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THE EFFECT OF NATURAL ELICITORS AND COLD STORAGE PERIOD ON QUALITY IMPROVEMENT OF UAE DATE PALM FRUITS (Phoenix dactylifera, CV. BARHI)

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This thesis is submitted in partial fulfilment of the requirements for the degree of Master of Science in Horticulture

Under the Supervision of Dr. Zienab Ahmed

November 2021

Declaration of Original Work

I, Fatima Yaaqoub Yousef Al Shaibani, the undersigned, a graduate student at the United Arab Emirates University (UAEU), and the author of this thesis entitled "*The Effect of Natural Elicitors and Cold Storage Period on Quality Improvement of UAE Date Palm Fruits* (*Phoenix dactylifera* L., cv. Barhi)**"**, hereby, solemnly declare that this thesis is my original research work that has been done and prepared by me under the supervision of Dr. Zainab Ahmed, in the College of Agriculture and Veterinary Medicine at UAEU. This work has not previously been presented or published nor formed the basis for the award of any academic degree, diploma or a similar title at this or any other university. Any materials borrowed from other sources (whether published or unpublished) and relied upon or included in my dissertation have been appropriately cited and acknowledged by appropriate academic conventions. I further declare that there is no potential conflict of interest concerning the research, data collection, authorship, presentation, and publication of this thesis.

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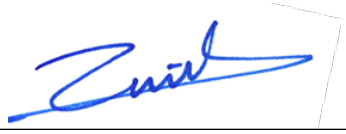
   Signature  Date 08/12/2021

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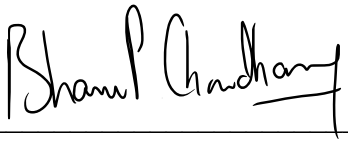
Signature  Date 04/01/2022

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Abstract

Date palm is an agricultural crop of economic importance in most Arab countries. Among the different varieties of date palms grown in the United Arab Emirates, the Barhi variety is extremely popular and is often consumed at the bisr stage (first edible stage). However, the major challenge for this fruit at bisr stage is to maintain its quality over a long period after harvest and during marketing. The objective of this study was to evaluate the synergistic effect of preharvest spraying with a natural elicitor, namely chitosan (Ch) 1%, alone and in combination with salicylic acid (SA) 2 mM and calcium chloride (Ca) 3%, on the quality parameters, shelf life and bioactive compound content of date fruit of cultivar 'Barhi' at harvest and during two months of cold storage. The results showed that all treatments significantly delayed the ripening and decay of date 'Barhi' compared to the control. Treatment Ch followed by Ch+ SA and Ch+ SA +Ca had the least weight loss. Treatments Ch + Ca, Ch + SA + Ca and Ch+ SA had significantly lower levels of total soluble solids compared to control fruits (TSS). The treatments Ch + Ca and Ch + Ca + SA did not exhibit rotten fruit after 60 days of cold storage. At the end of the storage period, the Ca treatment, followed by Ch + Ca + SA, had the highest levels of total phenols (TPC), flavonoids (TFC), and tannins (TC). Ch + SA + Ca, Ch + SA and Ch had significantly higher antioxidant and antimicrobial activities compared to the control. Based on these results, these treatments can be recommended to extend the shelf life of 'Barhi' date fruit. Our results suggest that the use of elicitor combinations to extend the shelf life of date fruit during cold storage is a promising strategy to maintain fruit quality and reduce postharvest pathogen damage.

Keywords: Barhi Date, Bisr Stage, Preharvest Protection, Natural Elicitor, Fruit Spoilage, Cold Storage, Long-term Quality, United Arab Emirates.

