JHPR

www.jhpr.birjand.ac.ir

Volume 8, Issue 1, March 2025, E-ISSN: 2588-6169

Journal of Horticulture and Postharvest Research

An International Journal

JOURNAL OF HORTICULTURE AND POSTHARVEST RESEARCH
2025, VOL. 8(1), 1-14

Journal of Horticulture and Postharvest Research

Journal homepage: www.jhpr.birjand.ac.ir

Influence of exogenously applied plant extracts on growth, certain physiological and morphological, as well as yield parameters of Gem squash (*Cucurbita pepo* L.)

Siphokuhle Mbuyisa[1,*], Isa Bertling[1] and Bonga Lewis Ngcobo[2]

1, School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal, P Bag X01 Scottsville 3209, Pietermaritzburg, South Africa
2, Department of Horticulture, Durban University of Technology, P.O. Box 1334, Durban, 4000, South Africa

ARTICLE INFO

Original Article

Article history:
Received 28 May 2024
Revised 19 August 2024
Accepted 25 August 2024

Keywords:
Cucurbitaceae
Natural biostimulant
Soil drench
Sustainable agriculture

DOI: 10.22077/jhpr.2024.7698.1384
P-ISSN: 2588-4883
E-ISSN: 2588-6169

***Corresponding author:**
School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal, P Bag X01 Scottsville 3209, Pietermaritzburg, South Africa.

Email: mbuyisasiphokuhle@gmail.com

© This article is open access and licensed under the terms of the Creative Commons Attribution License http://creativecommons.org/licenses/by/4.0/ which permits unrestricted, use, distribution and reproduction in any medium, or format for any purpose, even commercially provided the work is properly cited.

ABSTRACT

Purpose: The study was conducted to evaluate growth, physiological, morphological and yield response of gem squash plants following soil drench application of different plant extracts. **Research method:** A pot experiment conducted in the glasshouse was laid out following complete randomized design (CRD), with five replications. Thirty healthy, similar-sized gem squash plants were grown and treated with different treatments (plant extracts). Treatments included: *Ascophyllum nodosum* extract (ANE), aloe vera leaf extract (ALvE), garlic bulb extract (GBE), ginger rhizome extract (GRE), moringa leaf extract (MLE) and the control (no application). **Findings:** The soil drench application of plant extracts, especially ANE and MLE, had the best growth response of gem squash plants compared with other treatments and the control. Plants treated with ANE and MLE produced a greater number of leaves and branches and simultaneously produced broader leaf area compared to other plant extracts and the control. ANE-treated plants produced the highest leaf chlorophyll concentration, followed by ALvE and MLE. All plant extracts, ANE, MLE, ALvE and GBE, significantly increased the total dry biomass, except GRE was not significantly different from the control. The yield parameters, *viz.* total fruit yield, fruit mass and fruit diameter, were positively affected by all treatments applied, although ANE- and MLE-treated plants yielded the largest number of fruit/plants, heaviest fruit and biggest fruit compared to other treatments. **Research limitations:** There were no limitations identified. **Originality/Value:** Although further studies on plant extracts usage are still required, this study highlight the potential of plant extracts, especially ANE and MLE, as a natural biostimulants to improve growth and yield attributes of gem squash has been demonstrated.

INTRODUCTION

Plant-produced food, such as fruit and vegetables, has gained increasing popularity, because consuming such food as a component of a well-balanced diet is crucial to meet human dietary requirements, hence, sustaining a healthy body (Zhang et al., 2021). Among fruit vegetable crops, gem squash (*Cucurbita pepo* var. pepo) is a one of the most- produced Cucurbitaceae vegetable crops due to its high nutritive value (Paris, 2001). Judging its actual production is, however, challenging, as the Food and Agriculture Organization statistics (FAOSTAT) and agricultural statistics report the combined production of squash, pumpkins and gourds (Lust et al., 2016). According to FAO (2022), the world's production of pumpkins, squash and gourds was 22 806 million tons in 2022, whereas the South African production was 278 million tons. Gem squash is a seasonal vegetable consumed mashed, roasted or boiled, not only for its low caloric value, but also for its enhanced nutritional status, contributing to its medicinal properties (Blanco-Díaz et al., 2014). Besides carbohydrates, summer squash also contains various other phytonutrients, such as proteins, vitamins, amino acids, polysaccharides, phenolic acids, flavonoids, carotenoids (β-carotene), and minerals (especially K, P, Mg and Fe) (Aliu et al., 2012). All these phytochemicals are vital to human health due to their antioxidant, anti-radical, anti-carcinogenic, anti-inflammatory, antiviral and antimicrobial activities. These compounds, thus, play a pivotal role in reducing the risk of chronic diseases, such as diabetes, cancer, and heart disease (Oloyede et al., 2012).

The world's population is anticipated to rise enormously from the current 7.7 billion to exceed 9.7 billion by 2050 (Van Dijk et al., 2021). As a consequence of this exponentially increasing global population, food demand is also expected to increase by 59-98%, globally (Godfray et al., 2010; Elferink & Schierhorn, 2016). Given such increase in food demand, increasing agricultural production by approximately 60-70% is paramount in order to provide sufficient food for the global population in 2050 (Van Dijk et al., 2021). Agricultural industries are, therefore, facing a major challenge of increasing crop productivity and food production under unpredictable weather conditions due to climate change (Parajuli et al., 2019), contributing to biotic and abiotic constraints, including drought and salinity, as well as weed, pest and disease infestations (Zulfiqar et al., 2020). These constraints could potentially increase in the future and might pose a significant threat to the stability of agricultural crop production (Wheeler & Bruan, 2013). To produce sufficient food, synthetic pesticides and inorganic fertilizers have been traditionally used and became vital for agricultural production and crop protection against biotic and abiotic factors (Baweja et al., 2020). Conversely, complete reliance on chemical inputs to improve crop productivity can also jeopardize human health and compromise the environment (Sharma et al., 2019). In addition, the continuous, excessive utilization of such agrochemicals could result in the development of new resistant strains that could become difficult to control (Alewu & Nosiri, 2011; Ziatabar Ahmadi et al., 2024). In addition, most of these pesticides are expensive; hence, offering an economic incentive to reduce application of such agrochemicals, potentially making farming simpler and safer, partly as this would provide healthier and more sustainable food to consumers (Zulfiqar et al., 2020).

Modern-day agriculture aims to explore and develop alternative strategies of crop production as a stepping stone towards sustainable agriculture (Zulfiqar et al., 2020). Among several strategies proposed, use of plant extracts (like *Moringa oleifera*, *Ascophyllum nodosum,* and *Aloe barbadensis*) as natural biostimulants, has gained increasing interest in the agricultural research field to overcome the above-mentioned challenges (Carvalho et al., 2022; Goordeen & Mohammed, 2021). This emerging, promising, novel and eco-friendly approach has been extensively tested in a wide range of fruit vegetable crops, including

eggplant (*Solanum melongena* L.) (Ali et al., 2019), sweet pepper (*Capsicum annuum* L.) (Rajendran et al., 2022) and tomato (*Solanum lycopersicum* L.) (Basra et al., 2016; Ngcobo et al., 2024). Interestingly, this technique has also been tested in some species of the Cucurbitaceae family, such as cucumber (*Cucumis sativus* L.) (Abd-El Gawad & Osman, 2014) and watermelon (*Citrullus lanatus* L.) (Abdel-Mawgoud et al., 2010). Given the importance of safe, healthy and sustainable food, this study aims to evaluate the influence of soil drench-applied plant extracts on the growth, certain physiological, morphological and yield attributes of gem squash.

MATERIALS AND METHODS

Site, plant material and growing conditions

The experiment was conducted in a glasshouse at the University of KwaZulu Natal, Pietermaritzburg, South Africa (29°37'32.9"S 30°24'18.8"E). Environmental condition, such as temperature and relative humidity (RH) inside the glasshouse, was maintained at 25±2°C, 60 % RH during the day and 10±2°C, 75 % RH at night. With regards to irrigation, plants were irrigated using the manual irrigation method; water was carefully applied directly to the media surface to avoid water falling on the leave, creating a conducive environment for pathogens to attack. Plants were irrigated at the three-days interval. Summer squash was established directly from seeds bought from Blackwood Nursery, Pietermaritzburg, South Africa. These seeds were planted on the 10 L pots filled with a mixture of clay soil and Gromor® (Gromor, Cato Ridge, South Africa) growing medium. After planting, seeds took two weeks to emerge above the mixture of the soil and growing medium.

Collection of plant material and extract preparation

Plant materials used for extracts preparation were purchased from different suppliers. Fresh moringa (*Moringa oleifera*) leaf powder (MLP) was supplied by a commercial supplier (runKZN, Pietermaritzburg, South Africa), whereas brown algae (*Ascophyllum nodosum*) powder was purchased from a local supermarket (Dis-Chem pharmacy, Woodburn Mall, Pietermaritzburg, South Africa). Healthy aloe vera (*Aloe barbadensis*) plants were locally bought from Woodland nursery, (Pietermaritzburg, South Africa), while fresh Egyptian white garlic (*Allium sativum*) and ginger (*Zingiber officinale*) rhizome were purchased from a local supermarket. Plant extracts, *i.e., Ascophyllum nodosum* extract (ANE), aloe vera leaf extract (ALvE), garlic bulb extract (GBE), ginger rhizome extract (GRE) and moringa leaf extract (MLE) were prepared according to the protocol described by (Rajendran et al., 2022); Chumark et al. (2008), Ting-Ting et al. (2011); Amuji et al. (2012) and Ngcobo and Bertling (2021), with slight modifications. A mass of 10 g of each plant material was weighed out and homogenized in a volumetric flask (1 L) with 500 mL distilled water. The homogenates were then placed onto a hot plate, continuously agitated with an electromagnetic stirrer and allowed to boil for 15 min at 100°C. After 15 min, the solutions were allowed to stand for 2 hrs to cool down; then the supernatants were collected and filtered three times through muslin cloth. To make a final volume of 1 L, serial dilutions were then made with distilled water.

Experimental design and extract application

A pot experiment was laid out following a completely randomized design (CRD) with five replications. Five healthy, similar-sized summer squash (*Cucurbita pepo* L.) seedlings were randomly selected and assigned to each of the six treatments, namely control, ANE, ALvE, GBE, GRE and MLE, resulting in 30 experimental units. These treatments (extracts) were directly applied to the root zone via soil drench. The first soil drench application of the

treatments was carried out two weeks after seedling emergence and repeated weekly until harvesting. Each plant received 200 mL of the respective extract assigned to that specific treatment. After treatment application, plants were given three days (sufficient time) to absorb the active ingredients of the extracts before the next irrigation cycle to avoid leaching of the minerals and phytochemicals present in the extract.

Determination of dependent variables

Measurement of growth parameters

Total number of leaves and branches per plant were counted manually, whereas leaf area was measured using LICOR portable leaf area meter (LI-3000CAP). Data on the total number of leaves and leaf area were initially recorded (two weeks after emergence) before treatment application until fruiting, whereas total number of branches was only recorded at fruit set. Then the data was further collected in 14-day (bi-weekly) intervals until fruiting.

Determination of leaf chlorophyll concentration

Immediately after harvesting, the leaf chlorophyll concentration was determined following the destructive procedure described and improved by Lichtenthaler and Buschmann (2001), it was then calculated using the following equations (1, 2, 3, and 4):

$$Ca = 12.25\ A663.2 - 2.79\ A646.8 \quad (1)$$

$$Cb = 21.50\ A646.8 - 5.10\ A663.2 \quad (2)$$

$$Ca + b = 7.15\ A663.2 + 18.71\ A646.8 \quad (3)$$

$$Cx + c = (1000\ A470 - 1.82\ Ca - 85.02\ Cb)/198 \quad (4)$$

Measurement of total dry biomass

Immediately after harvesting, fresh plant residues were over-dried for four days at 80°C. After four days of oven drying, the total dry biomass was and then recorded.

Measurement of yield parameters

At full maturity stage, all summer squash fruits were harvested from all replicates. Total fruit yield (number of fruits/plant), fruits size/diameter (cm) and tuber mass (g) were recorded immediately after harvesting.

Statistical analysis

The data collected was subjected to GenStat statistical software (GenStat®, 21st edition, VSN International, UK), and graphs were plotted with Microsoft Excel. One-way analysis of variance (ANOVA) was used to analyse the obtained data. Mean separation and comparison was performed using Duncan's Multiple Range Tests at a 5 % ($p \leq 0.05$) level of significance.

RESULTS

Vegetative growth parameters (number of leaves and branches, and leaf area)

The analysis of variance indicated that the pre-harvest application of treatments (plant extracts) had a significant positive effect on the vegetative growth perspective of summer squash plants (Fig. 1, 2 and 3). Although all treatments ANE, GBE and MLE had the most profound effect on these growth parameters, hence, produced plants with the highest number

of leaves (50.50, 45.25 and 54.27, respectively) and greatest number of branches (1.05, 0.95 and 1.30, respectively) at week 8 of treatment application than the GRE and the control (Fig. 1 and 2). Soil drench application of plant extracts, especially ANE and MLE, considerably enhanced leaf area of summer squash plants, thus, recorded the larger leaf area (126.40 and 101.90 cm^2/cm^2, respectively) at week 8 of treatment application than other treatments ALvE, GBE, GRE and the control (Fig. 3).

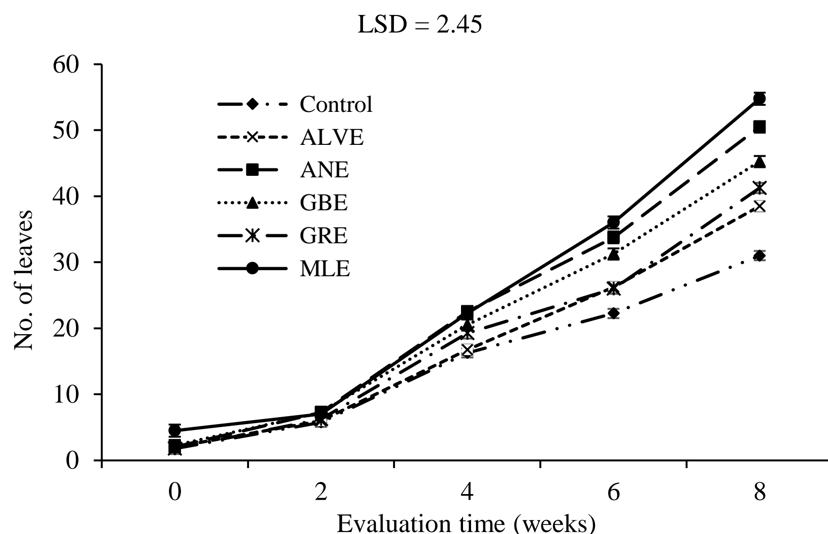


Fig. 1. Effect of pre-harvestly applied plant extracts on summer squash number of leaves during growth and development stage. Treatments were control = only distilled water, ANE = *Ascophyllum nodosum* extract, AL_vE = aloe vera leaf extract, GBE = garlic bulb extract, GRE = ginger rhizome extract, MLE = moringa leaf extract were analysed using Duncan's multiple range test at ($p \leq 0.05$) level of significance. LSD = 2.45 and F pr < 0.001.

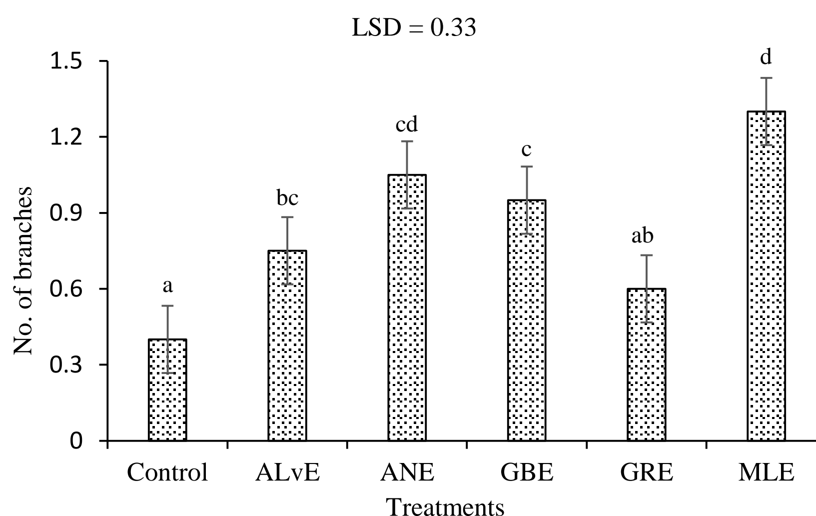


Fig. 2. Effect of soil drench application of plant extracts on the number of branches produced by summer squash plants. Treatments were control = only distilled water, ANE = Ascophyllum nodosum extract, AL_vE = aloe vera leaf extract, GBE = garlic bulb extract, GRE = ginger rhizome extract, MLE = moringa leaf extract were analysed using Duncan's multiple range test at ($P < 0.05$) level of significance. Bars of different lower-case letters in each column denote statistically significant differences. LSD = 0.33 and F pr < 0.001.

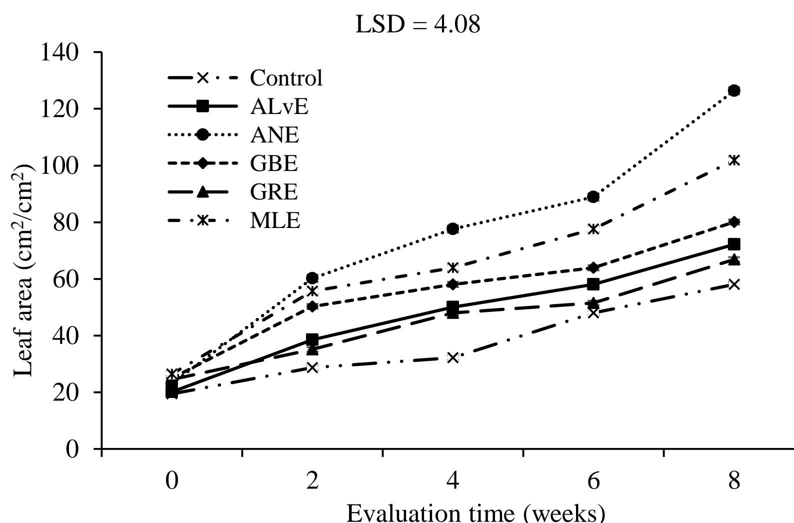


Fig. 3. Effect of pre-harvest application of various plant extracts on the leaf area borne by summer squash plants during growth and development stage. Treatments were control = only water, ANE = *Ascophyllum nodosum* extract, AL_vE = aloe vera leaf extract, GBE = garlic bulb extract, GRE = ginger rhizome extract, MLE = moringa leaf extract were analysed using Duncan's multiple range test at ($p \leq 0.05$) level of significance. LSD = 4.08 and F pr < 0.001.

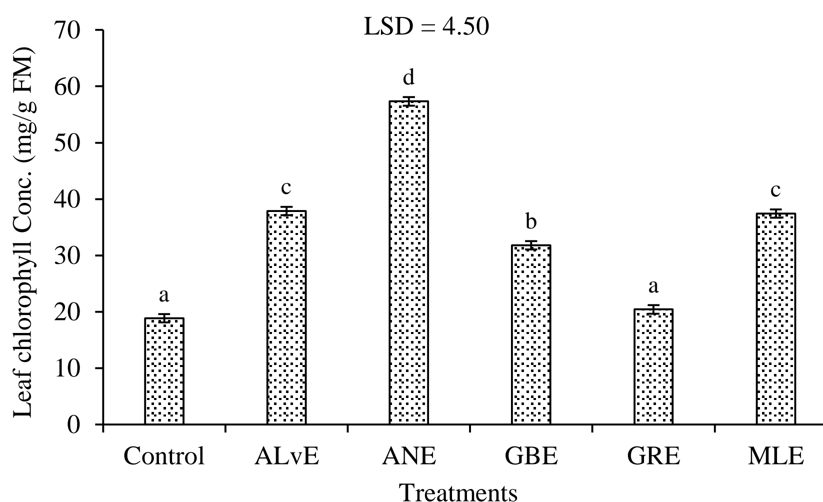


Fig. 4. Effect of pre-harvest application of plant extracts on summer squash leaf chlorophyll concentration. Treatments were control = only water, ANE = *Ascophyllum nodosum* extract, AL_vE = aloe vera leaf extract, GBE = garlic bulb extract, GRE = ginger rhizome extract, MLE = moringa leaf extract were analysed using Duncan's multiple range test at ($p \leq 0.05$) level of significance. Bars of different lower-case letters in each column denote statistically significant differences. LSD = 4.50 and F pr < 0.001.

Physiological parameter (leaf chlorophyll concentration)

The pre-harvest application of plant extracts on summer squash plants, as soil drench application, significantly improved the leaf chlorophyll concentration ($p \leq 0.05$). As a result, the highest leaf chlorophyll concentration was recorded in summer squash plants treated with ANE, ALvE and MLE (57.33, 37.87 and 37.43 mg/g FM) compared with other treatments GBE, GRE and the control (Fig. 4).

Morphological parameter (total dry biomass)

Variations on the morphological data indicated that soil drench application of plant extracts, as a pre-harvest treatment, significantly influenced ($p \leq 0.05$) the total dry biomass of summer squash plants. Unlike other treatments, ANE, MLE and GBE had a considerable effect on total dry biomass, as they recorded the heavier total dry biomass (57.67, 50.09 and 47.55 g, respectively) than ALvE, GRE and the control (Fig. 5).

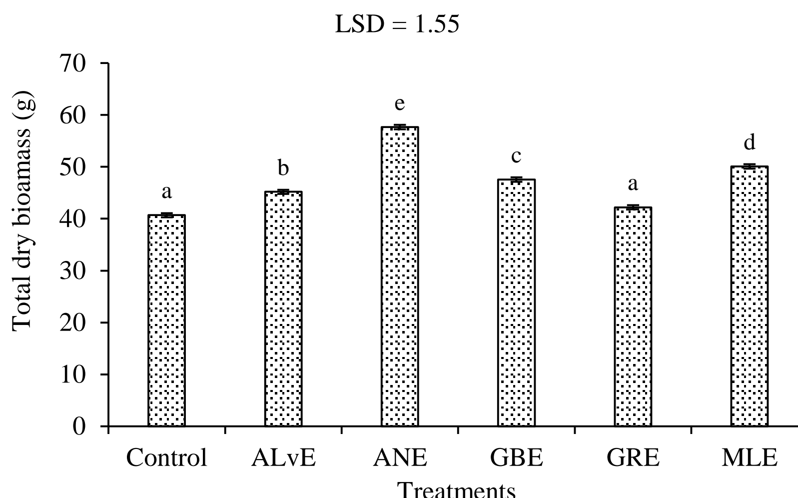


Fig. 5. Effect of pre-harvest application of plant extracts on the total dry biomass of summer squash plant residues. Treatments were control = only water, ANE = *Ascophyllum nodosum* extract, AL_vE = Aloe vera leaf extract, GBE = Garlic bulb extract, GRE = Ginger rhizome extract, MLE = Moringa leaf extract were analysed using Duncan's multiple range test at ($p \leq 0.05$) level of significance. Bars of different lower-case letters in each column denote statistically significant differences. LSD = 1.55 and F pr < 0.001.

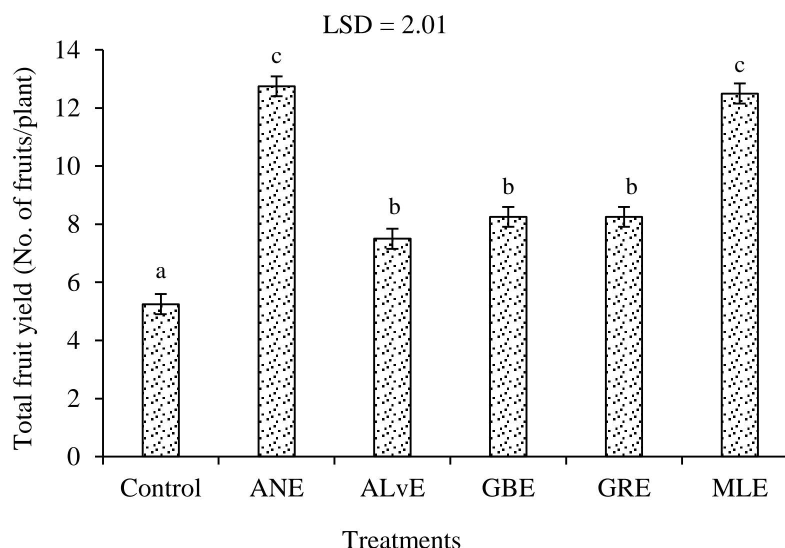


Fig. 6. Effect of soil drench-applied of plant extracts on the total fruit yield of summer squash. Treatments were control = only water, ANE = *Ascophyllum nodosum* extract, AL_vE = aloe vera leaf extract, GBE = garlic bulb extract, GRE = ginger rhizome extract, MLE = moringa leaf extract were analysed using Duncan's multiple range test at ($p \leq 0.05$) level of significance. Bars of different lower-case letters in each column denote statistically significant differences. LSD = 4.50 and F pr < 0.001.

Yield parameters (total fruit yield, fruit mass, and fruit diameter)

The obtained results revealed that the pre-harvest application of plant extracts, as soil drench, had the significant improvement ($p \leq 0.05$) on the yield attributes of summer squash. ANE- and MLE-treated plants notably had the pronounced effect on the measured yield attributes, hence, yielded the highest total fruit yield (12.75 and 12.5 fruits/plant, respectively), heaviest fruit mass (227 and 225.2 g, respectively) and largest fruit diameter (9.5 and 10 cm, respectively) compared to other treatments ALvE, GBE, GRE and the control (Fig. 6, 7 and 8).

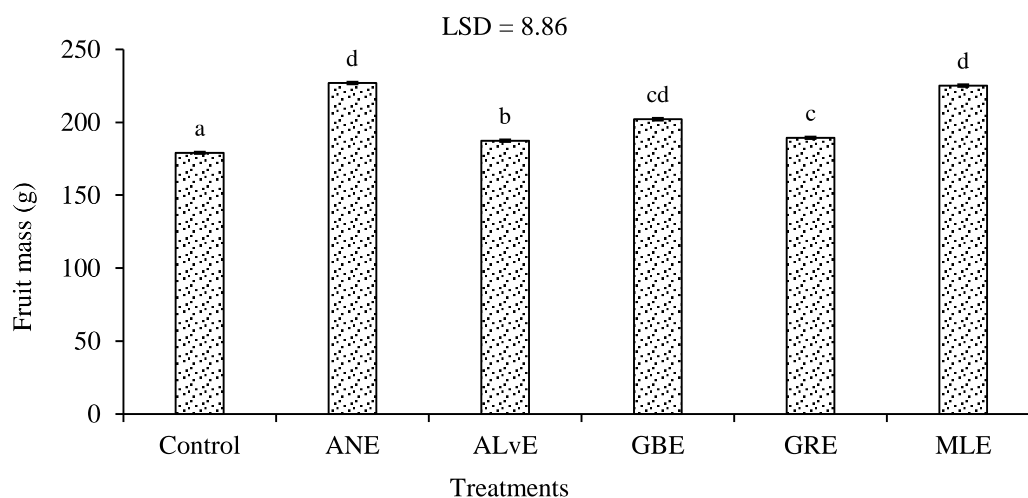


Fig. 7. Effect of soil drench-applied plant extracts on summer squash fruit mass. Treatments were control = only water, ANE = Ascophyllum nodosum extract, AL_vE = aloe vera leaf extract, GBE = garlic bulb extract, GRE = ginger rhizome extract, MLE = moringa leaf extract were analysed using Duncan's multiple range test at ($p \leq 0.05$) level of significance. Bars of different lower-case letters in each column denote statistically significant differences. LSD = 8.86 and F pr < 0.001.

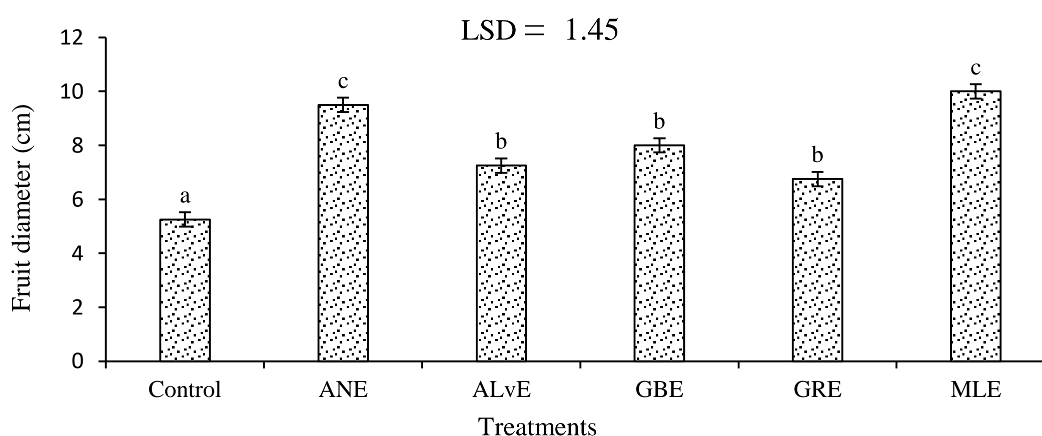


Fig. 8. Effect of pre-harvestly applied plant extracts on the fruit size-diameter of summer squash. Treatments were control = only water, ANE = *Ascophyllum nodosum* extract, AL_vE = aloe vera leaf extract, GBE = garlic bulb extract, GRE= ginger rhizome extract, MLE = moringa leaf extract were analysed using Duncan's multiple range test at ($p \leq 0.05$) level of significance. Bars of different lower-case letters in each column denote statistically significant differences. LSD = 1.45 and F pr < 0.001.

DISCUSSION

Despite the impact of climate change and its aligned factors, there is a need for agricultural industries to increase agricultural production in an eco-friendly and sustainable approach to produce safe and high nutritious food in order to feed the exponentially increasing global population and meet increasing food demand; hence, contributing to global food security and achieving sustainable development goals *viz.* zero hunger, as well as good health and well-being (FSIN, 2020). Pre-harvest application of plant extracts, as natural biostimulants, has received an increasing interest due to its positive effect on plant growth and yield potential of crops (Craigie, 2011; Hayat et al., 2018). Plant extract application has demonstrated its immense potential to increase plant vigour and yield attributes by interfering with plant physiological processes and increasing nutrient acquisition without endangering the environment (Zulfiqar et al., 2020).

The results presented in the present study indicate that soil drench application of plant extracts considerably improved vegetative growth, physiological and yield attributes of gem squash. These results are confirmed by Jang et al. (2021), who noted that application of various plant extracts as soil drench application significantly improved growth and yield attributes of cucumber. According to Kumari et al. (2011), vegetative growth, physiological, morphological and yield attributes of Cucurbitaceae species (*Cucurbita pepo* var. pepo) were enhanced by the application of plant extracts, this could possibly be due to their biofertilization effect. Plant extracts contain significant amounts of mineral nutrients, especially N, P, K, Mg, Ca, Zn, and Fe (Moore, 2004; Moyo et al., 2011). Hala et al. (2017) reported that the presence of such mineral elements in the extracts increases the availability and uptake of these mineral, thus, promoting vegetative growth, physiological, morphological and yield parameters.

The beneficial effect of these plant extracts could also be linked to the biostimulatory effect due to the growth-promoting phytohormones, such as auxins, gibberellins and cytokinins, present in the extracts (Rayorath et al., 2008; Yamaguchi, 2008). The exogenous application of these extracts, therefore, induced the endogenous biosynthesis of plant hormones, thereby promoting cell expansion through cell division and elongation, resulting in growth and yield improvement. Improved leaf chlorophyl concentration following plant extract applications could possibly be attributed to enhanced gene transcripts involved in photosynthesis, cell metabolism and stress response (Wang et al., 2009). Application of these extracts suppressed cysteine protease activity, thus, inhibiting chlorophyll degradation and delayed senescence (Buet et al., 2019). Enhanced leaf chlorophyl concentration due to ANE application could be ascribed to the presence of betaines, which also inhibited the breakdown of leaf chlorophyl (Blunden et al., 2009).

In addition to minerals, phytohormones and betaines, plant extracts contain several antioxidant compounds, including ascorbic acid, phenolics and tocopherols, which their presence induces the antioxidant biosynthesis, hence, triggering plant defence mechanisms against stress caused by reactive oxygen species (ROS) (Wang et al., 2009; Saini et al., 2016; Ngcobo & Mbuyisa, 2024). Plant extracts, especially ANE, are good source of alginic acids and polyuronides, which are responsible for the improvement of soil properties, including soil structure, water -holding capacity aeration, capillary action, hence, boosting soil microbial activity and soil organic matter, which ultimately increase mineral availability and absorption by plants (Moore, 2004). These compounds also increase the mobility and translocation of carbohydrates and other organic compounds, hence, improving fruit development. Rioux et al. (2007) revealed that ANE also contain polysaccharides (*viz.* fucoidan and laminarin), that exhibit radical scavenging antioxidant activity

The results obtained in the present study coincide with Mbuyisa et al. (2023), who reported that application of various plant extracts, especially ANE and MLE, positively influenced growth parameters, such as number of leaves, number of branches, leaf area, total dry biomass and yield parameters of potato (*Solanum tuberosum* cv. Sifra). Ngcobo and Bertling (2021) demonstrated MLE application to cherry tomato (*Solanum lycopersicum* var. cerasiforme) significantly increases vegetative growth parameters, which these results are in line with present study. In addition, these findings coincide with Hidangmayum and Sharma (2017), who reported a significant increase in vegetative growth and yield parameters of onions (*Allium cepa* var. N-53), following ANE application. Furthermore, Manna et al. (2012) also demonstrated that application of ANE to chilli plants increased leaf chlorophyll concentration. Our results are in accordance with Yaseen and Takacs-Hajos (2022), who indicated that applying MLE to different lettuce (*Lactuca sativa*) cultivars (*viz.* May King, Kobak and Great Lakes) improved leaf chlorophyll concentration. In addition, Hala et al. (2017) revealed that MLE application to remarkably improved growth and yield attributes sweet pepper, which their findings correspond with the results of the present study. Similarly, Rayorath et al. (2008) revealed that application of ANE positively affected vegetative growth parameters, such as number of leaves, plant height and root growth, of *Arabidopsis thaliana*. Moreover, the application of ANE enhanced vegetative growth and yield of leafy vegetables, such as spinach (*Spinacea oleracea*) (Fan et al., 2013) and lettuce (Chrysargyris et al., 2018).

CONCLUSION

The use of plant extracts, especially ANE and MLE, applied as natural biostimulants to increase crop productivity significantly improved growth, physiological and morphological, as well as yield parameters of gem squash. It can, therefore, be concluded that exogenous application of such plant extracts can effectively enhance plant growth and development, as well as yield, of horticultural crops. Hence, the results presented in this research are of high significance, particularly to small-scale cucurbit growers, as hot water extracts present a sustainable, cheap and environmentally friendly approach to increasing crop productivity, while reducing the utilization of synthetic and chemical-based, thereby sustaining the environment and reducing health concerns of consumers. To promote the adoption of such application, further studies on plant extracts, particularly ANE, GBE and MLE, are still required before recommendations for use in large-scale production can be made.

Conflict of interest
Authors reported no potential conflict of interest.

Data availability statement
The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

REFERENCES

Abd El-Gawad, H., & Osman, H. S. (2014). Effect of exogenous application of boric acid and seaweed extract on growth, biochemical content and yield of eggplant. *Journal of Horticultural Science & Ornamental Plants*, *6*(3), 133-143.

Abdel-Mawgoud, A. M. R., Tantaway, A. S., Hafez, M. M., & Habib, H. A. M. (2010). Seaweed extract improves growth, yield and quality of different watermelon hybrids. *Research Journal of Agriculture and Biological Sciences*, *6*(2), 161–168.

Alewu, B., & Nosiri, C. (Ed.). (2011). *Pesticides in the Modern World: Effects of Pesticides Exposure.* Mexico: *InTechOpen*.

Ali, M., CHENG, Z., Hayat, S., Ahmad, H., Ghani, M. I., & Tao, L. I. U. (2019). Foliar spraying of aqueous garlic bulb extract stimulates growth and antioxidant enzyme activity in eggplant (*Solanum melongena* L.). *Journal of Integrative Agriculture*, *18*(5), 1001-1013. https://doi.org/10.1016/S2095-3119(18)62129-X

Aliu, S., Rusinovci, I., Fetahu, S., Salihu, S., & Zogaj, R. (2012). Nutritive and mineral composition in a collection of *Cucurbita pepo* L grown in Kosova. *Food and Nutrition Sciences*, *3*(5), 634-638. https://doi.org/10.4236/fns.2012.35087

Amuji, C. F., Echezona, B. C., & Dialoke, S. A. (2012). Extraction fractions of ginger (*Zingiber officinale* Roscoe) and residue in the control of field and storage pests. *Journal of Agricultural Technology*, *8*(6), 2023-2031.

Basra, S. M. A., & Lovatt, C. J. (2016). Exogenous applications of moringa leaf extract and cytokinins improve plant growth, yield, and fruit quality of cherry tomato. *HortTechnology*, *26*(3), 327-337. https://doi.org/10.21273/HORTTECH.26.3.327

Baweja, P., Kumar, S., & Kumar, G. (Ed.). (2020). *Fertilizers and Pesticides: Their Impact on Soil Health and Environment* (pp. 265-285). https://doi.org/10.1007/978-3-030-44364-1_15

Blanco-Díaz, M. T., Del Río-Celestino, M., Martínez-Valdivieso, D., & Font, R. (2014). Use of visible and near-infrared spectroscopy for predicting antioxidant compounds in summer squash (*Cucurbita pepo* ssp pepo). *Food Chemistry*, *164*. https://doi.org/10.1016/j.foodchem.2014.05.019

Blunden, G., Currie, M., Mathe, J., Hohmann, J., & Critchley, A. T. (2009). Variations in betaine yields from marine algal species commonly used in the preparation of seaweed extracts used in agriculture. *Phycology*, *76*, 14. https://doi.org/10.1177/1934578X1000500418

Buet, A., Costa, M. L., Martínez, D. E., & Guiamet, J. J. (2019). Chloroplast protein degradation in senescing leaves: Proteases and lytic compartments. *Frontiers in Plant Science*, *10*, 451973. https://doi.org/10.3389/fpls.2019.00747

Carvalho, R. da S., Silva, M. A. da, Borges, M. T. M. R., & Forti, V. A. (2022). Plant extracts in agriculture and their applications in the treatment of seeds. *Ciência Rural*, *52*(5), 1-18. https://doi.org/10.1590/0103-8478cr20210245

Chrysargyris, A., Xylia, P., Anastasiou, M., Pantelides, I., & Tzortzakis, N. (2018). Effects of *Ascophyllum nodosum* seaweed extracts on lettuce growth, physiology and fresh-cut salad storage under potassium deficiency. *Journal of the Science of Food and Agriculture*, *98*(15), 5861–5872. https://doi.org/10.1002/jsfa.9139

Chumark, P., Khunawat, P., Sanvarinda, Y., Phornchirasilp, S., Morales, N. P., Phivthong-Ngam, L., Ratanachamnong, P., Srisawat, S., & Klai-upsorn, S. P. (2008). The in vitro and ex vivo antioxidant properties, hypolipidaemic and antiatherosclerotic activities of water extract of *Moringa oleifera* Lam. leaves. *Journal of Ethnopharmacology*, *116*(3), 439–446. https://doi.org/10.1016/j.jep.2007.12.010

Craigie, J. S. (2011). Seaweed extract stimuli in plant science and agriculture. *Journal of Applied Phycology*, *23*, 371–393. https://doi.org/10.1007/s10811-010-9560-4

Elferink, M., & Schierhorn, F. (2016). Global demand for food is rising. Can we meet it. *Harvard Business Review*, *7*(04), 2016.

Fan, D., Hodges, D. M., Critchley, A. T., & Prithiviraj, B. (2013). A commercial extract of brown macroalga (*Ascophyllum nodosum*) affects yield and the nutritional quality of spinach in vitro. *Communications in Soil Science and Plant Analysis*, *44*(12), 1873–1884. https://doi.org/10.1080/00103624.2013.790404

FAO (Food and Agriculture Organization). (2022). Online Statistical Database: Food and Agriculture Organization of the World. FAOSTAT. [2023-07-15] http://www.fao.org/faostat/en/?#data/QC.

Food security information network (FSIN). (2020, September). 2020 Global report on food crises. *Global Network against Food Crisis.* https://www.fsinplatform.org/global-report-food-crises-2020

Godfray, H. C. J., Beddington, J. R., Crute, I. R., Haddad, L., Lawrence, D., Muir, J. F., Pretty, J., Robinson, S., Thomas, S. M., & Toulmin, C. (2010). Food security: the challenge of feeding 9 billion people. *Science*, *327*(5967), 812–818. https://doi.org/10.1126/science.1185383

Goordeen, A., & Mohammed, M. (2021). Growth, development, maturation indices, proximate and mineral composition of moringa (*Moringa oleifera*). *Journal of Horticulture and Postharvest Research*, *4*(4), 427-438. https://doi.org/10.22077/jhpr.2021.4296.1205

Hala, H., El-Noor, A., & Ewais, N. A. (2017). Effect of *Moringa oleifera* leaf extract (MLE) on pepper seed germination, seedlings improvement, growth, fruit yield and its quality. *Middle East Journal of Agriculture Research*, *6*, 448–463.

Hayat, S., Ahmad, H., Ali, M., Ren, K., & Cheng, Z. (2018). Aqueous garlic extract stimulates growth and antioxidant enzymes activity of tomato (*Solanum lycopersicum*). *Scientia Horticulturae*, *240*, 139–146. https://doi.org/10.1016/j.scienta.2018.06.011

Hidangmayum, A., & Sharma, R. (2017). Effect of different concentrations of commercial seaweed liquid extract of *Ascophyllum nodosum* as a plant bio stimulant on growth, yield and biochemical constituents of onion (*Allium cepa* L.). *Journal of Pharmacognosy and Phytochemistry*, *6*(4), 658–663.

Jang, S. J., Park, H. H., & Kuk, Y. I. (2021). Application of various extracts enhances the growth and yield of cucumber (*Cucumis sativus* L.) without compromising the biochemical content. *Agronomy*, *11*(3), 505. https://doi.org/10.3390/agronomy11030505

Kumari, R., Kaur, I., & Bhatnagar, A. K. (2011). Effect of aqueous extract of *Sargassum johnstonii* Setchell & amp; Gardner on growth, yield and quality of *Lycopersicon esculentum* Mill. *Journal of Applied Phycology*, *23*(3), 623–633. https://doi.org/10.1007/s10811-011-9651-x

Lichtenthaler, H. K., & Buschmann, C. (2001). Chlorophylls and carotenoids: Measurement and characterization by UV-VIS spectroscopy. *Current Protocols in Food Analytical Chemistry*, *1*(1), F4-3. https://doi.org/10.1002/0471142913.faf0403s01

Lust, T. A., & Paris, H. S. (2016). Italian horticultural and culinary records of summer squash (*Cucurbita pepo*, Cucurbitaceae) and emergence of the zucchini in 19th-century Milan. *Annals of Botany*, *118*(1), 53–69. https://doi.org/10.1093/aob/mcw080

Manna, D., Sarkar, A., & Maity, T. K. (2012). Impact of biozyme on growth, yield and quality of chilli (*Capsicum annuum* L.). *Journal of Crop and Weed*, *8*(1), 40-43.

Mbuyisa, S., Bertling, I., & Ngcobo, B. (2023). Impact of foliar-applied plant extracts on growth, physiological and yield attributes of the potato (*Solanum tuberosum* L.). *Agronomy*, *14*(1), 38. https://doi.org/10.3390/agronomy14010038

Moore, K. K. (2004). Using seaweed compost to grow bedding plants. *BioCycle*, *45*, 43-44.

Moyo, B., Masika, P. J., Arnold, H., & Voster, M. (2011). Nutritional characterization of Moringa (*Moringa oleifera* Lam.) leaves. *African Journal of Biotechnology*, *10*(60), 12925-12933. https://doi.org/10.5897/AJB10.1599

Ngcobo, B. L., & Bertling, I. (2021, November). Influence of foliar *Moringa oleifera* leaf extract (MLE) application on growth, fruit yield and nutritional quality of cherry tomato. *II International Symposium on Moringa 1306.* Pretoria, South Africa (pp. 249-254). *Acta Horticulturae.* https://doi.org/10.17660/ActaHortic.2021.1306.31

Ngcobo, B. L., & Mbuyisa, S. (2023, November). Recent developments in environmentally friendly techniques for extracting bioactive compounds from moringa plant parts. *III International Symposium on Moringa 1394.* Aracaju, Sergipe, Brazil (pp. 93-98). *Acta Horticulturae*. https://doi.org/10.17660/ActaHortic.2024.1394.12

Ngcobo, B., Bertling, I., & Mbuyisa, S. (2024). Evaluating the efficacy of *Moringa oleifera* leaf extracts prepared using different solvents on growth, yield and quality of tomatoes and peppers. *Journal of Horticulture and Postharvest Research*, *7*(4), 389-406. https://doi.org/10.22077/jhpr.2024.7667.1383

Oloyede, F. M., Agbaje, G. O., Obuotor, E. M., & Obisesan, I. O. (2012). Nutritional and antioxidant profiles of pumpkin (*Cucurbita pepo* Linn.) immature and mature fruits as influenced by NPK fertilizer. *Food Chemistry*, *135*(2), 460-463. https://doi.org/10.1016/j.foodchem.2012.04.124

Parajuli, R., Thoma, G., & Matlock, M. D. (2019). Environmental sustainability of fruit and vegetable production supply chains in the face of climate change: A review. *Science of the Total Environment*, *650*, 2863-2879. https://doi.org/10.1016/j.scitotenv.2018.10.019

Paris, H. S. (2001). History of the cultivar-groups of *Cucurbita pepo*. *Horticultural Reviews-Westport Then New York-*, *25*, 71-170. https://doi.org/10.1002/9780470650783.ch2

Rajendran, R., Jagmohan, S., Jayaraj, P., Ali, O., Ramsubhag, A., & Jayaraman, J. (2022). Effects of *Ascophyllum nodosum* extract on sweet pepper plants as an organic biostimulant in grow box home garden conditions. *Journal of Applied Phycology*, *34*(1), 647-657. https://doi.org/10.1007/s10811-021-02611-z

Rayorath, P., Jithesh, M. N., Farid, A., Khan, W., Palanisamy, R., Hankins, S. D., Critchley, A. T., & Prithiviraj, B. (2008). Rapid bioassays to evaluate the plant growth promoting activity of *Ascophyllum nodosum* (L.) Le Jol. using a model plant, *Arabidopsis thaliana* (L.) Heynh. *Journal of Applied Phycology*, *20*, 423-429. https://doi.org/10.1007/s10811-007-9280-6

Rioux, L.-E., Turgeon, S. L., & Beaulieu, M. (2007). Characterization of polysaccharides extracted from brown seaweeds. *Carbohydrate Polymers*, *69*(3), 530-537. https://doi.org/10.1016/j.carbpol.2007.01.009

Saini, R. K., Sivanesan, I., & Keum, Y.-S. (2016). Phytochemicals of *Moringa oleifera*: a review of their nutritional, therapeutic and industrial significance. *3 Biotech*, *6*(2), 203. https://doi.org/10.1007/s13205-016-0526-3

Sharma, A., Kumar, V., Shahzad, B., Tanveer, M., Sidhu, G. P. S., Handa, N., Kohli, S. K., Yadav, P., Bali, A. S., Parihar, R. D., Dar, O. I., Singh, K., Jasrotia, S., Bakshi, P., Ramakrishnan, M., Kumar, S., Bhardwaj, R., & Thukral, A. K. (2019). Worldwide pesticide usage and its impacts on ecosystem. *SN Applied Sciences*, *1*(11), 1446. https://doi.org/10.1007/s42452-019-1485-1

Ting-Ting, W., Zhi-Hui, C., Khan, M. A., Qing, M., & Ling, H. (2011). The inhibitive effects of garlic bulb crude extract on Fulvia fulva of tomato. *Pakistan Journal of Botany*, *43*(5), 2575-2580.

Van Dijk, M., Morley, T., Rau, M. L., & Saghai, Y. (2021). A meta-analysis of projected global food demand and population at risk of hunger for the period 2010–2050. *Nature Food*, *2*(7), 494-501. https://doi.org/10.1038/s43016-021-00322-9

Wang, T., Jónsdóttir, R., & Ólafsdóttir, G. (2009). Total phenolic compounds, radical scavenging and metal chelation of extracts from Icelandic seaweeds. *Food Chemistry*, *116*(1), 240-248. https://doi.org/10.1016/j.foodchem.2009.02.041

Wheeler, T., & von Braun, J. (2013). Climate Change Impacts on Global Food Security. *Science*, *341*(6145), 508-513. https://doi.org/10.1126/science.1239402

Yamaguchi, S. (2008). Gibberellin metabolism and its regulation. *Annual Review of Plant Biology.*, *59*, 225-251. https://doi.org/10.1146/annurev.arplant.59.032607.092804

Yaseen, A. A., & Takacs-Hajos, M. (2022). Evaluation of moringa (*Moringa oleifera* Lam.) leaf extract on bioactive compounds of lettuce (*Lactuca sativa* L.) grown under glasshouse environment. *Journal of King Saud University - Science*, *34*(4), 101916. https://doi.org/10.1016/j.jksus.2022.101916

Zhang, D., Feng, Y., Li, N., & Sun, X. (2021). Fruit and vegetable consumptions in relation to frequent mental distress in breast cancer survivors. *Supportive Care in Cancer*, *29*, 193-201. https://doi.org/10.1007/s00520-020-05451-8

Ziatabar Ahmadi, S. R., Seifi, E., Varasteh, F., & Akbarpour, V. (2024). Effect of biofertilizer inoculation on the growth and physiological traits of Red Angel and Wonderful pomegranate plantlets under salinity stress. *Journal of Horticulture and Postharvest Research*, *7*(2), 171-182. https://doi.org/10.22077/jhpr.2024.7168.1356

Zulfiqar, F., Casadesús, A., Brockman, H., & Munné-Bosch, S. (2020). An overview of plant-based natural biostimulants for sustainable horticulture with a particular focus on moringa leaf extracts. *Plant Science*, *295*, 110194. https://doi.org/10.1016/j.plantsci.2019.110194

JOURNAL OF HORTICULTURE AND POSTHARVEST RESEARCH
2025, VOL. 8(1), 15-26

Journal of Horticulture and Postharvest Research

Journal homepage: www.jhpr.birjand.ac.ir

Bio-protective solutions for carrot spoilage: exploring the antifungal properties of ginger, garlic, onion, and *Moringa*

Eugenia Amaka Njoku[1], Florence Nwakaego Mbaoji[2], Justus Amuche Nweze[3,*], Bonaventure Chukwujindu Echezona[1] and Kayode Paul Baiyeri[1]

1, Department of Crop Science, Faculty of Agriculture, University of Nigeria, Nsukka, Nigeria
2, Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Nigeria
3, Department of Science Laboratory Technology, Faculty of Physical Sciences, University of Nigeria, Nsukka, Nigeria

ARTICLE INFO

Original Article

Article history:
Received 1 June 2024
Revised 14 August 2024
Accepted 5 September 2024

Keywords:
Antifungal activity
Carrot
Fungi
Plant extracts
Postharvest preservation

DOI: 10.22077/jhpr.2024.7713.1386
P-ISSN: 2588-4883
E-ISSN: 2588-6169

***Corresponding author:**
Department of Science Laboratory Technology, Faculty of Physical Sciences, University of Nigeria, Nsukka, Nigeria.

Email: justus.nweze@unn.edu.ng

© This article is open access and licensed under the terms of the Creative Commons Attribution License http://creativecommons.org/licenses/by/4.0/ which permits unrestricted, use, distribution and reproduction in any medium, or format for any purpose, even commercially provided the work is properly cited.

ABSTRACT

Purpose: To address postharvest losses in the carrot supply chain caused by pathogenic fungi, this study evaluates the antifungal potential of ethanol extracts from *Allium cepa*, *Zingiber officinale*, *Allium sativum*, and *Moringa oleifera* against carrot spoilage fungi, including *Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus, Fusarium oxysporum,* and *Fusarium solani*. **Research Method:** Filtered plant extracts were obtained using ethanol extraction method. This study evaluated the efficacy of various plant extracts in reducing microbial load and inhibiting fungal growth on carrot roots using standard microbiological procedures, including agar well diffusion and broth microdilution techniques. **Findings:** The study demonstrated that ethanol extracts, particularly from ginger, significantly ($p < 0.05$) reduced fungal load on carrot roots. The inhibition zone analysis revealed that ginger and *Moringa* extracts, along with ketoconazole, effectively inhibited *A. niger* and *A. fumigatus*, with ketoconazole producing the largest inhibition zones. Ginger showed the highest antifungal effectiveness, with minimal inhibitory concentrations ranging from 31.25 mg/ml to 250 mg/ml, particularly against *A. niger* and *A. fumigatus*, demonstrating higher antifungal activity compared to other treatments. Garlic consistently exhibited an MIC of 250 mg/ml against all test fungi. Additionally, the minimum fungicidal concentration results highlighted ginger extract's potent biocidal effects, especially against *A. flavus*, with an MIC of 62.5 mg/ml. **Research limitations:** The study is limited to *in vitro* assessments; field conditions may affect the efficacy of the extracts due to environmental factors. **Originality/Value:** This research highlights ginger's potential as a natural antifungal agent, offering practical applications for improving carrot preservation and reducing postharvest losses.

INTRODUCTION

Carrot (*Daucus carota*) is among the premium vegetable crops due to its high health and nutrient-packed quality. Carrot assists in regular maintenance of body needs through the provision of beta carotene, vitamin K1, fiber, and antioxidants (Ahmad et al., 2019; Motegaonkar et al., 2024). In sub-Saharan Africa, where the system is still developing, the loss of vegetables such as carrot is sustained before harvest, during harvest, and under storage. In Nigeria, for example, immeasurable losses are incurred in the vegetable sector due to zero to insufficient levels of post-harvest handling procedures (Odeyemi, 2019). Fungi are among the plant pathogens that account for about 30% loss in both quality and yield of horticultural crops along the food value chain (Xi et al., 2022; Moradinezhad & Ranjbar, 2023). *Fusarium* species (*F. oxysporum* and *F. solani*), known soil and waterborne diseases, cause both field and storage damage in various crop produce such as carrot and tomatoes (Rahimi Kakolaki et al., 2024). The shelf life of carrot is also affected by activities of other species of fungal pathogens, including *Aspergillus* species (*A. niger, A. fumigatius, A. flavus,* etc) (Alegbeleye et al., 2022). *Aspergillus* species contaminate edible materials through the secretion of toxic substances (mycotoxins), which have the potential to cause cancer. These substances (hepatocarcinogens) pose serious health risks to both humans and animals (Alshammari, 2023). These pre and postharvest pathogenic issues in carrot production are commonly treated with chemical fungicides. However, currently, due to concerns arising from the potential bio-disaster resulting from constant application of chemical fungicides in agriculture, there is a need for risk-free, antifungal, bio-alternatives with a broad spectrum of action (Rawal & Singh Adhikari, 2016).

Since the 1990s, extracts from plant materials and similar medicinal substitutes for ailment therapy have been widely used. Studies have shown that less than 10% of plant species in nature are utilized by humans and animals; however, a higher percentage are assumed to be used for therapeutic purposes (Sezer et al., 2024). Many of these medicinal plants have been studied, and their active phyto-constituents extracted and characterized (Habiba & Yasmeen, 2023). In a previous study, the antimicrobial activities of *Zingiber officinale* isolates against bacterial and fungal loads were confirmed (Santo Grace et al., 2017). *Moringa* species have been recognized by folk medicine practitioners to be effective in treating tumors (Harcourt, 2015) and it contains many phytochemical compounds that contributes to its biological efficacy (Bridgemohan et al., 2020; Goordeen & Mohammed, 2021). In addition to the nutritional benefits of the garlic plant, it has significant medicinal relevance (antioxidant and antimicrobial properties), making it a globally sought-after vegetable crop (Phan et al., 2019). Previous studies have demonstrated the effectiveness of plant extracts, such as those from ginger, garlic, and onion, in controlling spoilage pathogens, including fungi. These findings highlight the potential of these extracts for use in food preservation industries (Adl et al., 2024; Bridgemohan et al., 2020; El-Samawaty et al., 2021; Suharti et al., 2020).

Despite the large amount of research work on carrot, problems of postharvest handling chain still exist. There is a need for more research on the best agronomic treatment approach that will reduce post-harvest losses (Elik et al., 2019). In addition, considerable attention should be paid to the use of affordable and available indigenous botanical extracts to extend the shelf lives of edible carrot roots (Alegbeleye et al., 2022). Taking into account all the facts mentioned above, the present study was conducted to ascertain the protective effect of *Allium cepa, Z. officinale, Allium sativum,* and *Moringa oleifera* ethanol extracts against fungal growth during the storage of carrot roots and to determine which of the plant extracts, and the doses that will offer optimum protection against the fungal pathogens.

MATERIALS AND METHODS

The study was conducted in the Department of Microbiology Research Laboratory at the University of Nigeria, Nsukka (UNN). Freshly harvested Carrot Touchon roots, grown with 10 t/ha of poultry manure and 200 kg of 15 15 15 NPK fertilizer, were obtained from the Department of Crop Science, UNN. The botanicals (onion bulbs, ginger rhizomes, garlic cloves, and *Moringa* leaves) were purchased from a local market in Nsukka, Enugu State. The study adopted a completely randomized experimental design (CRD) with three replicates. The treatments included the four botanicals, a positive control drug (ketoconazole) at concentrations of 500, 250, and 125 mg/ml, two negative controls (water-treated root and untreated root), and three levels of plant extract concentrations (500, 250, and 125 mg/ml).

Preparation, extraction and reconstitution of plant extracts

An optimized cold maceration procedure was used for the extraction. The blended air-dried plant materials (500 g each) were separately macerated with 1000 ml of 95% ethanol for 96 hours with intermittent agitation (Ndu et al., 2008). The filtrate was concentrated using a rotary evaporator, weighed, and stored in a refrigerator at 4 ℃ for future use. The extracts were reconstituted by dissolving 25,000 mg (25 g) of each extract in 50 ml sterile distilled water to produce a 500 mg/ml stock solution; it was further serially diluted to obtain 250 and 125 mg/ml concentrations.

Preparation of culture media

SDA (65 g in liter demineralized water (dH_2O)) and Mueller Hinton Broth (MHB) (38 g in a litre dH_2O) were prepared according to the manufacturer's instructions. The agar was autoclaved at a temperature of 121°C for 20 minutes, cooled, and dispensed into sterile 15 × 100 mm Petri dishes.

The carrot-dip and spoilt tissue preparation

Freshly harvested carrot roots were dipped in the various treatments for two minutes, and each root was transferred to a sterile foil. The foils were placed on the bench at room temperature (26 °C) and monitored every 5 days for three weeks. Segments of tissue (One gram) from the spoiled areas of carrots were cut out with a sterile scalpel and introduced into flat-bottomed flasks containing 100 ml of previously prepared nutrient broth. The flasks were incubated at 28 °C for 72 hours, after which serial ten-fold dilutions of the enriched culture were made.

Enumeration and isolation of fungi

Approximately one-tenth of a milliliter of the serially diluted samples was introduced into plates containing sterile Saboraud Dextrose Agar (SDA) with chloramphenicol (0.05 mg/ml to inhibit bacterial growth). The plates were uniformly spread with a sterile glass rod and incubated in an inverted position at 28 °C for 5 days to allow the development of fungal colonies.

The test fungi (*A. niger, A. fumigatus, A. flavus, F. oxysporium,* and *F. solani)* were isolated from spoilt Carrot Touchon root tissue used for the study and were reserved as stock culture, maintained in SDA slants at 4 °C in a refrigerator.

Purification and maintenance of the microbial isolates
Distinct fungal colonies that developed on the plates were randomly picked and sub-cultured on SDA plates, before transferring to SDA slants. These stock isolates were stored at 4°C in a refrigerator.

Characterization and identification of the fungi isolates
The colonial and microscopic characteristics of the isolated fungi were determined using the lactophenol cotton blue staining method and the slide culture test.
Slide Culture Test: Fungal isolates were introduced on a clean slide and stained with two drops of lactophenol cotton blue solution, then observed under a microscope. According to the description by Oyeleke and Manga (2008), the isolates were identified. Slides containing SDA were inoculated with fragments of aerial mycelia and incubated at 28 °C for 42-72 h. Subsequently, they were stained with lactophenol cotton blue dye and viewed fewer than 100 × magnifications.

Antimicrobial susceptibility test
Agar well diffusion method
The method of Magaldi et al (2004) was used to determine the susceptibility of test fungi to the plant extracts, based on the ability of the extract to diffuse through the agar surface. Fungal isolates on SDA plates (65 g/L) were incubated for 48-72 h. The test fungi were collected in respective test tubes containing normal saline solution (0.85% m/v). The 0.5 McFarland standard was prepared by withdrawing 0.5 ml from a 1% concentration of H_2SO_4 (v/v) and replacing it with the same volume from a 1.1% $BaCl_2$ solution. The resulting turbid solution was adjusted to a 0.5 McFarland standard equivalent to 1-5.0 x 10^6 cfu/ml. The standardized test fungi were inoculated onto the entire MHA plates and allowed to stand for 15 min. A sterile cork-borer (6 mm in diameter) was used to create wells in the plates. Subsequently, 200 µL of various concentrations of the plant extracts were introduced into the wells using a micropipette. The plates were incubated for about 72 h at 28 °C. The diameter of the inhibition zone was recorded based on the observation of the clearance zone around the well.

