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Estimation of antioxidant levels and some productive traits of grapes treated with copper disperse under cold storage conditions

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ABSTRACT

Grapes are becoming an increasingly significant fruit because of their chemicals and nutritional qualities. Therefore, the importance of the best methods for preserving grapes fresh for as long as possible is growing. Hence, the current study was aimed at estimating the antioxidant levels and some productive characteristics of the grapes treated with copper disperse. The current experiment was conducted during two progressive seasons of 2024 and 2025 on three-year-old grape trees in a private orchard at college of Agriculture, University of Tikrit, Iraq. Grape fruits were treated with copper dispersion and some traits were evaluated, including Percentage of weight loss, Percentage of disintegration, Percentage of damage, Estimation of the percentage of total dissolved solids, Total acidity in juice, Fruit respiration rate and some antioxidant enzymes. The results showed that the lowest weight loss (1.156±0.003), lowest percentage of disintegration (1.156±0.003), percentage of damage (4.735±0.004), lowest total dissolved solids (38.29±0.22), the highest total soluble solids (TSS) (17.12 ±0.01), the highest total acidity (TA) (0.447±0.005) were observed in the group treated with copper dispersion with a coating containing 16 holes, which reached, which showed significant ($p \leq 0.05$) differences compared to other treated groups and the control group. for antioxidants, DPPH in the group treated with copper dispersion with a coating containing 16 holes (11.21±0.303) showed non-significant ($p \leq 0.05$) differences compared to the control group, which had DPPH (11.897±0.935). Other treatments showed significant ($p \leq 0.05$) differences with the control group, and the group that was immersed in copper dispersion without coating had the lowest levels of DPPH (8.878 ±0.332). FRAP in the group treated with copper dispersion with a coating containing 16 holes (445.8±11.45) showed non-significant ($p \leq 0.05$) differences compared to the control group, which had FRAP (487.19±13.92). Other treatments showed significant ($p \leq 0.05$) differences with the control group, and the group packaged with perforated film (16 perforations) had the lowest levels of FRAP (348.82±12.2). It was concluded from the current study that copper dispersion has different effects on some grape fruit traits, which include maintaining weight, reducing the percentage of spoilage, and preserving antioxidants.

Keywords: grape, FRAP, TSS, total acidity, antioxidant enzymes.

INTRODUCTION

Grapes are non-climatic fruits with low physiological activity. After harvest, they do not ripen further but are subject to losses such as water loss, which leads to loss of berry firmness and weight, berry segregation, and browning of the cluster stalk [1]. Storage and retailing grapes (*Vitis Vinifera* L.) provide a number of challenges [2]. Its quality flaws include fruit degradation, rapid softening, rachis browning, color changes, and weight loss. There are numerous issues with grapes (*Vitis Vinifera* L.) during storage and sale. Its quality flaws include fast softening, rachis browning, fruit deterioration, color changes, and weight loss [2, 3]. Anti-transpiration

agents protect the fruit peel by partially closing the lenticels and stomata on the peel. They also act as a barrier, restricting water transfer out of the fruit, reducing the rate of transpiration and evaporation, limiting weight loss, and thus delaying drying [4]. The demand for fresh grapes has increased recently, as has the awareness of the negative effects of chemicals on the environment and human health. Additionally, there is a growing interest in the creation and application of safe natural formulations, such as natural polymers, as pre-storage treatments. Chitosan is among the most palatable and potent forms of polymers [5, 6]. In nature, chitosan is abundant and common, second only to cellulose [7, 8]. It is a biodegradable, non-toxic biopolymer with strong antibacterial and biocompatible qualities. For a variety of foods, it serves as a safe preservative [9]. Its edible nature has led to its usage in post-harvest fruit physiology as a natural preservative for orchard crops during cold storage, as well as for its ability to keep fruit from rotting and extending its shelf life [10]. Because it lowers transpiration, respiration, and ethylene production [11], it can be applied as a coating on a variety of orchard crops. This extends the shelf life and marketing time of fruits, and it also acts as a barrier to lower respiration and sweating rates through surfaces [12]. Fruit is kept in an environment with a high relative humidity owing to the packaging techniques that minimize moisture loss [13]. A fundamental component of proteins, copper aids in the creation of enzymes that control the majority of plant biochemical reactions [14]. Because of their strong antibacterial and antioxidant qualities, which prevent spoiling and increase shelf life, copper nanoparticles (Cu NPs) are mainly used in edible coatings for fruits. These nanoparticles, which are frequently created utilizing biological agents in environmentally friendly ways, are distributed across natural polymer-based coatings, such as starch or chitosan to create a barrier that controls gas exchange, minimizes water loss, and stops microbes from entering. In order to create a barrier that prevents oxidation and microbial growth as well as keeps the fruit fresh for longer, the application usually entails submerging the fruit in the coating solution and then drying it [15]. For all of this, and given the importance of grapes to Iraqi producers and consumers, and the lack of studies on the effect of natural polymers during the storage of grapes in general, and the olive variety in particular, this study was conducted to determine the best storage practices that help preserve the nutritional value and quality of fruit during refrigerated storage until it reaches the consumer.