Title and Abstract (in Arabic)

تأثير بعض المحفزات الطبيعية والتخزين المبرد على تحسين جودة ثمار التمور (صنف البرحى) في دولة الإمارات العربية المتحدة

الملخص

يعتبر نخيل التمر من المحاصيل الزراعية ذات الأهمية الاقتصادية في معظم الدول العربية. من بين أصناف التمور المختلفة في دولة الإمارات، فإن صنف البرحى يحظى بشعبية كبيرة وغالبًا ما يتم استهلاكه في مرحلة البسر (المرحلة الأولى الصالحة للأكل). ومع ذلك، فإن التحدي الأكبر لهذه الفاكهة في مرحلة البسر هو الحفاظ على جودتها لفترة طويلة بعد الحصاد وأثناء التسويق. هدفت هذه الدراسة إلى تقييم التأثيرات الفسيوكيميائية والميكروبية التفاعلية للرش للثمار المحصودة في مرحلة البسر والمخزنة لمدة شهرين على درجة حرارة 2درجة مئوية ورطوبة 92-95% باستخدام مادة طبيعية محفزة كيتوسان بتركيز (1%) منفردا؛ ومع إضافة 2مل من حامض سايساك مع كلوريد الكالسيوم بتركيز (3%). وأظهرت النتائج أن جميع التفاعلات كان لها تأثير إيجابي في إنضاج تمور البرحى وتقليل الفاقد مقارنة بالمجموعة تحت التحكم. كان علاج كيتوسان متبوعًا بـ كيتوسان + سايساك، وكلوريد الكالسيوم + سايساك + كيتوسان قلل الخسارة في الوزن. كانت المعالجات وكلوريد الكالسيوم + كيتوسان، وكلوريد الكالسيوم + سايساك + كيتوسان، وسايساك + كيتوسان تحتوي على مستويات أقل بكثير من إجمالي المواد الصلبة الذائبة مقارنة بالفواكه الضابطة. لم تظهر المعاملتان كلوريد الكالسيوم + كيتوسان، وكلوريد الكالسيوم + سايساك + كيتوسان فواكه فاسدة بعد 60 يومًا من التخزين البارد، كان لمعالجة كلوريد الكالسيوم، متبوعًا بـمزيج كلوريد الكالسيوم + سايساك + كيتوسان، أعلى مستويات الفينولات الكلية، الفلافونويد والتانين. كان لدى وكلوريد الكالسيوم + سايساك + كيتوسان، وكيتوسان + سايساك وكيتوسان أنشطة مضادات الأكسدة ومضادات الميكروبات أعلى بشكل ملحوظ مقارنةً بالمجموعة تحت التحكم. بناءً على هذه النتائج، يمكن التوصية بهذه العلاجات لإطالة العمر الافتراضي لثمرة التمر "البرحى". أيضا، تشير نتائجنا إلى أن استخدام تركيبات المحفزات الطبيعية لإطالة العمر الافتراضي لفاكهة التمر أثناء التخزين البارد هي استراتيجية واعدة للحفاظ على جودة الفاكهة وتقليل أضرار مسببات الأمراض بعد الحصاد.

مفاهيم البحث الرئيسية: تمور البرحى، مرحلة البسر، حماية ما قبل الحصاد، المحفزات الطبيعية، تلف الفاكهة، التخزين البارد، الجودة طويلة الأمد، الإمارات العربية المتحدة.

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Dedication

To my beloved parents and family

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Chapter 1: Introduction

1.1 An Overview

Date palm (*Phoenix dactylifera* L.), the tree of life is an extremely important subsistence crop in most of the Arabian countries. Date palm tree is included in *Arecaceae* family (Angiosperms, monocotyledon) consisting of about more than 2,500 species and 200 genera. One of the genera is *Phoenix* (*Coryphoideae phoeniceae*) which is native to the tropical or subtropical regions of southern Asia or Africa. Among the 14 species of *Phoenix*, the prominent one is *Phoenix dactylifera* L (Siddiq et al., 2013; Eoin, 2016). Many fruit crops are unable to survive in the drastic environmental conditions of hot arid regions while the date palms grow well with most production concentrated in the Middle-East (Al-Shahib & Marshall, 2003).

Beyond the arid climates, date palm can also be grown in many other countries as an ornamental plant, including the continents of Americas, southern Europe, Asia, Africa, and Oceania (Dransfield et al., 2005). Date palm trees at an average age of 5 years produces 400–600 kg date fruit /tree/year and the fruiting continue up to 60 years. Date fruit is a one-seeded berry with a fleshy pericarp and terminal stigma. A membranous endocarp is present in between the seed and the flesh. The consistency of the fruit varies from soft to dry and the colour varies from yellow to black. The seed is ventrally grooved with a hard endosperm made of a cellulose deposit and usually oblong with a small embryo.