Determination of minimum inhibitory concentration (MIC) using broth macro-dilution method
This was done according to a modified method of Committee for Antimicrobial Susceptibility Testing of the European Society of Clinical Microbiology & Diseases (EUCAST, 2003). Standardized inoculums of half McFarland turbidity were further increased by two logs to produce a final working concentration of 10^4 cfu/ml. Two serial dilutions of plant extract (of 500 mg/ml stock concentration) were performed in Eppendorf tubes containing 200 µL sterile MHB (38 g/L). This resulted in final extract concentration gradients of 250, 125, 62.5, and 31.25 mg/ml. Each of the tubes was inoculated with equal volumes of the standard inoculum and incubated for 72 hours at 28 °C. The lowest concentration of the test plant extract that showed inhibition of the test fungi was considered the MIC. The positive control was 40 mg/L, while the negative control was the drug-free tube (containing the broth and test organism only).

Determination of minimum fungicidal concentration
The tube(s) indicating the MIC and other preceding tubes (also showing inhibition of the bioactive compound) were streaked on MHA plates and incubated for about 72 hours at 28 °C. The absence of (or very scanty) growth after incubation indicated the minimum fungicidal

concentration (MFC) of the plant extract (Committee for Antimicrobial Susceptibility Testing of the European Society of Clinical Microbiology & Diseases, 2003).

Statistical analysis

Data obtained were analyzed using a One-way Analysis of Variance (ANOVA), and the treatment means were compared using Fisher's Least Significant Difference (F-LSD) at a 5 % probability level (Hayter, 1986). Data collected were also described with descriptive statistics such as averages, standard deviations, percentages, and charts.

Table 1. Antifungal efficacy of plant extracts on carrot root microbial load over time.

Plant Extract	Conc. (mg/ml)	Initial load × 10 CFU	5 DUO × 10 CFU (%)	10 DUO × 10 CFU (%)	15 DUO × 10 CFU (%)	20 DUO × 10 CFU (%)
Onions	500	66.00	55.11 (16.5)	47.12 (28.60)	33.28 (49.57)	14.61 (77.87)
Garlic	500	76.33	55.04 (27.89)	39.17 (47.63)	30.57 (59.95)	18.00 (76.42)
Ginger	500	46.00	25.50 (44.57)	21.90 (52.39)	16.36 (64.44)	8.74 (80.99)
Moringa	500	59.00	49.97 (15.30)	44.70 (24.23)	38.20 (35.25)	19.10 (67.63)
Ketoconazole	500	71.00	37.89 (46.64)	28.14 (60.37)	17.02 (76.03)	11.07 (84.41)
LSD $_{(0.05)}$		NS	24.72	26.29	23.76	NS
Onions	250	60.33	53.43 (11.43)	46.39 (23.10)	34.76 (42.39)	20.60 (65.85)
Garlic	250	34.00	29.43 (13.45)	26.53 (21.98)	20.72 (39.06)	13.91 (59.10)
Ginger	250	48.00	35.61 (25.81)	27.12 (43.50)	20.08 (58.16)	16.20 (66.25)
Moringa	250	45.00	41.68 (7.38)	38.09 (15.35)	31.18 (30.71)	23.04 (48.80)
Ketoconazole	250	57.00	33.07 (41.99)	25.82 (54.71)	17.50 (69.30)	10.39 (81.77)
LSD $_{(0.05)}$		NS	22.13	25.84	18.58	17.66
Onions	125	67.00	60.73 (9.36)	52.29 (21.95)	45.06 (32.74)	30.37 (54.67)
Garlic	125	32.00	30.19 (5.66)	28.20 (11.88)	22.93 (28.33)	16.76 (47.64)
Ginger	125	41.00	37.15 (9.38)	34.65 (15.48)	32.50 (20.72)	30.82 (24.82)
Moringa	125	41.67	39.36 (5.55)	37.20 (10.73)	31.67 (24.01)	27.52 (33.96)
Ketoconazole	125	76.67	46.20 (39.74)	37.05 (51.68)	25.92 (66.19)	14.96 (80.49)
LSD $_{(0.05)}$	-	20.86	15.45	16.95	15.96	15.08
H_2O	-	31.00	32.37 (-4.42)	35.75 (-15.32)	39.74 (-28.2)	43.22 (-39.42)
Untreated cont.	-	45.00	55.25 (-22.78)	66.43 (-47.62)	72.45 (-61.01)	79.06 (-75.68)
LSD $_{(0.05)}$	-	9.44	NS	18.25	42.38	28.17

The table shows the percentage reduction of microbial load on carrot roots treated with various plant extracts at different concentrations (125, 250, and 500 mg/ml) and a positive control (Ketoconazole) over a period of 20 days. The negative controls include water-treated (H2O) and untreated roots. DUO represents Days Under Observation, and values in parentheses indicate the percentage reduction or increment of microbial load compared to the initial load. LSD refers to the Least Significant Difference at a 0.05 level of significance.

RESULTS

Microbial load reduction on carrot roots by plant extracts and ketoconazole

The plant extracts significantly ($p < 0.05$) reduced the microbial loads on carrot roots (Table 1). At 20 days under observation (DUO), the different extracts at the highest dose (500 mg/ml) did not differ in their response to microbial load reduction. Throughout the observation period, the positive control (Ketoconazole) had a better effect on the percentage bio load reduction, although, at five DUO, it had a similar response with ginger extract. However, *Moringa* poorly protected the carrot root against the pathogens throughout the study period, although, at 5 DUO, it significantly ($p < 0.05$) had a similar effect with onion extract. Furthermore, at 250 mg/ml, ketoconazole had a higher percentage microbial load reduction than the other treatments, with *Moringa* having the least antifungal activity. Interestingly, it had a similar effect with onions and garlic at five DUO, and with garlic at 15 and 20 DUO. Notably, throughout the observation period, all the extracts at 125 mg/ml had poorer antifungal activity than ketoconazole. It was also observed that the percentage reduction in colony-forming units increased with days across all the concentrations (Table 1). The same table revealed that the three concentrations significantly ($p < 0.05$) affected the percentage reduction in microbial load on carrot roots. All the three concentrations of positive control (Ketoconazole) did not react differently to microbial load reduction. Among the negative control treatments (H_2O treated roots and untreated roots), there was a significant effect on the bio load increment. The microbial load increased in the two negative controls, but the mean percentage bio load increment in untreated roots was significantly ($p < 0.05$) higher than the values for water-treated roots (Table 1).

Inhibition zones of plant extracts and ketoconazole against test fungi isolates

It was revealed that ginger and *Moringa* extracts, along with the positive drug control (Ketoconazole) at given concentrations, had clear zones of inhibition against the test fungi isolates (Table 2). Ketoconazole at concentrations of 500, 250, and 125 mg/ml produced mean inhibition zones of 22.5 mm, 22 mm, and 14 mm, respectively, against *A. niger*, while 500 mg/ml of *Moringa* extract had the least mean inhibition zone diameter (10 mm). The highest and intermediate concentrations (500 and 250 mg/ml) of ginger exhibited activity against *A. fumigatus* and produced mean inhibition zone diameters of 12 mm each. Ketoconazole, at all levels of concentrations, produced clear zones of inhibition against *A. fumigatus*, with the highest mean inhibition zone diameter (24.5 mm) at 500 mg/ml and the least diameter (15 mm) at the lowest concentration (125 mg/ml). The plant extracts provided no inhibition against *A. flavus* and *F. oxysporum,* while Ketoconazole produced the highest mean inhibition zone of 19 mm against *A. flavus* at 500 mg/ml and the least inhibition zone diameter (14 mm) at 125 mg/ml concentration. Similarly, the highest inhibition zone diameter of 22.5 mm against *F. oxysporum* was produced at 500 mg/ml by Ketoconazole, while the least microbial activity (9 mm IZD) was produced at 125 mg/ml concentration. The same table showed that the least inhibition zone diameter (8 mm) against *F. solani* was produced at 125 mg/ml by ginger, while ketoconazole had the highest clearance zone of 20 mm at 500 mg/ml.

Minimum inhibition concentrations of plant extracts and ketoconazole against fungal strains

The treatments inhibited the activities of all the test fungi at a given minimum inhibition concentrations (MIC) (Table 3). The extracts of ginger and *Moringa* had higher inhibitory activity against *A. niger* than the other treatments with MIC of 62.5 mg/ml each while garlic produced the least effect by inhibiting the reference test fungi at highest MIC value (250

mg/ml). Among the treatments, ginger and ketoconazole had the lowest MIC value (62.5 mg/ml each), and was more active while onions and garlic poorly inhibited *A. fumigatus* with MIC value of 250 mg/ml each. The inhibitory activity of ginger against *A. flavus* at MIC of 31.5 mg/ml was more than the other treatments whereas; garlic and onions had the least activity against *A. flavus* at higher MIC of 250 mg/ml each. Ketoconazole's inhibitory action against *F. oxysporum* at MIC of 31.5 mg/ml was more than the inhibition offered by all the plant extracts while, garlic and ginger had the least activity against the reference test fungi at a higher MIC (250 mg/ml) value. Ginger among the plant extracts and the positive control had higher inhibitory activity (i.e. at MIC 62.5 ml/ml each) against *F. solani* than the other treatments with their inhibitory activity at the same MIC (250 mg/ml) (Table 3).

Table 2. Inhibition zone diameter at a given concentration of botanical extracts and ketoconazole against test fungi.

		Test fungi/IZD (mm)				
Botanicals	Conc. (mg/ml)	*A. niger*	*A. fumigatus*	*A. flavus*	*F. oxysporum*	*F. solani*
Ginger	500	No zone	12.0 ± 00	No zone	No zone	10.0 ± 00
Ginger	250	No zone	12.0 ± 00	No zone	No zone	9.0 ± 1.41
Ginger	125	No zone	No zone	No zone	No zone	8.0 ± 00
Moringa	500	10.0 ± 2.83	No zone	No zone	No zone	No zone
Ketoconazole	500	22.5 ± 0.71	24.5 ± 2.12	19.0 ±1.41	22.5 ± 4.95	20.0 ± 00
Ketoconazole	250	22.0 ± 0	22.5 ± 3.54	18.5 ± 2.12	22.0 ± 2.12	18.0 ± 2.83
Ketoconazole	125	14.0 ± 2.83	19.0 ± 1.41	15.0 ± 00	16.0 ± 2.12	16.5 ± 4.95
Ketoconazole	62.5	No zone	18.0 ± 1.41	No zone	13.0 ± 2.83	12.0 ± 00
Ketoconazole	31.25	No zone	15.0 ± 2.12	No zone	9.0 ± 1.41	12.0 ± 00

The inhibition zone diameters (IZD) of ginger, *Moringa*, and ketoconazole at various concentrations against different test fungi. The data show the antifungal activities of these substances against test fungi.

Table 3. Minimum inhibitory concentration of botanical extracts against test fungi.

	Test fungi/MIC (mg/ml)				
Botanicals	*A. niger*	*A. fumigatus*	*A. flavus*	*F. oxysporum*	*F. solani*
Garlic	250	250	250	250	250
Ginger	62.5	62.5	31.25	250	62.5
Moringa	62.5	125	125	125	250
Onions	125	250	250	125	250
Ketoconazole	125	62.5	125	31.25	62.5

This table displays the MIC values (in mg/ml) of different botanical extracts and ketoconazole against various strains of test fungi. MIC refers to the lowest concentration of a substance required to inhibit the growth of a microorganism. The table shows that each treatment inhibited the activities of all the test fungi at a given minimum inhibition concentration (MIC).

Table 4. Minimum biocidal concentration of botanicals against test fungi.

	Test fungi/MBC (mg/ml)				
Botanicals	*A. niger*	*A. fumigatus*	*A. flavus*	*F. oxysporum*	*F. solani*
Garlic	>250	>250	>250	>250	>250
Ginger	62.5	125	31.25	250	250
Moringa	>250	>250	>250	>250	>250
Onions	>250	>250	>250	>250	>250
Ketoconazole	>250	>250	125	31.25	>250

This table presents the MBC values (in mg/ml) for various botanical extracts and ketoconazole against different strains of test fungi. The symbol ">" indicates that the MBC value was greater than the highest concentration tested.

Minimum fungicidal concentrations of plant extracts and ketoconazole against fungal strains

Among the treatments, only the extract of Ginger at 62.5 mg/ml and 125 mg/ml were fungicidal to *A. niger and A. fumigatus* respectively (Table 4). The extracts of ginger and ketoconazole were able to eliminate *A. flavus* at MBC of 62.5 mg/ml and 125 mg/ml, respectively while the other treatments had non-biocidal action on the same test fungi. Ketoconazole and extract of Ginger were equally biocidal to *F. oxysporum* at MBC of 31.25 mg/ml and 250 mg/ml respectively while the other treatments had no activity. However, among the treatments, only the ginger extract produced biocidal effect on *F. solani* at 250 mg/ml.

DISCUSSION

The phytoconstituents inherent in the various plant extracts were implicated in the reduction of bio-load seen in carrot roots. The present study however, established the effectiveness of the highest dose of ginger extract in reducing bio-load than the lowest dose. This result is line with the findings of (Xi et al., 2022) that ginger rhizome extract gave higher antifungal activity at higher dose and drastically reduced *F. solani* colony forming units and subsequent spores' advancement. Furthermore, the higher percentage reduction in bio-load by ginger extract over other plant extracts proved its higher potency and broad spectrum of activity. This present finding concurs with that of Onyeagba et al. (2004) where ginger rhizome extract was more effectiveness than other ethanol extracts of garlic and lime against isolates of *A. niger* and *A. flavus*, etc. This may be probably, due to numerous (over 400) bioactive compounds (zingerone, traces of monoterpnoid, shogoal, etc.) associated with ginger ingredient (Chrubasik et al., 2005; Grzanna et al., 2005). The fungal bio load reduction caused by highest dose (500 mg/ml) of ginger extract was comparable to that of standard drug (250 mg/ml). Interestingly, the efficacy of ginger extract is in tandem with the earlier report where it was implicated in effective retardation of infectious spore multiplication and growth as result of its unlimited fungicidal properties (Xi et al., 2022). However, the reverse was the case with negative controls (water treated control and untreated control), where there was massive microbial growth which was higher in the untreated roots. This may be because of absence of drug protection (treatment), immunity degeneration, and subsequent breakdown of the root tissues, following ageing and senescence in the course of the study. The degradation process is always associated with increased respiration and immunity collapse during prolonged storage. This is in concordance with Ciccares et al. (2013) that decay and death of stored produce may be traced to high rate of catabolic process at the expense of the reserved energy. Clearance zones of inhibition against test fungi were recorded in ginger and *Moringa*. These extracts at concentrations of 125 mg/ml and above, exhibited clearance zone of inhibition (8.00 mm to 10.00 mm) against *A. niger* and *A. fumigatus, F. solani*, respectively. Several studies established the anti-fungal activity of *Moringa* in arrays of extraction solutions: ethanol against *A. niger*, *Rhizopus stolonifera* and *Candida albican* (Aisha et al., 2016) inhibition against *A. niger*, *Sclerotium rolfsii, Botryodiplodia theobromae* etc. with petroleum ether extraction (Paray et al., 2018), and ethanol, methanol, water etc. extracts against *Aspergillus* spp, *Rhizopus* spp., *Penicillium* spp, and *Trichodema* spp (Oniha et al., 2021). Likewise, fungal inhibitory activity of ginger rhizome extract against *F. solani* was reported in a previous study where the activity of the extract was higher than the extract from other parts (stem and leaves) (Peng et al., 2022). The absence of inhibition zones observed in the onion and garlic extracts may be probably due to inability of the ingredients to diffuse freely into the agar media (MHA).

However, all the test fungi isolates displayed varying levels of susceptibility to all the plant extracts and control drug. The present study observed a higher susceptibility of all the isolates to garlic and onion extracts which reflected in the high MIC (125 to 250 mg/ml) and higher MBC (>250 mg/ml) values. This is line with report where the MIC and MBC of garlic and onion extracts were established, although against a bacterial pathogen, *Staphylococcus aureus* (Anyamaobi et al., 2020). Furthermore, in another study, the anti-fungal activity of aqueous extracts of garlic (MIC/MBC: 325 mg/ml) was more effective than that of onions (MIC/MBC >900 mg/ml) and Leek (MIC: 900 and MBC: >900 mg/ml) against *A. niger* (Irkin & Korukluoglu, 2007).

Surprisingly, *Moringa* inhibited the growth of test fungi but inhibited *A. niger* at MIC: 62.5 mg/ml) more. This result is similar to the result of postharvest study on onions rot where antifungal potency of *Moringa* was proved. In the same study, the ethanol leaf extract of the *Moringa* inhibited *A. niger* at 75% concentration in potato dextrose broth (Arowora & Adetunji, 2014).

Notably, among the botanicals tested in the study, ginger was the only extract biocidal to all the test fungi. This may be because, ginger possesses a wider range of organic compounds and metabolites that may have countered normal biological metabolic process rendering the cell membrane porous, destroying building up process of respiratory system, and compromising cell wall integrity (Liu et al., 2017; Mandal & Domb, 2024). Also, among the two fungi species *Aspergillus* and *Fusarium*, the ginger extract was more fungicidal (31.25 to 125 mg/ml) to the three isolates of *Aspergillus* than to the two isolates of *Fusarium* (250 mg/ml).

CONCLUSION

The study highlights the potential of plant extracts, particularly ginger, garlic, and *Moringa*, as alternative treatments for reducing fungal growth in stored carrots. Ginger extract was found to be the most effective against all test fungi, followed by garlic and onion extracts. *Moringa* extract inhibited the growth of test fungi but was more effective against *A. niger*. The results suggest that plant extracts could be a viable alternative to synthetic fungicides in the food preservation industry, although further research is needed to investigate their mechanisms of action and safety. Additionally, the study emphasizes the importance of controlling *F. oxysporum*, a more resistant pathogen, in postharvest carrot handling. Overall, these findings provide valuable insights into the use of plant extracts for reducing fungal growth in stored carrots and suggest potential avenues for future research.

Funding
No funding.

Conflicts of interest/Competing interests
The authors declare that they have no conflicts of interest or competing interests with respect to the research, authorship, and/or publication of this article. The authors have no personal, professional, or financial affiliations that could potentially influence the outcomes of this study.

Availability of data and material
The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Authors' contributions

EAN designed the experiment in collaboration with FNM, JAN, BCE, and KPB. FNM, JAN, BCE, and KPB provided guidance and expertise throughout the research process for EAN to conduct the research and write the initial manuscript. JAN & KPB revised the manuscript. All authors reviewed and approved the final version of the manuscript.

Acknowledgments

We would like to express our gratitude to Chidebelu Paul for his invaluable guidance and assistance throughout the course of this research project. His expertise and support were instrumental in the completion of this work.

REFERENCES

Adl, E., Jahani, M., & Aminifard, M. H. (2024). Investigating the antifungal effects of essential oils on *Aspergillus* sp. in strawberry (*Fragaria ananassa* Duchesne) fruit. *Journal of Horticulture and Postharvest Research*, *7*(2), 141–154. https://doi.org/10.22077/JHPR.2024.7065.1349

Ahmad, T., Cawood, M., Iqbal, Q., Ariño, A., Batool, A., Sabir Tariq, R. M., Azam, M., & Akhtar, S. (2019). Phytochemicals in *Daucus carota* and their health benefits—review article. *Foods*, *8*(9), 424. https://doi.org/10.3390/FOODS8090424

Aisha, Kutama, & Kabir. (2016). Phytochemical screening and antifungal activity of *Moringa oleifera* on some selected fungi in Dutse, Jigawa State. *Global Advanced Research Journal of Agricultural Science*, *5*(6), 243–248.

Alegbeleye, O., Odeyemi, O. A., Strateva, M., & Stratev, D. (2022). Microbial spoilage of vegetables, fruits and cereals. *Applied Food Research*, *2*(1), 100122. https://doi.org/10.1016/j.afres.2022.100122

Alshammari, N. (2023). Mycotoxin source and its exposure causing mycotoxicoses. *Bioinformation*, *19*(4), 348–357. https://doi.org/10.6026/97320630019348

Anyamaobi, P. O., Wokem, G. N., Bessie Enweani, I., Nwamaka Okosa, J., Emmanuel Opara, C., & Nwokeji, M. C. (2020). Antimicrobial effect of garlic and ginger on *Staphylococcus aureus* from clinical specimens in Madonna University Teaching Hospital, Nigeria. *International Journal of Recent Scientific Research*, *11*(3), 37840–37845. https://doi.org/10.24327/ijrsr.2020.1103.5189

Arowora, K. A., & Adetunji, C. O. (2014). Antifungal effects of crude extracts of *Moringa oleifera* on *Aspergillus niger* v. tieghem associated with post harvest rot of onion bulb. *SMU MEDICAL JOURNAL*. https://worldveg.tind.io/record/52606

Bridgemohan, P., Goordeen, A., Mohammed, M., & Bridgemohan, R. S. H. (2020). Review of the agro-ecology, phytochemistry, postharvest technology and utilization of moringa (*Moringa oleifera* Lam.). *Journal of Horticulture and Postharvest Research*, *3*(2), 311–332. https://doi.org/10.22077/JHPR.2020.3037.1116

Chrubasik, S., Pittler, M. H., & Roufogalis, B. D. (2005). *Zingiberis rhizoma*: a comprehensive review on the ginger effect and efficacy profiles. *Phytomedicine : International Journal of Phytotherapy and Phytopharmacology*, *12*(9), 684–701. https://doi.org/10.1016/J.PHYMED.2004.07.009

Committee for Antimicrobial Susceptibility Testing of the European Society of Clinical Microbiology, E., & Diseases, I. (2003). Determination of minimum inhibitory concentrations (MICs) of antibacterial agents by broth dilution. *Clinical Microbiology and Infection*, *9*(8), 9-15. https://doi.org/10.1046/j.1469-0691.2003.00790.x

El-Samawaty, A. E. R. M. A., El-Wakil, D. A., Alamery, S., & Mahmoud, M. M. H. (2021). Potency of plant extracts against *Penicillium* species isolated from different seeds and fruits in Saudi Arabia. *Saudi Journal of Biological Sciences*, *28*(6), 3294. https://doi.org/10.1016/J.SJBS.2021.02.074

Elik, A., Yanık, D. K., Yanik, D. K., Guzelsoy, N. A., Yavuz, A., & Gogus, F. (2019). Strategies to reduce post-harvest losses for fruits and vegetables. *International Journal of Scientific and Technological Research*. https://doi.org/10.7176/JSTR/5-3-04

Goordeen, A., & Mohammed, M. (2021). Growth, development, maturation indices, proximate and mineral composition of moringa (*Moringa oleifera*). *Journal of Horticulture and Postharvest Research*, *4*(4), 427-438. https://doi.org/10.22077/jhpr.2021.4296.1205

Grzanna, R., Lindmark, L., & Frondoza, C. G. (2005). Ginger--an herbal medicinal product with broad anti-inflammatory actions. *Journal of Medicinal Food*, *8*(2), 125–132. https://doi.org/10.1089/JMF.2005.8.125

Habiba, U., & Yasmeen, H. (2023). Article 7 Part of the Alternative and Complementary Medicine Commons Recommended Citation Recommended Citation Afzal, I. *Journal of Bioresource Management*, *10*(4). https://corescholar.libraries.wright.edu/jbm

Harcourt, P. (2015). Antimicrobial activity of the leaf and seed extracts of *Moringa Oleifera* on some bacteria isolates. *Journal of Medical Science and Clinical Research*, *3*(1), 3904–3912. https://worldveg.tind.io/record/53698

Hayter, A. J. (1986). The maximum familywise error rate of Fisher's least Significant difference test. *Journal of the American Statistical Association*, *81*(396), 1000. https://doi.org/10.2307/2289074

Irkin, R., & Korukluoglu, M. (2007). Control of *Aspergillus niger* with garlic, onion and leek extracts. *African Journal of Biotechnology*, *6*(4), 384–387. https://www.ajol.info/index.php/ajb/article/view/56223

Liu, K., Zhou, X., & Fu, M. (2017). Inhibiting effects of epsilon-poly-lysine (ε-PL) on *Pencillium digitatum* and its involved mechanism. *Postharvest Biology and Technology*, *123*, 94–101. https://doi.org/10.1016/J.POSTHARVBIO.2016.08.015

Magaldi, S., Mata-Essayag, S., Hartung De Capriles, C., Perez, C., Colella, M. T., Olaizola, C., & Ontiveros, Y. (2004). Well diffusion for antifungal susceptibility testing. *International Journal of Infectious Diseases*, *8*(1), 39–45. https://doi.org/10.1016/j.ijid.2003.03.002

Mandal, M. K., & Domb, A. J. (2024). Antimicrobial activities of natural bioactive polyphenols. *Pharmaceutics 2024, Vol. 16, Page 718*, *16*(6), 718. https://doi.org/10.3390/PHARMACEUTICS16060718

Moradinezhad, F., & Ranjbar, A. (2023). Advances in postharvest diseases management of fruits and vegetables: A review. *Horticulturae*, *9*(10), 1099. https://doi.org/10.3390/horticulturae9101099

Motegaonkar, S., Shankar, A., Tazeen, H., Gunjal, M., & Payyanad, S. (2024). A comprehensive review on carrot (*Daucus carota* L.): the effect of different drying methods on nutritional properties and its processing as value-added foods. *Sustainable Food Technology*, *2*(3), 667–688. https://doi.org/10.1039/D3FB00162H

Ndu, O. O., Akah, P. A., Ochiogu, I. S., Ezike, A. C., & Mbaoji, F. N. (2008). Effects of ethanolic root and leaf extracts of *Cissampelos mucronata* on length of gestation period. *Planta Medica*, *74*(09), PA131. https://doi.org/10.1055/S-0028-1084129

Odeyemi, O. (2019). Postharvest handling practices and losses of some major fruits sold in Abeokuta, South-western Nigeria. *Nigeria Journal of Horticultural Society*, *24*(1), 113–121.

Oniha, M., Eni, A., Akinnola, O., Omonigbehin, E. A., Ahuekwe, E. F., & Olorunshola, J. F. (2021). In vitro antifungal activity of extracts of *Moringa oleifera* on phytopathogenic fungi affecting *Carica papaya*. *Open Access Macedonian Journal of Medical Sciences*, *9*(A), 1081–1085. https://doi.org/10.3889/OAMJMS.2021.6794

Onyeagba, R. A., Ugbogu, O. C., Okeke, C. U., & Iroakasi, O. (2004). Studies on the antimicrobial effects of garlic (*Allium sativum* Linn), ginger (*Zingiber officinale* Roscoe) and lime (*Citrus aurantifolia* Linn). *African Journal of Biotechnology*, *3*(10), 552–554. https://doi.org/10.5897/AJB2004.000-2108

Oyeleke, S. B., & Manga, S. B. (2008). Essentials of laboratory practicals in microbiology. In *Minna, Nigeria: Tobest Publishers*.

Paray, A. R., Rasool, A., Bhakat, M., Mohanty, T. K., Behare, P., Lone, S. A., Parry, U. R., Kumar, R., Sinha, R., Yadav, H. P., Rahim, A., Danish, Z., & Shah, N. (2018). Antimicrobial activity of crude aqueous extracts of *Moringa oleifera*, *Azadirachta indica*, *Carica papaya*, *Tinospora cordifolia* and *Curcuma longa* against certain bacterial pathogens. *Journal of Pharmacognosy and Phytochemistry*, *7*(4).

Peng, H., Hu, H., Xi, K., Zhu, X., Zhou, J., Yin, J., Guo, F., Liu, Y., & Zhu, Y. (2022). Silicon nanoparticles enhance ginger rhizomes tolerance to postharvest deterioration and resistance to *Fusarium solani*. *Frontiers in Plant Science*, *13*, 816143. https://doi.org/10.3389/FPLS.2022.816143/BIBTEX

Phan, A. D. T., Netzel, G., Chhim, P., Netzel, M. E., & Sultanbawa, Y. (2019). Phytochemical characteristics and antimicrobial activity of Australian grown garlic (*Allium Sativum* L.) cultivars. *Foods 2019, Vol. 8, Page 358*, *8*(9), 358. https://doi.org/10.3390/FOODS8090358

Rahimi Kakolaki, M., Omidi, A., Rasooli, A., & Shekarforoush, S. S. (2024). *In vitro* antifungal activity of barberry fruit extract (*Berberis* spp.) against *Fusarium* spp.. *Journal of Horticulture and Postharvest Research, 7*(Special Issue - Postharvest Technologies), 47-60. http://doi.org/10.22077/jhpr.2023.6783.1333

Rawal, P., & Singh Adhikari, R. (2016). Evaluation of antifungal activity of *Zingiber officinale* against *Fusarium oxysporum* f. sp. lycopersici. *Advances in Applied Science Research*, *7*(2), 5–9. www.pelagiaresearchlibrary.com

Santo Grace, U., Sankari, M., & Gopi. (2017). Antimicrobial activity of ethanolic extract of *Zingiber officinale* – An *in vitro* study. *Journal of Pharmaceutical Sciences and Research*, *9*(9), 1417–1419.

Sezer, F., Deniz, S., Sevim, D., Chaachouay, N., & Zidane, L. (2024). Plant-derived natural products: A source for drug discovery and development. *Drugs and Drug Candidates 2024, Vol. 3, Pages 184-207*, *3*(1), 184–207. https://doi.org/10.3390/DDC3010011

Suharti, T., Nugraheni, Y. M. M. A., Suita, E., & Sumarni, B. (2020). Effect of plant extracts and chemical fungicide on viability and percentage of seed-borne fungal infection on calliandra (*Calliandra callothyrsus*) seed. *IOP Conference Series: Earth and Environmental Science*, *533*(1), 012040. https://doi.org/10.1088/1755-1315/533/1/012040

Xi, K. Y., Xiong, S. J., Li, G., Guo, C. Q., Zhou, J., Ma, J. W., Yin, J. L., Liu, Y. Q., & Zhu, Y. X. (2022). Antifungal activity of ginger rhizome extract against *Fusarium solani*. *Horticulturae*, *8*(11), 983. https://doi.org/10.3390/HORTICULTURAE8110983/S1

JOURNAL OF HORTICULTURE AND POSTHARVEST RESEARCH
2025, VOL. 8(1), 27-42

Journal of Horticulture and Postharvest Research

Journal homepage: www.jhpr.birjand.ac.ir

University of Birjand

Enhancing decay resistance and maintaining quality of stored apples (*Malus domestica* 'Golden Delicious') through essential oil-enriched edible coatings

Atiyeh Oraee[1], Yahya Selahvarzi[1 *], Mona Ghazimoghadam[1], Bahram Abedi[1] and Mohammad Ali Sabokkhiz[2]

1, Department of Horticultural Science and Landscape, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran
2, Department of Plant protection, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran

ARTICLE INFO

Original Article

Article history:
Received 29 May 2024
Revised 19 September 2024
Accepted 2 October 2024

Keywords:
Antioxidant activity
Blue mold decay
Fruit quality
Postharvest decay

DOI: 10.22077/jhpr.2024.7686.1385
P-ISSN: 2588-4883
E-ISSN: 2588-6169

***Corresponding author:**
Department of Horticultural Science and Landscape, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran.

Email: selahvarzi@um.ac.ir

© This article is open access and licensed under the terms of the Creative Commons Attribution License http://creativecommons.org/licenses/by/4.0/ which permits unrestricted, use, distribution and reproduction in any medium, or format for any purpose, even commercially provided the work is properly cited.

ABSTRACT

Purpose: Apples are susceptible to several diseases, which makes marketing and storage challenging. Therefore, it is critical to develop strategies that minimize weight loss while maintaining quality. **Research method:** Golden Delicious apples were coated with an edible mixture of *Aloe vera* gel (ALV) with or without *Zataria multiflora* essential oil (ZMO). Subsequently, the effects of these coatings on disease severity and incidence of *Botrytis cinerea* and *Penicillium expansum*, as well as storage quality features, were assessed. Inoculated apples with *B. cinerea* or *P. expansum*, the causative agents of apple postharvest gray and blue molds, were covered and stored at 25°C for 10 days and 2°C for 120 days. **Findings:** The antifungal ALV gel coatings significantly reduced the severity of gray and blue molds on inoculated apples, with the 150 $\mu L\ L^{-1}$ of ZMO-based coating showing the highest effectiveness. The ALV+ZMO coatings had the best results, exhibiting a lower decay index, and increased firmness, total phenol content, and antioxidant activity in the coated apples. The highest ripening index was observed in the control samples, which ranged from the initial value of 37.0 to 54.7 by the end of the storage period. Meanwhile, ALV alone was the most effective at decreasing weight loss. **Research limitations:** Thyme essential oil (EO) has limitations when used directly, inculding its strong odor and flavor, low stability, low water solubility, and high volatility**. Originality/Value:** Overall, ALV and ZMO edible coatings appear to be a viable solution for suppressing fungal infections while maintaining apple quality under both situations.

INTRODUCTION

The apple fruit plays a beneficial role in providing antioxidant protection to the human body due to its rich source of phytochemicals such as flavonoids and phenolic acids (Mannucci et al., 2017). Global apple production in 2023-2024 exceeded 83 M tons (FAS, 2024). There is a growing market demand for fresh fruit available year-around. However, despite the market demands for fresh apples throughout the year, their shelf-life diminishes rapidly due to physiological changes and fungal decay (Mostafidi et al., 2020). Postharvest apple losses are primarily caused by postharvest fungal pathogens such as *Botrytis cinerea* and *Penicillium expansum*, the agents responsible for gray and blue mold, which adversely affect fruit safety and quality, resulting in significant economic losses (Glos et al., 2022).

Developing eco-friendly alternative methods, including bio-control agents containing bioactive and chemical compounds (Li et al., 2017), sodium de-hydro acetate (Duan et al., 2016), and natural antimicrobial substances (Esmaeili et al., 2021; Rahimi Kakolaki et al., 2024) to inhibit postharvest fungal decay is becoming increasingly important (Moradinezhad & Ranjbar, 2023). In recent decades, edible films and coatings have been recognized as cost-effective and environmentally friendly techniques that can extend the shelf life of products, both during storage and when being sold in shops and marketplaces (Panchal et al., 2022).

Aloe vera (ALV) gel edible coating is a natural polysaccharide that contains a variety of biological components (Supa et al., 2024). It serves as a rich source of antimicrobial and antioxidant agents, such as phenolic compounds which include anthraquinones, emodin, and aloin (Habibi et al., 2022). Additionally, *Aloe vera* can trigger defense responses in plant tissues (Hassan et al., 2022). These properties of *Aloe vera* are attributed to its high polysaccharides content along with vitamins, soluble sugars, minerals (such as calcium, chromium, copper, selenium, magnesium, manganese, potassium, sodium and zinc), proteins, and a relatively low fat content (Pandey & Singh, 2016). To increase the lipid content, essential oils rich in fatty acids like *Zataria multiflora* essential oil (ZMO) can be incorporated into the treatment, which has been shown to effectively manage deterioration and prolong the overall quality and shelf life of fresh produce (Hashemi et al., 2021; Jahanshahi et al., 2023).

A combination of polysaccharides and lipids has been suggested to enhance the efficacy of the coating treatment. This study aimed to investigate the protective effect of ALV alone or in combination with ZMO in managing gray and blue mold caused by *B. cinerea* and *P. Expansum*. The influence of these treatments and fungal inoculation on fruit quality and physiological characteristics was also evaluated. Additionally, the effectiveness of ALV and ZMO as a novel coating was investigated concerning the physicochemical and sensory properties of the fruit during cold storage.

MATERIALS AND METHODS

'Golden Delicious' apple fruits (*Malus domestica* Brokh) were manually harvested at commercial maturity (140 days after full bloom) from an orchard in Chenaran, located in North-East Iran's Khorasan region. The collection of plant material adhered to applicable institutional, national, and international guidelines and legislation, with permission obtained for the plant material collection. The fruits were sterilized with 1% sodium hypochlorite for 2 min, followed by washing with tap water, disinfection with 70% ethanol, and air-drying at room temperature.

Inoculum preparation

The fungi used in this study were *Botrytis cinerea* ATCC (12481) and *Penicillium expansum,* obtained from the Iranian Research Organization for Science and Technology and the Agriculture Biotechnology Research Institute of Iran, respectively. The fungal strains were cultured on Potato Dextrose Agar (PDA) at 28±2°C for 5-10 days. Suspensions were prepared by immersing the culture in distilled water and filtered through two layers of cheesecloth. The inoculum size was assessed using a hemocytometer and adjusted to a concentration of 5×10 conidia ml^{-1} for each strain (Oliveira et al., 2015).

Preparation of *Aloe vera* gel (ALV) and *Zataria multiflora* essential oil (ZMO)

Mature and fresh leaves of the *Aloe vera* plant were harvested from a greenhouse located on Ferdowsi university of Mashhad's campus. The mucilaginous gel was extracted from the outer portion and blended using a blender. The gel's properties were determined as pH 4.63±0.02, total soluble solids=1.5±0.05%, and acidity=0.043±0.003% citric acid. The gel was stabilized by adjusting the pH to 3.75 with phosphoric acid (Navarro et al., 2011), then pasteurized by heating at 80°C for 10s, and cooled down to 5°C for further use (Jiwanit et al., 2018). The gel was diluted with deionized water to achieve a 60% (v/v) concentration. The Persian thyme plants, at a full bloom, were harvested from the major growing area of Khorasan in Mid-June of 2022. The essential oil was extracted through hydro-distillation utilizing a Clevenger apparatus for 3h. The obtained oil was then dried with anhydrous sodium sulfate and stored in sealed vials at 4°C for further use.

Treatment application of antifungal coating

The main experiment was divided into two parts; in the first part, the fruits were kept at 25°C for 10 days, and in the second part, the fruits were stored at 2°C for 120 days' post-treatment. Apple fruits were selected and randomly divided into 13 groups, each consisting of 12 samples. There were 12 replications per treatment (n=12). One group was used for analyzing the fruit characteristics at harvest. while the remaining samples were used for the following treatments: control (untreated), 60% ALV gel, 150 µL L^{-1} of ZMO, and 60%ALV+150 µL L^{-1} of ZMO (ALV+ZMO). Before the treatments, the fruits were artificially wounded (approximately 5 mm in depth) near the equatorial region of the fruit using a sterile cork borer. Three groups of 12 fruits each were treated for 10 min by immersing them in the respective treatment (distilled water, 60%ALV, 150 µL L^{-1} of ZMO, and 60%ALV+150 µL L^{-1} of ZMO) and then allowed to dry at room temperature. Twenty-four hours after the treatments, two groups of each treatment were inoculated with *B. cinerea* (gray mold) and *P. Expansum* (blue mold) by depositing 20 µl of the pathogen suspensions (5×10 conidia ml^{-1}) into the previously created wounds. One group from each treatment served as a control without fungus inoculation. All fruits were stored at 25°C and 90-95% relative humidity for 10 days. The experiment was replicated, and lesion diameter and disease severity were measured 10 days after treatment and storage at 25°C. The primarly factor considered was the types of essential oils (ALV and ZMO), while the secondary factor was the presence of two types of fungi (*Botrytis cinerea* and *Penicillium expansum*). Following the 10 days treatment and storage at 25°C, measurements were taken for antioxidant activities, firmness, and total phenol content. The primary factor considered here was the different types of essential oils (ALV and ZMO), and the secondary factor was present and absent of the two fungi strains (*Botrytis cinerea* and *Penicillium expansum*). Decay index, weight loss, antioxidant activities, firmness, and total phenol content were measured at a temperature of 2°C. The primary factor considered here was the different concentrations of essential oils, while the secondary factor was the timing of measurement.

Microbiological analysis infection

After 10 days, the disease incidence of gray and blue mold was calculated by measuring the average diameter of the damaged area (lesion diameter). Disease severity was assessed using a five-point scale based on the extent of damage, with the following categories: 1=0% of surface rotten per fruit, 2= 10-25%, 3= 26-50%, 4=51-75% and 5= 76-100% rotten (Maqbool et al., 2011).

Fruit quality

In the second part of the experiment, the effect of the edible ALV coatings enriched with ZMO on the decay index and quality of apple fruit during 120 days of storage at 2±1°C was investigated. The treatments included: control group (without coating), 60%ALV, 150 μL L^{-1} of ZMO, and 60%ALV+150 μL L^{-1} of ZMO. The fruits were divided into 64 groups with ten apple fruits per group, and there were four replications of each treatment. Each fruit was cut in half along the equator to assess internal symptoms of physiological disorders and decay, using the following scale: 0= no decayed fruit; 1= slight (<25% decay); 2= moderate symptoms (25%-50% decay); and 3= sever (>50% decay) (Laribi et al., 2013).

12 fruits per treatment were used to measure weight loss (WL), which was expressed as the percentage loss of initial weight. Firmness was assessed using a penetrometer model (Effegi-mod FT327). The ripening index was considered as TSS/TA ratio. Total soluble solid (TSS) was determined at 2 °C using a portable refractometer (Atago; Japan). Titratable acidity (TA) was measured through titrating 15 ml of diluted juice in 60 ml of distilled water with 0.1N sodium hydroxide until reaching the endpoint of 8.2, and expressed as malic acid percentage. The total phenol content was analyzed using the Follin-ciocalteu reagent (Singleton & Rossi, 1965). The antioxidant activity of apple juice was measured based on free radical scavenging capacity using the DPPH method (Gil et al., 2000).

Statistical analysis

Statistical analysis was performed using JMP 9 statistical software (SAS, Institute, and Cary, NC). The results were statically assessed via multi-factorial analysis of variance (ANOVA) with a 95% confidence level using Fisher's LSD procedure, where $P < 0.05$.

RESULTS AND DISCUSSION

The *Aloe* gel and ZMO treatments used in the current study showed a significant effect ($P<0.01$) on inhibiting the development of gray and blue mold decay (Table 1, Fig. 1A and B). However, the application of treatments (ALV alone, ALV combination with ZMO) resulted in greater reduction in lesion diameter and disease severity compared to untreated apples. Higher lesion diameters of *Botrytis cinerea* (48.5mm) and *Penicillium expansum* (24.5mm) were observed in the control group, whereas the lowest values were observed with 60%ALV+ZMO 150 μL L^{-1}. The treatments using ALV coating alone or in combination ZMO significantly reduced the lesion diameter caused by gray mold (54.2% and 61.03%, respectively) and blue mold (37.3% and 48.08%, respectively) compared to the control (Fig. 1A). When considering the interaction between treatments and fungi, the highest disease severity (5 scores) was observed in the control group, while the lowest disease severity (1.3 scores) was seen in fruit treated with coating (60%ALV+ZMO150 μL L^{-1}) and infected with *Penicillium expansum*. Additionally, there were no notable variations in disease severity between fruit infected with *Botrytis cinerea* and treated with 60% ALV alone and those treated with 60% ALV and 60%ALV+ZMO150 μL L^{-1} (Fig. 1B).

Table 1. ANOVA analysis of the impact of essential oil (ALV and ZMO) and fungal species (*Botrytis cinerea* and *Penicillium expansum)* on the avarage lesion diameter and disease severity of apple after 10 days at 25 °C.

Sources of variations	df	Mean Square	
		Avarage lesion diameter	Disease severity
Fungus	1	852**	0.24ns
Treatments	3	534**	1.72ns
Fungus×Treatments	3	74.8**	5.32**
Error	3	2.17	0.974

** Significance at the level of <0.01 probability, ns non significant.

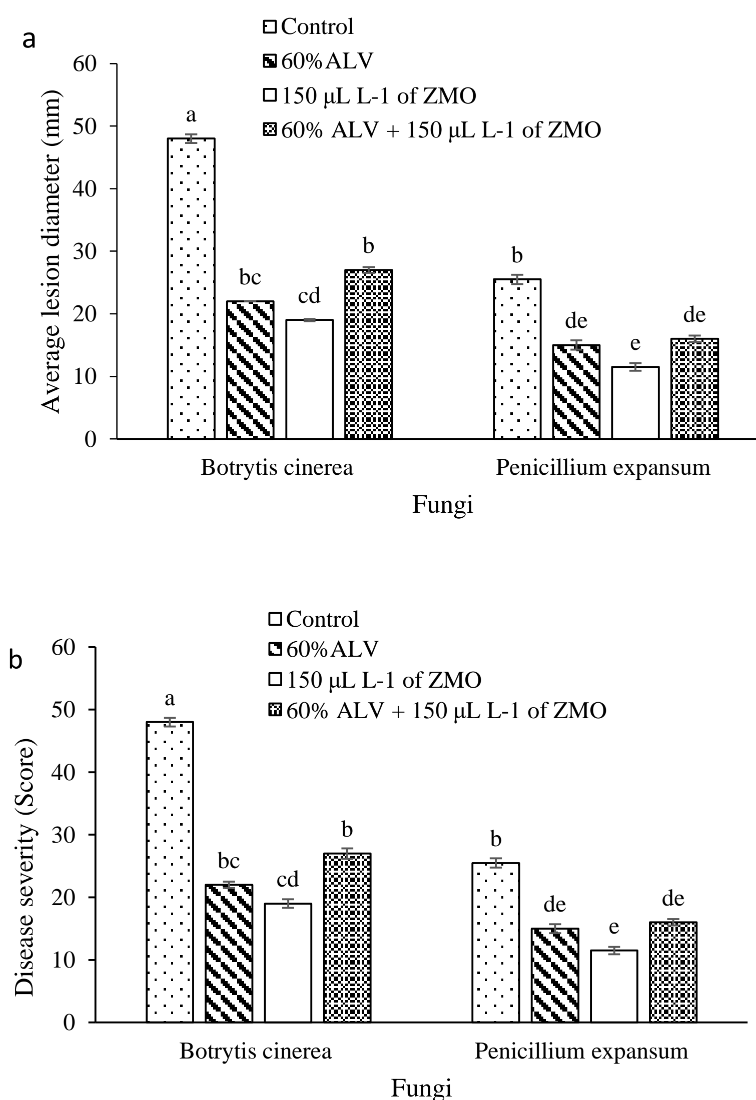


Fig. 1. Effect of 60% ALV alone or combination with ZMO on the lesion diameter (a) and disease severity of apple fruit caused *Botrytis cinerea* and *Penicillium expansum* (b), at 25 °C for 10 days. Error bars represent the mean ± SE.

The experiment was conducted under ideal conditions, as mature fruits were artificially inoculated with a high level of inoculum pressure to induce disease development, specifically without applying low temperatures. Previous studies have also shown that the use of ALV significantly reduced the lesion size (Navarro et al., 2011; Jiwanit et al., 2018). Al-Hassanavi et al. (2023) showed that basil and permint essential oils reduced the lesion diameter in apple fruits infected with *Penicillium expansum*. The main compounds of this ZMO are thymol and carvacrol, constituting 42.46% and 16.85%, respectively (Lahooji et al., 2010). The antifungal effects of ZMO are primarily atributable to its phenolic monoterpene constituents (Avaei et al., 2015). The ZMO is a rich source of oxygenated monoterpenes that cause destabilization and morphological damage to fungal cell membranes (Mohammadi et al., 2016), as well as disrupt various metabolic activities (Ramezanian et al., 2016). The ALV-based edible coating is a combination of hydrocolloids which provides excellent gas barrier properties (Luciano-Rosario et al., 2020). It also helps reduce oil-vapor diffusion and maintain essential oil on the fruit surface (Ali et al., 2015). Meanwhile, the antifungal properties of ALV are related to certain predominant anthraquinone such as aloin and barbaloin, which may effectively affect phospholipid membranes, leading to significant changes in the physical properties of the membrane. These alternations could include disruption of bilayer phospholipid membranes (Zapata et al., 2013).

The decay index, firmness, ripening index, total phenol content, and antioxidant activity of apples were significantly affected by the interaction of essential oil (ALV and ZMO) and the storage period at 2 °C (0, 30, 60, 90 and 120 days) (Fig. 2). The ALV and ZMO treatments during storage period had a significant ($P<0.01$) effect on the decay index. Uncoated apples showed the first signs of decay after 60 days of storage at low-temperature. By 120 days of storage, the control fruits exhibited decay indices at moderate and severe levels, while coated fruits showed either no decay or only slight signs (Fig. 2).

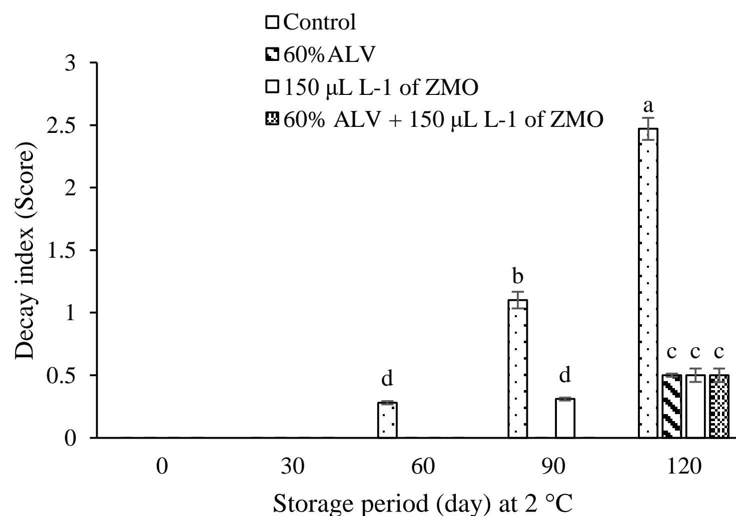


Fig. 2. Effect of 60% ALV alone or combination with ZMO on Decay index of apple fruit, followed by storage at low temperature (2 °C, 120 days). Error bars represent the mean ± SE.

Table 2. ANOVA analysis of the impact of essential oil (ALV and ZMO) and storage period at 2 °C (0, 30, 60, 90 and 120th) on the decay index, firmness, ripening index, total phenol content, and antioxidant activity of apple.

Sources of variations	df	Mean Square				
		Decay index	Firmness	Ripening index	Total phenol content	Antioxidant activity
Treatments	3	1.59**	11.6**	79.2**	5.41**	1962**
Time	4	2.14**	32.1**	297**	13.2**	4248**
Treatments×Time	12	0.546**	5.03**	15.9**	2.91**	884**
Error	40	0.03	0.019	0.925	0.007	0.62

** Significance at the level of <0.01 probability

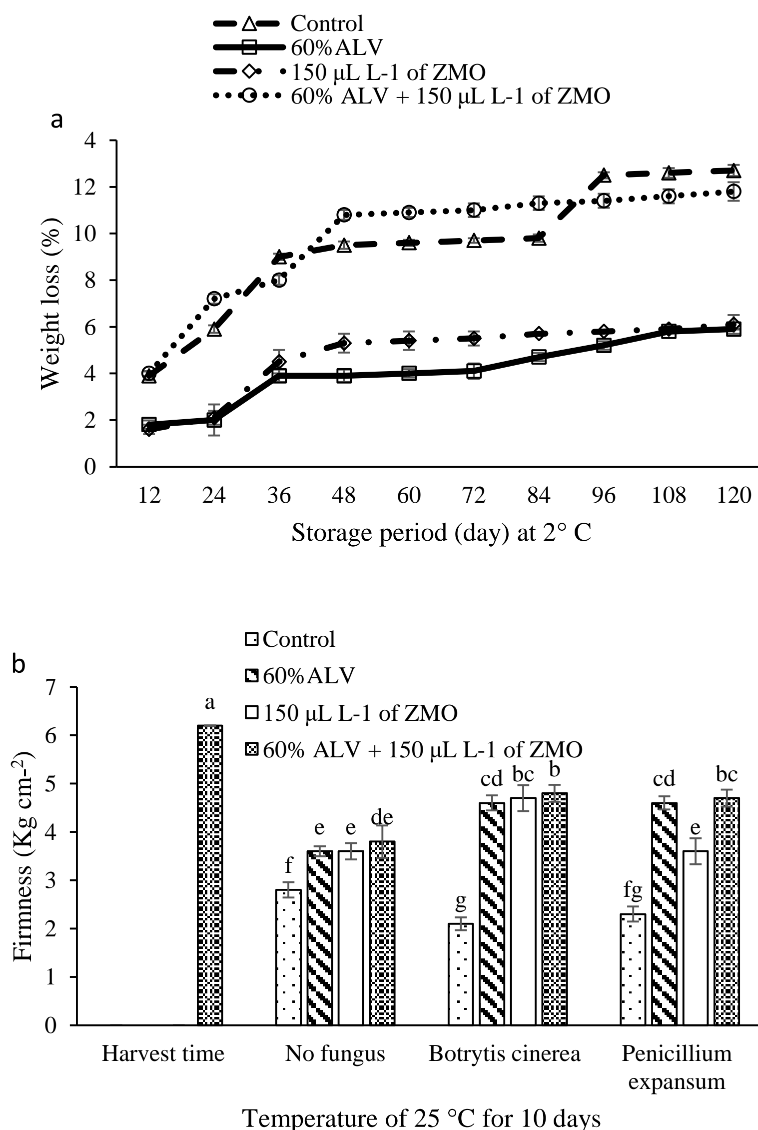
The ALV, ZMO, and ALV combined with ZMO coatings were effective in preserving the quality of apples during cold storage, probably due to their ability to reduce dehydration and shriveling of the coated apples. Previous studies have shown that ZMO has antifungal against postharvest fruit pathogens, such as *Phytophthora drechsleri* in cucumber (Ramezanian et al., 2016), and *Alternaria citri* in oranges (Mohammadi et al., 2016). ALV coatings have also been reported to enhance resistance against decay agents in raspberry (Hassanpour, 2015), and papayas (Mendy et al., 2019). ALV and ZMO can also increase tissue resistance to decay by increasing the ability to scavenge free radicals and improving the antioxidant system. The presence of phenolic compounds can influence antioxidant activity (Hidayati et al., 2020). Phenolic compounds and flavonoids, acting as key antioxidants, may have a significant play in absorbing and neutralizing free radicals, thereby preventing decay progression in fruit (Hassan et al., 2021). In apples coated with ALV and ZMO, the storage time was extended, as this treatment reduced the decay rate. Therefore, ALV with ZMO helps to maintain the quality of apples during storage.

The weight of apples inoculated with fungus did not show any statisticat differences (P≤0.05) after 10 days at room temperatures (data not shown). Figure 3 displays the changes in weight of coated and uncoated samples stored at 2°C for 120 days. There was an increase in weight loss across all treatments during storage. By day 120, apples treated with ALV, ALV+ZMO, and ZMO had weight losses of 5.9%, 11.6%, and 6.6%, respectively, which were lower than in the control group at 16.5%. Contrary to expectations, the weight loss of the ALV+ZMO treated fruits was higher than that of just ALV treated fruits (Figure 3A). The ALV and ZMO treatments during storage at 2 °C had a significant (P<0.01) effect on the firmness, total phenol content, ripening index and antioxidant activity of the apples (Table 1). Fruits treated with ALV (alone or with ZMO) and ZMO were significantly firmer than untreated fruits in both experimental conditions. The firmness of untreated fruits after 120 days of storage at 2°C and after 10 days at 25°C was 37.9%, and 58.3%, respectively, and 67.8%, 65.2% for fruit inoculation with *B. cinerea* and *P. expancum,* respectively with respect to the initial force value of fruit before treatment (6.1 kg cm^2). Meanwhile, firmness loss for all treated fruits was less than 38% under the same conditions. On the other hand, the fruit firmness of ALV alone, ALV+ZMO, and ZMO treatments decreased by 28.9%, 21.2%, 26.5%, and 29.7%, 25.7%, 41.2% when they were inoculated with *B. cinerea* and *P. expancum,* respectively. The application of ALV alone or combined with ZMO was most effective in reducing the softening process. Nevertheless, the type of fungal inoculation did not significantly affect the firmness retention (Fig. 3B and C).

Table 3. ANOVA analysis of the impact of essential oil (ALV and ZMO) and fungal species (*Botrytis cinerea* and *Penicillium expansum)* on the firmness, ripening index, and antioxidant activity of apple after 10 days at 25°C.

Sources of variations	df	Mean Square			
		Firmness	Total phenol content	Ripening index	Antioxidant activity
Treatments	3	18.6**	5.87**	79.2**	2690**
Fungus	3	5.2**	6.08**	297**	5673**
Treatments× Fungus	9	6.4**	4.04**	15.9**	1077**
Error	32	0.012	0.021	0.925	0.77

** Significance at the level of <0.01 probability.



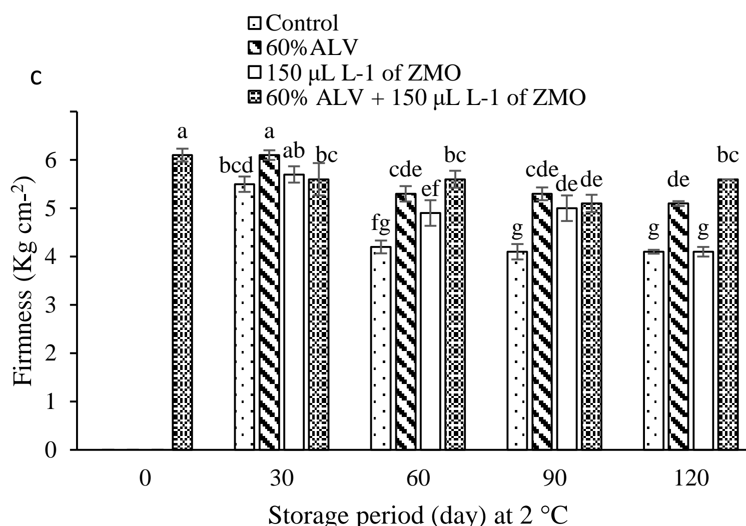


Fig. 3. Effect of 60% ALV alone or combination with ZMO on weight loss (%) of apple fruit, followed by storage at low temperature (2°C, 120 days) (a), firmness in artificially inoculated and non-inoculated apple fruit, followed by storage at room temperature (25°C, 10 days) (b) and low temperature (2°C, 120 days) (c). Error bars represent the mean ± SE.

The highest WL was observed for pears covered with a containing coating potassium sorbate on the 9th day, with the WL of fruits nearly double that of the control (Kowalczyk et al., 2017). Although polysaccharide coatings may not provide the best moisture barrier (Synowiec et al., 2014), ALV plays an important role in the reducing CO_2 production and preventing moisture loss in sweet cherry fruit during storage. The reduction of weight loss has also been observed in papaya coated with ALV gel (Mendy et al., 2019). On the other hand, Dashipour et al. (2015) demonstrated that adding ZMO to carboxymethylcellulose film actually enhanced its water vapor permeability. They provided reasons for this, pointing out that while the presence of ZMO increased the hydrophobicity ratio of films, it also decreased the cohesion of the film due to the microdroplets of ZMO. This can be attributed to the increased tortuosity and hydrophobicity caused by the essential oils. Changes in cell wall components and middle lamella were associated with the degradation of cellulose and hemicellulose as well as the depolymerization of pectin (Thakur et al., 2018), due to the activity of degrading enzymes such as polygalacturonase, pectin methylesterase, and β-galactosidase, which are main key factors in ethylene production and softening climacteric fruit postharvest (Valero et al., 2013). ZMO was found to help maintain the fruit firmness by reducing the activity of the endo polygalacturonase enzyme (Nasiri et al., 2017). ALV treatments have been shown to be an effective method for reducing the loss of firmness in various fruit and vegetables during storage (Khatri et al., 2020). The reduced water vapor in fruits coated with ALV gel results in maintaining turgor pressure in the cell walls. The *Aloe vera*-treated fruits with ZMO showed slightly higher strength, possibly due to the increased hydrophobic properties in this treatment (Hasan, et al., 2021).

The ripening index (TSS/TA) increased in all treatments over the storage period at 2°C (Fig. 4). The highest ripening index was observed in the control samples, which ranged from the initial value of 37.0 to 54.7 by the end of the storage period. The increase was lower with the ALV alone (43) and ALV+ZMO (46.9) treatments, on the 120th day of storage. However, there was no significant difference between ALV and ALV+ZMO treatments.

