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Research Article
Maintenance Quality and Reduce Chilling Injury of Naomi Mango Fruits During Cold Quarantine

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Abstract

Background and Objective: Quarantine treatment enables export of mango fruits to parts of the world that enforce quarantine against fruit fly. Chilling injuries (CI) are prevalent in the mango fruits stored at temperatures lower than 12°C during cold-quarantine. So, this research examined the potentially enable of cold-shock treatment and integrated of edible coating with packing in (EPE) foam net to enhance the resistance of Naomi mango fruits during cold export. **Materials and Methods:** The effect of cold quarantine was studied on fruit quality of 'Naomi' mango for 2016 and 2017 seasons. In this respect, mango fruits were coated with sodium alginate at 3%, semperfresh™ at 1% and packed in (EPE) foam net to reduce CI during cold quarantine. The treated fruits were stored for 4 h at 0°C, then transferred to 20°C for 20 h (cold-shock treatment) prior to store at 2±1°C and 90-95% RH for 15 days (quarantine treatment). While, the control fruit were directly stored at 2±1°C and 90-95% RH for 15 days. Thereafter, all fruits were stored at 20±2°C for and 70-75% RH for 7 days as shelf life period to simulate a marketing period. **Results:** All applied treatments significantly reduced fruits weight loss (%), respiration rate, retard the loss of soluble solid (%) titratable acidity (%), have a good potential in maintaining firmness (lb inch^{-2}), skin color h°, vitamin C, total phenol, flavonoids content, membrane stability index (MSI %), high rate of antioxidant capacity and retard the loss of shelf-life of fruits than the control. **Conclusion:** It was concluded that edible coatings used semperfresh™ at 1% with (EPE) foam net packing have a good potential in controlling postharvest chilling injury and maintaining the fruit quality being the most effective treatment on all parameters tested. It could potentially enable the export mango fruits to all quarantine-enforcing countries.

Key words: Naomi mango fruits, quarantine, chilling injuries (CI), sodium alginate, semperfresh™, (EPE) foam net packing, membrane stability index

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Mango (*Mangifera indica* L.) is one of the most important fruits facing problem in storage and transportation to the market over long distances due to its perishable nature. Mango is distributed worldwide due to its appealing taste, aroma and nutritional value[1]. The Naomi mango is a seedling of Palmer variety it can bloom twice a year and the first flowering ripens in August and September. In general, mango is infected with fruit flies (*Ceratitis capitata* Wiedemann, Diptera: Tephritidae) which reduced the possibility of export to various countries. Therefore, effective postharvest quarantine treatments for mangoes exported are essential to ensure fruit fly control without compromising fruit quality[2]. Several postharvest methods for quarantine treatment have been developed, such as radiation, heat and cold treatments. However, heat treatments can impair fruit sensory quality while, radiation is relatively expensive and its application is complicated. Cold storage of mango is used to prolong shelf life by slowing the metabolic rate of fruit. Since, mango is a tropical fruit that is susceptible to chilling injuries (CI) when stored at low temperature (below 12°C) after harvest, which decreases fruit quality and storage life and in turn reduces consumer acceptance. CI symptoms of mango fruits are showed on the peel as red and black spots, peel browning, abnormal ripening, reduced aroma and flavor and increased susceptibility to decay. The mango peel is more susceptible to CI than the pulp[3]. Typical CI symptoms may include unusual increase in firmness, external and internal tissue browning, poor aroma and flavor, surface pitting, uneven ripening and increased susceptibility to postharvest rot.

The USDA allowed to cold management for 18 days at 2.2°C as a quarantine treatment against fruit fly for many fruit types, including mango[4].

Mature mango fruit are susceptible to CI at storage temperatures below 12° and storage below this temperature can lead to the development of CI. Low-temperature conditioning (LTC) reduces external CI in mango[5]. Recently, the mango transcriptome's response to sub-optimal temperature storage was characterized. Interestingly, one of the main pathways that were elevated was sugar metabolism, where starch is metabolized to mono-saccharides and di-saccharides. The elevated sugar content probably increases osmolarity and reduces the fruit's freezing point[6].

Edible coatings protect food products from mechanical, physical, chemical and microbial damage and could be a new technological alternative to maintain fruit quality during cold storage[7]. Coatings on products create a semi-permeable barrier to external elements that can reduce moisture loss, solute migration, respiration and oxidative reactions and retard the natural physiological ripening process.

Alginate is a natural polysaccharide extracted from brown sea algae (Phaeophyceae) and composed of 2 uronic acids: β-d-mannuronic acid and α-l-guluronic acid. Sodium alginate (SA) is a salt of alginic acid, it is composed of block polymers of sodium poly (l-guluronate), sodium poly (d-mannuronate) and alternating sequences of both sugars. Sodium alginate is a hydrophilic biopolymer that has a coating function because of its unique colloidal properties, which include its use for thickening, suspension forming, gel forming and emulsion stabilizing[8].

The semperfresh™ (AgriCoat Industries Ltd, Berkshire, UK) was comprised of sucrose esters of fatty acids, sodium carboxymethyl cellulose and mono diglycerides of fatty acids obtained in liquid form (50/100 w/v). This sucrose esters polyester component of edible films is known as a major barrier for moisture loss which slowdown ripening and increase shelf life of produces. Semperfresh™ is coating, widely used in the fresh fruit and vegetable industry to reduce bruising, weight loss and preserves green color and fruit pressure in storage without delaying normal ripening processes for consumers. Semperfresh™ inhibits water loss while allowing gas exchange between the fruit and its environment. Semperfresh™ effect as the modified atmosphere reduced oxygen with slightly raised carbon, excess respiration, better color, delayed softening, fresh appearance, delayed breakdown in storage and reduced chilling injury[9].

Fruits packing in (EPE) foam net are also known as expanded polyethylene. EPE foam comprised of non-cross-linked closed-cell structures is a kind of new environmentally friendly packaging materials. It consists of many single bubbles of low-density.

The objective of this research was to examine the cold-shock treatment and integration of edible coating with sodium alginate (SA) and semperfresh™ treatment with packing Naomi mango fruit in (EPE) foam net to enhance fruits resistance under cold quarantine of 2°C for 15 days with the minimal development of CI during export.

MATERIAL AND METHODS

Mature green mangoes (*Mangifera indica* L., cv. Naomi) were picked full size and unripe in August[6] during seasons 2016 and 2017 from a commercial private orchard at El Salhia region Sharqia Government, Egypt. Fruits harvested from trees 7 years old grown in sandy soil and irrigated with drip

irrigation system planted at 2×5 m space. The fruit were selected uniform size, absence of defects, packed in plastic boxes and directly transferred to the laboratory. At the beginning of the experiments, samples of 15 fruits were taken to determine the initial fruits properties.

The experiment was laid out in completely randomized design with 3 replicate, 20 fruits/replicate (60 fruits in each treatment). The fruits free from physical damage and diseases with similar sizes, color and firmness were washed with tap water and air-dried, then received the following treatments:

- Edible coating with sodium alginate 3%
- Edible coating with sodium alginate 3% +packed in (EPE) foam net
- Edible coating with semperfresh™ 1%
- Edible coating with semperfresh™ 1% +packed in (EPE) foam net
- Packed in (EPE) foam net
- Control without coating or packing

The sanitized fruits were immersed in different coating solutions, where the fruits were maintained for 3 min and were then spread on nylon net until the surface was perfectly dried. The control treatment was done by immersing the fruits in aqueous solution without addition of any coating material.

Sodium alginate: Sodium alginate (Sigma-Aldrich Co., Steinheim, Germany) was dissolved in hot water (45°C) with continuous shaking until the solution became clear. After cooling to 20°C, glycerol at 20% v/v was added as a plasticizer. Fruits were dipped twice in the fresh coating solution for 1 min to assure the uniformity of the coating of the whole surface. After, fruits were dried for 30 min underan air-flow heater at 25°C. Control fruits were dipped in distilled water[10].

Semperfresh™: The semperfresh™ edible coating solution, composed of sucrose esters of fatty acids, sodium carboxymethyl cellulose and mono diglycerides of fatty acids, was obtained in concentrated liquid form (50.0%, w/v). It was diluted with water to obtain the recommended concentration for the mango fruits (1.0% w/v). The diluted solution was left for 30-45 min with occasional stirring and then applied to the fruits[11].

