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EVALUATION OF THE SENSITIVITY OF KUMQUAT FRUIT HARVESTED AT DIFFERENT STAGES OF COLOR MATURITY DURING COLD STORAGE

Dalia M. Hikal[1], Mohamed A. Abdein[2*], EL-Sayed M. Qaoud[3*], Azza S. Hussein[4], Sally A. Arafa[5], Safaa A. Limam[6], Nancy A. Taha[7], Ahmed Y. El-Saman[8], Amgad M. Elazazy[8], Dina S.M. Ibrahim[8], Lames Abd El-Ati[8], Randa E.Y. Habasy[8], Huda M.H. Ismaiel8, Suzan M. Hussien[9], A.A. Lo'ay[10], Mahmoud F. Maklad[11], Hesham S. Ghazzawy[12], Mostafa Gouda[13] and Amira S.A. Abd El-Rahman[14]

[1]Nutrition and Food Science, Home Economics Department, Faculty of Specific Education, Sohag University, Sohag 82524, Egypt

[2]Seeds Development Department, El-Nada Misr Scientific Research and Development Projects, Turrell, Mansoura 35511, Egypt. Email: abdeingene@yahoo.com*

[3]Horticultural Department, Faculty of Agriculture, Suez Canal University, Ismailia 41522, Egypt. Email: s_qaoud@agr.suez.edu.eg*

[4]Pomology Department, Faculty of Agriculture, Assiut University, Assiut 71526, Egypt

[5]Botany Department, Faculty of Agriculture, Mansoura University, Mansoura 35516, Egypt

[6]Food Science and Technology, Department, Faculty of Agriculture, Assiut University, Assiut 71515, Egypt

[7]Agricultural Chemistry Department, Faculty of Agriculture, Mansoura University, Mansoura 35516, Egypt

[8]Horticulture Research Institute, Agricultural Research Centre, Giza 12619, Egypt

[9]Botany Department, Faculty of Science, Mansoura University, Mansoura 35516, Egypt

[10]Pomology Department, Faculty of Agriculture, Mansoura University, Mansoura 35516, Egypt

[11]Horticulture Department, Faculty of Agriculture, Ain shams University, Cairo 11241, Egypt

[12]Date Palm Research Center of Excellence, King Faisal University, Al Hofuf 31982, Saudi Arabia

[13]College of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou 310058, China

[14]Horticulture Department, Faculty of Agriculture, Benha University, Moshtohor, Qalyoubia13736, Egypt

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Corresponding Author: Mohamed A. Abdein, EL-Sayed M. Qaoud (abdeingene@yahoo.com, s_qaoud@agr.suez.edu.eg)

ABSTRACT

Harvested fruit color maturity stages (HFCMs) are classified into three stages: green stage (Gs), yellow stage (Ys), and orange stage (Os). The purpose of the study was to evaluate how kumquat fruits harvested at different stages of color maturity behave under cold storage in order to fill the knowledge gap in postharvest fruit storage research and reduce food waste. For four weeks, fruit classes were kept at 4 ± 1°C and 95 ± 5% relative humidity. Principal component analysis (PCA) was combined with the findings of these assays to investigate the impact of HFCMs on the kumquat's overall response. Each week throughout the storage period, the fruit decay rate, fruit space color parameter, and chilling injury index were estimated. Fruit skin pigments and fruit chemical quality traits were assessed. The results proved that Gs presented low CI symptoms and more AEAs compared with other kumquat fruit maturity stages. Furthermore, the Gs can delay the decay of vitamin C contents and inhibit increases in malondialdehyde and electrolyte leakage. The experiment results, which showed that Gs may greatly slow the decay rate in the quality attributes of kumquat fruit and are superior to other maturities, were supported by a thorough analysis of the data using PCA (Ys and Os). The findings indicate that Gs have a strong chance of creating a technique for handling kumquat fruit in the future. Testing the response of different ages of kumquat fruit to storage at low temperatures is new.

KEYWORDS: Fortunella Margarita; Postharvest; Chilling Injury; Antioxidant Enzymes; Antimicrobial Activities; MALDI-TOF Analysis.

1. INTRODUCTION

Horticulture scholars have classified kumquat as a non-climacteric fruit (Brüggenwirth and Knoche, 2017). In addition to its nutritional value (Barreca et al., 2011) and medical benefits, it also captures the attention of consumers due to its look (Aloui et al., 2014). The shelf life of natural kumquats is limited by Penicillium (blue rot) fungus infection during the marketing chain (Chen et al., 2019, Mosallam et al., 2024a), resulting in economic losses (Liu et al., 2018). Consumers typically favor a full kumquat fruit with its skin. Therefore, attention must be exercised when exogenous chemicals are applied to fruits.

In general, low temperatures are utilized to manage fruit ripening, although some conditions, such as the maturity of the collected fruit, impact fruit reactions (Zhu et al., 2017). Some metabolic changes are known to occur when food is held at a low temperature for a long period. Additionally, antioxidant enzyme activity is decreased (Amaro et al., 2015, Abdelkader et al., 2022, Abdein et al., 2023), but ROS production pathways are accelerated. Kumquats held below 10°C exhibited chilling injury symptoms (CI) occurrence. ROS promotes oxidative processes, which contribute to CI clarity (Combrinck et al., 2006). When ROS production and antioxidant activity are unbalanced, homeostasis (an increase in oxidative reaction pathways) is set off (Dianat et al., 2016). Due to the susceptibility of kumquats to low temperatures, more enforcement is necessary for them to inhibit CI symptoms (Ferraz et al., 2016).

Several reports have stressed the need to use natural substances to reduce or limit fungal infestations (Demiray and Tulek, 2017). Many studies have also been conducted on the storage of kumquat fruit to preserve fruit quality during cold storage (Qaoud et al., 2024, Mosallam et al., 2024b). Preharvest spraying of kumquat fruits with gibberellic acid and its effect on endogenous hormones and fruit quality during cold storage (Cai et al., 2021). Treating fruit with ellagic acid to enhance the antioxidant system during cold storage (Horuz et al., 2017) or using hot water to enhance the activity of antioxidants (Avhad and Marchetti, 2016, Khalil et al., 2022) and cold storage performance (Yang et al., 2022). Furthermore, kumquat fruit can be preserved by coatings enriched with tarragon essential oils or by using UV to minimize decay (Hosseini et al., 2019).

Numerous industries, including the agricultural and food sectors, use nanoparticles because of their special functional qualities, and there are already a growing number of products on the market that contain nanomaterials. Enhancing food production processes, prolonging the shelf life of fresh products, enhancing product consistency, stability, and texture, and improving nutrient absorption and bioavailability are the primary motivations for the use of nanosized ingredients and additives in food as well as food-related products (Bouwmeester et al., 2014).

An essential plant nutrient needed for the construction of cell walls and membranes is calcium (Ca^{2+}). It can build a structure called the "egg-box" of pectin by cross-linking with de-esterified pectic residues. This increases the firmness of the fruit and stabilizes the plant's cell wall, shielding it from enzyme deterioration (White and Broadley, 2003). Consequently, calcium treatments prolong a fruit's storage life and postpone its softening. Additionally, calcium's ability to reduce physiological disorders, like internal browning, is another factor in preserving fruit quality while it is being stored (Martín-Diana et al., 2007).

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been used to identify enzymes and peptides (Böhme et al., 2012). Identification of microbial proteins with great specificity and comparison of peaks to data stored in a database. Using this method, it is possible to quickly identify specific peptide and protein mass spectral patterns in either microbial or plant cells (Ashfaq et al., 2022). Comparison of a collection of experimentally obtained peptide masses from trypsin digestion with a database of in silico digested peptide masses from known proteins is the basis for the peptide mass fingerprinting (PMF) method of protein identification (Jang and Kim, 2018).