MATERIALS AND METHODS

Experimental site

The study was conducted in a private refrigerated warehouse (Al-Mustafa Radiator) in Balad District, Salah al-Din Governorate, during the 2024 harvest season on fruits taken from grapevines treated with nano-calcium. In the field experiment, clusters were hand-picked at the orchard maturity stage, when the total soluble solids content reached 14–17% (Al-Shammari and Fadhel, 2021). Homogeneous clusters were selected in terms of size and color, free of pathogens and mechanical damage. Initial measurements of the studied traits were performed (Table 1).

FACTORS STUDIED

Fruits treated with nanocalcium:

- Level 1: 0 without treatment (control treatment).
- Level 2: Ca2 sprayed with 150 mg/L^{-1}.
- Packaging methods (B).
- Individually perforated paper bags with (16) holes were used (P1).
- Vacuum bags (P2) were not perforated.

Fruit treatment with some transpiration inhibitors (C):

- Without dipping (C0).
- Fruit dipping with 2% chitosan was prepared by dissolving 20 grams of chitosan in a liter of distilled water containing 10 ml of acetic acid, as the substance is insoluble in water, according to Tezotto-Uliana et al. (2014). The clusters were immersed in the solution for 5 minutes, ensuring that all parts were submerged. After extracting the clusters from the solution, they were left to dry for 30 minutes under a gentle air current to allow dew to evaporate at room temperature (Sabie et al., 2018).
- The fruits were dipped in copper dispersion (C2) at a concentration of 4 g/L^{-1} for two minutes. The active ingredient concentration in Cu^{max} dispersion was used.

The clusters were separated into two groups and placed in sterile plastic boxes. The first group was used to assess fruit features while it was being stored, while the second group was left to compute weight loss until the end of the experiment. Relative humidity ranged from 85 to 90% while the fruits were kept at 0 + -1 °C. From July 20th, 2024, to October 20th, 2024, the clusters were kept in storage.

Characteristics studied

1. Percentage of weight loss

Calculated according to the following equation:

$$percentage\ of\ weight\ loss = \\ = Weight\ of\ fruits\ before\ storage\ (g) - \\ - Weight\ of\ fruits\ after\ storage\ (g) \times \\ \times\ 100\ /\ Fruit\ weight\ before\ storage\ (g) \quad (1)$$

2. Percentage of disintegration: Calculated according to the following equation:

$$Percentage\ of\ disintegration = \\ = Weight\ of\ grains\ in\ the\ treatment\ \times \\ \times\ 100/\ total\ treatment\ weight \quad (2)$$

3. Percentage of damage

$$Percentage\ of\ damage\ (\%) = \\ = Weight\ of\ damaged\ grains\ in\ the\ treatment\ \times \\ \times\ 100/\ total\ treatment\ weight \quad (3)$$