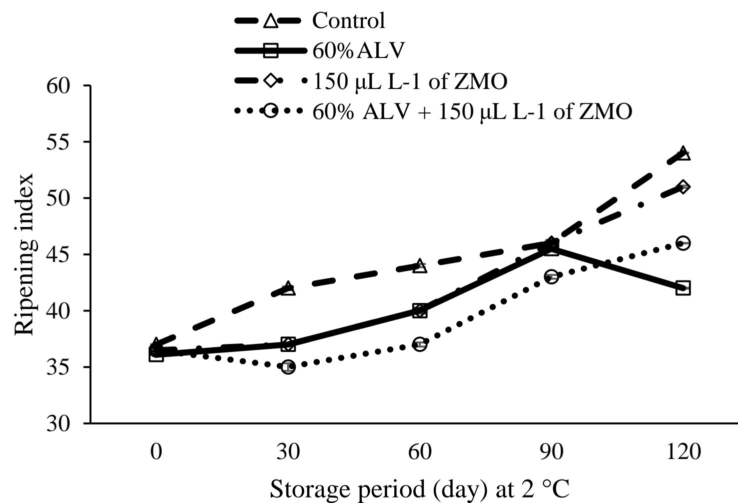
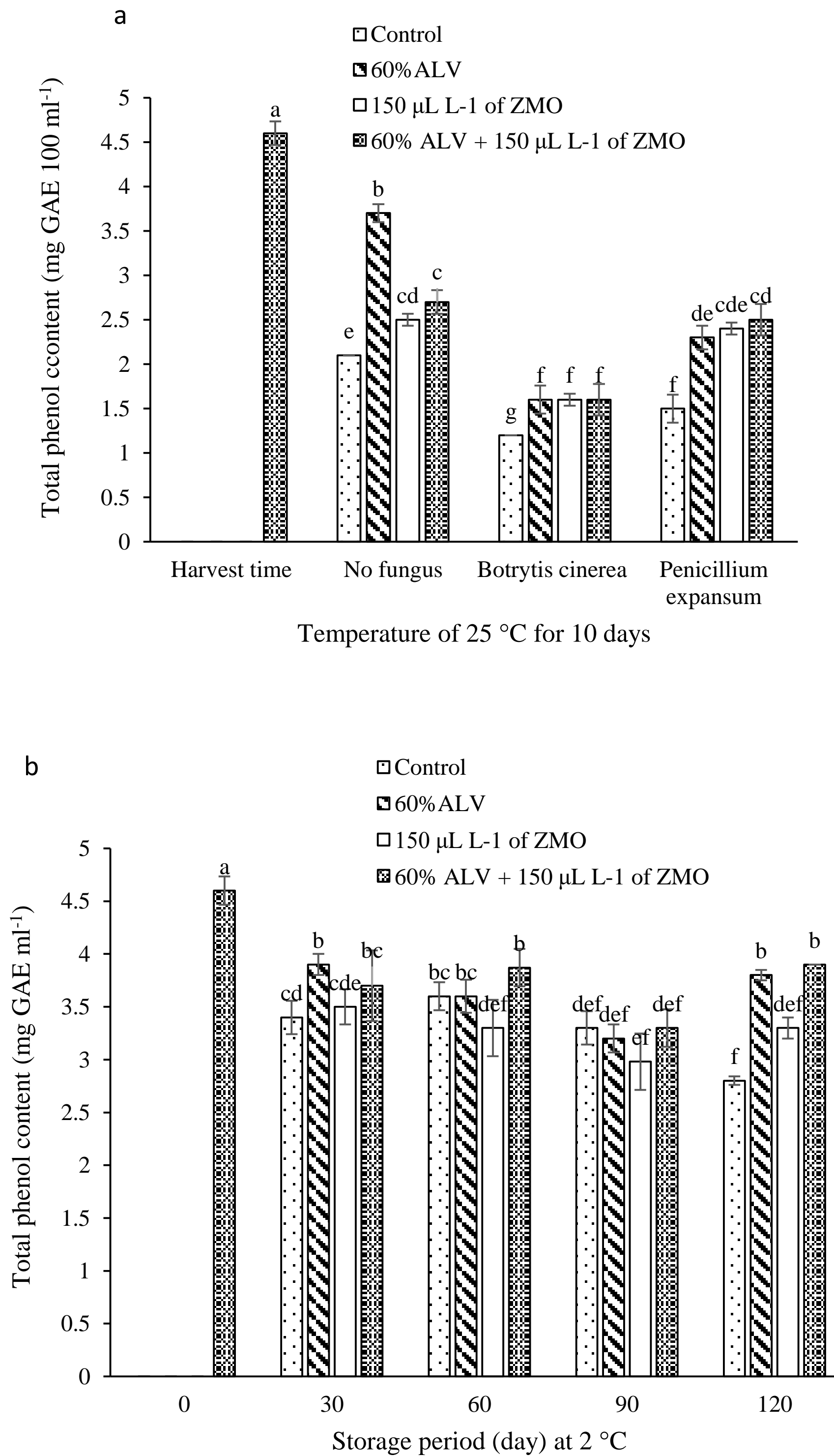


Fig. 4. Effect of 60% ALV alone or combination with ZMO on ripening index of apple fruit, followed by storage at low temperature (2°C, 120 days). Error bars represent the mean ± SE.

The most significant reduction in the ripening index was observed in the coating treatment ALV incorporation with ZMO throughout the storage period. The initial increase in TSS could be attributed to the hydrolysis of starch to sugars, while the subsequent decrease in TSS may be a result of the reduced respiration rate and metabolism of sugars to organic acids (Shehata et al., 2020). Lower TSS levels could be related to the hydrolysis of carbohydrates to sugars (Rehman et al., 2020). The amount of TA in fruit is directly related to the organic acid content (Shehata et al., 2020). The fruit acidity tends to decrease over time, possibly due to the oxidation of organic acids during fruit ripening. The edible fruit layers reduce the respiration rate, leading to a decrease in the consumption of organic acids in the respiration metabolism of the fruit (Dhital et al., 2018). The ALV coating could modify the internal atmosphere of coated fruits (Mendy et al., 2019); consequently, leading to a decline in ethylene biosynthesis, and acid and sugar metabolism in the fruit (Martínez-Romero et al., 2017). Overall, the ripening process was delayed using ALV coating alone or in combination with ZMO. Similar results have been reported in the case of apples coated with carnauba-shellac wax enriched with lemongrass oil (Jo et al., 2014), and guavas coated with both chitosan, and alginate enriched with pomegranate peel extract (Nair et al., 2018).

There were significant differences ($P \leq 0.05$) in the total phenol content between the control apples and those coated with ALV, ALV+ZMO, and ZMO throughout the storage period at both room and low temperatures. However, there were some fluctuations in the total phenol content observed during the storage of coated apples at 2°C. The total phenolic content was (4.7mg GAE 100 ml^{-1}) at harvest time and it decreased in all treatments after storage at 2°C and 25°C (Figures 5A and B). The inoculation of fruits with the two fungi exhibited a sharp decline in the total phenolic content compared with non-inoculated fruits (Fig. 5A). Coated apples generally had a higher total phenolic content than uncoated apples (Fig. 5A and B). In the most of samples, there is no significant difference between antioxidant activity of infected apples with *Penicillium expansum* compared to non-infected apple treated with essential oil. Although, after 10 days at 25 C, the antioxidant activity of ALV+ZMO apples infected by *Botrytis cinerea* increased significantly compared to non-infected apples (Fig. 5C). The antioxidant activity of all samples on 90th day significantly decreased compared to

30th day, although no significant difference was observed between treatments on 30th days and 120th days (Fig. 5D).



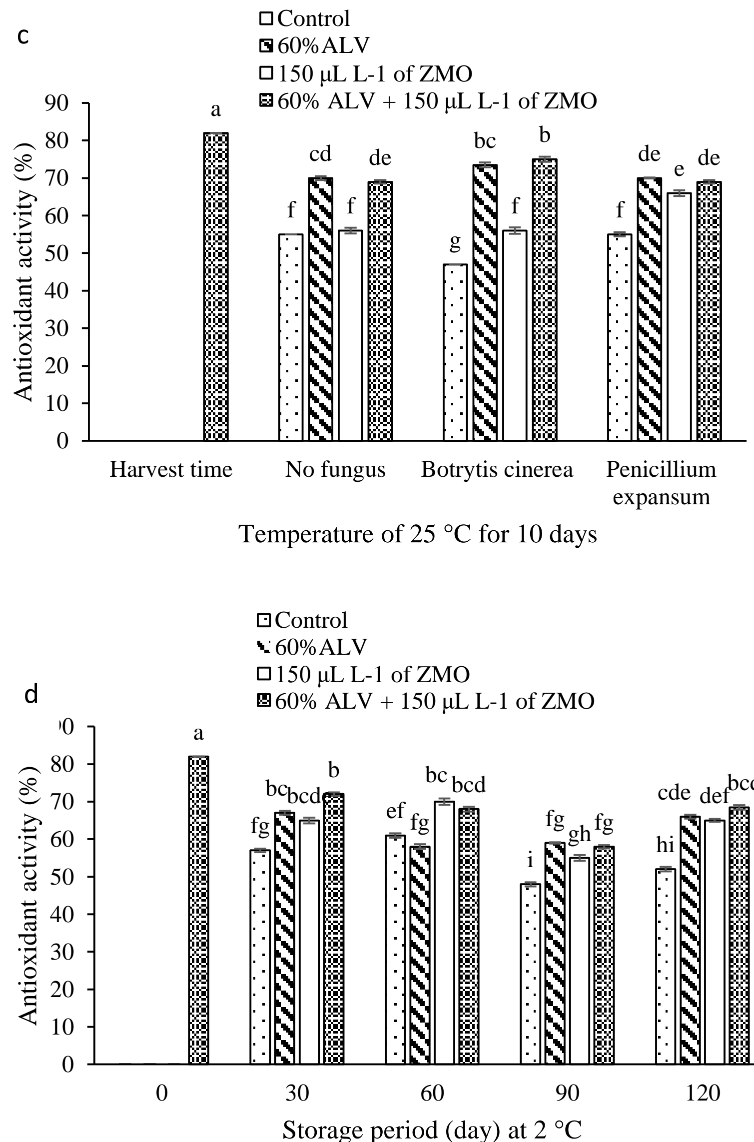


Fig. 5. Effect of 60% ALV alone or combination with ZMO on total phenolic content in artificially inoculated and non-inoculated apple fruit, followed by storage at room temperature (25°C, 10 days) (a) and low temperature (2°C, 120 days) (b), and antioxidant activity, followed by storage at room temperature (25°C, 10 days) (c) and low temperature (2°C, 120 days) (d). Error bars represent the mean ± SE

The ALV coating treatment effectively declined the loss of total phenol content, possibly by increasing phenylalanine ammonia-lyase enzyme (PAL) activity. Hassanpour (2015) observed that ALV coating can stimulate PAL enzyme activity. Jiwanit et al. (2018) reported that ALV coating enriched with *Pichia guilliermondii* efficiently increased total phenol content and reduced pathogen growth. In ZMO treatment, phenolic compounds such as thymol and carvacrol have been shown to be effective against pathogenic fungi (Nasiri et al., 2017). Total phenol content has been reported to be inducers of plant defense response (Kharchoufi et al., 2018). The postharvest application of ALV and ZMO increased resistance against fungal pathogens since they may act as an exogenous elicitor of host-defense response (Jiwanit et al., 2018). The reduction of total phenol content and antioxidant activity during storage could be related to fruit senescence and the breakdown of cell structures (Ghasemnezhad et al., 2013). Edible coatings play an inhibitory role in controlling the gas

exchange between the fruit and its surrounding areas, reducing oxygen intake and thus retarding oxidative processes (Motamedi et al., 2016). ALV show greater antioxidant capacity than synthetic BHT or α-tocopherol, where ALV could enhance the resistance of the tissue to decay by increasing its antioxidant system and its free radical scavenging capability (Hassanpour, 2015).

CONCLUSION

In this research, we examined the impact of using ALV gel with and without ZMO as a new type of edible coating on the quality of apple fruit postharvest. The application of the coating on the fruit surface resulted in a delay in the severity and occurrence of diseases caused by *Botrytis cinerea* and *Penicillium expansum* , with the most effective treatments found to be at 150 µL L-1 of ZMO. Additionally, the addition of ZMO essential oil to the *Aloe vera* gel showed positive effects on preserving the firmness, total phenol content, and antioxidant activity of the apples.

Conflict of interest

The authors at this moment hereby declare that there is no conflict of interest.

REFERENCES

Avaei, A., Mohamadi Sani, A., & Mahmoodzadeh Vaziri, B. (2015). Chemical composition and antimicrobial effect of the essential oil of Zataria multiflora Boiss endemic in Khorasan-Iran. *Asian Pacific Journal of Tropical Disease*, *5*(3), 181-185. https://doi.org/10.1016/S2222-1808(14)60649-6

Al-Hassanavi, H., Rostami Tobnag, G., & Shokri, R. (2023). Application of essential oils and optimizing storage conditions for control of postharvest diseases in apple. *Advances in Horticultural Science*, *37*(2). https://doi.org/10.36253/ahsc-13919

Ali, A., Noh, N. M., & Mustafa, M. A. (2015). Antimicrobial activity of chitosan enriched with lemongrass oil against anthracnose of bell pepper. *Food Packaging and Shelf Life*, *3*, 56-61. https://doi.org/10.1016/j.fpsl.2014.10.003

Dashipour, A., Razavilar, V., Hosseini, H., Shojaee-Aliabadi, S., German, J. B., Ghanati, K., Khakpour, M., & Khaksar, R. (2015). Antioxidant and antimicrobial carboxymethyl cellulose films containing Zataria multiflora essential oil. *International journal of biological macromolecules*, *72*, 606-613. https://doi.org/10.1016/j.ijbiomac.2014.09.006

Dhital, R., Mora, N. B., Watson, D. G., Kohli, P., & Choudhary, R. (2018). Efficacy of limonene nano coatings on post-harvest shelf life of strawberries. *Lwt*, *97*, 124-134. https://doi.org/10.1016/j.lwt.2018.06.038

Duan, X., Jing, G., Fan, F., & Tao, N. (2016). Control of postharvest green and blue molds of citrus fruit by application of sodium dehydroacetate. *Postharvest Biology and Technology*, *113*, 17-19. https://doi.org/10.1016/j.postharvbio.2015.10.015

Esmaeili, Y., Paidari, S., Baghbaderani, S. A., Nateghi, L., Al-Hassan, A. A., & Ariffin, F. (2022). Essential oils as natural antimicrobial agents in postharvest treatments of fruits and vegetables: A review. *Journal of Food Measurement and Characterization*, 16, *507–522*. https://doi.org/10.1007/s11694-021-01178-0

FAS, United States Department of Agriculture Foreign Agricultural Service https://fas.usda.gov/data/production/commodity/0574000 (accessed 2024).

Ghasemnezhad, M., Zareh, S., Rassa, M., & Sajedi, R. H. (2013). Effect of chitosan coating on maintenance of aril quality, microbial population and PPO activity of pomegranate (*Punica granatum* L. cv. Tarom) at cold storage temperature. *Journal of the Science of Food and Agriculture*, *93*(2), 368-374. https://doi.org/10.1002/jsfa.5770

Gil, M. I., Tomás-Barberán, F. A., Hess-Pierce, B., Holcroft, D. M., & Kader, A. A. (2000). Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *Journal of Agricultural and Food chemistry*, *48*(10), 4581-4589. https://doi.org/10.1021/jf000404a

Glos, H., Bryk, H., Michalecka, M., & Puławska, J. (2022). The recent occurrence of biotic postharvest diseases of apples in Poland. *Agronomy*, *12*(2), 399. https://doi.org/10.3390/agronomy12020399

Habibi, A., Yazdani, N., Chatrabnous, N., Koushesh Saba, M., & Vahdati, K. (2022). Inhibition of browning via aqueous gel solution of Aloe vera: a new method for preserving fresh fruits as a case study on fresh kernels of Persian walnut. *Journal of Food Science and Technology*, 59, 2784–2793 https://doi.org/10.1007/s13197-021-05301-3

Hasan, M. U., Riaz, R., Malik, A. U., Khan, A. S., Anwar, R., Rehman, R. N. U., & Ali, S. (2021). Potential of Aloe vera gel coating for storage life extension and quality conservation of fruits and vegetables: An overview. *Journal of Food Biochemistry*, *45*(4), e13640. https://doi.org/10.1111/jfbc.13640

Hashemi, M., Dastjerdi, A. M., Mirdehghan, S. H., Shakerardekani, A., & Golding, J. B. (2021). Incorporation of Zataria multiflora Boiss essential oil into gum Arabic edible coating to maintain the quality properties of fresh in-hull pistachio (Pistacia vera L.). *Food Packaging and Shelf life*, *30*, 100724. https://doi.org/10.1016/j.fpsl.2021.100724

Hassan, H. S., EL-Hefny, M., Ghoneim, I. M., El-Lahot, M. S. A., Akrami, M., Al-Huqail, A. A., ALI H.M., & Abd-Elkader, D. Y. (2022). Assessing the use of aloe vera gel alone and in combination with lemongrass essential oil as a coating material for strawberry fruits: HPLC and EDX analyses. *Coatings*, *12*(4), 489. https://doi.org/10.3390/coatings12040489

Hassanpour, H. (2015). Effect of Aloe vera gel coating on antioxidant capacity, antioxidant enzyme activities and decay in raspberry fruit. *LWT-Food Science and Technology*, *60*(1), 495-501. https://doi.org/10.1016/j.lwt.2014.07.049

Hidayati, J. R., Yudiati, E., Pringgenies, D., Oktaviyanti, D. T., & Kusuma, A. P. (2020). Comparative study on antioxidant activities, total phenolic compound and pigment contents of tropical *Spirulina platensis*, *Gracilaria arcuata* and *Ulva lactuca* extracted in different solvents polarity. In *E3S Web of Conferences* (Vol. 147, p. 03012). EDP Sciences.

Jahanshahi, B., Jafari, A., & Gholamnezhad, J. (2023). Effect of edible tragacanth coating on fruit quality of tomato cv. Falkato. *Journal of Horticulture and Postharvest Research*, *6*(1), 43-54. https://doi.org/10.22077/jhpr.2022.5478.1282

Jiwanit, P., Pitakpornpreecha, T., Pisuchpen, S., & Leelasuphakul, W. (2018). The use of Aloe vera gel coating supplemented with Pichia guilliermondii BCC5389 for enhancement of defense-related gene expression and secondary metabolism in mandarins to prevent postharvest losses from green mold rot. *Biological control*, *117*, 43-51. https://doi.org/10.1016/j.biocontrol.2017.08.023

Jo, W. S., Song, H. Y., Song, N. B., Lee, J. H., Min, S. C., & Song, K. B. (2014). Quality and microbial safety of 'Fuji'apples coated with carnauba-shellac wax containing lemongrass oil. *LWT-Food Science and Technology*, *55*(2), 490-497. https://doi.org/10.1016/j.lwt.2013.10.034

Kharchoufi, S., Parafati, L., Licciardello, F., Muratore, G., Hamdi, M., Cirvilleri, G., & Restuccia, C. (2018). Edible coatings incorporating pomegranate peel extract and biocontrol yeast to reduce *Penicillium digitatum* postharvest decay of oranges. *Food Microbiology*, *74*, 107-112. https://doi.org/10.1016/j.fm.2018.03.011

Khatri, D., Panigrahi, J., Prajapati, A., & Bariya, H. (2020). Attributes of Aloe vera gel and chitosan treatments on the quality and biochemical traits of post-harvest tomatoes. *Scientia Horticulturae*, *259*, 108837. https://doi.org/10.1016/j.scienta.2019.108837

Kowalczyk, D., Kordowska-Wiater, M., Zięba, E., & Baraniak, B. (2017). Effect of carboxymethylcellulose/candelilla wax coating containing potassium sorbate on microbiological and physicochemical attributes of pears. *Scientia Horticulturae*, *218*, 326-333. https://doi.org/10.1016/j.scienta.2017.02.040

Lahooji, A., Mirabolfathy, M., & Karami-Osboo, R. (2010). Effect of *Zataria multiflora* and *Satureja hortensis* essential oils, thymol and carvacrol on growth of *Fusarium gramineum* isolates and deoxynivalenol production. *Iranian Journal of Plant Pathology, 46, 37-50.*

Laribi, A. I., Palou, L., Taberner, V., & Pérez-Gago, M. B. (2012). Modified atmosphere packaging to extend cold storage of pomegranate cv.'Mollar de Elche'. In Proceedings of the International Conference of Agricultural Engineering—CIGR-AgEng.

Li, J., Lei, H., Song, H., Lai, T., Xu, X., & Shi, X. (2017). 1-methylcyclopropene (1-MCP) suppressed postharvest blue mold of apple fruit by inhibiting the growth of *Penicillium expansum*. *Postharvest Biology and Technology*, *125*, 59-64. https://doi.org/10.1016/j.postharvbio.2016.11.005

Luciano-Rosario, D., Keller, N. P., & Jurick, W. M. (2020). *Penicillium expansum*: Biology, omics, and management tools for a global postharvest pathogen causing blue mould of pome fruit. *Molecular Plant Pathology*, *21*(11), 1391-1404. https://doi.org/10.1111/mpp.12990

Mannucci, A., Serra, A., Remorini, D., Castagna, A., Mele, M., Scartazza, A., & Ranieri, A. (2017). Aroma profile of Fuji apples treated with gelatin edible coating during their storage. *LWT-Food Science and Technology*, *85*, 28-36. https://doi.org/10.1016/j.lwt.2017.06.061

Maqbool, M., Ali, A., Alderson, P. G., Mohamed, M. T. M., Siddiqui, Y., & Zahid, N. (2011). Postharvest application of gum arabic and essential oils for controlling anthracnose and quality of banana and papaya during cold storage. *Postharvest Biology and Technology*, *62*(1), 71-76. https://doi.org/10.1016/j.postharvbio.2011.04.002

Martínez-Romero, D., Zapata, P. J., Guillén, F., Paladines, D., Castillo, S., Valero, D., & Serrano, M. (2017). The addition of rosehip oil to Aloe gels improves their properties as postharvest coatings for maintaining quality in plum. *Food Chemistry*, *217*, 585-592. https://doi.org/10.1016/j.foodchem.2016.09.035

Mendy, T. K., Misran, A., Mahmud, T. M. M., & Ismail, S. I. (2019). Application of *Aloe vera* coating delays ripening and extend the shelf life of papaya fruit. *Scientia Horticulturae*, *246*, 769-776. https://doi.org/10.1016/j.scienta.2018.11.054

Mohammadi, A., Hashemi, M., & Hosseini, S. M. (2016). Integration between chitosan and *Zataria multiflora* or *Cinnamomum zeylanicum* essential oil for controlling Phytophthora drechsleri, the causal agent of cucumber fruit rot. *LWT-Food Science and Technology*, *65*, 349-356. https://doi.org/10.1016/j.lwt.2015.08.015

Moradinezhad, F., & Ranjbar, A. (2023). Advances in postharvest diseases management of fruits and vegetables: A review. *Horticulturae*, *9*(10), 1099. https://doi.org/10.3390/horticulturae9101099

Mostafidi, M., Sanjabi, M. R., Shirkhan, F., & Zahedi, M. T. (2020). A review of recent trends in the development of the microbial safety of fruits and vegetables. *Trends in Food Science & Technology*, *103*, 321-332. https://doi.org/10.1016/j.tifs.2020.07.009

Motamedi, E., Nasiri, J., Malidarreh, T. R., Kalantari, S., Naghavi, M. R., & Safari, M. (2018). Performance of carnauba wax-nanoclay emulsion coatings on postharvest quality of 'Valencia' orange fruit. *Scientia Horticulturae*, *240*, 170-178. https://doi.org/10.1016/j.scienta.2018.06.002

Nair, M. S., Saxena, A., & Kaur, C. (2018). Effect of chitosan and alginate based coatings enriched with pomegranate peel extract to extend the postharvest quality of guava (*Psidium guajava* L.). *Food chemistry*, *240*, 245-252. https://doi.org/10.1016/j.foodchem.2017.07.122

Nasiri, M., Barzegar, M., Sahari, M. A., & Niakousari, M. (2017). Tragacanth gum containing *Zataria multiflora* Boiss. essential oil as a natural preservative for storage of button mushrooms (*Agaricus bisporus*). *Food Hydrocolloids*, *72*, 202-209. https://doi.org/10.1016/j.foodhyd.2017.05.045

Navarro, D., Díaz-Mula, H. M., Guillén, F., Zapata, P. J., Castillo, S., Serrano, M., Valero, D., & Martínez-Romero, D. (2011). Reduction of nectarine decay caused by *Rhizopus stolonifer*, *Botrytis cinerea* and *Penicillium digitatum* with *Aloe vera* gel alone or with the addition of thymol. *International Journal of Food Microbiology*, *151*(2), 241-246. https://doi.org/10.1016/j.ijfoodmicro.2011.09.009

Oliveira, D. G. P., Pauli, G., Mascarin, G. M., & Delalibera, I. (2015). A protocol for determination of conidial viability of the fungal entomopathogens Beauveria bassiana and Metarhizium anisopliae from commercial products. *Journal of Microbiological Methods*, *119*, 44-52. https://doi.org/10.1016/j.mimet.2015.09.021

Panchal, N., Das, K., Prabhakar, P. K., & Ghanghas, N. (2022). Edible films and coatings for fruits and vegetables: Composition, functions, and regulatory aspects. *Edible Food Packaging:*

Applications, Innovations and Sustainability, 191-216. https://doi.org/10.1007/978-981-16-2383-7_10

Pandey, A., & Singh, S. (2016). Aloe Vera: A Systematic Review of its Industrial and Ethno-Medicinal Efficacy. *International Journal of Pharmaceutical Research & Allied Sciences*, *5*(1), 1-21.

Rahimi Kakolaki, M., Omidi, A., Rasooli, A., & Shekarforoush, S. S. (2024). *In vitro* antifungal activity of barberry fruit extract (*Berberis* spp.) against *Fusarium* spp.. *Journal of Horticulture and Postharvest Research, 7*(Special Issue - Postharvest Technologies), 47-60. http://dx.doi.org/10.22077/jhpr.2023.6783.1333

Ramezanian, A., Azadi, M., Mostowfizadeh-Ghalamfarsa, R., & Saharkhiz, M. J. (2016). Effect of *Zataria multiflora* Boiss and *Thymus vulgaris* L. essential oils on black rot of 'Washington Navel' orange fruit. *Postharvest Biology and Technology*, *112*, 152-158. https://doi.org/10.1016/j.postharvbio.2015.10.011

Rehman, M. A., Asi, M. R., Hameed, A., & Bourquin, L. D. (2020). Effect of Postharvest application of *Aloe vera* gel on shelf life, activities of anti-oxidative enzymes, and quality of 'Gola' guava fruit. *Foods*, *9*(10), 1361. https://doi.org/10.3390/foods9101361

Shehata, S. A., Abdeldaym, E. A., Ali, M. R., Mohamed, R. M., Bob, R. I., & Abdelgawad, K. F. (2020). Effect of some citrus essential oils on post-harvest shelf life and physicochemical quality of strawberries during cold storage. *Agronomy*, *10*(10), 1466. https://doi.org/10.3390/agronomy10101466

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American journal of Enology and Viticulture*, *16*(3), 144-158.

Synowiec, A., Gniewosz, M., Kraśniewska, K., Przybył, J. L., Bączek, K., & Węglarz, Z. (2014). Antimicrobial and antioxidant properties of pullulan film containing sweet basil extract and an evaluation of coating effectiveness in the prolongation of the shelf life of apples stored in refrigeration conditions. *Innovative Food Science & Emerging Technologies*, *23*, 171-181. https://doi.org/10.1016/j.ifset.2014.03.006

Supa, S., Howlader, P., Ali, M., Rupa, R., & Bose, S. (2024). Edible coatings maintained postharvest quality and increased shelf life of guava fruits. *Journal of Horticulture and Postharvest Research*, *7*(Special Issue - Postharvest Technologies), 15-34. https://doi.org/10.22077/jhpr.2023.6531.1324

Thakur, R., Pristijono, P., Golding, J. B., Stathopoulos, C. E., Scarlett, C. J., Bowyer, M., & Vuong, Q. V. (2018). Development and application of rice starch based edible coating to improve the postharvest storage potential and quality of plum fruit (*Prunus salicina*). *Scientia Horticulturae*, *237*, 59-66. https://doi.org/10.1016/j.scienta.2018.04.005

Valero, D., Díaz-Mula, H. M., Zapata, P. J., Guillén, F., Martínez-Romero, D., Castillo, S., & Serrano, M. (2013). Effects of alginate edible coating on preserving fruit quality in four plum cultivars during postharvest storage. *Postharvest Biology and Technology*, *77*, 1-6. https://doi.org/10.1016/j.postharvbio.2012.10.011

Zapata, P. J., Navarro, D., Guillén, F., Castillo, S., Martínez-Romero, D., Valero, D., & Serrano, M. (2013). Characterisation of gels from different Aloe spp. as antifungal treatment: Potential crops for industrial applications. *Industrial Crops and Products*, *42*, 223-230. https://doi.org/10.1016/j.indcrop.2012.06.002

JOURNAL OF HORTICULTURE AND POSTHARVEST RESEARCH
2025, VOL. 8(1), 43-66

Journal of Horticulture and Postharvest Research

Journal homepage: www.jhpr.birjand.ac.ir

Chitosan oligosaccharides maintained postharvest quality and increased shelf life of mango

Nishat Jahan Nitu[1], Md. Sefat Ullah[1], Prianka Howlader[1], Md. Nazmul Hasan Mehedi[1], Habiba Zannat Meem[1] and Santosh Kumar Bose[1*]

1, Department of Horticulture, Patuakhali Science and Technology University (PSTU), Dumki, Patuakhali-8602, Bangladesh

ARTICLE INFO

Original Article

Article history:
Received 25 July 2024
Revised 1 October 2024
Accepted 6 October 2024

Keywords:
Edible coating
Mangifera indica
Postharvest
Shelf life

DOI: 10.22077/jhpr.2024.7888.1395
P-ISSN: 2588-4883
E-ISSN: 2588-6169

***Corresponding author:**
Department of Horticulture, Patuakhali Science and Technology University (PSTU), Dumki, Patuakhali-8602, Bangladesh.

Email: santosh@pstu.ac.bd

© This article is open access and licensed under the terms of the Creative Commons Attribution License http://creativecommons.org/licenses/by/4.0/ which permits unrestricted, use, distribution and reproduction in any medium, or format for any purpose, even commercially provided the work is properly cited.

ABSTRACT

Purpose: Mango is one of the most important and widely cultivated climacteric fruit which ripens rapidly after harvesting. It exhibits very short shelf life mainly due to high respiration rate, susceptible to various storage pathogens and mechanical injuries at the time of postharvest management which lead to reduce the quality. However, the experiment was carried out to investigate the chitosan oligosaccharides (COS) coating effects on postharvest quality and shelf life of mango varieties. **Research Method:** Mango fruits of two selected varieties (Langra and Amropali) were collected at mature stage. Changes in different physico-chemical characteristics were studied at different days of storage under ordinary room condition through different COS concentration viz., control, COS 25 mg/L, COS 50 mg/L, COS 100 mg/L, COS 250 mg/L and COS 500 mg/L. The two factor-experiments were laid out in a completely randomized design with three replications. **Findings:** Results demonstrated that COS had a positive effect on retaining higher amount of anthocyanin content, total sugar and total soluble solid content. Moreover, COS treated fruits exhibited significant delays of firmness, weight loss percentage, titratable acidity, pH and vitamin C content compared to untreated fruits. In addition, between two varieties of mango, Langra exhibited better performance compared to Amropali when treated with COS 100 mg/L. **Research Limitations:** The study did not focus on ethylene biosynthesis and respiration rate determination. **Originality/Value:** COS 100mg/L have great potentiality to maintain postharvest quality and increase shelf life of mango which could be applied commercially for preservation of mango in an ecofriendly manner.

INTRODUCTION

Mango (*Mangifera indica*) belongs to the family Anacardiaceae, is one of the most delicious, attractive and extensively cultured tropical fruit in the world. It is known as king of fruits among all the fruits cultivated in the world (Kobra et al., 2012). In Bangladesh mango found to be grow in all districts and one of the most popular fruits. It has also strong economic impact on the economy of Bangladesh. Mango occupied an area of 121075 ha with production of 1948583 metric tons which contribute 28.22% of the area and 26.38% production of total fruit crops in Bangladesh (BBS, 2022). Nutritionally, mango is a great source of carbohydrates, nutritional fiber, vitamins (vit A, beta-carotene, vit C, vit K etc), minerals arid (iron, calcium, magnesium, manganese etc.) protein, low fats and it play vital role to save many human diseases (Sogi et al., 2013).

Mangoes, one of the climacteric fruits, ripen rapidly after harvest and are easily infected by several postharvest diseases, susceptible to mechanical injury which lead to considerable postharvest losses, and limits the storage, handling and transport. Post-harvest losses occur due to various factors such as the usage of inappropriate harvesting tools, inefficient handling and lack of suitable transport equipment, usage of inappropriate packaging materials, poor temperature management and rough handling of fresh fruits as well as substandard road infrastructure (Kefas et al., 2024). There are a number of fungi *(Colletotrium gloeosporoides, Botryodiplodia theobromae* etc.) attack mango fruits at maturity after collection from tree. These fungi cause infection during storage and transfer. Short shelf-life and lack of proper postharvest management has restricted mango export to distant markets. Therefore, various techniques such as use of a plant growth regulator, ionizing radiation, plant extracts, modified atmosphere, controlled atmosphere, and edible coatings were used to extend the postharvest life of mango (Perumal et al., 2017). The use of synthetic chemicals and different storage techniques may be leave residue to the fruit which have detrimental effect on both human and environment (Bose et al., 2021a). Nowadays, globally consumers prefer more natural, eco-friendly process product with high nutritional quality and long shelf life. Regarding this issue, the use of bio-preservatives is of growing interest for preserving quality and increasing shelf life of mango.

Nowadays the use of natural non-toxic substance including chitosan (Chaiprasart et al., 2006), chitosan oligosaccharides (He et al., 2019), alginate oligosaccharides for fruit storage and preservation, showing great attention. Chitosan oligosaccharide, degraded from chitosan, has been established as an effective plant elicitor, water soluble non-toxic compounds which influenced several secondary metabolites to improve fruit qualities. Considering the above scheme in mind, the present research work was undertaken in order to assess the effects of chitosan oligosaccharides as postharvest treatment on storage quality and shelf life of mango fruits.

MATERIALS AND METHODS

Experimental chemicals and materials

Fully mature uniform size and free from defects mango fruits cv. Langra and Amropali were harvested from commercial orchard (Chapai Nawabgonj, Bangladesh) and quickly transferred to the research laboratory. Chitosan oligosaccharide (degree of polymerization = 2~10; degree of deacetylation> 95 %) were collected from Dalian GlycoBio Co., Ltd. (Dalian, China). Two factor (Factor A: Variety- Langra (V_1) and Amrapali (V_2); Factor B: Postharvest treatments (6) T_0 = Control (no treatment); T_1= 25 mg/L COS; T_2= 50 mg/L COS; T_3 = 100 mg/L COS; T_4 =250 mg/L COS and T_5 =500 mg/L COS) experiment were carried out at Postharvest

laboratory, Department of Horticulture, Patuakhali Science and Technology University following completely Randomized Design (CRD) with three replications.

Preparation and application of postharvest treatment

For preparation of different concentration of COS, certain amount of COS powder was dissolved in 1000 ml distilled water and stored in a glass jar. Twenty-four fruits for each treatment were then individually dipped in prepared COS solution and the fruits were kept on brown paper of the laboratory table at ambient condition arranging at random by replication. For control, twenty-four fruits of each variety (Eight fruits were in each replication) were selected randomly from the mango lot and kept without treating COS solution.

Methods of studying physico-chemical properties

Firmness

Fruit firmness was determined by using digital penetrometer along with a measuring probe (5 mm diameter stainless steel). Fruit firmness was measured from two opposite side of each fruit by penetrating the probe at a depth of 5 mm into the fruit with pre- and post– test speed 1 mms- 1. The firmness was calculated as maximum penetration force reached during tissue breakage and expressed as Newton (N).

Weight loss

The fruits of each treatment were weighted with the help of electric balance at 2 days' interval and then weight loss percentage was calculated by using the following formula (1):

$$\text{Total weight loss (\%)} = \frac{\text{IW}-\text{FW}}{\text{IW}} \times 100 \quad (1)$$

Here IW= Initial (g); FW= Final weight (g)

Titratable acidity (TA) and pH determination

Titratable acidity was measured according to the method described by Ranganna (1977) with minor modification. Briefly, ten grams of mango pulp were homogenized for two minutes with 40 ml of distilled water by using a Kitchen blender (Vision, Bangladesh) and filtered through a Whatman filter paper No.2. Consequently, 5 ml of the extracted juice were taken in a 100 ml conical flask and two to three drops of phenolphthalein indicator solution were added and then the conical flask was shaken vigorously. The sample was titrated with 0.1M NaOH solution until the color turned into pink and insistent for 15 seconds. The titer volume was recorded and the result was expressed as percentage citric acid, which was calculated using the following formula (2):

$$\text{Citric acid (\%)} = \frac{\text{Titre (mL)} \times \text{NaOH normality (0.1 M)} \times \text{Vol.made up (50 mL)} \times \text{Citric acid eq.weight (64 g)} \times 100}{\text{Volume of sample for titrate (5 mL)} \times \text{Weight of sample taken (10 g)} \times 1000} \quad (2)$$

The remaining juice extract from TA measurement was used to measure the pH of the fruit pulp. The pH was determined by using a glass electrode pH meter.

Determination of vitamin C

Vitamin C content of mango was determined by titration method using 2, 6-dichlorophenol indophenol dye solution as described by Ranganna (1986). The method of determination involves the reduction of 2, 6-dichlorophenol indophenol dye to a colorless form by ascorbic acid in an alkaline solution. Then the vitamin C content of the sample was calculated by the following formula (3):

$$\text{Vitamin C (mg/100g fruit)} = \frac{T \times D \times V1}{V2 \times W} \times 100 \qquad (3)$$

(Here, T= Titre, D= Dye factor, V_2= Volume made up, V_1= Volume taken for titration, W= Weight of the sample taken for estimation)

Estimation of total anthocyanin content

Total anthocyanin content of mango peel was estimated according to the method described by Sims and Gamon (2002). Briefly, 5g pulp of mango were properly homogenized with 10ml (1:2) 80% cold acetone (80:20 vol:vol, pH = 7.8) and centrifuged for 4 minutes at 800 rpm maintaining 4^0C. The clear supernatant was diluted to a final volume of 5 ml by adding acetone and was used for the estimation of total anthocyanin content. The absorbance of the extract solutions was recorded at 665nm, 649nm, 646nm, 663nm, 470 nm, and 529nm and 650nm wave length by using double beam spectrophotometer (Dynamica HALO-DB-20S UV-VIS Double Beam Spectrophotometer). Content of chlorophyll-a and chlorophyll-b as well as anthocyanin was calculated by using the following formulae (4, 5, and 6):

$$\text{Chlorophyll a-a } (\mu g/ml) = 12.21\ A_{665} - 6.88\ A_{649} \qquad (4)$$
$$\text{Chlorophyll a-b } (\mu g/ml) = 20.13\ A_{646} - 5.03\ A_{663} \qquad (5)$$

$$\text{Anthocyanin } (\mu mol/ml) = A_{529} - 0.288\ A_{650}$$
$$\text{Anthocyanin } (\mu mol/g \times 207.247 = \mu g/g) = A_{529} - 0.288\ A_{650} \qquad (6)$$

Where, A is the absorbance of the extract solution in a 1-cm path length cuvette at wave length.

Total soluble solids (•Brix)

The total soluble solids of mango fruit pulp were determined by using hand refractometer (Model BS Eclipse 3-45) at room temperature. Fruit pulp was homogenized in a kitchen blender for two minutes and filtrated through four layers of muslin cloth. A drop of fruit juice was placed on the prism of refractometer and direct reading was noted. The results were expressed as percent soluble solids (°Brix).

Estimation of total sugar content

Total sugar content of mango pulp was estimated by using standard Fehling's solution. Fifty gram of fruits were used to calculate percent reducing, non-reducing and total sugar content using the following formulae (7, 8, and 9):

$$\%\ \text{Reducing sugar} = \frac{F \times D \times 100}{T \times W \times 100} \qquad (7)$$

(Where, I = mg of invert sugar required to reduce to known volume of Fehling's solution, D = Dilution, T = Titre and W = wt. of the sample)

% Non-reducing sugar = (% Total invert sugars - % reducing sugars originally present) × 0.95 (Conversion factor) (8)

$$\%\ \text{Total sugars} = \%\ \text{reducing sugar} + \%\ \text{non-reducing sugar} \qquad (9)$$

Shelf life

Shelf life of mango fruits was calculated by counting the days required to ripe fully as to retaining optimum marketing and eating qualities.

Statistical analysis

The collected data on different parameters under the study were statistically analyzed using SPSS software (IBM, New York, USA). The significant different among treatment means were separated and analysis of variance for all the parameters were performed by F-test followed by Duncan's Multiple Range Test (DMRT) at 1% level of probability (Gomez & Gomez, 1984).

RESULTS AND DISCUSSION

Changes of fruit firmness

Effect of varieties

The firmness fruit is one of the most important indices that govern the quality of fruit during storage. The firmness reduced with the advancement of storage period (Fig. 1). Changes of fruit firmness between mango varieties were significant at different days after storage. The changes of fruit firmness had higher in 'Langra' (9.39, 7.39, 5.85) at 3, 6, and 9 DAS, respectively than that of the variety 'Amropali' (7.26, 6.43, 5.13 respectively).

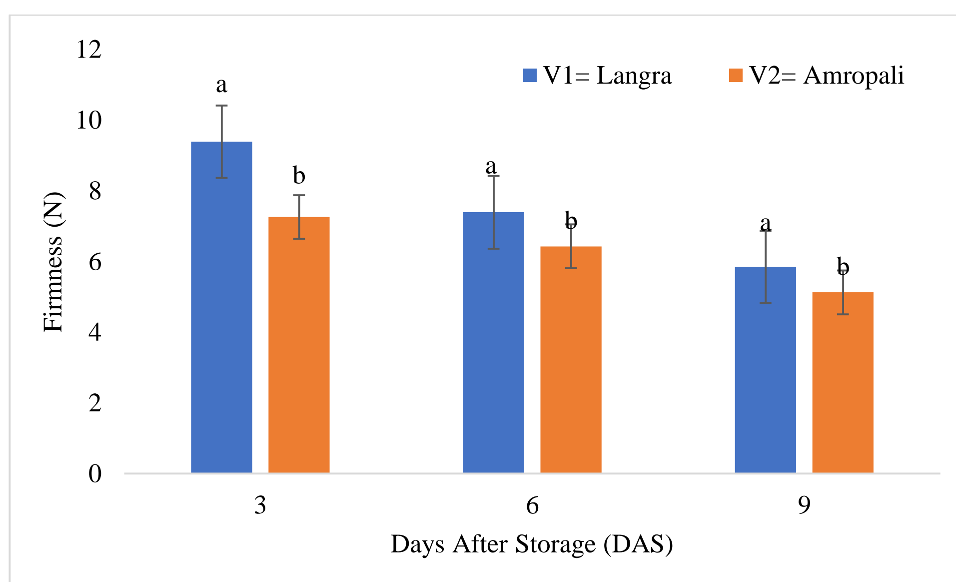


Fig. 1. Effect of variety on firmness of mango at different days after storage. Vertical bars represent standard error.

These results revealed that the changes of fruit firmness gradually decrease in increasing of storage period as well as the higher changes of firmness were obtained at 9 DAS. The fruits firmness declined due to the degradation of cell wall components and reduced the cell wall integrity. The finding of the present study is almost similar to Mustari et al. (2020) and Mondal et al. (2023), they reported that mango fruit firmness drastically reduced with advancement of storage period.

Effect of postharvest treatments

Firmness changes showed significant in case of different postharvest treatments during storage. It was observed that the firmness changes occurred at faster rate in control, whereas the rates were slower in those fruits are treated with COS 100 mg/L Fruit firmness was maximum (9.26) in COS 50 mg/L treated fruits and minimum (7.7) in control which was statistically similar with COS 100 mg/L treated fruits. Also at 6 and 9 DAS, the maximum firmness was observed in COS 50 mg/L and COS 100 mg/L (7.77 and 6.51) and the minimum firmness was shown in control (5.74 and 4.21) treated fruits, respectively (Fig. 2).

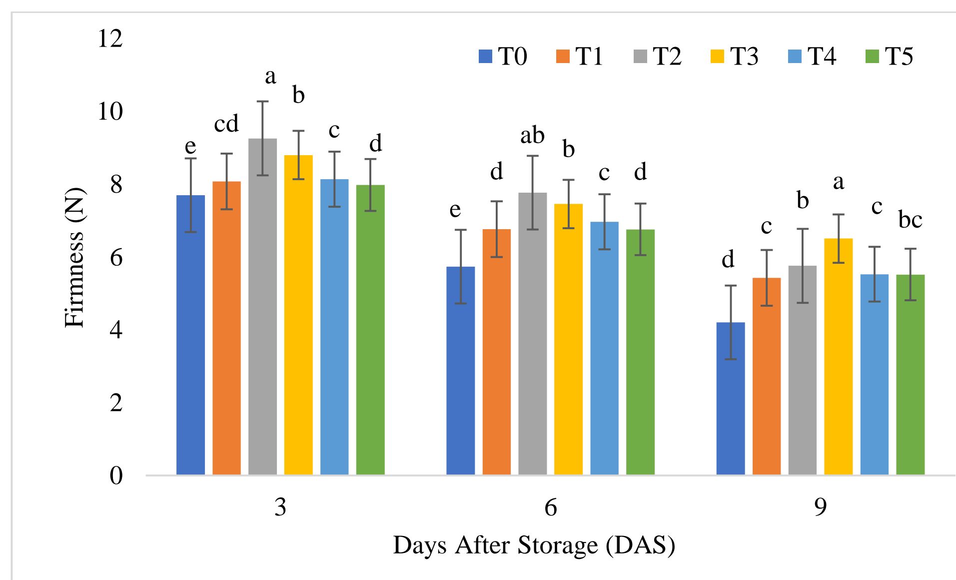


Fig. 2. Effect of treatments on firmness of mango at different days after storage. Vertical bars represent standard error. Here, T_0: Control, T_1: 25mg/L COS, T_2: 50 mg/L COS, T_3: 100 mg/L COS, T_4: 250 mg/L COS, T_5: 500 mg/L COS.

These results revealed that changes of fruit firmness significantly decreased among the all postharvest treatments in increasing of storage periods. Bose et al. (2021b) also found similar results when strawberry fruits treated with alginate oligosaccharides (AOS), they reported that postharvest application of AOS delayed the loss of firmness and suppressed the activity of N-glycan processing enzymes (a-Man and b-Hex) along with N-glycan processing enzymes associated genes expression resulting in delayed fruit softening.

Combined effect of variety and postharvest treatments

Significant variation was also obtained by the combined effect of studied mango varieties and different postharvest treatment during storage (Table 1). At 3, 6 and 9 days after storage, the highest firmness (10.03, 8.45 and 6.93 N) was recorded from treatment V_1T_3 and the lowest (6.65, 5.23 and 3.94 N) was recorded from treatment V_2T_0, respectively. The firmness decreased both in treated and untreated fruits during entire storage period. 100 mgL^{-1} COS treated fruit significantly delayed the loss of firmness compared to control fruits. Losses in firmness with the progress of storage period of mango fruits due to increased activities of cell wall hydrolysis enzymes such as pectinesterase, polygalacturonase pectin methylesterase and pectatelyases (Ali et al., 2004).

Table 1. Effect of varieties and postharvest treatments on firmness of mango during storage.

Variety × Treatment	Firmness (N)		
	3 DAS	6 DAS	9 DAS
V_1T_0	8.74e	6.25e	4.48f
V_1T_1	9.11d	7.27d	5.6cd
V_1T_2	9.76b	7.65bc	5.76bcd
V_1T_3	10.03a	8.45a	6.93a
V_1T_4	9.33cd	7.47cd	6.14b
V_1T_5	9.36c	7.24d	6.21b
V_2T_0	6.65h	5.23f	3.94g
V_2T_1	7.06g	6.26e	5.27de
V_2T_2	8.76e	7.9b	5.76bcd
V_2T_3	7.56f	6.46e	6.1bc
V_2T_4	6.96g	6.47e	4.91ef
V_2T_5	6.6h	6.29e	4.83ef
Level of Significance	**	**	**
LSD at 5%	0.15	0.27	0.27
LAD at 1%	0.23	0.37	0.52
CV (%)	1.21	2.35	4.13

** Significant at 1% level of probability, DAS = Days after Storage, V_1: Langra, V_2: Amropali, T_0: Control, T_1: 25mg/L COS, T_2: 50 mg/L COS, T_3: 100mg/L COS, T_4: 250 mg/L COS, T_5: 500 mg/L COS.

Weight loss (%)

Effect of varieties

In case of total weight loss, highly significant variation was observed between two mango varieties at different days after storage (Fig. 3). The variety 'Langra' showed the minimum weight loss (1.62%, 2.77%, 3.56%) compare to 'Amropali (1.83%, 2.70%, 3.5%) at 3, 6 and 9 days after storage, respectively. However, percentage of total weight loss of storage fruits showed significant increase with the advancement of storage period but it was lower in 'Langra' than 'Amropali' due to the keeping ability of moisture had higher. Weight loss is one of the most important indicators for maintaining the quality of any fruits during storage. The weight loss reduction is unsurprising to the physiological loss in weight due to water respiration and transpiration through peel tissue, and other organic changes taking place in the fruit (Atlaw, 2018).

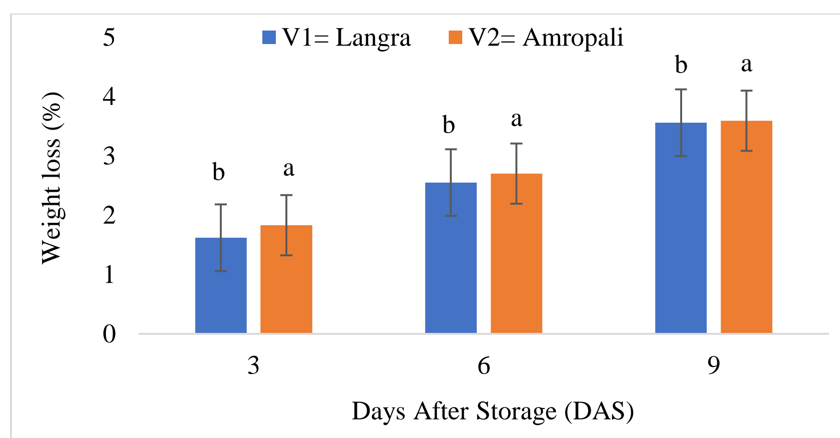


Fig. 3. Effect of variety on total weight loss of mango at different days after storage. Vertical bars represent standard error.

Effect of postharvest treatments

Significant variation was also recorded among the effect of postharvest treatments regarding to weight loss percentage at different days after storage (Fig. 4). The weight loss was lower (1.44%, 2.18%, 3.01%) in those fruits which were treated by COS 100 mg/L at 3, 6 and 9 days after storage, respectively while it was statistically differed from other treatments. In contrast, the higher weight loss (1.93%, 2.91%, 4.04%) was recorded in untreated fruits at 3, 6 and 9 days, respectively. These results appeared that all the treatments showed significant variation among them COS 100 mg/L recorded the greater performance. Similar results were also obtained by Bose et al. (2019), they reported that 100 mg/L AOS treated strawberry exhibited lower weight loss compared to untreated fruits and which may possibly be due to slow respiration and metamorphic activity of fruits. Natural elicitor postharvest treatment also delayed weight loss compared to control treatments (Rastgoo et al., 2024).

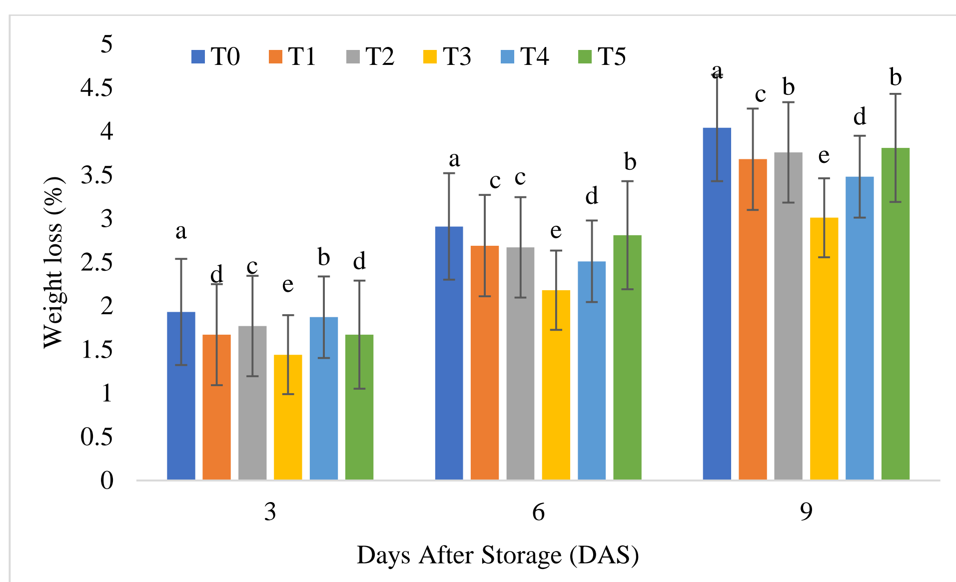


Fig. 4. Effect of treatments on total weight loss of mango at different days after storage. Vertical bars represent standard error. Here, T_0: Control, T_1: 25mg/L COS, T_2: 50 mg/L COS, T_3: 100 mg/L COS, T_4: 250 mg/L COS, T_5: 500 mg/L COS.

Combined effect of variety and postharvest treatments

A significant variation was also observed by the combined effect of studied mango varieties and different postharvest treatment during storage (Table 2). Among the treatment combinations, the minimum weight loss in (1.24%, 1.94%, 2.85%) was noted from the 'Langra' fruits treated by COS 100mg/L (V_1T_3) at 3, 6 and 9 DAS, respectively (Table 2) and the maximum weight loss (1.96%, 2.94%, 4.05%) was recorded from 'Amropali' (V_2T_0) fruits which was not subjected to any postharvest treatments. The main cause of weight loss of fruits and vegetables is the loss of water by transpiration and respiration processes (Elsabee & Abdou, 2013). The mango fruit weight loss increased during storage possibly due to the increased of the respiratory metabolism and exacerbate the loss of water absorbed by the chitosan coating on the fruit surface (Abbasi et al., 2009). Our results are in agreement with previous studies that postharvest treatments delayed the loss of fruit weight compared to untreated fruits during storage.

Table 2. Effect of varieties and postharvest treatments on weight loss of mango during storage.

Variety × Treatment	Weight loss (%)		
	3 DAS	6 DAS	9 DAS
V_1T_0	1.90ab	2.88b	4.02a
V_1T_1	1.60d	2.63d	3.72bc
V_1T_2	1.65d	2.56e	3.77b
V_1T_3	1.24f	1.93g	2.86g
V_1T_4	1.86b	2.46f	3.55d
V_1T_5	1.49e	2.78c	3.81b
V_2T_0	1.96a	2.95a	4.05a
V_2T_1	1.75c	2.76c	3.65cd
V_2T_2	1.9ab	2.78c	3.76b
V_2T_3	1.65d	2.43f	3.16f
V_2T_4	1.88b	2.56e	3.41e
V_2T_5	1.85b	2.81c	3.81b
Level of Significance	**	**	**
LSD at 5%	0.06	0.05	0.06
LSD at 1%	0.09	0.06	0.13
CV (%)	2.32	1.13	1.66

** Significant at 1% level of probability, DAS = Days after Storage, V_1: Langra, V_2: Amropali, T_0: Control, T_1: 25mg/L COS, T_2: 50 mg/L COS, T_3: 100mg/L COS, T_4: 250 mg/L COS, T_5: 500 mg/L COS. Values having same letters within the column do not differ significant at 5% level of probability.

Titratable acidity (%)

Effect of varieties

Varietals effect showed significant differences in respect of titratable acidity at different days after storage (Fig. 5). Between the varieties, 'Amropali' gave the maximum titratable acidity (0.37, 0.30 and 0.24%) to compare 'Langra' (0.36, 0.29 and 0.21%) at 3,6 and 9 DAS, respectively.

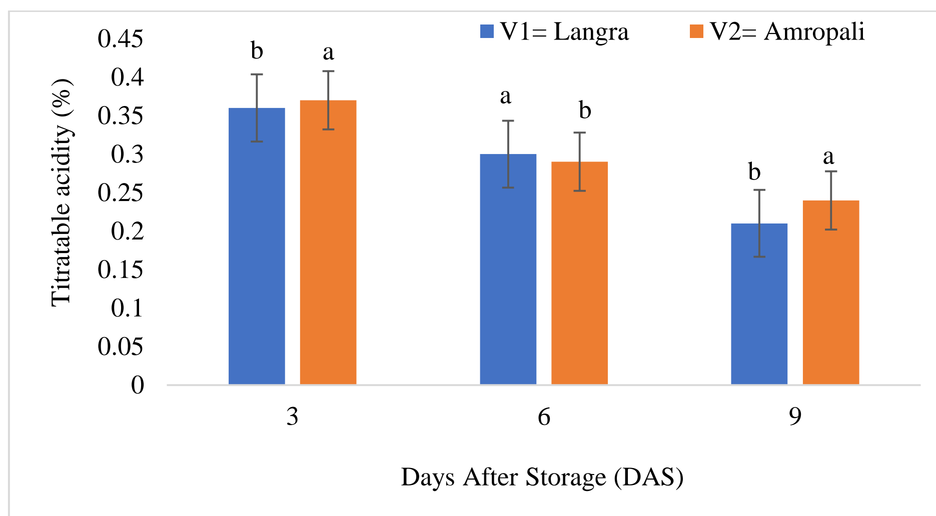


Fig. 5. Effect of variety on titratable acidity of mango at different days after storage. Vertical bars represent standard error.

Effect of postharvest treatments

Highly significant variation was observed among different postharvest treatments during storage (Fig. 6). COS 100 mg/L treated fruits had higher (0.4917, 0.361, 0.28 %) at 3, 6 and 9 DAS, respectively which was closely followed by COS 25 mg/L treated fruits of mango (0.42, 0.31 and 0.26 %) at 3, 6 and 9 DAS respectively. However, the lowest titratable acidity content (0.26, 0.21 and 0.117%) was obtained in those fruits which were untreated at 3,6 and 9 DAS respectively. The TA content decrease with increasing days after storage. Similar findings were reported by Supa et al. (2024), they noted that edible coating treatment retained higher TA content compared to control.

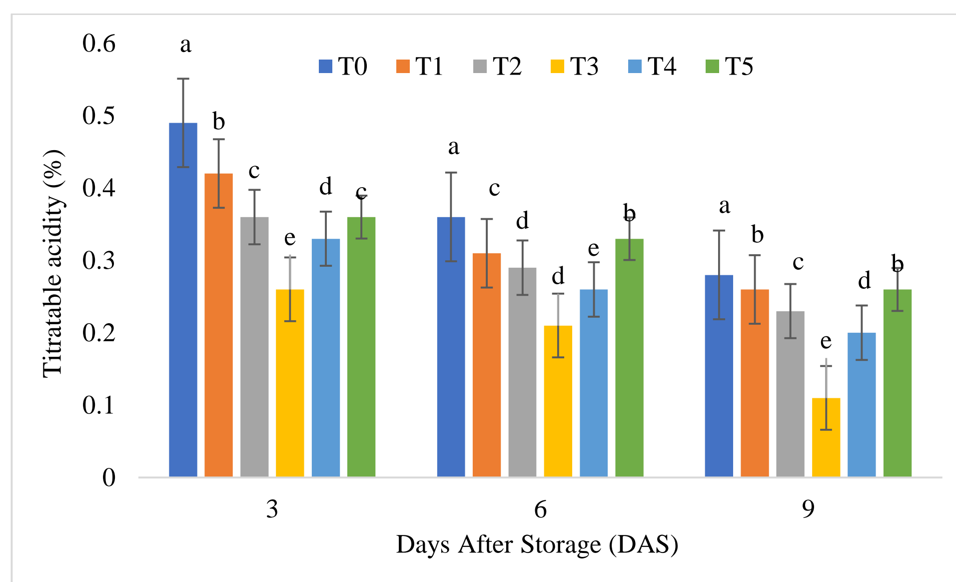


Fig. 6. Effect of treatments on titratable acidity of mango at different days after storage. Vertical bars represent standard error. Here, T_0: Control, T_1: 25mg/L COS, T_2: 50 mg/L COS, T_3: 100 mg/L COS, T_4: 250 mg/L COS, T_5: 500 mg/L COS.

Combined effect of variety and postharvest treatments

A highly significant variation was noted among the combined effect of varieties and various postharvest treatments in respect of titratable acidity at different days after storage (Table 3). The highest TA content (0.50, 0.37 and 0.28 %) was found in those fruits of 'Langra' which was treated by COS 100mg/L at 3, 6 and 9 DAS, respectively which was statistically differed forms other treatment combinations. The lowest TA content (0.24, 0.22 and 0.11%) was found from the untreated fruits of the variety 'Langra' at 3, 6 and 9 DAS. These results revealed that the TA content decrease gradually with the advancement of storage time among the all treatment combinations. The titratable acidity gradually declined with the increase in storage duration that is due to the consumption of organic acids in respiratory metabolism and conversion of citric acid into sugars (Rab et al., 2011; Rathore et al., 2007). Titratable acidity is the indicator of acidity of fleshy fruit and it is directly related to the amount of organic acid present in the fruit. During ripening of fruit, the devaluation of acidity may be due to the metabolic changes in fruit and use of organic acid in the respiratory process of fruit.

Table 3. Effect of varieties and postharvest treatments on titratable acidity of mango during storage.