Cold storage study: The coated and uncoated fruits were kept in one layer at carton boxes. All fruits except the control were stored for 4 h at 0°C, then transferred to 20°C for 20 h (cold-shock treatment[12]) prior to store at 2±1°C and 90-95% RH for15 days (quarantine treatment[6]). While, the control fruit were directly stored at 2±1°C and 90-95% RH for 14 days. Thereafter, all fruits were stored at 20±2°C for and 70-75% RH for 7 days as shelf life period to simulate a marketing period.

After completion of the respective storage duration fruits were analyzed for physical and chemical attributes were determined after 15 days intervals of cold quarantine storage and after 7 days at ambient temperature as described below.

Determination of physical and chemical properties:

- **Weight loss (%):** It was determined according to the following equation:

$$\text{Weight loss (\%)} = \frac{\text{Initial fruit weight - Final fruit weight}}{\text{Initial fruit weight}} \times 100$$

- **Decay (%):** All unmarketable fruits were considered as decayed and decay (%) was calculated according to the following equation:

$$\text{Decay (\%)} = \frac{a \times 100}{b}$$

Where:

a = Number of decayed fruits at time of sampling
b = Initial fruits number

- **Chilling injury (CI) index:** The chilling injury was indicated using the CI index as described by Zhao *et al.*[12] with slight modifications. Browning, surface pitting and lenticel discoloration of fruits were used as indicators for chilling injury. It was rated on a scale from 1-5 as, 1 = No chilling injury, 2 = 1-25%, 3 = 26-50%, 4 = 51-75% and 5 = 76-100% chilling injury
- **Respiration Rate (ml CO_2 kg^{-1} h^{-1}):** Respiration rate was measured by gas analyzer (Model 1450-Servomex 1400) according to McCollum *et al.*[13], the airtight glass jars (4 L) were used to fruit incubation under the same storage circumstances for 24 h, respiration rate was measured as mL of CO_2 kg^{-1} fruits h^{-1}
- **Skin color (hue angle h°):** The color of the peel was determined with a colorimeter Chroma Meter model CR-410® (Konica-Minolta, Japan). Measurements were made near the peduncle, in the middle of the fruit and

in the pedicel. Determinations were performed using the system of CIEL, a*, b* and the color tone was estimated using the methods described by McGuire[14] as the following equation:

$$(h°) = \tan^{-1}\left(\frac{b}{a}\right)$$

Where:

a = interval of colors between green (-) and red (+)
b = interval of colors between blue (+) and yellow (-)
h° = Skin hue color

- **Fruit firmness (lb inch^{-2}):** It was measured using a Magness-Taylor penetrometer (pressure tester). Readings were taken in 3 positions in each tested fruit, averaged and recorded in lb inch^{-2}

A homogeneous sample was prepared from these 5 fruit/replicate for measuring TSS, acidity, pH and vitamin C.

- **Total soluble solid (TSS) (%):** About 1 mL of mango pulp juice was dissolved in 40 mL double-distilled water. TSS (%) was determined at 22°C in each sample with hand refractometer Carl-Zeiss using 2-3 drops of juice obtained by squeezing the fruits and expressed as Brix[15]
- **Titratable acidity (TA) (%):** To determine the titratable acidity of the fruit, 10 g of pulp of each fruit were first diluted with sterile distilled water to achieve 50-10 mL of the dilution were then titrated with 0.1 N NaOH according to the process reported by the AOAC[16]. The results were expressed as a percentage of citric acid present in the samples (g citric acid/100 g fresh pulp weight)
- **Vitamin C (mg g^{-1} Fw):** Vitamin C was measured by the oxidation of ascorbic acid with 2, 6-dichlorophenol indophenol dye and the results expressed as mg g^{-1} on a fresh weight (FW) basis according to AOAC[16]

Leakage of ions, total phenols and flavonoids, free radical scavenging capacity and enzymes activities were measured in peel since the integrity of these tissues is critical for fruit storability and the biochemical changes in peel reflect the physiological of whole fruit including flesh.

- **Leakage of ions from fruit peel:** Leakage of ions from peel disks was measured according to Sairam *et al.*[17] with some modifications and was expressed as membrane stability index (MSI %). About 3 g of peel disks/replicate/treatment was randomly taken and placed in 30 mL of deionized water at ambient temperature for 4 h in a shaker. Conductivity before boiling (C1) was measured with an electrical conductivity digital meter (Orion 150A+, Thermo Electron Corporation, USA). The same disks were kept in a boiling water bath (100°C) for 30 min to release all electrolytes, cooled to 22±2°C with running water and conductivity after boiling was recorded (C2). MSI was expressed in percentage using the formula:

$$\text{Membrane stability index (\%)} = \left[1 - \frac{C_1}{C_2}\right] \times 100$$

Preparation of the methanol extract of fruit peel: About 2 g of fruit peel (randomly collected from 5 fruit/replicate) were extracted by shaking at 150 rpm for 1 2 h with 20 mL methanol (80%) and filtered through filter paper No. 1. The filtrate designated as methanol extract that was used for estimations of total phenols, total flavonoids and antioxidant activity.

- **Total phenol content:** Total phenols concentration was measured according to Chun *et al.*[18]. About 50 µL of the methanol extract was mixed with 100 µL Folin-Ciocalteu reagent, 850 µL of methanol and allowed to stand for 5 min at ambient temperature. A 500 µL of 20% sodium carbonate was added and allowed to react for 30 min. Absorbance was measured at 750 nm. Total phenols was quantified from a calibration curve obtained by measuring the absorbance of known concentrations of gallic acid and the results expressed as mg g^{-1} FW gallic acid equivalent
- **Total flavonoids content:** Total flavonoids concentration was determined using a modified colorimetric method described previously by Zou *et al.*[19]. Methanol extract or standard solution (250 µL) was mixed with distilled water (1.25 mL) and 5% $NaNO_2$ solution (75 µL). After standing for 6 min, the mixture was combined with 10% $AlCl_3$ solution (150 µL), 1 M NaOH (0.5 mL) and distilled water (275 µL) were added to the mixture 5 min later. The absorbance of the solutions at 510 nm was then measured. Total flavonoids was quantified from a calibration curve obtained by measuring the absorbance of known concentrations of quercetin equivalent (QE)and the results expressed as mg g^{-1} FW quercetin equivalent (QE)

- **Antioxidant capacity (DPPH radical scavenging assay of fruit peel):** The DPPH free radical scavenging activity of methanol extract of fruit peel was determined using the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) according to the method of Ao *et al.*[20]. A methanol extract (0.1 mL) was added to 0.9 mL of freshly prepared DPPH methanol solution (0.1 mM). An equal amount of methanol was used as a control. After incubation for 30 min at room temperature in the dark, the absorbance (Abs) was measured at 517 nm using a spectrophotometer. Activity of scavenging (%) was calculated using the following formula:

$$\text{DPPH radical scavenging (\%)} = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

The inhibition concentration (IC_{50}) was defined as µg phenolics of the test sample that decreases 50% of initial radical. The IC_{50} values were calculated from the dose responses curves.

Enzymes measurements of fruit tissue

Enzyme assays: To analysis superoxide dismutase (SOD), CAT and APX 5 g of frozen mango tissue was ground in10 mL of 100 mmol L^{-1} sodium phosphate buffer, pH 7.5. The homogenate was centrifuged at 12000 rpm for 20 min at 4°C and the resulting supernatants were used directly for assay.

- **Superoxide dismutase (SOD) activity:** It was determined by the method of Liu *et al.*[21] One unit of SOD was defined as the amount of enzyme that caused a 50% decrease of the SOD-inhibitable 4-nitroblue tetrazolium chloride (NBT) reduction. The SOD activity was expressed as U mg^{-1} protein
- **Catalase (CAT) activity:** It was assayed in a 2 mL reaction mixture containing 1.9 mL of 50 mM K_3PO_4 buffer, pH 7.0 containing 25 mM H_2O_2 and 0.1 mL of enzyme extract. The subsequent decomposition of H_2O_2 was observed at 240 nm (" = 39.4 M^{-1} cm^{-1}). The CAT activity was expressed as µmol H_2O_2 decomposed (mg^{-1} $protein^{-1}$) min^{-1}
- **Ascorbate peroxidase (APX) activity:** It was assayed by the method of Asada[22] in 2 mL reaction mixture, containing 0.5 mL of 100 mM K_3PO_4 buffer, pH 7.0, 0.5 mL of 1 mM L-ascorbic acid (AA), 0.5 mL of 0.4 mM EDTA, 0.02 mL of 10 mM H_2O_2, 0.38 mL of distilled water and 0.1 mL of enzyme extract. The subsequent decrease in ascorbic acid was observed at 290 nm (" = 2.8 mM^{-1} cm^{-1}). The APX activity was expressed as µmol AsA decomposed U min^{-1} mg^{-1} protein

Enzymes measurements of fruit peel: About 10 g of fruit peel (randomly collected from 5 fruit/ replicate) was homogenized with 20 mm M Tris-HCl buffer, pH 7.2 using homogenizer. The homogenate was centrifuged at 10,000 rpm for 10 min at 4°C. The supernatant was designed as crude extract and stored at -20°C for both α-amylase and peroxidase assay.

