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Preparation and Characterization of Zinc and Frankincense-Loaded Carboxymethyl Cellulose Nanoemulsions: Their Impact on Antifungal Activity, Biochemical Properties, and Shelf-Life Extension of Valencia Orange

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Abstract

Natural preservation methods have grown in prominence and demand as a means of preserving fruits and prolonging their shelf life rather than synthetic additions. Therefore, this study is directed to investigate carboxymethyl cellulose (CMC) loaded with frankincense and zinc ions nanoemulsion as food coating material to assess their antifungal activity and efficacy in protecting the quality of Valencia orange during storage. The nanoemulsions were synthesized using the solvent evaporation technique and characterized using transmission electron microscopy (TEM) and Fourier-transform infrared spectroscopy (FTIR). The antifungal activity was tested both in vitro and in vivo against the harmful fungus Penicillium commune and *Aspergillus niger*. Fresh mature oranges were coated with different prepared nanoemulsions. Biochemical parameters (total soluble solids percentage, total acidity, and ascorbic acid content), and physical parameters (weight loss, decay percentage, and firmness) were tested at different time intervals up to 180 days. The TEM results illustrate the presence of agglomerated nanoparticles of frankincense and the effect of zinc in the separation and formation of well-dispersed nanoparticles with an average diameter of 28.7±14nm. The *in vitro* antifungal activity results indicated that the nanoemulsion of CMC loaded with zinc and frankincense showed maximum activity against *Penicillium commune* with an inhibition efficiency of 33.3% followed by *Aspergillus niger* with an inhibition efficiency of 28.4%. Moreover, *in-vivo* antifungal activity showed the pronounced effect of CMC loaded with Zn and frankincense against the prevention of growth of both pathogens with weak growth of *Aspergillus niger* was observed after 30 days. The biochemical properties of Valencia orange, including total soluble solids (TSS), acidity, and vitamin C content, exhibited significant improvements when treated with the zinc and frankincense-loaded CMC nanoemulsion. Additionally, weight loss and decay percentage were significantly reduced, while fruit firmness was notably enhanced, indicating improved structural integrity and extended freshness. These improvements suggest that nanoemulsion effectively enhances cold storage preservation by maintaining the fruit's physicochemical quality over a storage period of up to 180 days. These findings underscore the potential of CMC-based nanoemulsions as a promising strategy for prolonging the shelf life and preserving the quality of Valencia orange under refrigerated conditions.

Keywords: Nanoemulsions; Antifungal Activity;Biochemical Properties; Fruit Preservation; Valencia Orange.

1. Introduction

Oranges, like other citrus fruits, are extremely popular and valued for their delicious, refreshing flavor and high nutritional worth [1]. Because it is a popular late-season cultivar, 'Valencia' fruits are commercially preserved for up to 4-5 months to lengthen the selling season [2, 3]. The most notable difficulties in preserving citrus fruits, especially orange, are controlling ethylene production and the potential for chilling harm, which can occur if oranges are kept at too low temperatures [4, 5]. Therefore, post-harvest care and storage are crucial for preserving citrus fruits' freshness and nutritional content [6]. Refrigerated storage conditions especially between 3°C and 9°C are often utilized to improve the shelf life of citrus fruits, particularly during the hot summer months [7, 8]. Recently, special direction has been given to applying a variety of natural and healthful edible coatings to minimize post-harvest loss, improve shelf life, and maintain fruit superiority [8].

One of the most valuable edible coatings is the derivatives of cellulose as CMC(CMC) which is composed of glucopyranose units that include a carboxymethyl group bound to the hydroxyl group [9].CMC is well known as an edible coating due to its outstanding film-forming properties, non-toxicity, and biodegradability [10]. It is effective in forming a semi-permeable barrier on the surface of fruits, reducing moisture loss, and retardation respiration rates through the control of gas exchange especially oxygen and carbon dioxide, all of these are important factors in extending the shelf life of fresh food [10–12]. The antimicrobial properties of CMC need to be reinforced by other materials that manifest an effect against *Penicillium commune*, a pathogen that causes common post-harvest diseases in citrus fruits [13]. In addition, Zinc(Zn) is a promising element in fruit preservation because of its antimicrobial characteristics [14, 15, 16], suppression ofethylene synthesis, a crucial hormone that may cause fruit

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ripening,[17], decrease of weight loss [18]and stabilization of fruit cell membranes, which aids in cell integrity and prevents enzymatic breakdown of cell walls, a usual cause of fruit softening and deterioration [19].

Frankincense (Fr) is a resinous extract that was obtained from the Boswellia tree. Its hopeful application in the field of food preservation may be due to the presence of different bioactive compounds in its structure that manifest various anti-inflammatory, antioxidant, and antimicrobial actions [20, 21]. Recent studies reveal thatoleogum resin of Fr may contain more than 200 various compounds such aspolyphenols, penta-and tetracyclic terpenoids, essential oils, saponins, sugars, alkaloids, and others [20. Fr essential oil includes bioactive components such as α-pinene, limonene, and p-cymene that have extraordinary antioxidant and antibacterial properties [21, 22]. These chemical components may vary amount different groups, seasons, and locations [20].

The present work aims to investigate carboxymethyl cellulose (CMC) loaded with frankincense and zinc ions nanoemulsion as food coating material to assess their antifungal activity and efficacy in protecting the quality of Valencia orange during storage. By researching these natural and possibly synergistic therapies, the study hopes to give an eco-friendly and cost-effective alternative to protecting the quality of Valencia oranges, eventually helping growers, distributors, and consumers by providing longer-lasting, high-quality fruit.

To our knowledge, up to now, there is no study applyingCMC functionalized with zinc and Fr in the subject of food preservation.*Khalil et al.,*[23] functionalized CMC with lemon essential oil for packaging cherry tomatoes and baby spinach leaves. The results show that the prepared films possess a significant antimicrobial effect, low moisture, and good preservation effect. *dos Santoset al.,*[24] enhance the properties of chitosan films with carnauba wax and ZnO nanoparticles to preserve the post-harvest quality of Passion fruit. They stated that the prepared films protect the fruits against weight loss and microorganism attacks.*Elabbasy et al.*,[25] investigated the effects of H_2O_2, oxalic acid, Gum Arabic, and Fr solutions on the quality and shelf life of Valencia oranges. Their findings showed that H_2O_2 and Fr manifest better results.

2. Materials and methods

2.1. Materials

CMC and Zinc acetate were purchased from Sigma Aldrich company(Germany). Fr was obtained from Somalia. Dichloro methane and distilled water were also applied in this work.

2.2. Preparation for frankincense extract

The obtained Fr was crushed with caution using the morter and then sieved by mesh at 150 µm to get a fine powder. 3gm of the obtained powder was soaked in 50 ml dichloro methane and stirred well in dark conditions for about 24 h at 40^{o}C. The resulting extract was centrifuged for about 30 min. at 3000 rpm and finally, the supernatant was collected and kept in the refrigerator until use.

2.3. Preparation of CMC emulsion loaded with zinc and frankincense extract

CMC with a concentration (o.5%) was dissolved in distilled water under stirring, homogenized for about 15 min., also sonicated for about 10 min, and then stirred for 2 h. This sample was denoted as a **CMC** sample. For the CMC emulsion loaded with Fr extract, the method was as follows; after the complete dissolution of CMC, the previously prepared Fr extract was dropped into the CMC emulsion with 0.03% of Fr. Then the CMC emulsion with Fr was stirred for about 2h, homogenized for about 15 min, sonicated for about 10min, and then stirred for about 24 h to allow complete exit of dichloro methane. This sample was termed as **CMC-Fr1**. Another concentration (0.06%) of Fr was prepared, that labeled **CMC-Fr2**. For the CMC emulsion loaded with Zinc, Zinc acetate (0.03%) was added. Then the same steps were applied. This sample that contains CMC loaded with zinc was labeled **CMC-Z**. The CMC emulsion loaded with both zinc and Fr was prepared as follows: Zinc acetate (0.03%) was added under stirring to the dissolved CMC solution. Then Fr extract was added with the ratio of 0.06% concerning the CMC solution. After that, the same steps mentioned forC-Fr samples were performed. This sample was denoted as **CMC-Z-Fr2**.