Depending on environmental conditions, field management and cultivars there are wide variations in characteristics of fruit and seed (Zaid & de Wet, 2002; Al-Yahyai & Kharusi, 2012). Whereas the UN Food and Agriculture Organization (FAO)

estimates that over 100 million date palm trees scatter on ca. 1.3 million hectare worldwide (FAO, 2018). Egypt, Iran, Algeria, Saudi Arabia, Iraq, Pakistan, Sudan, Oman, UAE, and Tunisia are the top date-producing countries as shown in Figure 1.

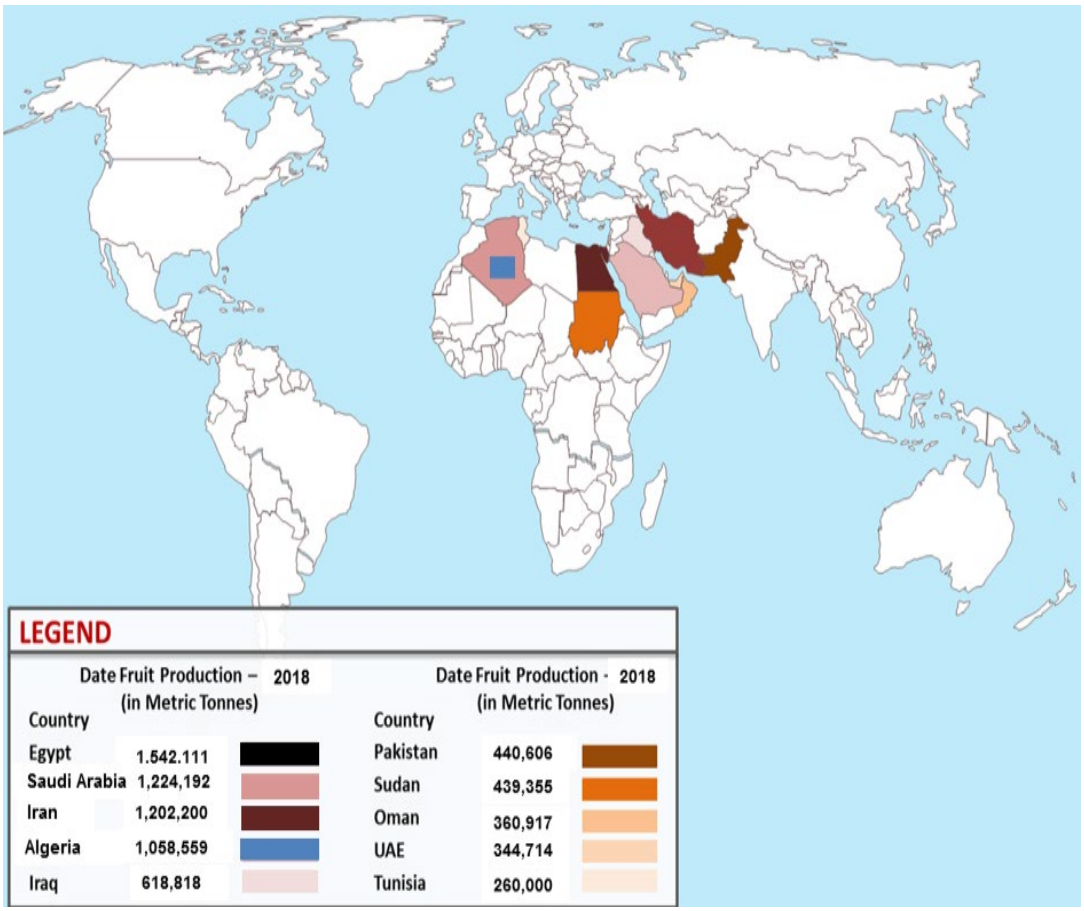


Figure 1: World map of the top ten date fruit producing countries in 2018
(Source: FAO, 2018; Al-Alawi et al., 2017)

In the UAE, date palm is a very remarkable and precious tree, which has strong religious, traditional, and nutritional significance to the local community. In the last two decades, date palm cultivation in the UAE has experienced remarkable growth, with over 250 varieties of crop being farmed in various part of the nation, especially in Abu Dhabi emirates, where Al Ain oasis Liwa are famous producing areas. The total growth rate of date fruit is paralleled by a high consumption rate in the UAE, with a

per capita daily intake of 10 to 200 g in Abu Dhabi Emirate alone (Habib & Ibrahim, 2011).