Variety × Treatment	Titratable acidity (%)		
	3 DAS	6 DAS	9 DAS
V_1T_0	0.24g	0.22h	0.12f
V_1T_1	0.42c	0.31cd	0.24c
V_1T_2	0.36d	0.28f	0.22d
V_1T_3	0.47b	0.35b	0.28a
V_1T_4	0.35d	0.26g	0.16e
V_1T_5	0.35d	0.37a	0.25b
V_2T_0	0.28f	0.21h	0.12f
V_2T_1	0.42c	0.32c	0.28a
V_2T_2	0.37d	0.30de	0.25b
V_2T_3	0.50a	0.37a	0.28a
V_2T_4	0.32e	0.25g	0.25b
V_2T_5	0.36d	0.29ef	0.28a
Level of Sig.	**	**	**
LSD at 5%	0.02	0.01	9.74
LSD at 1%	0.03	0.01	0.01
CV (%)	3.90	2.05	2.52

** Significant at 1% level of probability, DAS = Days after Storage, V_1: Langra, V_2: Amropali, T_0: Control, T_1: 25mg/L COS, T_2: 50 mg/L COS, T_3: 100mg/L COS, T_4: 250 mg/L COS, T_5: 500 mg/L COS.
Values having same letters within the column do not differ significant at 5% level of probability.

pH

Effect of varieties

pH value gradually increased with the advancement of storage period and observed significantly different between the varieties of mango. Initially the pH value of 4.67 and 4.75 were found from the variety Langra and Amrapali, respectively. The highest (5.02, 6.19 and 6.86) pH was noted from 'Langra' than 'Amrapali' (4.94, 5.99 and 6.73) at 3, 6 and 12 DAS, respectively (Fig. 7). These results revealed that the 'Langra' took the more pH than 'Amrapali' due to the adaptability range and their genetic variation between two varieties in storage condition.

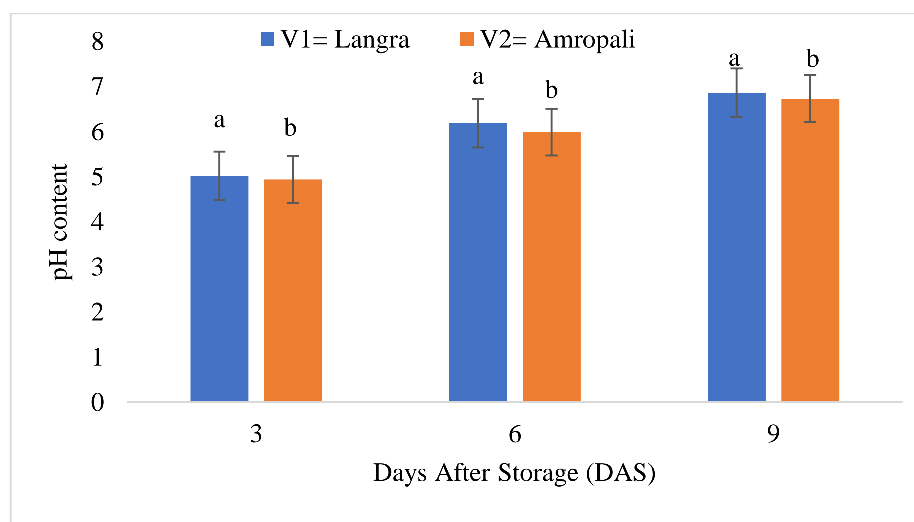


Fig. 7. Effect of variety on pH of mango at different days after storage. Vertical bars represent standard error.

Effect of postharvest treatments

Significant variation was found due to the effect of various postharvest treatments in respect of pH content at 3 to 12 DAS (Fig. 8).

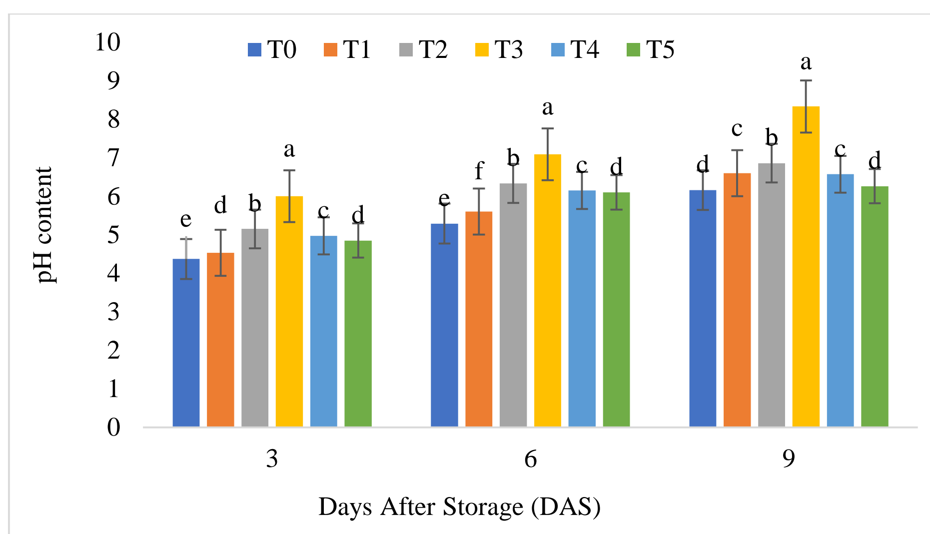


Fig. 8. Effect of treatments on pH of mango at different days after storage. Vertical bars represent standard error. Here, T_0: Control, T_1: 25mg/L COS, T_2: 50 mg/L COS, T_3: 100 mg/L COS, T_4: 250 mg/L COS, T_5: 500 mg/L COS.

Among the postharvest treatments, the higher values of pH content (6.00, 7.09 and 8.33) were recorded from the fruits treated with COS 100 mg/L at 3, 6 and 9 DAS, respectively which was statistically differed from other postharvest treatments. In contrast, the lowest values of pH content (4.37, 5.29, 6.16) were recorded while the fruits were not subjected to any postharvest treatments at 3, 9 DAS, respectively. Nasrin et al. (2008) also found significant variation among the postharvest treatments regarding to pH content of the storage fruits. These findings are closely related to He et al. (2018), they found that pH was higher in untreated fruits and lower in COS treated fruits.

Combined effect of variety and postharvest treatments

Combined effect of varieties and postharvest treatments were significantly different during entire storage period (Table 4). Among the treatment combinations, COS 100 mg/L treated fruit of 'Langra' (V_1T_3) record highest pH value (6.53, 7.55 and 8.8) at 3, 6 and 9 DAS, respectively while it was statistically differed from other treatment combinations. The lowest pH value (4.35, 5.26 and 6.16) was noted from the variety' Langra' (V_1T_0) while it was untreated at 3, 6, and 9 DAS, respectively. These results indicated that the values of pH increase with the advancement of storage time. These results are in agreement with those of Maftoonazad et al. (2008), who reported that a higher increase in pH was found in the control samples as compared to coated peaches.

Table 4. Effect of varieties and postharvest treatments on pH of mango during storage.

Varieties × Treatments	pH at different DAS		
	3	6	9
V_1T_0	4.35f	5.26h	6.16f
V_1T_1	4.40f	5.77f	6.57d
V_1T_2	5.14c	6.32c	6.85c
V_1T_3	6.53a	7.55a	8.80a
V_1T_4	4.88d	6.18d	6.51dc
V_1T_5	4.83d	6.09e	6.28ef
V_2T_0	4.38f	5.32h	6.16f
V_2T_1	4.67e	5.43g	6.64cd
V_2T_2	5.16c	6.34c	6.867c
V_2T_3	5.47b	6.64b	7.86b
V_2T_4	5.07c	6.13de	6.63cd
V_2T_5	4.88d	6.10e	6.24f
Level of Significance	**	**	*
LSD at 1%	0.20	0.09	0.32
CV (%)	1.77	0.63	2.02

*&** Significant at 5% & 1% level of probability, DAS = Days after Storage, V_1: Langra, V_2: Amropali, T_0: Control, T_1: 25mg/L COS, T_2: 50 mg/L COS, T_3: 100mg/L COS, T_4: 250 mg/L COS, T_5: 500 mg/L COS.
Values having same letters within the column do not differ significant at 5% level of probability.

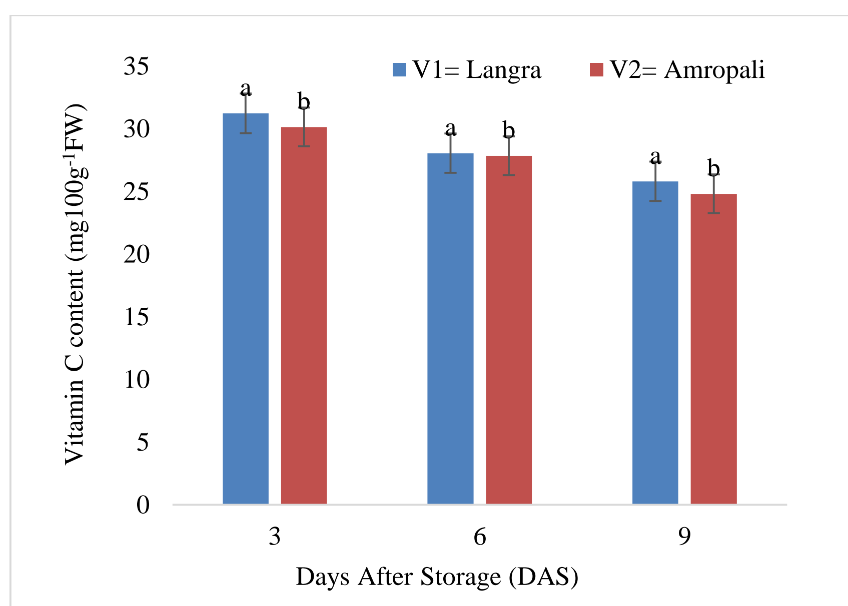


Fig. 9. Effect of variety on vitamin C content of guava at different days after storage. Vertical bars represent standard error.

Vitamin C content

Effect of varieties

Varietal effects were highly significant in relation to vitamin C content during storage of mango (Fig 9). Between two varieties, the variety 'Langra' produced significantly the maximum vitamin C content (31.24, 28.06 and 25.81 mg 100 g¹ FW) compared to 'Amropali' (30.16, 27.85 and 24.82 mg 100 g^{-1} FW) at 3,6 and 9 DAS, respectively. These results indicated that the vitamin C content significantly decreased with the advancement of storage period. Similar findings were also obtained by Supa et al. (2024), they observed varietal difference in respect of vitamin C content during storage, this may possibly due to genetical differences.

Effect of postharvest treatments

Vitamin C content was significantly influenced by the effect of various postharvest treatments at different days after storage. It was recorded that the maximum vitamin C content (38.41, 36.96 and 35.12 mg 100 g^{-1} FW) was found from the mango fruits stored in ambient temperature while it was treated by COS 100 mg/L at 3, 6 and 9 DAS, respectively. In contrast, the lowest vitamin C content (24.25, 21 and 18.96 mg 100 g^{-1} FW) was noted from untreated storage mango fruits which were statistically different from other treatments at 3,6 and 9 DAS, respectively (Fig. 10). These results revealed that the vitamin C content was significantly decreased with the advancement of storage period due to the conversion of acid to sugar with the activity of ascorbic dehydrogenase which results was fully agreed by Caron et al. (2013).

Combined effect of variety and postharvest treatments

Maximum vitamin C content (33.67, 31.16 and 35.62 mg 100 g^{-1}FW) was obtained by the variety 'Langra' while it was treated by COS 100 mg/L at 3, 6 and 9 DAS and whereas the lowest vitamin C content (23.21, 21 and 17.26 mg 100 g^{-1} FW) was found in untreated fruits of 'Amropali' (V_2T_0) which was statistically different from other at the 3, 6 and 9 DAS, respectively (Table 5). The results revealed that vitamin C content of mango were gradually decreased with advancement of storage period and reached the lowest values at the end of storage period. The findings of this study are similar to the findings obtained by Supa et al. (2024). The reduction of vitamin C content during storage of fruits might possibly due to retardation of oxidation process and consequently slow rate of conversion of L-ascorbic acid into dehydroascorbic acid by ascorbic acid oxidase. Similar observation has also been recorded in mango (Jain & Mukherjee, 2011).

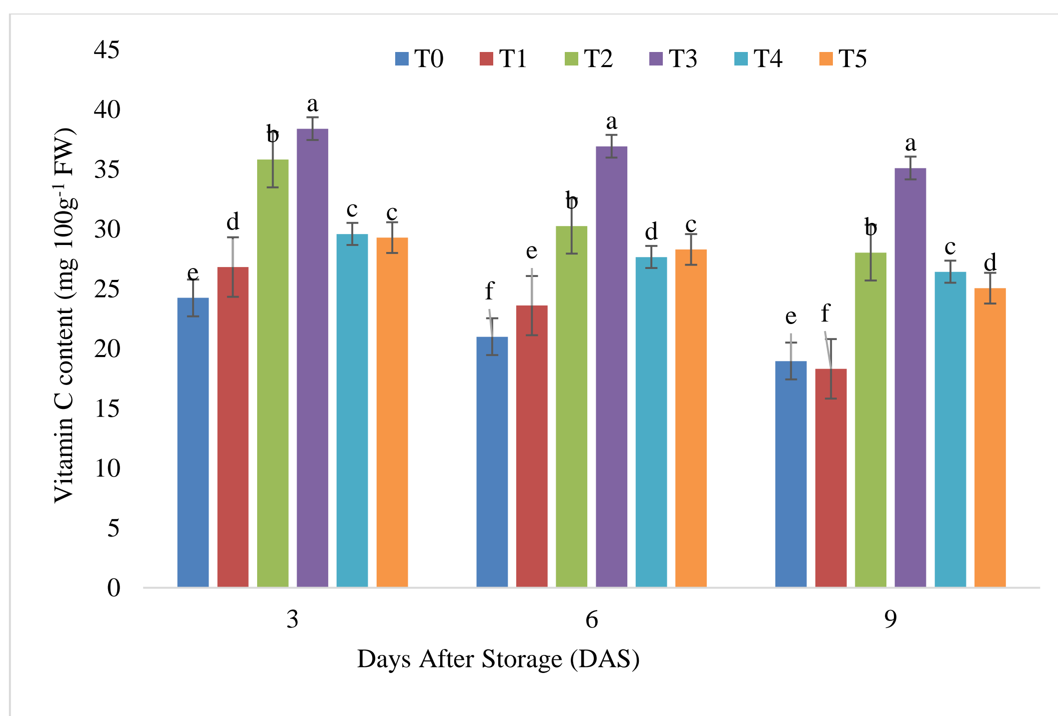


Fig. 10. Effect of treatments on vitamin C content of guava at different days after storage. Vertical bars represent standard error. Here, T_0: Control, T_1: 25mg/L COS, T_2: 50 mg/L COS, T_3: 100 mg/L COS, T_4: 250 mg/L COS, T_5: 500 mg/L COS.

Table 5. Effect of varieties and postharvest treatments on vitamin C content of mango during storage.

Variety × Treatment	Vitamin C (mg100g^{-1} FW)		
	3 DAS	6 DAS	9 DAS
V_1T_0	25.16g	21.0h	19.73f
V_1T_1	25.47g	23.6g	19.37f
V_1T_2	33.67c	31.17c	29.77c
V_1T_3	39.25a	37.47a	34.6b
V_1T_4	29.18e	28.13e	26.33d
V_1T_5	28.2f	27.03f	25.1e
V_2T_0	23.3h	21.0h	18.2g
V_2T_1	28.2f	23.6g	17.27h
V_2T_2	37.99b	29.37d	26.3d
V_2T_3	37.57b	36.4b	35.63a
V_2T_4	30.0d	27.2f	26.53d
V_2T_5	30.37d	29.57d	25.03e
Level of Significance	*	**	*
LSD at 5%	0.72	0.38	0.67
LSD at 1%	0.97	0.51	0.91
CV (%)	1.38	0.08	1.56

*&** Significant at 5% & 1% level of probability, DAS = Days after Storage, V_1: Langra, V_2: Amropali, T_0: Control, T_1: 25mg/L COS, T_2: 50 mg/L COS, T_3: 100mg/L COS, T_4: 250 mg/L COS, T_5: 500 mg/L COS.
Values having same letters within the column do not differ significant at 5% level of probability.

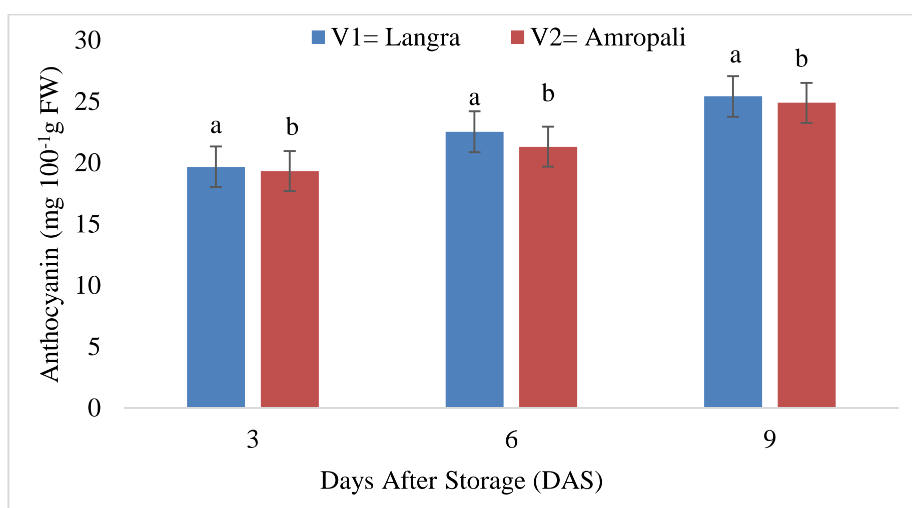


Fig. 11. Effect of variety on anthocyanin content of mango at different days after storage. Vertical bars represent standard error.

Anthocyanin Content

Effect of varieties

A highly significant variation was observed due to the effect of mango varieties in respect of anthocyanin content at different days after storage (Fig. 11).

The maximum anthocyanin content was recorded in ‘Langra’ (19.67. 22.53 and 25.42 ml) compared to 'Amrapali' (19.33, 21.31 and 24.89 ml) after 3, 6 and 9 DAS. These results indicated that the anthocyanin content significantly increased with the advancement of storage period.

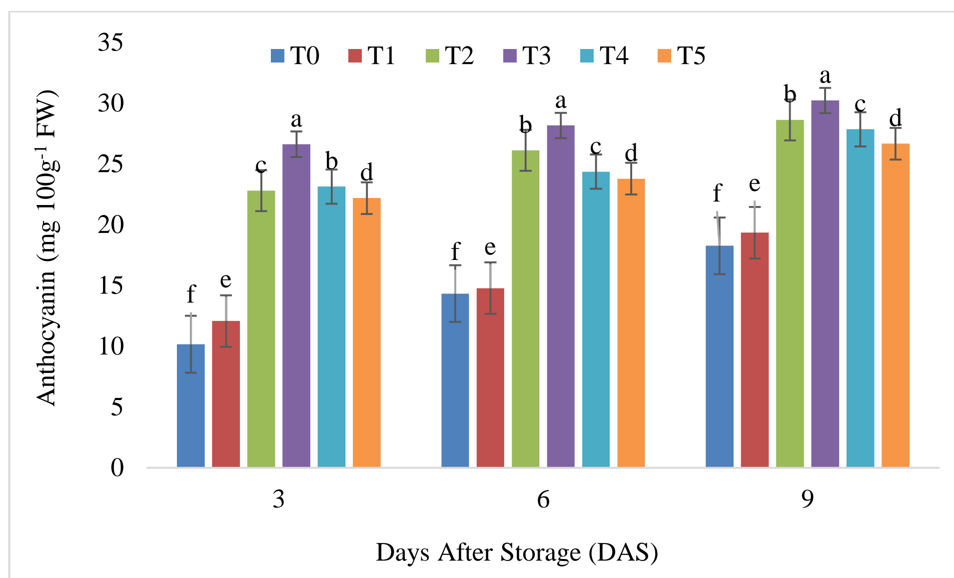


Fig. 12. Effect of treatments on anthocyanin content of mango at different days after storage. Vertical bars represent standard error. Here, T_0: Control, T_1: 25mg/L COS, T_2: 50 mg/L COS, T_3: 100 mg/L COS, T_4: 250 mg/L COS, T_5: 500 mg/L COS.

Effect of postharvest treatments

Anthocyanin content was significantly influenced by the effect of various postharvest treatments at different days after storage (Fig. 12). Maximum anthocyanin content (26.62, 28.18 and 30.223 mg/100 g FW) was found from the mango fruits stored in ambient temperature while it was treated with COS 100 mg/L at 3, 6 and 9 DAS. In contrast, the lowest anthocyanin content (10.17, 14.33 and 18.27 mg/100 g FW) was found from untreated storage mango fruits which were statistically different from other treatments at 3, 6 and 9 DAS, respectively. These results revealed that the anthocyanin content was significantly increased. Similar results were observed in case of strawberry fruit that AOS treated fruit retained higher anthocyanin content compared to control treatment (Bose et al., 2019).

Combined effect of variety and postharvest treatments

Combined effect of the mango varieties and various postharvest treatments were significantly significant in case of anthocyanin content at different days after storage (Table 6). It was found that the maximum anthocyanin content (27.53, 28.56 and 30.9 mg/100 g FW) was obtained by the variety 'Langra' while it was treated with COS 100 mg/L at 3, 6 and 9 DAS and whereas the lowest anthocyanin content (9.76, 12.43 and 18.75 mg/100 g FW) was found in untreated fruits of 'Amrapali' which was statistically different from other treatments at the 3, 6 and 9 DAS respectively. These results are supported by the findings of Bose et al. (2019), who reported AOS 100 mg/L treated strawberry fruit demonstrated higher anthocyanin content compared to untreated fruit at the end of storage.

Total soluble solids

Effect of varieties

Significant variation was observed between two varieties of mango in respect of total soluble solid (TSS) content during storage (Fig. 13). Between the varieties, 'Langra' recorded the maximum TSS (10.42, 12.24 and 13.94 % Brix) than 'Amrapali' (9.11, 10.45 and 12.60 % Brix) at 3, 6 and 9 DAS, respectively. These results revealed that the TSS content significantly increased with the increasing of storage time.

Table 6. Effect of varieties and postharvest treatments on anthocyanin content of mango during storage.

Varieties × Treatments	Anthocyanin ($mg100g^{-1}$ FW) at different DAS		
	3	6	9
V_1T_0	10.56i	16.23f	17.75h
V_1T_1	12.40g	15.76f	19.93f
V_1T_2	23.09d	26.12c	28.61c
V_1T_3	27.53a	28.56a	30.90a
V_1T_4	22.72e	24.78d	27.90d
V_1T_5	21.73f	23.73e	26.49e
V_2T_0	9.76j	12.43h	17.80h
V_2T_1	11.76h	13.77g	18.75g
V_2T_2	22.53e	26.09c	28.64c
V_2T_3	25.70b	27.79b	29.54b
V_2T_4	23.57c	23.95e	27.80d
V_2T_5	22.65e	23.82e	26.84e
Level of Significance	*	**	**
LSD at 1%	0.44	0.81	0.55
CV (%)	0.98	1.60	0.94

*&** Significant at 5% & 1% level of probability, DAS = Days after Storage, V_1: Langra, V_2: Amropali, T_0: Control, T_1: 25mg/L COS, T_2: 50 mg/L COS, T_3: 100mg/L COS, T_4: 250 mg/L COS, T_5: 500 mg/L COS.
Values having same letters within the column do not differ significant at 5% level of probability.

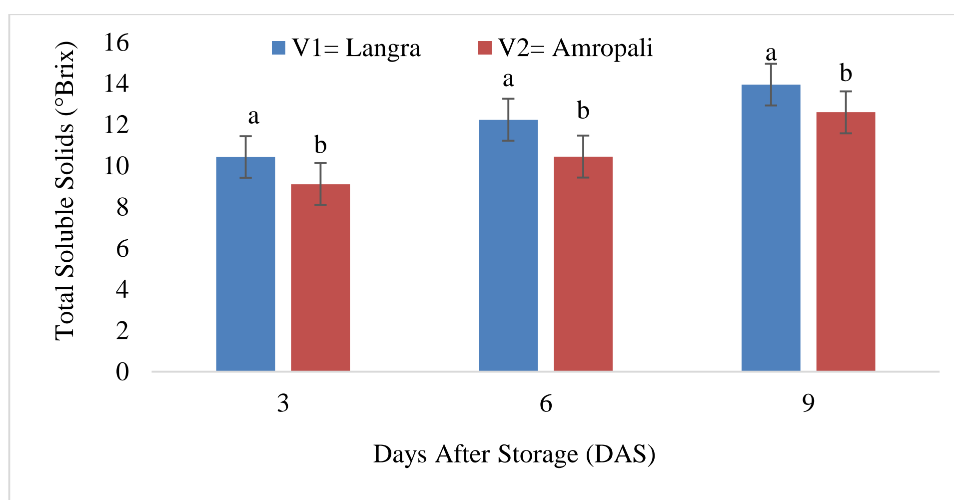


Fig. 13. Effect of variety on TSS of mango at different days after storage. Vertical bars represent standard error.

Effect of postharvest treatments

Highly significant variation was found due to the effect of various postharvest treatments in respect of TSS content at different days after storage (Fig. 14). The fruits treated with COS 100 mg/L was found the lowest TSS (8.7, 10.45 and 11.9 % Brix), and untreated fruits recorded highest TSS (10.93, 13.26 and 15.73 % Brix) at 3, 6 and 9 DAS, respectively. Present studied results supported the findings of Alhassan and Ndomakaah (Alhassan & Ndomakaah, 2024), they reported that postharvest treatment of aloe vera significantly delayed the TSS production compared to untreated banana during storage,

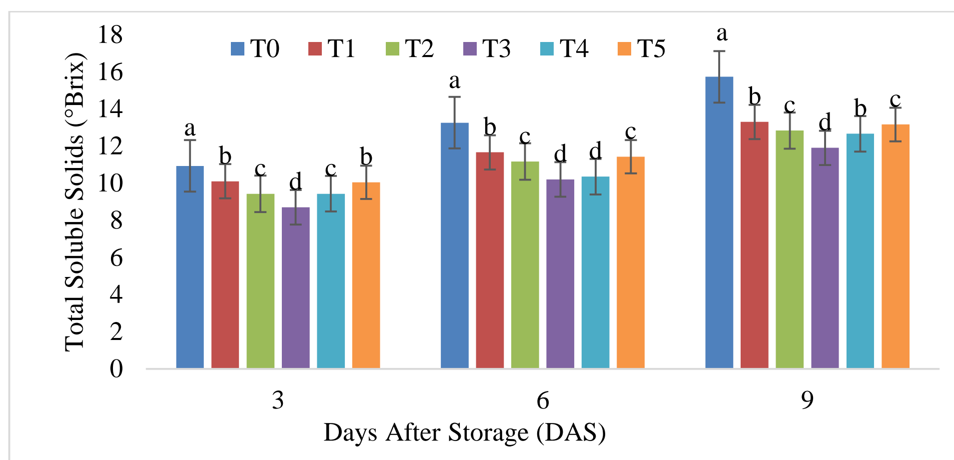


Fig. 14. Effect of treatments on TSS of mango at different days after storage. Vertical bars represent standard error. Here, T_0: Control, T_1: 25mg/L COS, T_2: 50 mg/L COS, T_3: 100 mg/L COS, T_4: 250 mg/L COS, T_5: 500 mg/L COS.

Table 7. Effect of varieties and postharvest treatments on TSS content of mango during storage

Varieties × Treatments	TSS (° Brix) at different DAS		
	3	6	9
V_1T_0	11.3a	14.73a	17.46a
V_1T_1	10.93ab	12.96b	14.3b
V_1T_2	9.83d	12.03c	12.8ef
V_1T_3	9.0ef	10.36ef	11.9g
V_1T_4	10.23cd	11.13d	13.56d
V_1T_5	11.3a	12.23c	13.63cd
V_2T_0	10.56bc	11.8c	14.0bc
V_2T_1	9.26e	10.36ef	12.4f
V_2T_2	9.03ef	10.3ef	12.86e
V_2T_3	8.4g	10.03f	11.9g
V_2T_4	8.63fg	9.56g	11.76g
V_2T_5	8.8fg	10.63e	12.7ef
Level of Significance	*	**	**
LSD at 1%	0.56	0.62	0.58
CV (%)	2.47	2.37	1.89

*&** Significant at 5% & 1% level of probability, DAS = Days after Storage, V_1: Langra, V_2: Amropali, T_0: Control, T_1: 25mg/L COS, T_2: 50 mg/L COS, T_3: 100mg/L COS, T_4: 250 mg/L COS, T_5: 500 mg/L COS.
Values having same letters within the column do not differ significant at 5% level of probability.

Combined effect of variety and postharvest treatments

Combined effect of the mango varieties and various postharvest treatments were significantly varied in case of TSS (%Brix) content during entire storage period. The TSS contents were observed in COS 100 mg/L treated fruits of 'Amrapali' (7.4, 9.03 and 11.9 % Brix) at 3, 6 and 9 DAS, respectively whereas the highest TSS (11.31, 14.73 and 17.46% Brix) was recorded from untreated fruits of 'Langra' at 3, 6 and 9 DAS (Table 7). The observation indicate that the total soluble solids contents increased significantly during storage period and it was the minimum in both varieties of 'langra' and 'Amropali' were treated with COS 100 mg /L. However, this increasing TSS is due to the conversion of complex carbohydrates into simple sugars and it was also correlated with hydrolytic changes in starch and conversion of starch to sugar being an important index of ripening process in mango and other climacteric fruits. These results are also in line with the results reported by Ali et al. (2011) and Gol and Rao (2011), who assigned the probable reasons for the reducing levels of TSS accumulation in the

chitosan alone coated fruit to the slowing down of respiration and metabolic activity, hence retarding the ripening process.

Total sugar (%)

Effect of varieties

Changes of total sugar were significantly affected by the effect of studied mango varieties at 9 DAS while it did not vary at 3 and 6 DAS (Fig. 15). Between two varieties, the variety 'Langra' had higher total sugar content (11.98%) than 'Amropali' (11.92%) at 9 DAS. At 3 and 6 DAS, both varieties were statistically identical in case of total sugar content.

Effect of postharvest treatments

Various postharvest treatments were varied significantly in respect of total sugar content of mangoes during the storage (Fig. 16). Among the postharvest treatments, COS 100 mg/L treated storage fruits recorded the lowest total sugar content (2.63 and 6.15 and 10.21%) at 3, 6 and 9 DAS, respectively whereas it was statistically differed from other postharvest treatments. On the other hand, untreated fruits showed the highest total sugar content (4.7, 9.14 and 14.13%) at these stages, respectively. From the above results, it was observed that the total sugar content gradually increased with the increasing storage period which might be due to rapid conversion of polysaccharides into sugars. The result is also similar to the findings obtained by Bose et al. (2019) and Mondal et al. (2023).

Combined effect of variety and postharvest treatments

Combined effect of the studied mango varieties and various postharvest treatments were significant in respect of total sugar content at different days after storage (Table 8). It was found that COS 100 mg/L treated fruits of 'Amrapali' recorded the lowest total sugar content (5.6 and 10.20%) at 6 and 9 DAS, and at 3 DAS the lowest total sugar content (2.6%) was recorded in 'Langra', respectively which was statistically differed from other treatment combinations. Similarly, the highest total sugar content (9.2 and 14.36%) was noted in those fruits of 'Amrapali' at 6 and 9 DAS and (5.20%) was found in those fruits of 'Langra' at 3 DAS which was not subjected to any postharvest treatments. Results demonstrated that, total sugar of mango gradually increased with the prolonged of storage period. This remarkable increase in total sugars might be attributed to the conversion of organic acid to starch during ripening and storage of fruits. Our results are in agreement with Islam et al. (2018) they reported that postharvest treated fruit exhibited higher total sugar content than untreated strawberry fruit.

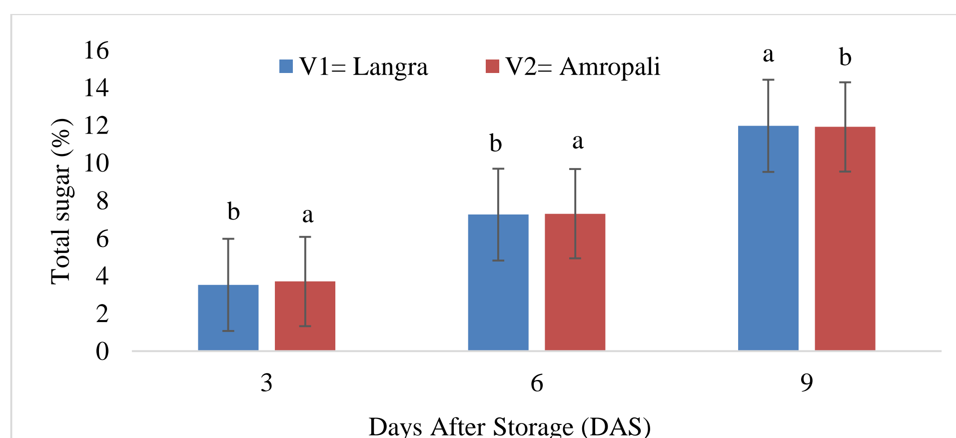


Fig. 15. Effect of variety on total sugar content of mango at different days after storage. Vertical bars represent standard error.

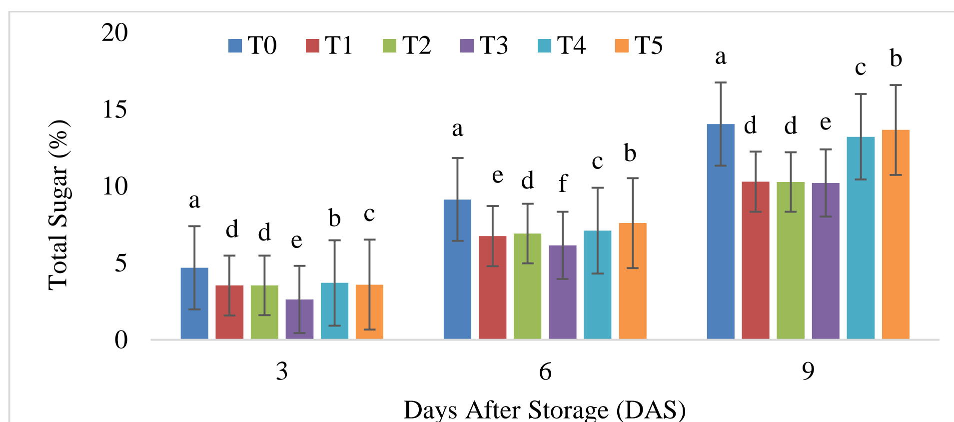


Fig. 16. Effect of treatments on total sugar content of mango at different days after storage. Vertical bars represent standard error. Here, T_0: Control, T_1: 25mg/L COS, T_2: 50 mg/L COS, T_3: 100 mg/L COS, T_4: 250 mg/L COS, T_5: 500 mg/L COS.

Shelf life

Effect of varieties

Significant variation was observed between two varieties of mango during storage period (Fig 17). Longest shelf life (12.56 days) was observed in the variety 'Langra' compare to 'Amrapali' (11.98 days).

Effect of postharvest treatments

Shelf life extension of mango has been one of the most important concerns of the researchers. These results revealed that the longest shelf life (17.66 days) of mango was recorded form COS 100 mg/L treated whereas the shortest shelf life of mango (8.5 days) was recorded from the untreated fruits (Fig. 18). Among other postharvest treatments, the shelf life 11.33, 12.83 days were noted form COS 25 mg/L, COS 50 mg/L treated fruits respectively. Islam et al. (2018) reported that shelf life of mango was higher in treated fruits and lower in untreated fruits during storage.

Table 8. Effect of varieties and postharvest treatments on total sugar content of mango during storage

Variety × Treatment	Total sugar (%) content (DAS)		
	3	6	9
V_1T_0	4.18b	9.23a	14.37a
V_1T_1	3.52fg	6.67h	10.21i
V_1T_2	3.63dc	7.05f	10.23i
V_1T_3	2.6i	5.66j	10.21i
V_1T_4	3.65d	7.01f	13.08f
V_1T_5	3.64d	7.87c	13.81b
V_2T_0	5.21a	9.06b	13.73c
V_2T_1	3.58ef	6.77h	10.4g
V_2T_2	3.48g	6.82g	10.31h
V_2T_3	2.67h	6.65i	10.21i
V_2T_4	3.78c	7.22e	13.37e
V_2T_5	3.57f	7.36d	13.54d
Level of significance	**	**	*
LSD at 5%	0.058	0.052	0.059
LSD at 1%	0.079	0.071	0.080
CV (%)	0.95	1.12	1.19

*&** Significant at 5% & 1% level of probability, DAS = Days after Storage, V_1: Langra, V_2: Amropali, T_0: Control, T_1: 25mg/L COS, T_2: 50 mg/L COS, T_3: 100mg/L COS, T_4: 250 mg/L COS, T_5: 500 mg/L COS.
Values having same letters within the column do not differ significant at 5% level of probability.

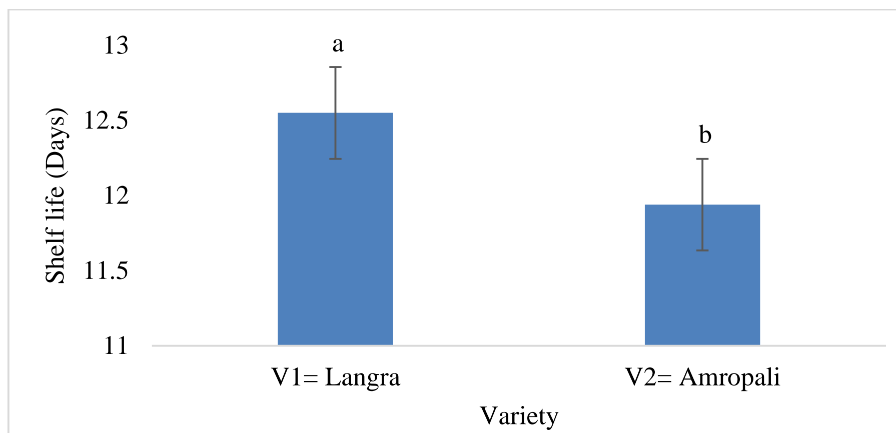


Fig. 17. Effect of variety on shelf life of mango at different days after storage. Vertical bars represent standard error.

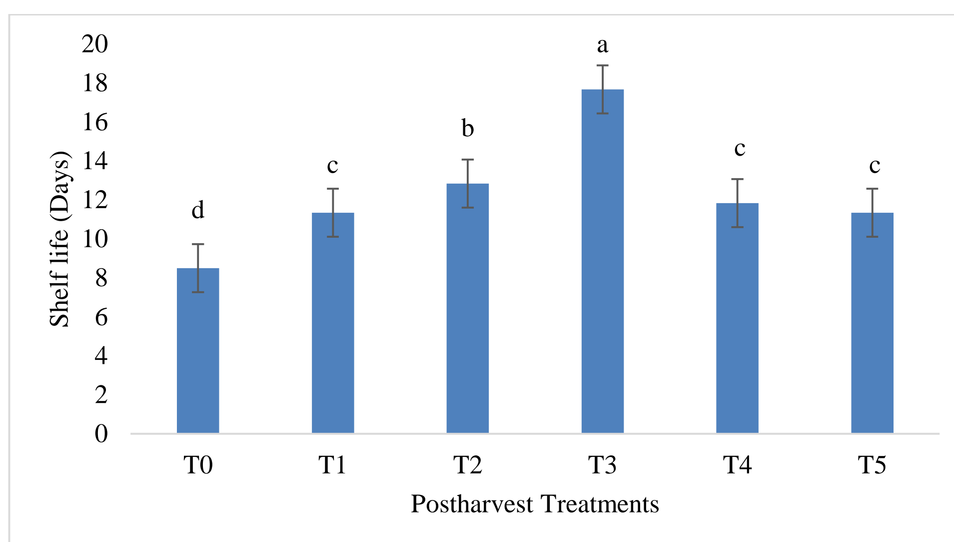


Fig. 18. Effect of treatments on shelf life of mango at different days after storage. Vertical bars represent standard error. Here, T_0: Control, T_1: 25mg/L COS, T_2: 50 mg/L COS, T_3: 100 mg/L COS, T_4: 250 mg/L COS, T_5: 500 mg/L COS.

Combined effect of variety and postharvest treatments

The longest shelf life (18.67 days) was observed in those fruits of 'Langra' treated by COS 100mg/L, which was statistically differed from other treatment combinations. In contrast, the shortest shelf life (8.33 days) was observed in those fruits of 'Langra' and ‘Amrapali’ which was not subjected to any postharvest treatment (Table 9) which was also statistically differed from other treatment combinations. The maximum shelf life of mango was obtained from the fruits of 'Langra' which was treated by COS 100 mg/L due to the lower weight loss, less disease incidence, disease severity, higher titratable acidity and vitamin C content were recorded under this treatment combination. From the above investigation, it was revealed that the variety 'Langra' had highly efficient than 'Amrapali' among the whole studied characteristics while COS 100mg/L treated fruits obtained the similar results. Their combined effect had also more effective to extend the more shelf life. So, the variety 'Langra' and postharvest treatment of COS 100 mg/L alone or their combination would be highly effective for extend the shelf life of mango.

Table 9. Effect of varieties and postharvest treatments on shelf life of mango during storage.

Varieties × Treatments	Shelf life (DAS)
V_1T_0	8.33f
V_1T_1	12.33cd
V_1T_2	13.33c
V_1T_3	18.66a
V_1T_4	11.33de
V_1T_5	11.33de
V_2T_0	8.66f
V_2T_1	10.33e
V_2T_2	12.33cd
V_2T_3	16.66b
V_2T_4	12.33cd
V_2T_5	11.33de
Level of Significance	**
LSD at 1%	1.373
CV (%)	4.87

*&** Significant at 5% & 1% level of probability, DAS = Days after Storage, V_1: Langra, V_2: Amropali, T_0: Control, T_1: 25mg/L COS, T_2: 50 mg/L COS, T_3: 100mg/L COS, T_4: 250 mg/L COS, T_5: 500 mg/L COS.
Values having same letters within the column do not differ significant at 5% level of probability.

CONCLUSION

The present study illustrated that, postharvest coating of chitosan oligosaccharide applications is the promising strategy for the management postharvest fruit quality of mangoes cv. "Langra and Amropali" during storage. Between two varieties, the variety 'Langra' had highly efficient than 'Amrapali' among the whole studied characteristics while COS 100 mg/L treated fruits obtained the similar results. Their combined effect had also more effective as well as the more shelf life were recorded. So, therefore, the variety 'Langra' and postharvest treatment COS 100 mg/L alone or their combination would be highly effective to the physico-chemical properties and extend shelf life of mango. Further studies with other variety are needed in this regard before confirmation and recommendation.

Conflict of interest

Author declared no conflict of interests.

Acknowledgment

I am grateful to the Ministry of Science and Technology of Bangladesh for financial support to conduct the research work. I am also grateful to Prof. Heng Yin, Natural Products and Glyco-biology Laboratory, Dalian Institute of Chemical Physics, University of Chinese Academy of Sciences for providing COS.

REFERENCES

Abbasi, N.A., Iqbal, Z., Maqbool, M., & Hafiz, I.A. (2009). Postharvest quality of mango (*Mangifera indica* L.) fruit as affected by chitosan coating. *Pakistan Journal of Botany, 41*(1), 343-357.

Alhassan, N., & Ndomakaah, A. (2024). Aloe vera gel coating maintains physicochemical parameters, extends the storage life, and preserves the qualities of Lantundan and Cavendish bananas. *Journal of Horticulture and Postharvest Research*, *7*(3), 287-300. https://doi.org/10.22077/jhpr.2024.7190.1357.

Ali, A., Muhammad, M.T.M., Sijam, K., & Siddiqui, Y. (2011). Effect of chitosan coatings on the physicochemical characteristics of Eksotika II papaya (*Carica papaya* L.) fruit during cold storage. *Food Chemistry, 124*(2), 620-626. https://doi.org/10.1016/j.foodchem.2010.06.085

Ali, Z.M., Chin, L.H., & Lazan, H. (2004). A comparative study on wall degrading enzymes, pectin modifications and softening during ripening of selected tropical fruits. *Plant Science, 167*(2), 317-327. http://doi.org/10.1016/j.plantsci.2004.03.030

Atlaw, T.K. (2018). Preparation and utilization of natural Aloe vera to enhance quality of mango fruit. *Journal of Food and Nutrition Sciences, 6*(3), 76-81. http://dx.doi.org/10.11648/j.jfns.20180603.12

BBS 2022: Year Book of Agricultural Statistics of Bangladesh. Bangladesh Bureau of Statistics, Statistics Division, Ministry of Planning, Govt. of People's Republic of Bangladesh, 28th series. 204.

Bose, S.K., Howlader, P., Wang, W., & Yin, H. (2021a). Oligosaccharide is a promising natural preservative for improving postharvest preservation of fruit: A review. *Food Chemistry, 341*, 128178. https://doi.org/10.1016/j.foodchem.2020.128178

Bose, S.K., He, Y., Howlader, P., Wang, W., & Yin, H. (2021b). The N-glycan processing enzymes β-DN-acetylhexosaminidase are involved in ripening-associated softening in strawberry fruit. *Journal of Food Science and Technology, 58*, 621-631. https://doi.org/10.1007/s13197-020-04576-2

Bose, S.K., Howlader, P., Jia, X., Wang, W., & Yin, H. (2019). Alginate oligosaccharide postharvest treatment preserve fruit quality and increase storage life via Abscisic acid signaling in strawberry. *Food Chemistry, 283*, 665-674. https://doi.org/10.1016/j.foodchem.2019.01.060.

Caron, V.C., Tessmer, M.A., Mello, S.C., & Jacomino, A.P. (2013). Quality of mini tomatoes harvested at two maturity stages and kept chilled in three packages. *Horticultura Brasileira, 31*, 279-286. http://dx.doi.org/10.1590/S0102-05362013000200017

Chaiprasart, P., Handsawasdi, C., & Pipattanawong, N. (2006). The effect of chitosan coating and calcium chloride treatment on postharvest qualities of strawberry fruit (*Fragaria× ananassa*). Acta Horticulture, 708, 337-342. https://doi.org/10.17660/ActaHortic.2006.708.58

Elsabee, M. Z., & Abdou, E. S. (2013). Chitosan based edible films and coatings: A review. *Materials Science and Engineering: C, 33*(4), 1819-1841. https://doi.org/10.1016/j.msec.2013.01.010

Gol, N.B., & Rao, R.T. (2011). Banana fruit ripening as influenced by edible coatings. *International Journal of Fruit Science, 11*(2), 119-135. https://doi.org/10.1080/15538362.2011.578512

Gomez, K.A., & Gomez, A.A. (1984). *Statistical procedures for agricultural research*: 2nd Edition, John Wiley and Sons, New York, 680 p.

He, Y., Bose, S.K., Wang, M., Liu, T., Wang, W., Lu, H., & Yin, H. (2019). Effects of chitosan oligosaccharides postharvest treatment on the quality and ripening related gene expression of cultivated strawberry fruits. *Journal of Berry Research, 9*(1), 11-25. http://doi.org/10.3233/JBR-180307.

He, Y., Bose, S.K., Wang, W., Jia, X., Lu, H., & Yin, H. (2018). Pre-harvest treatment of chitosan oligosaccharides improved strawberry fruit quality. *International Journal of Molecular Sciences, 19*(8), 2194. https://doi.org/10.3390/ijms19082194.

Islam, T., Afrin, N., Parvin, S., Dana, N., Rahman, K., Zzaman, W., & Islam, M. (2018). The impact of chitosan and guava leaf extract as preservative to extend the shelf-life of fruits. *International Food Research Journal, 25*(5). 2056-2062.

Jain, S.K., & Mukherjee, S. (2011). Enhancing keeping quality of fruits in mango cv. *Langra. Indian Journal of Horticulture, 68*(1), 142-144.

Kefas, E.B., Aliyu, B., & Tashiwa, Y.I. (2024). Postharvest losses, causes and mitigation in tomato transportation: a systematic review. *Journal of Horticulture and Postharvest Research*, *7*(3), 223-236. https://doi.org/10.22077/jhpr.2024.7460.1370

Kobra, K., Hossain, M., Talukder, M., & Bhuyan, M. (2012). Performance of twelve mango cultivars grown in different agro-ecological zones of Bangladesh. *Bangladesh Journal of. Agricultural Research*. 37(4): 691-710. http://dx.doi.org/10.3329/bjar.v37i4.14394

Maftoonazad, N., Ramaswamy, H.S., & Marcotte, M. (2008). Shelf-life extension of peaches through sodium alginate and methyl cellulose edible coatings. *International Journal of Food Science & Technology, 43*(6), 951-957. https://doi.org/10.1111/j.1365-2621.2006.01444.x

Mondal, D.R., Malek, M.A., Bose, S.K., and Rahman, M.M. (2023). Effect of postharvest treatments on shelf life and physico-chemical properties of mango cv. *Amrapali*. *International Journal of Innovative Research, 8(1): 20–25.*

Mustari, N., Monir, M.D.R., Sani, M.D.N.S., Hasan, M.D.M., Biswas, S., Hossain, M.D.S. and Malek, M.A. (2020). Effect of indegenous technique on post harvest quality and shelf life of mango. Journal of Biology and Nature, *11*(3), 22-31.

Nasrin, T., Molla, M., Hossaen, M.A., Alam, M., & Yasmin, L. (2008). Effect of postharvest treatments on shelf life and quality of tomato. *Bangladesh Journal of Agricultural Research, 33*(4), 579-585. https://doi.org/10.3329/bjar.v33i4.2291

Perumal, A.B., Sellamuthu, P.S., Nambiar, R.B., & Sadiku, E.R. (2017). Effects of essential oil vapour treatment on the postharvest disease control and different defence responses in two mango (*Mangifera indica* L.) cultivars. *Food and Bioprocess Technology, 10*, 1131-1141. https://link.springer.com/article/10.1007/s11947-017-1891-6

Rab, A.R., Sajid, M.M. S., Saeeda, S., & Najia, N. (2011). Effects of wet heat treatment (WHT) durations on the quality of sweet orange stored at room temperature. Sarhad Journal of Agriculture, *27*(2), 189-194.

Ranganna, S. (1977). Manual of analysis of fruit and vegetable productsMcGraw-Hill. *New Delhi: Tata*.

Ranganna, S. (1986). *Handbook of analysis and quality control for fruit and vegetable products*: Tata McGraw-Hill Education.

Rastgoo, N., Rastegar, S., & Rohani, A. (2024). Maintaining quality of Lisbon lemon (*Citrus limon*) in cold storage using natural elicitors. *Journal of Horticulture and Postharvest Research, 7*(2), 99-114. https://doi.org/10.22077/jhpr.2024.7125.1352

Rathore, H.A., Masud, T., Sammi, S., & Soomro, A.H. (2007). Effect of storage on physico-chemical composition and sensory properties of mango (*Mangifera indica* L.) variety Dosehari. *Pakistan Journal of Nutrition, 6*(2), 143-148. http://doi.org/10.3923/pjn.2007.143.148

Sims, D.A., & Gamon, J.A. (2002). Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. *Remote Sensing of Environment, 81*(2-3), 337-354. https://doi.org/10.1016/S0034-4257(02)00010-X

Sogi, D.S., Siddiq, M., Greiby, I., & Dolan, K.D. (2013). Total phenolics, antioxidant activity, and functional properties of 'Tommy Atkins' mango peel and kernel as affected by drying methods. *Food Chemistry, 141*(3), 2649-2655. http://doi.org/10.1016/j.foodchem.2013.05.053

Supa, S.A., Howlader, P., Ali, M., Rupa, R.A., & Bose, S.K. (2024). Edible coatings maintained postharvest quality and increased shelf life of guava fruits. *Journal of Horticulture and Postharvest Research, 7*(Special Issue), 15-34. http://doi.org/10.22077/jhpr.2023.6531.1324

JOURNAL OF HORTICULTURE AND POSTHARVEST RESEARCH
2025, VOL. 8(1), 67-88

Journal of Horticulture and Postharvest Research

Journal homepage: www.jhpr.birjand.ac.ir

Evaluation of engineering, physiochemical and nutritional properties of three different varieties of pomelo fruit

Simple Sharma[1,*], Barinderjit Singh[1] and Yashi Srivastava[2]

1, Department of Food Science and Technology, I. K. Gujral Punjab Technical University, Kapurthala, Punjab-144603, India
2, Department of Applied Agriculture, Central University of Punjab, Bathinda, Punjab-151401, India

ARTICLE INFO

Original Article

Article history:
Received 13 June 2024
Revised 18 September 2024
Accepted 2 October 2024

Keywords:
Citrus maxima
Engineering properties
Geometric properties
Mineral analysis
Texture

DOI: 10.22077/jhpr.2024.7757.1388
P-ISSN: 2588-4883
E-ISSN: 2588-6169

***Corresponding author:**
Department of Food Science and Technology, I. K. Gujral Punjab Technical University, Kapurthala, Punjab-144603, India.

Email: simplesharma966@gmail.com

© This article is open access and licensed under the terms of the Creative Commons Attribution License http://creativecommons.org/licenses/by/4.0/ which permits unrestricted, use, distribution and reproduction in any medium, or format for any purpose, even commercially provided the work is properly cited.

ABSTRACT

Purpose: The aim of this study was to identify the engineering, physiochemical, and nutritional properties of selected varieties of pomelo fruit. **Research method:** The study was carried out using a one-way analysis of variance with three replications on selected varieties of pomelo fruit. The experiment consisted of three cultivars, namely red, pink, and white pomelo to analyze the engineering, physiochemical, and nutritional properties. **Findings:** The results revealed that the geometrical and gravimetric analysis showed variation among different parameters of varieties of pomelo fruit. Textural property, such as the puncture resistance test was highest for the pink variety at 20.19 N. The color analysis in the optical parameter showed the highest values for the white variety of pomelo. The identification of functional compounds done by Fourier transform infrared spectroscopy provides advancement for the production of different functional products. The assessment of physicochemical and nutritional properties provides knowledge of nutrients, essential minerals (boron, magnesium, aluminum, silicon, phosphorous, potassium, iron, copper, zinc), and quality of fruit, making it an expert functional food ingredient and can be utilized for various applications in food industries. The physicochemical and nutritional properties indicated significant variation ($p<0.05$) among different parts of selected varieties of pomelo fruit. **Research limitations:** There was no limitation. **Originality/Value:** Pomelo is an underutilized fruit with a rich source of bioactive compounds, has a favorable nutritional profile, and has health-improving effects. With its great nutritive value, utilization of this fruit is still very limited because of a lack of information regarding its physicochemical, nutritional, and processing technologies. This research work on different food properties provides a broad area of knowledge regarding designing, processing, storage, transportation, product development and is useful to encourage commercialization.

INTRODUCTION

Pomelo, known as *Citrus maxima,* is one of the largest and most exotic fruits of the citrus family. It is a significant citrus fruit grown in India and accounts for approximately 30% of the total area used for citrus cultivation. Pomelo fruit is blessed with a large number of vitamins, minerals, pectins, dietary fibers, and also polyphenols (flavonoids and phenolics) (Li et al., 2022). The presence of these active constituents in pomelo fruit is considered to exhibit health benefits such as anti-inflammatory, antitumor, anticlotting, antimicrobial, and antioxidant activities. Known for its aroma, flavor, and tangy taste, it is commonly used to extract juice but is also consumed in raw form (Li et al., 2022; Tocmo et al., 2020). Pomelo fruit and its different fruit parts such as peel, segment peel, seeds, juice, and pomace are composed of an array of physicochemical and nutritional components. The waste discarded during production and processing contains a huge number of useful active compounds and presents valuable prospects in the fields of technology and health promotion (Yin et al., 2023; Lin et al., 2021). Despite all health-encouraging and therapeutic benefits, the pomelo fruit is regarded as underutilized due to the lack of processing techniques that further because several post-harvest losses. During agricultural processing to avoid post-harvest damages, information related to engineering properties such as physical, frictional, mechanical and optical properties are valuable for effective industrial processing of pomelo fruit, benefit with designing and manufacturing of various processing equipment, for handling, cleaning, conveying, grading, transportation, and storage (Lawal et al., 2023; Ihrahim & Hamed, 2018; Sirisomboon & Lapchareonsuk, 2012). It is necessary to know about how the external and internal properties of pomelo vary ultimately affecting the transportation and storage.

Information regarding the engineering properties of pomelo fruits is sparse, so there is a need to evaluate the properties that can benefit with designing and manufacturing of various processing equipment (Shravan & Shere, 2018). For the identification of functional groups fourier transform infrared spectroscopy (FTIR) is a rapid method for the estimation of different absorption bands and further provides a vision for the advancement of several functional foods. The physicochemical properties of fruits are important to determine the final quality of the product as well as evaluate the inner and outer structure of the fruit (Sirisomboon & Theamprateep, 2012). Furthermore, the assessment of these characteristics is crucial to design and ensure the quality of the food during its processing. The nutritional value and health benefits of pomelo fruit cause popularity among consumers because of the development of emerging food products from the waste of pomelo fruit (Gupta et al., 2021; Motie et al., 2014). Mineral elements are vital for the growth and development of living organisms, primarily binding to proteins to create metalloproteins, particularly enzymes. Mineral elements typically play crucial roles in maintaining human health, and a lack of these elements can result in unfavorable clinical disorders. However, these conditions can be prevented or reversed with appropriate supplementation. The majority of mineral elements found in the human body are derived from daily food consumption, with fruits being a particularly significant source (Zhang & Rui, 2012). Figure 1 depicts the overview of the engineering, physiochemical, and nutritional properties of pomelo fruit. From the literature, the engineering properties were mainly studied on orange, mandarin, tangerine, kinnow, and murcott varieties of citrus. No specific paper was available on pomelo varieties regarding all the engineering properties, and only the textural properties of pomelo were available. Also, less data availability related to the physicochemical, nutritional, and mineral properties of different varieties of pomelo fruit. Hence, the present study was undertaken to evaluate the engineering, physiochemical, and nutritional properties of different varieties of pomelo fruit

to build essential information for food researchers, processors, and scientists regarding industrial processing and applications in food industries.

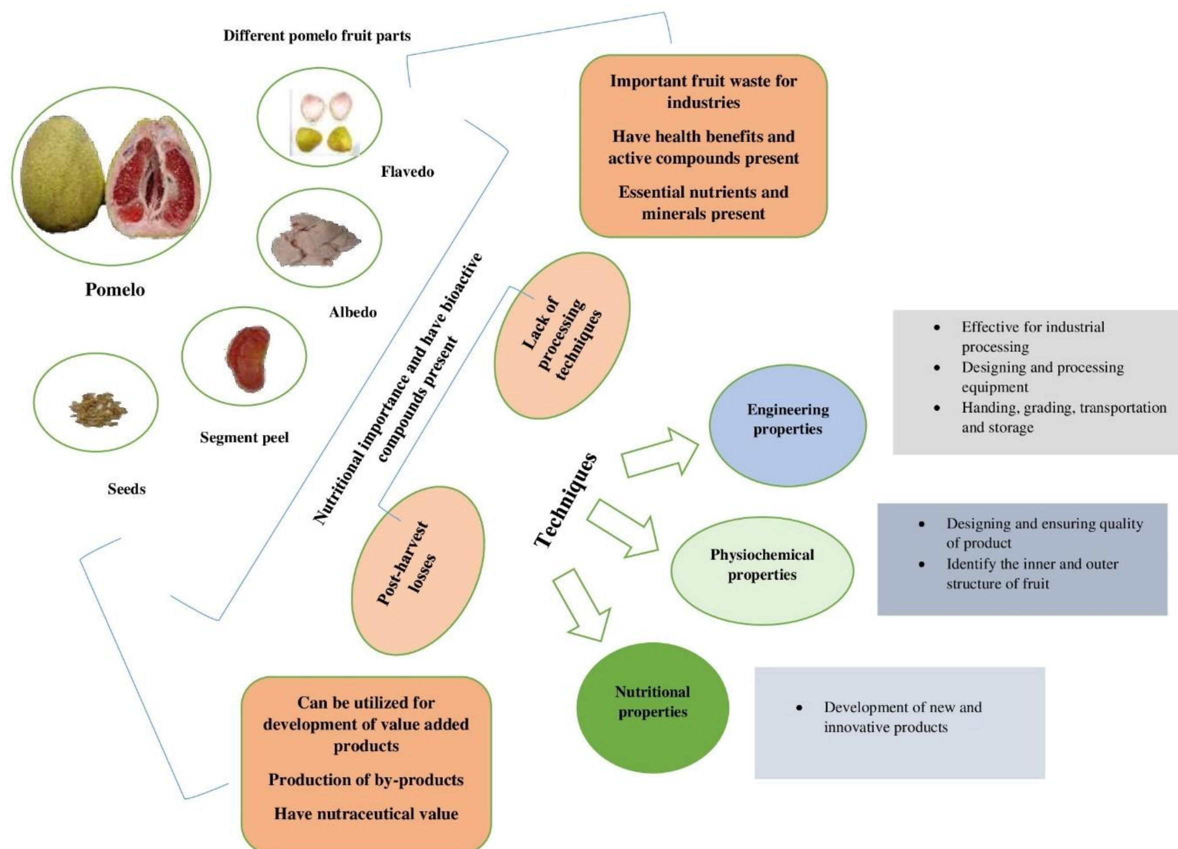


Fig. 1. Overview of engineering, physiochemical and nutritional properties of pomelo fruit.

MATERIALS AND METHODS

Raw material selection

Pomelo fruits were procured from the Department of Fruit Science, Punjab Agricultural University, Ludhiana, India for the year 2024. To provide an accurate representation of the gathered produce, random sampling was used. Only healthy fruits were evaluated for the study; all damaged and diseased fruits were thrown away. The selected fruits were washed, cleaned, and used for different analysis. For FTIR and mineral analysis lyophilized samples of pomelo fruit were used (Pomelo fruits lyophilized at -50°C, as dried samples are required for test). The evaluation of engineering, physiochemical, and nutritional properties were assessed using fresh commodity.