2.4. Characterization

The morphology of the prepared samples was investigated using TEM (JeolJem-1230, JEOL, Tokyo, Japan. The chemical composition of the prepared samples was examined by Fourier Transform Infrared Spectroscopy (FTIR)(model FT/IR-6100 type A). All samples' spectra were recorded at 4000- 400 cm^{-1} wavenumbers.

2.5. Antifungal activity of the targeted prepared emulsions

2.5.1. *In Vitro* antifungal activity assay

The efficiency of the synthesized emulsion samples (CMC, CMC-Z, CMC-Fr 1, CMC-Fr2, and CMC-Z-Fr2) to represent as fungicidal agents could open a new trend to utilize them against different phytopathogenic fungi. In this respect, multicellular fungal pathogens like, *Penicillium commune* and *Aspergillus niger* were investigated in the presence of the targeted samples *in-vitro*. For the *in-vitro* examination, fungal pathogens were initially pre-activated using Potato dextrose broth medium (Codalab, Spain) at 28^{o}C for 48 h. under shaking conditions. The justification of inoculum size for each fungal pathogen was conducted using serial dilution method and accurately quantified by Colony Forming Unite (CFU) to ensure the constant concentration of each fungal pathogen throughout all performed tests. Screening of the targeted molecules towards the phytopathogenic fungi was implemented using the agar-well diffusion technique according to the CLSI Protocols [26]. A fixed concentration of each compound was used (25 µg/mL) in comparison to the standard antifungal agents (Amphotericin B, Nizoral, and Fluconazole). After the incubation period, the observed results of the selected molecules and standard antifungal agentswere measured based on the inhibition zone diameter (mm) around each targeted molecule [27].

2.5.2. *In Vivo* antifungal activity assay

The contribution of the tested emulsions to increasing the shelf life of fruits was assessed using infected orange samples with the phytopathogenic fungi, *Penicillium commune and Aspergillus niger in-vivo*. In this regard, in two different sites around the end of the blossom of fresh oranges, a hole or piercing with a depth of 5 mm has been done with a 1.25 mm diameter needle [28].

Inoculation of the activated fungal spores was immediately carried out in the two holes at 50 µl. The test compounds were divided into 5 groups, CMC, CMC-Z, CMC-Fr 1, CMC-Fr2, and CMC-Z-Fr2, the control was represented by Tween 80 solutions (0.05% w/v). Each group was coated on the surface of the fruit before and after inoculated with fungal spores. Evaluation of disease development after fungal infections appeared in the case of the untreated sample (named control). Each treatment was performed in triplicate and the results were measured as a percentage of inhibition disease.

2.6. Sample collections

At ripening, fresh Valencia oranges (*Citrus sinensis* L. Osbeck) were harvested from a private plantation in Wadi El Natrun, Beheira Governorate, Egypt, during the 2022 and 2023 seasons. The trees were 12 years old and budded on Volkamer lemon (*Citrus volkameriana* Ten. and Pasq.) rootstock. They were cultivated in sandy loam soil with a drip watering system and followed regular agricultural practices. Fruits were gathered during the ripening stage in the second week of March each season and then accurately labeled. Fruits with good quality, uniform size, and free of damage or sickness were selected.

2.7. Sample preparation and experimental design

Fruits were rinsed with distilled water and cleaned with a 1% sodium hypochlorite solution to remove any foreign matter such as dust, dirt, or other contaminants before being air-dried for usage. After treatment, the fruits were air-dried in plastic boxes for 180 days at 5°C ± 2°C and 85 ± 2 percent humidity. The fruits were randomly separated into two groups: one for testing the fruit's physical qualities and the other for measuring their chemical parameters. Each group had 450 fruits, which were separated into five subgroups to mimic the five treatments employed. Each treatment had three replicates, with 30 fruits per replicate, laid out in a Randomized Complete Block Design (RCBD).Fruits were tested for a range of factors every 15 days until their storage time ended, each of which received the following treatments:

T1. Control (untreated fruits).
T2. Fruits treated with CMC emulsion.
T3. Fruits treated with CMC-Z emulsion.
T4. Fruits treated with CMC-Fr2 emulsion.
T5. Fruits treated with CMC-Z-Fr2 emulsion.

2.8. Measurements and determinations

2.8.1. Fruit biochemical properties

2.8.1.1. Total Soluble Solids (T.S.S. %)

The total soluble solid percentage was measured in 10 mL of fruit juice filtrate using the refractometer equipment (TAGO 9099, Tokyo, Japan) according to AOAC [29].

2.8.1.2. Total acidity percentage

The acidity of 10 milliliters of fruit juice was determined. A titration technique was utilized. The berry extract was combined with 100 mL of distilled water. The total acidity percentage was calculated by titration with 0.1 N NaOH according to AOAC [29].

2.8.1.3. T.S.S./acid ratio

The T.S.S./acid ratio was recorded by dividing the T.S.S. value by the total acidity value.

2.8.1.4. Ascorbic acid content

The ingredients required are prepared as follows: Ingredients: 4% oxalic acid, dye solution (2,6-dichlorophenol indophenol), stock standard solution of ascorbic acid (1 mg/mL), and working standard solution (100 µg/mL). The samples are cleaned with distilled water, crushed, combined with 10 mL of 4% oxalic acid, filtered through muslin cloth, and adjusted to a volume of 25 mL. A 5 mL aliquot of the working standard solution is titrated with 10 mL of 4% oxalic acid in the dye solution until a pale pink hue develops, followed by 5 mL of the extracted sample. The amount of ascorbic content (mg/100g) is determined using the following formula:

$$\text{Amount of ascorbic content (mg/100g)} = \frac{500 \times V2 \times 25 \times 100}{V1 \times 5 \times 5} \quad \text{equ. (3).}$$

Where: 500 = µg of standard ascorbic acid taken for titration. V1 = Volume of dye consumed by 500µg of standard ascorbic acid. V2 = Volume of dye consumed by 5 mL of test sample. 25= Corresponds to total volume of the extract. 100 = Ascorbic acid content/100g of the sample.5 = Weight of sample taken for extraction. 5 = Volume of the test sample taken for titration.The ascorbic acid content of the samples was calculated according to [30].

2.8.2. Fruit physical properties

2.8.2.1. Weight loss (%)

The percentage of weight loss was calculated from the following equation:

$$\text{Weight loss \%} = \frac{Wi(g) - Ws(g)}{Wi(g)} \times 100 \quad \text{equ. (1)}$$

Where Wi = The initial weight of the fruit before cold storage, Ws = The weight of the fruit at a period of sampling. *Interval = 15 days for refrigerator.

2.8.2.2. Decay %

Skin appearance, shriveling, fungi, bacteria, and infections were all evaluated. During each inspection, damaged fruits were destroyed, and the weight of fruits per replicate was utilized to compute the decay percentage using the formula:

$$\text{Decay \%} = \frac{\text{Total number of decayed fruits}}{\text{Initial number of stored fruits}} \times 100 \quad \text{equ. (2)}$$

2.8.2.3. Fruits firmness: Fruit firmness (Kg/cm^2) was determined on two opposite sides of the flesh of each fruit using a Magness–Taylor pressure tester and a penetrometer (Turoni Decco Iberica Penetrometer model. FT 011) [31].