The use of post-harvest physical and chemical technologies to maintain the quality of fresh fruits and vegetables and reduce post-harvest losses has been the subject of intensive research in recent years. However, the efficiency and benefits of quality assessment using several contactless and nondestructive technologies are not discussed and contrasted with traditional methods. Therefore, the aim of this research was to evaluate the behavior of different harvest color maturity stages (Gs, Ys, and Os) of kumquat fruits treated with nano-calcium under cold storage.

2. MATERIALS AND METHODS

2.1. Fruit Materials

Fortunella margarita (Lour.) Swingle, an oval 'Kumquat' from the Rutaceae family, was harvested from three HFCMs. As soon as 90 days have passed since the fruit's flowering, its rind changes from green to dark orange (Figure 1). An SSC:TA ratio > 3 is considered commercially mature for kumquats (Liu et al., 2018). Fruits of uniform size and free of insect damage, disease, and mechanical damage were selected. Kumquat plants were grown in private farms in Nobaria, a region south of Alexandria, Egypt, in sandy soil with permission and under the supervision of the Faculty of Agriculture, Mansoura University, Mansoura, Egypt. Kumquat fruit materials used after harvest in 2024 and 2025 success seasons. It was planted approximately 3 × 3.5 meters. At 13oC and 90% RH, the fruit was transported to the lab (Agricultural Chemistry Department, Faculty of Agriculture, Mansoura University, Mansoura, Egypt) in about four hours. All chemicals used in the study were obtained from Sigma-Aldrich, St. Louis, MO, USA, with permission and under supervision from the Agricultural Chemistry Department, Faculty of Agriculture, Mansoura University, Mansoura, Egypt. Approximately 1800 fruits were used in this study. The fruits were divided into two main batches for grading and storage purposes. There were 900 fruits in the first batch, which was divided into three batches of 300 fruits each. Three hundred fruits were distributed equally into three replicates, with 100 fruits in each replication. All fruits treated with the three HFCMs were treated with Nano-calcium at the optimum concentration (Chen and Wang, 2012). Physical parameters measured include CI symptoms, water loss percentage (WL %), and kumquat color layout hue angle (ho). Among the most common types of damage, diseases, and mechanical damage were assessed. The distribution of the second batch (900 fruits) was the same as that of the first batch. Chemical determinations were divided into sections for ascorbic acid (AsA), total soluble solids (SSC %), titratable acidity (TA %), and SSC:TA ratio. Carotene (Car) and total chlorophyll (Chl a+b) were identified in the fruit skin pigments. Among the antioxidant enzyme activities (AEAs) were measured superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR), and catalase (CAT). The fruits were then maintained at 4 ± 1°C and 95 ± 5% RH.

2.2. CI-Index, Water Loss%, Decay Percentage, And Fruit Color (Ho)

After storage at 4 ± 1°C for four weeks, the kumquat showed CI damage. It was described as a brown patch with pit-like depressions on a kumquat. The severity of browning-color patches and the rate of kumquat skin degradation were used to visually measure the degree of damage each week. It is separated into five groups: Skin damage can range from none to mild, moderate, severe, and extremely severe (Lafuente et al., 1997). Based on the first value at harvest time, fruit WL% was measured as a percentage, and fruit decay was also measured as a percentage (Yu et al., 2008). Using a colorimeter (Minolta CR-300) and the CIE L* a* b* system interoperate given by the Commission Internationale de l'éclairage, a weekly sketch of the kumquat's skin tone was taken (CIE). The value was obtained using the b*/a* equation, and the results are presented in terms of the Minolta camera read (Yuan et al., 2016).

2.3. Ascorbic Acid, And Photosynthetic Pigments

To calculate AsA while safeguarding it throughout the tests, 2,6-dichlorophenolindophenol reagent and (6%) oxalic acid were used in a titrimetric method (Jiang and Zhang, 2001). The AsA concentration was estimated as mg kg-1 on a fresh weight basis. Car and Chl (a+b) were extracted by using 2.5 g of kumquat skin, utilizing 10 mL of the N, N-dimethylformamide solvent (Kalaji et al., 2017). Both Car and Chl a+b were expressed as g kg-1 fresh weight.

2.4. SSC (%), TA (%), And SSC: TA Ratio

The SSC% of kumquat juice was calculated in a laboratory setting using a Refractmeter (PR-32 ALAGO Co., Japan). Kumquat juice (10 mL) was used to titrate NaOH (0.1 N) based on citric acid for TA%. The percentage was used to represent the outcomes. Nevertheless, the SSC:TA ratio was used to determine the maturity of kumquat fruit (Pelletier et al., 2010).

2.5. Antioxidant Enzymes Activities (Aeas)

Potassium phosphate (20 mL, 100 mM KH2PO4) was used to produce 2 g of kumquat as a flavedo, and CAT activity was then calculated. The mixture was subjected to two centrifugations at 25000 g for 25 min. at 4°C. 200 µL of the extract was needed to evaluate the CAT activity in a final volume of 5 mL, which contained 1 mL of catalyst extraction (400–800 mg protein). The volume of the mixture, which at 25°C transforms one mmol of H_2O_2 every second, serves as the basis for the designation of the CAT activity unit. Enzyme activity was expressed in units of moles per kilogram µmol s-1 kg-1. 1 g of Kumquat flavedo skin was used to extract the APX, which was then combined with 10 mL of 50 mM KH2PO4, EDTA, 1 mM ascorbic acid, and 1% polyvinylpolypyrrolidone (PVP) in a 5°C reaction mixture. The mixture was centrifuged at 20,000 g for 35 min at 5°C in order to determine the activity at the end of 3 mL. Apply clear supernatants frequently. It was discovered that the pattern of an enzyme that used 1 mmol of ascorbate every second at 25°C served as the unit of enzyme activity (Zhu et al., 2017, Albalwe et al., 2024). Enzyme activity was expressed in moles per kilogram. Enzyme activity is presented as µmol s-1 kg-1.

1 g of Kumquat flavedo skin was combined with 10 ml of 100 mM KH2PO4 and 0.5 mM EDTA at 4°C to produce GR. Centrifuging the mixture twice for 30 minutes at a speed of 30,000 g. 200 L of supernatant were needed to measure the activity in 3 mL (Re et al., 1999).

To measure SOD activity, one gram of flavedo kumquat skin was added to 10 ml of 50 mM KH_2PO_4 and 1.33 mM diethylenetriamine penta-acetic acid at 5°C. Unexpectedly, the substance underwent 20000 g, 25-minute centrifugations at 5°C. 60-70 milliliters of chemical concentration were present in three milliliters of the supernatant (24–56 mg protein). Activity is proxied by the chemical concentration, which causes a half-maximal decline. The chemical concentration that resulted in a half-maximal drop served as a proxy for activity. Enzyme activity is presented as µmol s-1 kg-1. Total protein was prepared and analyzed as a basis for calculating catalyst activities (Wang et al., 2010).

2.6. Lipid Peroxidation Malondialdehyde Accumulation (MDA), And Electrolyte Leakage%

Kumquat flavedo malondialdehyde (MDA) accumulation was determined by the TABRS test. 2.5 g of kumquat flavedo tissue is mixed with 0.5 mL of butylated hydroxytoluene, 25 mL of metaphosphoric acid (5%; w/v of HPO3), and 0.5 mL of BHT (2%; w/v of C15H24O). To calculate the MDA buildup in kumquat peel during storage, tetraethoxypropane (C7H16O4; Sigma-Aldrich) was used to generate a standard curve with a range of 0-2 mM (TBARS), which was comparable to 0-1 mM MDA. Using fresh weight, MDA content was reported as mol Kg-1 (de Dios Alché, 2019, El-Gioushy et al., 2022). Estimated (EL%) for each sample at different times while it was kept cold. The peel (2 g) was mixed with mannitol (10 mL of mannitol at 6 mol) for 3 h. A conductivity meter was used to measure conductivity (M1). The peel tissue was then obliterated by placing all cuvettes in a water bath and boiling them for an hour at 100°C. All cuvettes were then kept under laboratory conditions for cooling, and conductivity was evaluated as the total leakage (M2). The proportion of relative EL was calculated (Chen et al., 2019).