- **Peroxidase assay:** Peroxidase (EC 1.11.1.7) activity (POD) was assayed according to Miranda *et al.*[23]. The reaction mixture containing in 1 mL: 0.008 mL of 0.97 M H_2O_2, 0.08 mL of 0.5M guaiacol, 0.25 mL of 0.2 M sodium acetate buffer, pH 5.5 and least amount of enzyme preparation. The change in absorbance at 470 nm due to guaiacol oxidation was followed for 1 min using a spectrophotometer. One unit of enzyme activity was defined as the amount of enzyme which increases the O.D. 1.0 min^{-1} under standard assay conditions
- **α-amylase assay:** α-amylase (EC 3.2.1.1) activity was assayed by determining the liberated reducing end products using maltose as standards[24]. The reaction mixture (0.5 mL) containing 5 mg substrate, 0.25 mL of 0.2 M sodium acetate buffer pH 5.5 and a suitable amount of crude extract. Assays were carried out at 37°C for 1 h. Then 0.5 mL dinitrosalicylic acid reagent was added to each tube and heated in a boiling water bath for 10 min. After cooling to room temperature, the absorbance was measured at 560 nm. One unit of enzyme activity was defined as the amount of enzyme which liberated 1 µM of reducing sugar/min under standard assay conditions

Statistical analysis: Data of both seasons of the study were analyzed using analysis of variance (ANOVA). Differences among treatment means were statistically compared using Duncan's multiple tests at a level 0.05, using the CoStat V6.4 program.

RESULTS AND DISCUSSION

This study estimated the effect of edible coating with sodium alginate (SA) and semperfresh™ treatment with packing Naomi mango fruit in (EPE) foam net to enhance mango fruit's resistance to cold-shock and low temperature with the minimal development of CI during quarantine.

Weight loss (%): The results showed that, the weight loss (%) of Naomi mangoes increased after cold storage at 2°C and through marketing at 20°C. In both seasons, all treatments used significantly reduced weight loss (%) compared to the control. From Table 1 there was a significant

Table 1: Effect of coating with sodium alginate, semperfresh™ and packed in (EPE) foam net on weight loss and decay (%) of Naomi mango fruits during cold storage and under market conditions during 2016 and 2017 seasons

	Weight loss (%)			Decay (%)		
Treatments	At harvest	15 days at 2°C	7 days at 20°C	At harvest	15 days at 2°C	7 days at 20°C
Season 2016						
Coating with sodium alginate 3%	0.00^{j}	2.27^{gh}	4.15^{cd}	0.00^{g}	0.00^{g}	5.00^{c}
Coating with sodium alginate 3%+packed in (EPE) foam net	0.00^{j}	2.24^{h}	4.19^{c}	0.00^{g}	0.00^{g}	4.69^{d}
Coating with semperfresh™ 1%	0.00^{j}	2.15^{i}	4.11^{d}	0.00^{g}	0.00^{g}	4.49^{e}
Coating with semperfresh™ 1%+packed in (EPE) foam net	0.00^{j}	2.10^{i}	4.00^{e}	0.00^{g}	0.00^{g}	3.89^{f}
Packed in (EPE) foam net	0.00^{j}	2.32^{g}	4.89^{b}	0.00^{g}	0.00^{g}	5.60^{b}
Control	0.00^{j}	3.58^{f}	6.92^{a}	0.00^{g}	0.00^{g}	10.97^{a}
LSD at 5%	-	0.071	0.079	-	-	0.078
Season 2017						
Coating with sodium alginate 3%	0.00^{l}	2.30^{j}	4.33^{c}	0.00^{g}	0.00^{g}	5.12^{c}
Coating with sodium alginate 3%+packed in (EPE) foam net	0.00^{l}	2.30^{j}	4.25^{d}	0.00^{g}	0.00^{g}	4.87^{d}
Coating with semperfresh™ 1%	0.00^{l}	2.22^{j}	4.16^{e}	0.00^{g}	0.00^{g}	4.59^{e}
Coating with semperfresh™1%+packed in (EPE) foam net	0.00^{l}	2.13^{k}	4.10^{f}	0.00^{g}	0.00^{g}	3.96^{f}
Packed in (EPE) foam net	0.00^{l}	2.36^{h}	5.00^{b}	0.00^{g}	0.00^{g}	5.80^{b}
Control	0.00^{l}	3.78^{g}	7.02^{a}	0.00^{g}	0.00^{g}	11.16^{a}
LSD at 5%	-	0.053	0.067	-	-	0.035

Means followed by the same letters are not significantly different by Duncan multiple range test at 0.05 levels

difference in percent weight loss during storage due to the treatments. In fact, significant reduction in weight loss value obtained after 15 days of cold storage at 2°C (2.10 and 2.13%) for Naomi mango fruits coated with semperfresh™ 1% +packed in (EPE) foam net, while ranged 4.00 and 4.10 % after 7 days during marketing at 20°C. However, the maximum weight loss was recorded from untreated control fruits (3.58 and 3.78%) after 15 days of cold storage at 2°C and (6.92 and 7.02) after 7 days during marketing at 20°C in both seasons, respectively. From our data it is clear that, weight loss increase during cold storage of mango fruits. In this regard, it was reported that increased weight loss is caused by reduced metabolic activity and moisture evaporation through skin[25]. It is also worth mentioning that the rate at which water is lost depends on the storage temperature and water pressure gradient between the fruit tissue and the surrounding atmosphere.

In addition, edible coatings with semperfresh™ act as an extra layer, which also covers the stomata, leading to a decrease in transpiration and in turn, reduction in the weight loss[11].

Decay (%): Results presented in Table 1 showed that, all the investigated postharvest treatments significantly affected decay percentage of Naomi mango fruits during cold storage. Regardless of storage period, all treatments did not apply any decayed fruits during cold storage at 2±1°C a for15 days (quarantine treatment). On the other hand, decay was first recorded, after 7 days through marketing at 20°C in both seasons. While, all the treatments applied, significantly reduced decay compared to the untreated fruits. The minimum decay was recorded for mango fruits coated with semperfresh™ 1% +packed in (EPE) foam net (3.89 and 3.96%) through marketing at 20°C in the 2 seasons, respectively. While, the maximum decay was recorded for untreated mango fruits (10.97 and 11.00%) through marketing at 20°C in the 2 seasons, respectively.

In the current experiment, both weight loss and decay incidence significantly increased during storage (Table 1). Mango fruit is a typical climacteric type of fruit with a relatively high rate of metabolic activity such as high ethylene production and respiration rate that accelerate the ripening processes following harvest. These processes are coincided with an increase in weight loss, rapid softening, peel browning and decay that shorten fruit storability and storage. Cold quarantine requires 18 days of storage at 2.2°C to eradicate the mediterranean fruit fly from various fruit types[6]. "Semperfresh™" contains a carbohydrate-based medium, which could have provided nourishment to the fungus. This would explain that "semperfresh™" treated fruits had a lower disease level than control fruit[9].