2.9. Statistical analysis

All physical, physiochemical, microbiological, and phytochemical data acquired from the whole random design were statistically evaluated using ANOVA (2 Way Randomized Complete Design) using Costat V6-303 statistical software following *Snedecor and Cochran*[32]. The means of each treatment were compared using the least significant differences (LSDs) at $p < 0.05$, both across various storage periods and within the same time point.

3. Results and discussion

3.1. Structural observation by TEM

TEM was applied to visualize the internal structure of the prepared materials. For CMC-Fr1, as shown in Fig (1. A& D), the Fr was loaded as agglomerated irregular nanoparticles into CMC with an average particle diameter of 210±52 nm. This agglomeration was increased for CMC-Fr2 which contains a high ratio of Fr as present in Fig (1.B&E). The average particle diameter for CMC-Fr2 nanoemulsion was 323±88 nm. However, for CMC-Z-Fr2 which contains Zn and Fr, the existence of Zn (Fig.1.C&F) separates the agglomerated Fr particles and enhances the formation of nanospheres with an average diameter of 28.7±14 nm.

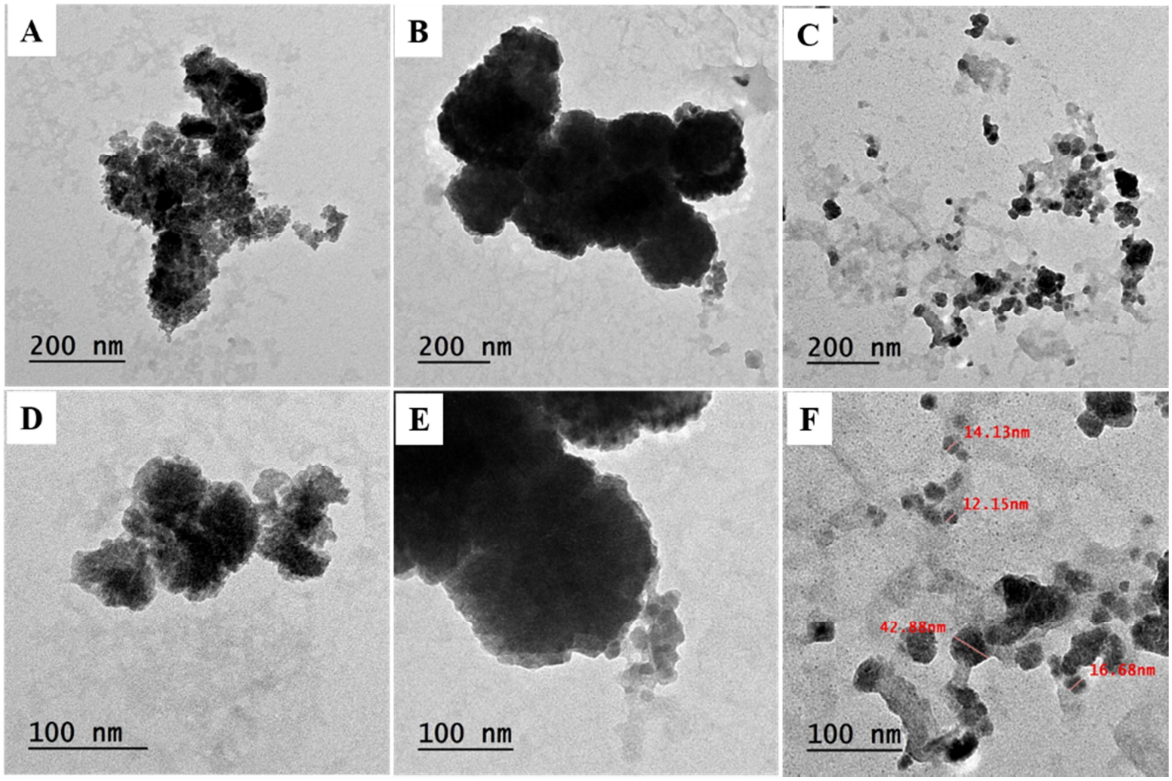


Figure 1: TEM images at different magnifications of, CMC-Fr1(A&D), CMC-Fr2(B&E), and CMC-Z-Fr2(C&F).

3.2. Structural investigation by FTIR

Fig 2. illustrates the FTIR spectra of different samples. As presented in Fig.(2.A), the CMC spectrum shows a broad band that appeared at around 3300 cm^{-1} ascribed to the O-H stretching vibration [33]. While, the peaks seen at about 1594 cm^{-1} and 1419 cm^{-1} are correlated to the carboxylate (COO^-) stretching vibrations [34] and the CH bending vibrations, respectively [35]. Moreover, the peaks at 1320 cm^{-1} and 1038 cm^{-1} are due to the bending vibration of C-H and C-O-C pyranose ring vibration, respectively [36]. In the same context, the CMC-Z spectrum exhibits the same characteristic peaks as CMC with no presence of any other peaks. In the FTIR spectrum of Fr, the peak at around 3186 cm^{-1} is attributed to N-H stretching vibrations of amine groups, which are frequently present in *Frankincense* essential oils and resins [22]. The peaks that appeared at about 2925 cm^{-1} and 1560 cm^{-1} are related to C-H and N-H stretching vibrations, respectively [37]. At about 1415 cm^{-1} the peak is connected to C-H bending vibrations ofaromatic rings. Fr structure contains a large number of aromatic molecules, which support both its stability and bioactivity [22]. The peaks at 1037 cm^{-1} and 886 cm^{-1} are related toC–O–C stretching vibration and H bending of aromatic rings, respectively [38]. As illustrated in Fig (2. B), the CMC-Fr1 nanoemulsion spectrum exhibits some peaks related to Fras peaks at 2924 cm^{-1} and 2850 cm^{-1}, while peaks present at around 1600 cm^{-1} and at 1320 cm^{-1} are related to CMC. At 1420 cm^{-1} and 1037 cm^{-1}, there is an overlap between peaks of CMC and Fr. However; the same characteristic peaks were also

presented in the spectrum of CMC-Fr2 nanoemulsion, as shown in Fig (2. B). There were two new peaks at 1710 cm^{-1} and 1220 cm^{-1} reflecting a type of interaction that may be present between CMC and Fr. On the other hand, the CMC-Z-Fr2 nanoemulsion spectrum shows an obvious reduction in peak intensities. The peak that corresponds to the carboxylate group shifts from 1594 cm^{-1} to 1589 cm^{-1}. Also, the addition of zinc to CMC-Fr nanoemulsion also affects the N-H and C=O stretching vibrations in Fr, which means that it interacts with Fr.

