetate buffer 0.5 mL with 4.5 pH. The reactants were incorporated into the test tubes and left for incubation for 60 min at 37 °C. After the incubation, the samples were heated at 100 °C for 5 min and dinitrosalicylic acid was added. Thereafter, absorbance was noted at 540 nm.

2.13.3. Assay of CX enzyme activity

The CX enzyme activity was assayed in 'Kinnow' fruits flesh with the assay of Deng et al. [42]. In brief, 0.5 mL of 0.1 mol L^{-1} acetate (pH 5.0) buffer, 0.1 mL extract of enzyme and 0.4 mL of carboxymethyl cellulose (1 %) were mixed and left for a reaction for 5 min. After that, the reactants were heated for 5 min at 100 °C and following the heating, assay samples were cooled rapidly at 20 °C. Finally, after cooling the absorbance was read at 540 nm in triplicates.

2.13.4. Assay of PME enzyme activity

The detailed assay of Saleem et al. [15] was used for the determination of PME activity in 'Kinnow' fruits flesh at each period of sampling. The sample assay contained 0.75 mL deionized water, 0.1 mL crude enzymes extract, 2 mL pectin (2 %) and 0.1 of 0.01 % concentrated bromothymol blue in test tubes. Finally, the assay mixture absorbance of all samples was noted at 620 nm and the activity of PME enzyme was calculated.

2.13.5. Assay of β-Gal enzyme activity

The activity of β-Gal enzyme was determined by following the comprehensive protocol of Wei et al. [43]. The mixtures of the assay contained 0.5 mol L^{-1} (2 mL) concentrated sodium carbonate (5.2 pH) buffer, 0.1 mL of 3 mmol L^{-1} *p*-nitrophenyl-α-d-arabinofuranoside and 0.5 mL extract of crude enzymes. The reaction assay was left at 37 °C for 30 min for the purpose of incubation. Finally, β-Gal enzyme activity was determined after noting the absorbance at 400 nm in triplicates.

2.14. Determination of antioxidant enzymes activity

2.14.1. Assay of APX activity

The method of Nakano and Asada [44] was utilized for the determination of APX activity in the flesh of 'Kinnow'. In brief, 0.2 mL phosphate buffer (pH 7.0 and 0.5 mol L^{-1}), 0.1 mL enzymes extract, 0.1 mL H_2O_2 (0.1 mmol L^{-1}), 0.5 mmol L^{-1} (0.1 mL) L-ascorbate and 0.1 mmol L^{-1} Na_2-EDTA were reacted in the assay. The reaction in the assay mixture was started by adding H_2O_2. After that, absorbance was noted at 290 nm and U mg^{-1} protein was used to express the activity of APX enzyme.

2.14.2. Assay of CAT activity

This was determined in the flesh of 'Kinnow' fruits by adopting the assay of Ali et al. [45]. Briefly, 5.9 mmol L^{-1} concentrated 0.1 mL of H_2O_2, 0.1 mL crude extract of the enzyme and 7.0 pH based 2.4 mL phosphate buffer (50 mmol L^{-1}) were reacted in the assay. The assay reaction was performed under 4 °C conditions and 240 nm wavelength was used for the absorbance reading and the activity of the enzyme was expressed as a U mg^{-1} protein.

2.14.3. Assay of SOD activity

The method of Ali et al. [45] was used for the determination of SOD activity. The enzyme assays mixture consisted of 200 μL of methionine (22 μmol L^{-1}), 100 μL of crude extract, 800 μL deionized H_2O, NBT 100 μL (20 μmol L^{-1}), 500 μL of buffer (50 mmol L^{-1}, 7.8 pH), 200 μL of Triton-X (0.1 μmol L^{-1}) and 100 μL of riboflavin (0.6 μmol L^{-1}). The assay mixture absorbance was noted at 560 nm. Before absorbance reading, the mixtures were subjected to UV-light illumination for 15 min and activity was derived as U mg^{-1} protein.

2.14.4. Assay of POD activity

The protocol of Ali et al. [45] was used for activity determination of POD enzyme in 'Kinnow' fruits flesh. There were 50 mmol L^{-1} pH 7.0 phosphate buffer, 0.5 mL (20 mmol L^{-1}) guaiacol and 0.3 mL (40 mmol L^{-1}) H_2O_2 in the assay mixture. The incubation of the assay reaction was carried out for 5 min and 470 nm wavelength was used for measuring the absorbance of the samples for enzyme activity which was expressed as a U mg^{-1} protein.

2.15. Determination of non-enzymatic antioxidants

2.15.1. Determination of DPPH scavenging activity

This was assayed with the protocol of Shimada et al. [46]. Briefly, DPPH (0.1 mmol L^{-1}) solution was prepared in MeOH and 1 mL of this solution was collected and 4 mL of 'Kinnow' fruits juice was mixed with it. After the incorporation of the juice sample, the resultant mixture was vigorously shaken and incubated for 15 min in dark. Finally, DPPH scavenging activity (as a percentage) was computed after taking absorbance at 571 nm.

2.15.2. Determination of total phenolics

Total phenolics were analyzed by using the detailed assay of Kaushik et al. [47]. The blue color was developed with FC reagent in 20 % sodium carbonate (alkaline medium) and absorbance was read at 750 nm on UV-VIS spectrophotometer (UV-1900, Shimadzu, Japan). A standard curve was made with gallic acid. Finally, the concentration of phenolics was expressed in terms of gallic acid equivalents as mg GAE kg^{-1}.

2.15.3. Determination of ascorbic acid content

The concentration of ascorbic acid content (AAC) in the flesh of 'Kinnow' fruits was analyzed with the protocol of Ali et al. [45]. Briefly, 10 mL of juice was taken in a flask and the volume was made 100 mL with 0.4 % oxalic acid. An aliquot (5 mL) was collected and titration was performed with 2,6 dichloroindophenol. Finally, AAC was derived as mg 100 g^{-1} FW.

2.16. Biochemical attributes and ethanol content in juice

2.16.1. Titratable acidity (TA) determination

TA was determined from fruit juice. The juice was titrated against 0.1 N NaOH. A curve of citric acid was used as a standard to calculate TA in percent [45].

2.16.2. Total soluble solids (TSS) determination

The juice of 'Kinnow' flesh was extracted and thoroughly homogenized in a beaker. Then the TSS content was determined with a hand refractometer (Atago PAL—I) and expressed as percent [45].

2.16.3. Determination of ripening index

The ripening index was determined by taking TSS and TA values at each interval of sampling. Briefly, the concentration of TSS was divided by TA to determine the ripening index of 'Kinnow' fruits [45].

2.16.4. Ethanol content

The ethanol content in 'Kinnow' mandarin fruits juice was determined with the protocol of Valencia-Chamorro et al. [48]. Briefly, 5 mL juice (taken from a composite sample of 15 fruits) was transferred to 10 mL vial containing silicon septum seals/crimp-top caps. The one mL sample from the headspaces of the vials was taken (from the previously equilibrated vials for 60 min in the water bath at 20 °C followed by incubation for 15 min at 40 °C) to establish the equilibrium in the headspaces and then injected in gas chromatograph with auto-sampler (HS-2000, Thermo Fisher Scientific) with flame ionization detector. The retention times were used for ethanol identification by comparing with the standard and it was expressed as mg L^{-1} of juice.

2.17. Sensory evaluation

The sensory analysis was carried out with a hedonic scale. The scale ranged from 1 to 9 and sensory attributes were assessed on each period of sampling. The mandarin fruits were peeled manually and presented to the panelists in a randomized form. The sensory attributes such as taste, flavor, aroma and overall quality were evaluated. The result ratings were then presented as a score [45].

2.18. Data analysis

The data obtained were statistically analyzed by one-way analysis of variance. The mean comparison among the treatments was carried out by using the least significant difference (LSD) test. The differences among the various treatment means were considered significant at 5 % probability level. Heat map clustering was performed with ClustVis online software and principal component analysis (PCA) was carried out with XLSTAT (Addinsoft, NY, USA).

3. Results and discussion

3.1. Fruit weight loss

Fruit weight loss (FWL) was significantly increased with progressed storage period irrespective of treatments (Fig. 1A). Nevertheless, the increase in FWL of mandarins was substantially higher in controls in comparison with GABA and AG coating treatments as well as in AG + GABA combination. The AG coating and AG + GABA combination treatments were most efficient in reducing the weight loss of mandarins. In general, the differences regarding FWL between AG coating and GABA treatments were non-significant at all sampling intervals. After 60 days of storage, FWL in AG + GABA treated fruits was lower as compared with controls (Fig. 1A). The excessive FWL generally results in the symptoms of wrinkling/shriveling on the fruits [14]. This ultimately results in a significantly increased economic loss to growers and supply chain stakeholders that deal with fresh fruits [49]. The AG forms a thin layer/film on the coated fruits and acts as an effective barrier ultimately resulting in inhibited desiccation which thereby leads to a significant reduction in mass loss eventually leading to substantially lower FWL. The AG-coated group exhibited reduced FWL probably owing to a coating-based barrier formed between the fruits and the keeping environment [50]. On the other side, GABA suppresses metabolic acidities i.e. respiration rate and ethylene biosynthesis which reduces the senescence of treated produce and inhibits FWL. The combined AG + GABA treatment exhibited the lowest FWL in 'Kinnow' which could be due to GABA-induced suppression of metabolic activities and AG-based desiccation reduction in the treated mandarins.