Chilling Injury (CI) index: Treatment with semperfresh™ 1% +EPE packing was effective for delaying the increase in CI symptoms, since the CI index was less about 60-65% compared with the control fruits during 15 days of cold storage (Table 2, Fig. 1). Since, chilling injury in this treatment ranged 0.75 and 0.85 during the 2 seasons, respectively. Low temperature conditioning is an alternative technique for horticultural commodities to increase chilling

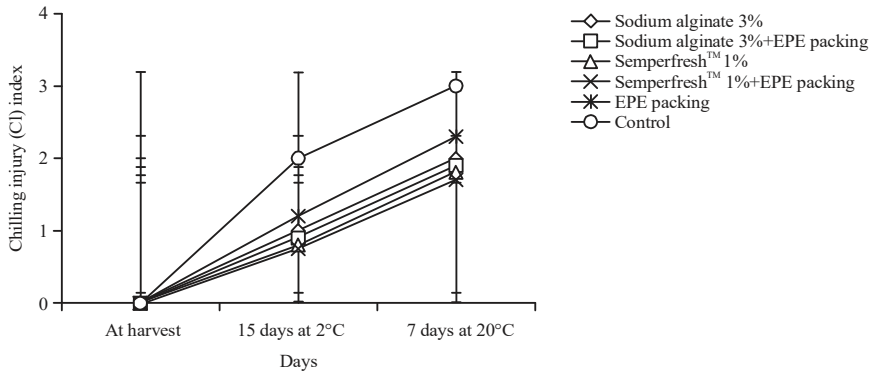

Fig. 1: Chilling injury in Naomi mango at harvest, after 15 days at 2°C and after 7 days at 20°C as a mean of 2016-2017 seasons

Table 2: Effect of coating with sodium alginate, semperfresh™ and packed in (EPE) foam net on chilling injury index and respiration rate (mg CO_2 kg^{-1} h^{-1}), of Naomi mango fruits during cold storage and under market conditions during 2016 and 2017 seasons

	Chilling injury index			Respiration rate (mg CO_2 kg^{-1} h^{-1})		
Treatments	At harvest	15 days at 2°C	7 days at 20°C	At harvest	15 days at 2°C	7 days at 20°C
Season 2016						
Coating with sodium alginate 3%	0.00^{l}	1.00^{h}	2.00^{c}	12.00^{c}	6.60^{j}	11.30^{d}
Coating with sodium alginate 3%+packed in (EPE) foam net	0.00^{l}	0.90^{j}	1.90^{d}	12.00^{c}	6.40^{k}	11.00^{e}
Coating with semperfresh™ 1%	0.00^{l}	0.80^{j}	1.80^{e}	12.00^{c}	6.30^{l}	10.50^{f}
Coating with semperfresh™ 1%+packed in (EPE) foam net	0.00^{l}	0.75^{k}	1.70^{f}	12.00^{c}	5.90^{m}	10.20^{g}
Packed in (EPE) foam net	0.00^{l}	1.20^{g}	2.30^{b}	12.00^{c}	9.00^{i}	17.60^{b}
Control	0.00^{l}	2.00^{c}	3.00^{a}	12.00^{c}	9.90^{h}	18.00^{a}
LSD at 5%	-	0.031	0.050	-	0.081	0.079
Season 2017						
Coating with sodium alginate 3%	0.00^{l}	1.12^{i}	2.05^{d}	11.60^{c}	6.30^{j}	11.10^{d}
Coating with sodium alginate 3%+packed in (EPE) foam net	0.00^{l}	1.00^{j}	1.94^{e}	11.60^{c}	6.20^{k}	11.00^{e}
Coating with semperfresh™ 1%	0.00^{l}	0.98^{j}	1.86^{f}	11.60^{c}	6.10^{l}	10.40^{f}
Coating with semperfresh™ 1%+packed in (EPE) foam net	0.00^{l}	0.85^{k}	1.75^{g}	11.60^{c}	5.70^{m}	10.00^{g}
Packed in (EPE) foam net	0.00^{l}	1.24^{h}	2.40^{b}	11.60^{c}	8.90^{i}	17.40^{b}
Control	0.00^{l}	2.27^{c}	3.24^{a}	11.60^{c}	9.60^{h}	17.80^{a}
LSD at 5%	-	0.021	0.033	-	0.087	0.105

Means followed by the same letters are not significantly different by Duncan multiple range test at 0.05 levels

tolerance. This involves holding cold sensitive tissue at temperatures just above critical temperature to induce chilling tolerance. The concept is similar to that used in acquired thermo tolerance where treating fruit or other tissues with permissive (non damaging) high temperatures induce thermo tolerance[3]. The cold-shock treatment at 0°C for 4 h significantly inhibited chilling injury in mango fruit by 59.7% lower than the control. Furthermore enhancing the level of non enzymatic antioxidants, such as phenolic compounds may also be a part of the mechanisms involved in the enhancement of chilling tolerance in mango fruit treated by cold shock[12]. CI symptoms such as skin pitting, scalding, uneven ripening, loss of color and increased decay were evident in mango stored for 7 days at 20°C showed a slight to moderate pitting or scalding rate of 1.70 and 1.75 during both seasons, respectively. Since, the untreated fruits showed higher symptoms of CI after 7 days at 20°C ranged 3.00 and 3.24 during both seasons respectively. Accumulation of those CI symptoms was correlated to severe lipid peroxidation[3].

Respiration rate (mg CO_2 kg^{-1} h^{-1}): As presented in Table 2 and Fig. 2, significant differences in respiration rates were recorded in response to storage periods and treatments investigated in this study. Regardless of storage period, all treatments in both seasons significantly inhibited respiration rate compared to control. Presented results showed that respiration rates recorded after 15 days of cold storage declined and this declination was followed by increase after 7 days of marketing at the end of the investigated storage period. This delay was recorded for semperfresh™ 1% +packed in (EPE) foam net (5.90 and 5.70 mg CO_2 kg^{-1} h^{-1}) after 15 days of cold storage at 2°C and through marketing net (10.20 and 10.00 mg CO_2 kg^{-1} h^{-1}) after 7 days at 20°C in the 2 seasons, respectively. The thickness of the barrier and

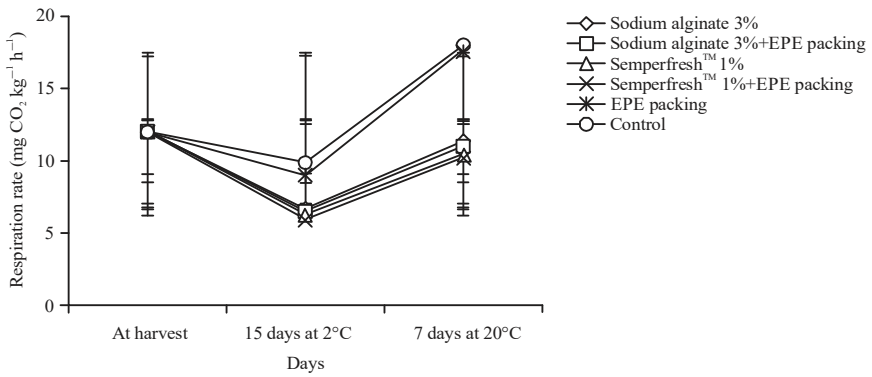

Fig. 2: Respiration rate in Naomi mango at harvest, after 15 days at 2°C and after 7 days at 20°C as a mean of 2016-2017 seasons

Table 3: Effect of coating with sodium alginate, semperfresh™ and packed in (EPE) foam net on skin hue color (h°) and fruit firmness (lb inch^{-2}) of Naomi mango fruits during cold storage and under market conditions during 2016 and 2017 seasons

	Skin hue color (h°)			Fruit firmness (lb inch^{-2})		
Treatments	At harvest	15 days at 2°C	7 days at 20°C	At harvest	15 days at 2°C	7 days at 20°C
Season 2016						
Coating with sodium alginate 3%	131.00^{a}	120.00^{e}	103.00^{j}	17.20^{a}	16.00^{d}	11.90^{j}
Coating with sodium alginate 3%+packed in (EPE) foam net	131.00^{a}	122.00^{d}	107.00^{i}	17.20^{a}	16.20^{c}	12.20^{i}
Coating with semperfresh™ 1%	131.00^{a}	124.00^{c}	110.00^{h}	17.20^{a}	16.30^{c}	12.60^{h}
Coating with semperfresh™ 1%+packed in (EPE) foam net	131.00^{a}	126.00^{b}	115.00^{f}	17.20^{a}	16.90^{b}	12.80^{g}
Packed in (EPE) foam net	131.00^{a}	114.00^{g}	97.00^{k}	17.20^{a}	15.70^{e}	11.10^{k}
Control	131.00^{a}	107.00^{i}	87.00^{l}	17.20^{a}	14.00^{f}	10.70^{l}
LSD at 5%	-	1.253	0.894	-	0.148	0.190
Season 2017						
Coating with sodium alginate 3%	136.00^{a}	123.00^{d}	110.00^{h}	17.40^{a}	16.10d^{e}	12.20^{i}
Coating with sodium alginate 3%+packed in (EPE) foam net	136.00^{a}	124.00^{d}	115.00^{g}	17.40^{a}	16.40c^{d}	12.50^{i}
Coating with semperfresh™ 1%	136.00^{a}	127.00^{c}	118.00^{f}	17.40^{a}	16.60^{c}	12.90^{h}
Coating with semperfresh™ 1%+packed in (EPE) foam net	136.00^{a}	129.00^{b}	120.00^{e}	17.40^{a}	17.00^{b}	13.30^{g}
Packed in (EPE) foam net	136.00^{a}	118.00^{f}	104.00^{i}	17.40^{a}	15.80^{e}	11.60^{j}
Control	136.00^{a}	111.00^{h}	92.00^{j}	17.40^{a}	14.50^{f}	11.00^{k}
LSD at 5%	-	1.549	1.286	-	0.231	0.230

Means followed by the same letters are not significantly different by Duncan multiple range test at 0.05 levels

moisture permeability of coatings is important factors from the view point of mass transfer rate. On the other hand, respiration causes a weight reduction because of the loss of a carbon atom from the fruit in each cycle. Damage of the plasma membrane is the primary reason for CI in fruit during cold storage as the membrane damage sets off a cascade of secondary reactions including increased respiration, ethylene production and enzymatic browning as well as accumulation of toxic compounds and altered cellular structure[26]. Semperfresh™ coating was found to be more effective in reducing the respiration rates of fruit because of the fact that it is more efficient in restricting the gas exchange between fruit and the atmosphere during storage[27].