2.7. H_2O_2, And O_2•–Production Rate and DPPH Reduction

For the H_2O_2 test, the kumquat flavedo sample (1 g) was combined with 6 mL of acetone $(CH_3)_2CO$ solution. 20 minutes of centrifuging the mixture at 15000 g at 5°C. The 100 mL of extraction received 200 mL of NH4OH and 100 l of 5% Ti $(SO_4)_2$. The titanium-peroxide (TiO_2) complex started the process, and the deposit was removed by adding 4 mL of 2 M H_2SO_4 after centrifugation at 5000 × g for 15 min. A spectrophotometer set at 415 nm and H_2O_2 level were used to calculate the amount (Anuyahong et al., 2020). The results are displayed in mol Kg-1 based on fresh weight. One gram of kumquat flavedo and three milliliters of KH_2PO_4 (50 mM, pH 7.8) were combined at room temperature and filtered to calculate the O_2 content. After adding the polyvinylpyrrolidone (PVP, 1% w/v) reagent, the sample was centrifuged at 15000 g for 20 min. at 5°C (Taher et al., 2022). By measuring the NO_2 lifetime of hydroxylamine (H_3NO), which produces O_2•, the rate of O_2•. Standard curve that quantifies O_2• production from hydroxylamine using NO_2. Based on fresh weight, the O_2• value was expressed as mol Kg-1 (Yang et al., 2011). The DPPH radical assay, which is based on the dismutation of radical activity, was used to evaluate the antioxidant activity of the kumquat flavedo. The DPPH radical quenching mechanism was studied using a spectrophotometer that measures the DPPH decrease at 517 nm. Based on the fresh weight, DPPH* was expressed as a percentage.

2.8. MALDI-TOF Analysis

The plant tissues were prepared for protein extraction in 200 µL of extraction buffer containing 8 M urea, 0.1 ml tris-HCl pH 7.8, and 1 × protease inhibitor mix (-20 °C). At 15,000 rpm, the cell suspension was centrifuged for 30 min. The proteins were precipitated by adding ice-cold acetone. 1 µg of sequencing grade trypsin was added to the samples, followed by incubation at 37ºC for 16-18 hrs (Lo'ay and Ameer, 2019, Lo'ay et al 2021a &b).

2.9. Statistical Analysis

The experiment included four degrees of storage time in weeks, three FCM treatments, and three replicates of each treatment. It was just random. The JMP Pro 16 application was used to conduct Duncan's test; statistical significance was determined as P<0.05. (SAS Institute, Cary, NC, USA).

3. RESULTS

3.1. CI-Index, Weight Loss%, Fruit Skin Color Hue, And Fruit Decay Percentage

The effects of HFCMs on physical characteristics during cold storage are shown in (Figure 2). It was found that CI started after the 2nd week and developed thereafter until the end of the storage period. The kumquats at Gs presented less damage than those at yellow stage (Ys) and orange stage (Os) through 4 W of cold storage. Differentiation among HFCMs was visibly clear after the 2^{nd} week and became clear in the 4th week (Figure 2A). Regarding fruit WL%, fruit harvested at Os showed higher WL% than at the Ys and Gs stages (Figure 2B). This is reflected in the color hue angle and decay of fruit during the cold storage period, especially with fruit harvest at Os. Increased WL% from the fruit is associated with increased cold symptoms, which also decreases the ho of the fruit (Figure 2C). Furthermore, Gs fruit showed less fruit decay than the other fruit maturities (Figure 2D). The other fruit color space parameters were indicated by the effects of low-temperature storage on kumquat fruit during cold storage. The results pointed to a significant ($P<0.05$) effect of HFCMs in response to low temperatures. Changes in a*, b*, and L* parameters for different harvest fruit color maturity stages during the storage period were clearly observed (Figure 3). The color-space parameters a* and b* increase. However, L* decreased until the end of the storage period compared to other fruit color maturities.

3.2. Ascorbic Acid and Photosynthetic Pigments

Both Chl a+b and Car are the greatest pigments, in addition to the AsA content found in kumquat fruit, which has a major impact on the degree of appearance of the visual color and quality of the fruit. The interaction between HFCMs and storage duration (in weeks) is shown in Figure 4. There were substantial variations across HFCMs in terms of Chl a+b, Car, and AsA concentrations at harvest. Initially, the HFCMs recorded a high value during the initial cold storage time. Furthermore, the values were graded according to the color maturity stage of the fruit. The Gs fruit had more Chl a+b = 0.350 g Kg-1 and AsA = 68 g Kg-1 whereas in Car = 75 g Kg-1 at the initial week of cold storage. Moreover, Chl a+b and AsA decreased, but Car increased independently in response to the increase in HFCMs during the cold storage period. This was related to the concentrations of Chl, Car, and AsA in the fruit.

3.3. SSC%, TA%, And SSC: TA Ratio

The SSC%, TA%, and SSC:TA ratio is displayed as functions of storage time in weeks in Figure 5. These variations were investigated in three HFCMs slated for four weeks of storage. There was a significant interaction ($P<0.05$) between storage duration at weekly intervals and HFCMs that has been obtained. It was observed that SSC% increased considerably and gradually throughout four weeks of cold storage, whereas TA% decreased autonomously. Consequently, the SSC:TA ratio was affected by the HFCMs at the start of the experiment. Where the fruit was recorded according to HFCMs.: Gs; SSC, 5.52 (%); TA, 0.66 (%); and SSC:TA ratio, 8.36, Ys; SSC, 6.92 (%); TA, 0.53 (%); and SSC:TA ratio, 13.06, and Os; SSC, 8.33 (%); TA, 0.41 (%); and SSC:TA ratio, 20.29.

3.4. Antioxidants Enzyme Activities (Aeas) Performance

The impact of storage time in weeks and HFCMs on the antioxidant activities of fruits stored in cold storage is shown in Figure 6. Initially, it was found that the AEAs of fruits differed independently according to HFCMs. The AEAs capacity increased gradually during cold storage for four weeks. The maximum activity was observed up to the 2nd week for all fruit color maturities, and significant differences were found ($P<0.05$). After two weeks of cold storage, the AEAs of all kumquats (G, Y, and O stages) and the antioxidant activities declined independently until the end of the storage period.

3.5. Estimation Malondialdehyde (MDA), And Electrolyte Leakage (EL%)

Fluctuations in MDA and EL (%) are shown in Figure 7. Our research revealed that kumquat was picked from HFCMs (Gs, Ys, and Os) and stored for four weeks. When storage duration and HFCMs were taken into consideration, an interaction between MDA and EL (%) was found analytically ($P<0.05$). The increasing values of MDA and EL (%) extended during the duration of the storage period, which is relevant to the main alterations in fruit held at 4 ± 1°C.