3.2. Disease incidence

The fruits of the treatments including alone GABA and AG as well as

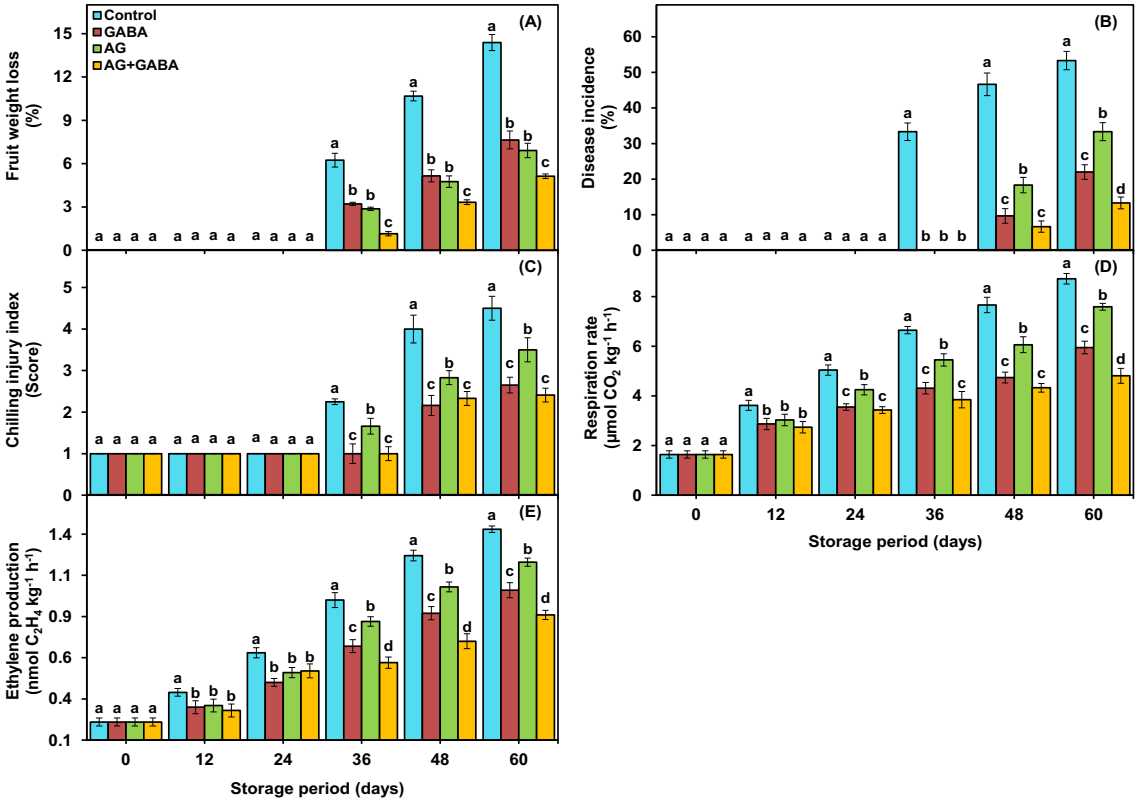

Fig. 1. The effect of combined application of Arabic gum and γ-aminobutyric acid on weight loss (A), disease incidence (B), chilling injury index (C), respiration rate (D) and ethylene production (E) of 'Kinnow' fruits. The different letters represent significant ($P \leq 0.05$) differences based on least significant difference (LSD) test. Data are mean of three replications and vertical bars show standard error of the means.

AG + GABA combination did not show disease incidence till day-36 of the storage time (Fig. 1B). In contrast, non-coated controls showed disease incidence after 36 days. In contrast to controls, GABA-treated and AG-coated fruits showed a substantially lower percentage of disease incidence but it was higher in contrast with AG + GABA combined treatment. On day-60, disease incidence in AG + GABA treated fruits was significantly lower as compared to the non-treated group (Fig. 1B). Postharvest diseases are the leading reasons of high economic losses during supply chains of fruits [51]. The coating acts as a barricade between the produce and pathogens eventually resulting in a suppressed incidence of disease in treated commodities [1]. The edible coatings based on polysaccharides form a thin layer on the surface ultimately resulting in the repulsion of the disease-causing pathogens [2]. The AG coating probably acted as a barricade that protected the coated 'Kinnow' from the disease-causing pathogen and suppressed disease infection. The GABA acts directly in the induction of the host-mediated resistance against the postharvest fungal pathogens of fruits [52]. In our case, GABA probably enhanced the resistance of 'Kinnow' and AG acted as a barrier between treated fruits and pathogens. So, the better control of disease incidence could be attributed to the antifungal activity of GABA [53] and the barricade potential of AG coating thereby substantially reducing the number of disease-infected fruits.

3.3. CI symptoms

The CI incidence was progressively increased in untreated and AG-coated fruits and reached to the highest level on day-60 (Fig. 1C). However, no CI was observed in GABA and AG + GABA treated 'Kinnows' till 36th day and progressively increased thereafter from 4th to 60th day. Generally, CI index was substantially lower in fruits treated with alone GABA and AG + GABA treatment after 60 days compared with controls (Fig. 1C). Cold storage leads to CI symptoms which deleteriously influences fresh horticultural produce such as 'Kinnow' mandarin and results in the reduction of visual quality [1]. The AG edible coating protects against CI disorder due to the formation of a protective layer over the treated fruits due to protection of the membrane and inhibition of lipid peroxidation [11]. Etemadipoor et al. [54] also found that AG coating reduced the incidence of CI in guava fruits. It is well established that GABA possesses anti-chilling properties and protects the treated fresh horticultural produce against CI generally by acting as an osmolyte and membrane protector under low-temperature conditions [21]. The GABA protects the cell membranes by reducing lipid peroxidation and leakage of the electrolytes due to suppression of PLD and LOX enzyme activity which degrade membrane lipids structure [21]. In this work, GABA markedly reduced CI and the impact was significantly better in combined AG + GABA treated 'Kinnow' fruits which could be attributed to suppressed membrane permeability, reduced oxidative damage and higher antioxidative system.

3.4. RPR and EPR

The RPR and EPR progressively increased in untreated fruits and reached to a maximum on day-60 (Fig. 1D and E). On the other side, AG coating, GABA and AG + GABA treatments showed lower RPR and EPR throughout the storage. Interestingly, alone GABA treated 'Kinnow' exhibited substantially lower RPR and EPR in comparison with AG coated group. On day-60, RPR and EPR in 'Kinnow' fruits were significantly lower in the combined AG + GABA treated group as compared with the control (Fig. 1D and E).

The gums-based edible coatings generally alter the internal gaseous composition of the treated fruits [51,55,56]. The coating application reduces the availability of O_2 thereby reducing the biosynthesis of ethylene (as this process needs O_2 for the ethylene biosynthesis reaction at the final step). The reduced ethylene production ultimately suppresses the respiratory rate which eventually helps in storage life extension [57]. On the other side, GABA reduces ethylene production by down-regulating its biosynthesis [57]. The down-regulation of ethylene ultimately reduces the respiration rate of the treated fruits [57]. The lower RPR and EPR in AG + GABA treatment could be attributed to the modified internal 'Kinnow' atmosphere which helped to suppress ethylene biosynthesis that subsequently reduced respiration rate. On the other side, GABA acted as an ethylene-antagonist which inhibited its (ethylene) biosynthesis and reduced the rate of respiration in contrast with the control. The reduced RPR and EPR were also observed in AG-coated mango [51] and guar gum-coated 'Valencia' oranges [55], as well as GABA, treated apple [57], pistachio [58] and kiwifruit [59].

3.5. REL and MDA

The REL of treated fruits was increased at a significantly higher rate during storage and reached a maximum concentration on day-60 (Fig. 2A). In general, AG-coated, GABA treated and fruits subjected to the combination of AG and GABA showed significantly less REL as compared with control. The fruits of 'Kinnow' treated with a combined application of AG and GABA exhibited substantially lower REL in contrast with untreated control after 60 days (Fig. 2A). Oxidative stress in terms of overproduction of H_2O_2 and $O_2^{\bullet-}$ damages the cellular membranes and eventually results in the stimulated REL in chilling stressed fruits [21] and vegetables [60]. The exogenous AG coatings-based treatment inhibits REL due to reduced oxidative damage [18]. In addition, osmolytes such as GABA-based exogenous application safeguard the cold-stored fruits from chilling stress and reduces the REL [21]. The GABA-induced lower REL shows better chilling tolerance with extended storage potential of produce [29]. In the present work, REL was significantly lower in alone GABA treatment as well as in GABA-+AG-treated 'Kinnow' fruits. The reduced REL could be ascribed to AG and GABA induced inhibited oxidative stress which ultimately conserved membrane integrity and showed better chilling tolerance in treated 'Kinnow' fruits.

MDA content showed a progressive increase in the peel of untreated fruits throughout 60 days of storage (Fig. 2B). Nonetheless, fruits treated with AG, GABA and combined AG + GABA revealed a markedly less MDA concentration increase in contrast with control. The alone AG coating was not much effective in inhibiting the increase of MDA as compared with GABA and a combination of AG and GABA treatment. Compared with the control, the increase in MDA was markedly less in AG + GABA treated fruits after 60 days of storage (Fig. 2B). The increased ROS including $O_2^{\bullet-}$ and H_2O_2 generation enhances the production of MDA due to membrane lipids disintegration. The MDA generally increases owing to chilling-induced damages to cell membranes under cold stored conditions [11]. Therefore, to suppress the accumulation of MDA, some exogenous treatments which could conserve membrane integrity are required in stored horticultural produce under chilling conditions. In the earlier reports, the edible coatings-based treatment efficiently reduced the MDA generation in 'Kinnow' fruits which could be attributed to the protection provided by the coatings [1]. Similarly, AG coating inhibited MDA production as noted in coated mango [14] and apricot [18]. The inhibited lipid peroxidation could be attributed to AG coating-induced suppressed oxidative damage which thereby reduced MDA production. On the other side, GABA protects the membranes of cold-stored fresh horticultural produce by suppressing the activity of LOX and PLD enzymes thereby inhibiting the increase in MDA accumulation [21]. In our present work, alone GABA treatment and combination of AG with GABA substantially suppressed the MDA accumulation in stored 'Kinnow' fruits which could be attributed to the protective effect of GABA (as an osmoprotectant and membrane stabilizer) that was further improved possibly due to the improved effect of AG coating.