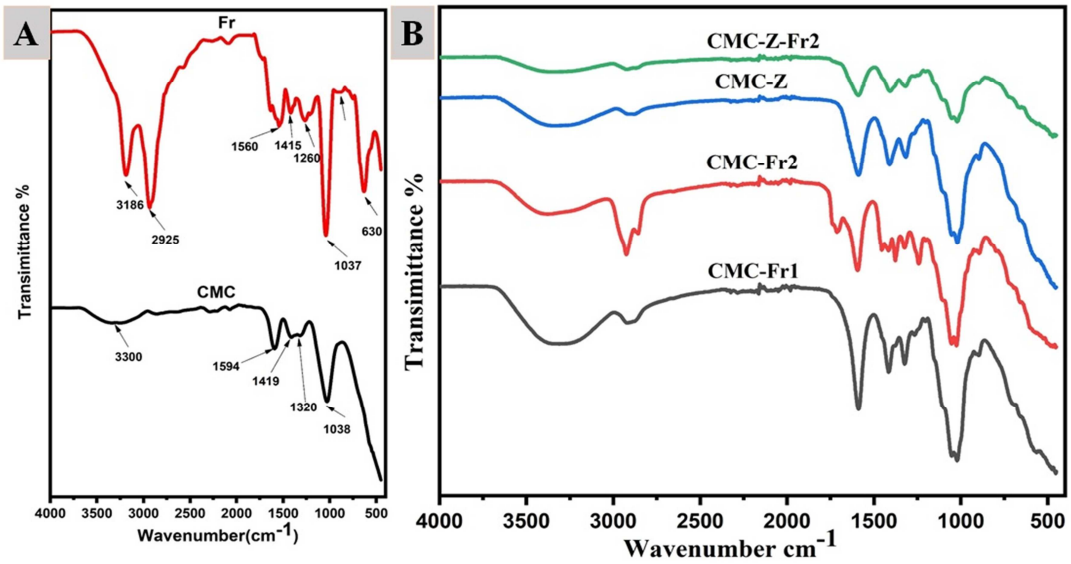


Figure 2: FTIR spectra of A; CMC and *Frankincense* (Fr) and B; of CMC- Fr, CMC-Zn, and CMC-Z-Fr2 respectively.

3.3. Antifungal activity assay

3.3.a. *In Vitro* antifungal activity

The capability of the Frto inhibits the proliferation of some phytopathogenic fungi, *Aspergillus niger,* and *Penicillium commune* was carried out. The Fr at two different concentrations, 25 and 50 µg/mL, (Fr1 &Fr2 respectively), was subjected to the screening test using the agar well diffusion. Each fungal pathogen was investigated and compared to the standard antifungal agents. In this respect, *A. niger* was found to be inhibited by all tested antifungal agents, while *P. commune* was found to be more resistant toward Nizoral (Table 1). Otherwise, the activity of the tested molecules was proven to have a strong ability to inhibit the proliferation of *P. commune*. In which, the inhibition zone diameter was recorded as 8 and 10 mm for Fr1 and Fr2, respectively (Fig.3). A moderate activity was detected in the case of *A. niger*, particularly towards Fr2 (3 mm). The antifungal activity of Fr may result from the presence of various active biological molecules such as boswellic acids, polyphenols, alkaloids, and essential oils that exhibit significant antimicrobial influence [39, 40]. The mechanism of action may be due to the interaction between the active molecules included in Fr as Limonene and, αpinene and enzymes, cell wall, proteins, and nucleic acids of the fungal pathogens [39, 41, 42]. Several studies have been conducted to investigate the activity of Fr against different pathogens. In the present work, Fr exhibits lower activity against *A. niger* which matches with the results obtained by *Miloš Čet al*.,[43]. Otherwise, *Ljaljevi´et al.,*[44] showed that; Fr has high activity against *Penicillium commune*. These variations of the activity of Fr may be due to differences in chemical composition, solubility, diffusion in growth media, and also extraction method [45].

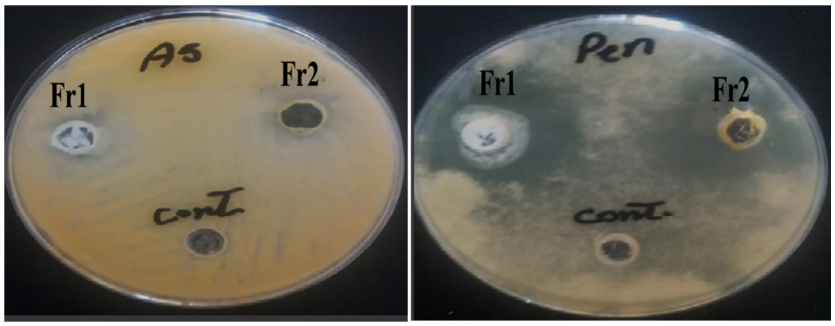


Figure 3: Antifungal activity of the targeted molecules using agar-well diffusion.

Table 1: Antifungal activity of the targeted molecules using agar-well diffusion

Organism	Diameter of inhibition zone (mm)				
	Fr1	Fr2	Fluconazole [a]	Amphotericin B	Nizoral
Aspergillus niger	4±0.15	3±0.22	2±0.25	4±0.22	3±0.04
Penicillium commune	8±0.18	10±0.52	4±0.31	7±0.05	ND [b]

[a] Fluconazole, Nizoral, and Amphotericin B were used as standard antifungal agents at 20 µg/ mL, [b] ND: not determined.

On the other hand, the efficiency of the CMC, CMC-Z, CMC-Fr1, CMC-Fr2, and CMC-Z-Fr2 prepared nanoemulsions against *Aspergillus niger* and *Penicillium commune* pathogens was also evaluated according to the reduction in CFU using Potato dextrose broth medium (PDB) [26]. As summarized in (Table 2), the inhibition of survival activity was almost negligible for CMC emulsions that contain CMC alone and weak for CMC-Z emulsions in which zinc is loaded into CMC. Loading Fr into CMC promotes inhibition of survival activity against both pathogens and a high concentration of Fr (CMC-Fr2) exhibits more inhibition than a low concentration(CMC-Fr1). However, the inhibition of fungal survival was strongly noticed for the CMC-Z-Fr2 sample that reached 28.4% toward *A. niger*, while causing a 33.3% reduction in the *P. commune* growth. While, loading of Fr alone into CMC promotes the inhibition against *A. niger* than for *Penicillium commune,* the presence of both Fr and Zn (CMC-Z-Fr2) shows a higher effect for *P. commune* than for *Aspergillus niger*. *Helmiyati etal.,*[46] infuse Zn into CMC-PVA films and illustrate that; the films exhibit antibacterial influence against *E. coli* and *S. aureus* with the inhibition zone of about 2.15 ± 0.42 mm and 3.25 ± 0.47 mm, respectively for both pathogens. *Ramsi et al.,* [47] coated cotton fabrics with Zn NPs formulated from *Boswellia serrata* and mentioned; the prepared formulation shows an antibacterial effect against *Acinetobacter baumanii, Klebsiella oxytoca, Psuedomonas aeruginosa, Staphylococcus aureus, Escherichia coli, and Klebsiella pneumonia.*

Table 2: Antifungal activity of the prepared compounds along with antifungal agents using CFU procedure.

Sample Code.	**Inhibition in Fungal survival (%)**	
	Aspergillus niger	***Penicillium commune***
CMC	0.26±0.12	0.8±0.09
CMC-Z	3.7±1.33	5.5±2.61
CMC-Fr1	11.5±1.15	6.9±0.55
CMC-Fr2	13.9±3.02	7.2±2.15
CMC-Z-Fr2	28.4±1.25	33.3±3.35
Fluconazole a	16.5±1.55	31.8±2.77
Amphotericin B	36.7±4.31	44.8±2.11
Nizoral	25.8±3.2	ND

a Fluconazole, Nizoral, and Amphotericin B were used as standard antifungal agents at 20 µg/ mL, b ND: not determined.