Chemicals

All the chemicals used in the analysis were of high purity AR grade obtained from Sigma Aldrich, USA.

Determination of the physical properties of pomelo fruit

Physical properties play a significant role in the production of processing technology and the quality of final products. The quality aspect of any fruit can be indicated by the physical parameter. The physical properties include geometric and gravimetric properties:

Geometric properties

The length and width of the pomelo fruit were measured using a digital Vernier caliper 0.01 mm. The diameter of the fruit was evaluated at three different points, i.e., the major, middle, and minor positions from the top, middle, and bottom of the fruit. Fruit weight was evaluated using 5 pomelo fruits chosen at random, and their weights were recorded on an electronic scale with a precision of 0.01 g (Shravan & Shere, 2018). The fruit parts' weight including the rind, flavedo, albedo, segment peel, seeds, pulp, and pomace were determined by using an electronic weighing balance with a precision of 0.01 g as described by Shravan & Sere, (2018).

The arithmetic, geometrical mean diameter, and sphericity of pomelo fruit were measured by standard formulas (1 to 3) Yadav et al. (2019); Selvan et al. (2021).

$$\mathrm{AMD} = \frac{\mathrm{L} + \mathrm{W} + \mathrm{H}}{3} \quad (1)$$

Where L= Length, W= Width, H= Height (mm)

$$\mathrm{GMD} = (\mathrm{L} \times \mathrm{W} \times \mathrm{H})^{1/3} \quad (2)$$

Where L= length, W= width, H = height (mm)

$$\mathrm{Sphericity} = \mathrm{D/H} \quad (3)$$

Where D = diameter of fruit (mm), and H = height (mm)

The shape can be predicted by evaluating the nature of the fruit and using its sphericity value. The rind thickness of citrus fruit was measured using a digital Vernier caliper with a precision of 0.01 mm (Mukhim et al., 2015). Number of seeds was measured for three fruits by manually separating and counting, after which the average was determined and expressed as a number (Shravan & Shere, 2018). The length and width of the pomelo fruit seeds were measured using an electronic Vernier caliper with a 0.01 mm accuracy. One seed and the hundred seed weight were measured by using an electronic weighing balance with a precision of 0.01 (Deivasigamani & Swaminathan, 2018).

Gravimetric properties

The surface area and volume of the sample were determined using formulas (4 to 5) Selvan et al. (2021):

$$\mathrm{S} = \pi \times (\mathrm{GMD})^2 \quad (4)$$

$$\text{Volume of sample (V)(ml)} = \text{Final toluene level} - \text{Initial toluene level (ml)} \quad (5)$$

The bulk density was calculated using a cylindrical container and balance. Fruits were placed into a jar to the top of its upper border. Fruits that protruded half of their part above the container's top border were removed. The mass of the fruits packed in the container was

measured using an electronic balance, and bulk density was calculated as explained by Rafiee et al. (2007).

$$\text{Bulk density } (\rho b) = \frac{m_b}{V} \quad (6)$$

Where ρ_b = Bulk density (kg/m^3), m_b = Mass of fruits (kg), V = Volume of cylindrical container (m^3)

True density is defined as the samples mass divided by its actual volume. Using the toluene displacement technique, the true density was determined. A single pomelo fruit was carefully placed into a 1000 ml measuring cylinder that was half-filled with toluene and its mass was measured using an electronic balance with a resolution of 0.0001 g (Selvan et al., 2021). The fruit's displacement of toluene was measured, and the real density was determined as follows:

$$\text{True density } (\rho t) = \frac{m}{V_{td}} \quad (7)$$

Where ρ_t = True density (kg/m^3); m = Mass of individual fruit (kg); V_{td} = Volume of toluene displaced (m^3)

Porosity (ε) was computed according to Singh et al. (2019) using the following relationship from the average values of bulk and actual densities.

$$\text{Porosity } (\varepsilon) = \frac{\rho_t - \rho_b}{\rho_t} \times 100\% \quad (8)$$

The projected area was used to estimate the fruit-projected areas perpendicular to dimensions (PA1, PA2, and PA3) using the equation given by Mahawar et al. (2019).

$$\text{CPA} = \frac{PA_1 + PA_2 + PA_3}{3} \quad (9)$$

Where PA_1 = Projected area perpendicular to the length (mm^2), PA_2 = Projected area perpendicular to the width (mm^2), PA_3 = Projected area perpendicular to the thickness (mm^2)
The L-D and L-M ratio was calculated by standard equations (10 to 11) Mahawar et al. (2019).

$$\text{L} - \text{D ratio} = \text{L/D} \quad (10)$$

Where L = length; D = diameter

$$\text{L} - \text{M ratio} = \text{L/M} \quad (11)$$

Where L = length; M = mass

The flesh/seed ratio was measured by the method outlined by Ercisli et al. (2015). The mass and seeds are separated from the fruits after cutting them. To calculate the flesh-to-seed ratio, the two portions were first individually weighed and divided into their component parts to determine the flesh/seed ratio.

Frictional properties

The frictional properties are related to friction and are important in the design of handling and conveying equipment of produce. The frictional properties involve the angle of repose of seeds and the coefficient of static friction.

Angle of repose of seeds

The method used by Fathollahi et al. (2021) involved filling a hollow cylinder with seeds and gently removing the cylinder upward to allow the seeds to run down the closed container and form a conical shape, which was used to determine the angle of repose. The apex height was measured, and the repose angle was calculated using the trigonometry rule as given:

$$\theta = \tan^{-1}(h/r) \quad (12)$$

Coefficient of static friction

This is the ratio of the force required to begin sliding the sample across a surface and the sample's weight. The method described was used to determine the coefficient of static friction on four distinct structural surfaces, including steel sheet, iron sheet, glass, and plywood (Dhineshkumar & Siddharth, 2015). Each fruit was set on the floor and lifted gradually with a screw until it started to slide. When sliding starts, the inclined surface's angle with the horizontal is measured. The following expression was used to compute the coefficient of static friction (s).

$$\text{Coefficient of static friction } (\mu s) = \tan\theta \quad (13)$$

Where θ = Angle that the incline makes with the horizontal when sliding begins

Textural/mechanical property

The texture analyzer was equipped with a 5 mm cylindrical probe to the probe carrier to assess the puncture resistance test. The stem calyx axis of the pomelo was aligned with the flat plate before it was set on top of it. The test was run with the probe moving at a speed of 5 mm/s. Average values are presented for the three fruits (replications) used to measure the puncture resistance as determined by Singh et al. (2019).

Optical properties

The Hunter lab colorimeter was utilized to access the color parameters of the fruit samples by measuring L*, a* and b* values using the CIE system (Lab Scan XE spectro-colorimeter), which were assessed in terms of CIE L (lightness), a (redness and greenness), and b (brightness) (yellowness and blueness). A white tile and a black tile were used to normalize the sensor's color measurement. Each fruit was placed over the colorimeter's sample measurement port's 8 mm aperture to measure its color. At each place, measurements were taken three times, and then they were averaged Hongwiangjan et al. (2015).

Identification of functional groups

FTIR

The different functional groups present in different parts of selected varieties of pomelo fruit were evaluated using FTIR (PerkinElmer, USA). The absorption spectra of the samples were analyzed in the range of 450–4000 cm^{-1} with 4 cm^{-1} resolutions (Deng et al., 2021).

Physiochemical properties

Different physiochemical properties such as pH, TSS, titratable acidity, ascorbic acid, and reducing, non-reducing, and total sugars of pomelo varieties are studied. pH was measured using method 981.12 (AOAC, 2005). The total soluble solid of the fruit was determined with a refractometer (LABOLAN, SL, Mod 301, Navarra, Spain) using method 932.12 (AOAC, 2005). Titratable acidity was measured according to method 942.15 (AOAC, 2005). Ascorbic acid (AA) was determined based on the quantitative discoloration of 2,6-dichlorophenol

indophenol titrimetric method as described in method 967.21 (AOAC, 2005). The reducing, non-reducing, and total sugars were estimated using Lane and Eynon method (Khan et al., 2021). All observations were taken in triplicates.

Nutritional properties

Each part of selected varieties of pomelo fruit is utilized for the determination of nutritional properties such as moisture content, total ash, crude fat, crude fiber, crude protein, and carbohydrate content. Moisture content was determined according to method 930.04 AOAC (2005). Total ash assessed the quality of food products for the presence of inorganic substances in it by using method 930.05 (AOAC, 2005). Crude fat was determined in accordance with the Soxhlet extract method using petroleum ether as the extracting agent (60-80 °C) according to method 930.09 (AOAC, 2005). Crude fiber was determined according to the method of Ani & Abel (2018). Crude protein content (N × 6.25) was determined following the Kjeldahl method (method 978.04) (AOAC, 2005). The carbohydrate content was determined by a difference method (Khan et al., 2021). The sum of the percentage moisture, ash, protein, fat, and crude fiber was subtracted from 100.

$$\text{Percentage (\%) carbohydrate} = 100 - (\%\text{moisture} + \%\text{ash} + \%\text{protein} + \%\text{fat} + \text{crude fiber}) \quad (14)$$

Mineral elements were identified using Inductive Coupled Plasma mass spectrometry (ICP-MS, 7900, Agilent), and pomelo samples were prepared by microwave digestion with similar conditions and operating requirements as described by (Zhang & Rui, 2012).

Statistical analysis

The means and standard deviations were computed for the different properties of pomelo fruit. All experiments were performed in triplicate, and final values were reported as the mean ± standard deviation (SD). The data were analyzed using one-way analysis of variance (ANOVA) using IBM SPSS Statistics version 22 software (Armonk, NY: IBM Corp.). To assess the significant values ($p \leq 0.05$), the Tukey range HSD test was performed.

RESULTS AND DISCUSSION

Physical properties of pomelo fruit

The physical properties include both geometric and gravimetric properties. Obtained results for the tested geometric properties are presented in Table 1. Geometric properties indicate dimensional characteristics and are vital for designing mechanisms for the storage and transportation of harvested produce. Length is a geometric property used in safeguarding solids by separating non-native particles and defining thermal and mass transfer (Khan et al., 2021; Shravan & Shere, 2018). The variation in fruit length and width varies with varieties of pomelo fruit. The red pomelo attained the highest length and width of 143.63 and 128.19 mm as compared to pink and white pomelo, respectively. The fruit diameter of pomelo fruits was measured from the top, middle, and bottom portions. The red pomelo had the highest diameters from the middle and bottom portions at 116.38 and 122.22 mm, with a low mean value at the top portion of 89.15 mm. The white pomelo had an eminent diameter of 97.93 mm at the top portion, whereas low average values of 115.66 mm and 104.71 mm were attained from the middle and bottom areas, respectively. The pink pomelo had a small diameter of fruit at all three top, middle, and bottom portions. The highest fruit weight was observed in pink pomelos at 1238.17 g followed by red and white pomelos. Primary dimensions such as length, width, and thickness are critical in the design of processing

equipment such as graders, sorters, and cleaners. The weights of different parts, including the rind, flavedo, albedo, segment peel, seeds, pulp, and pomace of pomelo, were measured. The pink pomelo had the highest rind weight, flavedo, albedo, segment peel, seeds, pulp, and pomace at 326.17, 156.87, 235.57, 110.47, 23.20, 674.20, and 174.50 g, respectively. The arithmetic and geometric mean diameters were highest in red pomelo at 129.43 and 128.94 mm, respectively. Sphericity values are used to size equipment and design separators, and the aspect ratio shows how oblong the fruit is. The sphericity was higher in red pomelo at 1.12%, whereas pink and white pomelo had values of 1.02 and 1.08%, respectively. It showed that the red variety of pomelo fruit can be considered a spherically shaped fruit. The shape of the red pomelo was obovate, the pink pomelo was round and the white pomelo was oblate in shape due to the variation in diameter and values of sphericity of all three varieties from the top, middle, and bottom portions, respectively. The rind thickness protects the fruit from external environmental stresses. The rind thickness was highest in the pink pomelo at 14.48 mm. Red pomelo showed the highest average seeds per fruit, i.e. 67.67, whereas pink and white indicated fewer seeds per fruit at 62.67 and 25.33. The seed length was highest in the red pomelo at 27.74 mm and seed breadth was large in the white pomelo at 18.91 mm. One seed weight indicated the highest in red pomelo at 0.41 g, whereas the 100 seed weight was highest in pink pomelo at 44.93 g. The mean values for the geometric properties of the three varieties of pomelo showed significant ($P<0.05$) differences for all the studied properties. The results of the geometric properties of pomelo cultivars are close to the values determined by Mahawar et al. (2019) in Kinnow mandarin and Rehal et al. (2017) in Murcott mandarin.

Gravimetric properties are imperative for the design of packaging material and transportation systems. The data related to gravimetric properties are indicated in Table 2. The surface area of the red pomelo had the highest mean value of 52317.80 mm^2. The volume of fruit was determined with the displacement method. The volume of the white pomelo fruit was the highest at 578.67 ml. These volumes help in the estimation of density and heat transfer rates during fruit drying and cooling. The values of surface area and volume of fruit were higher than the values evaluated by Shahbazi and Rahmati (2013) in grapefruit. The bulk and true densities were higher for pink pomelo fruit at 565.97 and 1318.33 kg/m^3. The results of the bulk and true densities of pomelo fruit were higher than the values depicted by Rehal et al. (2017) in Murcott mandarin. The porosity was higher in white pomelo at 69.73%, and red and pink pomelo had porosities of 54.11 and 57.06%, respectively. The bulk density, true density, and porosity are useful factors for constructing the hopper and managing the flow rate in fruit processing, grading, transporting, and packaging equipment (Ani & Abel, 2018; Shahbazi & Rahmati, 2013). The projected area serves as an accurate model for heat and mass transfer analysis during drying and cooling unit operations and serves as a suitable indicator of mass and specified information regarding projected areas that generate the design of grading units (Rehal et al., 2017). Red pomelo had the highest projected area of 41677.46 mm^2. The results related to porosity and projected area of pomelo fruit were higher in relation to the results of Miraei Ashtiani et al. (2014) in lime and Singh et al. (2019) in sweet orange and sweet lemon. The L-D ratio was higher in the red pomelo at 1.24, whereas the L-M ratio was higher in the white pomelo at 0.19. The flesh/seed ratio was highest at 64.85 in red pomelo. The mean values for all the gravimetric properties of pomelo varieties defined significant ($P<0.05$) differences for the studied properties. The results derived for L-D, L-M, and flesh/seed ratio of pomelo cultivars were higher compared to results evaluated by Mahawar et al. (2019) in Kinnow mandarin and Rehal et al. (2017) in Murcott mandarin.

Table 1. Geometric properties of different varieties of pomelo fruit.

Geometric parameters		Red pomelo	Pink pomelo	White pomelo
Fruit length (mm)		143.63±12.11^{a}	100.75±4.68^{b}	116.45±2.22^{b}
Fruit width (mm)		128.19±1.46^{a}	101.90±9.01^{b}	122.55±7.81^{a}
Fruit diameter (mm)	Top	89.15±2.45^{b}	86.64±8.34^{ab}	97.93±5.34^{a}
	Middle	116.38±2.43^{a}	102.84±7.59^{b}	115.66±8.78^{a}
	Bottom	122.22±3.53^{a}	97.71±7.01^{c}	104.71±12.21^{b}
Fruit weight (g)		932.50±78.76^{ba}	1238.17±165.19^{a}	621.00±71.17^{c}
Fruit parts weight (g)	Rind	325.93±0.84^{b}	362.17±0.59^{a}	216.80±31.61^{c}
	Flavedo	126.50±1.05^{b}	156.87±0.42^{a}	58.18±0.59^{c}
	Albedo	206.30±2.25^{b}	235.57±0.74^{a}	126.17±0.65^{c}
	Segment peel	82.00±0.87^{b}	110.47±0.64^{a}	37.60±0.85^{c}
	Seeds	7.28±0.06^{ba}	23.20±0.20^{a}	5.33±0.04^{cb}
	Pulp	472.13±2.10^{b}	674.20±0.20^{a}	218.10±0.56^{c}
	Pomace	157.70±1.30^{b}	174.50±0.70^{a}	65.81±0.46^{c}
Arithmetic mean diameter (mm)		129.43±0.03^{a}	101.84±0.02^{c}	118.24±0.02^{b}
Geometrical mean diameter (mm)		128.94±0.02^{a}	101.85±0.02^{c}	118.17±0.03^{b}
Sphericity (%)		1.12±0.02^{a}	1.02±0.02^{b}	1.08±0.02^{a}
Shape		Obovate	Round	Oblate
Rind thickness (mm)		9.95±2.43^{cb}	14.48±2.08^{a}	10.06±1.86^{b}
Number of seeds per fruit (Seeds/fruit)		67.67±1.53^{a}	62.67±0.58^{b}	25.33±0.58^{c}
Seed	Length (mm)	27.74±0.67^{a}	16.28±1.24^{c}	23.76±1.12^{b}
	Breadth (mm)	18.73±0.63^{a}	9.66±0.85^{b}	18.91±0.69^{a}
One seed weight (g)		0.41±0.05^{a}	0.38±0.01^{ba}	0.28±0.05^{c}
100 seed weight (g)		41.67±1.45^{b}	44.93±0.31^{a}	19.26±0.02^{c}

Values are expressed as Mean ± SD (n=3). Values having different superscripts in the same row indicated significant difference ($p<0.05$).

Frictional properties of pomelo fruit

The data related to frictional properties are depicted in Table 3. The angle of repose of seeds was higher in the pink pomelo at 73.55, whereas the red and white pomelo had 65.56 and 59.05. For the measurement of the coefficient of static friction, different structural surfaces, such as steel sheets, iron sheets, glass, and plywood were utilized. The steel sheet, glass, and plywood had the highest coefficient of static friction of 0.30, 0.42, and 0.41 under red pomelo. The iron sheet had the highest static friction of 0.35 under the white pomelo. The mean values for all the frictional properties of pomelo varieties defined significant ($P<0.05$) differences for the studied properties. The coefficient of friction and angle of repose parameters aid in determining shearing forces between fruits and surfaces for conveyor design. They can also be used to determine the natural rest position of fruit during storage

(Singh et al., 2019; Miraei Ashtiani et al., 2014). The results of the angle of repose of seeds and the coefficient of static friction parameters of frictional properties are close to the values depicted by Miraei Ashtiani et al. (2014) in lime and Singh et al. (2019) in sweet orange and sweet lemon.

Textural/Mechanical property of pomelo fruit

The textural properties indicated an important role in the quality of the produce. These properties are beneficial to design a machine that can puncture the fruit with ease. For textural properties, a puncture resistance test was examined. The data related to textural properties are indicated in Table 4. The puncture resistance test was higher in pink pomelo at 20.19 N, whereas red and white pomelo had 11.81 N and 15.27 N puncture resistance. The mean values of the textural properties of pomelo varieties defined a significant (P<0.05) difference for the puncture resistance test. The results determined that the pink pomelo variety had more strength and therefore, the machine required more energy to break the fruit. But, as literature revealed the energy required to break the fruit should be minimal to cut costs and enhance machine efficiency. The result of puncture resistance was close to the values reported by Sirisomboon and Theamprateep (2012) in pomelo fruit.

Table 2. Gravimetric properties of different varieties of pomelo fruit.

Gravimetric parameters	Red pomelo	Pink pomelo	White pomelo
Surface area (mm^2)	52317.80±16.90^a	32643.00±9.79^c	43945.15±19.69^b
Volume of fruit (ml)	512.00±102.14^a	299.67±14.50^b	578.67±70.47^a
Bulk density (kg/m^3)	487.14±0.99^b	565.97±1.87^a	284.28±2.02^c
True density (kg/m^3)	1061.67±3.51^b	1318.33±3.51^a	941.67±60.93^c
Porosity (%)	54.11±0.21^b	57.06±0.05^b	69.73±1.70^a
Projected area (mm^2)	41677.46±3044.87^a	24140.98±2179.89^c	32966.62±1994.83^b
L-D ratio	1.24±0.13^a	0.98±0.07^b	1.01±0.10^b
L-M ratio	0.16±0.02^a	0.08±0.01^b	0.19±0.03^a
Flesh/seed ratio	64.85±0.25^a	29.06±0.25^c	40.92±0.43^b

Where; L-D ratio = Length-diameter ratio, L-M ratio = Length-mass ratio. Values are expressed as Mean ± SD (n=3). Values having different superscripts in the same row indicated significant difference (p<0.05).

Table 3. Frictional properties of different varieties of pomelo fruit.

Frictional parameters		Red pomelo	Pink pomelo	White pomelo
Angle of repose (°) of seeds		65.56±0.58^b	73.55±1.15^a	59.05±2.97^c
	Steel sheet	0.30±0.05^a	0.18±0.05^b	0.29±0.06^a
	Iron sheet	0.34±0.11^a	0.22±0.03^b	0.35±0.07^a
Coefficient of static friction (°)	Glass	0.42±0.12^a	0.24±0.03^b	0.38±0.12^a

Values are expressed as Mean ± SD (n=3). Values having different superscripts in the same row indicated significant difference (p<0.05).

Table 4. Textural/Mechanical property of different varieties of pomelo fruit.

Textural/Mechanical parameter	Red pomelo	Pink pomelo	White pomelo
Puncture resistance test (N)	11.81±3.56^{b}	20.19±0.68^{a}	15.27±1.31^{ab}

Values are expressed as Mean ± SD (n=3). Values having different superscripts in the same row indicated significant difference (p<0.05).

Table 5. Optical properties of different varieties of pomelo fruit.

Optical parameters		Red pomelo	Pink pomelo	White pomelo
Color	L	65.82±1.20^{b}	69.02±0.63^{a}	71.24±0.60^{a}
	a	6.86±0.79^{a}	3.21±0.30^{b}	6.17±0.41^{a}
	b	32.52±1.36^{b}	31.06±0.41^{b}	36.33±0.29^{a}
	L*	71.77±1.07^{b}	74.59±0.55^{a}	76.53±0.52^{a}
	a*	7.49±0.83^{a}	3.48±0.32^{c}	6.58±0.45^{b}
	b*	54.92±3.32^{b}	48.80±1.09^{c}	61.06±1.39^{a}
	dE*	90.71±2.56^{b}	89.21±0.58^{b}	98.13±0.48^{a}

L* = Brightness, a* = Greenness, b* = Yellowness. Values are expressed as Mean ± SD (n=3). Values having different superscripts in the same row indicated significant difference (p<0.05).

Optical properties of pomelo fruit

The optical properties indicated that the L* (brightness), a* (greenness), and b* (yellowness) parameters were positively associated with the energy absorption and the deformation ratio of the whole fruit Olabinjo et al. (2017). The data related to the color parameter of optical properties are depicted in Table 5. The values for all the parameters of optical properties defined significant (P<0.05) differences. The L, L*, b, b* and dE* values were highest for the white pomelo at 71.24, 76.53, 36.33, 61.06 and 98.13, respectively. The and a* mean values were higher for red pomelo at 6.86 and 7.49. The color values of the pomelo cultivars are close to the values reported by Hongwiangjan et al. (2015) and Terdwongworakul et al. (2009) in the pomelo fruit. Color values are extremely useful in determining the maturity and browning of pomelo fruit. It is also a useful indicator of product quality.

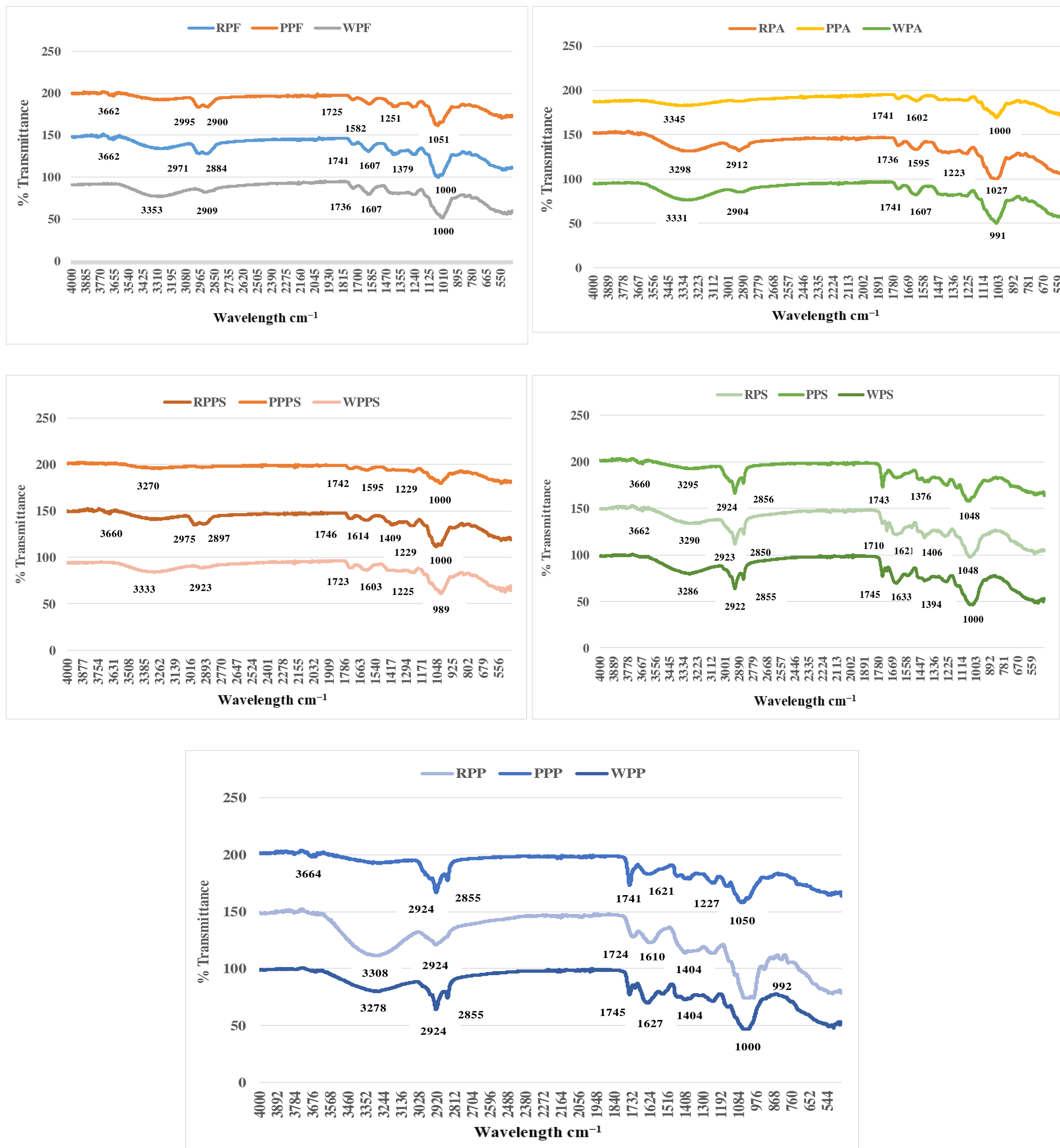


Fig. 2. FTIR spectrum of different parts of selected varieties of pomelo fruit. RPF = Red pomelo flavedo, PPF = Pink pomelo flavedo, WPF = White pomelo flavedo; RPA = Red pomelo albedo, PPA = Pink pomelo Albedo, WPA = White Pomelo Albedo; RPPS = Red pomelo peel segment, PPPS = Pink pomelo peel segment, WPPS = White pomelo peel segment; RPS = Red pomelo seeds, PPS = Pink pomelo seeds, WPS = White pomelo seeds; RPP = Red pomelo pomace, PPP = Pink pomelo pomace, WPP = White pomelo pomace.

FT-IR spectroscopy

The red, pink and white pomelo varieties were utilized to identify the presence of functional groups of polyphenolic compounds based on the intensity of peak in the region of infrared radiations. FTIR spectrum for different varieties of pomelo fruit for different polyphenols is depicted in Figure 2. The graphs showed the existence of polyphenolic compounds that have

been given further approval by several polyphenolic standards. In the flavedo portion, FTIR spectra of the pink variety of pomelo showed a peak at 3662 cm^{-1} which indicated the presence of O-H stretching of the alcohol group. The peak at 2995 cm^{-1} indicated the presence of C-H stretching of the alkane group. C-H stretching was observed at 2900 cm^{-1} attributed to the alkane group for the red variety of pomelo. The peak at 1725 and 1582 cm^{-1} represented the presence of C=O stretching of aliphatic ketone and N-H bending of the amine group. The peaks at 1251 and 1051 cm^{-1} depicted the presence of strong C-O stretching of alkyl aryl ether and C-O stretching of primary alcohol (Gupta et al., 2021). The flavedo portion of red and white pomelo also indicated quite similar peaks. The red variety showed a peak at 3662 cm^{-1} which represents the O-H stretching of the alcohol group. The peak at 2971 and 2884 cm^{-1} indicated the presence of C-H stretching of alkane. The peaks at 1741, 1607, 1379, and 1000 cm^{-1} indicated the presence of C=O stretching of esters, C=C stretching of alkene, O-H bending of phenols, and C-F stretching of fluoro compounds. For white pomelo 3353 cm^{-1} showed a peak of O-H stretching of alcohol. The peaks at 2909, 1736, 1607, and 1000 cm^{-1} indicated the presence of C-H stretching of alkane, C=O stretching of esters, C=C stretching of conjugated alkene, and C-F stretching of fluoro compounds. In the albedo portion, the FTIR spectra of pink variety showed a peak at 3345 cm^{-1} indicating the presence of O-H stretching of alcohol. The peaks at 1741, 1602, and 1000 cm^{-1} showed the presence of C=O stretching of esters, C=C stretching of conjugated alkene, and C-F stretching of fluoro compounds. The red and white pomelo of albedo showed similar peaks (Deng et al., 2024).

In the peel segment portion, the FTIR spectra of pink pomelo represented peaks at 3270, 1742, 1595, 1229, and 1000 cm^{-1} indicating the presence of O-H stretching of alcohol, C=O stretching of esters, N-H bending of amines, C-O stretching of alkyl aryl ether and C-F stretching of fluoro compounds. The red pomelo showed peaks at 3660, 2975-2897, 1746, 1614, 1409, 1229 and 1000 cm^{-1} depicting the presence of O-H stretching of alcohol, C-H stretching of alkane, C=O stretching of esters, C=C stretching of α,β-unsaturated ketone, O-H bending of carboxylic acid, C-O stretching of alkyl aryl ether and C-F stretching of fluoro compounds (Gupta et al., 2021). The white pomelo of the peel segment showed peaks at 3333, 2923, 1723, 1603, 1225 and 989 cm^{-1} indicating the presence of N-H stretching of aliphatic primary amine, C-H stretching of alkane, C=O stretching of aliphatic ketone, C=C stretching of α,β-unsaturated ketone, C-O stretching of vinyl ether and C=C bending of alkene 36) (Ouyang et al., 2023). In the seeds portion, the FTIR spectra of pink pomelo showed peaks at 3660 and 3295 cm^{-1} indicating the presence of O-H stretching of alcohol groups. The peaks at 2924 and 2856 cm^{-1} indicated the presence of C-H stretching of alkane. The stretching of peaks at 1743, 1376, and 1048 cm^{-1} defined the presence of C=O stretching of esters, O-H bending of phenols and CO-O-CO stretching of anhydride. The red pomelo seeds showed peaks at 3662-3290 and 2923-2850 cm^{-1} indicating the presence of O-H stretching of the alcohol group and C-H stretching of alkane. The presence of peaks at 1710, 1621, 1406, and 1048 cm^{-1} indicated the presence of C=O stretching of carboxylic acid, C=C stretching conjugated alkene, S=O stretching of sulfonyl chloride and CO-O-CO stretching of anhydride (Deng et al., 2024). The white pomelo seeds showed peaks at 3286 and 2922-2855 cm^{-1} indicating the presence of O-H stretching of alcohol group and C-H stretching of alkane. The peaks at 1745, 1633, 1394, and 1000 cm^{-1} showed the presence of C=O stretching of esters, C=C stretching conjugated alkene, O-H bending of phenols and C-F stretching of fluoro compounds. In the pomace portion, the FTIR spectra of pink pomelo showed the peaks at 3664 and 2924-2855 cm^{-1} indicating the presence of O-H stretching of alcohol group and C-H stretching of alkane. The peaks at 1741, 1621, 1227, and 1050 cm^{-1} indicated the presence of C=O stretching of esters, C=C stretching conjugated alkene, C-O stretching of alkyl aryl ether, and CO-O-CO stretching of anhydride (Zheng et al., 2022). The presence of peaks at

red and white pomelo in seeds showed not much variation in peaks. Thus, concluded that pomelo is a wealthy resource of phytoconstituents and the different portions of varieties of pomelo showed peaks on specific wavelengths due to the presence of functional groups of polyphenolic compounds.

Physiochemical properties

Results for the physiochemical properties of different varieties of pomelo fruit are presented in Table 6. Physiochemical properties including pH, TSS, titratable acidity, ascorbic acid, reducing, non-reducing, and total sugars were determined in the juice portion of red, pink, and white pomelo. The pH level serves as an indicator of the acidity of the final product. White pomelo indicated highest pH (3.82) as compared to red (3.69) and pink (3.59) pomelo. Total soluble solids (TSS) refer to the amount of sugars that can be extracted from the product. TSS value showed maximum value in pink pomelo (10.10 °B) as compared to red (9.90 °B) and white pomelo (9.17 °B). The titratable acidity and ascorbic acid of white pomelo were more as compared to red and pink pomelo. The reducing, non-reducing, and total sugars were indicated more in pink pomelo than in red and white pomelo. Pink pomelo was found to have more sugar content or is sweet in flavor among other varieties of pomelo. Results showed that there was a significant difference ($P<0.05$) in the physiochemical parameters of pomelo fruit. Similar, findings were approved by Balmori et al. (2023) and Yin et al. (2023) in pomelo fruit.

Table 6. Physiochemical properties of different varieties of pomelo fruit.

Parameters	Fruit part	Physiochemical properties		
		Red pomelo	Pink pomelo	White pomelo
pH	Juice	3.69±0.01^{b}	3.59±0.01^{c}	3.82±0.02^{a}
TSS (°brix)		9.90±0.10^{b}	10.10±0.10^{a}	9.17±0.29^{c}
Titratable acidity (%)		0.69±0.04^{b}	0.56±0.02^{b}	0.85±0.10^{a}
Ascorbic acid (mg/100g)		45.31±1.97^{b}	34.93±0.58^{c}	56.74±0.02^{a}
Reducing sugars (%)		6.37±0.01^{b}	6.67±0.02^{a}	5.82±0.02^{c}
Non-reducing sugars (%)		4.13±0.01^{b}	4.24±0.02^{a}	3.14±0.02^{c}
Total sugars (%)		10.50±0.02^{b}	10.91±0.03^{a}	8.96±0.03^{c}

Values are expressed as Mean ± SD (n=3) . Values having different superscripts in the same row indicated significant difference ($p<0.05$).

Nutritional properties

Nutritional properties define the quality and health value of the commodity. Results related to nutritional properties are presented in Table 7. Moisture content, total ash, crude fat, crude protein, and carbohydrate content were studied under the nutritional properties of pomelo fruit. The juice portion of pomelo showed maximum moisture content followed by flavedo, pomace, albedo, segment peel, and seeds. The moisture content in the flavedo portion varied from 85.34, 85.31 and 83.87% in red, white, and pink pomelo respectively. The albedo portion showed moisture content of 77.65, 76.67 and 75.13% in red, white, and pink pomelo respectively. The segment peel and seeds showed more moisture content in white pomelo as 78.35 and 8.35% as compared to red and pink pomelo. The juice and pomace portions of red pomelo illustrated maximum moisture content (87.32 and 82.78%) respectively. Significant variation ($P<0.05$) was observed in moisture content among pomelo varieties. Total ash was found more in flavedo followed by seeds, pomace, segment peel, albedo, and juice. Flavedo (3.26%) and albedo (1.86 %) showed more total ash in red pomelo as compared to pink and white pomelo. Other portions of pomelo including segment peel (2.04%), seeds (3.12%), and pomace (2.24%) showed more total ash in the red variety in comparison to pink and white. Crude fat was found to be more in seeds followed by flavedo, pomace, segment peel, albedo, and juice. Seeds have more crude fat in red (38.34%) followed by pink (38.28%) and white (38.22%) pomelo. Red pomelo showed more crude fat content in flavedo (11.31%), albedo (6.34%), and segment peel (8.33%) in comparison to pink and white pomelo. Similarly, juice (0.03%) and pomace (10.32%) showed more crude fat in red pomelo. A significant difference ($P<0.05$) was observed in the total ash and crude fat of pomelo varieties. The pomelo fruit's high fiber content makes it a promising candidate for enhancing the texture, flavor, and nutritional value of bakery and other value-added products. Additionally, it offers significant health benefits by preventing gastrointestinal issues like constipation and is considered a natural anti-colon cancer agent. Red pomelo seeds (32.11%) showed the highest crude fiber in comparison to pink (32.09%) and white (32.05%) pomelo. Among flavedo and albedo, the outer portion (flavedo) had more crude fiber. Crude fiber of red pomelo (25.23%) was found to be more when compared to pink (25.14%) and white (25.19%) pomelo. Segment peel and pomace depicted no significant difference ($P<0.05$) among selected varieties of pomelo. In the juice portion, red pomelo (5.66 %) had more crude fiber compared to pink (5.62%) and white (5.56%) pomelo.

Crude protein is beneficial in food for enhancing the health potential and quality. Seeds showed more crude protein followed by flavedo, albedo, pomace, segment peel, and juice. Among red, pink, and white pomelo the red pomelo showed more crude protein in flavedo (11.24%), albedo (10.73%), and segment peels (10.61%), seeds (12.48%), juice (6.25%) and pomace (10.65%), respectively. The significant carbohydrate content found in pomelo fruit can serve as a cost-effective alternative to other expensive sources of carbohydrates, making it particularly beneficial for low-income people, especially in developing nations. Carbohydrate content was higher in seeds followed by juice, albedo, segment peel, pomace, and flavedo of pomelo. Pink pomelo was found to have more carbohydrate content in flavedo (66.89%), albedo (65.68%), segment peel (67.92%), seeds (179.05%), and pomace (66.93%) in comparison to red and white pomelo, respectively. The white pomelo showed more carbohydrate content in juice (26.67%) in comparison to red and pink pomelo. Overall, the results proclaimed that there was a significant difference ($P<0.05$) in the nutritional properties of pomelo. Similar, data was investigated by Yin et al. (2023) in pomelo and Ayona & Athira (2017) in citrus fruit.

Table 7. Nutritional properties of different varieties of pomelo fruit.

Parameters	Fruit parts	Nutritional properties		
		Red pomelo	Pink pomelo	White pomelo
Moisture (%)	Flavedo	85.34±0.02^{a}	83.87±0.56^{b}	85.31±0.03^{a}
	Albedo	77.65±0.03^{a}	75.13±0.76^{b}	76.67±0.39^{a}
	Segment peel	77.04±0.31^{b}	76.09±0.65^{b}	78.35±0.03^{a}
	Seeds	7.43±0.02^{b}	6.87±0.40^{c}	8.35±0.03^{a}
	Juice	87.32±0.09^{a}	86.97±0.45^{ab}	85.81±0.53^{b}
	Pomace	82.78±0.59^{a}	79.52±0.11^{b}	81.94±0.52^{a}
Total ash (%)	Flavedo	3.26±0.01^{a}	3.20±0.01^{b}	3.18±0.01^{b}
	Albedo	1.86±0.02^{a}	1.82±0.01^{a}	1.76±0.02^{b}
	Segment peel	2.04±0.01^{a}	2.01±0.01^{b}	1.97±0.01^{c}
	Seeds	3.12±0.01^{a}	3.12±0.01^{a}	3.09±0.02^{b}
	Juice	0.78±0.01^{a}	0.76±0.01^{b}	0.72±0.01^{c}
	Pomace	2.24±0.01^{a}	2.18±0.02^{b}	2.21±0.01^{ab}
Crude fat (%)	Flavedo	11.31±0.02^{a}	11.22±0.01^{b}	11.24±0.02^{b}
	Albedo	6.34±0.02^{a}	6.25±0.03^{b}	6.29±0.02^{ab}
	Segment peel	8.33±0.01^{a}	8.27±0.02^{b}	8.32±0.02^{a}
	Seeds	38.34±0.01^{a}	38.28±0.04^{ab}	38.22±0.04^{b}
	Juice	0.03±0.01^{a}	0.02±0.01^{b}	0.02±0.00^{b}
	Pomace	10.32±0.01^{a}	10.26±0.02^{b}	10.30±0.01^{a}
Crude fiber (%)	Flavedo	25.23±0.01^{a}	25.14±0.02^{c}	25.19±0.02^{b}
	Albedo	22.13±0.01^{a}	22.06±0.01^{b}	22.08±0.02^{b}
	Segment peel	23.23±0.01^{a}	23.17±0.01^{b}	23.21±0.01^{a}
	Seeds	32.11±0.01^{a}	32.09±0.01^{b}	32.05±0.02^{c}
	Juice	5.66±0.02^{a}	5.62±0.01^{b}	5.56±0.03^{c}
	Pomace	23.54±0.02^{a}	23.46±0.03^{b}	23.53±0.02^{a}
Crude protein (%)	Flavedo	11.24±0.01^{a}	11.19±0.02^{b}	11.16±0.01^{b}
	Albedo	10.73±0.01^{a}	10.69±0.03^{ab}	10.65±0.03^{b}
	Segment peel	10.61±0.03^{a}	10.56±0.04^{b}	10.54±0.03^{b}
	Seeds	12.48±0.01^{a}	12.42±0.03^{ab}	12.37±0.05^{b}
	Juice	6.25±0.01^{a}	6.22±0.03^{ab}	6.19±0.03^{b}
	Pomace	10.65±0.01^{a}	10.54±0.03^{b}	10.48±0.03^{c}
Carbohydrate content (%)	Flavedo	65.70±0.01^{b}	66.89±0.52^{a}	65.46±0.03^{b}
	Albedo	63.57±0.30^{c}	65.68±0.81^{a}	64.12±0.46^{b}
	Segment peel	67.17±0.29^{b}	67.92±0.61^{a}	65.70±0.08^{c}
	Seeds	178.61±0.07^{ab}	179.05±0.34^{a}	177.38±0.15^{b}
	Juice	25.40±0.07^{b}	25.64±0.10^{b}	26.67±0.48^{a}
	Pomace	63.97±0.62^{c}	66.93±0.17^{a}	64.57±0.57^{b}

Values are expressed as Mean ± SD (n=3). Values having different superscripts in the same row indicated significant difference ($p<0.05$).

Mineral analysis

Mineral analysis of different parts of selected varieties of pomelo fruit are presented in Table 8. Ten different mineral elements including boron, magnesium, aluminum, silicon, phosphorous, potassium, iron, copper, zinc, and strontium are identified. A significant difference ($P<0.05$) was observed in mineral elements of selected varieties of pomelo fruit. The results indicated that pomelo fruit contains significant quantities of important minerals, often surpassing the recommended dietary allowance (RDA). This suggests that consuming pomelo fruit may help to maintain the proper balance and ratios of these elements in the bodies of those in need. Boron element was observed more in pink pomelo as compared to red and white pomelo. Pink pomelo showed a boron content maximum in flavedo (1.176 ppb) followed by albedo (1.309 ppb), segment peel (1.399 ppb), seeds (1.350 ppb), and pomace (1.004 ppb), respectively. The daily recommended dose of allowance (RDF) of boron is 1-1.5

mg and results revealed a range of little more than that. Boron plays an important role in preventing calcium loss and bone demineralization by influencing the development and activity of steroid hormones (Czech et al., 2020). Magnesium element showed variation among varieties of pomelo. Red pomelo had more magnesium in albedo (69.34 ppb) and seeds (169.371). Pink pomelo had more magnesium in flavedo (130.172 ppb), juice (130.511 ppb), and pomace (87.607 ppb). However, white pomelo showed more magnesium content in the segment peel (80.820 ppb). Magnesium is an essential mineral for the activation of over 300 different enzymes in the body. It is beneficial for the absorption of certain vitamins and minerals and essential for the proper functioning and composition of the arteries, heart, kidney, bone, and neuromuscular system (Roghini & Vijayalakshmi, 2018). Aluminum is an important mineral used to combat diseases such as liver, heart, and brain. Results revealed that white pomelo was found to have more aluminum content in flavedo (10.677 ppb), albedo (7.036 ppb), and segment peel (5.435 ppb). Red pomelo in seeds (8.583 ppb) and pink pomelo in juice (6.318 ppb) and pomace had more aluminum (8.970 ppb) content. Silicon showed considerable differences among pomelo varieties. Red pomelo showed more silicon in flavedo (19.913 ppb), segment peel (50.582 ppb), and pomace (61.624 ppb). Pink showed more silicon in albedo (44.640 ppb) and juice (61.369 ppb), whereas white pomelo had more silicon in seeds (54.466 ppb). Silicon is an important mineral that has health advantages that are crucial in preventing specific ailments like atherosclerosis, tuberculosis, sleeping difficulties, and skin disorders (Silva et al., 2017). Phosphorus is essential for the body to synthesize protein, which is crucial for the growth, maintenance, and repair of cells and tissues. Red pomelo was found to have more phosphorus in seeds (430.881 ppb). Pink pomelo had more phosphorus in flavedo (180.629 ppb), juice (431.057 ppb), and pomace (282.364 ppb). White pomelo had more phosphorus in albedo (109.756 ppb) and segment peel (127.647 ppb). Potassium is a vital mineral that is necessary for the proper functioning of the entire body. It facilitates the optimal functioning of neurons, muscles, and the heart, as well as the transportation of nutrients in the body (Hong et al., 2019). The daily recommended dietary allowance (RDA) of potassium is 4300 mg. Results revealed that red pomelo had more potassium in albedo (671.928 ppb). Pink pomelo had more potassium in seeds (762.865 ppb), juice (2,204.666 ppb), and pomace (1,137.967 ppb), whereas white pomelo had more potassium in flavedo (882.49 ppb) and segment peel (574.637 ppb).

Similarly, minerals including Iron (Fe), copper (Cu), and Zinc (Zn) are also been reported as essential minerals for combating deficiency diseases. Iron is an essential component of hemoglobin, which facilitates the transportation of oxygen to the tissues of the body. Additionally, it is a constituent of various other proteins and enzymes. Copper acts as an antioxidant and maintains healthy nerve cells, the immunological system, and red blood cell production (Neshovska, 2023). Zinc being an important mineral helps to maintain the immune system, growth of cells, and heals damaged tissues. However, the strontium is a heavy element and results showed considerable differences in different parts of varieties of pomelo fruit (Hong et al., 2019). Generally, the results revealed that minerals present in pomelo fruit showed a range more than RDA (Recommended dietary allowance) and had considerable differences among varieties. It is the best fruit for someone deficient in these specific minerals. Hence, pomelo fruit is a good source of essential minerals and helps individuals to promote growth and encourage health benefits.

Table 8. Mineral analysis of different varieties of pomelo fruit.

Variety	Fruit parts	Mineral analysis									
		11B [ppb]	24Mg [ppb]	27Al [ppb]	28Si [ppb]	31P [ppb]	39K [ppb]	56Fe [ppb]	63Cu [ppb]	64Zn [ppb]	88Sr [ppb]
Red pomelo	Flavedo	1.273	97.326	5.343	19.913	104.372	637.204	23.716	0.651	2.067	3.766
	Albedo	1.284	69.34	3.102	4.041	89.028	671.928	9.028	0.557	2.734	3.815
	Segment peel	1.188	72.312	5.049	50.582	103.931	480.794	17.795	0.311	2.271	5.775
	Seeds	0.234	169.371	8.583	BDL	430.881	714.007	7.458	10.319	46.982	11.472
	Juice	BDL	60.829	3.745	55.456	193.345	781.106	11.518	0.574	2.335	0.663
	Pomace	0.513	63.516	1.451	41.624	202.314	804.828	17.600	0.703	2.694	2.096
Pink pomelo	Flavedo	1.176	130.172	3.409	2.361	180.629	796.111	7.227	0.604	2.294	3.753
	Albedo	1.309	60.390	1.837	44.640	73.891	635.343	15.317	0.652	1.872	3.666
	Segment peel	1.399	61.448	2.291	14.554	115.388	548.792	4.605	0.316	1.332	4.205
	Seeds	1.350	146.910	8.387	30.977	401.427	762.865	13.965	0.952	2.453	2.667
	Juice	BDL	130.511	6.318	61.369	431.057	2,204.666	12.738	1.475	2.512	1.334
	Pomace	1.004	87.607	8.970	9.892	282.364	1,137.967	7.826	0.494	0.979	2.234
White pomelo	Flavedo	1.460	118.968	10.677	17.449	169.31	882.49	10.809	0.381	2.462	5.437
	Albedo	0.641	68.386	7.036	4.337	109.756	586.961	8.439	0.296	1.703	4.340
	Segment peel	0.862	80.820	5.435	17.739	127.647	574.637	9.653	5.606	54.142	8.945
	Seeds	0.274	140.699	2.005	54.466	318.069	485.280	18.278	1.073	3.606	2.139
	Juice	BDL	104.030	5.550	25.427	315.807	1,355.77	5.132	0.773	1.742	0.909
	Pomace	0.262	63.503	7.504	0.043	206.908	889.122	11.188	0.473	1.767	1.650

BDL – Below Detectable Limit (Less than 1 ppb).

CONCLUSION

Pomelo fruit has very high nutraceutical and functional potential values. The difficulty in processing and lack of knowledge of its usefulness, this exotic fruit has been kept unexplored. The limited availability of this fruit is the primary factor contributing to its lack of appeal. To uncover the different benefits and to explore the pomelo fruit's different food properties including the engineering, physiochemical, and nutritional properties of selected varieties of pomelo fruit. The different food properties of pomelo fruit are studied to generate information that could be applied for the designing of various machinery and to determine the final quality of the product. The results described the significant difference ($p<0.05$) in engineering, physiochemical, and nutritional properties among the different parts of varieties of pomelo fruit. The results revealed that pomelo fruit is a good source of essential minerals including boron, magnesium, aluminum, silicon, phosphorous, potassium, iron, copper, and zinc. The variation in results was attained due to varietal differences, environmental conditions' effect on variety, soil conditions, and genotypic relation that bring changes in the varieties of pomelo providing differences in values. Overall, pomelo possesses vital chemical and nutritional properties that are considered for different applications in the food industry. These food properties are important sources of information for food processing industries to enhance the post-harvest processing of pomelo fruit and have become a perfect and long-lasting resource for the agriculture and food industry.

Contribution

Simple Sharma: Conceptualization, methodology, investigation, resources, and writing (original draft, review, and editing). Barinderjit Singh: Writing (original draft, review, and editing). Yashi Srivastava: Writing (review and editing). All authors approved the final version of the manuscript.

Conflict of interest

The authors declare no conflict of interest, financial or otherwise.

Acknowledgments
All individuals listed as authors have contributed substantially to the design, performance, and analysis of literature for this review, and the authors are thankful to Punjab Technical University for providing infrastructure for making this research contribution possible.

REFERENCES

Ani, P. N., & Abel, H. C. (2018). Nutrient, phytochemical, and antinutrient composition of *Citrus maxima* fruit juice and peel extract. *Food Science & Nutrition*, *6*(3), 653-658. https://doi.org/10.1002/fsn3.604

AOAC. (2005) Official methods of analysis (17th ed.). Arlington: Association of Official Analytical Chemists (2005).

Ayona, J., & Athira, U. (2017). Comparative analysis of nutritional and anti-nutritional components of selected citrus fruit species. *International Journal for Research in Applied Science and Engineering Technology*, *5*(10), 309-312. https://doi.org/10.22214/ijraset.2017.10047

Balmori, V., Marnpae, M., Chusak, C., Kamonsuwan, K., Katelakha, K., Charoensiddhi, S., & Adisakwattana, S. (2023). Enhancing phytochemical compounds, functional properties, and volatile flavor profiles of pomelo (*Citrus grandis* (L.) Osbeck) juices from different cultivars through fermentation with *Lacticaseibacillus paracasei*. *Foods*, *12*(23), 4278. https://doi.org/10.3390/foods12234278

Czech, A., Zarycka, E., Yanovych, D., Zasadna, Z., Grzegorczyk, I., & Kłys, S. (2020). Mineral content of the pulp and peel of various citrus fruit cultivars. *Biological Trace Element Research*, *193*, 555-563. https://doi.org/10.1007/s12011-019-01727-1

Deivasigamani, S., & Swaminathan, C. (2018). Evaluation of seed test weight on major field crops. *International Journal of Research Studies in Agricultural Sciences*, *4*(1), 8-11. http://dx.doi.org/10.20431/2454-6224.0401001

Deng, M., Lin, Y., Dong, L., Jia, X., Shen, Y., Liu, L., Chi, J., Huang, F., Zhang, M. & Zhang, R. (2021). Physicochemical and functional properties of dietary fiber from pummelo (*Citrus grandis* L. Osbeck) and grapefruit (*Citrus paradisi* Mcfad) cultivars. *Food Bioscience*, *40*, 100890. https://doi.org/10.1016/j.fbio.2021.100890

Deng, M., Ye, J., Zhang, S., Zhang, R., Lu, Q., Dong, L., Huang, F., Jia, X. & Zhang, M. (2024). Composition, structural, physicochemical and functional properties of dietary fiber from different tissue parts of Shatianyu (*Citrus grandis* L. Osbeck). *LWT*, *191*, 115581. https://doi.org/10.1016/j.lwt.2023.115581

Dhineshkumar, V., & Siddharth, M. (2015). Studies on physical properties of orange fruit. *Journal of Food Research and Technology*, *3*(4), 125-130.

Ercisli, S., Yanar, M., Sengul, M., Yildiz, H., Topdas, E. F., Taskin, T., Zengin, Y., & Yilmaz, K. U. (2015). Physico-chemical and biological activity of hawthorn (*Crataegus spp.* L.) fruits in Turkey. *Acta Scientiarum Polonorum. Hortorum Cultus*, *14*(1), 83-93.

Fathollahi, I., Farmani, J., Kasaai, M. R., & Hamishehkar, H. (2021). Some physical properties of Persian lime (*Citrus latifolia*) seeds and physicochemical properties of the seed oil as affected by solvent extraction and cold pressing methods. *Journal of Food Measurement and Characterization*, *15*, 1169-1178. https://doi.org/10.1007/s11694-020-00712-w

Gupta, A. K., Dhua, S., Sahu, P. P., Abate, G., Mishra, P., & Mastinu, A. (2021). Variation in phytochemical, antioxidant and volatile composition of pomelo fruit (*Citrus grandis* (L.) osbeck) during seasonal growth and development. *Plants*, *10*(9), 1941. https://doi.org/10.3390/plants10091941

Hong, Y. S., Choi, J. Y., Nho, E. Y., Hwang, I. M., Khan, N., Jamila, N., & Kim, K. S. (2019). Determination of macro, micro and trace elements in citrus fruits by inductively coupled plasma–optical emission spectrometry (ICP-OES), ICP–mass spectrometry and direct mercury analyzer. *Journal of the Science of Food and Agriculture*, *99*(4), 1870-1879. https://doi.org/10.1002/jsfa.9382

Hongwiangjan, J., Terdwongworakul, A., & Nakawajana, N. (2015). Assessment of pomelo maturity using optical properties and characteristics of its peel. *Journal of Advanced Agricultural Technologies Vol*, *2*(2), 88-91.

Ibrahim, H. M., & Hamed, A. A. (2018). Some physicochemical and functional properties of lemon and orange peels. *International Journal of Current Microbiology and Applied Sciences*, *7*, 4871-4885. https://doi.org/10.20546/ijcmas.2018.708.513

Khan, A., Azam, M., Shen, J., Ghani, M. A., Khan, A. S., Ahmad, S., Iqbal, M.A., Anjum, N., Zhang, J., Anjum, M.A. & Javed, A. (2021). Overall quality maintenance of grapefruit during cold storage using pre-storage neem leaf extract dipping. *Journal of Food Measurement and Characterization*, *15*, 1727-1736. https://doi.org/10.1007/s11694-020-00752-2

Lawal, K. G., Riaz, A., Mostafa, H., Stathopoulos, C., Manikas, I., & Maqsood, S. (2023). Development of carboxymethylcellulose based active and edible food packaging films using date seed components as reinforcing agent: physical, biological, and mechanical properties. *Food Biophysics*, *18*(4), 497-509. https://doi.org/10.1007/s11483-023-09793-8

Li, Q., Yao, S., Deng, L., & Zeng, K. (2022). Changes in biochemical properties and pectin nanostructures of juice sacs during the granulation process of pomelo fruit (*Citrus grandis*). *Food Chemistry*, *376*, 131876. https://doi.org/10.1016/j.foodchem.2021.131876

Lin, L. Y., Huang, C. Y., Chen, K. C., & Peng, R. Y. (2021). Pomelo fruit wastes are potentially valuable antioxidants, anti-inflammatories, antihypertensives, and antihyperglycemics. *Horticulture, Environment, and Biotechnology*, *62*, 377-395. https://doi.org/10.1007/s13580-020-00325-8

Mahawar, M. K., Bibwe, B., Jalgaonkar, K., & Ghodki, B. M. (2019). Mass modeling of kinnow mandarin based on some physical attributes. *Journal of Food Process Engineering*, *42*(5), e13079. https://doi.org/10.1111/jfpe.13079

Miraei Ashtiani, S. H., Baradaran Motie, J., Emadi, B., & Aghkhani, M. H. (2014). Models for predicting the mass of lime fruits by some engineering properties. *Journal of Food Science and Technology*, *51*, 3411-3417. https://doi.org/10.1007/s13197-012-0862-1

Motie, J. B., Ashtiani, S. M., Abbaspour-Fard, M. H., & Emadi, B. (2014). Modeling physical properties of lemon fruits for separation and classification. *International Food Research Journal*, *21*(5), 1901-1909.

Mukhim, C., Nath, A., Deka, B. C., & Swer, T. L. (2015). Changes in physico-chemical properties of Assam lemon (*Citrus limon* Burm.) at different stages of fruit growth and development. *The Bioscan*, *10*(2), 535-537.

Neshovska, H. (2023). Determination of the chemical and mineral composition of citrus by-products in relation to its utilization as a feed raw material. *Bulgarian Journal of Animal Husbandry/Životnov Dni Nauki*, *60*(4), 42-48.

Olabinjo, O. O., Ogunlowo, A. S., Ajayi, O. O., & Olalusi, A. P. (2017). Analysis of physical and chemical composition of sweet orange (*Citrus sinensis*) peels. *International Journal of Environment, Agriculture and Biotechnology*, *2*(4), 2201-2206. http://doi.org/10.22161/ijeab/2.4.80

Ouyang, H., Wu, L., Hu, Y., Li, L., Li, Z., He, H., Jiang, Z., Li, Q., Ni, H. & Zheng, M. (2023). Effect of steam explosion treatment on physicochemical, functional and structural properties of pomelo fruitlets. *Lwt*, *184*, 114963. https://doi.org/10.1016/j.lwt.2023.114963

Rafiee, S., Jahromi, M. K., Jafari, A., Sharifi, M., Mirasheh, R., & Mobli, H. (2007). Determining some physical properties of bergamot [*Citrus medica*]. *International Agrophysics*, *21*(3), 293-297.

Rehal, J., Kaur, G. J., & Bons, H. K. (2017). Studies on physico-mechanical properties of W. Murcott mandarin. *Journal of Applied and Natural Science*, *9*(1), 80-84. https://doi.org/10.31018/jans.v9i1.1154

Roghini, R., & Vijayalakshmi, K. (2018). Phytochemical screening, quantitative analysis of flavonoids and minerals in ethanolic extract of *Citrus paradisi*. *International Journal of Pharmaceutical Sciences and Research*, *9*(11), 4859-4864. http://doi.org/10.13040/IJPSR.0975-8232

Selvan, S. S., Edukondalu, L., Kumar, A. A., & Madhava, M. (2021). Determination of engineering properties of sweet orange (*Citrus sinensis* L.) fruits. *The Pharma Innovation Journal, 10*(3), 786-790.

Shahbazi, F., & Rahmati, S. (2013). Correlation the mass of grapefruit to some physical attributes. *Journal of Agro-alimentary Processes and Technologies*, *19*(1), 43-47.

Shravan, R., & Shere, D. M. (2018). Study of physico-chemical characteristics of sweet orange (*Citrus sinensis*) fruit. *Journal of Pharmacognosy and Phytochemistry*, *7*(6), 1687-1689.

Silva, J. G. S., Orlando, E. A., Rebellato, A. P., & Pallone, J. A. L. (2017). Optimization and validation of a simple method for mineral potential evaluation in citrus residue. *Food Analytical Methods*, *10*, 1899-1908. https://doi.org/10.1007/s12161-016-0748-3

Singh, S. S., Abdullah, S., Pradhan, R. C., & Mishra, S. (2019). Physical, chemical, textural, and thermal properties of cashew apple fruit. *Journal of Food Process Engineering*, *42*(5), e13094. https://doi.org/10.1111/jfpe.13094

Sirisomboon, P., & Lapchareonsuk, R. (2012). Evaluation of the physicochemical and textural properties of pomelo fruit following storage. *Fruits*, *67*(6), 399-413. https://doi.org/10.1051/fruits/2012034

Sirisomboon, P., & Theamprateep, C. (2012). Physicochemical and textural properties of pomelo (*Citrus maxima* Merr. cv. Kao Nam Pueng) fruit at preharvest, postharvest and during the commercial harvest period. *Philippine Agricultural Scientist*, *95*(1), 43-52.