3.6. H_2O_2 And $O_2^{\bullet-}$ Production and DPPH Reduction%

As a function of storage time, there was a significant interaction between the fluctuations in the rates of $O_2^{\bullet}$ and H_2O_2 radical generation and scavenging (measured by DPPH reduction %) (4 W). When storage intervals and HFCMs were used as experimental components, the differences were significant ($P<0.05$) (Figure 7). From the beginning until the fourth week of storage, the kumquat flavedo $O_2^{\bullet}$ and H_2O_2 radical accumulation rates evolved and grew continuously; thus, both radicals developed independently associated with HFCMs until the end of storage. Positively, Gs showed the lowest radical formation, as opposed to other HFCMs, at the initial time. Notably, radical generation/accumulation was enhanced independently by HFCMs until the end of the storage period. However, the estimation of scavengers of DPPH reduction presented both independent and gradual overall HFCMs during the storage intervals.

3.7. Multivariate Analysis of Kumquat Parameters

A principal component analysis (PCA) for physiological and biochemical metric data from kumquats held under shelf-life conditions was carried out to ascertain how various phases of HFCMs were affected. Fruits were stored for four weeks at 4 ± 1°C and 95 ± 5% RH. PCA was used to isolate the effects of HFCMs on kumquat fruits held at low temperatures. The variability of the data was explained by PC1 (73.2%) and PC2 (13.4%), (Figure 8A). The CI index was negatively associated with each factor, as shown in Figure 8B. The AEAs and CI index had a favorable correlation. MDA and EL% demonstrated a positive association with these three valuables (APX, CAT, SOD, and GR). The SSC: TA ratio and TA% displayed a negative correlation. However, AsA showed a positive correlation with the AEAs and TA% while displaying a negative association with other assets such as SSC% and SSC: TA. The correlations and these findings are shown by Pearson's correlation matrix among the investigated factors (Table 1).

3.8. Optimization Of A MALDI TOF-TOF Mass Spectrometer Matrix

HCCA was dissolved in a saturated solution containing 0.9, 0.8, 0.7, and 0.6% of TFA, respectively. Figure 9 shows the histograms of crystallization between the matrix and solvents. When 70% of HCCA was used, homogeneity of the spot was observed regardless of the amount of TFA. Good mass spectra were only available with laser scanning at 6000 with a standard deviation of the peak number between 4-8 for 0.9% TFA, 4-11 for 0.8% TFA, 4-12% for 0.7% TFA, and 5-12% for 0.6% TFA, respectively. The most homogenous surface of the sample matrix was obtained when using 10% HCCA and 0.6% TFA, with a sharp and low number of peaks as the lowest standard deviation. To obtain the high repeatability and sharp peaks, 10% HCCA with 0.6% TFA was used as the final matrix solvent in the present study.

3.9. MALDI-TOF Analysis for Antioxidant Enzymes

The closest matches to the purified proteins found in the database were obtained from MASCOT search results. The first was matched to glutathione peroxidase from Citrus japonica (Kumquat) with the accession number: (UGT708G1). The second was the closest match to superoxide dismutase from Citrus japonica (Kumquat) with the accession number (CITJP). Catalase activity was not detected in Citrus japonica. Analysis of the peaks at 390.7, 519.7, 402.5, 619.8, 848, 977.1, 989.2, and 2109 m/z resulted in the identification of the peptide sequences SIATR, AIGMGK, GIQTVTI, PDIPYDYSAIEPAISGEIMQIHHQK, AVEQIGQAINK, IQSAI, NIAPANEGGGEPPK, MSAEGAAIQGS, and GWVWIGIDTEFK. Analysis of the peaks at m/z 3912.5, 1041.6, 1013.1, 1007.8, 962.5, and 501.4 resulted in the identification of glutathione peroxidase and yielded the sequences IMK, SFSAK, SIYDFTVK, GNDVS, ISGYR, VIIVVNVASK, ELNV, IYEK, NQDFEVIAFPCNQFAGQEPGSNEE, IQEVACTMFK, and IDVNGK (Figure 10).

4. DISCUSSION

The different responses obtained between fruits harvested at different color variations (G, Y, and Os) may be due to the age differences in the cells of the fruit in size or volume and their development during the growth stages (Brüggenwirth and Knoche, 2017). Accordingly, it can be assumed that the kumquat fruit harvested at the green stage had a lower cell volume than the Y and O stages. Furthermore, the kumquat skin thickness at Gs was greater than at Ys or Os (de Dios Alché, 2019). From scientific information confirmed by an anatomical study with an electron microscope, it can be concluded that water loss from kumquat fruit increases with a decrease in the thickness of the peel (pericarp). Consequently, cell wall thickening increases during growth in form layers, which decreases in thickness with age (Jang and Kim, 2018). Rapid and robust MALDI-TOF MS techniques for microbial identification: Peel thickness during cold storage may play an important role in preventing kumquat water loss. The CI appearance during cold storage is connected to peel water loss that may be related to long-term ROS production under low temperature stress (Yang et al., 2011). Hence, cell membrane dysfunction can occur. Consequently, under cold stress conditions, an imbalance between the capacity of antioxidant scavengers and ROS producers causes an increase in oxidative stress and cell death. The appearance of chilling symptoms also reflects the hue of fruit color throughout cold storage. It was found that Chl decreased and Car was gradually synthesized, as previously demonstrated (Yu et al., 2008). These changes in fruit color under cold storage are also taken into consideration, while, AsA content in kumquat declines for two reasons: fruit growth and development and fruit ripening. Second, the cold storage temperature has an influence. Many studies have been conducted on mandarins, lemons, and raspberries. The explanation for the reduction in h° values give context to a darker color. The h° levels decreased when the fruits were stored at a low temperature of 5oC. In addition to pigment breakdown, enzymatic and non-enzymatic activities can also result in browning areas (Lafuente et al., 1997). Depending on the harvest kumquat color development stages, the changes achieved and illustrated in Figure 3 may be caused by an increase in the rates of water vapor in the fruit. There is also some evidence that inorganic acid concentration decreases with increasing fruit maturity stage (Gs to Ys) (Yu et al., 2008). The differences in antioxidant activities during storage may be due to kumquats being harvested at different ages. Antioxidant activity may decrease as the fruit ripens. Our study proved this trend at the beginning and end of the experiment, as shown in Figure 6. Additionally, in what is known as "homeostasis," a balance could exist between the production of free radicals and the quantity of antioxidants, which reduces the symptoms of cold (Figure 8). The results showed that fruit maturity is an influencing factor in the response to cold storage. Thus, perhaps as a result of different responses, the kumquat fruit led to a difference in antioxidant activity of the antioxidants (Kalaji et al., 2017). That is, an increase in ROS leads to increased fat oxidation under cold storage, considering the increased activity of antioxidants; therefore, there was less oxidative response, followed by less cell death (Liu et al., 2018). This could be a result of decreased O_2• and H_2O_2 radical generation during the cold storage period due to decreased aerobic respiration. Additionally, according to Anuyahong et al. (2020), O_2• and H_2O_2 radicals linked to increased SOD activity may diminish or scavenge raisin AEAs. The scavenging of both O_2• and H_2O_2 radicals produced by cold storage is greatly aided by the action of CAT and APX enzymes in conjunction with other antioxidants (de Dios Alché, 2019). Industrial kumquat protein isolate extracts were analyzed using MALDI-TOF mass spectra collected in linear positive ion mode (Horneffer et al., 2007). The requirement for a quick study is justified by the fact that different isolates in the food sector produce food items with various characteristics, such as flavor and consistency. The storage proteins glycinin and β-conglycinin are prevalent in soybeans. The tested samples were subjected to decreasing conditions during extraction to identify complicated protein subunits that vanish during hydrolysis and have an impact on the isolate's functionality and performance (Careri et al., 2003). CHCA was used as a matrix to acquire mass spectra in linear positive mode (m/z 2000-20,000). There were four marker peaks, and models could compare day 0 to the others and recognize them as such (3–11) ranged from 96.2% to 98.8%. Correct recognition rates of almost 90% were obtained from the total external validation using a separate sample set. However, at the end of the storage period, being able to differentiate between days was a significant constraint. However, this strategy might be a useful replacement for well-established sensory and spectroscopic techniques, which are adversely affected by inherent variations in the hue of fish tissues (de Oliveira et al., 2018). The capacity to track more types of molecules in a single sample is promising in the near future. For instance, it has been demonstrated that proteins, peptides, and TAGs can all be measured in diluted milk utilizing various matrices and improved procedures. Additionally, sample fractionation based on consecutive extractions and ultrafiltration is also available (Calvano et al., 2012). The identification or quantification of particular marker biomolecules is not necessary to acquire the MALDI-TOF MS profile spectra. The basis for their differentiation, which is used for classification/regression purposes or to detect fraud and adulteration, is the comparison of the peak position and intensity patterns. Such objectives necessitate the use of software tools for sophisticated data analyses. PCA is the most frequently used method, although supervised statistical approaches with training and testing datasets are advised (Ling et al., 2021).