3.3. b. *In Vivo* disease assessment on fruits

Subsequently, assessment of the prepared emulsions against the infected oranges with a consortium of fungal pathogens, *Aspergillus niger* and *Penicillium commune* was established for 30 days at room temperature. The occurrence of the full infection with both fungal pathogens for the uncoated sample showed rapidly penetrated puncture wounds within one week. As can be seen in (Fig. 4), the investigation of the prepared compounds was divided into CMC, CMC-Z, CMC-Fr1, CMC-Fr2, and CMC-Z-Fr2 groups and controls. A significant effect of all groups was shown on *P. commune* except for the CMC group; in which the two fungal pathogens were growing at the same time as the untreated samples. In addition, the appearance of *A. niger* in the case of CMC-Fr1 and CMC-Fr2 was started after 20 days of the control. Furthermore, the CMC-Z groupwas preventing of *A. niger* to proliferate for 12 days, after that the growth initially appeared. Efficiently, the growth of both fungal pathogens was completely prevented from each hole in the case of the CMC-Z-Fr2 group, a weak growth of *A. niger* was observed after 30 days. Therefore, the inhibition of the colonized region of *P. commune* was carried out in all groups except for the CMC group by more than 85% after the incubation period. Moreover, the inhibition of colonization area for both fungal pathogens caused by CMC-Z-Fr2 was also increased by 97% and 88% for *P. commune* and *A. niger*, respectively.

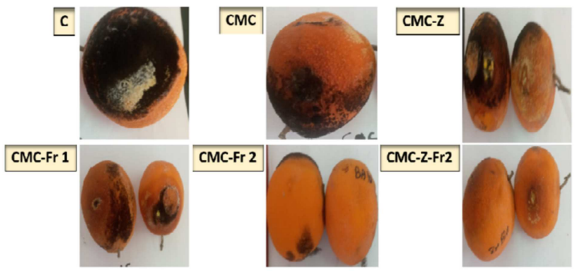


Figure 4: *In vivo* phytopathogenicity of the consortium from *Aspergillus niger* and *Penicillium commune* on Citrus fruits.

3.4. Fruit biochemical properties

3.4.1. Total Soluble Solids (T.S.S. %)

Fig. 5. clearly illustrates that adding CMC, zinc, and Fr to Valencia orange fruits during postharvest refrigeration significantly enhanced the total soluble solids percentage. The application of orange fruits with CMC-Z-Fr2 nanoemulsion was the best treatment for T.S.S. (%), followed by CMC- Fr2 nanoemulsion and CMC-Z emulsion, respectively, while the control treatment had the lowest values in both seasons. Furthermore, fruit stored for 15 days had the least T.S.S. (%) value when compared to other storage periods. In terms of the interaction effect between postharvest treatments and storage days, the results showed that the control treatment gave the highest value of T.S.S. (%) (11.99 and 12.09 in first and second season, respectively) at 135 days; the application of fruits with CMC emulsion resulted in the highest value (12.15 and 12.20 in first and second season, respectively) at 165 days of storage, while CMC-Z, CMC-Fr2, and CMC-Z-Fr2 applications showed the maximum value (12.26, 12.37, and 12.50, respectively, in the first season and 12.40, 12.47, and 12.69, respectively, in the second season) at 180 days of storage under refrigeration.

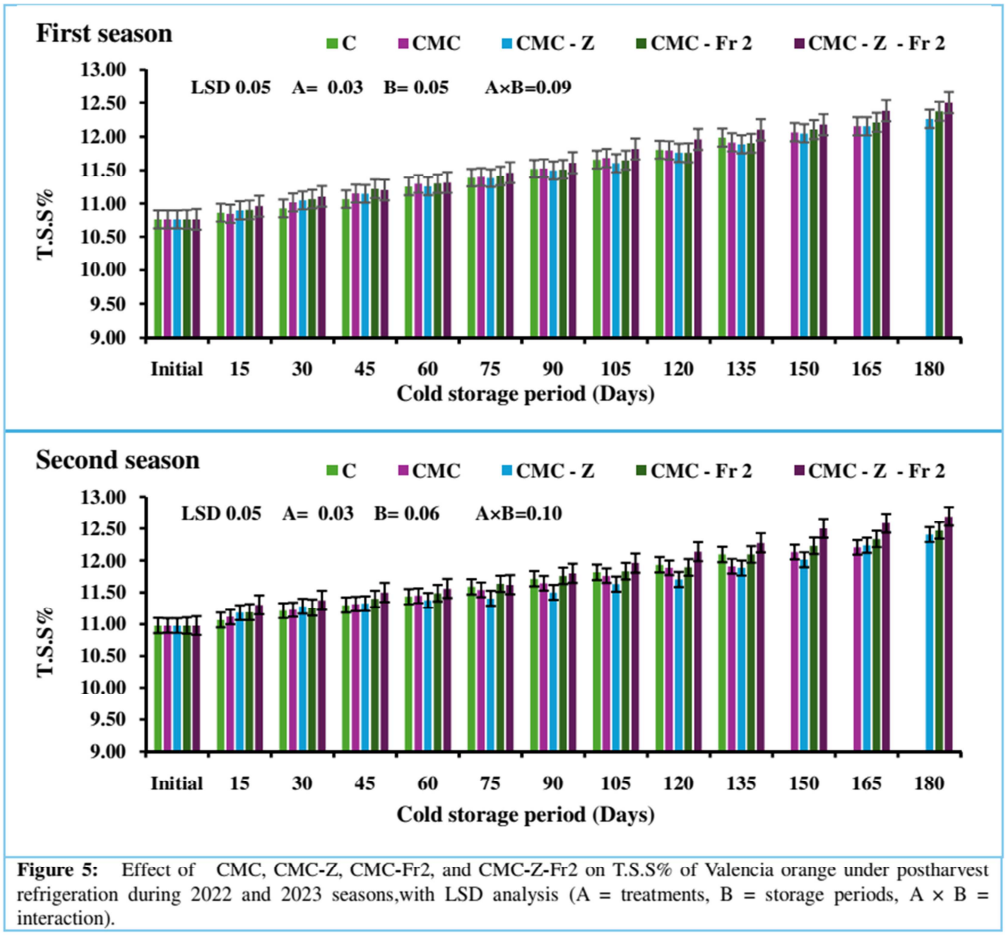
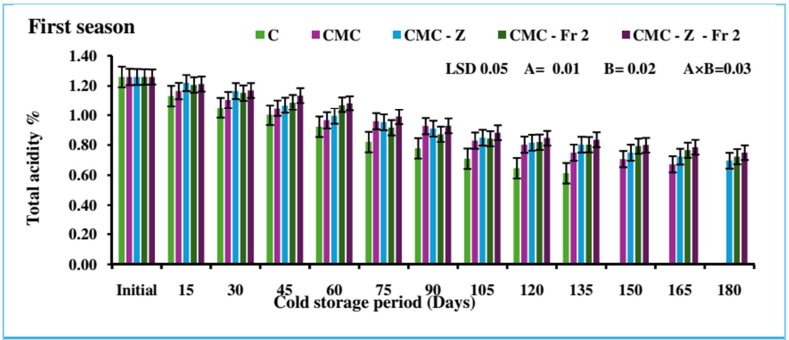


Figure 5: Effect of CMC, CMC-Z, CMC-Fr2, and CMC-Z-Fr2 on T.S.S% of Valencia orange under postharvest refrigeration during 2022 and 2023 seasons,with LSD analysis (A = treatments, B = storage periods, A × B = interaction).