Skin hue color (h°): As shown in Table 3, both treatments and storage period significantly affected skin hue color (h°) in both investigated seasons. The data presented that, all applied treatments delayed the development of fruits skin color when compared with the untreated fruits. In control fruit, hue color decreased rapidly during storage indicating a losing green color, either after 15 days of cold storage (107.00 and 111.00 h°) or during marketing after 7 days (87.00 and 92.00 h°). Furthermore, green color in mango fruits decreased with storage period advanced either during cold storage or through marketing. Whereas, the values of green color during shelf life were almost lower than those obtained at cold storage during the both seasons of study. Moreover, semperfresh™ 1% +EPE packing net maintained a higher skin hue color (h°) than all treatments or the control after 15 days of cold storage and 7 days during marketing in both season, because the hue color decreased slowly during all of the storage period. The increment due using these treatment reached about 126.00 and 129.00 h° after 15 days of cold storage during both seasons, respectively. While, after 7 days

Table 4: Effect of coating with sodium alginate, semperfresh™ and packed in (EPE) foam net on TSS (%) and titratable acidity (%) of Naomi mango fruits during cold storage and under market conditions during 2016 and 2017 seasons

	TSS (%)			Titratable acidity (%)		
Treatments	At harvest	15 days at 2°C	7 days at 20°C	At harvest	15 days at 2°C	7 days at 20°C
Season 2016						
Coating with sodium alginate 3%	8.50^{k}	10.50^{h}	13.10^{c}	1.66^{a}	1.25^{d}	0.97^{i}
Coating with sodium alginate 3%+packed in (EPE) foam net	8.50^{k}	10.40^{hi}	13.00^{cd}	1.66^{a}	1.25^{d}	1.00^{h}
Coating with semperfresh™ 1%	8.50^{k}	10.25^{ij}	12.90^{d}	1.66^{a}	1.30^{c}	1.02^{h}
Coating with semperfresh™ 1%+packed in (EPE) foam net	8.50^{k}	10.10^{j}	12.60^{e}	1.66^{a}	1.34^{b}	1.09^{g}
Packed in (EPE) foam net	8.50^{k}	10.80^{g}	13.40^{b}	1.66^{a}	1.20^{e}	0.90^{j}
Control	8.50^{k}	11.40^{f}	14.40^{a}	1.66^{a}	1.15^{f}	0.87^{k}
LSD at 5%	-	0.233	0.152	-	0.036	0.024
Season 2017						
Coating with sodium alginate 3%	8.40^{k}	10.70^{h}	12.90^{bc}	1.72^{a}	1.29^{e}	1.03^{k}
Coating with sodium alginate 3%+packed in (EPE) foam net	8.40^{k}	10.50^{i}	12.80^{cd}	1.72^{a}	1.31^{d}	1.05^{j}
Coating with semperfresh™ 1%	8.40^{k}	10.30^{j}	12.70^{d}	1.72^{a}	1.35^{c}	1.09^{i}
Coating with semperfresh™ 1%+packed in (EPE) foam net	8.40^{k}	10.20^{j}	12.50^{e}	1.72^{a}	1.40^{b}	1.16^{h}
Packed in (EPE) foam net	8.40^{k}	11.00^{g}	13.00^{b}	1.72^{a}	1.22^{f}	0.92^{l}
Control	8.40^{K}	12.00^{f}	14.00^{a}	1.72^{a}	1.18^{g}	0.90^{m}
LSD at 5%	-	0.230	0.136	-	0.025	0.017

Means followed by the same letters are not significantly different by Duncan multiple range test at 0.05 levels

during marketing the values averaged 115.00 and 120.00 h° in both seasons, respectively.

The color changes in plums were delayed by alginate edible coating, suggesting a delay in the maturation and ripening of the fruits and suppression of the metabolic activities[28].

Fruit firmness (lb inch^{-2}): As shown in Table 3, firmness was significantly affected by storage period and postharvest treatments investigated in both seasons. Regardless of storage period, treatments maintained firmness significantly compared to untreated control fruits. On the other hand and regardless of treatments, significant drops in fruit firmness were recorded as storage time proceeded. After 15 days of cold storage, all investigated treatments recorded values that were significantly different than those recorded at the beginning of storage, in both seasons. In this respect, the highest firmness was obtained at fruits treated with semperfresh™ 1%+EPE packing net (16.90 and 17 lb inch^{-2}) after cold storage at 2°C and through marketing at 20°C (12.89 and 13.30 lb inch^{-2}) in the 2 seasons, respectively. Low temperature conditioning treatments reduce external chilling injury and tissue breakdown[29].

Changes in mango texture during ripening have been previously attributed to the degradation of pectic compounds by pectic enzymes, which activity significantly increases as the fruit ripens[30]. Increase softness of Naomi mango stored at 2°C might have resulted from the water soaking of the tissues that often occurs when the fruits are exposed to chilling temperatures. Some previous studies have also reported similar results of delaying fruit softening by semperfresh™ coatings. These effects of edible coatings on fruit firmness are primarily due to the fact that coatings act as an extra layer on the fruit surface, which coats the stomata and pores, leading to a decrease in transpiration and thereby the moisture loss, which is ultimately responsible for maintaining the firmness of the fruits.

Moreover, retention of firmness by semperfresh™ coating can explained by retarded degradation of insoluble protopectins to the more soluble pectic acid and pectin. During fruit ripening, depolymerization or shortening of chain length of pectin substances occurs with an increase in pectin esterase and polygalacturonase activities. Low oxygen and high carbon dioxide concentrations as provided by application of coatings reduce the activities of these enzymes and allow retention of the firmness of fruits during storage[27].

Total soluble solid (TSS %): As shown in Table 4, both, treatments and storage periods had a significant effect on TSS (%) in cold stored mango fruits. In both seasons and regardless of treatments, significant increases in TSS (%) have been recorded along the periods of storage. TSS concentration was lower at all treatments used than the control. It was also noticed that TSS% increased as storage proceeded, for each treatment in both investigated seasons. Increased TSS could be due to increased activity of enzymes responsible for starch hydrolysis to soluble sugars and can be caused by the decline in the amount of carbohydrates, pectin, partial hydrolysis of protein and decomposition of glycosides into subunits during respiration[29].

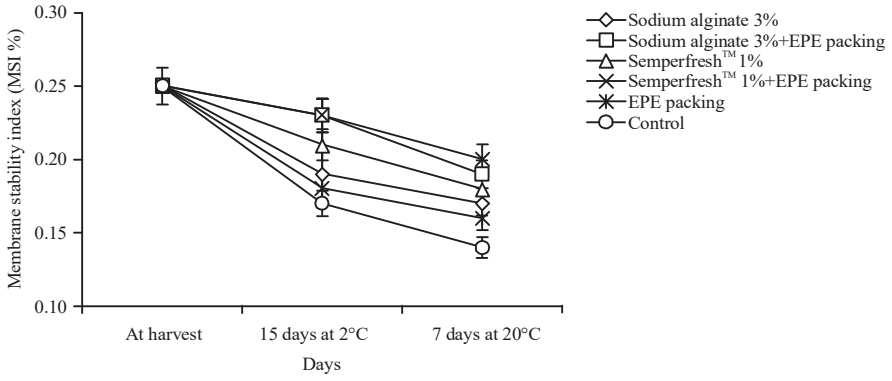

Fig. 3: Membrane stability index (MSI %) in Naomi mango at harvest, after 15 days at 2°C and after 7 days at 20°C

TSS % was higher for control fruits than all treatments either after 15 days at cold storage at 2°C (11.40 and 12.00%) or after 7 days of marketing at 20°C (14.40 and 14.00%) in both seasons, respectively. The lowest significant TSS% was recorded for mango fruits coated with semperfresh™ 1%+packed in (EPE) foam net (10.10 and 10.20%) after cold storage at 2°C and after 7 days through marketing at 20°C (12.60 and 12.50 %) in the 2 seasons, respectively.