3.6. H_2O_2 and $O_2^{\bullet-}$ contents

The H_2O_2 and $O_2^{\bullet-}$ contents of untreated fruits were increased at a

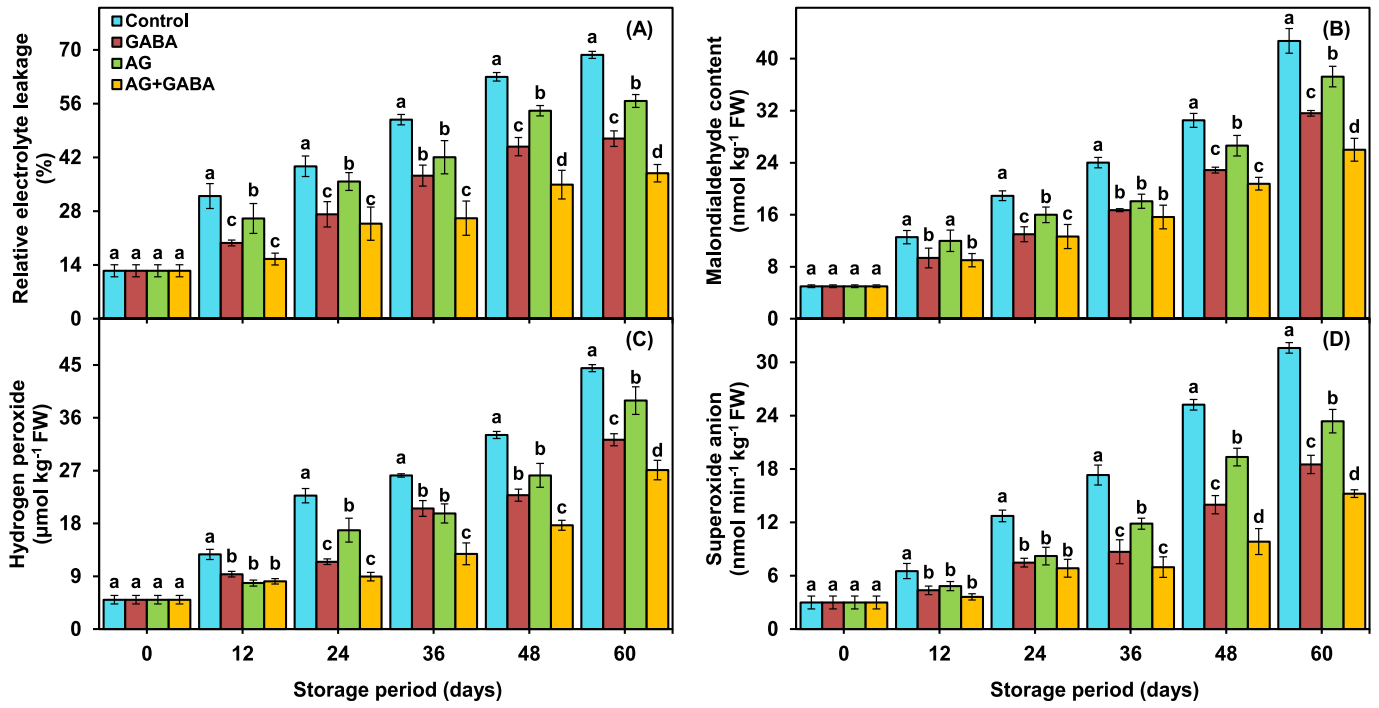

Fig. 2. The effect of combined application of Arabic gum and γ-aminobutyric acid on relative electrolyte leakage (A), malondialdehyde (B), hydrogen peroxide (C) and superoxide anion (D) contents in flesh of 'Kinnow' fruits. The different letters represent significant ($P \leq 0.05$) differences based on least significant difference (LSD) test. Data are mean of three replications and vertical bars show standard error of the means.

significantly higher rate from day-12 to day-60 (Fig. 2C and D). On the whole, AG coated, GABA treated and fruits subjected to combinational treatment based on AG coating and GABA showed significantly reduced H_2O_2 and $O_2^{\bullet-}$ as compared with control. The fruits of 'Kinnow' treated with AG + GABA showed markedly lower H_2O_2 and $O_2^{\bullet-}$ compared with untreated control after 60 days of storage (Fig. 2C and D).

The increased H_2O_2 and $O_2^{\bullet-}$ accumulation causes damage to cell membranes and leads to oxidative stress [14,21]. In addition, increased production of H_2O_2 and $O_2^{\bullet-}$ stimulates membrane peroxidation of lipids which has been found associated with symptoms of CI resulting due to integrity loss of the membranes [61]. Therefore, these alterations have been found to correlate with the senescence of the fruits and generally appear as a physiological disorder (CI) during chill storage. The application of AG coating reduces the production of ROS due to the safeguard of the membrane as noted in mango [14] and apricot [18] fruits. GABA is a non-protein amino acid and falls under the category of solutes of compatible nature which generally delays ROS production and enhances endogenous GABA accumulation under chilling stress [21]. The increased biosynthesis and/or maintenance of higher endogenous GABA ultimately protect the treated produce from ROS-induced oxidative stress [21]. In addition, GABA up-regulates the antioxidant enzymes along with enhanced non-enzymatic antioxidants which collectively help in oxidative stress (H_2O_2 and $O_2^{\bullet-}$) alleviation [21,61]. In this work, combined AG + GABA treatment was most effective in alleviating CI symptoms in 'Kinnow' fruits and imparted better chilling tolerance in comparison with their sole applications.

3.7. LOX and PLD activity

The activity of LOX and PLD enzymes was increased at a significantly higher rate in untreated fruits of 'Kinnow' as compared with alone GABA and AG coating treatments as well as in the combination of AG + GABA during storage (Fig. 3A and B). Generally, alone AG coating application exhibited higher LOX and PLD activity in contrast with sole GABA and combined AG and GABA treatment. Overall, the combined AG + GABA treated fruits group showed lower activity of LOX and PLD enzymes

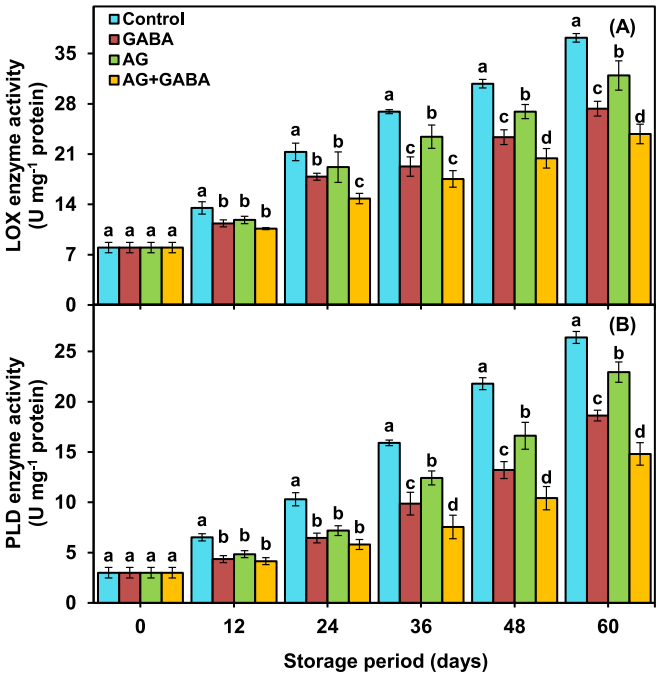

Fig. 3. The effect of combined application of Arabic gum and γ-aminobutyric acid on LOX (A) and PLD (B) enzymes activity in flesh of 'Kinnow' fruits. The different letters represent significant ($P \leq 0.05$) differences based on least significant difference (LSD) test. Data are mean of three replications and vertical bars show standard error of the means.

throughout the storage. After 60 days, LOX and PLD activity of 'Kinnow' fruits was substantially lower in combined AG + GABA treatment as compared with control (Fig. 3A and B).

Different enzymes such as LOX and PLD play a critical role in the degradation of the membrane lipids [62]. The increased PLD and LOX

activity ultimately contributes to increased CI occurrence owing to membrane lipids degradation and stimulated membrane permeability [63]. In this work, AG coating and GABA treatments (alone and in combined form) substantially reduced the activities of LOX and PLD enzymes. The lower LOX and PLD activity in GABA+AG treated 'Kinnow' fruits might be due to reduced CI and subsequently inhibited senescence as chilling stress negatively affects the membrane integrity and increases the contact of degrading enzymes (LOX and PLD) with their substrates (lipids) and increases the membrane leakage. So, the reduced PLD and LOX activity ultimately resulted in conserved membrane stability (indicated by reduced membrane permeability) and suppressed lipid peroxidation (expressed by lower MDA content) which subsequently enhanced the chilling tolerance of the treated 'Kinnow' fruits.