3.4.2. Total acidity percentage

Fig. 6. shows that the application of CMC, zinc, and Fr considerably improved the total acidity (%) of Valencia orange fruits in postharvest refrigeration. With lengthy storage, the proportion of total acidity steadily decreases. Similarly, increasing the storage duration reduced overall acidity (%). The treatment of orange fruits with CMC-Z-Fr2 nanoemulsion was the best treatment in terms of reducing total acidity, followed by treatment with CMC-Fr2 and CMC-Z emulsions respectively, when compared to the control in both seasons. The impact of storage length, as shown in Fig (9), was that fruits held for 15 days had the highest value when compared to other storage days for both seasons. Furthermore, the interaction effect between postharvest treatments and storage days results revealed that the control treatment gave the lowest value of total acidity (%) (0.61 and 0.52 in first and second season, respectively) at 135 days, the application of fruits with CMC emulsion resulted in the lowest value (0.67 and 0.64 in first and second season, respectively) at 165 days, while CMC-Z, CMC-Fr2, and CMC-Z-Fr2 applications showed the minimum value (0.70, 0.72, and 0.75, respectively, in the first season and 0.65, 0.67, and 0.71, respectively, in the second season) at 180 days under refrigeration conditions.



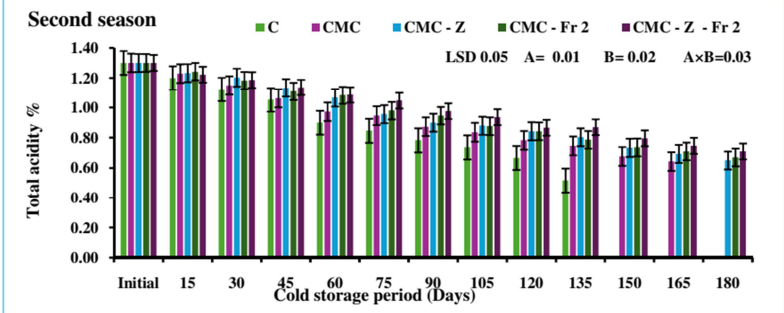
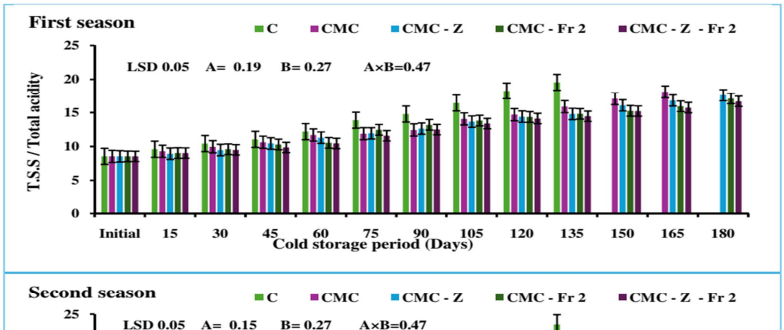


Figure 6.: Effect of CMC, CMC-Z, CMC-Fr2, and CMC-Z-Fr2 nanoemulsions on total acidity % of Valencia orange under postharvest refrigeration during 2022 and 2023 seasons, with LSD analysis (A = treatments, B = storage periods, A × B = interaction).

3.4.3. T.S.S./acid ratio

Fig. 7. shows that treatments of Valencia orange fruits with CMC, zinc, and Fr during postharvest refrigeration altered the T.S.S./acid ratio. The application of orange fruits with CMC-Z-Fr2 nanoemulsion resulted in the lowest T.S.S./acid value, followed by treatments with CMC-Fr2 and CMC-Z emulsions respectively, while the control treatment yielded the highest value in both seasons. Furthermore, fruit held for 15 days had the lowest T.S.S./acid value when compared to other storage periods. The interaction effect between postharvest treatments and storage days results showed that the control treatment gave the highest value of T.S.S./acid (19.55 and 23.45 in the first and second seasons, respectively) at 135 days, the application of fruits with CMC emulsion resulted in the highest value (18.11 and 18.97 in the first and second seasons, respectively) at 165 days, while CMC-Z , CMC-Fr2 ,and CMC-Z-Fr2 applications showed the highest values (17.60, 17.11, and 16.68, respectively, in the first season and 19.09, 18.61, and 17.88, respectively, in the second season) at 180 days under refrigeration conditions. Furthermore, research indicated that extending the storage duration of Valencia orange fruits enhanced their T.S.S./acid content in both seasons.



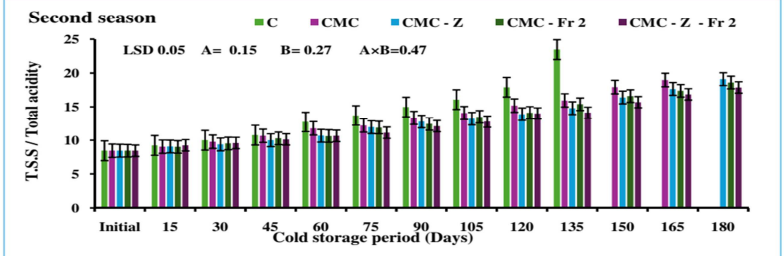


Figure 7: Effect of CMC, CMC-Z, CMC-Fr2, and CMC-Z-Fr2 nanoemulsions on T.S.S / total acidity of Valencia orange under postharvest refrigeration during 2022 and 2023 seasons, with LSD analysis (A = treatments, B = storage periods, A × B = interaction).

3.4.4. Ascorbic acid content

Fig. 8. Clearly shows that applying CMC, zinc, and Fr to Valencia orange fruits during postharvest refrigeration increased ascorbic acid concentration considerably. The application of orange fruits with CMC-Z-Fr2 nanoemulsion was the best ascorbic acid treatment, followed by CMC-Fr2 and CMC-Z emulsions respectively, while the control treatment had the lowest values in both seasons. In terms of the interaction effect between postharvest treatments and storage days, the results showed that the control treatment gave the lowest value of ascorbic acid (43.23 and 43.06 mg/100g in first and second season, respectively) at 135 days, the application of fruits with CMC emulsion resulted in the lowest value (44.15 and 43.67 mg/100g in first and second season, respectively) at 165 days, while CMC-Z, CMC-Fr2, and CMC-Z-Fr2 applications showed the lowest values (44.54, 45.07, and 45.18 mg/100g, respectively, in the first season and 43.93, 44.29, and 44.48 mg/100g, respectively, in the second season) at 180 days under refrigeration conditions. Furthermore, fruit kept for 15 days had the highest level of ascorbic acid when compared to other storage times.

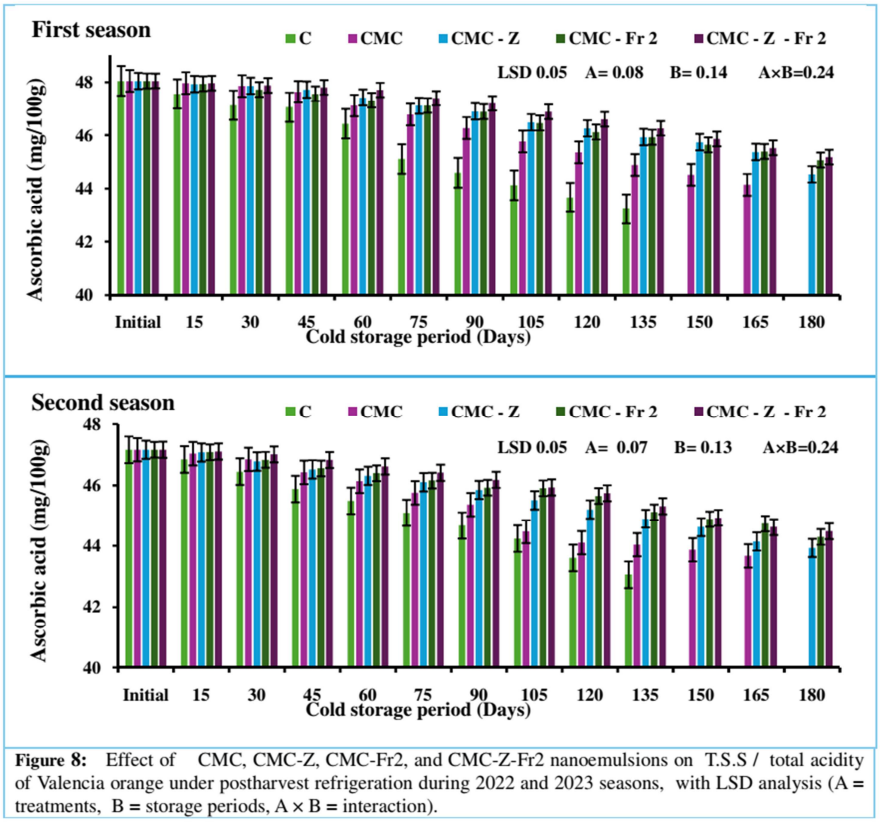


Figure 8: Effect of CMC, CMC-Z, CMC-Fr2, and CMC-Z-Fr2 nanoemulsions on T.S.S / total acidity of Valencia orange under postharvest refrigeration during 2022 and 2023 seasons, with LSD analysis (A = treatments, B = storage periods, A × B = interaction).