Mango fruit stored at sub-optimal temperature, starch is degraded to soluble sugars. Degradation of starch to mono- and di-saccharides such as sucrose, fructose and glucose increases the osmolarity and these compounds act as cryo-protectants to reduce the freezing point. Indeed, mango fruit that is harvested during the late season have increased TSS (osmolarity) and can be stored at lower temperatures[3].

Semperfresh™ attained higher total soluble solids in fruit coated. This increasing trend of TSS with advancement of storage might be due to hydrolysis of starch to simple sugars[26].

Titratable acidity (TA): Results in Table 4 showed that TA % in cold stored mango fruits was significantly affected in response to extended cold storage periods and investigated postharvest treatments. Regardless of applied treatment, significant declines in TA have been recorded throughout this study from cold storage period to marketing. On the other hand and regardless of storage period, investigated treatments had a significant effect on this character in both investigated seasons. Control treatment led to significant increment in TA (%) compared with all treated in both seasons either after cold storage at 2°C (1.15 and 1.18%) or after 7 days through marketing at 20°C (0.87 and 0.90%) in the 2 seasons, respectively.

Contrarily, treated mango with semperfresh™ 1%+packed in (EPE) foam net led to delay the decrease of TA% (1.34 and 1.40%) after cold storage at 2°C and after 7 days through marketing at 20°C (1.09 and 1.16%) in the 2 seasons, respectively.

This decrease might be due to the reduction of organic acids due to their consumption or conversion to sugars during respiratory metabolism[31]. In addition[26], semperfresh™ and alginate[28] treatments were able to retain significantly higher titratable acidity in fruits.

Membrane stability index (MSI %): Another index of cell injury is ion leakage, which is a commonly used technique to assess cell damage or viability. Membrane stability index (MSI) of peel decreased during storage and showed lower values than initial (Fig. 3). All treatments used significantly delayed the decrease of MSI % than the control. Moreover, membrane stability index was higher at fruits coated with semperfresh™ 1% +EPE packing and fruits coated with sodium alginate at 3% ranged 0.23 and 0.22% after 15 days of cold storage at 2°C while, ranged 0.20 and 0.19% after 7 days through marketing at 20°C during both seasons, respectively. The maintenance of higher membrane stability index by these treatments might be attributed to the antioxidant actions[27] of semperfresh™ and sodium alginate[28].

Vitamin C (mg/100 g FW): Vitamin C concentration was higher for all treatments than control as showed in (Table 5) either after 15 days of cold storage or 7 days through marketing. The amount of ascorbic acid decreased continuously with storage time. At the end of storage, vitamin C content at control treatments declined by 53.50% compared with harvest day. The contents of vitamin C in control treatments declined to 30.00 and 30.60 mg g^{-1} after cold storage at 2°C and ranged 21.40 and 21.70 mg g^{-1} after 7 days through marketing at 20°C during both seasons, respectively.

All treatments used significantly delayed the decrease of vitamin C than the control. The higher amounts of vitamin C

Table 5: Effect of coating with sodium alginate, semperfresh™ and packed in (EPE) foam net on vitamin C (mg g^{-1} FW) and total phenolic contents (mg g^{-1} FW) of Naomi mango fruits during cold storage and under market conditions during 2016 and 2017 seasons

	Vitamin C (mg g^{-1} FW)			Total phenolic contents (mg g^{-1} FW)		
Treatments	At harvest	15 days at 2°C	7 days at 20°C	At harvest	15 days at 2°C	7 days at 20°C
Season 2016						
Coating with sodium alginate 3%	40.00[a]	37.20[e]	27.00[k]	22.80[a]	19.90[d]	19.00[h]
Coating with sodium alginate 3%+packed in (EPE) foam net	40.00[a]	37.70[d]	27.70[j]	22.80[a]	20.00[d]	19.30[g]
Coating with semperfresh™ 1%	40.00[a]	38.20[c]	28.00[i]	22.80[a]	20.45[c]	19.70[e]
Coating with semperfresh™ 1%+packed in (EPE) foam net	40.00[a]	39.00[b]	29.80[h]	22.80[a]	20.80[b]	19.90[d]
Packed in (EPE) foam net	40.00[a]	32.40[f]	24.60[l]	22.80[a]	19.60[f]	18.00[i]
Control	40.00[a]	30.00[g]	21.40[m]	22.80[a]	19.00[h]	17.60[j]
LSD at 5%	-	0.199	0.187	-	0.130	0.051
Season 2017						
Coating with sodium alginate 3%	42.00[a]	37.70[e]	27.80[k]	23.00[a]	20.10[e]	19.40[j]
Coating with sodium alginate 3%+packed in (EPE) foam net	42.00[a]	38.00[d]	27.90[j]	23.00[a]	20.70[d]	19.60[i]
Coating with semperfresh™ 1%	42.00[a]	38.70[c]	28.70[i]	23.00[a]	20.80[c]	19.90[g]
Coating with semperfresh™ 1%+packed in (EPE) foam net	42.00[a]	39.70[b]	30.00[h]	23.00[a]	21.00[b]	20.00[f]
Packed in (EPE) foam net	42.00[a]	32.50[f]	24.90[l]	23.00[a]	19.90[g]	18.90[k]
Control	42.00[a]	30.60[g]	21.70[m]	23.00[a]	19.70[h]	17.90[l]
LSD at 5%	-	0.145	0.148	-	0.060	0.037

Means followed by the same letters are not significantly different by Duncan multiple range test at 0.05 levels

contents were obtained in semperfresh™ 1% +EPE packing treated fruits during the entire storage period. The contents of vitamin C in this treatment were 39.00 and 39.70 after cold storage at 2°C and were 29.80 and 30.00 after 7 days through marketing at 20°C during both seasons, respectively.

In addition, semperfresh™ and sodium alginate treatments retained higher vitamin C and delayed fruit ripening as reflected by a higher firmness and a lower TSS than other treatment. Vitamin C in fruits is primarily regulated by the phenol oxidase and ascorbic acid oxidase enzymes whose activities are influenced by the storage temperature and the oxygen content in the storage condition. This inhibition of vitamin C loss in treated fruits may be due to the lower oxygen permeability of the edible coatings with alginate which might have lowered the activity of the these enzymes and retard the oxidation of ascorbic acid[28]. Semperfresh™ coatings were effective in reducing the ascorbic acid loss for both ambient (20°C) at 70-75% relative humidity and cold storage (0°C) at 90-95% relative humidity conditions. The reduction of ascorbic acid loss in coated fruits was due to the low oxygen permeability of sucrose polyester coating which lowered the activity of the enzymes and prevented oxidation of ascorbic acid. The effect of low temperature significantly reduced the ascorbic acid loss. This showed the effect of temperature on the activities of the related enzymes. Ascorbic acid is lost due to the activities of phenol oxidase and ascorbic acid oxidase enzymes during storage[29].

Total phenolic contents: The interaction effects between treatment and storage period on total phenols revealed that, all treatments used significantly delayed the decrease in total phenolic content of Naomi mango during storage (Table 5). Just after harvest initial total phenol content was observed at 22.80 and 23.00 mg g^{-1} fresh weight during both seasons, respectively. The data showed the relationship between treatments was observed during the entire storage period and partially induced accumulation of phenol compounds to record maximum significant values in fruits treated with semperfresh™ 1%+EPE packing. The contents of total phenolic compounds in this treatment reached 20.80 and 21.00 mg g^{-1} after cold storage at 2°C and were 19.90 and 20.00 mg g^{-1} after 7 days through marketing at 20°C during both seasons, respectively. Moreover, control treatment induced the lower phenol content reaching 19.00 and 19.70 mg g^{-1} after cold storage at 2°C and was 17.60 and 17.90 mg g^{-1} after 7 days through marketing at 20°C during both seasons, respectively. Levels of phenolic compounds in mango fruit gradually decreased during the storage and this was inhibited by cold-shock treatment. The content of phenolic compounds in the fruit treated with cold-shock was 66% higher than that in control fruit on day 12 of storage[12]. The decrease of phenols concentration during storage might be due to breakdown of cell structure because of the senescence phenomena during ripening and the action of polyphenol oxidase.