3.8. GAD and GABA-T activity and endogenous GABA content

The endogenous GABA content and GAD enzyme activity increased in all treatments from day-12 to day-36 and then declined till day-60 (Fig. 4A and B). The increase in the content of endogenous GABA and GAD activity was substantially higher in all treated fruits of 'Kinnow' as compared with the control (Fig. 4A and B). The highest increase was noted in AG + GABA combination treated fruits and the lowest increment was observed in alone AG coating treatment. After 60 days, fruits

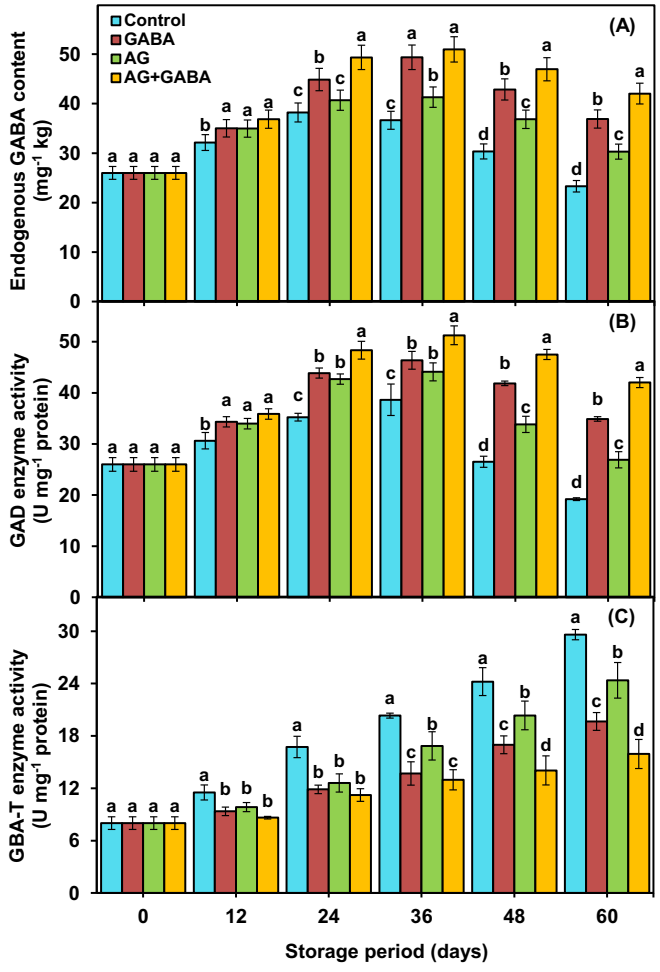

Fig. 4. The effect of combined application of Arabic gum and γ-aminobutyric acid on endogenous GABA content (A), GAD (B) and GABA-T (C) enzymes activity in flesh of 'Kinnow' fruits. The different letters represent significant ($P \leq 0.05$) differences based on least significant difference (LSD) test. Data are mean of three replications and vertical bars show standard error of the means.

of 'Kinnow' treated with AG + GABA combinational treatment showed the highest endogenous GABA concentration and activity of GAD enzyme in contrast with the untreated group (Fig. 4A and B).

The activity of GABA-T enzyme was increased in untreated fruits at a significantly higher rate and reached to a maximum on day-60 (Fig. 4C). In general, GABA and AG + GABA treatments showed lower GABA-T activity and alone AG coating exhibited relatively higher GABA-T activity throughout the storage. On day-60, the activity of GABA-T enzyme in fruits of 'Kinnow' was substantially lower in AG + GABA combination treated group as compared with the control (Fig. 4C).

It has been reported that GABA is an important metabolite in response to multiple types of stresses including chilling stress [21]. It protects the produce from oxidative damage and maintains osmotic balance. The increased GABA accumulation normally protects the plasma membranes from the chilling-induced negative effects ultimately showing reduced CI symptoms [25]. The catalytic conversion of glutamate into GABA is mediated by GAD enzyme. In contrast, GABA-T mediates the conversion (reversible) between succinic semialdehyde and GABA [64]. In this work, GAD activity was stimulated and GABA-T activity was suppressed in response to exogenous GABA application which resulted in a continuous supply of endogenous GABA generation and helped to alleviate CI in 'Kinnow' fruits. In addition, the AG application also resulted in the suppression of GABA-T and induction of higher GAD activity. The effect was significantly increased with the combination of GABA with AG coating. This suggests that the suppression of GABA-T and activation of GAD activity was directly mediated in response to exogenous GABA treatment as reported previously [54]. Based on these findings, we very cautiously speculate that exogenous GABA and AG coating probably conserved the cell membrane integrity and alleviated CI symptoms through the regulation of endogenous GABA metabolism.

3.9. Cell walls components

The WSP content was increased in untreated fruits at a significantly higher rate throughout the storage from 12th to 60th day (Fig. 5A). However, it was significantly lower in AG, GABA and combined AG + GABA treatments. Averagely, GABA and AG + GABA treatments exhibited a lower increase in WSP compared with alone AG coating treatment. On day-60, WSP content in fruits of 'Kinnow' treated with AG + GABA combination was substantially lower than control (Fig. 5A).

The concentration of CSP, NCSP and PRP was decreased significantly in untreated fruits as compared with alone GABA and AG coating as well as in combined AG + GABA treatment during storage (Fig. 5BCD). Generally, alone AG coating application exhibited lower suppression in the decrease of CSP, NCSP and PRP in contrast with sole GABA application and combination of AG and GABA. After 60 days, CSP, NCSP and PRP contents were markedly higher in 'Kinnow' fruits treated with AG + GABA combination as compared with control (Fig. 5BCD).

3.10. Firmness and activity of cell wall degrading enzymes

Firmness was decreased in untreated fruits at a significantly higher rate throughout the storage of 60 days (Fig. 6A). On the other side, the reduction in firmness was substantially lower in AG, GABA and combined AG + GABA treated fruit groups. On an average, GABA and AG + GABA treated fruits of 'Kinnow' exhibited the lowest decrease in firmness as compared with alone AG coating treatment. After 60 days, firmness of 'Kinnow' fruits treated with AG + GABA combination was substantially higher in comparison with the control (Fig. 6A).

The activity of CX, PG and β-Gal enzymes was significantly increased in untreated fruits of 'Kinnow' as compared with alone GABA and AG coating as well as combined AG + GABA treatment (Fig. 6BCD). The AG coating treatment exhibited higher CX, PG and β-Gal activity in contrast with alone GABA and combined AG and GABA treatments throughout the storage. After 60 days, CX, PG and β-Gal enzymes activity of 'Kinnow' fruits was substantially lower in combined AG + GABA treatment

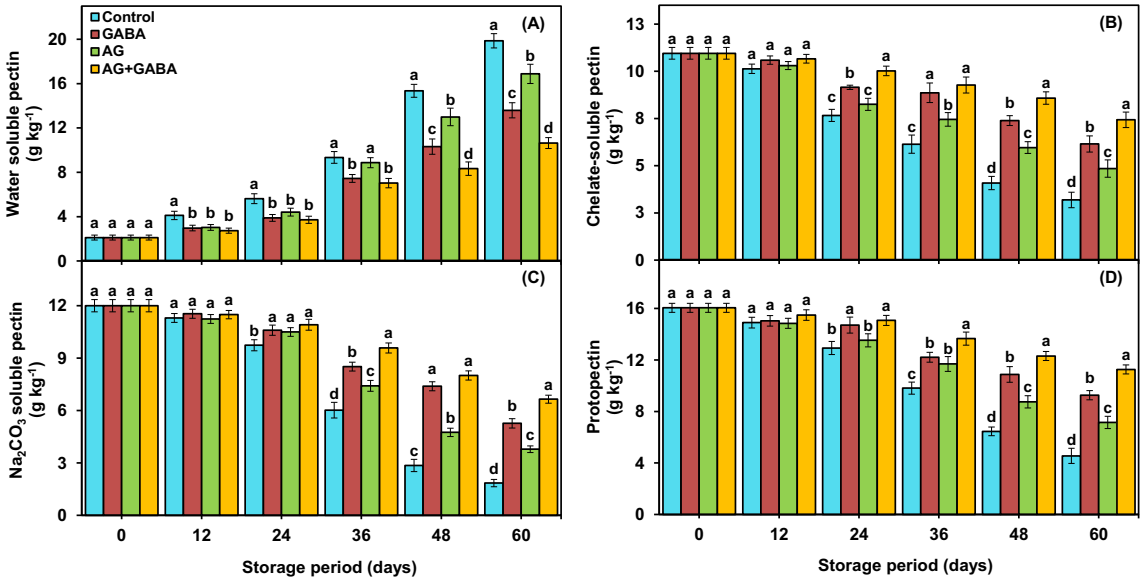

Fig. 5. The effect of combined application of Arabic gum and γ-aminobutyric acid on water-soluble pectin (A), chelate-soluble pectin (B), Na_2CO_3-soluble pectin (C) and protopectin (D) contents in flesh of 'Kinnow' fruits. The different letters represent significant ($P \leq 0.05$) differences based on least significant difference (LSD) test. Data are mean of three replications and vertical bars show standard error of the means.