Terdwongworakul, A., Puangsombat, A., & Pathaveerat, S. (2009). Qualitative and quantitative evaluation of pomelo maturity using multivariate combination of chemical and physical properties. *Journal of Texture Studies*, *40*(5), 584-605. https://doi.org/10.1111/j.1745-4603.2009.00199.x

Tocmo, R., Pena-Fronteras, J., Calumba, K. F., Mendoza, M., & Johnson, J. J. (2020). Valorization of pomelo (*Citrus grandis* Osbeck) peel: A review of current utilization, phytochemistry, bioactivities, and mechanisms of action. *Comprehensive Reviews in Food Science and Food Safety*, *19*(4), 1969-2012. https://doi.org/10.1111/1541-4337.12561

Yadav, N. K., Ali, A., Dev, R., Asabe, M. P., & Abass, M. (2019). Evaluation of physical properties of different grades of kinnow mandarin. *Journal of Pharmacognosy and Phytochemistry*, *8*(1), 1414-1417.

Yin, J., Hu, X., Hou, Y., Liu, S., Jia, S., Gan, C., Ou, Y. & Zhang, X. (2023). Comparative analysis of chemical compositions and antioxidant activities of different pomelo varieties from China. *Food Chemistry Advances*, *2*, 100180. https://doi.org/10.1016/j.focha.2022.100180

Zhang, H., & Rui, Y. K. (2012). Determining mineral elements in four kinds of grains from Beijing market by ICP-MS simultaneously. *Journal of Saudi Chemical Society*, *16*(1), 31-33. http://dx.doi.org/10.1016/j.jscs.2010.10.014

Zheng, M., Hong, J., Li, M., He, H., Jiang, Z., Ni, H., & Li, Q. (2022). Effects of particle sizes on structural and physicochemical properties of pomelo peel powders. *Journal of Food Processing and Preservation*, *46*(1), e16124. https://doi.org/10.1111/jfpp.16124

JOURNAL OF HORTICULTURE AND POSTHARVEST RESEARCH
2025, VOL. 8(1), 89-104

Journal of Horticulture and Postharvest Research

Journal homepage: www.jhpr.birjand.ac.ir

University of Birjand

Potential impact of different LED light spectra on callus induction, regeneration and plantlet growth of two cultivars of *Caladium bicolor*

Maryam Dehestani-Ardakani[1,2*], Mohsen Karimi Dorche[1] and Maryam Rahmati[1]

1, Department of Horticultural Sciences, Faculty of Agriculture & Natural Resources, Ardakan University, Ardakan, Iran
2, Medicinal and Industrial Plant Research Institute, Ardakan, Iran

ARTICLE INFO

Original Article

Article history:
Received 19 June 2024
Revised 10 September 2024
Accepted 2 October 2024

Keywords:
Hyperhydric shoot
Indoor plant
In vitro
Light-emitting diode
Proliferation

DOI: 10.22077/jhpr.2024.7787.1391
P-ISSN: 2588-4883
E-ISSN: 2588-6169

***Corresponding author:**
Department of Horticultural Sciences, Faculty of Agriculture & Natural Resources, Ardakan University, Ardakan, Iran.
Email: mdehestani@ardakan.ac.ir

© This article is open access and licensed under the terms of the Creative Commons Attribution License http://creativecommons.org/licenses/by/4.0/ which permits unrestricted, use, distribution and reproduction in any medium, or format for any purpose, even commercially provided the work is properly cited.

ABSTRACT

Purpose: *Caladium bicolor* is highly valued as both a landscape and indoor plant, primarily for its decorative appeal stemming from its diverse leaf shapes and vibrant, multicolored foliage. LED (light-emitting diode) lighting serves as a cost-efficient and potent means of promoting plant growth and development. The impact of different LED lights was investigated on callus induction, regeneration, and plantlet growth of two cultivars of *Caladium bicolor* ('White' and 'Red'). **Research Method**: Leaf explants were cultured on Murashige and Skoog (MS) medium supplemented with 1.5 mg L^{-1} IBA and 1 mg L^{-1} BA and moved to racks equipped with various LED lighting (100% red lights (R), 100% blue lights (B), 50% blue + 50% red lights (B+R), and 100% white fluorescent lamps (W)). **Findings**: Results showed W light was the best for maximum callus induction, leaf number, and plantlet height in both cultivars. Red + blue LED light spectrum motivated proliferation percentage of callus in both cultivars as compared to other light spectra. Conservation of 'White' caladium plantlets in R and B light spectra resulted in no hyperhydric micro shoot formation incidences. When examining various growth characteristics, it was evident that the B+R light spectrum of 'Red' caladium showed the best performance, while the B light spectrum in both cultivars had the least favorable outcomes compared to all other light spectra. **Research limitations**: There was no limitation. **Originality/Value**: Our findings offer a deeper understanding of how the quality of LED light impacts the *in vitro* propagation of caladium, potentially enhancing the cultivation of these plantlets through specific spectral exposure.

INTRODUCTION

Caladiums, specifically *Caladium bicolor*, are versatile plants that can thrive both indoors and outdoors. They are commonly used indoors as potted florists' plants due to their colorful foliage (Zhang et al., 2019). Caladiums are prized for their vibrant leaves and are known for their ability to add a pop of color to indoor spaces with relatively low light levels (Stamps & Savage, 2011). Their adaptability to different light conditions and the availability of various cultivars with unique leaf colors make caladiums a popular choice for indoor plant enthusiasts looking to brighten up their living or working spaces (Deng & Harbaugh, 2006). *In vitro* studies have shown successful micropropagation of caladium using specific hormone concentrations, leading to efficient shoot and root regeneration for propagation purposes (Kokalis-Burelle et al., 2017). Overall, caladium is not only a visually appealing house plant but also a subject of genetic and cytogenetic interest for breeding and cultivation advancements.

Different light spectrums have a significant impact on callus formation and germination in plant tissue cultures. Studies on wheat and *Prunella vulgaris* callus cultures have shown that specific light wavelengths influence biomass accumulation, secondary metabolite production, and antioxidant activity (Blidar et al., 2021; William & Carpenter, 1990). It has been reported that different light wavelengths have the ability to regulate various plant processes, including photosynthesis, germination, flowering, biomass accumulation, and phytochemical synthesis (Jafari et al., 2023). Additionally, research on lettuce, cucumber, and sweet pepper seedlings revealed that red and blue LEDs promote better growth responses compared to cool-white fluorescent light, affecting parameters like shoot length, root collar diameter, and dry matter content (Fazal et al., 2016). Furthermore, investigations on *Capsicum annuum* tissue culture demonstrated that red, white, blue, and green light can induce callus formation, with red light being optimal for bud differentiation while blue and green light inhibits it (da Silva et al., 2016). Therefore, the choice of light spectrum is crucial for optimizing callus formation and germination in different plant species.

Different light spectra have diverse effects on callus formation. Blue and purple light induce increased red coloration in callus, while yellow light promotes the greatest callus proliferation. White light enhances biomass production, total phenolic contents, and flavonoid contents in *Moringa oleifera* callus cultures (Bajwa et al., 2023). Red LED light significantly contributes the biomass accumulation in *Passiflora* calluses and induces the highest production of bioactive substances (Santos-Tierno et al., 2021). *Scutellaria baicalensis* calluses exposed to blue light show increased flavone content, particularly baicalin (Costine et al., 2022). *Eclipta alba* callus cultures under red light exhibit maximum dry weight and enhanced phenolics and flavonoids content, crucial for therapeutic potential (Khurshid et al., 2020). Overall, different light spectra play a significant role in modulating callus formation and the accumulation of bioactive compounds in various plant species.

Red-blue light and darkness were found to be optimal for biomass, protein content, and cell viability in *Hyoscyamus reticulatus* callus, while red and blue lights induced oxidative damage and altered cell morphology (Hassanpour, 2021). In *Hyptis marrubioides* callus, blue light negatively impacted on phenolic compound synthesis, while red light stimulated specific metabolite production, and darkness led to increased accumulation of certain bioactive compounds (Dantas et al., 2021). *Eutrema salsugineum* callus lines showed lower oxidative stress under red light than blue light, which induced higher antioxidant enzyme activities (Pashkovskiy et al., 2018). Blue light was crucial for enhancing flavone content in *Scutellaria baicalensis* callus tissue (Stepanova et al., 2020). Kazemi et al. (2023) demonstrated that using solely 100% blue light was unsuitable for optimizing the growth and biophysical

characteristics of the electron transport chain in begonias. For all three tested begonia cultivars, a combination of red, blue, and white light proved beneficial, promoting better growth and improving chlorophyll fluorescence parameters in the plants.

This study aimed to determine the optimal proportions of blue, red, blue + red LED, and white fluorescent lights suitable for callus induction, regeneration, and plant growth of two cultivars of *Caladium bicolor*. In this study, morphological and physiological changes were evaluated under different lighting conditions. We aim to achieve a more environmentally friendly production method for *C. bicolor* with low energy consumption and high efficiency.

MATERIALS AND METHODS

Plant material and culture establishment

This research was conducted 2023 at Ardakan University, Ardakan, Iran. In this research, vigorous, healthy young leaves from two distinct *C. bicolor* plant cultivars, by two different color 'White' and 'Red', were used as the initial explant. 'Red' cultivars typically feature vibrant red or pink variegated leaves, often with deep green margins. The leaves may exhibit a striking pattern of veins and spots, enhancing their decorative appeal. 'White' cultivars are characterized by striking white or cream-colored leaves, often with green margins or veins. Similar to 'red' cultivars, 'white' varieties may have unique patterns, including speckles or splashes of green, which add visual interest. The leaves are also heart-shaped, but the overall appearance may appear more delicate due to the lighter colors. To eliminate any potential contaminants, a thorough surface sterilization protocol was performed. The procedure began with triple washing of the leaves in sterile distilled water, followed by rinsing the leaves in a disinfectant solution containing 0.05% citric acid and 0.1% mercuric chloride for 3 minutes. To finalize the sterilization, the leaves were subjected to three additional washes in a solution with 0.05% citric acid to ensure contaminant-free explants for subsequent experimental procedures (Rezaie et al., 2018). Three explants were meticulously placed into a glass jar, measuring a height of 10 cm and a diameter of 6 cm. The explants underwent cultivation in MS (Murashige & Skoog, 1962) medium, which was supplemented with the addition of Indole-3-butyric acid and 6-benzyladenine (IBA at 1.5 mg L^{-1} and BA at 1 mg L^{-1}), to induce callus formation. Each individual jar was filled with 25 ml of MS medium and contained three leaf explants. Agar was added into the MS basal culture media at a density of 7 g L^{-1}, while sucrose was supplemented at a rate of 30 g L^{-1}. Explants were cultured under different light treatments in the controlled conditions in the growth chamber, each culture underwent a cyclical exposure to 16 hours of light and 8 hours of darkness. During the dark period, the temperature inside the chamber was regulated around 18 ± 2 °C, while it was adjusted to approximately 23 ± 2 °C during the light period. Furthermore, the photosynthetic photon flux density (PPFD) ranged from 34 to 40 µmol m^{-2} s^{-1}.

Light treatments

Explants were grown in growth chambers with various light treatments including 100% red LED (660 nm) lights (R), 100% blue (450 nm) LED lights (B), 50% red (660 nm) + 50% blue (450 nm) LED lights (RB), with 40 µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) and 100% white fluorescent lamps (W) with 100 µmol m^{-2} s^{-1} PPFD (measured with LI-250A; LI-COR Biosciences, Lincoln, NE, USA). These spectra were selected because of their relevance to leaf photosynthesis. Wavelengths of R and B lights were considered the main absorption spectra of chlorophyll (Aalifar et al., 2020), and the W light (WL, containing all light spectra in the range of photosynthetic active radiation) was used. The spectral distributions were estimated using Sekonic C7000 SpectroMaster spectrometer (Sekonic

Corp., Japan) in the wavelength range of 300–800 nm. LEDs were fixed in the number of aluminum boxes (100 × 110 × 50 cm^3) (Iranian Grow Light company, Iran). The distance between the source of light and plant jars were 50 cm. Adjustment of the light intensity in all mentioned growth chambers to the PPFD of 250 ± 10 µmol m^{-2} s^{-1} was performed. Three glass jars (each containing three explants) from each caladium cultivar were grown under each light spectrum, following a light/dark cycle of 16/8 hours, at temperatures of 23 and 20 ± 2 °C, and relative humidity of 20 ± 2 %. Plants were grown at five different light spectra and evaluated after 16 weeks in regard to their growth and biophysical parameters.

Following eight weeks, each jar was individually checked to record “the percentage of callus induction”, the percentage of indirect regeneration, and finally “the percentage of shoot/bud regeneration (i.e., direct regeneration). Furthermore, “the percentage of hyperhydric micro shoots formation” was also documented.

Proliferation and rooting

For the proliferation phase, individual shoot explants, which were cultivated in the preceding stage and measured about 2-3 cm in height, were employed. In this context, each individual shoot explant was cultivated using MS basal salts and vitamins, supplemented with 30 g L^{-1} sucrose, 7.0 g L^{-1} agar, and 0.05 mg L^{-1} Gibberellic acid (GA3; specifically for proliferation). These conditions were maintained for a growth period of four weeks. In the following, the resultant 4-week-old shoots were transferred into the plant growth regulator-free MS medium for about 2 weeks. Lastly, various parameters such as plantlet height, leaf number, shoot number, root number, root length, were documented for all the 10-week shoots. The length of each shoot and root was measured from the agar starting point to the tip using a ruler in centimeters. The number of shoots and roots was counted for each jar.

Statistical analysis

All statistical analyses were carried out using a factorial experiment with two main factors including two individual cultivars (‘White’ and ‘Red’) and four different light treatments (Red, blue, red+blue, and white spectra). In this research, it was used of the factorial test based on a completely random design. The means was analyzed with ANOVA test using SPSS (version 26.0, SPSS Inc., Chicago, IL, USA) software. The significant variation between the treatments data was computed using Duncan’s multiple-range tests (DMRT) at $p \leq 0.05$. To cluster all the treatment(s), hierarchical cluster analysis (HCA) combined with heatmap visualization was applied using the CIMMiner web tool. CIMMiner generates color-coded Clustered Image Maps (CIMs) ("heat maps") to represent "high-dimensional" data sets. The principal component analysis (PCA) was also applied to recognize the associations among the variables using the Paleontological Statistics Software (PAST version 4.03).

RESULTS AND DISCUSSION

Callus induction and regeneration

All treatments successfully stimulated callus induction in both cultivars. Significantly, the R spectrum demonstrated the greatest efficacy in callus induction across both cultivars, achieving a complete success rate of 100% (Fig. 1A). Also, both W and B+R spectra effectively stimulated callus formation in the 'White' and 'Red' cultivars. The lowest percentage of callus induction (30.33 and 20.00%) was observed in the B spectrum applied to both cultivars (Fig. 1A).

Regeneration rates displayed considerable variation across all four spectra, ranging from 20% to 80%, as depicted in Figure 1B. The 'Red' caladium showed maximum callus regeneration

under the B+R spectrum, while its minimal regeneration was noted in the B spectrum (Fig. 1B). Inducing callus is a crucial phase in reverting differentiated plant tissues to undifferentiated, facilitating *in vitro* morphogenesis to generate plantlets (Tůmová et al., 2010). Nonetheless, the process of callogenesis *in vitro* is influenced by multiple variables, including the nature and concentration of plant growth regulators, the age and kind of the explant, the composition of the growth media, and physical factors such as pH, temperature, and lighting conditions within the growth chamber (Khan et al., 2020). Light signaling pathways play a crucial role in controlling growth, differentiation, and metabolic processes in callus cultures. Distinct wavelengths of light elicit varied responses that promote biomass production in *in vitro* callus cultures (Ali & Abbasi, 2014; Fazal et al., 2016; Khan et al., 2019). The percentage of callus induction under constant white light can be attributed to the heightened levels of energy (100 μmol m^{-2} s^{-1} PPFD), which significantly influence the physiological activities, morphogenesis, and biochemical pathways in plants (Bajwa et al., 2023). This includes the synthesis of both primary and secondary metabolites. Comparable outcomes were noted in callus cultures of *Moringa oleifera* when subjected to continuous white light (24 hours) succeeded by exposure to yellow light (Bajwa et al., 2023). Additionally, comparable results were noted in cell cultures of *Withania somnifera* when exposed to continuous white light, which led to a significant increase in biomass accumulation (Adil et al., 2019). Previous research has demonstrated that *Artemisia absinthium* L. callus cultures exhibit significantly higher biomass accumulation under continuous white light compared to other lighting conditions. Similarly, callus cultures of *Lepidium sativum* L. exposed to continuous white light (24 hours) also showed enhanced biomass growth (Ali & Abbasi, 2014; Ullah et al., 2019).

Proliferation and elongation of the adventitious shoots

The percentage of proliferation varied among four spectra, ranging from 15% (B light treatment on 'Red' caladium) to 100% (B+R treatment on 'Red' cultivar, Fig. 2B). During the proliferation phase, which involves an increase in plant and internode length, additional explants are collected from the plantlet. This practice enhances productivity in commercial tissue culture operations, as was also observed in the current study.

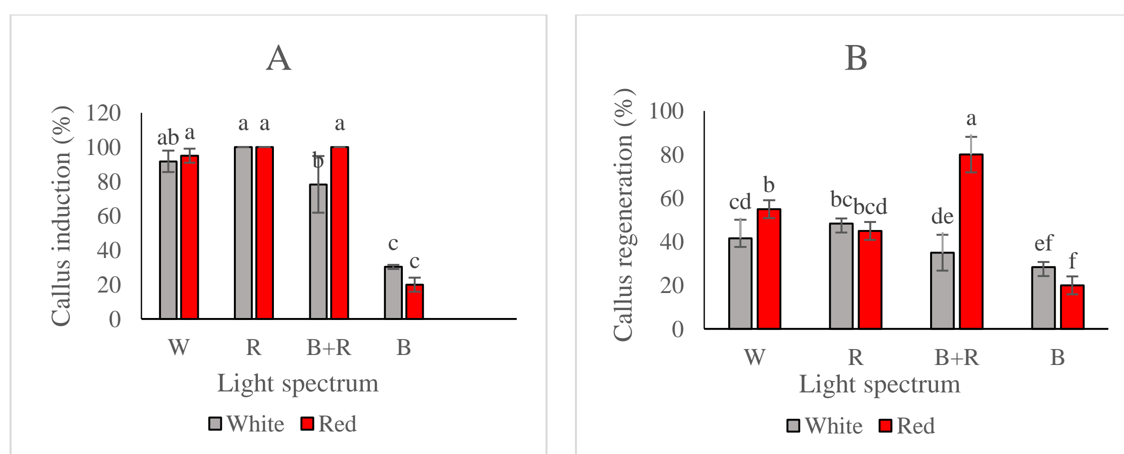


Fig. 1. Interaction effect of cultivars and different light spectra on A) callus induction and B) regeneration of two cultivars of *Caladium bicolor* on the MS medium. The distinct letter(s) indicated a significant difference ($p \leq 0.05$) as determined by Duncans multiple range test.

In this research, the application of combined blue and red LED on tissue-cultured leaf explants of 'White' and 'Red' caladium varieties resulted in an increase in proliferation rates (134.80% and 81.81%, respectively), when compared to traditional white fluorescent light. This finding aligns with similar outcomes observed in studies conducted on Vanilla (Bello-Bello et al., 2016). They discovered that the growth of *Vanilla planifolia* was hindered when exposed solely to blue and red LED. Conversely, the most effective propagation was achieved under white LED, and combine of blue and red LEDs, as well as under fluorescent light. The growth rate of *Panax vietnamensis* was found to be twice as high under red: blue LED lights at a 60:40 ratio, with an observed value of 11.21. In contrast, fluorescent lights resulted in a growth rate of 5.8. (Nhut et al., 2015). The effectiveness of using combined LEDs for the growth and development of plants, as opposed to monochromatic lighting, has been documented for various species including *Prunus domestica* (Nacheva et al., 2023), *Chrysanthemum* sp. (Kim et al., 2004), and *Lycium barbarum* L. (de Oliveira Prudente et al., 2019).

Plantlet height

The maximum plantlet height (10.33, 11.66, and 11.00 cm) was achieved in both cultivars in W treatment and ‘White’ caladium in R light spectrum. Other treatments did not show a significant difference (Fig. 6B).

In this research, it was found that the spectrum of light significantly affects the shoot length produced. It has been observed that plantlets of *Prunus domestica* subsp. *insititia* cultivated under red LED exhibited the longest average stem length, a finding that diverges from the outcomes presented in our study (Nacheva et al., 2023). Throughout the propagation stage of *Pyrus communis* ‘Arbi’, red LED displayed important advantages: it produced optimal shoot height and leaf surface (Lotfi, 2022). Red light typically promotes the elongation of stems and the lengthening of the spaces between nodes (Poudel et al., 2008; Li et al., 2010). Furthermore, blue light impedes cellular proliferation and modulates gene expression to restrict the elongation of stems (Lin et al., 2013). Enhanced internode observed under red and white light spectra can be attributed to augmented cell elongation and cell proliferation. These processes, which involve both the division and elongation of cells, correlate with elevated levels of gibberellic acid (GA) (Nacheva et al., 2023). The findings suggest that GA signaling is essential for the elongation of shoots in caladium *in vitro* explants under red and white light conditions.

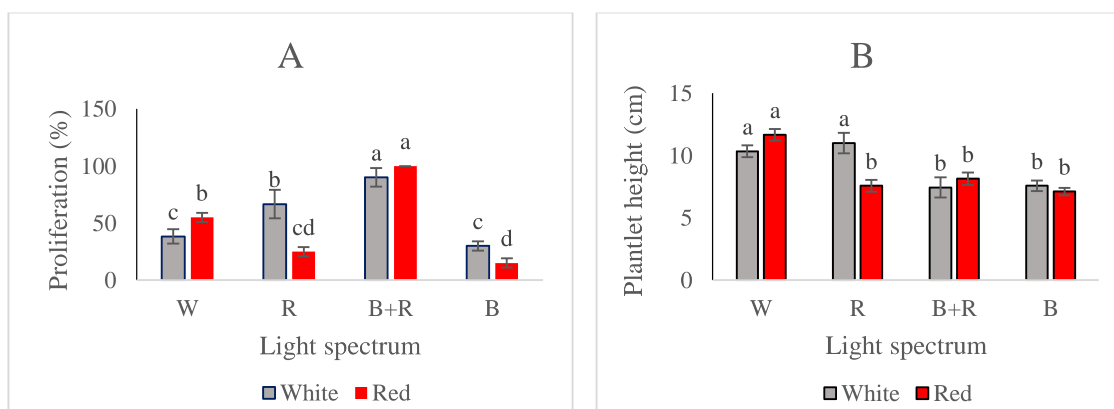


Fig. 2. Interaction effect of cultivars and different light spectra on (A) proliferation and (B) plantlet height of two *Caladium bicolor* cultivars on MS medium. The distinct letter(s) indicated a significant difference ($p \leq 0.05$) as determined by Duncans multiple range test.

Shoot and leaf number of regenerated plantlets

There was a significant difference in shoot proliferation among the various light spectra (Fig. 3A). The maximum of average shoot number of 6.33 shoots per jar was observed in R light treatment in 'White' cultivar (Fig. 3A). The optimum light spectrum for leaf production was evident in W treatment in both cultivars (Fig. 3B). In the W light spectrum used, 80 and 85 leaves were observed per jar in both 'White' and 'Red' caladium cultivars, respectively (Fig. 3B). However, B light for both cultivars was not successful in leaf production, resulting in the lowest average number of leaves (6.66 and 5.66 leaves per jar, respectively).

In the commercial propagation of caladium via tissue culture, the quantity of lateral shoots produced from a single explant is crucial. Our study demonstrated that utilizing red LED lighting enhanced the shoot multiplication in *in vitro*. This augmentation in shoot numbers facilitated a greater yield of explants during the proliferation phase and boosted the rate of proliferation. In 'White' caladium, the proliferation rate of lateral shoots experienced a 26.6% increase under red LED when compared to fluorescent lighting. Red light resulted in an 88.66% reduction in the number of shoots for 'Red' caladium when compared to white fluorescent light. The findings indicate that red LED light has a beneficial impact on the development of lateral shoots in tissue-cultured nodal shoots of the 'White' caladium cultivar. Seminally, research performed on Stevia (Ramirez-Mosqueda et al., 2017) and plum plantlets demonstrated that exposure to red LED light spectra enhanced the shoot proliferation per explant (Nacheva et al., 2023). Conversely, studies have shown that employing a mix of blue and red LED light spectra can lead to a significant increase in the number of shoots per explant in both Canola and Dendrobium (Lin et al., 2011; Lin et al., 2013).

The impact of differential light spectra on plants varies considerably depending on the intensity and quality of the light used, the duration of exposure, and the specific type of plant involved (Fazal et al., 2016). Nacheva et al. (2023) observed that the maximum leaf count occurred under red light, a finding that diverges from the results presented in this study.

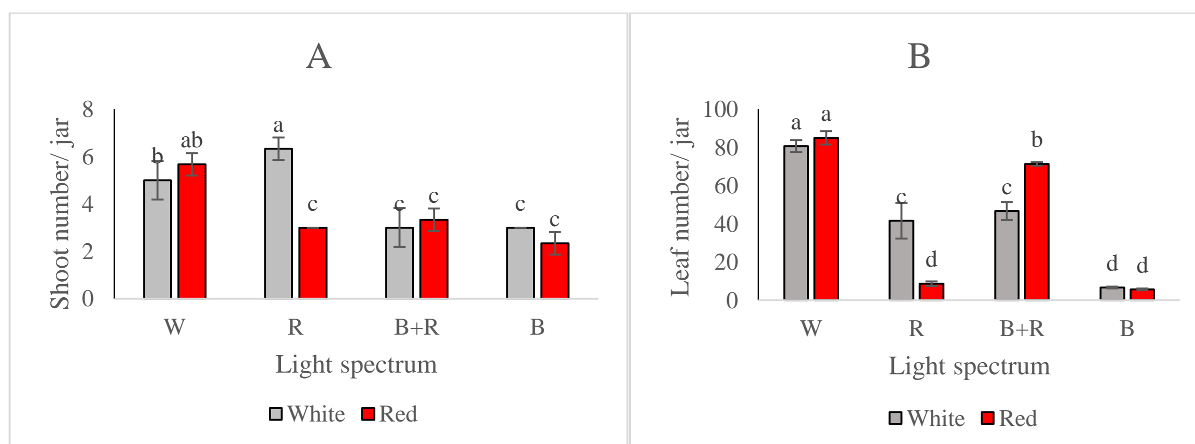


Fig. 3 Interaction effect of cultivars and different light spectra on (A) shoot and (B) leaf number of two cultivars of *Caladium bicolor* on the MS medium. The distinct letter(s) indicated a significant difference ($p \leq 0.05$) as determined by Duncans multiple range test.

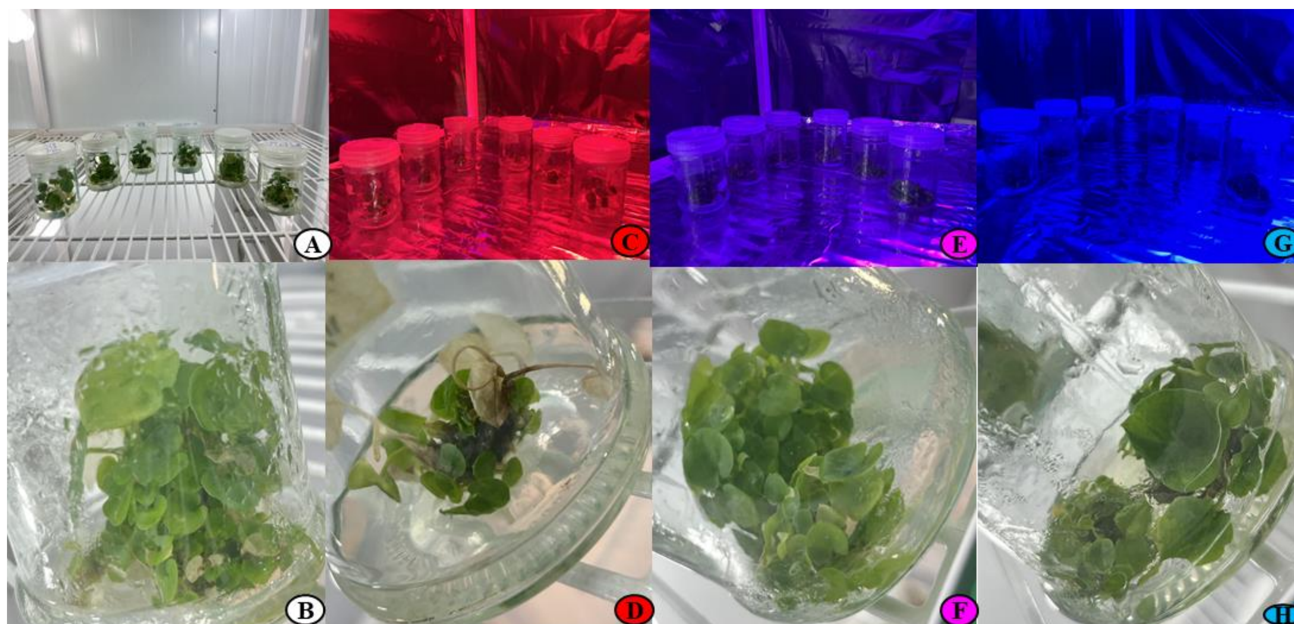


Fig. 4. Effects of different light spectra on the shoot regeneration and proliferation of *Caladium bicolor* cv. ‘Red’. (A) and (B) 100% White fluorescent lamp, (C) and (D) 100% Red LED light spectrum, (E) and (F) 50% Blue+ 50% Red LED and (G) and (H) 100% Blue LED spectrum.

Rooting of shoot and plantlet development

Eight weeks into the experiment, the initiation of root induction was started, with the associated data being documented after a period of 10 weeks. Next, the following parameters were measured and recorded.

Number of roots and root length

Figure 4 illustrates that the number and length of root systems are significantly influenced by various lighting conditions. The greatest root count observed was in the 'Red' caladium under the W light spectrum, averaging 7.00 roots per jar as shown in Figure 6A. The minimum root number was recorded for both varieties under B treatment (‘White’ and ‘Red’ 7.00 and 6.00 roots/jar, respectively) and for the 'Red' variety under R light was recorded 8.33 roots per jar (Fig. 6A).

The 'Red' cultivar exposed to W light (5.53 cm), as well as both varieties under the B+R light spectrum (‘White’ and ‘Red’ 5.83, and 6.00 cm, respectively), exhibited the greatest root lengths, measuring. In contrast, the shortest root lengths were recorded in the B light spectrum for both cultivars, with measurements of 3.66 and 3.33 cm, as illustrated in Figure 5B. The root system exhibited growth under all lighting conditions, as illustrated in Figure 4. The greatest quantity of roots was observed under W light, surpassing those grown under combined B+R light. Similarly, the longest average root lengths were achieved with the B+R light, notably exceeding those under W light in ‘Red’ caladium. These results diverge from the findings of Elsabaa et al. (2022) regarding potato *in vitro* culture, where an increase in both root number and length was noted predominantly under red light, followed by blue light.

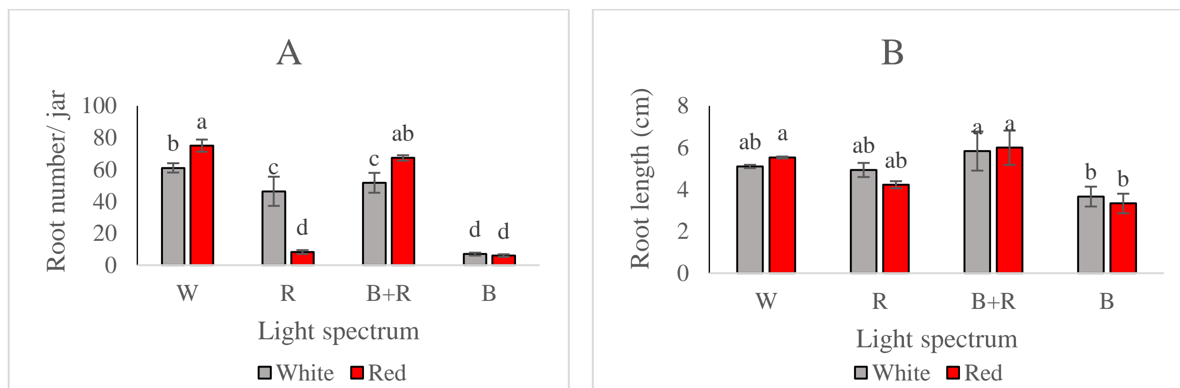


Fig. 5. Interaction effect of cultivars and different light spectra on (A) root number and (B) length of two cultivars of *Caladium bicolor* on the MS medium. The distinct letter(s) indicated a significant difference ($p \leq 0.05$) as determined by Duncans multiple range test.

Hyperhydric micro shoots formation

Hyperhydricity is a morpho-physiological disorder that manifests as diminished structural integrity in plantlets, stemming from excessive tissue hydration, insufficient lignification, and compromised stomatal functionality. These alterations can decrease the efficiency of *in vitro* regeneration and lower the survival rates of regenerates during the acclimatization process (Cassel & Curry, 2001; Gao et al., 2018). In this research, the preservation of 'White' caladium plantlets under R and B light spectra diminished the occurrence of hyperhydric micro shoots formation (Fig. 6).

In this research, 'White' caladium plantlets exposed to R and B light spectra showed minimal hyperhydric micro shoot formation, with no evidence of the disorder. The greatest proportion of hyperhydrated shoots was recorded in 'Red' plantlets under R light spectrum, reaching a percentage of 30% (Fig. 6). Insufficient chlorophyll and elevated moisture levels can disrupt physiological functions, potentially resulting in the translucency of shoots and leaves characteristic of hyperhydricity (Cassel & Curry, 2001). Elevated relative humidity, reduced lighting, gas build-up within the culture vessel, prolonged subculture periods and frequency, variations in the type and concentration of gelling agents, and hormonal imbalances contribute to hyperhydricity in plantlets. This condition can result in significant losses during the micropropagation process (Rojas-Martinez et al., 2010; Barbosa et al., 2013; Isah, 2015).

Observations indicate a decrease in chlorophyll a and b levels in the hyperhydric leaves of strawberry (Barbosa et al., 2013) and vanilla (Sreedhar et al., 2009) micro shoots. Isah (2019) demonstrated that the incidence of hyperhydricity in *C. bicolor* was higher in liquid medium cultures compared to solid ones, likely because the unrestricted diffusion of the medium into plant tissues induced conditions akin to anoxia. Incorporating 7.5 µM of silver nitrate effectively decreased the occurrence of the condition compared to other tested concentrations. However, alterations in the concentration of the gelling agent and variations in the light exposure duration did not yield effective results.

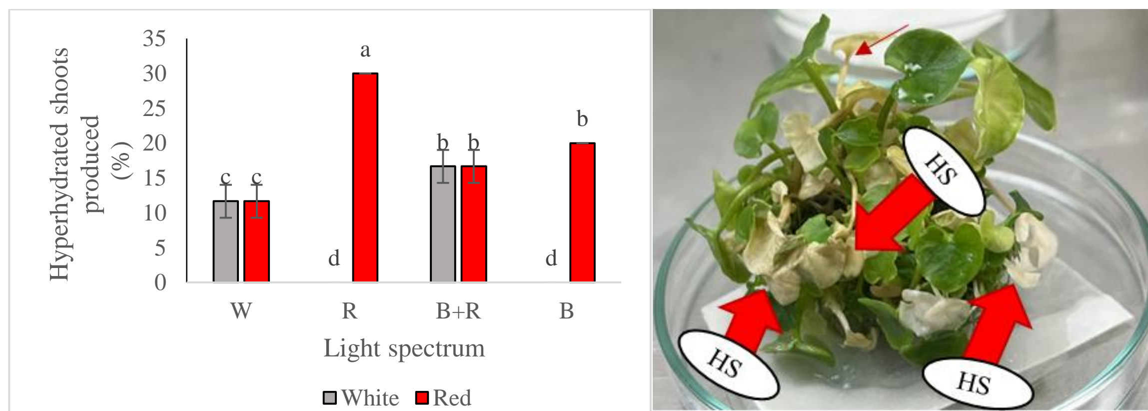


Fig. 6. Interaction effect of cultivars and different light spectra on hyperhydrated shoots (HS) of two cultivars of *C. bicolor* on the MS medium. The distinct letter (s) indicated a significant difference ($p \leq 0.05$) as determined by Duncans multiple range test.

Multivariate analyses

Heatmap-based cluster analysis

To enhance our comprehension of the ways in which traits react to various light spectrum treatments, we employed a heatmap-based clustering analysis (Fig. 7). The dendrogram illustrates the clustering of various light spectra according to their comparable efficacy. This also demonstrates how similar morphological traits are grouped, mirroring their distinct responses to varying degrees of radiation (Fig. 7). The horizontal axis of the heatmap represents different growth indicators, whereas the vertical axis displays varying degrees of light spectra. In the diagram, the deepest blue hue indicates the lowest values, while the brightest red denotes the highest values. Values that fall between these extremes are depicted using a gradient that transitions smoothly from one to the other.

The clustered heatmap revealed that the four distinct treatments formed two separate clusters. The first group divided into two subgroups consisted of the B+R spectrum of ‘Red’ cultivar and W light of both cultivars and R and B+R light spectra of ‘White’ caladium in the second subgroup. The B+R light spectrum outperformed the other treatments in terms of the majority of characteristics, demonstrating the significant impact of B+R light on the observed traits. The second major group was also divided into two subgroups consisting of R light in ‘Red’ caladium as first subgroup and B light spectrum in both caladium cultivars as the second subgroup. Among these treatments, only B light spectrum in both cultivars had the minimum values for most attributes.

In analyzing different growth traits, it became clear that the B+R light spectrum yielded superior results for the 'Red' caladium cultivar. Conversely, the B light spectrum resulted in the least favorable growth across both examined cultivars when compared to other light spectra. Conversely, characteristics including the number of roots and leaves, the percentage of regeneration and proliferation, and the induction of callus were categorized within the primary group. This classification suggests that the plants exhibited uniform reactions across varying light spectra concerning these particular traits. Within the secondary group, two distinct subgroups were established. The first subgroup encompassed measurements related to root length, shoot count, and the height of plantlets. Conversely, the second subgroup specifically grouped instances of hyperhydrated shoots (Fig. 7).

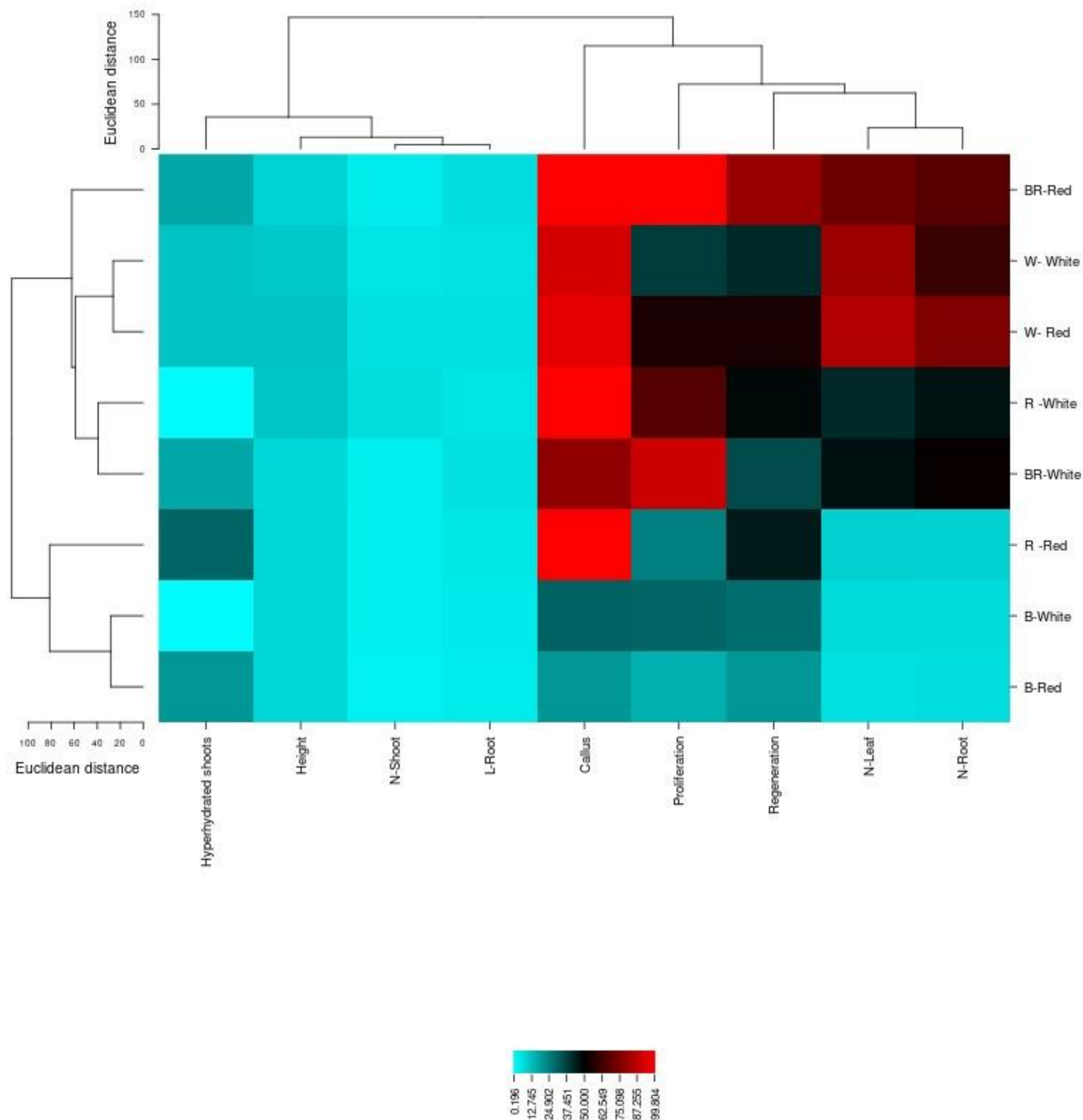


Fig. 7. Clustered heatmap for grouping of all 4 light spectra treatments (W, B, BR and R) in terms of the studied traits obtained from the *in vitro*-raised plantlets of both *Caladium bicolor* cultivars. Rows were clustered using *Euclidean distance* and average linkage. Columns were clustered using *maximum distance* and *McQuitty linkage.*

Principal Component Analysis (PCA)

PCA, or Principal Component Analysis, is a technique used in unsupervised learning to reduce the dimensionality of large data sets, thereby simplifying the complexity of the data. This method simplifies the comprehension of data while maintaining its informational integrity, thereby facilitating the graphical depiction of complex data sets. In this study, PCA was employed to explore the association and influence of various spectral light treatments on two varieties of *C. bicolor* (Fig. 8). According to the PCA, the first component accounted for 73.92% of the overall variance, while the second component represented 11.28%. Consequently, two distinct groups emerged from this analysis. Considering the first component, it was shown that the variables related to the traits are mainly includes length of

root, number of shoots and plantlet height. So, B+R light spectrum in 'Red' caladium had the highest values for these traits (Fig. 8). Number of leaf, root and proliferation percentage is grouped together. So, B+R light spectrum in 'White' caladium and W light spectrum in both cultivars had the highest values for these traits (Fig. 8). Callus induction, regeneration percentage and hyperhydrated shoots were clustered together and R light spectrum in 'White' cultivar showed the highest values for these traits (Fig. 8). The orientation of the vectors indicates the degree of association between the pertinent characteristics. In this study, exposure to the B light spectrum resulted in the lowest attribute values for both caladium cultivars compared to other treatments. Additionally, the results from the hierarchical clustering heat map corroborated the principal component analysis (PCA) findings. In both analyses demonstrated the B+R light spectrum in 'Red' caladium had the highest values. Also B light spectrum in both cultivars showed the lowest value.

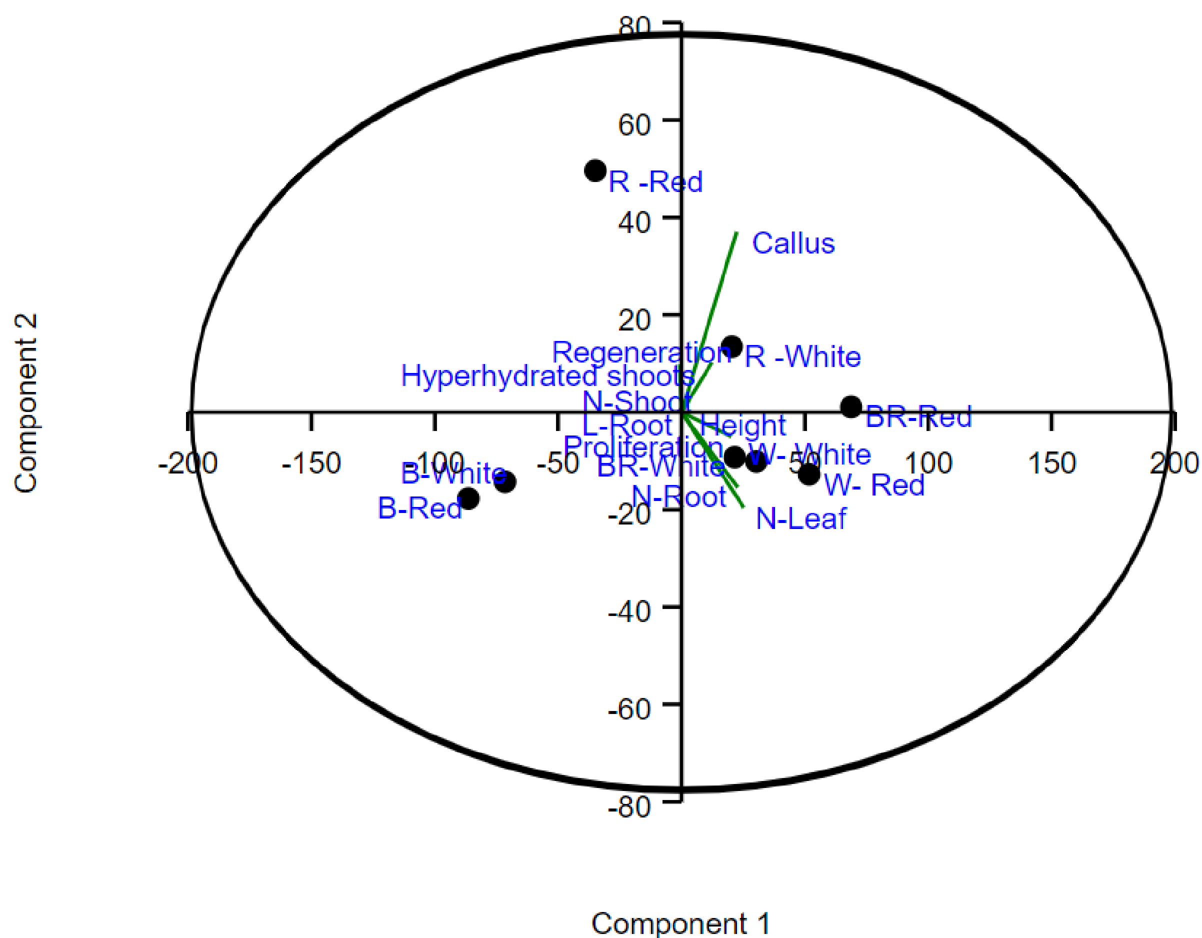


Fig. 8. Principal component analysis (PCA) of all 4 light spectra treatments (W, B, BR and R) in terms of the studied traits obtained from the *in vitro*-raised plantlets of both *Caladium bicolor* cultivars.

CONCLUSION

The current research showed that varying light spectra influenced all measured variables in the course of *in vitro* cultivation. Employing light-emitting diodes (LEDs) may offer significant benefits in addressing common issues encountered in *in vitro* cultures under fluorescent lighting, such as shoot and root elongation and elevated levels of photosynthetic pigments. So, it could be concluded that, W light were the best for maximum callus induction, leaf number, and plantlet height in both cultivars. Red + blue LED light spectrum motivated the proliferation percentage of callus in both cultivars as compared to other light spectra. Conservation of 'White' caladium plantlets in R and B light spectra resulted in no hyperhydric micro shoot formation incidences. It was shown that the B+R light spectrum yielded the most favorable results for the 'Red' caladium, whereas the B light spectrum resulted in the least favorable performance across both cultivars when compared to other light spectra.

Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

Aalifar, M., Aliniaeifard, S., Arab, M., Mehrjerdi, M. Z., & Serek, M. (2020). Blue light postpones senescence of carnation flowers through regulation of ethylene and abscisic acid pathway-related genes. *Plant Physiology and Biochemistry, 151,*103–112

Adil, M., Abbasi, B. H., & ul Haq, I. (2019). Red light controlled callus morphogenetic patterns and secondary metabolites production in *Withania somnifera* L. *Biotechnology Reports*, *24*, e00380. https://doi.org/10.1016/j.btre.2019.e00380

Ali, M., & Abbasi, B. H. (2014). Light-induced fluctuations in biomass accumulation, secondary metabolites production and antioxidant activity in cell suspension cultures of *Artemisia absinthium* L. *Journal of Photochemistry and Photobiology B: Biology*, *140*, 223-227. https://doi.org/10.1016/j.jphotobiol.2014.08.008

Bajwa, M. N., Khanum, M., Zaman, G., Ullah, M. A., Farooq, U., Waqas, M., Ahmad, N., Hano, C. & Abbasi, B. H. (2023). Effect of wide-spectrum monochromatic lights on growth, phytochemistry, nutraceuticals, and antioxidant potential of in vitro callus cultures of *Moringa oleifera*. *Molecules*, 28(3), 1497-1497. https://doi.org/10.3390/molecules28031497

Barbosa, L. M. P., Paiva Neto, V. B. D., Dias, L. L. C., Festucci-Buselli, R. A., Alexandre, R. S., Iarema, L., Finger, F.L., & Otoni, W. C. (2013). Biochemical and morpho-anatomical analyses of strawberry vitroplants hyperhydric tissues affected by BA and gelling agents. *Revista Ceres*, *60* (2), 152-160, https://doi.org/10.1590/S0034-737X2013000200002.

Bello-Bello, J. J., Martinez-Estrada, E., Caamal-Velazquez, J. H., & Morales-Ramos, V. (2016). Effect of LED light quality on in vitro shoot proliferation and growth of vanilla (*Vanilla planifolia* Andrews). *African Journal of Biotechnology, 15*, 272–277. https://doi.org/10.5897/AJB2015.14662

Blidar, C. F., Chis, R. S., Bota, S. R., Serban, G., & Stanasel, O. D. (2021). Effect of light quality on in vitro germination, seedling growth and photosynthetic pigments production in wheat (*Triticum aestivum* L.). *African Journal of Biotechnology*, *20*(7), 300-307. doi: 10.5897/AJB2021.17329

Cassel, A., & Curry, R. (2001). Oxidative stress and physiological, epigenetic and genetic variability in plant tissue culture: implications for micropropagators and genetic engineers, *Plant Cell Tissue Organ Culture, 64* (2–3), 145–157. https://doi.org/10.1023/A:1010692104861.

Costine, B., Zhang, M., Pearson, B., & Nadakuduti, S. S. (2022). Impact of blue light on plant growth, flowering and accumulation of medicinal flavones in *Scutellaria baicalensis* and *S. lateriflora*. *Horticulturae*, *8*(12), 1141.

da Silva, E. M., da Costa, G. G. S., Andrade, A. F., Ferreira, H. C. P., & Steiner, F. (2016). Light spectral quality on production of lettuce, cucumber and sweet pepper seedlings. *Scientia Agraria Paranaensis*, *15*(4), 446-452. https://doi.org/10.18188/SAP.V15I4.13415

Dantas, L. A., Faria, P. S. A., de Melo, A. M., Rosa, M., Resende, E. C., Pereira, P. S., Silva, F. G., & Neto, A. R. (2021). Spectral quality as an eliciting agent in the production of phenolic compounds in the callus of *Hyptis marrubioides* Epling. *Research, Society and Development*, *10*(9), 1-17. https://doi.org/10.33448/RSD-V10I9.18472

de Oliveira Prudente, D., de Souza, L. B., Paiva, R., Domiciano, D., Aparecida de Carvalho, P., & Nery, F. (2019). Goji berry (*Lycium barbarum* L.) *in vitro* multiplication improved by light-emitting diodes (LEDs) and 6-benzylaminopurine. *In Vitro Cellular & Developmental Biology Plant, 55,* 258–264.

Deng, Z., & Harbaugh, B. K. (2006). Dynamite Red'-A red fancy-leaved caladium for sunny landscapes and containers. *HortScience*, *41*(2), 471.

Elsabaa, N.A., Ahmed, M.E., Younes, N.A., & Shehata, M.N. (2022). Effect of Led Spectrum on Potato Growth *in Vitro. Journal of Plant Production, 13* (6), 273-279.

Fazal, H., Abbasi, B. H., Ahmad, N., Ali, S. S., Akbar, F., & Kanwal, F. (2016). Correlation of different spectral lights with biomass accumulation and production of antioxidant secondary metabolites in callus cultures of medicinally important *Prunella vulgaris* L. *Journal of Photochemistry and Photobiology B: Biology*, *159*, 1-7. https://doi.org/10.1016/j.jphotobiol.2016.03.008

Gao, H., Li, J., Ji, H., An, L., & Xia, X. (2018). Hyperhydricity-induced ultrastructural and physiological changes in blueberry (*Vaccinium* spp.), *Plant Cell, Tissue and Organ Culture.* 1–12, https://doi.org/10.1007/s11240-017-1361-x.

Hassanpour, H. (2021). Potential impact of red-blue LED light on callus growth, cell viability, and secondary metabolism of *Hyoscyamus reticulatus*. *In Vitro Cellular & Developmental Biology – Plant*, 1-10. https://doi.org/10.1007/S11627-021-10232-X

Isah, T. (2015). Adjustments to *in vitro* culture conditions and associated anomalies in plants, *Acta Biologica Cracoviensia Series Botanica, 57*(2), 9–28. https://doi.org/10.1515/ abcsb-2015-0026.

Isah, T. (2019). Changes in the biochemical parameters of albino, hyperhydric and normal green leaves of Caladium bicolor cv.“Bleeding hearts” in vitro long-term cultures. *Journal of Photochemistry and Photobiology B: Biology*, *191*, 88-98.

Jafari, S., Nikbakht, A., Haghighi, M., & Shahin Varnousfaderani, S. (2023). Optimizing of the quality of rose grown with varying ratios and periods of Red: Blue light-emitting diodes in commercial greenhouse. *Journal of Horticulture and Postharvest Research*, *6*(4), 331-348. https://doi.org/10.22077/jhpr.2023.6524.1322

Kazemi, D., Dehestani-Ardakani, M., Hatami, M., & Ghorbanpour, M. (2024). Research on the differences in phenotypic and photosynthetic biophysical parameters of begonias (Begonia rex) cultivars under various light spectral compositions. *Journal of Plant Growth Regulation*, *43*(1), 106-121. https://doi.org/10.1007/s00344-023-11059-z

Khan, M. A., Ali, A., Mohammad, S., Ali, H., Khan, T., Mashwani, Z. U. R., Asif, J., & Ahmad, P. (2020). Iron nano modulated growth and biosynthesis of steviol glycosides in Stevia rebaudiana. *Plant Cell, Tissue and Organ Culture*, *143(1)*, 121-130. https://doi.org/10.1007/s11240-020-01902-6

Khan, T., Ullah, M. A., Garros, L., Hano, C., & Abbasi, B. H. (2019). Synergistic effects of melatonin and distinct spectral lights for enhanced production of anti-cancerous compounds in callus cultures of *Fagonia indica*. *Journal of Photochemistry and Photobiology B: Biology*, *190*, 163-171. https://doi.org/10.1016/j.jphotobiol.2018.10.010

Khurshid, R., Ullah, M. A., Tungmunnithum, D., Drouet, S., Shah, M., Zaeem, A., Hameed, S., Hano, C. & Abbasi, B. H. (2020). Lights triggered differential accumulation of antioxidant and antidiabetic secondary metabolites in callus culture of *Eclipta alba* L. *Plos one*, *15*(6), e0233963.

Kim, S. J., Hahn, E. J., Heo, J. W., & Paek, K. Y. (2004). Effects of LEDs on net photosynthetic rate, growth and leaf stomata of chrysanthemum plantlets in vitro. *Scientia Horticulturae*, *101*(1-2), 143-151. https://doi.org/10.1016/j.scienta.2003.10.003

Kokalis-Burelle, N., Brito, J. A., & Hartman, R. D. (2017). Susceptibility of Seven Caladium (*Caladium × hortulanum*) Cultivars to Meloidogyne arenaria, M. enterolobii, M. floridensis, M. incognita, and M. javanica.. *Journal of Nematology, 49*(4), 457.

Li, H., Xu, Z., & Tang, C. (2010). Effect of light-emitting diodes on growth and morphogenesis of upland cotton (*Gossypium hirsutum* L.) plantlets in vitro. *Plant Cell, Tissue and Organ Culture, 103*(2), 155-163. https://doi.org/10.1007/s11240-010-9763-z

Lin, K.-H., Huang, M.-Y., Huang, W.-D., Hsu, M.-H., Yang, Z.-W., & Yang, C.-M. (2013). The effects of red, blue, and white light-emitting diodes on the growth, development, and edible quality of hydroponically grown lettuce (*Lactuca sativa* L. var. Capitata). *Scientia Horticulturea. 150,* 86-91. https://doi.org/10.1016/j.scienta.2012.10.002

Lin, Y., Li, J., Li, B., He, T., & Chun, Z. (2011). Effects of light quality on growth and development of protocorm-like bodies of *Dendrobium officinale* in vitro. *Plant Cell, Tissue and Organ Culture, 105*(3), 329-335. https://doi.org/10.1007/s11240-010-9871-9

Lotfi, M. (2022). Effects of monochromatic red and blue light-emitting diodes and phenyl acetic acid on *in vitro* mass production of *Pyrus communis* 'Arbi'. *Journal of Horticulture and Postharvest Research*, *5*(2), 119-128. https://doi.org/10.22077/jhpr.2021.4517.1229

Nacheva, L., Dimitrova, N., Koleva-Valkova, L.H., Stefanova, M., Ganeva, T., Nesheva, M., Tarakanov, I., & Vassilev, A. (2023). In vitro multiplication and rooting of plum rootstock 'Saint Julien' (*Prunus domestica* subsp. insititia) under fluorescent light and different LED spectra. *Plants, 12*(11), 21-25.

Nhut, D. T., Huy, N. P., Tai, N. T., Nam, N. B., Luan, V. Q., Hien, V. T., Tung, H. T., Vinh, B. T., & Luan, T. C. (2015). Light-emitting diodes and their potential in callus growth, plantlet development and saponin accumulation during somatic embryogenesis of *Panax vietnamensis* Ha et Grushv. *Biotechnology & Biotechnological Equipment*, *29*(2), 299-308. https://doi.org/10.1080/13102818.2014.1000210

Pashkovskiy, P.P., Soshinkova, T.N., Korolkova, D.V. Kartashov, A. V., Zlobin, I. E., Lyubimov, V. Yu., Kreslavski, V. D., & Kuznetsov, V. V. (2018). The effect of light quality on the pro-/antioxidant balance, activity of photosystem II, and expression of light-dependent genes in *Eutrema salsugineum* callus cells. *Photosynthesis Research, 136*, 199-214. https://doi.org/10.1007/s11120-017-0459-7

Poudel, P. R., Kataoka, I., & Mochioka, R. (2008). Effect of red-and blue-light-emitting diodes on growth and morphogenesis of grapes. *Plant Cell, Tissue and Organ culture, 92*(2), 147-153. https://doi.org/10.1007/s11240-007-9317-1

Ramirez-Mosqueda, M. A., Iglesias-Andreu, L. G., & Bautista-Aguilar, J. R. (2017). The effect of light quality on growth and development of in vitro plantlet of *Stevia rebaudiana* Bertoni. *Sugar Tech*, *19*(3), 331-336. https://doi.org/10.1007/s12355-016-0459-5

Rezaie, S., Dehestani-Ardakani, M., & Kamali, K. (2018). A new protocol for direct regeneration of stevia plant (*Stevia rebaudiana* Bertoni) by tissue culture techniques. *Journal of Horticulture and Postharvest Research*, *1*(2), 97-104. https://doi.org/10.22077/jhpr.2018.1273.1010

Rojas-Martinez L., Visser R.G., & de Klerk G.J. (2010). The hyperhydricity syndrome: waterlogging of plant tissues as a major cause. *Propagation of Ornamental Plants, 10*(4), 169-175.

Santos-Tierno, R., Garcia, R., Fonseca, E. Santos-Tierno, R., Garcia, R., Fonseca, E., Faleiro, F., Moreira, D., Pacheco, G., & Mansur, E. (2021). Light quality and explant type modulate growth, antioxidant properties and bioactive compounds production of calluses of *Passiflora setacea* cv BRS Pérola do Cerrado. *Plant Cell, Tissue and Organ Culture, 147*, 635–646. https://doi.org/10.1007/s11240-021-02188-y

Sreedhar, R.V., Venkatachalam, L., & Neelwarne, B., (2009). Hyperhydricity-related morphologic and biochemical changes in Vanilla (*Vanilla planifolia*), *Journal of Plant Growth Regulator, 28,* 46-57. https://doi.org/10.1007/s00344-008-9073-4.