5. CONCLUSIONS

Testing the response of kumquat fruit of different ages to storage at low temperatures is new. Hence, it is active during postharvest fruit preservation. In existing research, Gs fruit has been shown to be resistant to chilling temperature O_2•− and H_2O_2 radical quenching activities. Gs were also able to significantly maintain the quality of fruit by minimizing tissue death, deterioration in physiological and biochemical functions, and storage capacity loss compared to Ys and Os. Gs also inhibited the increase in MDA and EL% content and enhanced antioxidant capacity. By using these measures, kumquat growers and exporters can increase their marketability and consumer satisfaction, which will contribute to the sustainability of the kumquat industry.

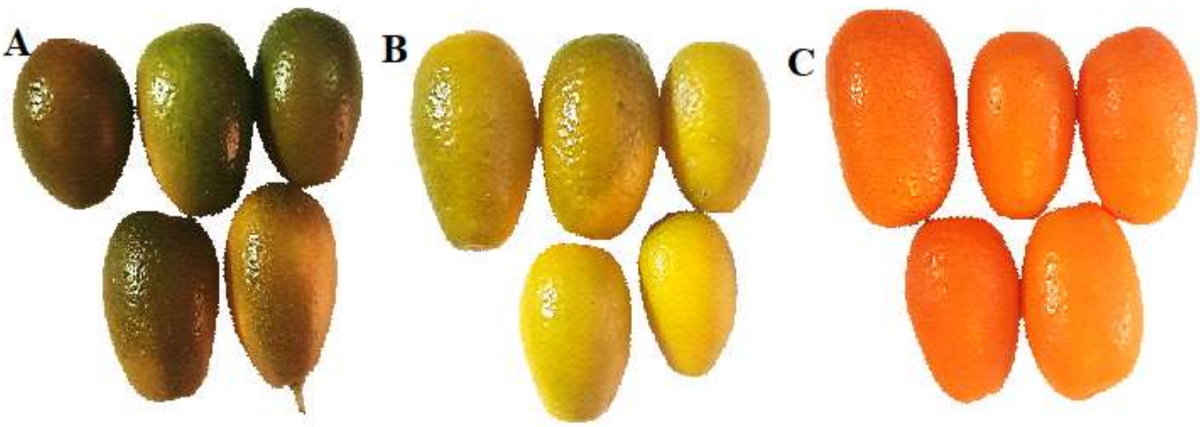
Abbreviations

Harvested fruit color maturity stages	HFCMs
Green stage	Gs
Yellow stage	Ys
Orange stage	Os
Antioxidant enzyme activity	AEAs
Chilling injury symptoms	CI
Principal component analysis	PCA
Malondialdehyde	MDA
Electrolyte leakage	EL%
Matrix-assisted laser desorption/ionization Time-of-flight mass spectrometry	MALDI-TOF MS

Table 1: Pearson's Correlation Matrix Between the Studied Parameters of Kumquat During Shelf-Life.

	CI-index	WL%	*L**	*a**	*b**	h°	Decay%	Chl $_{a+b}$	Car	SSC%	TA%	SSC:TA ratio	AsA	APX	CAT	GR	SOD	H_2O_2	O_2.-	MDA	EL%	DPPH*
CI-index	1.00*																					
WL%	0.85	1.00																				
L*	-0.74	-0.84	1.00																			
a*	0.40	0.69	-0.71	1.00																		
b*	0.63	0.75	-0.91	0.78	1.00																	
h°	-0.21	-0.44	0.50	-0.87	-0.65	1.00																
Decay%	0.75	0.94	-0.90	0.70	0.79	-0.44	1.00															
Chl a+b	-0.59	-0.67	0.94	-0.64	-0.90	0.54	-0.79	1.00														
Car	0.71	0.75	-0.97	0.61	0.85	-0.42	0.86	-0.94	1.00													
SSC%	0.52	0.54	-0.89	0.51	0.81	-0.40	0.70	-0.97	0.94	1.00												
TA%	-0.58	-0.61	0.93	-0.55	-0.85	0.42	-0.76	0.98	-0.96	-0.99	1.00											
SSC:TA ratio	0.62	0.61	-0.91	0.51	0.80	-0.36	0.75	-0.94	0.98	0.98	-0.98	1.00										
AsA	-0.78	-0.89	0.97	-0.72	-0.93	0.55	-0.93	0.92	-0.93	-0.83	0.88	-0.86	1.00									
APX	-0.46	-0.51	0.67	-0.53	-0.70	0.54	-0.52	0.71	-0.60	-0.64	0.66	-0.59	0.70	1.00								
CAT	-0.32	-0.17	0.50	0.12	-0.36	-0.19	-0.29	0.60	-0.55	-0.70	0.67	-0.63	0.42	0.49	1.00							
GR	-0.33	-0.10	0.50	0.05	-0.43	-0.05	-0.15	0.56	-0.56	-0.68	0.64	-0.64	0.39	0.50	0.81	1.00						
SOD	-0.59	-0.50	0.83	-0.27	-0.70	0.17	-0.61	0.87	-0.86	-0.92	0.91	-0.90	0.77	0.70	0.86	0.84	1.00					
H_2O_2	0.80	0.93	-0.95	0.74	0.90	-0.52	0.95	-0.85	0.89	0.76	-0.81	0.79	-0.98	-0.63	-0.34	-0.31	-0.69	1.00				
$O_2^{.-}$	0.87	0.93	-0.95	0.68	0.86	-0.46	0.94	-0.84	0.91	0.77	-0.82	0.83	-0.97	-0.60	-0.36	-0.35	-0.72	0.97	1.00			
MDA	0.83	0.91	-0.95	0.61	0.80	-0.38	0.94	-0.84	0.92	0.79	-0.83	0.84	-0.95	-0.56	-0.45	-0.35	-0.74	0.95	0.96	1.00		
EL%	0.85	0.92	-0.92	0.59	0.79	-0.37	0.91	-0.82	0.87	0.75	-0.80	0.79	-0.93	-0.60	-0.48	-0.36	-0.75	0.95	0.95	0.97	1.00	
DPPH*	0.72	0.92	-0.88	0.86	0.87	-0.60	0.91	-0.74	0.79	0.62	-0.68	0.67	-0.91	-0.56	-0.13	-0.11	-0.50	0.93	0.91	0.87	0.85	1.00

*: Values express average values of cold storage duration and FCMs. CI-Index – chilling injury index; WL%—Water loss percentage; *L**—Lightness; a*—Red/green value; b* - Blue/Yellow value; h° – Hue angle; Car – Carotene content; SSC%— Total soluble solids percentage; TA%— Total acidity percentage; SSC:TA ratio—The ratio between SSC to TA%; AsA—Ascorbic acid content; APX – Ascorbate peroxidase; Cat – Catalase; GR – Glutathione reductase; SOD – Superoxide dismutase; H_2O_2 – Hydrogen peroxide; $O_2^{.-}$ - Superoxide oxygen; MDA—Malondialdehyde accumulation; EL%-Electrolyte leakage percentage. DPPH*- free radical scavenging.