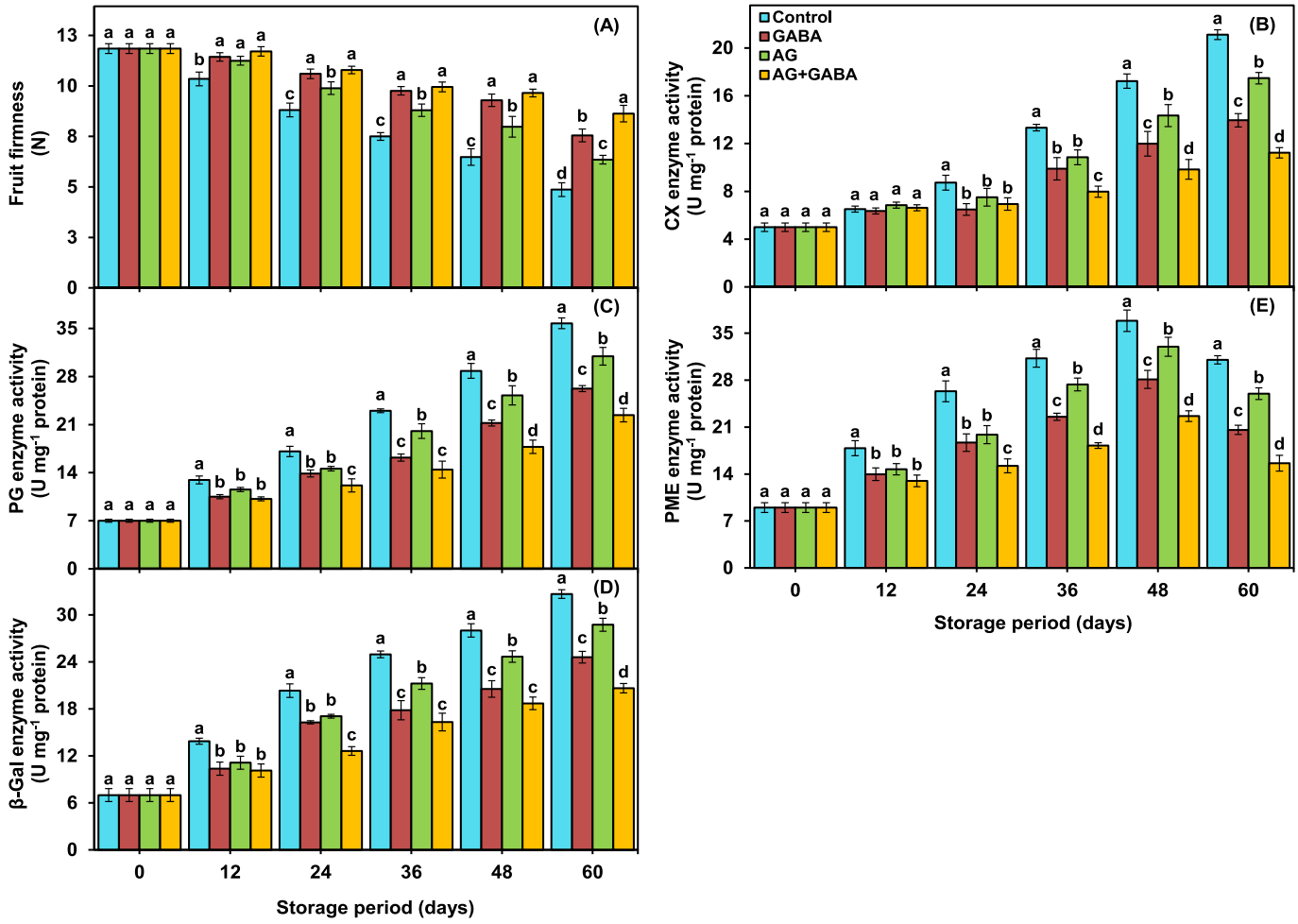

Fig. 6. The effect of combined application of Arabic gum and γ-aminobutyric acid on firmness (A), CX (B), PG (C), β-Gal (D) and PME (E) enzymes activity in flesh of 'Kinnow' fruits. The different letters represent significant ($P \leq 0.05$) differences based on least significant difference (LSD) test. Data are mean of three replications and vertical bars show standard error of the means. CX = Cellulase, PG = Polygalacturonase, β-Gal = β-galactosidase and PME = Pectin methylesterase.

as compared with control (Fig. 6BCD). On the other hand, PME activity was increased from day-12 to day-48 in all treatments and then decreased on day-60 (Fig. 6E). Nevertheless, the increase was substantially higher in the untreated group compared with AG, GABA and AG + GABA treatments. The results exhibited that AG alone treatment inhibited the increase in PME activity at a lower rate compared with GABA and AG + GABA treatments (Fig. 6E). After 60 days, the activity of PME enzyme was significantly lower in fruits of 'Kinnow' treated with AG + GABA combination in contrast with control (Fig. 6E).

The chilling stress alters the normal metabolic activity of stored fruits thereby disrupting the structural integrity (owing to pectin degradation) of the membrane tissues [25,65]. Citrus fruits are a rich source of pectin and it is well-established that pectin is a primary component of cell membranes and these are considered crucial for the structural integrity of the fruit tissues under chilling stress [7,66]. It has been reported that reduced pectin is closely associated with fruit softening and the degradation of pectin content. Different enzymes such as CX, PG, β-Gal and PME collectively work to degrade pectin and cell walls during postharvest storage [2,67]. The pre-storage coating such as AG application suppresses the hydrolases enzymes (CX, PG, β-Gal and PME) activity and delays softening [18]. In the same way, GABA also inhibits the activity enhancement of hydrolases thereby protecting the pectin from degradation. In the current work, AG coating, GABA and a combination of GABA with AG substantially suppressed the activity of hydrolase enzymes which protected the cell wall polysaccharides (such as CSP, NCSP and PRP) from the disassembly thereby resulting in suppressed cell walls degradation and exhibited reduced CI symptoms owing to the conserved structural integrity of cell walls in stored 'Kinnow' fruits.

3.11. Antioxidative enzymes activities

The activity of APX, CAT and SOD enzymes was decreased progressively in all treatments under storage (Fig. 7ABC). Nevertheless, the decrease was markedly higher in untreated fruits as compared with alone AG and GABA treatments as well as in the combined AG and GABA treated group (Fig. 7ABC). The activities of all these antioxidative enzymes generally decreased at a higher rate in the alone AG-coated group as compared with GABA and combined AG + GABA treated 'Kinnow' fruits throughout the storage. After 60 days, APX, CAT and SOD activity of 'Kinnow' fruits was substantially lower in combined AG + GABA treatment as compared with control (Fig. 7ABC).

The activity of POD enzyme increased in all treatments from day-12 to day-36 and then declined till day-60 (Fig. 7D). The increase in POD activity was substantially higher in all treated fruits of 'Kinnow' as compared with the control (Fig. 7D). The highest POD activity increase was noted in AG + GABA combination treated fruits and lowest increment was noted in alone AG coated group. After 60 days, 'Kinnows' treated with AG + GABA combination showed the highest POD enzyme activity in contrast with the untreated group (Fig. 7D).

The antioxidative enzyme system is considered very critical to suppress oxidative damage in citrus fruits in response to chilling stress-induced senescence [68]. The exogenous application of edible coatings on fruits reduces senescence along with the stimulation of antioxidative enzyme activities to suppress oxidative stress [18,69,70]. In addition, edible coating such as AG reduces the ripening and delays the senescence of the coated fruits [18]. The delayed senescence ultimately helps in the maintenance of higher activity of the antioxidative enzymes [14]. Similarly, GABA treatment stimulates antioxidative activities ultimately resulting in significantly enhanced activity of the antioxidant enzymes [21]. In this study, the activity of antioxidative enzymes enhanced in response to both AG and GABA application but the increment was much higher in the combined treatment. This could be attributed to the GABA and AG-induced stimulation in the activity of these enzymes which ultimately helped in the alleviation of ROS-based oxidative damage and chilling tolerance of cold stored fruits of 'Kinnow' mandarin with conserved quality.

3.12. Non-enzymatic antioxidants

3.12.1. DPPH scavenging activity

DPPH scavenging activity (DPPH-SVA) in the flesh of fruits progressively decreased with increased storage period regardless of the