Stamps, R. H., & Savage, H. M. (2011, December). Effects of production shade levels and cultivar selection on potted and cut caladium performance in interiorscapes. In *Proceedings of the Florida State Horticultural Society* (Vol. 124, pp. 264-267).

Stepanova, A. Y., Solov'yova, A. I., & Salamaikina, S. A. (2020). Influence of spectral light composition on flavones formation in callus culture of *Scutellaria baicalensis* Georgi. *Pharmacognosy Magazine*, *16*(67), 156-160.

Tůmová, L., Tůma, J., Megušar, K., & Doležal, M. (2010). Substituted pyrazinecarboxamides as abiotic elicitors of flavolignan production in *Silybum marianum* (L.) gaertn cultures in vitro. *Molecules*, *15*(1), 331-340. https://doi.org/10.3390/molecules15010331

Ullah, M. A., Tungmunnithum, D., Garros, L., Hano, C., & Abbasi, B. H. (2019). Monochromatic lights-induced trends in antioxidant and antidiabetic polyphenol accumulation in in vitro callus cultures of *Lepidium sativum* L. *Journal of Photochemistry and Photobiology B: Biology*, *196*, 111505. https://doi.org/10.1016/j.jphotobiol.2019.05.002

William, J., & Carpenter. (1990). Light and temperature govern germination and storage of caladium seed. *HortScience, 25*(1), 71-74. https://doi.org/10.21273/HORTSCI.25.1.71

Zhang, Y. S., Gu, S. J., Chen, J. J., & Cai, X. D. (2019). Effects of different nutrient solutions on the acclimatization of in vitro Caladium plantlets using a simplified hydroponic system. *Sains Malays*, *48*, 1627-1633. https://doi.org/10.17576/JSM-2019-4808-08

JOURNAL OF HORTICULTURE AND POSTHARVEST RESEARCH
2025, VOL. 8(1), 105-124

Journal of Horticulture and Postharvest Research

Journal homepage: www.jhpr.birjand.ac.ir

University of Birjand

Chitosan based coating enriched with *Spirulina platensis* and moringa leaf extracts preserved the postharvest quality of Mexican Lime (*Citrus aurantifolia*)

Soheila Aghaei Dargiri[1], Somayeh Rastegar[1,*] and Mahbobeh Mohammadi[1]

1, Department of Horticultural Sciences, Faculty of Agriculture and Natural Resources, University of Hormozgan, Bandar Abbas, Iran

ARTICLE INFO

Original Article

Article history:
Received 26 July 2024
Revised 17 September 2024
Accepted 2 October 2024

Keywords:
Antioxidant
Color
Enzyme
Phenolic compound

DOI: 10.22077/jhpr.2024.7961.1405
P-ISSN: 2588-4883
E-ISSN: 2588-6169

***Corresponding author:**
Department of Horticultural Sciences, Faculty of Agriculture and Natural Resources, University of Hormozgan, Bandar Abbas, Iran.

Email: rastegarhort@gmail.com; s.rastegar@hormozgan.ac.ir

© This article is open access and licensed under the terms of the Creative Commons Attribution License http://creativecommons.org/licenses/by/4.0/ which permits unrestricted, use, distribution and reproduction in any medium, or format for any purpose, even commercially provided the work is properly cited.

ABSTRACT

Purpose: The limited shelf life of Mexican lime fruits when stored under ambient conditions is a significant challenge. The progressive color alteration and loss of freshness can lead to reduced marketability and increased its waste. **Research Method**: The objective of this research was to preserve the storage quality of Mexican lime fruit by employing chitosan 1.5%, chitosan 1.5% + spirulina algae (Sp) (1%), and *Moringa oleifera* (Mo) leaf extracts (1%) at 20 ± 2 °C and 50-60% relative humidity for 24 days. **Findings**: The findings indicated that the samples coated with chitosan + Sp experienced a significantly lower weight loss compared to the control (19.8%) fruit after 24 days of storage, with a weight loss of 16.4%. A significant difference was observed between the control and treated fruit in terms of *a** color parameter, with the highest value found in the control group (2.5) and the lowest value found in the chitosan-treated group (-6.7). The treated fruit exhibited significantly higher levels of phenol and flavonoid content compared to the control group. After the 24 days of storage, the chitosan 1.5% + Sp treatment displayed the highest antioxidant activity (88.66%), followed closely by the chitosan + Mo treatment (88.76%), while the control group exhibited the lowest antioxidant activity (78.75%). The treatments exhibited a significant decrease in polyphenol oxidase (PPO) enzyme activity compared to the control group, accompanied by an increase in the activity of peroxidase (POD) and catalase (CAT) enzymes. **Research limitations**: There was no limitation. **Originality/value**: Generally, the utilization of chitosan edible coatings, specifically chitosan combined with spirulina algae, has shown promising results in preserving the quality and extending the shelf life of Mexican lime fruit stored at 20 ± 2 °C.

INTRODUCTION

Lime (*Citrus aurantifolia*) is a citrus fruit that is widely grown after oranges and mandarins. It has many economic, nutritional, and health benefits. Mexican lime is native to Southeast Asia and countries such as India, Mexico, Brazil, China, and Iran are recognized as major producers of limes (Khan et al., 2017). The appearance of the fruit is a key factor that determines its quality and marketability (Raddatz-Mota et al., 2019). However, green lime easily turns into yellow and green uneven fruits after harvest, which seriously affects the appearance quality of the fruit (Zhang & Zhou, 2019). External color is one of the key factors that define the external quality of citrus fruits (Wang et al., 2022). The challenges that restrict the postharvest life of limes, such as the reduction in weight and the discoloration of the peel, are indeed significant factors contributing to postharvest losses (Khan et al., 2017). It is important to address these challenges to minimize losses and extend the shelf life of limes. By addressing these challenges through appropriate postharvest treatments, the postharvest life of limes can be extended, reducing losses, and ensuring a consistent supply of high-quality fruit to the market. Research has explored the use of melatonin, methyl jasmonate, gamma-aminobutyric acid, and commercial wax enriched with tea seed oil as treatments to improve the postharvest quality of citrus fruits (Firozi et al., 2021; Rastgoo et al., 2024)

Recent studies have revealed that the use of edible coatings can effectively slow down the physiological processes associated with fruit ripening. These coatings create a barrier that partially blocks the pores of the fruit, leading to a reduction in respiration and transpiration rates (Maringgal et al., 2020). It has been reported that a wax treatment containing palm oil, guar gum, sorbitol, and glycerol preserved the quality of lime fruits stored under cold conditions (13 ± 2°C and 85% RH) by reducing weight loss and maintaining other important characteristics (Wijewardane, 2022).

Chitosan edible coating is a promising technology for postharvest preservation of fruits and vegetables, as it is environmentally friendly, biologically safe, and cost-effective (Shiekh et al., 2013). Chitosan edible coating is a type of biodegradable and biocompatible film that can be applied to the surface of fruits and vegetables to extend their postharvest shelf life and quality. It has many beneficial properties, such as antimicrobial, antioxidant, anti-browning, and moisture barrier effects. Chitosan edible coating can prevent or delay the microbial decay, enzymatic browning, water loss, softening, and ripening of fruits and vegetables during storage and transportation (Kore et al., 2017). Chitosan edible coating can be used alone or in combination with other natural additives, such as essential oils, plant extracts, nanoparticles, or wax (Saxena et al., 2020). Plant extracts contain a wide range of bioactive compounds such as antioxidants, antimicrobials, and phytochemicals, which can provide numerous benefits when incorporated into edible coatings. Spirulina is a nutrient-rich blue-green algae that is known for its high protein content, as well as its vitamins, minerals, and antioxidants (Ashoush & Mahdy, 2019) . In addition, spirulina is widely recognized as a natural and healthy ingredient, and its addition to edible coatings can enhance the perceived value and appeal of the coated products. Ramji and Vishnuvarthanan (2022) have proposed that due to its significant phytonutrient value and the presence of salicylic, chlorogenic, caffeic acids, and tocopherol, this microalga demonstrated great potential for its application as a pharmaceutical agent and nutritional supplement. In addition, the chitosan film spirulina exhibited promising features such as high tensile strength, low oxygen and water vapor transmission rate (Ramji & Vishnuvarthanan, 2022). There have been numerous reports on *Moringa oleifera* that have shed light on its considerable protein content, β-carotene, vitamins, phenolics, flavonoids, fatty acids, and other bioactive compounds (Saucedo-Pompa et al., 2018). Moringa leaves contain a high concentration of phenolic acids, flavonoids, glucosinolates, and

isothiocyanates. Multiple investigations have demonstrated the potential of moringa leaves as a functional additive in food products and food applications (Kubheka et al., 2020). By incorporating spirulina and moringa extract into edible coatings, the products can benefit from the nutritional value and health-promoting properties associated with spirulina. This can make coated products more attractive to health-conscious consumers who are seeking functional foods with added nutritional benefits (Budak & Sarıkaya, 2022). Spirulina algae and moringa extract were added to chitosan to produce a coating with enhanced functionality and improved properties.

In our previous experiments, we demonstrated that the application of guar (Ebrahimi & Rastegar, 2020) and alginate (Rastegar & Atrash, 2021) edible coatings enriched with spirulina effectively preserved the quality of mango fruit during storage. It has been demonstrated that gum Arabic (GA) 15% + moringa and Carboxy methylcellulose (CMC) 1% + moringa retained fruit firmness and lowered weight loss of avocado fruit (Kubheka et al., 2020). Previous research has provided evidence that the implementation of CMC coatings can effectively contribute to the preservation of Kinnow mandarin fruit quality throughout cold storage. Specifically, these coatings have been shown to mitigate weight loss, sustain desirable textural attributes, and minimize adverse physiological changes (Baswal et al., 2020).

Given the recent advancements in utilizing edible coatings for preservation of fruit quality, this study seeks to employ an innovative method by incorporating chitosan with moringa and spirulina extracts to maintain the quality of Mexican lime fruits (*Citrus aurantifolia*) during storage. To the best of our knowledge, there is limited research conducted on the combination of chitosan with spirulina and moringa extracts, highlighting a gap in combinatorial studies in this specific context. The primary objective is to examine the impact of this formulation on prolonging the shelf life of the Mexican lime fruits at a temperature of 20 ± 2 °C. Building upon existing scientific discussions highlighting the potential advantages of edible coatings in preserving fruit quality and reducing postharvest losses, we aim to explore a novel combination of chitosan, spirulina, and moringa extracts to enhance the mechanical and barrier properties of the coatings.

MATERIALS AND METHODS

Preparation of chitosan and fruit materials

Mature green Mexican lime fruit was collected from a commercial orchard in Mosafr-Abad plain (27 °N, 57 °E), Ziarat-Ali village, Rodan City, Hormozgan province, Iran. In terms of climate, this region has an average temperature of 37 °C and humidity of 64%. The fruits were packed in a plastic box and transported to the laboratory within 2 hours. Fruits were selected based on the similarity in size, absence of physical damage, and microbiological contamination. The 120 fruits were separated into groups of four, for treatment in triplicate. Edible coating based on chitosan (Sigma-Aldrich) was prepared as described by Wang & Gao (2013). Chitosan solutions were made by dissolving 1.5 g chitosan in acetic acid (0.5 mL acetic acid/100 mL de-ionized H_2O). The pH values for control and all chitosan solutions were adjusted to 5.6 with 1.0 mol/L NaOH. Extracts of spirulina (1%) and moringa (1%) were also prepared. The aqueous extracts prepared by stirring 40 g of the *Spirulina platensis* and *Moringa oleifera* leave powders in 100 ml of distilled water as describe by Ebrahimi & Rastegar, (2020). The treatments consisted of immersing fruits for 5 min at room temperature (20°C) in: (a) chitosan 1.5% (b) chitosan (1.5%) + spirulina (Sp) (c) chitosan (1.5%) + moringa (Mo). The control fruits also were immersed in 0.5 mL acetic acid/100 mL de-ionized H_2O. Following the air-dried of fruits at 20 °C for 1 h, 10 fruits for each replication

(3n) were packed in transparent plastic container with lid at a temperature of 20 ±2 °C and 50-60% relative humidity (RH) for 24 days.

Determination of physiological loss in weight (PLW)

The mass difference method determined the physiological weight loss of lime fruits (coated and controlled). In this context, coated lime fruits were weighed on the first and last day of storage using a weighing scale (Singh & Reddy, 2006). The reduction in lime mass (as percentage reduction) was calculated using formula 1:

$$PLW\ (\%) = \frac{w_0 - w_1}{w_0} \times 100 \qquad (1)$$

Where w_0 represents the initial mass and w_1 represents the final mass at 24 days.

Total soluble solids (TSS) and titratable acidity (TA)

The fruit juice was examined utilizing a DBR95 handheld refractometer, which was manufactured in Thailand. The analysis was conducted at a temperature of 25 °C, and the TSS content of the juice was expressed as a °Brix (Gupta et al., 2022). For the determination of TA, the research team utilized a titration method involving a 0.1 M NaOH solution, adjusting the pH of the lime juice samples to 8.2.

Fruit color

The color of both the control and coated lime fruits was assessed using the CIE Lab color space, which includes three coordinates: L^* (lightness), a^* (red-green axis), and b^* (blue-yellow axis). The measurements were carried out using a Chroma meter CR-400 (Konica, Tokyo, Japan) (McGuire, 1992).

Total phenolic content (TPC), total flavonoid content (TFC) and radical scavenging activity (RSA)

The extraction process involved homogenizing fruit juice samples with an 80% methanol solution. Afterward, the homogenate was centrifuged at a speed of 4000 × *g* for 10 min at room temperature. The supernatant was further utilized for analysis to quantify the phenol, and flavonoid content and evaluate antioxidant activity (Mohammadi et al., 2023).

TPC content in the lime fruit was measured using the standard method with the Folin–Ciocâlteu (FC) reagent with some changes (Ordonez et al., 2006). The methanol extract (0.3 mL) was mixed with the diluted Folin–Ciocalteu reagent (1.5 mL) and after 5 min, the sodium carbonate solution (7 %, 1.2 mL) was added to prepare the reaction mixture. The absorbance at 750 nm was measured using a UV spectrophotometer after the reaction mixture was incubated for 90 min. The phenolic content was expressed as the equivalent of gallic acid (mg/g FW) in the lime fruit extract.

The aluminum chloride spectrophotometric method followed by Chang et al. (2002) was used to measure the TFC of the control and coated fruits that were stored. The methanol extract (0.5 ml) and the $AlCl_3$ solution (10 %, 0.1 mL) were mixed with the acetate potassium solution (1 mM, 0.1 mL). The mixture was left for 30 min at room temperature. The absorbance was measured at 415 nm using a UV spectrophotometer. The flavonoid content of fruit extract was calculated using quercetin as a standard and expressed in mg/g FW.

The lime fruit RSA was measured using the standard DPPH 2, 2-diphenyl-1-picryl-hydroxyl) assay method with some changes (Brand-Williams et al., 1995). The lime fruit extract (30 µL) was mixed with the DPPH solution (150 µL DPPH) that was made by dissolving 0.025 g DPPH in 100 mL of the 85 % methanol. The mixture was left in the dark

for 40 min and then the absorbance was measured at 517 nm using a UV spectrophotometer. The equation 2 was used to calculate the results and they are expressed as the inhibition percentage.

$$I_n\ (\%) = \frac{C_a - S_a}{C_a} \times 100 \quad (2)$$

Where C_a and S_a represent the control absorbance and sample absorbance, respectively.

The activity of antioxidant enzymes

The modified method based on Aebi (1984) was used for measuring catalase (CAT) and peroxidase (POD) activity. Initially, a 2.0 g sample was homogenized in 20 mL of phosphate buffer (50 mM and pH 7) containing 1 mM EDTA and 4% (W/V) PVPP, while maintaining the temperature in an ice bath. Subsequently, the solution was subjected to centrifugation at 6000 ×*g* for 10 min at 4 °C, and the resulting supernatant was collected for the CAT, POD and PPO activity assay.

For CAT assay, a reaction mixture containing 0.2 enzyme extract in 50 mM sodium phosphate buffer (pH 7.0) and 150 µL of 20 mM hydrogen peroxide (H_2O_2). The CAT enzyme in the solution catalyzes the breakdown of H_2O_2, leading to a reduction in absorbance at 240 nm. The results were expressed as U mg^{-1} FW.

For the assay POD, the reaction cuvette was prepared by adding 60 µL of 0.05 M guaiacol, 20 µL of the enzyme solution, and 20 µL of 0.05 M hydrogen peroxide. The changes in absorbance of the mixture were recorded at 15 s intervals over 2 min at a wavelength of 470 nm and the results were expressed as U mg^{-1} FW (Mohammadi et al., 2023).

The peroxidase (PPO) activity extraction and assay were performed following the protocol outlined by Serradell et al. (2000). The resulting reaction mixture consisted of 80 µL of 0.5 M catechol and 100 µL of 0.05 M phosphate buffer (pH 6.5). This mixture was incubated at 35 °C for 5 min, following which 20 µL of enzyme extract PPO was added. The increase in absorbance was read at 420 nm for 3 min and the results were expressed as U mg^{-1} FW.

Statistical analysis

Experiments were conducted factorially in the form of a completely randomized design (CRD) with two factors (coatings and time (0 and 24 days)) for this study. The experiment was done with three repetitions and each repetition included 10 fruits. The data were analyzed using analysis of variance (ANOVA), and the mean values were compared with the LSD test at ($P < 0.05$) significance level using the SAS software (version 9.1). Principal component analysis (PCA) and Pearson correlation were calculated using R v3.4.3 9 software (R Core Team, 2022).

RESULTS

Weight loss

Figure 1 describes the assessment of weight loss in both the control and coated samples throughout the storage period. Throughout the storage period at 20 ± 2 °C, the weight loss rate of each sample progressively increased and reached its peak at 24 days of storage. The coated samples demonstrated lower weight loss compared to the control samples, with the chitosan + Sp coating exhibited the minimum weight loss of 16.4% (Fig. 1).

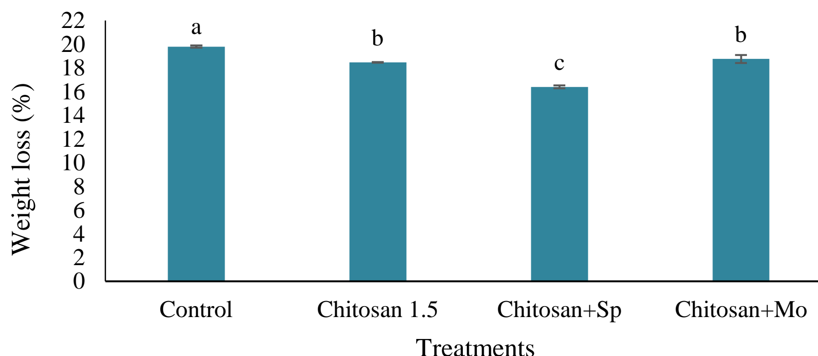


Fig. 1. Effect of treatment of chitosan, chitosan 1.5, chitosan + spirulina algae, and chitosan + moringa extract on a) weight loss of fruit juice of lime juice (*citrus aurantifolia*) stored at 20 ±2 °C. Vertical bars indicate the means' standard error (S.E.) (n = 3). The means with the same LSD test letters in each column are not statistically significant (P ≤ 0.05).

TSS and TA

The interaction effect of time and treatment on TSS and TA is shown in Fig. 1. During the storage period, the TSS content increased in all samples. However, after 24 days of storage, the fruit treated with chitosan +SP exhibited significantly lower (6.8 °Brix) TSS compared to the other treatments and the control group (7.9 °Brix) (Fig. 2a).

Fruit TA decreased over the storage period, but this decline was less pronounced in the treated fruits. Consequently, after 24 days of storage, a significant difference in TA was observed between the control (6.7%) and treatment groups (approximately 10.5%) (Fig. 2b).

Color parameters

The interaction effect of time and treatment on color parameters is shown in Fig. 3. Changes in the color parameters (L^*, a^*, and b^*) of lime fruit during storage were determined ($p < 0.05$). The characteristics of the color of the fruit at the time of harvest were L*=14.7, a*= 7.16 and b*=15.4. During storage, there was a significant increase in the L^* value of the fruit. However, after 24 days of storage, there was no significant difference found between the treated fruit and the control group (Fig. 3a).

The value of a^* increased gradually with increasing storage time. After 24 days of storage, the a^* value of control showed a significant increased rather than other samples. The minimum changes in the a^* was observed in the chitosan + Sp treatment, which also showed a significant difference compared to the other two treatments (Fig. 3b). The highest (most positive) level of a^* was observed in control (2.5) fruits. Similarly, the trend observed in the changes of the L^*, the b^* also gradually increased over time. There were no notable distinctions observed between the control and treatment groups concerning the b^* at final day (Fig. 3c).

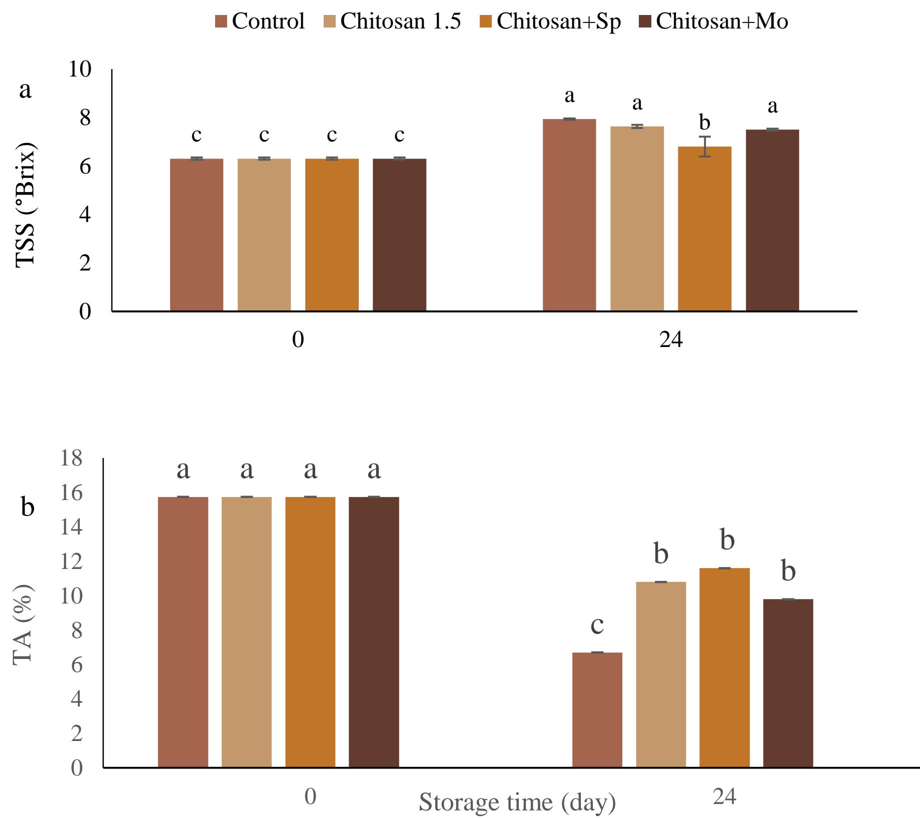


Fig. 2. Interaction effect of time and treatment (chitosan, chitosan 1.5, chitosan + spirulina algae, and chitosan + moringa leaf extract) on a) TSS, and b) TA of lime juice (*citrus aurantifolia*) stored at 20 ±2 °C. Vertical bars indicate the means' standard error (S.E.) (n = 3). The means with the same LSD test letters in each column are not statistically significant ($P \leq 0.05$).

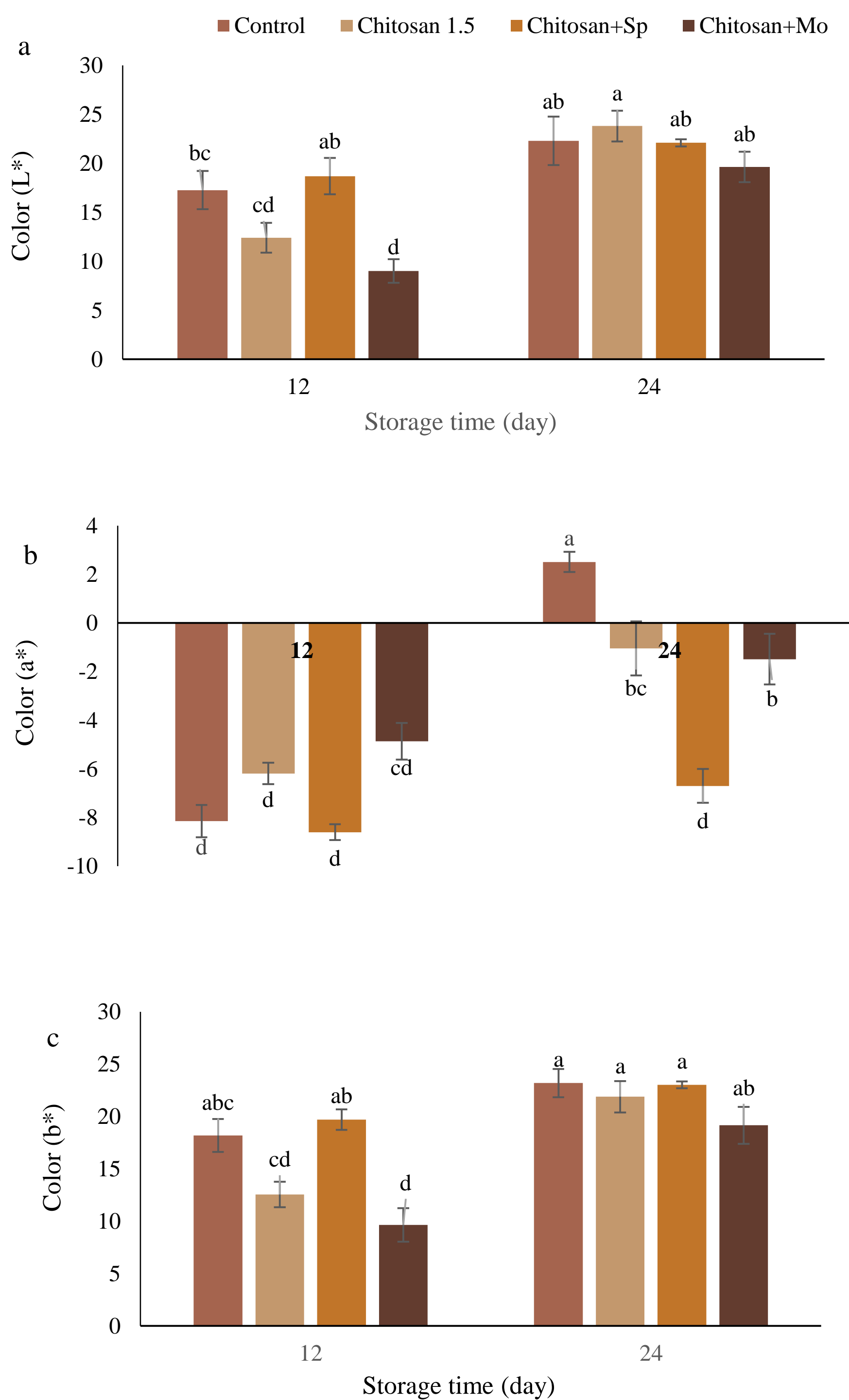


Fig. 3. Interaction effect of time and treatment (chitosan, chitosan 1.5, chitosan + spirulina algae, and chitosan + moringa leaf extract) on a) *L**, b) *a**, and c) *b** of lime juice (*citrus aurantifolia*) stored at 20 ±2 °C. Vertical bars indicate the standard error (S.E.) of the means (n = 3). The means with the same LSD test letters in each column are not statistically significant ($P \leq 0.05$).

TPC, TFC and RSA

The interaction effect of time and treatment on TPC, RSA and TFC is shown in Fig 4. As shown in Figure 4a, TPC content did not change significantly in the treated fruits, while a significant decrease was observed in the control fruits during storage.

The TFC in the fruits significantly decreased during storage, but the rate of reduction was higher in the control (reached 1.5 to 0.57 mg/g) group compared to the treated groups. A significant difference was observed between the control and treatment groups after 24 days of storage (Fig. 4b).

The trend in RSA reflected that of phenols, as a notable decrease was observed in the control group, while no significant changes were observed in the treated groups when compared to the initial day (Fig. 4c).

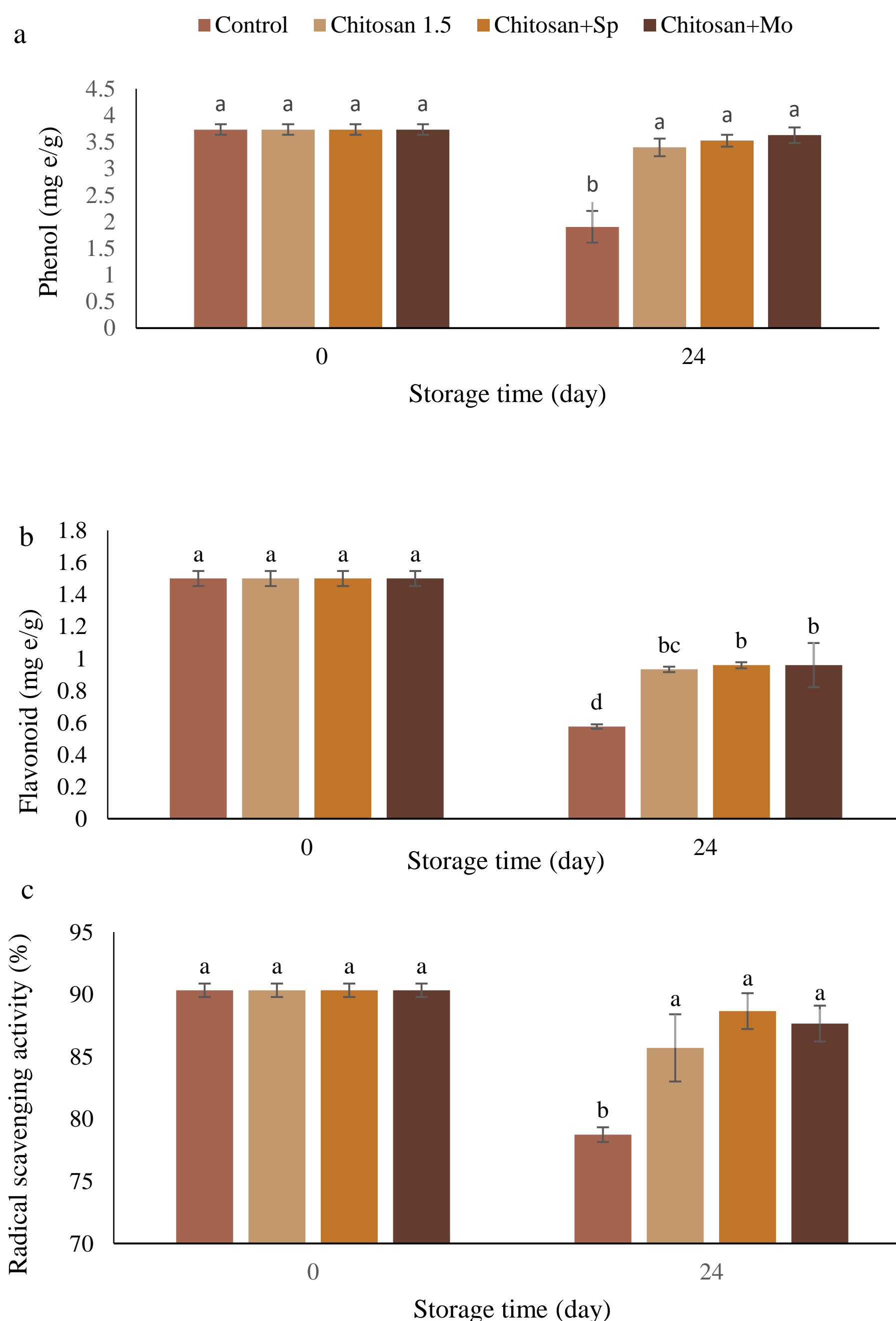


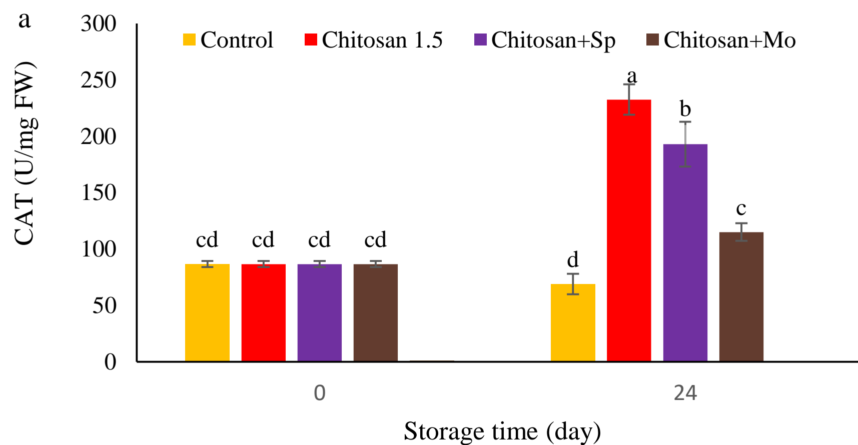
Fig. 4. Interaction effect of time and treatment (chitosan, chitosan 1.5, chitosan + spirulina algae, and chitosan + moringa leaf extract) on a) phenol, b) flavonoid, and c) radical scavenging activity of lime juice (*citrus aurantifolia*) stored at 20 ±2 °C. Vertical bars indicate the standard error (S.E.) of the means (n = 3). The means with the same LSD test letters in each column are not statistically significant (P ≤ 0.05).

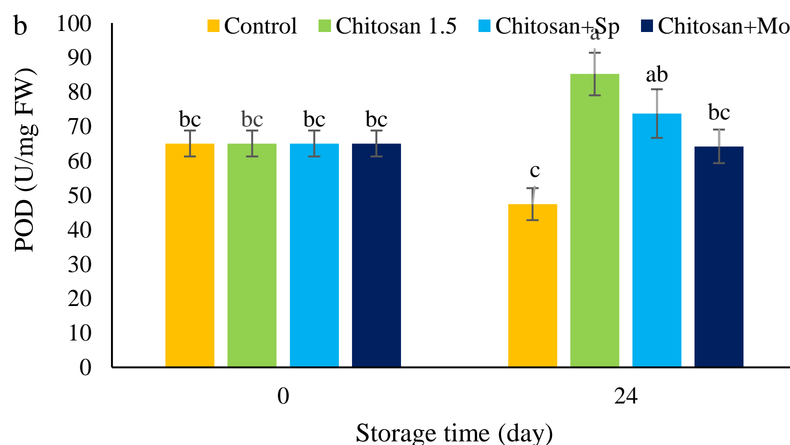
CAT, POD, and PPO

The interaction effect of time and treatment on CAT, POD, and PPO is shown in Figure 5. During storage, the activity of the CAT enzyme increased in the treated groups, while it remained relatively constant in the control group (Fig. 5a). The maximum enzyme activity was observed in the chitosan 1.5% treatment with a percentage increase of 168.6%.

The activity of the POD enzyme, unlike the treated samples that showed an increase during storage, decreased in the control samples. It changed from 65 U/mgFW to 47.5 U/mgFW value. Chitosan treatment exhibited the highest (85.2 U/mgFW) POD enzyme activity after 24 days of storage (Fig. 5b).

The activity of the PPO enzyme increased in most of the fruit, but the increase in enzyme activity was significantly advanced in the control group compared to the treated groups. After 24 days of storage, the enzyme activity in the control group was approximately 1.5 times higher than the enzyme activity in the chitosan 1.5%-treated group (Fig. 5c).





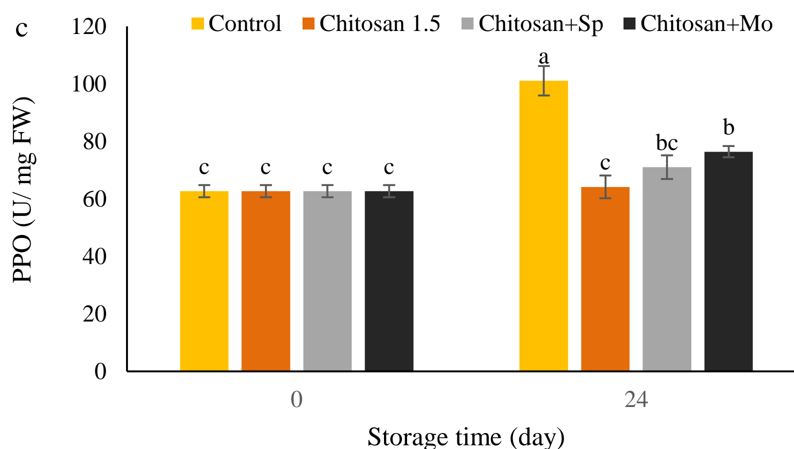


Fig. 5. Interaction effect of time and treatment (chitosan, chitosan 1.5, chitosan + spirulina algae, and chitosan + moringa extract) on a) CAT, b) POD, and c) PPO of lime juice (*Citrus aurantifolia*) stored at 20 ±2 °C. Vertical bars indicate the standard error (S.E.) of the means (n = 3). The means with the same LSD test letters in each column are not statistically significant (P ≤ 0.05).

Correlation and principal component analysis (PCA)

The heat map obtained from Hierarchical Cluster Analysis (HCA) showed that the different treatments and measured parameters were divided into two groups (Fig. 6). The group included: (I): DDPH, POD, TPC, TFC, TSS, pH, and CAT and (II): L^*, WL, b^*, a^*, and PPO. On the other hand, clustering into two groups that include: (IV): Chitosan 1.5-T2, Chitosan+Sp-T2, Control-T2 and Chitosan +Mo-T2 (V): Chitosan+Sp-T1, Control-T1, Chitosan 1.5-T1, and Chitosan +Mo-T1 (T1 and T2 represent the initial and subsequent time of storage, respectively) (Fig. 6a). The results showed that with the passage of time, chitosan + Sp and chitosan + Mo showed the highest positive correlation with L^*. In the observed control, it showed a positive correlation with L^* and it showed more darkness (darkness) of lime color. It showed a positive correlation of weight loss with the treatments of chitosan +SP over time (24 days). At the end of the experiment, the control showed a positive correlation with b^*, which can be said that the lime fruit in the control tended to be green to yellow in comparison to the treatments. Chitosan 1.5 showed a positive correlation with PPO at 24 days of storage time in fruit. The results showed a positive correlation in the treatment of chitosan 1.5 at the end of the experiment with the CAT enzyme, which indicates an increase in the activity of the CAT enzyme.

All 12 physiological and biochemical traits were loaded into two principal components (PC1 and PC2), which explained 1.87% of the total variances (Fig. 6a). While a lower proportion of variance (9.7%) was represented by PC2 (Fig. 6b). The most similarity in treatments was observed in CAT, DPPH, POD, PPO, b^* and L^* treatments. In the next group, TFC parameter, pH, TPC and TSS were placed in the same group in terms of similarity. Weight loss and a^* showed more similarity in another group.

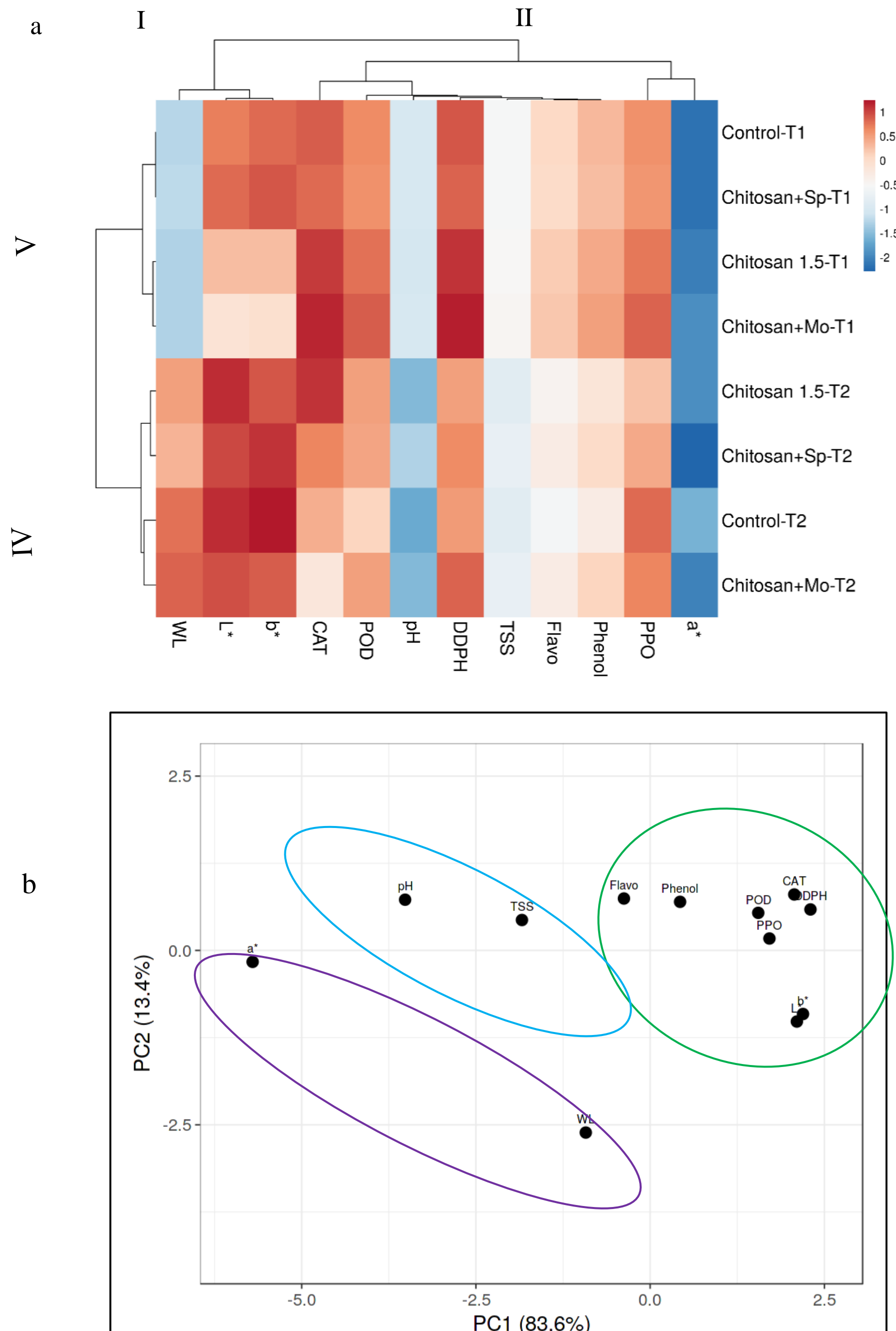


Fig. 6. a) Hierarchical cluster analysis (HCA) of cover treatments and variable trait relationships in lime fruit over time. Heat map of Pearson's correlation coefficient (r) values of variable traits, where the color scale that indicates r coefficient values (r=0.5 to 2) indicates positive (red) and negative (blue) correlations. (b) Dendrogram clustering of coating treatments in lime fruit and dose times 0 and 24 in all treatments except for fruit color. b) Principal component analysis (PCA) of variable trait relationships in lime fruit. PCA loading diagrams of the examined variable traits, and the circles show the highest correlation of the variables. PCA loading graphs of variable traits were examined. The tested variables include Flavo: Flavonoid, pH, Phenol, TSS: The Soluble Sugar, Color: L^*, a^*, b^*, WL: Weight Loss, PPO: Polyphenol Oxidase, POD: Peroxidase, CAT: Catalase, DDPH: Antioxidant activity.

DISCUSSION

The weight loss in fresh fruits and vegetables holds significant importance with respect to economic losses (Iftikhar et al., 2022). Weight loss during the storage of fresh fruits is a prevalent issue that significantly impacts their quality and shelf life. Weight loss in fruits is primarily attributed to moisture loss or dehydration, which can occur over time due to respiration, transpiration, and other physiological processes. During the storage of fruits, the loss of water content can lead to noticeable changes such as shrinkage, wilting, softening, and the development of a dull appearance (Kritzinger, 2019). Furthermore, weight loss can also lead to a decline in flavor and nutritional value, further compromising the overall quality of the fruits. Therefore, managing and minimizing weight loss is crucial to maintaining the freshness, appearance, and taste of fruits throughout their storage period (Artés et al., 2006). Edible coatings, which create a semi-permeable layer around fruit, can act as a barrier to water vapor, reducing transpiration and slowing down moisture loss from the fruit. This can help maintain the fruit's turgidity and prevent wilting or shrinkage, ultimately reducing weight loss (Sapper & Chiralt, 2018). In addition, edible coatings modulate the exchange of gases (such as oxygen and carbon dioxide) between the fruit and its external environment. This can help to slow down the respiration rate of the fruit, which in turn reduces weight loss associated with metabolic processes. Chitosan coating has shown a significant effect on weight loss of different fruits during storage, such as pear (Iftikhar et al., 2022) and apple (Zeb et al., 2020). The results exhibited that chitosan coating could decrease weight loss by up to 65% compared to the uncoated control (Parvin et al., 2023). In addition, the coated samples showed a lower weight loss than the control samples, with the chitosan + Sp coating showing a minimum weight loss of 16.4%. It has been reported that *Spirulina platensis* is rich in carbohydrates, mineral salts, high protein content, vitamins, antioxidants, and unsaturated fatty acids, which allow the formation of mechanical and interactive structural chains on the surface of the coated fruit, regulating transpiration and fruit mass loss (Santos et al., 2023). The incorporation of spirulina into films and coatings can improve their tensile strength and mechanical properties due to solid intermolecular interactions with the polymer matrix (Nakamoto et al., 2023). The inclusion of moringa in an edible carboxymethyl cellulose coating has also been documented to have a positive impact on the preservation of guava fruit. This was achieved by mitigating the loss of fruit moisture and reducing the rate of fruit respiration, thus enhancing the overall quality of the fruit during storage (Tesfay & Magwaza, 2017).

The TSS of fruits is known to influence their sweetness, which is an important factor in determining fruit quality and is extremely correlated with maturity in most fruits. In the current study the TSS values showed an increase in both coated and uncoated fruits, with uncoated fruits exhibited higher values (Thakur et al., 2019). The TSS content of citrus fruits tends to increase during storage periods due to various breakdown processes (Sun et al., 2019). However, the application of direct coatings has been observed to inhibit the synthesis and utilization of substances within the fruits, leading to decreased respiration rates and a subsequent decline in TSS concentration (Kou et al., 2014). By creating a modified atmosphere around the fruit, edible coatings can influence the physiological and biochemical processes that affect TSS. There have been varying reports on the effect of edible coatings on the TSS of different fruits. It has been reported that a polysaccharide-based edible coating did not significantly affect the TSS levels of juice in various citrus fruits, including mandarins, oranges, and grapefruit (Arnon et al., 2014). After 24 days of storage, the fruit treated with chitosan + Sp showed significantly lower TSS compared to the control group. Chitosan-based coatings have the potential to control fruit ripening, particularly when combined with

Spirulina. Bioactive substances present in spirulina, including vitamins, minerals, and phytohormones, may have an impact on the processes involved in fruit ripening (Hadiyanto et al., 2019). The fruit's shelf life might be prolonged and overripening could be delayed by the chitosan + Sp treatment by reducing ethylene production, the respiration rate of fruits and other ripening-related activities (Rastegar & Atrash, 2021) .

Citrus fruits are known to undergo color changes during storage, and the peel color is one of the parameters that can be affected. The citrus fruit color changes because of the breakdown of chlorophyll and the accumulation of carotenoids pigments in the outer layer of the fruit (Keawmanee et al., 2022). Some of the studies found that chitosan coating delayed color changes in fruit skin and pulp during storage. Coatings can help to extend the shelf life and color preservation, improve the visual quality, prevent shriveling and wilting, and maintain biochemical properties of fruits (Firdous et al., 2022). Similar to current study Krishna and Rao (2014) reported a lower b^* value in guava fruits coated with chitosan (1 and 2%) in comparison to fruits treated with acetic acid and those that were left untreated. Coatings can help prevent chlorophyll breakdown by lowering O_2 and raising CO_2 in the storage atmosphere. This has been shown to be beneficial in previous studies (Olawuyi et al., 2019). Contrary to the results of the present research, a study on mangoes found that applying a composite of 10% Arabic gum and 1% chitosan as an edible coating did not significantly affect the color of the fruit skin or pulp (Handojo et al., 2022). A study on blueberries found that chitosan coating plus silicon dioxide nanoparticles and nisin helped to control the color parameters of the fruit during storage (Eldib et al., 2020). Overall, the effect of chitosan coating on the color parameters of fruit during storage seems to depend on the specific fruit and the type of coating used. However, in general, chitosan coating appears to delay color changes and help maintain the quality of fruit during storage. The combined treatment of chitosan with Spirulina improved fruit color during storage in this study. Fruit color generally improves during storage by combining chitosan and spirulina treatment. This is achieved by lowering oxidative stress, stabilizing pigments, preventing microbial development, preserving the water status, and postponing senescence processes. Together, these systems preserve the aesthetic appeal and freshness of the fruit throughout the storage period (Santos et al., 2023). One of the effects of chitosan edible coating on fruits and vegetables is the modulation of their phenolic and flavonoid compounds. Phenolics and flavonoids are secondary metabolites that have antioxidant, anti-inflammatory, anticancer, and antimicrobial activities. They also contribute to the color, flavor, and nutritional value of fruits and vegetables (Esmaeili, 2024). Chitosan edible coating can reduce the oxygen permeability and water loss of fruits and vegetables, thus preventing oxidative stress and enzymatic browning that degrade phenolics and flavonoids (Sarengaowa et al., 2022). Edible coatings can be designed to have selective permeability, allowing certain gases, such as oxygen and carbon dioxide, to pass through while limiting the entry of other gases. This controlled gas exchange can help regulate the enzymatic activity involved in phenol metabolism, thus impacting the phenolic content of the fruit (García-Betanzos et al., 2017). In addition, Chitosan edible coating can induce the expression of genes related to flavonoid 3-hydroxylase (F_3H), phenylalanine ammonia-lyase (PAL), which increase the synthesis of phenolics and flavonoids in fruits and vegetables (Adiletta et al., 2021).

It has been reported that phenolic compound and the antioxidant activity in stored figs were significantly improved by the chitosan-based coating (Adiletta et al., 2019). Chitosan-based coating with rosemary essential oil increased the total phenolic content, antioxidant activity, color retention, and sensory quality of strawberries during storage (Quintana et al., 2021).

Both phenols and flavonoids contribute to the antioxidant capacity of fruits and vegetables, and their consumption has been linked to various health benefits. Phenolic compounds found in fruits and vegetables serve as crucial antioxidants that play a vital role in eliminating free radicals and safeguarding cells from damage (Toor & Savage, 2005). During the ripening process, flavonoids can undergo conversion into secondary phenolic compounds. Additionally, certain enzymes may act on these compounds as substrates specifically during the ripening stage. In both situations, there is a tendency for the concentration of these compounds within the fruit to decline (Howard et al., 2003). It has been suggested that the edible coating plays a role in inhibiting oxygen penetration, which leads to a significant delay in the breakdown of flavonoids (Ruzaina et al., 2017). The edible coating may cause the coated fruit to have a higher level of phenolic compounds. This is because the edible coating lowers the activity of polyphenol oxidase, which leads to less breakdown of phenolic compounds (Kerch, 2015). The observed reduction in flavonoid content in control samples could plausibly be attributed to their higher respiration rate, thereby leading to the breakdown of total phenolics. The fruits treated in this study showed higher phenol, flavonoid, and antioxidant content than the control. It has been reported that leaf extracts of moringa plants possess antimicrobial and antioxidant activities due to a high concentration of phenolics, vitamins, and carotenoids (Saucedo-Pompa et al., 2018). In addition, it has been reported that spirulina has antioxidant properties, which can help in the development of antioxidant coatings for various applications (Nakamoto et al., 2023). Moringa and spirulina, which are rich in antioxidants, flavonoids, and phenols, increase the protective ability of coatings by supplying more antioxidants that scavenge free radicals and stop the oxidation of phenolic components (Saucedo-Pompa et al., 2018; Budak & Sarıkaya, 2022).

The results of this research showed that during storage, the maximum activity of CAT and POD enzymes was observed in 1.5% chitosan treatment. Postharvest oxidative stress is a condition that can happen during the storage of fruit, which means that there is an imbalance between the production and elimination of reactive oxygen species (ROS) within the fruit tissues. ROS, such as H_2O_2, O^{2-}, and hydroxyl radicals, are generated as natural byproducts of cellular metabolism and can cause damage to cellular components if their levels are not properly regulated. The degree of protection against oxidative injury in fruit cells is closely related to the activity levels of antioxidant enzymes, particularly CAT and POD (Meitha et al., 2020). Numerous studies have provided evidence that the application of chitosan coating on fruit helps maintain the balance of intracellular oxidation metabolism. This is achieved through the efficient clearance of cytotoxic compounds via enzymatic antioxidants. Additionally, non-enzymatic antioxidants including glutathione (GSH), phenols, ascorbic acid (AA), anthocyanins, and flavonoids also contribute to this balance. By employing these antioxidants, chitosan-coated fruit effectively mitigates oxidative injury produced by reactive oxygen species, confirming the preservation of fruit quality, and extending its shelf life (Adiletta et al., 2021). Research has indicated that the application of chitosan coating on fig fruit leads to an increase in the activity of important antioxidant enzymes, including SOD, POD, and glutathione reductase (GR). By enhancing the activity of these antioxidant enzymes, chitosan coating contributes to the preservation of fig fruit quality during storage (Adiletta et al., 2019). The utilization of chitosan enriched with arginine nanoparticles in plum fruit has shown beneficial outcomes in maintaining fruit quality. Specifically, the application of this formulation has been observed to result in a decrease in the activity of the polyphenol oxidase (PPO) enzyme within the fruit, contributing to the preservation of its quality (Mahmoudi et al., 2022). It has been reported that the application of chitosan coating in pear fruit has been found to effectively restrict the activity of PPO enzyme. This restriction of PPO

activity helps prevent browning and consequently extends the storage life of the pear fruit (Adhikary et al., 2022).

In general, the coating of chitosan + Sp showed the best appearance (color and freshness) of the fruit. It has been reported that spirulina contains chlorogenic acid, which can bind and permeabilize the cell membrane of microorganisms, leading to the loss of membrane potential and inhibition of bacterial growth (Nakamoto et al., 2023). Spirulina is recognized for its abundance of bioactive compounds including proteins, vitamins, minerals, and antioxidants. These compounds play a significant role in the potential benefits of spirulina for the postharvest quality of fruits. By incorporating spirulina, several positive effects were observed, including increase of soluble solids, enhanced flesh firmness, increased ascorbic acid content, and a reduction in weight loss (Nakamoto et al., 2023).

CONCLUSION

In conclusion, the findings of this research demonstrate that the application of chitosan-based coatings, particularly when combined with spirulina algae, has demonstrated to be an effective method for preserving the storage quality and extending the shelf life of Mexican lime fruit stored at 20 ± 2 °C (Fig. 7). The coated fruit exhibited less weight loss (16.4%), improved color stability, maintained phenol and flavonoid content, and higher antioxidant activity compared to the control group. Furthermore, the treatments led to a decrease in PPO enzyme activity (36.6% rather than control) and an increase in the activity of POD (1.8-fold) and CAT (3-fold) enzymes, suggesting a positive impact on fruit freshness and quality. These results highlight the potential of chitosan coatings, in combination with natural extracts such as spirulina algae, as a viable solution to address the challenge of limited shelf life in Mexican lime fruits. Studying the impact of different combinations of chitosan-based coatings and natural extracts on the shelf life and health benefits of Mexican lime fruit to enhance consumer experience should be considered. Additionally, further research and optimization of coating formulations and application techniques may provide even more significant benefits in terms of fruit preservation and waste reduction in the future.

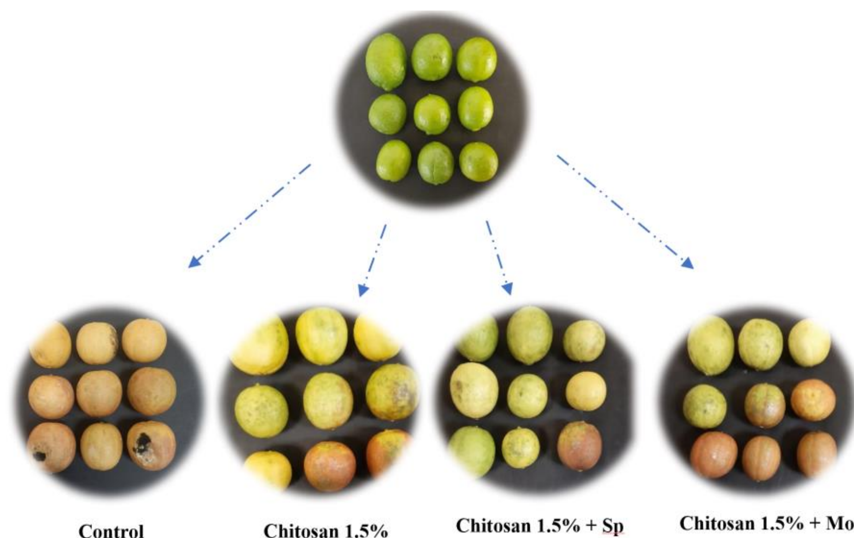


Fig .7 The effect of different treatments on maintaining the quality of Mexican lime fruit during 24 days of storage at 20 ± 2 °C.

CRediT authorship contribution statement

Soheila Aghaei Dargiri: Writing – original draft, Formal analysis, Data curation. Somayeh Rastegar: Review & editing, Validation, Methodology, Investigation, Project administration, Supervision. Mahbobeh Mohammadi: Performed experiment, 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.

Acknowledgments

The authors express their gratitude for the assistance provided by University of Hormozgan, Iran, in facilitating this research project.