4. Estimation of the percentage of total dissolved solids – using the same method as the field experiment.
5. Total acidity in juice was estimated using the same method as in the field experiment, as stated by A.O.A.C (2000).
6. Total acidity/TSS ratio was estimated using the same method as in the field experiment.
7. Fruit respiration rate: Fruit respiration rate (mg CO_2 kg^{-1} h^{-1}) was measured using the closed system method at room temperature, as reported in Al-Ani (1985). 20 ml of 0.1 N NaOH was placed in the base of the dissector, followed by 1 kg of fruit. The dissector was closed for 24 hours. The fruit was then extracted, and the NaOH base was sieved after adding a drop of phenolphthalein indicator with 0.1 N HCl until the base color changed from pink to colorless. The volume of the sieve was determined by applying the following equation:

$$mg\ CO2\ kg - 1\ h\ = \\ = 22\ \times\ (HCl\ consumed\ \times\ NaOH\ used\ > \\ \times\ NaOH\ used)/\ kg\ \times\ hour \quad (4)$$

Antioxidant enzymes

1. Free radical scavenging activity by DPPH assay

According to the procedure recommended by Brand-Williams et al. [16], the antiradical capacity of the sample extract was assessed. The color solution disappears during reduction, and a spectrophotometer set to 515 nm is used to track the progress of the reaction. Sample extract (15 mg/ml) was added to 3.9 ml of DPPH (0.025 g/l) in methanol (0.1 ml) and the absorbance was measured at 515 nm using a double beam UV-VIS Spectrophotometer (Biobase, China). The absorbance was monitored until the reaction stabilized at a plateau.

2. Ferric-reducing antioxidant power (FRAP)

Ferric-reducing antioxidant power (FRAP)-free solution without the extract using the Brand-Williams et al. [16] technique, antioxidant activity was evaluated using the FRAP method. The FRAP reagent was prepared by mixing 100 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl, and 20 mM ferric chloride in a 10:1:1 (by volume) ratio. 4.9 ml of FRAP reagent was combined with 100 microliters of samples, and the mixture was incubated for 15 minutes at 35 °C. At 593, the absorbance of the samples was measured. The FRAP-value was calculated using the $FeSO_4 \cdot 7H_2O$ standard curve, and the results were given as µmol Fe^{2+}/100g FW.

Experimental design and statistical analysis

The experiment was carried out as a factorial experiment according to a C.R.D design with three factors. The first factor was the fruit (calcium treatment), the second factor was packaging (individually perforated paper bags with (16) holes were used, and the second factor was vacuum bags without perforations). The third factor was fruit treatment with some transpiration inhibitors (without immersion, 2% chitosan, and 4% copper dispersion). Thus, there were 12 treatments resulting from the different interactions of the study factors. The treatments were randomly distributed into three replicates, so that the experiment included 36 experimental units, and each experimental unit was a box (weight loss group, measurement group) weighing 2 kg. Measurements were taken after 3 months of storage. After collecting the data, they were statistically analyzed according to the design used using the SAS program (2003). The averages were compared according to Duncan's multiple range test at a probability level of 0.05 [17].

RESULTS

Table 1 shows the weight loss in grape fruits between groups, where it is noted that the lowest weight loss was observed in the group treated with copper dispersion with a coating containing 16 holes, which reached (1.177±0.005), followed by the treatment with copper dispersion with a coating (1.774±0.002), which reached, which showed significant ($p \leq 0.05$) differences compared to the control group, which had weight loss (2.794±0.002), as showed in Figure 1.

Table 2 shows the percentage of disintegration in grape fruits between groups, where it is noted that the lowest percentage of disintegration was observed in the group treated with copper dispersion with a coating containing 16 holes, which reached (1.156±0.003), followed by the treatment with copper dispersion with a coating (1.364±0.004), which reached, which showed significant ($p \leq 0.05$) differences compared to the control group, which had percentage of disintegration (2.027±0.007).

Table 3 and Figure 2 show the percentage of damage in grape fruits between groups, where it is noted that the lowest percentage of damage was observed in the group treated with copper dispersion with a coating containing 16 holes, which reached (4.735±0.004), followed by the treatment with copper dispersion with a coating (4.875±0.577), which reached, which showed significant differences compared to the control group, which had a percentage of damage (7.418±0.006), as shown in Figure 3.