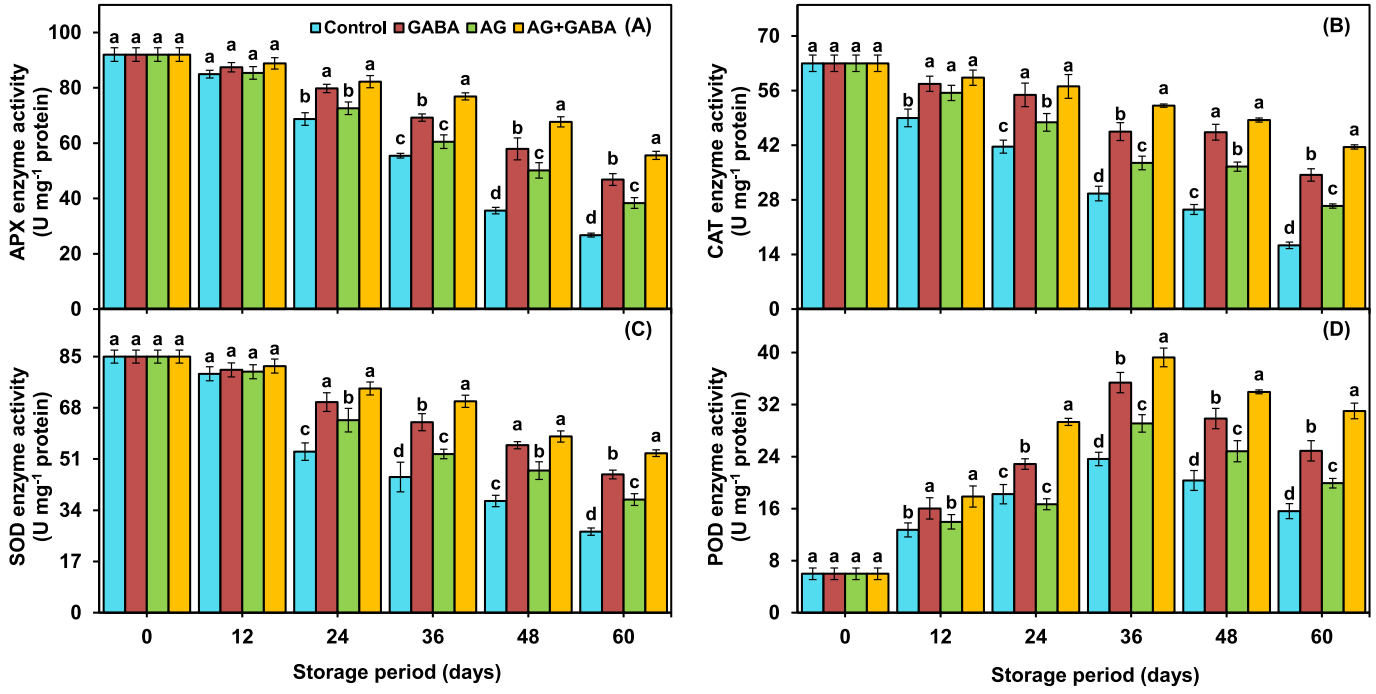

Fig. 7. The effect of combined application of Arabic gum and γ-aminobutyric acid on APX (A), CAT (B), SOD (C) and POD (D) enzymes activity in flesh of 'Kinnow' fruits. The different letters represent significant ($P \leq 0.05$) differences based on least significant difference (LSD) test. Data are mean of three replications and vertical bars show standard error of the means. AXP = Ascorbate peroxidase, CAT = Catalase, SOD = Superoxide dismutase and POD = Peroxidase.

treatments (Fig. 8A). The reduction in DPPH-SVA was significantly higher in untreated control in contrast with AG, GABA and AG + GABA treated fruits. The AG alone coating was relatively less effective in inhibiting the reduction of DPPH-SVA in contrast with GABA and AG + GABA treatments. After 60 days, DPPH-SVA in AG + GABA treated fruits was substantially higher in comparison with the untreated group (Fig. 8A). The DPPH-SVA generally decreases due to the oxidation and consumption of antioxidant compounds during the scavenging of ROS in the stored fruits [2,68]. Higher production of free radicals leads to a marked decline in antioxidant compounds upon rapid senescence [14]. A substantially higher phenolics and AAC reflect the higher DPPH-SVA [51,69]. In the present work, the concentration of AAC and total phenolics remained higher in AG and GABA-treated fruits. In addition, both AG and GABA suppressed the production of ROS and conserved higher antioxidative enzymes activity. The reduced ROS production and higher conservation of enzymatic and non-enzymatic antioxidants in AG + GABA treated 'Kinnow' probably contributed to higher DPPH-SVA. The higher DPPH-SVA thereby positively contributed to the improved chilling stress tolerance of 'Kinnow' possibly by contributing to ROS scavenging in combination with antioxidative enzymes.

3.12.2. Total phenolics

The concentration of total phenolics exhibited a gradual reduction from day-12 to day-60 compared with its initial concentration on day-0 (Fig. 8B). However, the reduction in total phenolics concentration was markedly higher in the untreated control. The AG alone coating was relatively less effective in impeding the total phenolics reduction in contrast with AG + GABA and GABA treatments. On day-60, the total phenolics concentration in AG + GABA treated fruits of 'Kinnow' mandarin was considerably higher compared with the untreated group (Fig. 8B).

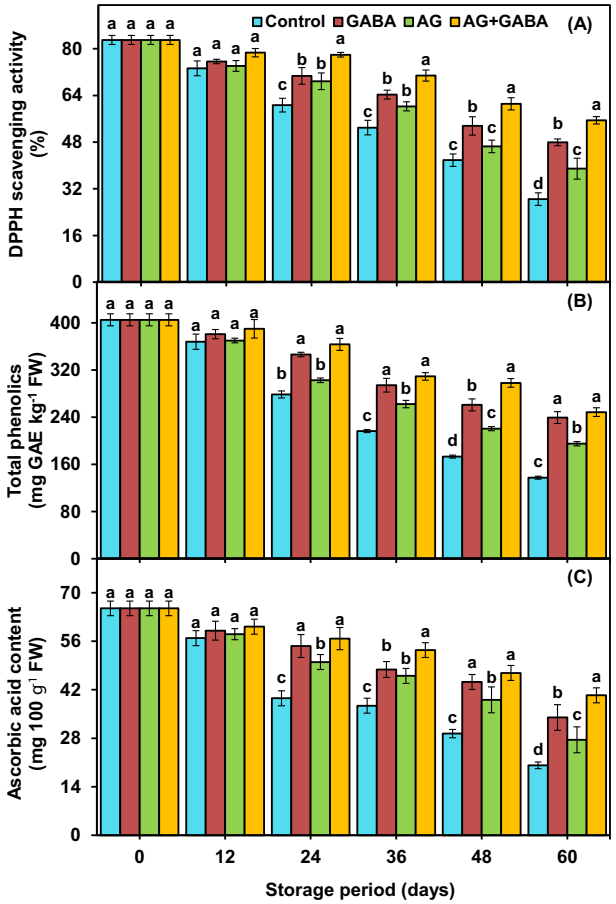

Fig. 8. The effect of combined application of Arabic gum and γ-aminobutyric acid on DPPH scavenging activity (A), total phenolics (B) and ascorbic acid content (C) in flesh of 'Kinnow' fruits. The different letters represent significant ($P \leq 0.05$) differences based on least significant difference (LSD) test. Data are mean of three replications and vertical bars show standard error of the means.

The concentration of total phenols declines under postharvest conditions in vegetables and fruits upon their oxidation [2,60]. The AG coating treatment offers a protective barrier and suppresses the availability of oxygen which ultimately reduces enzymes based process of oxidation to suppress the degradation of phenolic compounds [18]. Similarly, the treatment based on GABA also conserves higher phenolics concentration due to the suppression of auto-oxidation which is considered the main factor in the oxidative reduction of phenols [21]. Therefore, in the current findings, the higher conservation of total phenols in AG + GABA treated fruits of 'Kinnow' could be due to suppressed oxygen presence (due to AG coating-based barrier development) and inhibited oxidation.

3.12.3. Ascorbic acid content

Ascorbic acid content (AAC) in 'Kinnow' fruits was progressively reduced with extended storage time (Fig. 8C). Nevertheless, the decrease in AAC was considerably higher in untreated control in contrast with AG, GABA and AG + GABA treated fruits. The combined AG + GABA application was most effective in suppressing AAC decline as compared with GABA and AG treatments. On day-60, AAC in AG + GABA treated fruits was substantially higher than the untreated control (Fig. 8C). AAC is a non-enzymatic antioxidant that is susceptible to auto-oxidation based reduction in the presence of oxygen [71]. The coating treatment reduces AAC reduction due to inhibited availability of oxygen for auto-oxidation due to thin semipermeable layer formation which in turn decreases the oxygen availability [18,72]. The AG coating-induced reduction in the oxidation ultimately helps to conserve its (AAC) higher levels due to suppressed oxidative breakdown [18]. On the other side, GABA also inhibits AAC oxidation and increases its accumulation in different fruits [21,26]. So, in our case higher AAC retention in 'Kinnow' fruits could be due to AG and GABA application as the said treatments probably suppressed oxidation-based degradation of AAC till the end of storage.

3.13. Biochemical attributes and ethanol content in juice

3.13.1. Titratable acidity

Titratable acidity (TA) significantly decreased in the juice of untreated fruits with the progressed storage time from day-12 to day-60 (Fig. 9A). However, the decrease in TA of AG coated as well as GABA and AG + GABA treated fruits was significantly lower as compared with untreated control. After 60 days, juice TA in combined AG + GABA treated fruits was substantially higher as compared with the control (Fig. 9A). The content of TA is imperative in maintaining the general eating quality of citrus fruits such as mandarins and tangerines [1,68]. TA declines due to a decrease in organic acids and oxidation-induced reactions under postharvest conditions [43]. The overall concentration of TA generally decreases under chilled storage as the organic acids are normally used to sustain the metabolic activities in citrus fruits [7,66,72]. The coatings application on fruits impedes the reduction of organic acids and conserves higher TA content [18,73]. The delayed reduction of organic acids takes place owing to AG-based suppression of metabolic activity (such as ethylene and respiration rates) which eventually helps in the conservation of higher TA concentration. On the other hand, the osmolytes-based treatment such as GABA application also conserves higher TA by reducing the decline of organic acids consumed during metabolic activity [21]. In our current research, combined AG and GABA-based treatment proved to be most efficient in delaying the decline of TA in fruits of 'Kinnow' mandarins throughout the storage. The reduced degradation of TA in alone AG, GABA and combined AG and GABA treatments could be ascribed to delayed breakdown or