3.5. Fruit Physical Properties

3.5. 1. Weight Loss (%)

The results in Fig. 9. show that applying CMC, zinc, and Frconsiderably decreased weight loss (%) of Valencia orange fruits in postharvest refrigeration. During the two trial seasons, the treatment of orange fruits with CMC-Z-Fr2 nanoemulsion was the most effective in terms of weight loss reduction, followed by the CMC-Fr2. Furthermore, data in Fig. 5. showed that increasing the storage period under refrigeration conditions increased Valencia orange fruit weight loss (%), with fruits stored for 180 days recording the highest values compared to those stored for 15 days, which had the lowest value of fruit weight loss (%) in both seasons. The control showed the highest fruit weight loss rate(8.08 % and 8.97 % in the first and second season respectively) at 135 days, and the application of fruits with CMC resulted in the highest rate (7.72% and 7.63% in the first and second season respectively) at 165 days, while CMC-Z, CMC-Fr2, and CMC-Z-Fr2 applications showed the highest rate (7.51%,7.11%, and 6.85% respectively in the first season and 7.59 %, 7.17%, and 7.05 % respectively in the second season) at 180 days under refrigeration.

3.5.2. Decay (%)

Fig. 10. reveals that the decay percentage of Valencia orange fruits at harvest under refrigerated conditions was considerably altered by CMC, zinc, and Frapplications. Data indicated that the percentage of Valencia orange fruit degradation rose as the storage duration increased. The control treatment had the highest value of fruit decay (%), while the application of orange fruits by CMC-Z-Fr2 nanoemulsion was the best treatment in terms of reducing fruit decay (%), followed by the treatments CMC-Fr2 and CMC-Z in both seasons. Fig. 10. shows that the untreated Valencia orange fruits (control) had the highest fruit decay rate (10.07% and 11.23% in the first and second season respectively) at 135 days, and the application of fruits with CMC resulted in

the highest rate (8.69% and 9.06% in the first and second season respectively) at 165 days, while CMC-Z, CMC-Fr2, and CMC-Z-Fr2 applications showed the highest rate (8.16%, 8.00%, and 7.88% respectively in the first season and 8.24%, 8.08%, and 8.00% respectively in the second season) at 180 days under refrigeration.

3.5.3. Fruits firmness

Fruit firmness steadily declines with prolonged storage. Furthermore, Fig. 11. showed that the application with CMC-Z-Fr2 nanoemulsion resulted in the highest fruit firmness content of Valencia orange at harvest under refrigeration conditions, followed by application with CMC-Fr2. The control treatment had the lowest values of fruit firmness compared to all treatments in both seasons. The treatment of fruits with CMC-Z, CMC-Fr2, and CMC-Z-Fr2 resulted in the lowest values (2.13, 2.22, and 2.31 kg/cm^2, respectively, in the first season and 2.18, 2.18, and 2.22 kg/ cm^2, respectively, in the second season) at a storage age of 180 days, and the application of fruits with CMC emulsion resulted in the lowest value (2.11 and 2.16 kg/cm^2 in the first and second seasons, respectively) at a storage age of 165 days, while the control had the lowest fruit firmness value (1.74 and 1.66 kg/cm^2 in the first and second seasons, respectively) at 135 days. Fruit held for 15 days had the greatest rating of fruit firmness compared to other storage times.

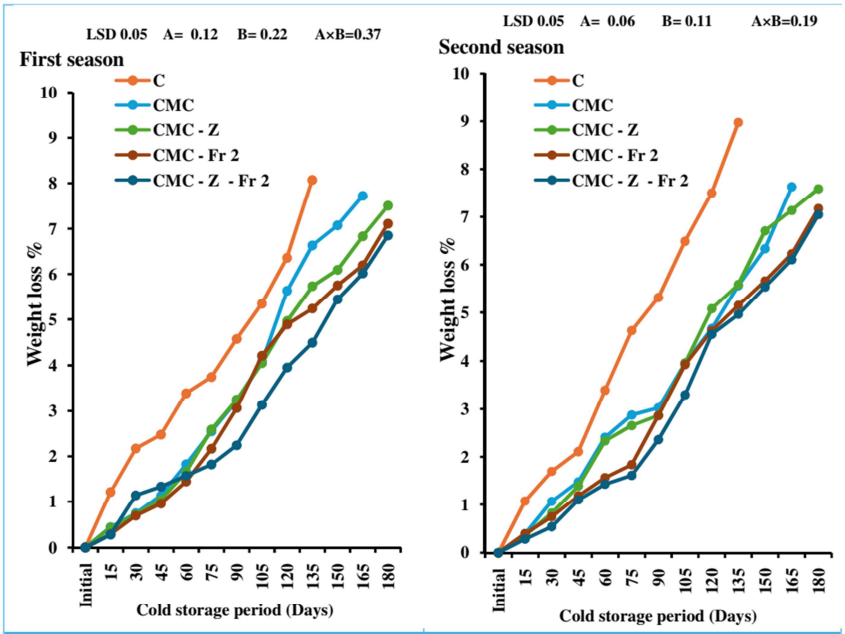


Figure 9: Effect of CMC, CMC-Z, CMC-Fr2, and CMC-Z-Fr2 nanoemulsions on weight loss % of Valencia orange under postharvest refrigeration during 2022 and 2023 seasons, with LSD analysis (A = treatments, B = storage periods, A × B = interaction).

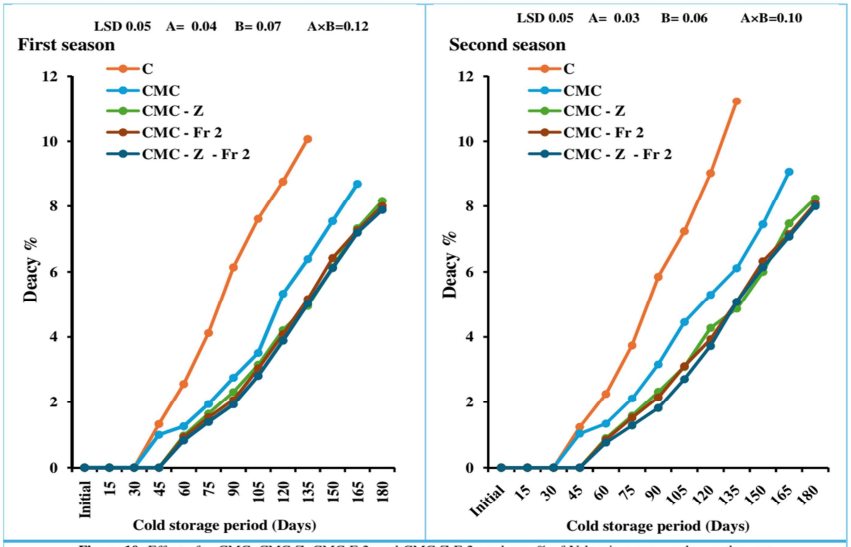


Figure 10: Effect of CMC, CMC-Z, CMC-Fr2, and CMC-Z-Fr2 on decay % of Valencia orange under postharvest refrigeration during 2022 and 2023 seasons, with LSD analysis (A = treatments, B = storage periods, A × B = interaction).