Table 1. The weight loss in grape fruits among groups

Groups	Mean	Standard deviation (SD)
Group A	2.794 a	0.002
Group B	2.576 b	0.004
Group C	2.227 c	0.003
Group D	1.996 d	0.002
Group E	1.774 e	0.002
Group F	1.177 f	0.005

Note: Different letters indicate that the treatments differ significantly, while similar letters indicate that there are no notable differences.

Table 4 shows the total dissolved solids in juice between groups, where it is noted that the lowest total dissolved solids was observed in the group treated with copper disperse with a coating containing 16 holes, which reached (38.29±0.22), followed by the treatment with copper disperse with a coating (38.67±0.208), which reached, which showed significant ($p \leq 0.05$) differences compared to the control group, which had total dissolved solids (39.61±0. 248), as shown in Figure 4.

Table 5 shows the total soluble solids (TSS) in juice between groups, where it is noted that the highest TSS was observed in the group treated with copper dispersion with a coating containing 16 holes, which reached (17.12 ±0.01), followed by the treatment with copper dispersion with a coating (16.813 ±0.15), which reached, which showed significant ($p \leq 0.05$) differences compared to the control group, which had TSS (15.66±0.026), as shown in Figure 5.

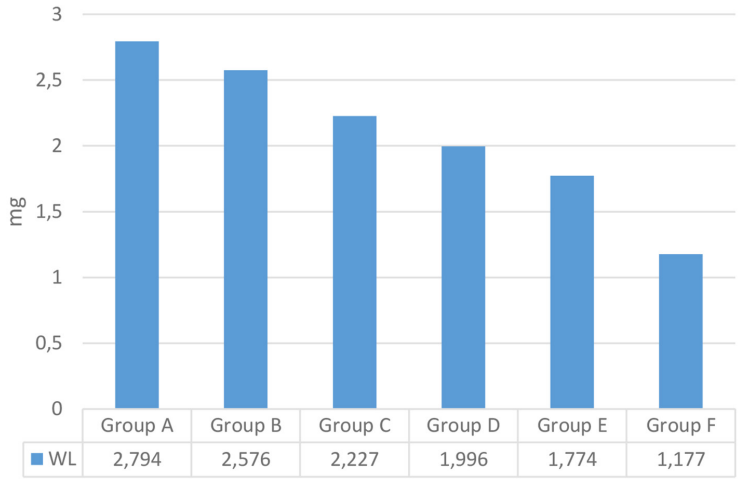

Figure 1. The weight loss in grape fruits among groups

Table 2. The percentage of disintegration in grape fruits among groups

Groups	Mean	Standard deviation (SD)
Group A	2.027 a	0.007
Group B	2.329 a	0.011
Group C	1.753 b	0.004
Group D	1.556 c	0.004
Group E	1.364 d	0.004
Group F	1.156 e	0.003

Note: Different letters indicate that the treatments differ significantly, while similar letters indicate that there are no notable differences.

Table 3. The percentage of damage in grape fruits among groups

Groups	Mean	Standard deviation (SD)
Group A	7.418 a	0.006
Group B	7.112 a	0.003
Group C	6.516 b	0.005
Group D	5.116 c	0.004
Group E	4.875 cd	0.577
Group F	4.735 d	0.004

Note: Different letters indicate that the treatments differ significantly, while similar letters indicate that there are no notable differences.

Table 6 shows the total acidity (TA) in grape fruits between groups, where it is noted that the highest TA was observed in the group treated with copper dispersion with a coating containing 16 holes, which reached (0.447±0.005), followed by the treatment with copper dispersion with a coating (0.435±0.004), which reached, which showed significant ($p \leq 0.05$) differences compared to the