REFERENCES

Adhikary, T., Gill, P. P. S., Jawandha, S. K., & Sinha, A. (2022). Chitosan coating modulates cell wall degrading enzymes and preserved postharvest quality in cold-stored pear fruit. *Journal of Food Measurement and Characterization*, *16*(2), 1395–1403. https://doi.org/10.1007/s11694-021-01169-w

Adiletta, G., Di Matteo, M., & Petriccione, M. (2021). Multifunctional role of chitosan edible coatings on antioxidant systems in fruit crops: A review. *International Journal of Molecular Sciences*, *22*(5), 2633. https://doi.org/10.3390/ijms22052633

Adiletta, G., Zampella, L., Coletta, C., & Petriccione, M. (2019). Chitosan coating to preserve the qualitative traits and improve antioxidant system in fresh figs (*Ficus carica* L.). *Agriculture*. https://doi.org/10.3390/agriculture9040084

Aebi, H. (1984). Catalase in vitro. In *Methods in enzymology,* Elsevier. Vol. 105, 121-126. https://doi.org/10.1016/S0076-6879(84)05016-3

Arnon, H., Zaitsev, Y., Porat, R., & Poverenov, E. (2014). Effects of carboxymethyl cellulose and chitosan bilayer edible coating on postharvest quality of citrus fruit. *Postharvest Biology and Technology*, *87*, 21–26. https://doi.org/10.1016/j.postharvbio.2013.08.007

Artés, F., Gómez, P. A., & Artés-Hernández, F. (2006). Modified atmosphere packaging of fruits and vegetables. *Stewart Postharvest Review*, *2*(5), 1-13. https://doi.org/10.2212/spr.2006.2.2

Ashoush, I. S., & Mahdy, S. M. (2019). Nutritional evaluation of cookies enriched with different blends of *Spirulina platensis* and *Moringa oleifera* leaves powder. *Journal of Food and Dairy Sciences*, *10*(3), 53–60. https://doi.org/10.21608/jfds.2019.36154

Baswal, A. K., Dhaliwal, H. S., Singh, Z., Mahajan, B. V. C., Kalia, A., & S Gill, K. (2020). Influence of carboxy methylcellulose, chitosan and beeswax coatings on cold storage life and quality of Kinnow mandarin fruit. *Scientia Horticulturae*, *260*, 108887. https://doi.org/10.1016/j.scienta.2019.108887

Brand-Williams, W., Cuvelier, M.E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology*, *28*(1), 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5

Budak, B., & Sarıkaya, S. B. Ö. (2022). Spirulina: properties, benefits and health-nutrition relationship. *Gümüşhane Üniversitesi Sağlık Bilimleri Dergisi*, *11*(4), 1654-1662. https://doi.org/10.37989/gumussagbil.1200004

Chang, C. C., Yang, M. H., Wen, H. M., & Chern, J. C. (2002). Estimation of total flavonoid content in propolis by two complementary colometric methods. *Journal of Food and Drug Analysis*, *10*(3), 178–182. https://doi.org/10.38212/2224-6614.2748

Ebrahimi, F., & Rastegar, S. (2020). Preservation of mango fruit with guar-based edible coatings enriched with *Spirulina platensis* and Aloe vera extract during storage at ambient temperature. *Scientia Horticulturae*, *265*. https://doi.org/10.1016/j.scienta.2020.109258

Eldib, R., Khojah, E., Elhakem, A., Benajiba, N., & Helal, M. (2020). Chitosan, nisin, silicon dioxide nanoparticles coating films effects on blueberry (*Vaccinium myrtillus*) quality. *Coatings*, *10*(10), 962. https://doi.org/10.3390/coatings10100962

Esmaeili, A. (2024). Polyphenols for antioxidant application: biochemistry of polyphenols. In *Science and engineering of polyphenols: Fundamentals and Industrial Scale Applications*. John Wiley & Sons, Inc. 436-451. https://doi.org/10.1002/9781394203932.ch17

Firdous, N., Moradinezhad, F., Farooq, F., & Dorostkar, M. (2022). Advances in formulation, functionality, and application of edible coatings on fresh produce and fresh-cut products: a review. *Food Chemistry*, 135186. https://doi.org/10.1016/j.foodchem.2021.135186

Firozi, M., Amiri, M., & Kahneh, E. (2021). Effect of tea seed oil on post-harvest quality of Moro blood orange. *Journal of Horticulture and Postharvest Research*, *4*(1), 115–126. https://doi.org/10.22077/jhpr.2020.3355.1143

García-Betanzos, C. I., Hernández-Sánchez, H., Bernal-Couoh, T. F., Quintanar-Guerrero, D., & Zambrano-Zaragoza, M. de la L. (2017). Physicochemical, total phenols and pectin methylesterase changes on quality maintenance on guava fruit (*Psidium guajava* L.) coated with candeuba wax solid lipid nanoparticles-xanthan gum. *Food Research International*, *101*, 218–227. https://doi.org/10.1016/j.foodres.2017.08.065

Gupta, A. K., Pathak, U., Tongbram, T., Medhi, M., Terdwongworakul, A., Magwaza, L. S., … Mishra, P. (2022). Emerging approaches to determine maturity of citrus fruit. *Critical Reviews in Food Science and Nutrition*, *62*(19), 5245–5266. https://doi.org/10.1080/10408398.2021.1883547

Hadiyanto, H., Christwardana, M., Suzery, M., Sutanto, H., Nilamsari, A. M., & Yunanda, A. (2019). Effects of carrageenan and chitosan as coating materials on the thermal degradation of microencapsulated phycocyanin from *spirulina sp*., *15*(5–6). https://doi.org/10.1515/ijfe-2018-0290

Handojo, L. A., Shofinita, D., Evelina, G., & Nasution, A. N. (2022). Edible coating development to extend shelf life of mangoes (*Mangivera indica* L.). In *IOP Conference Series: Earth and Environmental Science* (Vol. 980, p. 12046). IOP Publishing. https://doi.org/10.1088/1755-1315/980/1/012046

Howard, L. R., Clark, J. R., & Brownmiller, C. (2003). Antioxidant capacity and phenolic content in blueberries as affected by genotype and growing season. *Journal of the Science of Food and Agriculture*, *83*(12), 1238–1247. https://doi.org/10.1002/jsfa.1532

Iftikhar, A., Rehman, A., Usman, M., Ali, A., Ahmad, M. M., Shehzad, Q., Shabbir, M. A. (2022). Influence of guar gum and chitosan enriched with lemon peel essential oil coatings on the quality of pears. *Food Science & Nutrition*, *10*(7), 2443–2454. https://doi.org/10.1002/fsn3.2880

Keawmanee, N., Ma, G., Zhang, L., Yahata, M., Murakami, K., Yamamoto, M., Kato, M. (2022). Exogenous gibberellin induced regreening through the regulation of chlorophyll and carotenoid metabolism in Valencia oranges. *Plant Physiology and Biochemistry*, *173*, 14–24. https://doi.org/10.1016/j.plaphy.2022.04.016

Kerch, G. (2015). Chitosan films and coatings prevent losses of fresh fruit nutritional quality: a review. *Trends in Food Science & Technology*, *46*(2), 159–166. https://doi.org/10.1016/j.tifs.2015.08.003

Khan, M. M., Al-Yahyai, R., & Al-Said, F. (2017). *The lime: botany, production and uses*. CABI. https://doi.org/10.1079/9781780647846.0000

Kore, V. T., Tawade, S. S., & Kabir, J. (2017). Application of edible coatings on fruits and vegetables. *Imperial Journal of Interdisciplinary Research*, *3*(1), 591–603.

Kou, X., Wang, S., Zhang, Y., Guo, R., Wu, M., Chen, Q., & Xue, Z. (2014). Effects of chitosan and calcium chloride treatments on malic acid-metabolizing enzymes and the related gene expression in post-harvest pear cv. 'Huang guan'. *Scientia Horticulturae*, *165*, 252–259. https://doi.org/10.1016/j.scienta.2013.11.008

Krishna, K. R., & Rao, D. V. S. (2014). Effect of chitosan coating on the physiochemical characteristics of guava (*Psidium guajava* L.) fruits during storage at room temperature. *Indian Journal of Science and Technology*, *7*(5), 554. https://doi.org/10.17485/ijst/2014/v7i5.20

Kritzinger, I. (2019). Postharvest moisture loss in Japanese plums. Doctoral dissertation. Stellenbosch: Stellenbosch University.

Kubheka, S. F., Tesfay, S. Z., Mditshwa, A., & Magwaza, L. S. (2020). Evaluating the efficacy of edible coatings incorporated with moringa leaf extract on postharvest of 'Maluma' avocado fruit quality and its biofungicidal effect. *HortScience*, *55*(4), 410–415. https://doi.org/10.21273/HORTSCI14721-19

Mahmoudi, R., Razavi, F., Rabiei, V., Palou, L., & Gohari, G. (2022). Postharvest chitosan-arginine nanoparticles application ameliorates chilling injury in plum fruit during cold storage by enhancing ROS scavenging system activity. *BMC Plant Biology*, *22*(1), 555. https://doi.org/10.1186/s12870-022-03939-4

Maringgal, B., Hashim, N., Mohamed Amin Tawakkal, I. S., & Muda Mohamed, M. T. (2020). Recent advance in edible coating and its effect on fresh/fresh-cut fruits quality. *Trends in Food Science and Technology*, *96*, 253–267. https://doi.org/10.1016/j.tifs.2019.12.024

McGuire, R. G. (1992). Reporting of objective color measurements. *HortScience*, *27*(12), 1254-1255. https://doi.org/10.21273/HORTSCI.27.12.1254

Meitha, K., Pramesti, Y., & Suhandono, S. (2020). Reactive oxygen species and antioxidants in postharvest vegetables and fruits. *International Journal of Food Science*, *2020*(1), 8817778. https://doi.org/10.1155/2020/8817778

Mohammadi, M., Rastegar, S., & Rohani, A. (2023). Enhancing shelf-life and quality of Mexican lime (*Citrus aurantifolia* cv.) fruit: utilizing edible coating from wild sage seeds enriched with pomegranate seed oils. *Journal of Food Measurement and Characterization, 18*(1), 331-344. https://doi.org/10.1007/s11694-023-02176-0

Nakamoto, M. M., Assis, M., de Oliveira Filho, J. G., & Braga, A. R. C. (2023). Spirulina application in food packaging: gaps of knowledge and future trends. *Trends in Food Science & Technology*, *133*, 138–147. https://doi.org/10.1016/j.tifs.2023.02.001

Olawuyi, I. F., Park, J. J., Lee, J. J., & Lee, W. Y. (2019). Combined effect of chitosan coating and modified atmosphere packaging on fresh-cut cucumber. *Food Science & Nutrition*, *7*(3), 1043–1052. https://doi.org/10.1002/fsn3.930

Ordonez, A. A. L., Gomez, J. D., & Vattuone, M. A. (2006). Antioxidant activities of sechium edule (Jacq.) Swartz extracts. *Food Chemistry*, *97*(3), 452–458. https://doi.org/10.1016/j.foodchem.2005.05.024

Parvin, N., Rahman, A., Roy, J., Rashid, M. H., Paul, N. C., Mahamud, M. A., … Molla, M. E. (2023). Chitosan coating improves postharvest shelf-life of mango (*Mangifera indica* L.). *Horticulturae*, *9*(1), 64. https://doi.org/10.3390/horticulturae9010064

Quintana, S. E., Llalla, O., García-Risco, M. R., & Fornari, T. (2021). Comparison between essential oils and supercritical extracts into chitosan-based edible coatings on strawberry quality during cold storage. *The Journal of Supercritical Fluids*, *171*, 105198. https://doi.org/10.1016/j.supflu.2021.105198

Raddatz-Mota, D., Franco-Mora, O., Mendoza-Espinoza, J. A., Rodríguez-Verástegui, L. L., de León-Sánchez, F. D., & Rivera-Cabrera, F. (2019). Effect of different rootstocks on Persian lime (*Citrus latifolia* T.) postharvest quality. *Scientia Horticulturae*, *257*, 108716. https://doi.org/10.1016/j.scienta.2019.108716

Ramji, V., & Vishnuvarthanan, M. (2022). Chitosan ternary bio nanocomposite films incorporated with MMT K10 nanoclay and *Spirulina*. *Silicon*, *14*(3), 1209–1220. https://doi.org/10.1007/s12633-021-01045-z.

Rastegar, S., & Atrash, S. (2021). Effect of alginate coating incorporated with Spirulina, Aloe vera and guar gum on physicochemical, respiration rate and color changes of mango fruits during cold storage. *Journal of Food Measurement and Characterization*, *15*(1), 265–275. https://doi.org/10.1007/s11694-020-00636-4

Rastgoo, N., Rastegar, S., & Rohani, A. (2024). Maintaining quality of Lisbon lemon (*Citrus limon*) in cold storage using natural elicitors. *Journal of Horticulture and Postharvest Research*, *7*(2), 99–114. https://doi.org/10.22077/jhpr.2024.7125.1352

R Core Team. (2022). R: A language and environment for statistical computing. Retrieved from https://www.r-project.org

Ruzaina, I., Zhong, F., Abd. Rashid, N., Jia, W., Li, Y., Zahrah Mohamed Som, H., … Zahid Abidin, M. (2017). Effect of different degree of deacetylation, molecular weight of chitosan and palm

stearin and palm kernel olein concentration on chitosan as edible packaging for cherry tomato. *Journal of Food Processing and Preservation*, *41*(4), e13090. https://doi.org/10.1111/jfpp.13090

Saucedo-Pompa, J. Torres-Castillo, C. Castro-López, R. Rojas, E. Sánchez-Alejo, M. Ngangyo-Heya, & G. Martínez-Ávila. (2018). Moringa plants: Bioactive compounds and promising applications in food products. *Food Research International*, *111*, 438–450. https://doi.org/10.1016/j.foodres.2018.05.061

Santos, L. C. dos, Silva, I. J. da, Santos, A. V. D. dos, Sousa, E. P. da R., Oliveira, A. M. F. de, Sousa, V. F. de O., Araújo, R. H. C. R. (2023). Edible coating with microalgae and modified atmosphere packaging for post-harvest conservation of tomatoes. *Horticultura Brasileira, 41*, e2503. https://doi.org/10.1590/s0102-053620230101

Sapper, M., & Chiralt, A. (2018). Starch-based coatings for preservation of fruits and vegetables. *Coatings*, *8*(5), 152. https://doi.org/10.3390/coatings8050152

Sarengaowa, Wang, L., Liu, Y., Yang, C., Feng, K., & Hu, W. (2022). Screening of essential oils and effect of a chitosan-based edible coating containing cinnamon oil on the quality and microbial safety of fresh-cut potatoes. *Coatings*, *12*(10), 1492. https://doi.org/10.3390/coatings12101492

Saxena, A., Sharma, L., & Maity, T. (2020). Enrichment of edible coatings and films with plant extracts or essential oils for the preservation of fruits and vegetables. In *Biopolymer-based formulations.* Elsevier, 859–880. https://doi.org/10.1016/B978-0-12-816897-4.00028-1

Serradell, M. de los A., Rozenfeld, P. A., Martínez, G. A., Civello, P. M., Chaves, A. R., & Añón, M. C. (2000). Polyphenoloxidase activity from strawberry fruit (*Fragaria ananassa,* Duch., cv Selva): Characterisation and partial purification. *Journal of the Science of Food and Agriculture*, *80*(9), 1421–1427. https://doi.org/10.1002/1097-0010(200007)80:9<1421::AID-JSFA649>3.0.CO;2-K

Shiekh, R. A., Malik, M. A., Al-Thabaiti, S. A., & Shiekh, M. A. (2013). Chitosan as a novel edible coating for fresh fruits. *Food Science and Technology Research*, *19*(2), 139–155. https://doi.org/10.3136/fstr.19.139

Singh, K. K., & Reddy, B. S. (2006). Post-harvest physico-mechanical properties of orange peel and fruit. *Journal of Food Engineering*, *73*(2), 112–120. https://doi.org/10.1016/j.jfoodeng.2005.01.010

Sun, Y., Singh, Z., Tokala, V. Y., & Heather, B. (2019). Harvest maturity stage and cold storage period influence lemon fruit quality. *Scientia Horticulturae*, *249*, 322–328. https://doi.org/10.1016/j.scienta.2019.01.056

Tesfay, S. Z., & Magwaza, L. S. (2017). Evaluating the efficacy of moringa leaf extract, chitosan and carboxymethyl cellulose as edible coatings for enhancing quality and extending postharvest life of avocado (*Persea americana* Mill.) fruit. *Food Packaging and Shelf Life*, *11*, 40–48. https://doi.org/10.1016/j.fpsl.2016.12.001

Thakur, R., Pristijono, P., Bowyer, M., Singh, S. P., Scarlett, C. J., Stathopoulos, C. E., & Vuong, Q. V. (2019). A starch edible surface coating delays banana fruit ripening. *LWT*, *100*, 341–347. https://doi.org/10.1016/j.lwt.2018.10.070

Toor, R. K., & Savage, G. P. (2005). Antioxidant activity in different fractions of tomatoes. *Food Research International*, *38*(5), 487–494. https://doi.org/10.1016/j.foodres.2004.10.016

Wang, H., Iqbal, A., Murtaza, A., Xu, X., Pan, S., & Hu, W. (2022). A review of discoloration in fruits and vegetables: formation mechanisms and inhibition. *Food Reviews International*, 1–22.

Wang, S. Y., & Gao, H. (2013). Effect of chitosan-based edible coating on antioxidants, antioxidant enzyme system, and postharvest fruit quality of strawberries (*Fragaria x aranassa* Duch.). *LWT - Food Science and Technology*, *52*(2), 71–79. https://doi.org/10.1016/j.lwt.2012.05.003

Wijewardane, R. M. N. A. (2022). Evaluation of the effect of fruit coating on shelf life extension of lime (Citrus aurantifolia) under different storage condition. *Journal of Horticulture and Postharvest Research*, *5*(4), 337–348. https://doi.org/10.22077/jhpr.2022.4949.1261

Zeb, S., Sajid, M., Shah, S. T., Ali, M., Ali, S., Nawaz, Z., … Shah, S. A. A. (2020). 34. Influence of post-harvest application of chitosan on physico-chemical changes of apple fruit during storage. *Pure and Applied Biology*, *9*(4), 2554–2562. https://doi.org/10.19045/bspab.2020.90272

Zhang, P., & Zhou, Z. (2019). Postharvest ethephon degreening improves fruit color, flavor quality and increases antioxidant capacity in 'Eureka'lemon (*Citrus limon* (L.) Burm. f.). *Scientia Horticulturae*, *248*, 70–80. https://doi.org/10.1016/j.scienta.2019.01.056

JOURNAL OF HORTICULTURE AND POSTHARVEST RESEARCH
2025, VOL. 8(1), 125-138

Journal of Horticulture and Postharvest Research

Journal homepage: www.jhpr.birjand.ac.ir

Improving fruit traits of 'Braeburn' apples in low-altitude regions: The impact of foliar spray and rootstock interactions

Leili Habibzadeh[1], Mahdi Alizadeh[1*] and Mohammad Mehdi Sharifani[1]

1, Department of Horticulture, Faculty of Plant Production, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran

ARTICLE INFO

Original Article

Article history:
Received 27 July 2024
Revised 12 November 2024
Accepted 14 November 2024

Keywords:
Anthocyanin
Firmness
Pigment
Potassium
Prohexadione calcium

DOI: 10.22077/jhpr.2024.7970.1406
P-ISSN: 2588-4883
E-ISSN: 2588-6169

***Corresponding author:**
Department of Horticulture, Faculty of Plant Production, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.

Email: mahdializadeh@gau.ac.ir

© This article is open access and licensed under the terms of the Creative Commons Attribution License http://creativecommons.org/licenses/by/4.0/ which permits unrestricted, use, distribution and reproduction in any medium, or format for any purpose, even commercially provided the work is properly cited.

ABSTRACT

Purpose: The ‘Braeburn’ apple, cultivated in Mashhad, Iran, has poor red coloration that affecting its marketability. This research studied the effects of mono-potassium phosphate (MKP) and calcium prohexadione (ProCa) on red pigment development and biochemical traits, as well as interactions with two rootstocks used for grafting. **Research method:** The experimental treatments included four concentrations of MKP (0, 0.1, 1.0, and 2.0 g/L) sprayed in three periods: 20 days after petal fall, 45 and 30 days before commercial fruit harvest. Furthermore, ProCa was also applied in four concentrations (0, 125, 250, and 500 mg/L), one month before harvest (three times with 10-day intervals). **Findings:** The results showed that M.9 rootstock led to larger fruit diameters compared to MM.111, but overall physical traits remained unchanged. Chemical applications significantly affected fruit diameter and firmness, with ProCa treatments (250 and 500 mg/L) yielding the highest firmness levels. Rootstock type influenced total acidity (TA), total soluble solids (TSS), flavor index, and vitamin C content. M.9 rootstock combined with MKP spray resulted in the best TA, TSS, and flavor index. However, higher ProCa concentrations negatively impacted color development and anthocyanin levels. Thus, cultivating red apple cultivars in low-altitude regions like Mashhad is not recommended due to environmental factors affecting pigmentation. **Research limitations:** There was no apple orchard of the same variety located in a higher altitude within the same region for a comparative analysis. **Originality/Value:** The article clearly emphasizes that the orchard establishment of the ‘Braeburn’ apple is not technically authorized for low altitude places (lower than 1000 m).

INTRODUCTION

Apple (*Malus* × *domestica* Borkh.), belongs to the Rosaceae family, is one of the first known fruits since prehistoric times, it is the most widespread fruit species in the world (Robinson et al., 2001; Harris et al., 2002). The apple originated in Central Asia and was brought to North America by European immigrants after thousands of years of cultivation in Asia and Europe. The genus *Malus* currently has 23 primary species and more than 7500 diverse genotypes (Elzebroek, 2008). After bananas, citrus, and grapes, the apple is the fourth most important fruit in the world as well as the most important fruit in temperate regions. In recent years, the global production of apples has increased from 83.33 million tons in 2018 to 86.44 million tons in 2020 (FAOSTAT, 2022).

Apple's skin color plays an important role in consumer appeal and strives for that bright red color to entice the buyer (Dar et al., 2019). There are three major pigments found in apple skin, and the concentration of all three pigments changes during the season. Chlorophylls, which are green and exist in chloroplasts; carotenoids which are yellow, orange, or red and observed in chromoplasts; and anthocyanins that are red, blue, or purple in the vacuole (Jahangir et al., 2019; Matsuoka, 2019).

The color of the apple is primarily due to the background color of the skin, and secondarily due to the anthocyanin pigments. The main anthocyanin pigment in apples is cyanidin-3-galactoside, which is called "idaein" belongs to the class of organic compounds known as anthocyanidin-3-o-glycosides. These are phenolic compounds containing one anthocyanidin moiety which is O-glycosidically linked to a carbohydrate moiety at the C3 position (Jahangir et al., 2019; Dar et al., 2019). In other words, these natural pigments are glycosides in which a sugar, usually glucose, is attached to carbon number 3 (Saure, 1990).

A literature review (Castle, 1995; Plunkett et al., 2019; Jahangir et al., 2019; Dar et al., 2019; Chen et al., 2019) revealed that there are six main factors affect apple skin color development. These factors can be mentioned as genetic factors and mutations; physiological stage of the plant; light and temperature of the growing site; tree nutrition management; crop load; and stresses.

Fruit color and anthocyanin biosynthesis can be adjusted by using shade net, light, ethylene, temperature, radiant open foil, and the use of different chemicals (Blanke, 2008; Whale et al., 2008; Gouws & Steyn, 2014; Dar et al., 2019). Anthocyanins and other pigments such as flavonols and carotenoids, mineral compounds, vacuole acidity, and cell shape are known to be effective in fruit phenotype and color (Kim et al., 2003, Moradinezhad et al., 2024).

The biosynthesis of anthocyanins in the skin of the fruit is suppressed and stopped at warmer temperatures. Currently, the formation of weak and less pigments in colored cultivars has become a major problem due to global climate changes in some regions (Iglesias et al., 2008). Recently, apples with red flesh, well-liked in Japan, new cultivars with red flesh and low acidity have been registered, including "Rose Peal" and "Ruby Sweet" (Abe et al., 2017). Temperature during the ripening period is an important factor in anthocyanin synthesis in the flesh and skin of apple fruit (Honda et al., 2017). Sunlight is not critical for anthocyanin biosynthesis in the red flesh of some apple cultivars, unlike the apple skin. Fruits that were covered with a bag, almost 70% of fruits that were exposed to light had anthocyanin accumulation in the skin (Honda et al., 2017). Furthermore, for some cultivars, sunlight is needed for the maximum accumulation of anthocyanin in the skin and flesh.

Besides natural coloration induced by geographical and climatic factors, apple growers are always seeking the application of some chemicals to improve skin color and to attain fruits with intense red color (Brighenti et al., 2017). It has been reported that potassium (K) is

effective in increasing the coloring of apples and grapes with the accumulation of anthocyanins (Neilsen et al., 2004; Nava et al., 2008). Among the macro elements, calcium, nitrogen, potassium, and phosphorus are more related to the quality characteristics of the fruit (Fallahi et al., 2010). Compounds containing phosphorus have been recorded to increase the concentration of anthocyanin and improve the color of the fruit (Li et al., 2002). Also, calcium stabilizes the cell wall and maintains the uniformity of the cell membrane, which is closely related to the firmness of the fruit flesh (Solhjoo et al., 2017; Musacchi & Serra, 2018). Potassium is the most important component of the fruit, however, any excess should be avoided and the proper ratio of potassium to calcium should be obtained in order to prevent pre-harvest and post-harvest disorders (Brunetto et al., 2015; Jahani et al., 2024). Also, the development of fruit color depends on the regular supply of sugar in the fruit (Lueangprasert et al., 2010).

In a preliminary survey, we have emphasized the importance of selecting the right geographical location and proper elevation for cultivating colored apples (Habibzadeh et al., 2022). So, we have highlighted the key factors influencing anthocyanin synthesis, which is closely tied to light and temperature. It was revealed that apples grown at lower altitudes, such as Mashhad (Mazraeh Nemooneh at 982 meters), experienced a significant reduction in external quality. This decline particularly affected the intensity of skin color (anthocyanin accumulation) and the level of soluble solids (sugars). In contrast, apples harvested from higher elevations like Jang village (1350 meters) and Kardeh (1550 meters) displayed vibrant colors and higher red pigment density (Fig. 1). Karagiannis et al. (2020) recently found that several key color parameters, such as redness and color index, were significantly increased by high altitude. Supporting this observation, higher levels of anthocyanins and other phenolic compounds were also identified in the peels of apples cultivated at elevated altitudes.

The 'Braeburn' apple was also recently cultivated in eastern Iran (Mashhad), with low-altitude conditions. Hence, the deficiency in red coloration is a significant drawback for its marketability as well as apple orchard development in this area. The present research aimed to investigate the foliar application of mono-potassium phosphate (MKP) and calcium pro-hexodione (ProCa) on the development of red pigments and certain biochemical traits of apple fruits. Furthermore, as the 'Braeburn' cultivar was grafted on two different rootstocks, the interaction of chemical spray and rootstock was also studied.

Fig. 1. Role of growing site on Gala apple color intensity. The apples grown at: (a) lower altitudes (982 meters; Mashhad, Mazraeh Nemooneh) as compared to fruits harvested from (b) Jang village (1350 meters) and (c) Kardeh (1550 meters).

MATERIALS AND METHODS

The study was carried out in a commercial orchard, with 8-year-old 'Braeburn' apple trees grafted on either M.9 (intensive; 0.8 × 3.2 m) or MM.111 (semi-intensive; 2.5 × 4.0 m) rootstocks trained to a scaffold-pyramid system. The orchard was located in Eastern Iran, Mazraeh Nemooneh, Razavi Agro-Industrial, Khorasan-e-Razavi province, Mashhad (59° 43' 48.26" E, 36° 11' 20.13" N; altitude of 982 m). The orchards were equipped with an intelligent drip irrigation system, mist sprinkler, and net shading system against hail and sunburn (Fig. 2).

The experimental treatments included control, three concentrations of MKP (0.1, 1.0, and 2.0 grams per liter; g/L) sprayed in three periods (20 days after petal fall, 45 and 30 days before commercial harvest fruit), and three concentrations of ProCa (125, 250, and 500 mg/L) was applied one month before harvest (three times with 10-day intervals). It is important to note that the concentrations of MKP and ProCa are determined by the volume of water used for spraying. The trees were sprayed from May 1, 2022 until August 27, 2022.

The six fruits per tree were harvested at the commercial maturity stage for analysis. The maturity index was determined by assessing the fruit color and drawing on the expertise of local apple producers. Fruits were immediately transferred to the laboratory and the following traits were measured.

Morphological traits include fruit weight, length, diameter, volume, and firmness of fruit texture. Fruit biochemical characteristics: sugars (including sucrose, glucose, fructose, total sugars), total phenol, total soluble solids (TSS), pH, titratable acidity (TA), fruit flavor index, and vitamin C. Pigments: chlorophyll (chlorophyll a, b, and total), carotenoids, anthocyanin, and color indices (a^*, b^*, L^*).

Fig. 2. Apple orchard equipped with an intelligent drip irrigation system, mist sprinkler, and net shading system against hail and sunburn. Mazraeh Nemooneh, Razavi Agro-industrial Co., Mashhad, Iran.

The fruit weight was measured using a digital desktop scale with an accuracy of 0.001 g. The fruit length from the pedicle cavity up to the calyx, and the fruit diameter (from the waist of the fruit) were measured with a digital caliper of 0.01 mm accuracy. The fruit volume was evaluated by immersion in water and direct displacement of the water with a graduated cylinder. The firmness of the fruit tissue was assessed using a hand-held penetrometer with an 8 mm diameter tip (Model 327 FT, manufactured in Italy). This involved removing the skin along with a thin layer of flesh, after which pressure was applied and measured at the area corresponding to the fruit's greatest diameter. The firmness of the fruit tissue was expressed in kilograms per square centimeter. The pH of fruit juice with Labtron-110 pH meter, TA by titration with sodium hydroxide, vitamin C by Kashyap et al. (2012) method, phenol by Singleton & Rossi (1965) method, TSS with a Belgian refractometer model 060279, were measured.

The extraction of sugars by the Omokolo et al. (1996) method was performed and glucose by the Miller (1959) method, and fructose by the Ashwell (1957) method were estimated.

To measure pigments, the anthocyanin was measured with Wagner's (1979) method in 520 nm wavelength. The method of Barnes et al. (1992) was used for chlorophylls and carotenoids which are a spectrophotometric method based on DMSO solvent and 480, 510, 645, and 663 nm wavelengths. The fruit samples were also subjected to Hunterlab device to obtain the color parameters.

Statistical analysis

This research work was conducted as factorial experiment based on a randomized complete block design, with three replications. Each tree was considered a replication. The first factor was rootstock type (M.9 or MM.111) and the second factor was chemical treatments in seven aforementioned levels. The collected data were analyzed using SAS software, and mean comparisons were conducted using Duncan's test at both the 1% and 5% significance levels.

RESULTS AND DISCUSSION

Fruit physical traits

Table 1 displays the ANOVA results for the physical characteristics of apple fruits as affected by rootstock and chemical spray. While the rootstock did not impact the overall physical traits of the apple fruit, it only did influence the diameter of the fruits. Specifically, using the M.9 rootstock resulted in a higher fruit diameter compared to MM.111.

Among the fruit physical traits, the fruit diameter and firmness were significantly affected by the application of chemical compounds. Table 2 shows that the fruits treated with both MKP or ProCa were greatly firmer than the control. The trees sprayed with ProCa (250 and 500 mg/L) had the highest fruit firmness among the treatments. Calcium is used in large amounts by plants after nitrogen and potassium. It is a component of the middle lamellae, (Ca-pectates) of the cell wall, which strengthens the cell, increasing the length of the walls, and cell division, membrane permeability and the activation of several vital enzymes in nitrogen and protein metabolism (Njira & Nabwami, 2015; Solhjoo et al., 2017).

Table 1. The ANOVA results for rootstock type (M.9 and MM.111) and chemical compounds (mono-potassium phosphate and calcium prohexadione) on some physical characteristics of 'Braeburn' apple.

Source	df	Mean Square				
		Firmness	Weight	Volume	Diameter	Length
Block	2	1.87**	168.8ns	47.25ns	1.27ns	2.15ns
Rootstock (RS)	1	0.06ns	8.9ns	139.58ns	43.03**	12.77ns
Treatment (T)	6	13.16**	246.62ns	439.75ns	25.62**	8.99ns
RS×T	6	2.13**	81.84ns	80.4ns	9.96*	5.55ns
Error	-	0.22	183.37	216.16	2.98	7.48
CV	-	7.69	12.2	12.44	2.7	5.09

*, ** and ns are significant at 1%, 5% and non-significant levels, respectively.
Abbreviation: df, degree of freedom; CV, coefficient of variation.

Table 2. The interaction of rootstock type (M.9 and MM.111) and chemical compounds (mono-potassium phosphate and calcium prohexadione) on some physical characteristics of 'Braeburn' apple.

Rootstock	Treatment	Fruit physical traits				
		Firmness (kg/cm^2)	Weight (g)	Volume (cm^3)	Diameter (mm)	Length (mm)
M.9	Control	4.0±0.52 i*	106±3.8b	108.2±2.75bc	62.7±0.5b-e	51.5±0.5b
	KH_2PO_4 (0.1 mg/L)	5.1±0.58gh	111±20ab	122.2±21abc	65.4±0.28ab	63.1±2.78ab
	KH_2PO_4 (1.0 mg/L)	5.2±0.4gh	110±12.1ab	123.6±8abc	60.3±1.96e	53.6±2.97ab
	KH_2PO_4 (2.0 mg/L)	5.7±0.2de	130±15.7a	137.5±18.5a	66.7±2.29a	57.4±2.46a
	ProCa (125 g/L)	7.7±1.8bcd	105±14.6b	127.2±6.96abc	61.4±2.84ed	52.6±3.15b
	ProCa (250 g/L)	8.9±1.05a	106±20.7b	110±9.64bc	57.0±2.18f	51.4±6.26b
	ProCa (500 g/L)	8.06±0.7ab	106±7.49 b	110.5±3.46bc	60.4±1.39e	52.3±1.11b
MM.111	Control	4.4±0.3ih	107±1.42ab	118.3±2.88abc	62.9±1.99b-e	53.4±0.46ab
	KH_2PO_4 (0.1 mg/L)	5.1±0.57gh	114±24.27ab	115.5±6.9abc	67.7±2.07abc	55.8±2.94ab
	KH_2PO_4 (1.0 mg/L)	5.6±0.26fe	113±5.7ab	112.7±7.5bc	64.0±0.8a-d	54.8±1.73ab
	KH_2PO_4 (2.0 mg/L)	5.9±0.7de	118±5.14ab	128.8±6.73ab	65.4±0.42ab	54.3±1.52ab
	ProCa (125 g/L)	7.5±0.2def	116±6.35ab	120.5±13.5abc	65.0±1.7abc	53.9±0.53ab
	ProCa (250 g/L)	6.86±0.45dce	102±8.82b	105±6.6c	62.4±0.82cde	53.9±0.86ab
	ProCa (500 g/L)	8.3±0.6a	109±17.5ab	112.7±25.6bc	63.7±1.39bcd	53.5±3.27ab

* The data in each column followed by the same letter(s) are not significantly different at 5%. The ± values represent standard deviation.

Foliar application is one of the common methods of meeting the nutritional needs of plants, which is more effective than soil application of fertilizer when the soil conditions are unsuitable for the access of elements. Calcium also has little mobility and foliar application of Ca compounds can improve the product quality (Njira & Nabwami, 2015). It has already reported that foliar application of ProCa on apple trees increases fruit calcium content (De freitas et al., 2010; Amarante et al., 2021). Consequently, the enhanced calcium levels contribute to maintaining higher fruit flesh firmness, aligning with the findings of our current research. Other studies proved that calcium reduces the incidence of many postharvest physiological disorders (De freitas et al., 2016; Solhjoo et al., 2017).

Potassium is a crucial nutrient absorbed by plants that remains in ionic form and serves as an activator for cellular enzymes. It plays a key role in plant nutrition and growth, regulating metabolic processes like photosynthesis, and ultimately influencing plant performance and quality (Njira & Nabwami, 2015; Xu et al., 2020). Potassium is essential for plant growth, regulating water balance, enhancing stress resistance, activating critical enzymes, and maintaining optimal metabolic functions. Potassium deficiency inhibits protein synthesis, leading to stunted growth and reduced protein content in plants (Dzida et al., 2018; Yaldız et al., 2018). The fruits harvested from the trees treated with MKP (2.0 g/L) showcased enhanced physical characteristics such as weight, volume, diameter, and length. This

enhancement was particularly noticeable in trees grafted onto M.9 rootstock, suggesting that the combination of MKP treatment and M.9 rootstock resulted in even more pronounced improvements in fruit quality. Potassium plays a crucial role in improving fruit physical traits due to its various functions in plant physiology. Potassium helps regulate water uptake and distribution within the plant, which contributes to enhanced fruit size, weight, and volume. Additionally, potassium is involved in the activation of enzymes responsible for carbohydrate metabolism, which is essential for fruit growth and development (Kuzin et al., 2020). By promoting proper cell division and expansion, potassium can lead to larger and more uniform fruits. Furthermore, potassium contributes to the overall health and vigor of the plant, resulting in better nutrient uptake and utilization, all of which directly impact the physical characteristics and quality of the fruits. The presence of an adequate amount of potassium in the plant's system is essential for optimizing fruit physical traits and ensuring healthy and productive fruit production.

Fruit biochemical traits

A different pattern emerged when examining the biochemical traits of the apple fruits (Table 3). The rootstock had a significant impact on many of these traits, particularly TA, TSS, flavor index, and vitamin C content. The type of rootstock played a very significant role in determining the levels of these biochemical traits. Additionally, with the exception of TSS, the biochemical traits were significantly influenced by the application of chemical compounds. The data in Table 4 revealed that when M.9 rootstock was utilized the TA, TSS, flavor index, and vitamin C significantly improved. It is clear that TA, TSS, and flavor index were at the highest level in fruits picked from trees onto M.9 rootstock that received MKP spray.

Rootstocks are generally important in the improvement of the fruit quality for crops like apples, grapes, and citrus (Castle, 1995). Rootstocks influence the morphological, biochemical, and physiological characteristics of the scion portion (Kumar et al., 2024). Some researchers emphasized the influence of rootstock on improving fruit quality through TSS, reducing the sugar and acidity content of the fruits (Shahkoomahally et al., 2020). The TSS, also known as Brix, refers to the concentration of sugars, organic acids, and other soluble solids in the fruit juice. Rootstock selection plays a crucial role in influencing the TSS content and overall quality of apple fruits by affecting nutrient uptake, growth, root system efficiency, scion compatibility, and stress tolerance (Habibzadeh, 2022; Kumar et al., 2024).

In the present study, specific findings from the sugar analysis were not deemed statistically significant, thus the data is omitted from the presentation. However, a notable variance was observed for the amount of fructose in fruits harvested from M.9 rootstocks. The application of chemicals spray, specifically about total sugars and glucose levels, had a significant impact. Conversely, no significant effects were noted for other sugars. Especially, the use of MKP at 1 g/L led to the highest total sugar levels in M.9 rootstocks, a trend that was similarly evident for glucose.

Pigments and fruit color indices

The apple skin pigments and color indices (a^*, b^*, L^*) were influenced by both the rootstock and chemical spraying. It is crucial to note that a* value corresponds to the Red/Green color component. The b* value stands for the Blue/Yellow color component, and the "L*" value represents Lightness. While Table 5 presents the analysis of variance for the color indices, the analysis of variance for pigments is not shown. Instead, only the average comparison graphs for pigments are provided (Fig. 3). What is certain is that the application of MKP (2 g/L) on MM.111 rootstock led to the greatest increase in the a* index (redness). In both rootstocks,

calcium treatments yielded higher values of the b* index, indicating a shift towards a brighter green-blue color spectrum and a lack of red color development. Furthermore, ProCa treatments increased the L^* index. The highest L^* index was observed in ProCa 500 mg/L when using M.9 rootstock. All MKP concentrations had a lower L^* index than the control trees. These findings align with the research by Amarante et al. (2021), who demonstrated that calcium treatments delayed ripening, decreased anthocyanin levels, and reduced skin pigmentation in apple fruit.

The alterations in chlorophyll, carotenoid, and anthocyanin levels in apple skin are depicted in Figure 3. These pigment changes align with the evaluated color indices. The greatest quantity of anthocyanin was noted in both rootstocks following MKP spraying. Conversely, when ProCa was applied, regardless of the concentration, the anthocyanin levels were markedly low. If we judge only based on the changes of anthocyanin pigment, these two substances (ProCa and MKP) have opposite effects in the formation of fruit color. However, the fluctuations in other pigments also further validate this phenomenon. The negative effect of ProCa on apple fruit red coloration was also already demonstrated by Bizjak et al. (2012). Their findings revealed that late application of ProCa to ripening apple cultivar 'Braeburn' fruit results in decreased anthocyanin levels and changes in the phenolic content. Furthermore, warnings about high concentrations of ProCa and fruit color degradation have already been given by Cline et al. (2008).

The formation of red color in fruits is the result of anthocyanin production and accumulation, often accompanied by the breakdown of chlorophyll in the fruit peel (Tijskens et al., 2011; Kamiab et al., 2023). In apples, the red color development is influenced by genetic, environmental, and developmental factors, as well as agricultural practices (Saure, 1990). In the present research, foliar application of MKP has been found to effectively boost anthocyanin production and create a more vibrant red coloration. Specifically, the application of MKP at a concentration of 1.0 g/L proved to be the most successful treatment, as illustrated in Figure 3. The high temperatures can negatively affect the anthocyanin biosynthesis process, with warmer temperatures hindering adequate red color development (Gouws & Steyn, 2014; Honda & Moriya, 2018), a concern exacerbated by the global trend of rising temperatures. In areas with low altitude, like the study location, high temperatures may impede the development of red color in fruits. Planting the same cultivar at higher altitudes would circumvent this issue and removing the necessity for chemical sprays or similar treatments. Nevertheless, in such regions, MKP can serve as a straightforward strategy to slightly enhance the color.

Table 3. The ANOVA for rootstock type (M.9 and MM.111) and chemical compounds (mono-potassium phosphate and calcium prohexadione) on chemical characteristics of 'Braeburn' apple.

Source	df	Mean Square				
		pH	TA	TSS	Test Index	Vitamin C
Block	2	0.008 ^{ns}	0.16^{ns}	0.15^{ns}	0.92 ^{ns}	0.03^{ns}
Rootstock (RS)	1	0.039 ^{ns}	10.7**	7.45**	8.6**	1.22**
Treatment (T)	6	0.043**	0.43*	1.16 ^{ns}	1.57*	0.34**
RS×T	6	0.023**	0.67**	0.99^{ns}	3.5**	0.18*
Error	-	0.005	0.13	0.5	0.62	0.06
CV	-	2.04	17.6	4.8	18.3	30.06

*, ** and ns are significant at 1%, 5% and non-significant levels, respectively.
df, degree of freedom; CV, coefficient of variation.

Table 4. The interaction of rootstock type (M.9 and MM.111) and chemical compounds (mono-potassium phosphate and calcium prohexadione) on some physical characteristics of ' Braeburn ' apple.

Rootstock	Treatment	Fruit biochemical traits					
		pH	TA (mg /100 ml)	TSS (%)	Flavor Index	Vitamin C (mg /100 ml)	Total phenol (mg/g FW)
M.9	Control	3.54±0.04 cd*	1.93±0.39 c	14.66±0.4 bcd	3.97±0.9 c	0.58±0.12 b	0.57±0.07 ab
	KH_2PO_4 (0.1 mg/L)	3.43±0.032 bc	2.58±0.37 b	15.8±0.1 a	5.72±0.84 b	1.61±0.25 a	0.43±0.03 dc
	KH_2PO_4 (1.0 mg/L)	3.6 ±0.07abc	3.42±0.36 a	14.83±0.4 abc	7.11±0.25 a	0.88±0.44 b	0.48±0.01 bc
	KH_2PO_4 (2.0 mg/L)	3.53±0.03 abc	2.17±0.3 bc	15.4±0.3 ab	4.7±1.1 bc	0.88±0.44 b	0.58±0.05 a
	ProCa (125 g/L)	3.68±0.04 a	1.93±0.04 c	14.4±0.5 bcd	3.55±0.1 c	0.88±0b	0.41±0.02 dc
	ProCa (250 g/L)	3.64±0.04 abc	1.93±0.27 c	14.1±0.4 cde	3.8±0.3 c	0.58±0.25 b	0.58±0.07 a
	ProCa (500 g/L)	3.65 ±0.1abc	1.83±0.23 c	14.01±0.6 cde	3.6±0.4 c	1.46±0.25 a	0.56±0.06 ab
MM.111	Control	3.63±0.06 abc	1.62±0.16 c	14.03±0.65 cde	3.7±0.46 c	0.76±0.1 b	0.4±0.03 dc
	KH_2PO_4 (0.1 mg/L)	3.58±0.07 ed	1.73±0.04 c	14.2±0.7 cde	3.43±0.09 c	0.79±0.4 b	0.4±0.01 cd
	KH_2PO_4 (1.0 mg/L)	3.3±0.01 e	1.85±0.05 c	13.66±1.4 de	3.54±0.47 c	0.57±0.13 b	0.61±0.1 a
	KH_2PO_4 (2.0 mg/L)	3.64±0.11 cd	1.96±0.23 c	14.36±0.32 e	3.68±0.4 c	0.49±0.05 b	0.42±0.01 dc
	ProCa (125 g/L)	3.7±0.04 ab	1.15±0.2 bc	14.66±0.45 cde	4.3±0.53 c	0.52±0.17 b	0.42±0.01 dc
	ProCa (250 g/L)	3.67±0.05 ab	1.98±0.35 bc	14.13±0.35 bcd	4.03±0.65 c	0.58±0.1 b	0.37±0.05 d
	ProCa (500 g/L)	3.67±0.06 ab	1.79±0.14 c	14.16±1.05 abc	3.71±0.16c	0.76±0.1 b	0.34±0.05 d

* The data in each column followed by the same letter(s) are not significantly different at 5%. The ± values represent standard deviation.

Table 5. The ANOVA results for rootstock type (M.9 and MM.111) and chemical compounds (mono-potassium phosphate and calcium prohexadione) on colorimetric indices of 'Braeburn' apple.

Source	df	Mean Square		
		a *	b *	L *
Block	2	0.009 ns	0.32ns	0.008ns
Rootstock (RS)	1	0.84 ns	40.69**	372.5**
Treatment (T)	6	7.07**	6.69**	17.12**
RS×T	6	0.53ns	1.24 ns	9.05**
Error	-	0.34	1.24	1.17
CV	-	9.04	11.4	1.64

*, ** and ns are significant at 1%, 5% and non-significant levels, respectively.
Abbreviation: df, degree of freedom; CV, coefficient of variation.

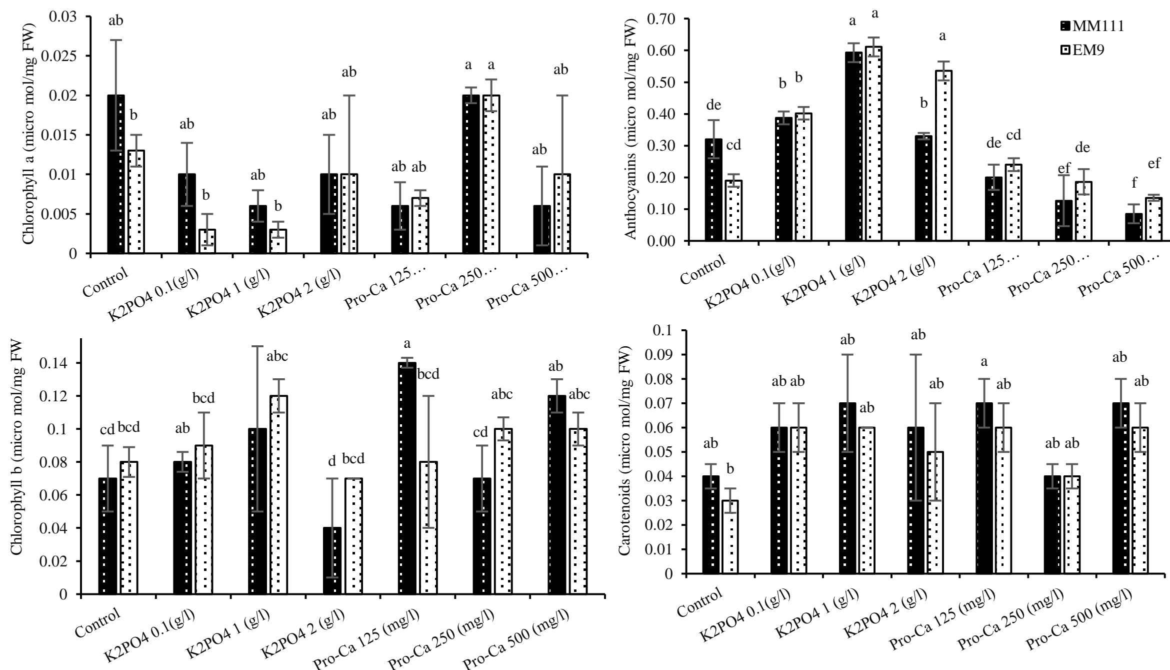


Fig. 3. Changes in chlorophylls (left), anthocyanins (top, right), and carotenoids (bottom, right) of Braeburn apples on M.9 and MM.111 rootstocks following spray with mono-potassium phosphate and calcium prohexadione. Columns followed by a different letter (s) indicate statistical significance at 1% probability.

CONCLUSION

The findings from this study demonstrated that applying MKP foliar spray at a concentration of 1.0 g/L during three key stages (20 days after flowering, 45 days prior to harvest, and 30 days before harvest) yielded positive outcomes in enhancing fruit quality and promoting the anthocyanin pigmentation in the skin of "Braeburn" apples. Furthermore, the use of ProCa positively influenced fruit firmness. However, an increase in ProCa concentration was found to have a negative impact on color development and anthocyanin levels. Based on the observations and results of this study, it is not advisable to grow bi-colored apple varieties in locations like Mashhad and other similar low-altitude regions (specifically below 1000 m). While chemical applications may enhance certain fruit characteristics, they cannot fully replace the influence of environmental factors, particularly low temperatures, in the development of red pigmentation.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgement

The cooperation received from Mazraeh Nemooneh, Razavi Agro-Industrial, Khorasan-e-Razavi province, is fully acknowledged.

REFERENCES

Abe, K. J., Soejima, Iwanami H, Kotoda N, Moriya S, Bessho H, Komori S, Ito Y, Takahashi S, Okada K, Kato H, Haji T, Ishiguro M, Masuda T & Tsuchiya S. (2017). *New apple cultivar 'Rose Pearl'*. Bull. NARO Inst. Fruit Tree & Tea Science, 1, 9–18 (In Japanese with English abstract). https://www.cabidigitallibrary.org

Amarante, C. V. T. D., Silveira, J. P. G., Freitas, S. T. D., Steffens, C. A., & Mitcham, E. J. (2021). Fruit quality of 'Braeburn'apple trees sprayed at post-bloom and preharvest with prohexadione-calcium and GA4+7. *Revista Brasileira de Fruticultura*, *43*(1), 653. https://doi.org/10.1590/0100-29452021653

Ashwell, G. (1957). Colorimetric analysis of sugars. *Methods in Enzynzology*, (3), 73-105.

Barnes, J. D., Balaguer, L., Manrique, E., Elvira, S., & Davison, A. W. (1992). A reappraisal of the use of DMSO for the extraction and determination of chlorophylls a and b in lichens and higher plants. *Environmental and Experimental Botany*, *32*(2), 85-100. https://doi.org/10.1016/0098-8472(92)90034-Y

Bizjak, J., Weber, N., Mikulic-Petkovsek, M., Slatnar, A., Stampar, F., Alam, Z., & Veberic, R. (2013). Influence of Phostrade Ca on color development and anthocyanin content of 'Braeburn'apple (*Malus domestica* Borkh.). *HortScience*, *48*(2), 193-199. https://doi.org/10.21273/HORTSCI.48.2.193

Blanke, M. M. (2008). Alternatives to reflective mulch cloth (Extenday™) for apple under hail net?. *Scientia Horticulturae*, *116*(2), 223-226. https://doi.org/10.1016/j.scienta.2007.12.004

Brighenti, A. F., Würz, D. A., Pasa, M. D. S., & Rufato, L. (2017). Plant growth regulators to enhance fruit color of 'Gala' apples. *Pesquisa Agropecuária Brasileira*, *52*(11), 1118-1122. https://doi.org/10.1590/S0100-204X2017001100018

Brunetto, G., MELO, G. W. B. D., Toselli, M., Quartieri, M., & Tagliavini, M. (2015). The role of mineral nutrition on yields and fruit quality in grapevine, pear and apple. *Revista Brasileira de Fruticultura*, *37*, 1089-1104. https://doi.org/10.1590/0100-2945-103/15

Castle, W. S. (1995). Rootstock as a fruit quality factor in citrus and deciduous tree crops. *New Zealand Journal of Crop and Horticultural Science*, *23*(4), 383-394. https://doi.org/10.1080/01140671.1995.9513914

Chen, W., Zhang, M., Zhang, G., Li, P., & Ma, F. (2019). Differential regulation of anthocyanin synthesis in apple peel under different sunlight intensities. *International Journal of Molecular Sciences*, 20(23), 6060. https://doi.org/10.3390/ijms20236060

Cline, J. A., Embree, C. G., Hebb, J., & Nichols, D. S. (2008). Performance of prohexadione-calcium on shoot growth and fruit quality of apple: Effect of spray surfactants. *Canadian Journal of Plant Science*, *88*(1), 165-174. https://doi.org/10.4141/CJPS07087

Dar, J. A., Wani, A. A., Ahmed, M., Nazir, R., Zargar, S. M., & Javaid, K. (2019). Peel colour in apple (*Malus domestica* Borkh.): An economic quality parameter in fruit market. *Scientia Horticulturae*, *244*, 50-60. https://doi.org/10.1016/j.scienta.2018.09.029

De Freitas, S. T., do Amarante, C. V., Labavitch, J. M., & Mitcham, E. J. (2010). Cellular approach to understand bitter pit development in apple fruit. *Postharvest Biology and Technology*, *57*(1), 6-13. https://doi.org/10.1016/j.postharvbio.2010.02.006

Dzida, K., Michałojć, Z., Jarosz, Z., Pitura, K., & Skubij, N. (2018). Effect of potassium fertilization on yield, growth and chemical composition of basil herb. *Acta Scientiarum Polonorum Hortorum Cultus, 17*(6), 135-145.

Elzebroek, A. T. G. (2008). *Guide to cultivated plants*. CABI. UK, 540 p.

Fallahi, E., Arzani, K., & Fallahi, B. (2013). Long-term leaf mineral nutrition in 'Pacific Gala'apple (*Malus domestica* Borkh.) as affected by rootstock type and irrigation system during six stages of tree development. *The Journal of Horticultural Science and Biotechnology*, *88*(6), 685-692. https://doi.org/10.1080/14620316.2013.11513025

Fallahi, E., Neilsen, D., Neilsen, G. H., Fallahi, B., & Shafii, B. (2010). Efficient irrigation for optimum fruit quality and yield in apples. *HortScience*, *45*(11), 1616-1625. https://doi.org/10.21273/HORTSCI.45.11.1616

FAOSTAT, F. (2022). Food and agriculture organization of the United Nations. *Statistical database*. Available at: http://faostat. Fao.Org/336.default.Asp.

Gouws, A., & Steyn, W. J. (2014). The effect of temperature, region and season on red colour development in apple peel under constant irradiance. *Scientia Horticulturae*, *173*, 79-85. https://doi.org/10.1016/j.scienta.2014.04.040

Habibzadeh L. (2022). Effect of Rootstock and application of some chemicals on physico-chemical parameters and red color development of Braeburn apple fruits in Khorasan-e- Razavi. MSc Thesis, Horticulture Department, Faculty of Plant Production, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.

Habibzadeh L., Alizadeh M, Sharifani M. (2022). Effective factors in the development of red color of apple fruit (*Malus domestica*). 4th International Congress of Agricultural Engineering, Natural Resources, Environment, 16th December, 2022, Tehran, Iran.

Harris, S. A., Robinson, J. P., & Juniper, B. E. (2002). Genetic clues to the origin of the apple. *TRENDS in Genetics*, *18*(8), 426-430. https://doi.org/10.1016/s0168-9525(02)02689-6.

Honda, C., & Moriya, S. (2018). Anthocyanin biosynthesis in apple fruit. *The Horticulture Journal*, *87*(3), 305-314. https://doi.org/10.2503/hortj.OKD-R01

Iglesias, I., Echeverría, G., & Soria, Y. (2008). Differences in fruit colour development, anthocyanin content, fruit quality and consumer acceptability of eight 'Gala' apple strains. *Scientia Horticulturae*, *119*(1), 32-40. https://doi.org/10.1016/j.scienta.2008.07.004

Jahani, M., Sayyari Zohan, M., Moradinezhad, F., Mirzaee, M. R., & Khayyat, M. (2024). Effectiveness of potassium spraying on mitigation of aril paleness disorder in different pomegranate cultivars. *Journal of Plant Nutrition*, *47*(18), 3024-3034. https://doi.org/10.1080/01904167.2024.2376232

Kamiab, F., Tavassolian, I., & Hosseinifarahi, M. (2023). Changes in the quantitative and qualitative characteristics of seedless barberry (*Berberis Vulgaris* L.) fruit as influenced by fruit thinning. *Journal of Horticulture and Postharvest Research*, *6*(1), 77-92. https://doi.org/10.22077/jhpr.2022.5416.1279

Karagiannis, E., Michailidis, M., Tanou, G., Scossa, F., Sarrou, E., Stamatakis, G., & Molassiotis, A. (2020). Decoding altitude-activated regulatory mechanisms occurring during apple peel ripening. *Horticulture Research*, *1*(7), 120. https://doi.org/10.1038/s41438-020-00340-x

Kashyap, G., & Gautam, M. D. (2012). Analysis of vitamin C in commercial and naturals substances by iodometric titration found in nimar and malwa regeion. *Journal of Scientific Research in Pharmacy*, *1*(2), 77-78.

Kim, S. H., Lee, J. R., Hong, S. T., Yoo, Y. K., An, G., & Kim, S. R. (2003). Molecular cloning and analysis of anthocyanin biosynthesis genes preferentially expressed in apple skin. *Plant Science*, *165*(2), 403-413. https://doi.org/10.1016/S0168-9452(03)00201-2

Kumar, A., Bhuj, B. D., & Dhar, S. (2024). New approaches of rootstocks in fruit production: a review. *Open Access Journal of Botanical Insights*, *2*(1), 000109.

Kuzin, A. I., Kashirskaya, N. Y., Kochkina, A. M., & Kushner, A. V. (2020). Correction of potassium fertigation rate of apple tree (*Malus domestica* Borkh.) in central Russia during the growing season. *Plants*, *9*(10), 1366. https://doi.org/10.3390/plants9101366

Li, Z., Gemma, H., & Iwahori, S. (2002). Stimulation of 'Fuji' apple skin color by ethephon and phosphorus–calcium mixed compounds in relation to flavonoid synthesis. *Scientia Horticulturae*, *94*(1-2), 193-199. https://doi.org/10.1016/S0304-4238(01)00363-6

Lueangprasert, K., Uthaibutra, J., Saengnil, K., & Arakawa, O. (2010). The effects of sugar application on the concentrations of anthocyanin and flavonol of 'Mahajanaka' mango (*Mangifera indica* Linn. cv. Mahajanaka) fruit. *Chiang Mai Journal of Science*, *37*, 355-362.

Matsuoka, K. (2019). Anthocyanins in apple fruit and their regulation for health benefits. *Flavonoid–a Coloring Model for Cheering Up Life*. IntechOpen; Available from: http://dx.doi.org/10.5772/intechopen.85257.

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry, 31*(3), 426-428.

Moradinezhad, F., Dorostkar, M., Niazmand, R., & Doraki, G. (2024). A comprehensive study of qualitative and biochemical characteristics of dried seedless barberry fruits from different regions

of South Khorasan Province, Iran. *Journal of Horticulture and Postharvest Research*, *7*(4), 345-360. https://doi.org/10.22077/jhpr.2024.7912.1399

Musacchi, S., & Serra, S. (2018). Apple fruit quality: Overview on pre-harvest factors. *Scientia Horticulturae*, *234*, 409-430. https://doi.org/10.1016/j.scienta.2017.12.057

Nava, G., Dechen, A. R., & Nachtigall, G. R. (2007). Nitrogen and potassium fertilization affect apple fruit quality in southern Brazil. *Communications in Soil Science and Plant Analysis, 39*(1-2), 96-107. https://doi.org/10.1080/00103620701759038

Neilsen, G. H., Neilsen, D., Herbert, L. C., & Hogue, E. J. (2004). Response of apple to fertigation of N and K under conditions susceptible to the development of K deficiency. *Journal of the American Society for Horticultural Sciences, 129*(1), 26-31.

Njira, K. O., & Nabwami, J. (2015). A review of effects of nutrient elements on crop quality. African *Journal of Food, Agriculture, Nutrition and Development, 15*(1), 9777-9793. https://doi.org/10.18697/ajfand.68.13750

Omokolo Ndoumou, D., Tsala Ndzomo, G., & Djocgoue, P. F. (1996). Changes in carbohydrate, amino acid and phenol contents in cocoa pods from three clones after infection with phytophthora megakarya Bra. and Grif. *Annals of Botany*, *77*(2), 153-158.

Plunkett, B. J., Henry-Kirk, R., Friend, A., Diack, R., Helbig, S., Mouhu, K., ... & Allan, A. C. (2019). Apple B-box factors regulate light-responsive anthocyanin biosynthesis genes. *Scientific Reports*, *9*(1), 17762. https://doi.org/10.1038/s41598-019-54166-2.

Robinson, T., & Lopez, S. (2010, August). Crop load affects'Honeycrisp'fruit quality more than Nitrogen, Potassium, or irrigaion. *In XXVIII International Horticultural Congress on Science and Horticulture for People (IHC2010): International Symposium on the 940 (pp. 529-537).* https://doi.org/10.17660/ActaHortic.2012.940.76

Saure, M. C. (1990). External control of anthocyanin formation in apple. *Scientia Horticulturae, 42*(3), 181-218. https://doi.org/10.1016/0304-4238(90)90082-P

Shahkoomahally, S., Chaparro, J. X., Beckman, T. G., & Sarkhosh, A. (2020). Influence of rootstocks on leaf mineral content in the subtropical peach cv. UFSun. *HortScience*, *55*(4), 496-502. https://doi.org/10.21273/HORTSCI14626-19

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American journal of Enology and Viticulture*, *16*(3), 144-158.

Solhjoo, S., Gharaghani, A., & Fallahi, E. (2017). Calcium and potassium foliar sprays affect fruit skin color, quality attributes, and mineral nutrient concentrations of 'Red Delicious' apples. *International Journal of Fruit Science*, *17*(4), 358-373. https://doi.org/10.1080/15538362.2017.1318734

Tijskens, L. M. M., Unuk, T., Tojnko, S., Hribar, J., & Simcic, M. (2011). Colour development in the apple orchard. *Journal of Fruit and Ornamental Plant Research*, *19*(1), 113-121.

Wagner, G. J. (1979). Content and vacuole/extravacuole distribution of neutral sugars, free amino acids, and anthocyanin in protoplasts. *Plant Physiology, 64*(1), 88-93. https://doi.org/10.1104/pp.64.1.88

Whale, S. K., Singh, Z., Behboudian, M. H., Janes, J., & Dhaliwal, S. S. (2008). Fruit quality in 'Cripp's Pink'apple, especially colour, as affected by preharvest sprays of aminoethoxyvinylglycine and ethephon. *Scientia Horticulturae*, *115*(4), 342-351. https://doi.org/10.1016/j.scienta.2007.10.015

Xu, X., Du, X., Wang, F., Sha, J., Chen, Q., Tian, G., ... & Jiang, Y. (2020). Effects of potassium levels on plant growth, accumulation and distribution of carbon, and nitrate metabolism in apple dwarf rootstock seedlings. *Frontiers in Plant Science*, *11*, 904. https://doi.org/10.3389/fpls.2020.00904

Yaldız, G., Özen, F., Çamlıca, M., & Sönmez, F. (2018). Alleviation of salt stress by increasing potassium sulphate doses in four medicinal and aromatic plants. *Acta Agriculturae Scandinavica, Section B—Soil & Plant Science*, *68*(5), 437-447. https://doi.org/10.1080/09064710.2017.1420214