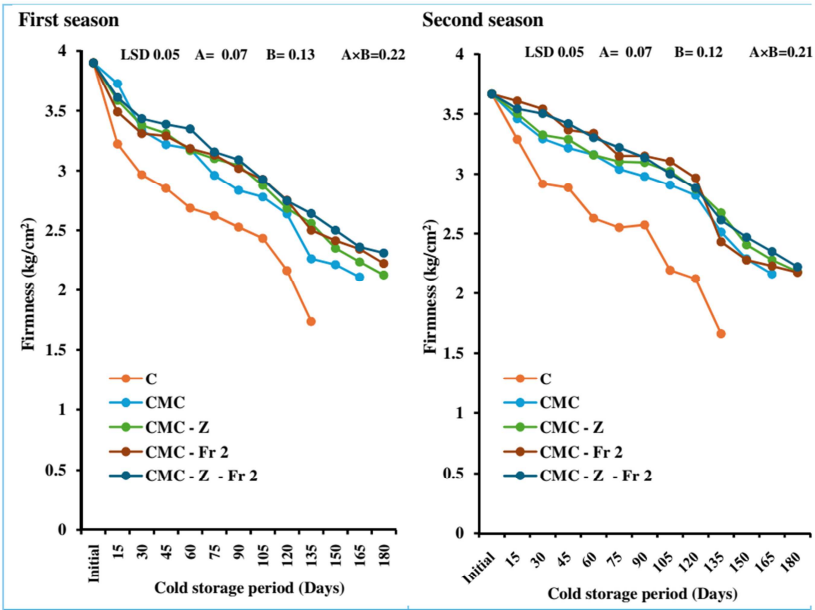


Figure 11: Effect of CMC, CMC-Z, CMC-Fr2, and CMC-Z-Fr2 nanoemulsions on firmness (kg/cm^2) of Valencia orange under postharvest refrigeration during 2022 and 2023 seasons, with LSD analysis (A = treatments, B = storage periods, A × B = interaction).

CMC is well known for its capacity to produce coating on the surface of fruits, serving as a barrier to humidity loss and gas exchange. CMC's film-forming possessions, along with its biodegradability and non-toxicity, make it an excellent choice for use in edible coatings. CMC has been confirmed in studies to considerably decrease fruit weight loss by limiting water evaporation and lowering respiration rate, which is a key informant of weight loss during storage. CMC avoids degradation by acting as a physical barrier to microorganisms [10, 48, 49]. The application of zinc to the coating amplifies this effect due to its recognized antifungal qualities [50]. CMC produces a protective layer on the orange surface, slowing the value of water loss through transpiration and respiration. This barrier lowers the interaction of gases and moisture between the fruit and the environment, which minimizes weight loss during storage [51]. CMC decreases the fruit's exposure to oxygen, reducing the oxidation of ascorbic acid. This protective action improves and preserves the ascorbic acid content during cold storage [52].CMC coating can aid in preserving fruit's titratable acidity by slowing down respiration and lowering the consumption of organic acids. The coating effectively delays the metabolic processes that lead to acid degradation, preserving the fruit's natural acidity during cold weather. Additionally, the CMC coating improves the sugar content by reducing the fruit's respiration rate, which slows the conversion of sugars into other compounds. This helps to keep the fruit sweet throughout storage [48, 53].

Zinc can help reduce weight loss in Valencia oranges during storage by improving the structural integrity of cell walls, hence minimizing moisture loss [54]. Zinc's role in strengthening cell membranes and in the creation of proteins that regulate cell turnover helps to inhibit water loss. Zinc also possesses antibacterial characteristics that help prevent the formation of spoilage microorganisms, lowering the risk of deterioration [55]. The utilization of zinc can provide a less favorable habitat for pathogens, increasing the shelf life of the fruit and boosting quality parameters such as total soluble solids and titratable acidity [14, 56, 57]. Furthermore, zinc helps to preserve fruit by portraying an important role in the formation of cell wall components such as pectin. It also helps to decrease the activity of enzymes that break down cell walls, retaining firmness during cold storage [19]. These coatings may be modified with varied amounts of zinc to achieve the ideal balance between prolonging shelf life and preserving the fruit's taste qualities [18].

Frankincense includes naturally occurring chemicals that can create a thin protective covering on the fruit's surface. This layer reduces moisture loss by restricting water vapor transport, thus lowering weight loss during storage [58]. Furthermore, frankincense is recognized for its antibacterial and antifungal qualities, which can inhibit the growth of microorganisms that cause spoilage and slow the pace of decay during storage [25]. The protective coating generated by frankincense helps to keep fruit firm by preventing moisture loss and inhibiting the action of enzymes that destroy cell walls, which generally leads to softening [59]. This led to preserving structural integrity over time. Furthermore, avoiding microbial decomposition benefits to retain the fruit's natural acidity [21].

CMC with zinc and frankincense has a complex protective action that improves fruit quality during cold storage. This technique not only decreases weight loss and decay, but it also preserves firmness, acidity, sugar levels, and ascorbic acid content, guaranteeing that the fruit remains fresh and healthy for an extended duration. CMC creates a semi-permeable coating on the fruit's surface, limiting moisture loss and gas exchange. When zinc is integrated into this coating, it improves the structural

integrity of the fruit's cell walls while also supporting antibacterial qualities. Frankincense, which has high antibacterial and antioxidant assets, helps to reduce microbial spoilage and oxidative stress.

4. Conclusion

The present study successfully demonstrated the preparation and application of Fr and Zn-loaded CMC nanoemulsions using solvent evaporation technique as novel bioactive food coating materials to improve Valencia orange shelf life and quality. The morphological analyses using TEM showed spherical nano size aggregates of Fr with mean average diameter 210± 52 for CMC-Fr 1 and 323±88 nm for CMC-Fr2. While the incorporation of Zinc ions resulted in less aggregated nanospheres with an average diameter of 28.7±14 nm. The antifungal activity, evaluated using the disk diffusion method, showed that Fr-based nanoemulsions demonstrated superior activity against *Penicillium commune* with maximum inhibition efficiency of 33.3% for CMC-Z-Fr2 emulsions. In addition, the study shows that CMC-Z-Fr2 emulsions were the best coating materials at retaining biochemical properties like total soluble solids, titratable acidity, and ascorbic acid content, as well as at in vivo antifungal activity assay. It also helped with weight loss and made the material firmer. Together, the study findings imply that using CMC coatings, especially when combined with zinc and frankincense, are a viable, natural, and cost-effective technique for increasing the shelf life and preserving the quality of Valencia orange. Future studies should look at expanding the use of this method to additional fruit kinds and storage circumstances.

Data Availability

The data sets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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.

Author Contribution

Adel F. Ahmed, Safaa Saleh, Emad Tolba, Mohamed Abdelraof, Ola M. Awad & Omali Youssef El-Khawaga: Conceptualization, Methodology, Formal Analysis, Investigation, Funding, Visualization, Writing – original draft preparation.

Funding Declaration

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