SSC:TA ratio = 6	9	17
Hue = 113.59	69.51	21.05
a* = 0.67	1.01	3.49
L* = 54.11	48.26	43.64
b* = 76.11	73.23	73.49
90-days after booming	125 days	170 days

Fig 1: Presents The Color Profile of Fruits Harvested at Different Color Maturity Stages (Hfcms). The Hfcms Are Categories into Three Classes: A—The Green Stage (Gs), B—The Yellow Stage (Ys), And C—The Orange Stage (Os).

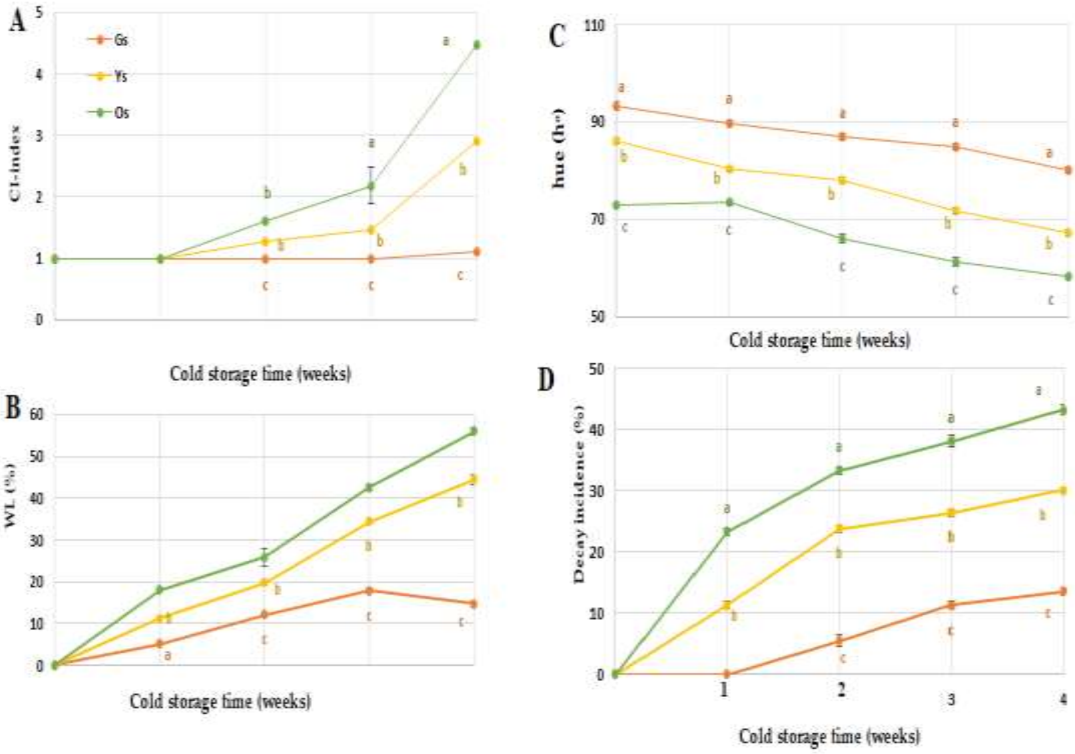


Fig. 2: CI-Index (A), WL (%) B, Hue (Ho) C, And Decay Incidence (%) D Of Kumquat Fruit Harvested at Different Color Maturity Stages (Gs, Ys, And Os), Stored At 4 ± 1°C With 95 ± 5% RH For 4 W. The Mean Values in Graphs with Various Letter Combinations Are Significantly Different (P<0.05). SE Of Means, Represented by Vertical Bars, For N = 300 (100 Fruit × 3 Replicates)

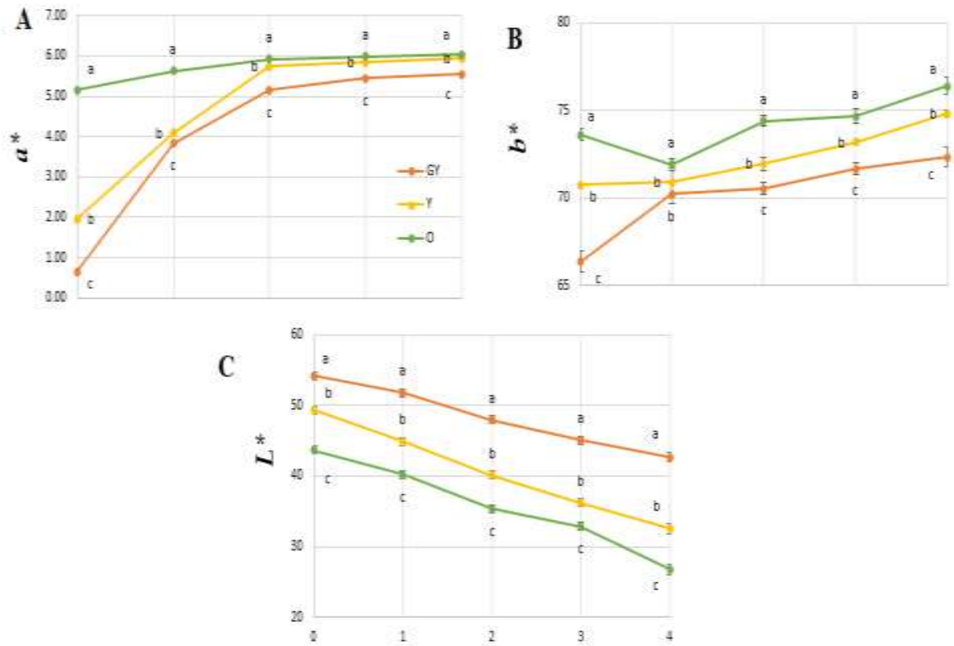


Fig 3: A, B*, And L* Color Parameters of Kumquat Skin Fruit Harvested at Different Color Maturity Stages (Gs, Ys, And Os), Stored At 4 ± 1 °C With 95 ± 5% RH For 4 W. The Mean Values in Graphs with Different Letters Are Significantly Different by Duncan's Test (P<0.05). Vertical Bars Represent ± SE Of Means, N = 300 (100 Fruit × 3 Replicates).*