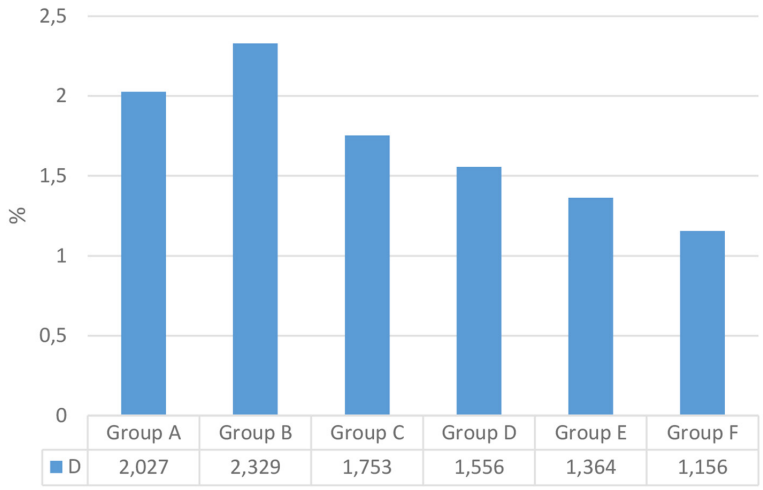

Figure 2. The percentage of disintegration in grape fruits among groups

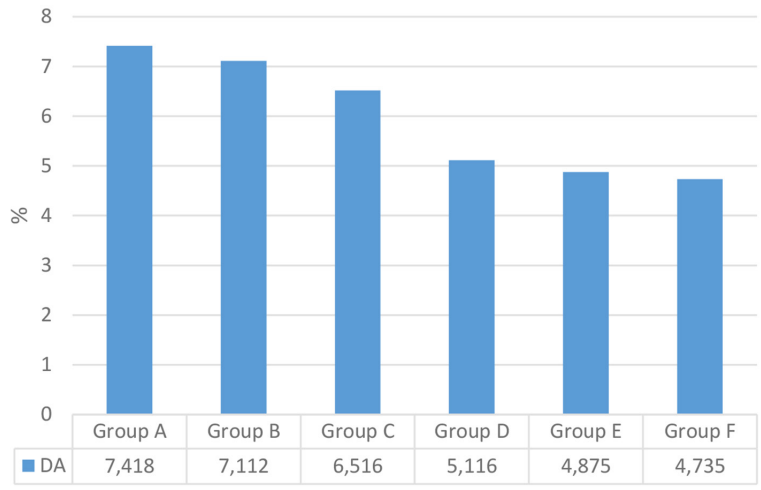

Figure 3. The percentage of damage in grape fruits among groups

Table 4. The total dissolved solids in grape fruits among groups

Groups	Mean	Standard deviation (SD)
Group A	39.61 ab	0.248
Group B	41.28 a	0.426
Group C	40.68 ab	0.239
Group D	38.66 c	0.165
Group E	38.67 c	0.208
Group F	38.29 d	0.22

Note: Different letters indicate that the treatments differ significantly, while similar letters indicate that there are no notable differences.

Table 5. The total soluble solids in grape fruits among groups

Groups	Mean	Standard deviation (SD)
Group A	15.66 f	0.026
Group B	15.467 e	0.032
Group C	16.043 d	0.042
Group D	16.51 c	0.02
Group E	16.813 b	0.15
Group F	17.12 a	0.01

Note: Different letters indicate that the treatments differ significantly, while similar letters indicate that there are no notable differences.

control group, which had TSS (0.395±0.003), as shown in Figure 6.

Table 7 shows the fruit respiration rate in grape fruits between groups, where it is noted that the lowest fruit respiration rate was observed in the F group, which reached (2.976±0.002), followed by the treatment with copper dispersion with a coating (3.013±0.002), which reached, which showed significant ($p \leq 0.05$) differences compared to the