Phenolic synthesis is affected in general by different biotic and abiotic stress including chilling. Under low temperature stress, the increase synthesis of phenolic compounds is a response of plants to overcome. Chilling injury through synthesizing poly phenolic phytotoxins, by an increase of the activity of phenylalanine ammonia lyase added to low level of

Table 6: Effect of coating with sodium alginate, semperfresh™ and packed in (EPE) foam net on flavonoids content (mg g^{-1}) and antioxidant capacity (%) of Naomi mango fruits during cold storage and under market conditions during 2016 and 2017 seasons

	Flavonoids content (mg g^{-1} FW)			Antioxidant capacity (DPPH IC_{50} values)		
Treatments	At harvest	15 days at 2°C	7 days at 20°C	At harvest	15 days at 2°C	7 days at 20°C
Season 2016						
Coating with sodium alginate 3%	1.80^{a}	1.57^{e}	1.17^{j}	21.70^{a}	13.00^{e}	8.10^{j}
Coating with sodium alginate 3%+packed in (EPE) foam net	1.80^{a}	1.65^{d}	1.20^{i}	21.70^{a}	13.90^{d}	7.90^{k}
Coating with semperfresh™ 1%	1.80^{a}	1.70^{c}	1.28^{h}	21.70^{a}	12.30^{g}	7.20^{l}
Coating with semperfresh™ 1%+packed in (EPE) foam net	1.80^{a}	1.75^{b}	1.32^{g}	21.70^{a}	12.40^{f}	7.10^{m}
Packed in (EPE) foam net	1.80^{a}	1.40^{f}	1.12^{k}	21.70^{a}	19.00^{c}	10.00^{i}
Control	1.80^{a}	1.34^{g}	1.03^{l}	21.70^{a}	19.40^{b}	11.80^{h}
LSD at 5%	-	0.038	0.021	-	0.102	0.089
Season 2017						
Coating with sodium alginate 3%	1.89^{a}	1.64^{e}	1.27^{j}	22.00^{a}	13.20^{e}	8.30^{j}
Coating with sodium alginate 3%+packed in (EPE) foam net	1.89^{a}	1.75^{d}	1.30^{i}	22.00^{a}	14.00^{d}	8.00^{k}
Coating with semperfresh™ 1%	1.89^{a}	1.80^{c}	1.35^{h}	22.00^{a}	12.70^{f}	7.40^{l}
Coating with semperfresh™ 1%+packed in (EPE) foam net	1.89^{a}	1.83^{b}	1.40^{g}	22.00^{a}	12.50^{g}	7.20^{m}
Packed in (EPE) foam net	1.89^{a}	1.47^{f}	1.22^{k}	22.00^{a}	19.30^{c}	10.20^{i}
Control	1.89^{a}	1.39^{g}	1.09^{l}	22.00^{a}	19.80^{b}	11.90^{h}
LSD at 5%	-	0.024	0.017	-	0.131	0.099

Means followed by the same letters are not significantly different by Duncan multiple range test at 0.05 levels

poly phenol oxidase activity as a trial to reduce the oxidation of phenolic substrates to quinones[32].

Total flavonoids content: Estimated values of total flavonoid content (TFC) were given in Table 6 which revealed that, all treatments applied produced higher insignificant values of TFC in the peel than the control fruits during both seasons. Since, coated fruits with semperfresh™ retarded the decline of TPC during cold storage or marketing. The highest content of total flavonoids content in fruits coated with semperfresh™ 1%+EPE packing fruits ranged 1.75 and 1.83 mg (QE) g^{-1} FW after cold storage at 2°C and were 1.32 and 1.40 mg (QE) g^{-1} FW after 7 days through marketing at 20°C during both seasons, respectively.

Whereas the lowest values were obtained in control fruits ranged 1.34 and 1.39 mg (QE) g^{-1} FW after cold storage at 2°C and were 1.03 and 1.09 mg (QE) g^{-1} FW after 7 days through marketing at 20°C during both seasons, respectively. In this respect, phenolic are responsible for the high antioxidant capacity. It has been observed that flavonoid contents correlate with the reduction of deteriorative reactions[33]. The higher flavonoid content present in 'Ataulfo' mangoes could be associated with their long shelf life as it has been reported in other important products[34].

Antioxidant capacity (%):

The initial antioxidant capacity of fruit peel extract measured by the DPPH method (IC_{50} values) ranged 21.70 and 22.00 µg phenolic concentration during both seasons, respectively (Table 6). It was lower rate (higher IC_{50} values) for control fruits after cold storage (19.40 and 19.80 µg). The high rate (lower IC_{50} values) of antioxidant capacity were noticed by coating with semperfresh™+EPE packing (12.40 and 12.50 µg) after cold storage while, after 7 days (7.10 and 7.20 µg) during both seasons, respectively.

During ripening, the total antioxidant activity increases and this increase are mainly due to change into the lipophilic antioxidant activity. The maintenance of higher membrane stability index by semperfresh™ and sodium alginate treatments compared to control might be attributed to the antioxidant actions of these compounds. These results might be also attributed to the concept that the edible coatings are selective barriers to O_2 and CO_2 modifying internal atmospheres and decreased respiration rate and fruit ripening[35].

The increase in the antioxidant capacity (lower IC_{50} values) during storage confirm those of Kondo *et al.*[36] where DPPH-radical scavenging activity (IC_{50} values) of mangoes peel increased during 10 days storage at 6 and 12°C. Fernando *et al.*[37] reported that total antioxidant activities (mmol TE/100 g FW) measured by DPPH and FRAP of 'Hom Thong' and 'Khai' bananas flesh during ripening at 25°C for 10 days increased with ripening but rapidly decreased with senescence. However, the decrease in total phenols concentration with the increase in antioxidant capacity during ripening might suggest qualitative changes in phenolic classes toward higher antioxidant potential. Accordingly, excessive reactive oxygen species production could participate in the oxidation of lipids and proteins of cell membrane that are involved in mango ripening. Indeed a steady decrease in

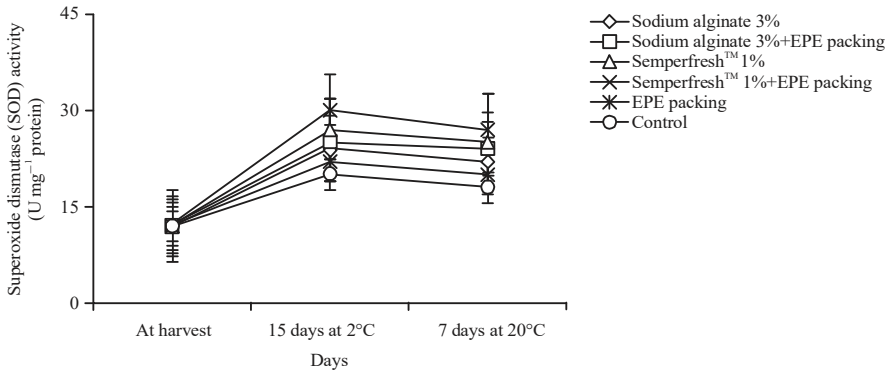

Fig. 4: Superoxide dismutase (SOD) activity (U mg^{-1} protein) in Naomi mango at harvest, after 15 days at 2°C and after 7 days at 20°C

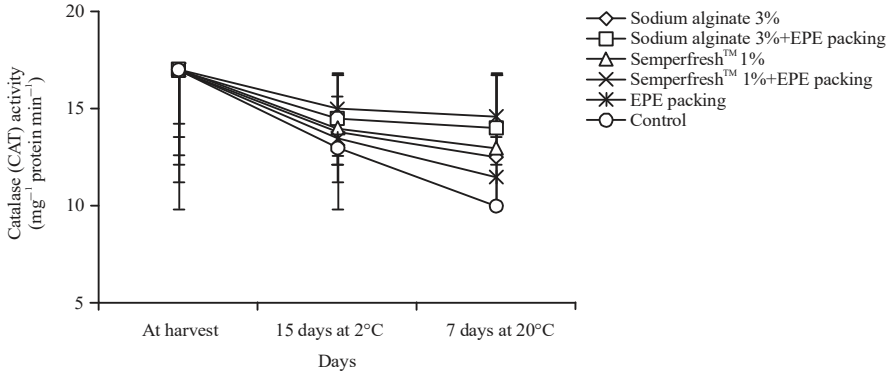

Fig. 5: Catalase (CAT) activity (μmol H_2O_2 decomposed mg^{-1} protein min^{-1}) in Naomi mango at harvest, after 15 days at 2°C and after 7 days at 20°C as a mean of 2016-2017 seasons

membrane stability index as measured by the leakage of ions, was observed upon the progression of fruit ripening, indicates a gradual loss of membrane's stability due to changes occurring in the biochemical and biophysical properties of cell membranes.