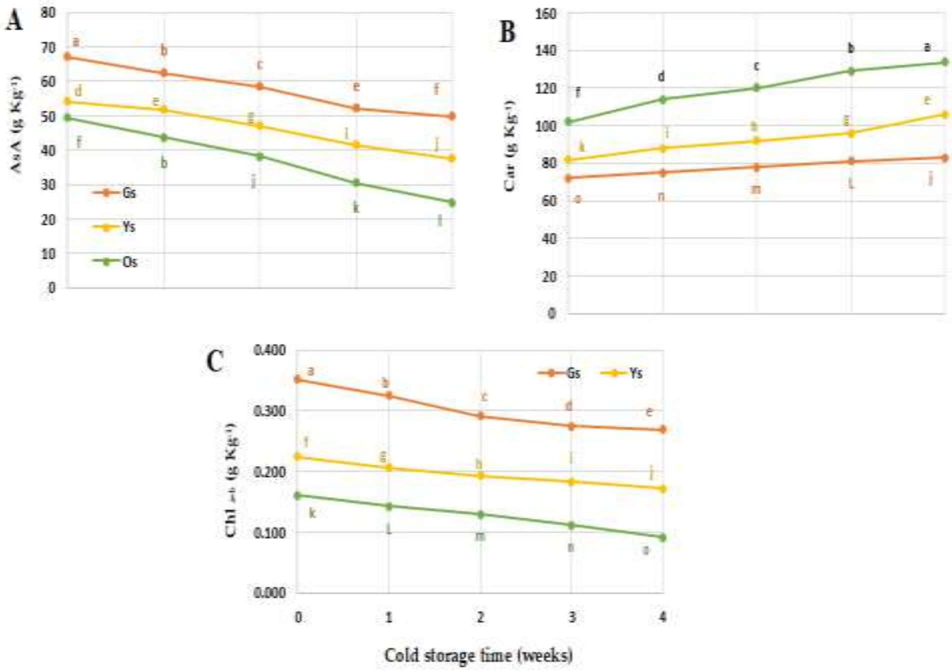


Fig. 4: Ascorbic Acid (A), Car (B), And Total Chlorophyll (C) Of Kumquat Fruit Harvested at Different Color Maturity Stages (Gs, Ys, And Os), Stored At 4 ± 1°C With 95 ± 5% RH For 4 W. According To Duncan's Test, Mean Values in Graphs with Various Letter Combinations Are Significantly Different (P<0.05). SE Of Means, Represented by Vertical Bars, For N = 3.

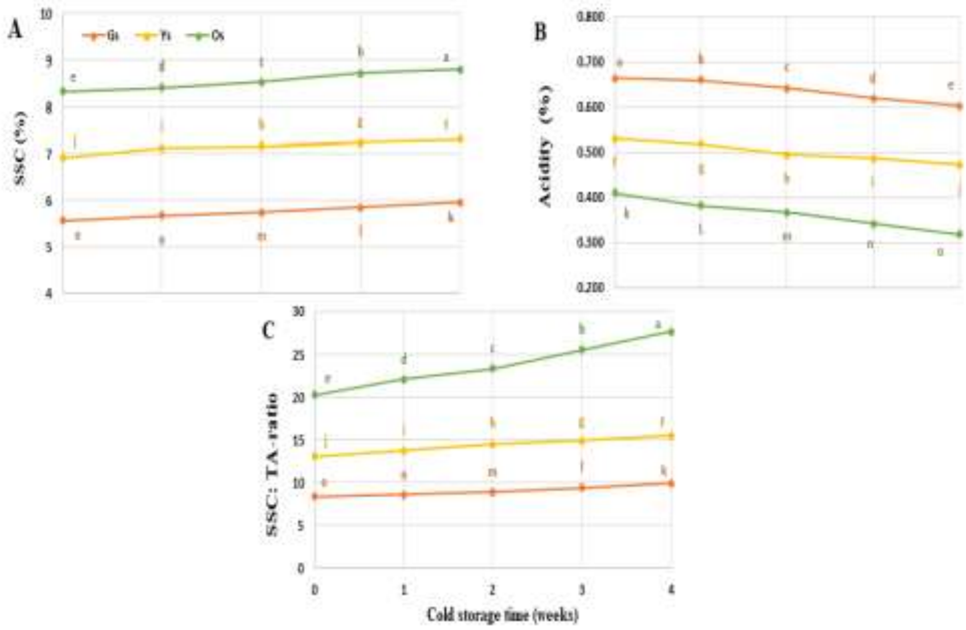


Fig. 5: SSC (A), TA (B), And SSC: TA Ratio (C) Of Kumquat Fruit Harvested at Different Color Maturity Stages (Gs, Ys, And Os), Stored At 4 ± 1°C With 95 ± 5% RH For 4 W. Mean Values in Graphs with Different Letters Are Significantly Different by Duncan's Test (P<0.05). Vertical Bars Represent ± SE Of Means, N = 3.

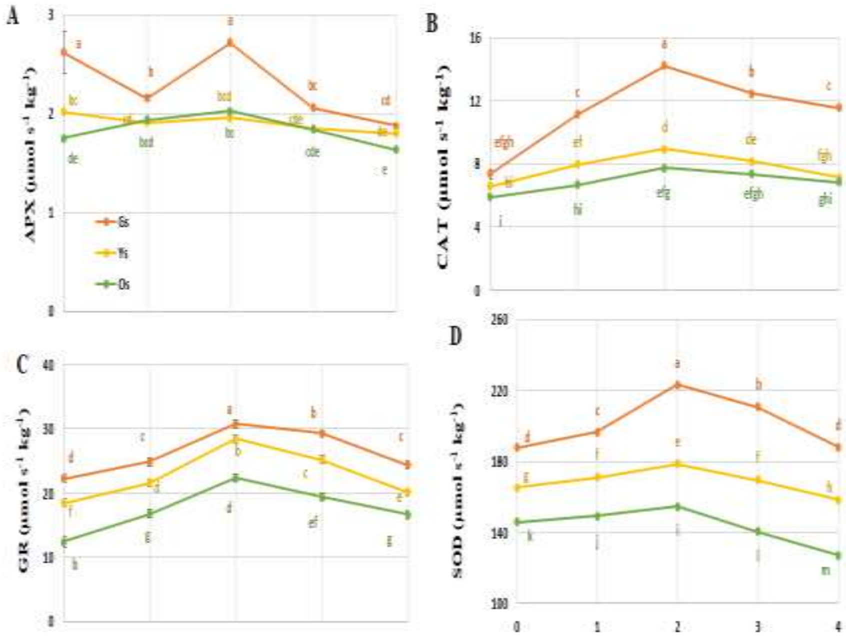


Fig. 6: APX, CAT, GR, And SOD Activities of Kumquat Fruit Harvested at Different Color Maturity Stages (Gs, Ys, And Os), Stored At 4 ± 1°C With 95 ± 5% RH For 4 W. Mean Values in Graphs with Different Letters Are Significantly Different by Duncan's Test (P<0.05). Vertical Bars Represent ± SE Of Means, N = 3.

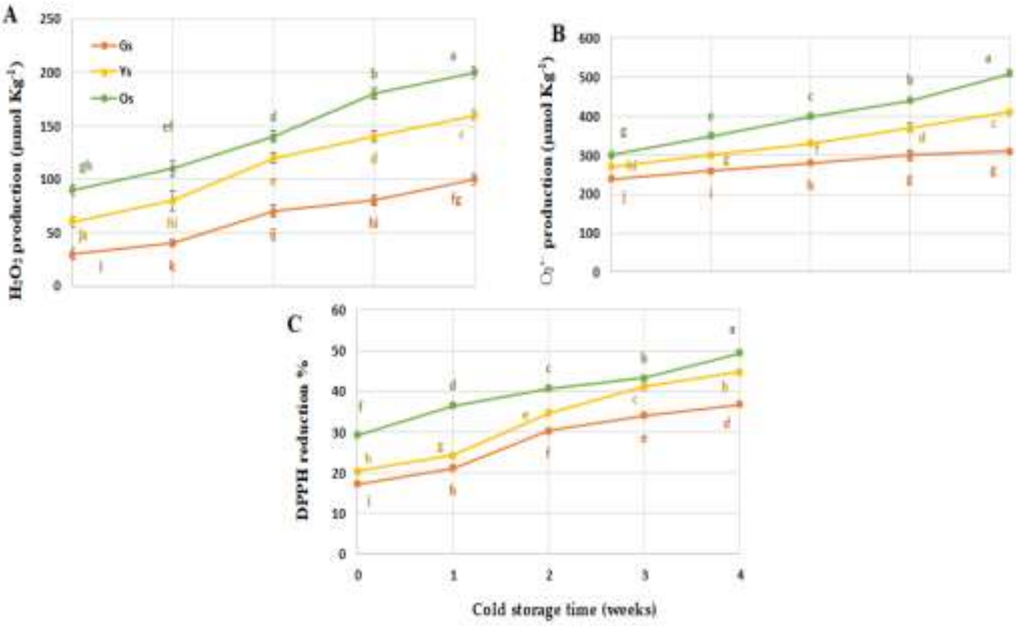


Fig. 7: Exhibits The Responses of Different Color Maturity Stages of Kumquat Fruit (Gs, Ys, And Os) On H_2O_2, O_2•− Production Rates, And DPPH Reduction Percentage, Which Were Stored at Low Temperature (4 ± 1°C And 95 ± 5% RH) For Four Weeks. Error Bars Reflect Standard Errors, And Different Letters Indicate Changes That Are Statistically Significant At P<0.05 Between Treatments Throughout Each Storage Period.