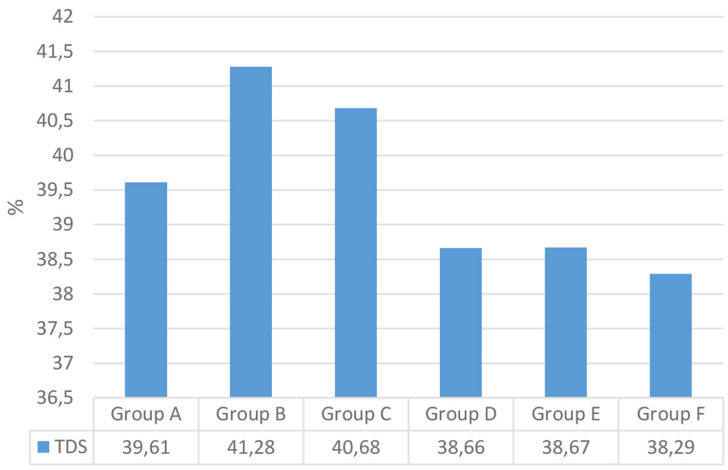

Figure 4. The total dissolved solids in grape fruits among groups

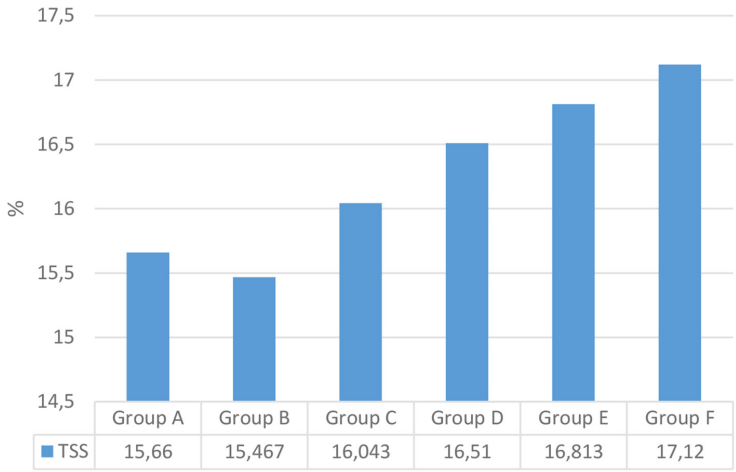

Figure 5. The total soluble solids in grape fruits among groups

Table 6. The total acidity in grape fruits among groups

Groups	Mean	Standard deviation (SD)
Group A	0.395 e	0.003
Group B	0.375 d	0.004
Group C	0.394 c	0.002
Group D	0.427 b	0.007
Group E	0.435 b	0.004
Group F	0.447 a	0.005

Note: Different letters indicate that the treatments differ significantly, while similar letters indicate that there are no notable differences.

Table 7. The fruit respiration rate in grape fruits among groups

Groups	Mean	Standard deviation (SD)
Group A	5.225 a	0.004
Group B	4.117 b	0.002
Group C	3.126 c	0.003
Group D	3.025 d	0.002
Group E	3.013 e	0.002
Group F	2.976 f	0.002

Note: Different letters indicate that the treatments differ significantly, while similar letters indicate that there are no notable differences.

control group, which had fruit respiration rate (5.225±0.004), as shown in Figure 7)

Table 8 shows the free radical scavenging activity by DPPH assay in grape fruits between groups, where it is noted that the DPPH in group treated with copper dispersion with a coating containing 16 holes (11.21±0.303) showed non-significant ($p \leq 0.05$) differences compared to the control group, which had DPPH (11.897±0.935). The other treatments showed significant ($p \leq 0.05$) differences with