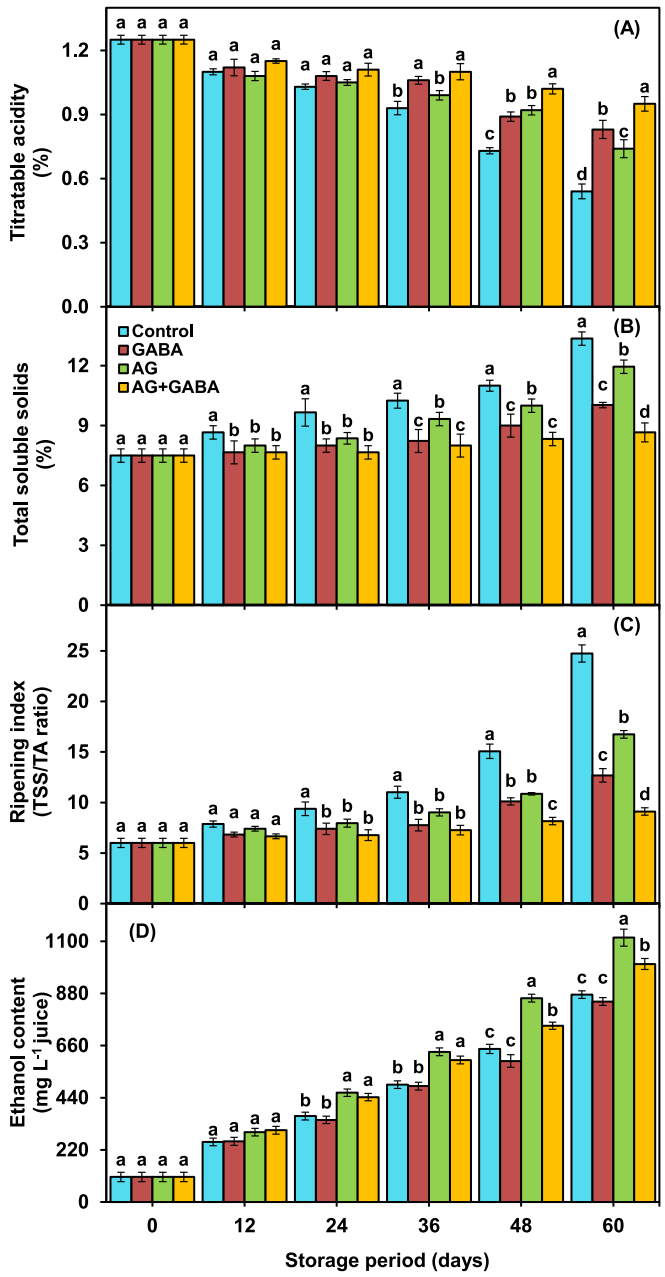

Fig. 9. The effect of combined application of Arabic gum and γ-aminobutyric acid on titratable acidity (A), total soluble solids (B), ripening index (C) and ethanol content (D) in flesh of 'Kinnow' fruits. The different letters represent significant ($P \leq 0.05$) differences based on least significant difference (LSD) test. Data are mean of three replications and vertical bars show standard error of the means.

degradation of the organic acids and decreased metabolic activities which eventually resulted in conserved TA of 'Kinnow' fruits.

3.13.2. Total soluble solids

Total soluble solids (TSS) exhibited an increasing trend in untreated control fruits and reached to the highest concentration on day-60 (Fig. 9B). On the other hand, no significant difference regarding the TSS was noted in AG, GABA and AG + GABA treatments till day-24. On the whole, GABA and AG + GABA treatments were appropriate in suppressing the TSS increase compared with AG coating application (Fig. 9B). After 60 days, AG + GABA treated 'Kinnow' fruits exhibited the lowest TSS concentration as compared with the untreated group. The TSS has been found to increase due to the conversion of complex sugars (carbohydrates) into simple units [7,66,68,74]. The AG edible coating application impedes the breakdown of these complex sugars and starch, therefore delaying the TSS increase in the coated fruits [11,18]. In the same way, GABA application also suppresses the complex starch breakdown into sugars thereby eventually resulting in a lower increase in TSS of the treated fruits [21]. The delayed increase in TSS has generally been found associated with suppressed ripening and senescence of AG-coated [11,18] and GABA-treated fruits [21]. In the present work, AG and GABA application markedly reduced the increase in TSS content. It was observed that combined AG and GABA treatment showed the most promising result in suppressing the TTS content increase. This could be attributed to a better efficacy of combined AG and GABA treatment which efficiently worked to delay the increase in TTS content of the 'Kinnow' fruits kept under chilling conditions.

3.13.3. Ripening index

The ripening index exhibited incessant increase during the whole period of 60 days in all treatments (Fig. 9C). However, the ripening index increase was substantially higher in untreated control in contrast with AG, GABA and combined AG + GABA treatments (Fig. 9C). The combination of AG + GABA treatment was most efficient in reducing the increase of ripening index as compared with their alone treatments. After 60 days, the ripening index in AG + GABA treatment was substantially lower as compared with untreated 'Kinnow' fruits (Fig. 9C).

The ripening index is an imperative parameter that shows the ripening extent and postharvest senescence during the storage of fruits including mandarins and tangerines [1,5,59]. It shows an increase owing to the reduction of organic acids and increment of sugars in stored fruits [59]. Its rapid increase shows the prompt ripening and subsequent senescence. The advanced stage of ripening and senescence ultimately leads to excessive sweetness and altered taste of citrus fruits [1,68]. The AG coating and GABA application significantly suppressed the increase in TSS and degradation of TA. The delayed TSS increase in AG + GABA treated 'Kinnow' could be attributed to suppressed senescence and higher TA content might be owing to reduced organic acids reduction due to suppressed metabolic activity. It has been reported that AG and GABA treatments inhibit the decrease in TA and suppress increment in TSS content, thereby leading to a substantially lower increase in the ripening index of the stored fruits as noted in banana and aonla [11,21]. Therefore, the lower increase in ripening index could be attributed to AG and GABA-induced suppression of TSS increase along with delayed degradation of TA content.

3.13.4. Ethanol content

No statistically significant difference was noted regarding the ethanol content of 'Kinnow' fruits on day-12. Thereafter, ethanol content progressively increased in the juice of 'Kinnow' fruits from 24th to 60th day (Fig. 9D). Generally, the highest ethanol content increase was noted in AG coated group in contrast with the untreated control. Interestingly, GABA-treated fruits exhibited the lowest ethanol content as compared with the untreated control and AG + GABA group (Fig. 9D). It is important to mention that it (GABA) even suppressed ethanol increase in AG + GABA treated 'Kinnow' group. On day-60, ethanol in the juice of 'Kinnow' was lowest in GABA treated group as compared with other treatments (Fig. 9D).

Ethanol is a volatile compound that has been found to change greatly in edible coating-treated citrus during storage [75]. The production of ethanol generally increases in the stored citrus and has been found associated with the development of off-flavor if exceeds the optimal (2000 mg L^{-1}) limit. In our case, AG-coated and AG + GABA-treated mandarins showed significantly higher ethanol content in comparison with untreated control and alone GABA-treated 'Kinnow'. The higher ethanol content in AG alone and AG + GABA treated 'Kinnow' showed the development of a possible modified atmosphere inside the coated fruits [55,76,77]. It is important to mention that none of the treatments showed higher ethanol content beyond the maximum permissible limit

(2000 mg L^{-1}). Interestingly, GABA application suppressed the ethanol buildup but this is not clear how it reduced its (ethanol) production therefore needs further investigation.

3.14. Sensory quality

The treatment of 'Kinnow' fruits with AG, GABA and AG + GABA combined application showed substantially better taste, aroma, flavor and overall quality compared with the untreated group (Fig. 10A-D). The untreated control exhibited a significantly higher reduction in taste, aroma, flavor and overall quality (Fig. 10A-D). Overall, the alone AG-coated group showed significantly lower scores of taste, aroma, flavor and overall quality in contrast with GABA and AG + GABA combined treatment. After 60 days, taste, aroma, flavor and overall quality scores were significantly higher in AG + GABA combination treated 'Kinnow' fruits as compared with the untreated group (Fig. 10A-D).

Sensory quality is generally assessed in the commodities treated with edible coatings to investigate the possible accumulation of off-flavor-related changes [2,7]. On the other hand, natural elicitors-based treatment can conserve a characteristic sensory quality by delaying metabolic changes [7]. In our current study, no significant development regarding off-flavor was observed in any of the treatments applied. The untreated fruits showed significantly lower flavor scores despite the fact that the ethanol content was higher in AG alone and AG + GABA treated 'Kinnow'. The reduced flavor in the untreated 'Kinnow' could be ascribed to chilling-induced prompt senescence which was significantly suppressed in AG, GABA and AG + GABA treated fruit groups. It has been reported that coating application suppresses senescence and conserves the sensory properties of treated 'Kinnow' fruits [2–4]. In general, the combination of AG and GABA was markedly effective to conserve the characteristic sensory quality of the fruits under cold storage which could be due to the alleviation of CI and suppressed chilling-induced senescence.

3.15. Heat map clustering and PCA

Due to a significantly large data set regarding the storage times and applied treatments a heat map clustering was developed for visualization, clarity and association/relationship among the different studied attributes (Fig. 11). The heat map clustering showed that the results correlated and clear discrimination of the treatment was noted as two main cluster groups. The first main cluster consisted of untreated control (Fig. 11). On the other side, the second main cluster contained AG coating, GABA and AG + GABA groups. In the second main cluster, three sub-clusters were further discriminated (Fig. 11). The first sub-cluster was AG coating, the second was GABA treatment and the third sub-cluster contained AG + GABA group (Fig. 11). The PCA was constructed for further validation and association among the data set. In general, the treatments were separated into four groups and it was observed that the control and AG coating were closer to each other and grouped together at different times of storage (Fig. 12A). On the other side, GABA and combined AG + GABA were more closer to each other (Fig. 12A). The two PCA plots with >1.0 Eigenvalues (F1: 86.89 % and F2: 9.70 % accounted for 96.59 % variability in the data set) exhibited that the first PCA component (F1) showed a positive correlation with PME, β-Gal, PG, PME, CX, LOX, PLD, POD, MDA, REL, EPR, RPR, H_2O_2, $O_2^{\bullet}$, WSP, PWL, GABA-T, TSS, RI, DI and CI (Fig. 12B). On the other side, the second PCA component (F2) had a positive correlation with GAD, GABA, firmness, TA, CAT, PRP, CAT, APX, SOD, ascorbic acid, phenolic contents, NCSP, CSP and DPPH-SVA (Fig. 12B).