Effect on activities of enzymes (SOD, CAT and APX): The activities of SOD, CAT and APX increased and reached the highest peak on 15 days of cold storage and then decreased gradually during marketing.

From Fig. 4 showed the variation of the superoxide dismutase activity (SOD activity U mg^{-1} protein) as a function of storage time (days) in the peel of Naomi mango during cold storage at 2°C). Clearly, the maximum of SOD activity is thereafter decreases gradually till the end of storage period.

CAT activity Fig. 5 showed the variation of catalase (CAT) activity (μmol H_2O_2 decomposed mg^{-1} protein min^{-1})as a function of storage time (days) in the peel of Naomi mango. Generally, the CAT activity increased at the time of harvest with increasing maturity stage. After 15 days of cold storage, significant reduction in CAT activity was associated with control fruits (13.00). In addition, semperfresh™ 1%+EPE packing induced great enhancement in CAT activity of treated fruits (15) after cold storage at 2°C and (14.00) after 7 days through marketing at 20°C. However, CAT value was significantly higher with little difference in fruits treated with sodium alginate +EPE packing (14.50) after cold storage at 2°C and (14) after 7 days through marketing at 20°C.

APX activity Fig. 6 displays the variation of APX activity (μmolAsA decomposed mg^{-1} protein min^{-1}) as function of storage time in the peel of Naomi mango. In fact, the APX activity was increased with increasing maturity stage. APX increases during storage to a maximum activity after 15 days of storage.

It has also been thought that chilling temperature during storage induces oxidative stress through accumulation of reactive oxygen species (ROS), which results in membrane damage and consequently CI in fruit[38]. The ROS can be scavenged by both non enzymatic antioxidants and by an enzymatic system comprising enzymes, such as SOD, CAT and APX[39]. The application of semperfresh™ and sodium alginate

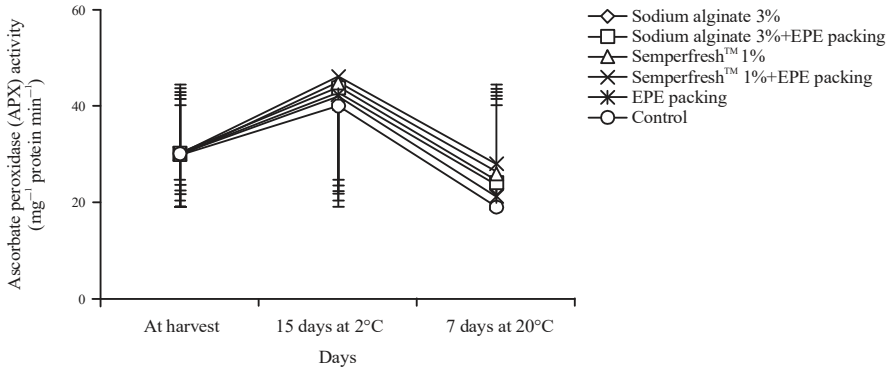

Fig. 6: Ascorbate peroxidase (APX) activity (μmol AsA decomposed mg^{-1} protein min^{-1}) in Naomi mango at harvest, after 15 days at 2°C and after 7 days at 20°C

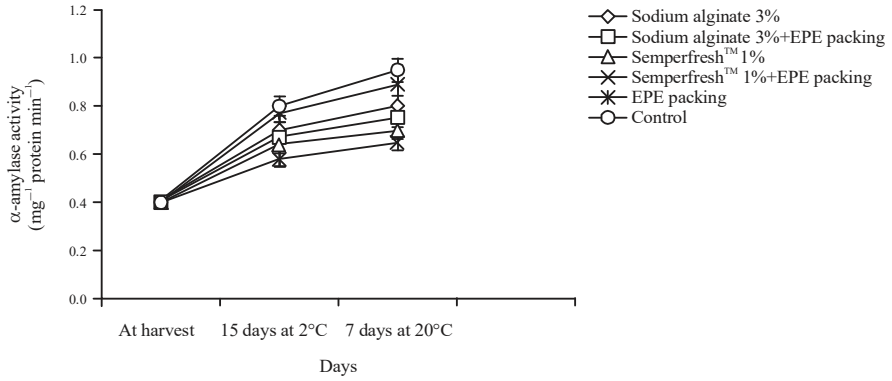

Fig. 7: α-amylase activity (μmol AsA decomposed mg^{-1} protein min^{-1}) in Naomi mango at harvest, after 15 days at 2°C and after 7 days at 20°C

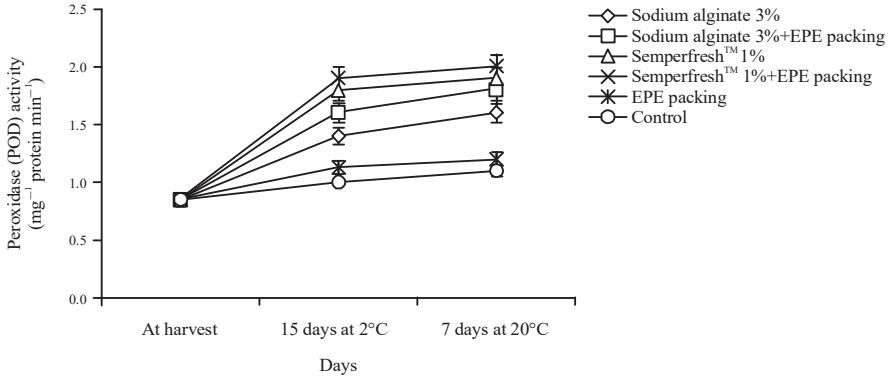

Fig. 8: Peroxidase (POD) activity (μmol AsA decomposed mg^{-1} protein min^{-1}) in Naomi mango at harvest, after 15 days at 2°C and after 7 days at 20°C

alleviated CI in fruit during cold storage and such treatments are associated with increases in those enzymatic activities that reduce ROS accumulation with accompanying lower levels of H_2O_2 and $O_2^{-}\cdot$, which could protect membranes from oxidative stress and subsequently contribute to decreased CI development during storage at low temperature.

α-amylase activity: Data in Fig. 7 showed that, α-amylase activity was lower at all treatments than control. The α-amylase activity increased during cold storage and showed higher values than initial. The significant interaction effects between treatment and storage period on α-amylase activity revealed that, after 7 days of shelf life at 20°C, all the

treatments decreased α-amylase activity compared to control. In addition, semperfresh™ 1%+EPE packing showed the lowest α-amylase activity compared with other treatments.

In the current study, α-amylase activity of fruit peel significantly increased during storage and decreased by the applied treatments compared to control, suggesting a role of this enzyme in fruit ripening. In confirmation, the climacteric rising in Tommy Atkins mangoes associated with a remarkable increase in amylase activity, reducing and non-reducing sugars contents and decrease in the starch content of fruit pulp[40].

Peroxidase activity increased during storage and showed higher value than initial (Fig. 8). Moreover, peroxidase activity was higher for all applied treatments than control. Our results showed that peroxidase activity was higher at semperfresh™ 1%+EPE packing than other treatments including control. POD is considered as an important antioxidant and defense-related enzymes. Fruit ripening and senescence is possibly an oxidative process in which the transition from mature stage into ripening to senescence stage is accompanied by a progressive shift toward an oxidative state[41].

CONCLUSION

Cold storage is the best known technique to extend postharvest fruit life. However, chilling injury limits the application of cold treatment. In conclusion, semperfresh™ and sodium alginate might enhance CI resistance in Naomi mango fruit by maintaining membrane integrity associated with enhanced antioxidant activity and regulation of energy metabolism. Application of semperfresh™ 1% plus EPE packing to mango fruits before low temperature storage be beneficial in controlling postharvest chilling injury.

SIGNIFICANT STATEMENT

"This study discovered the potentially enable of cold-shock treatment and edible coating with sodium alginate, semperfresh™ and EPE foam net packing that can be beneficial for enhancing the resistance of Naomi mango fruits during cold export. This study will help the researchers to uncover the critical areas of maintaining the quality of mango fruits which potentially enable the export to all quarantine-enforcing countries that many researchers were not able to explore. Thus a new theory of cold-shock treatment and integrated of edible coating with packing in (EPE) foam net that are beginning to be adopted on a commercial scale which may be arrived at retain cold-quarantine of mango fruits without any adverse effect on quality parameters."

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