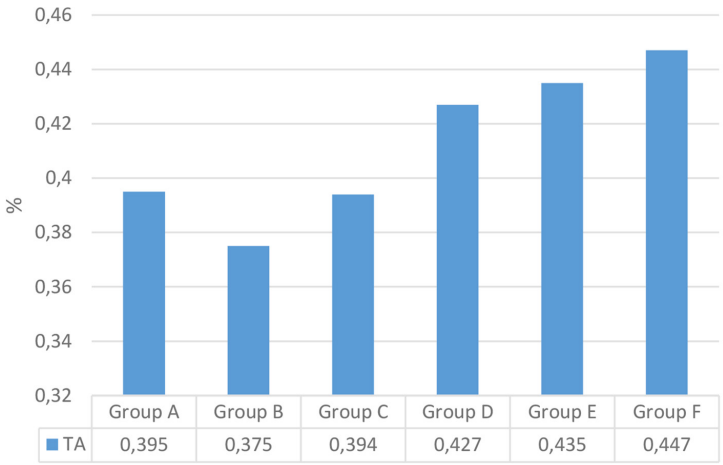

Figure 6. The total acidity in grape fruits among groups

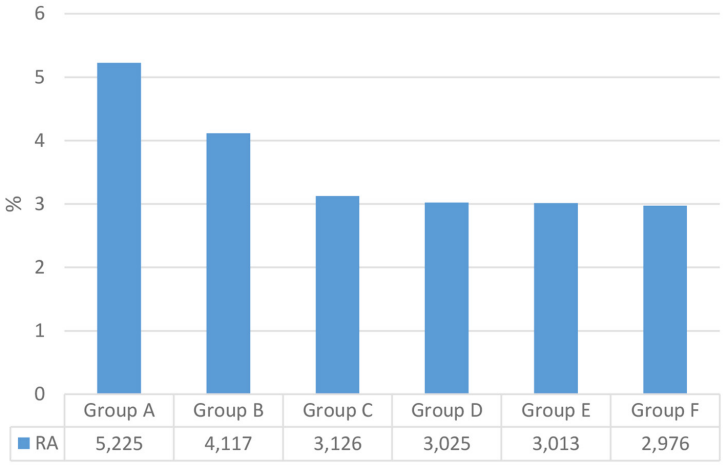

Figure 7. The fruit respiration rate in grape fruits among groups

Table 8. The levels of DPPH (µmol /g) in grape fruits among groups

Groups	Mean	Standard deviation (SD)
Group A	11.897 a	0.935
Group B	9.693 b	0.739
Group C	9.635 b	0.459
Group D	8.878 c	0.332
Group E	9.213 b	0.133
Group F	11.21 a	0.303

Note: Different letters indicate that the treatments differ significantly, while similar letters indicate that there are no notable differences.

Table 9. FRAP (µmol Fe+2/100 g FW) levels in grape fruits among groups

Groups	Mean	Standard deviation (SD)
Group A	487.19 e	13.92
Group B	378.71 c	24.81
Group C	348.82 d	12.2
Group D	402.46 b	14.64
Group E	367.53 c	22.07
Group F	445.8 a	11.45

Note: Different letters indicate that the treatments differ significantly, while similar letters indicate that there are no notable differences.

the control group, and the group that was immersed in copper dispersion without coating had the lowest levels of DPPH (8.878 ±0.332), as shown in Figure 8.

Table 9 shows the FRAP in grape fruits between groups, where it is noted that the lowest fruit FRAP was observed in the group F, which reached (445.8±11.45), followed by group D (402.46±14.64), which reached, which showed