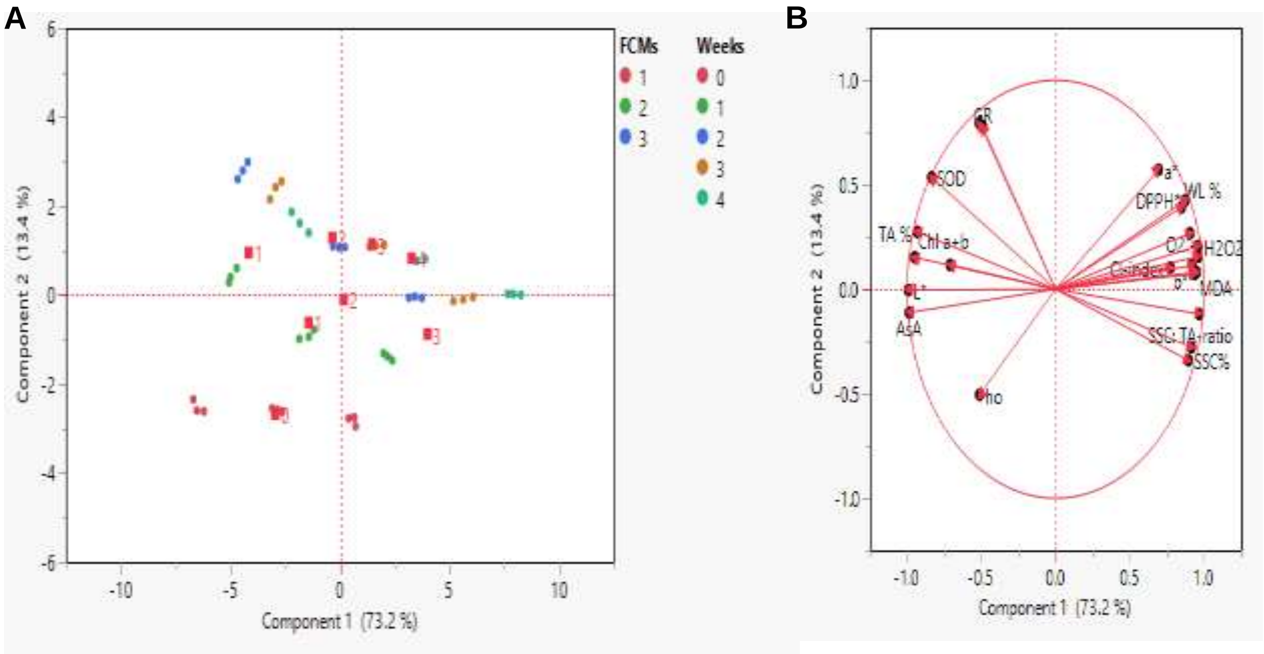


Fig. 8: All Of the Physiological and Biochemical Measurements Made in Bunches During the Storage Period, As Well As the Shelf-Life Duration of Kumquat Fruit in Weeks and Fcms (Three Stages), Are Displayed in a With the Contributions of Each Parameter on the Four PCA Axes (B) Principal Components Analysis (PCA). Variable Correlation.

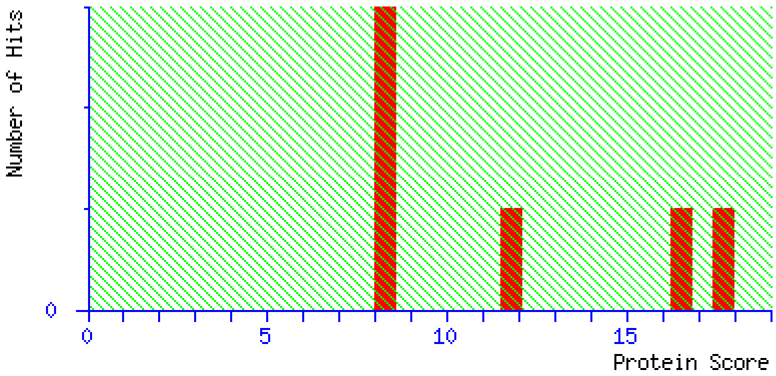


Fig. 9: The Optimization of Matrix 70% HCCA With 0.6% TFA.

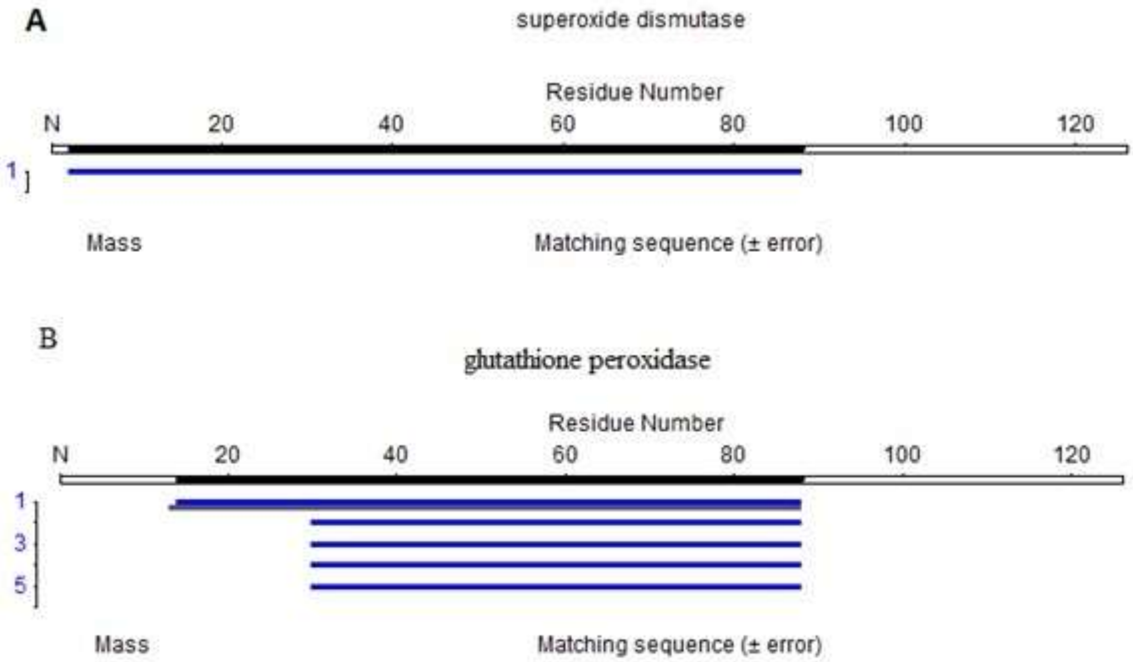


Fig. 10: The Mass List of Antioxidant Enzymes Shows the Similarity Matching Between the Onlind Data Published A) Superoxide Dismutase B) Glutathione Peroxidase from Citrus Japonica.

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