4. Conclusion

The combined postharvest treatment of 'Kinnow' fruits with AG + GABA conserved the quality attributes of fruits under cold storage. The AG + GABA treatment significantly suppressed symptoms of CI and reduced mass loss and disease incidence along with lower RPR and EPR. The combined treatment significantly conserved membrane integrity by reducing lipid peroxidation and electrolyte leakage together with

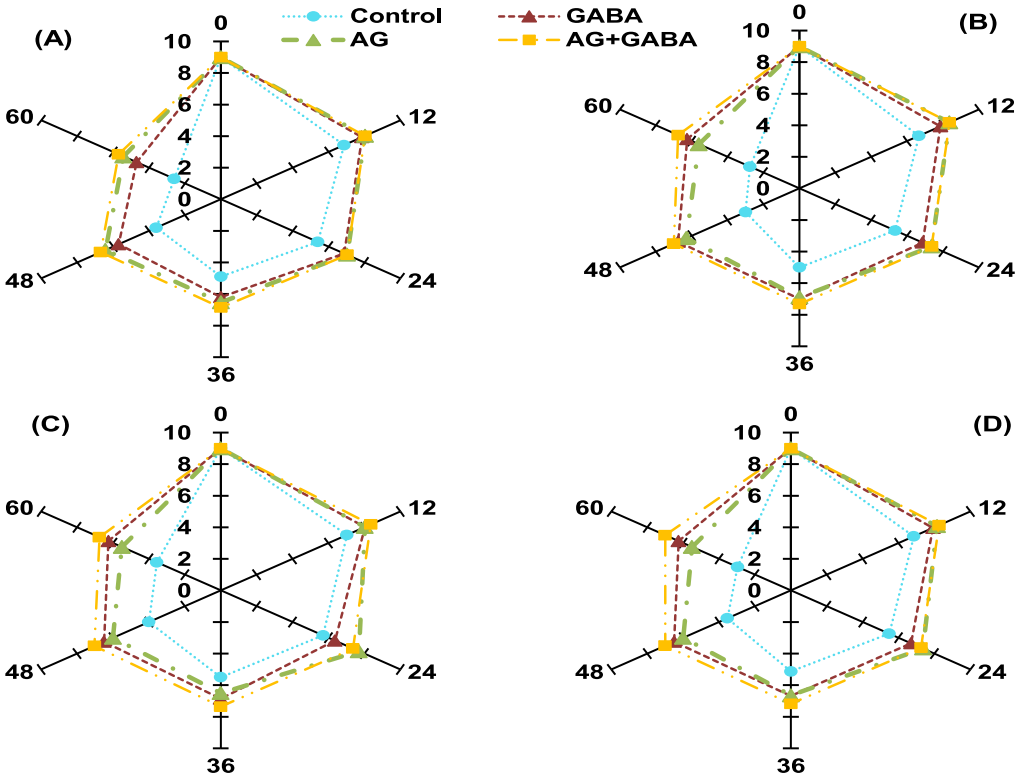

Fig. 10. The effect of combined application of Arabic gum and γ-aminobutyric acid on taste (A), aroma (B), flavor (C) and overall quality (D) of 'Kinnow' fruits.

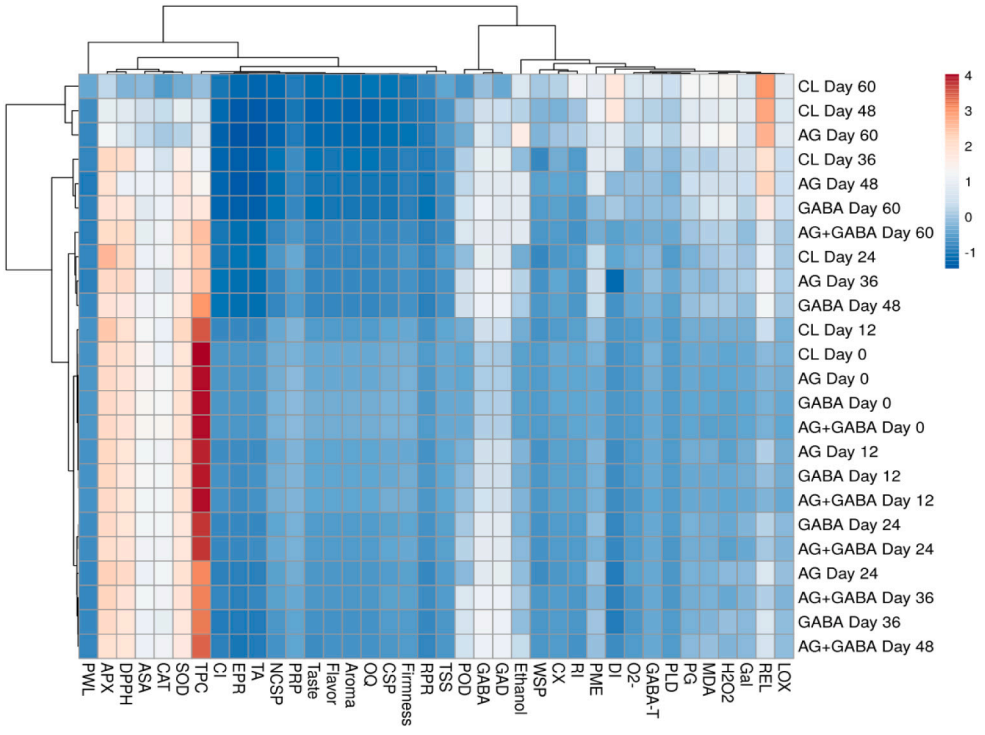

Fig. 11. Heat map clustering corresponding to quality attributes of 'Kinnow' mandarin fruits of control, AG coating, GABA and AG + GABA treatments.

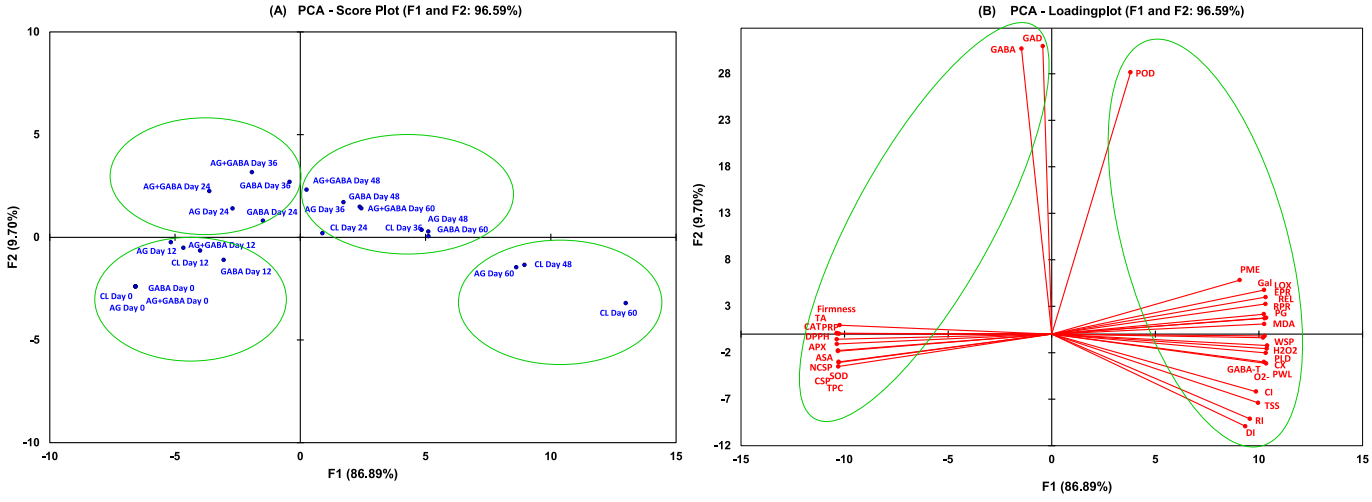

Fig. 12. Principal component analysis corresponding to quality attributes of 'Kinnow' mandarin fruits of control, AG coating, GABA and AG + GABA treatments. PCA score plot (A) and PCA loading plot (B).

suppressed oxidative stress indicators such as H_2O_2 and $O_2^{\bullet-}$ production. The fruits of combinational treatment exhibited lower GABA-T and higher GAD activity which enhanced the net accumulation of endogenous GABA content. In addition, combined AG + GABA applied fruits showed higher firmness probably owing to lower WSP and higher NCSP, CSP and PRP probably due to inhibition in the increase of cell walls degrading enzymes such as CX, PME, PG and β-Gal. The antioxidative activities were significantly higher in AG + GABA treated 'Kinnows' along with higher total phenols and AAC concentrations. In addition, the combination of AG with GABA delayed the reduction in TA and suppressed the increase in TSS and ripening index which subsequently helped in the conservation of sensory quality. The significantly better performance of the combined AG + GABA could be attributed to their possible synergistic effect which could not be attained in their alone application. In conclusion, AG + GABA combination-based treatment could be used for mitigation of CI and quality maintenance of harvested 'Kinnow' fruits.

CRediT authorship contribution statement

Sajid Ali: Conceptualization, Project administration, Methodology, Resources, Investigation, Writing – original draft. **Ahmad Sattar Khan:** Writing – review & editing, Visualization. **Aamir Nawaz:** Writing – review & editing, Visualization. **Safina Naz:** Formal analysis, Writing – review & editing. **Shaghef Ejaz:** Formal analysis, Writing – review & editing. **Anis Ali Shah:** Formal analysis, Writing – review & editing. **Muhammad Wasim Haider:** Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijbiomac.2023.123966.

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