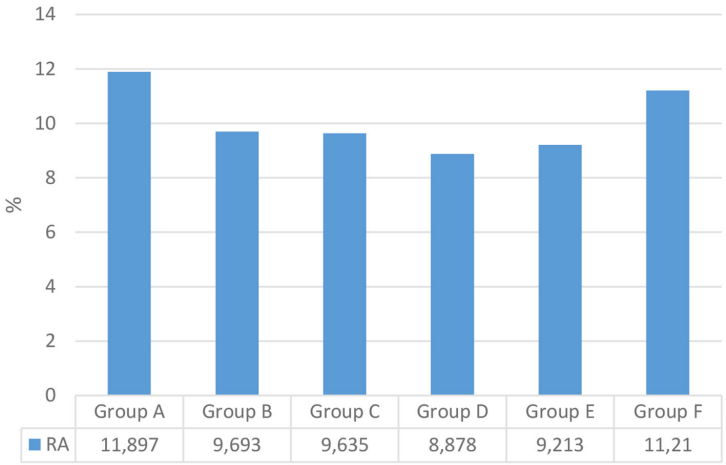

Figure 8. The levels of DPPH (µmol /g) in grape fruits among groups

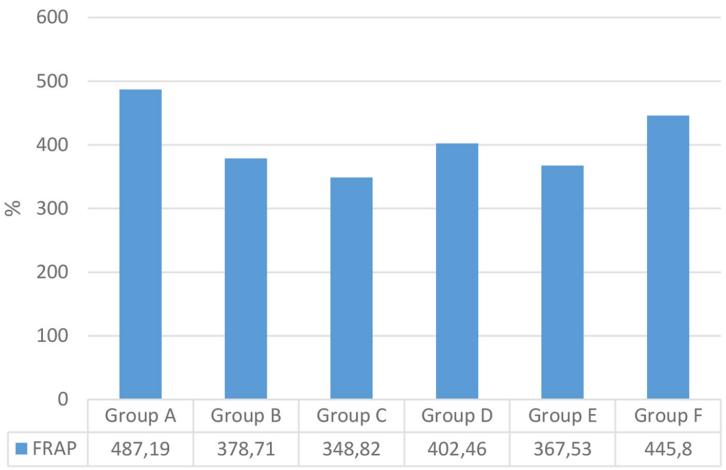

Figure 9. FRAP (µmol Fe+2/100 g FW) levels in grape fruits among groups

significant ($p \leq 0.05$) differences compared to the control group, which had FRAP (487.19 ±13.92), as shown in Figure 9.

DISCUSSION

Significant post-harvest losses of fruits and vegetables are a serious concern for any nation with an agricultural economy. This phenomena, however, is widespread and occurs in practically all emerging nations. Since fruits and vegetables are highly perishable goods, handling them carefully is necessary to reduce losses. Particularly in tropical climates, horticultural crops are particularly susceptible to deterioration due to their high moisture content [18]. Because they are biologically active, they engage in biochemical processes, including transpiration, respiration, ripening, and others that degrade quality. In underdeveloped nations, post-harvest losses resulting from incorrect handling and storage might reach up to 50 percent [19]. Water loss via transpiration and respiration is the primary cause of weight loss, the percentage of disintegration, and the total soluble solids in fresh fruits and vegetables. The obtained findings are consistent with a study by Hernández-Munoz et al. [20] which showed that applying a 1.5% Nanochitosan-Based Coating with Copper Loaded to strawberries was successful in providing a physical barrier against moisture loss, hence postponing fruit dryness and shrinkage. According to reports, chitosan with copper works better than starch and cellulose derivatives to postpone weight loss in bananas, mangos, and strawberries [21, 22]. Increased water loss in uncoated grapes may be the cause of the slower rate of pH and acidity changes in both covered grapes during storage as compared to the control. Soluble solids, however, did not undergo any notable changes while being stored. In fact, the fruits that lost the most water had the most changes in soluble solids. These findings were consistent with the research by Vargas et al. [23]. Additionally, Tanada-Palmu and Grosso [24] noted that during storage, the amount of soluble solids in coated and control strawberries increased. They explained this observation by pointing out that grapes lose a significant amount of water while being stored. A statistical study showed that the copper-coated samples differed significantly. It is done in copper dispersion packaging throughout storage in order to preserve the quality of grape. Adding ZnO has a benefit in preserving anthocyanin and antioxidant chemicals, as well as reducing a drop in weight, hardness, percentage of decay, and ascorbic acid content [25]. Research has also shown that the samples coated with nanocomposite films are effective at altering their mechanical, chemical, and physical characteristics while being stored [26, 27]. Nanochitosan with a copper-loaded covering has been shown to improve fruit antioxidant activity, according to Wang and Gao [28]. Antioxidant activity in uncoated grapes declined in the conducted investigation. When the fruits were stored, their antioxidant capacity decreased more quickly when they were coated without copper than when they were covered with copper. Reduced antioxidant capability may result from the pro-oxidant impact of copper, which may explain the higher conservation of antioxidant components in the copper-coated samples. On the basis of the obtained findings, when compared to the uncoated samples, the copper ions in the coating formula increase the levels of antioxidant enzymes, such as glutathione and catalase in grapes. Such an observation may be connected to significant contribution of copper to the ascorbate oxidase activity [29].

CONCLUSIONS

It was concluded from the current study that copper dispersion has different effects on some grape fruit traits, which include maintaining weight, reducing the percentage of spoilage, and preserving antioxidants. This confirms the necessity of using it to preserve the safety of fruits and vegetables for the longest possible storage period.

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