As a climacteric fruit, the time from ripening to senescence stage in “Barhi” is truly short and ultimately leads to marketability loss. Therefore, slowing down the ripening process and retarding fruit senescence is critical for enhancing the marketability and storage life. To the best of our knowledge, there are no reports on the synergistic effects of different natural elicitors on *Barhi* fruit particularly as preharvest spray treatments.

1.2 Dates Palm Fruit

Several physical and chemical changes are involved in the growth and development of date palm fruit. The five stages in the development of date fruit that are internationally denominated by Arabic terms such *Hababouk*, *Kimri* (also known as *Khimri* or *Jimri*), *Khalal* (also known as *Balah* or *Bisr*), *Rutab* and *Tamr* (or *Tamar*) (Fayadh & Al-Showiman, 1990; Kader & Yahia, 2011).

After fertilization, the *Hababouk* stage starts, and the colour of the fruit is creamy to light green. The *Hababouk* stage is characterized by the loss of two unfertilized carpels. Followed by the *Hababouk* stage is the *Kimri*, green stage which lasts about 9 weeks depending on cultivar and location. At *Kimri* stage the size of the fruit is big with high water content. The third stage is *Khalal* (*Bisir*), it lasts about 4 to 5 weeks. The fruit attains physiological maturity with slight decrease in size, weight, and starch content at *Khalal* stage (Al-Mssallem et al., 2013).

Depends on the cultivar, the colour of the fruit changes from green to yellow, pink, or red, or yellow spotted with red. The fruit starts to accumulate reducing sugars and loses weight during the *Rutab* stage. At this stage, the fruit is softer with light brown colour.

The fruit progressively loses water (30–45%), and starch is converted to sugars during the *Khalal* and *Rutab* stages. *Tamer* stage is characterised by highly sweet, dark brown and soft fruit with exceptionally low moisture content and therefore are ideal for long-term storage to be consumed during off season. (Kader & Hussein, 2009). Dates are often consumed as fresh mainly at the ripe stage (*Rutab*). However, in some cultivars, date fruits are consumed at the physiological maturity stage (*Khalal*) as fresh, dried, or in various processed forms. The growth stages of date fruit are shown in Figure 2.

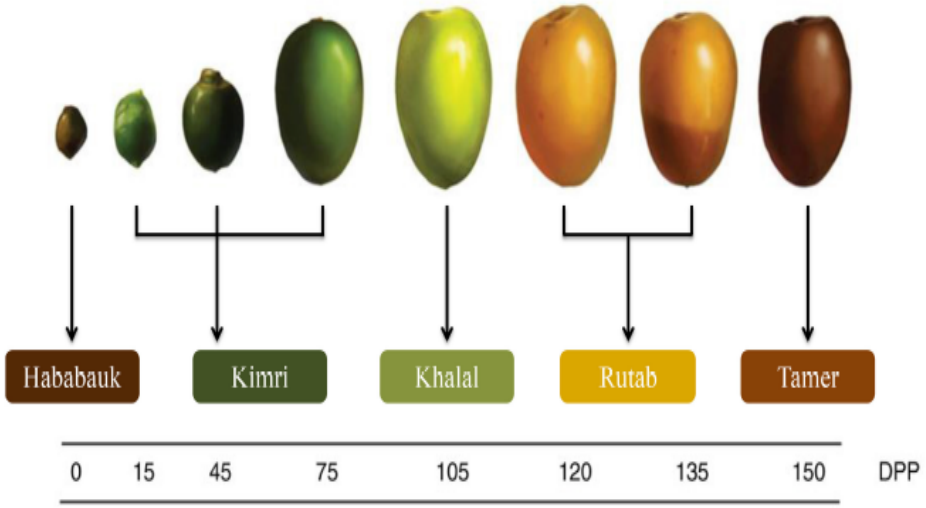


Figure 2: The growth stages of date fruit

(Source: Al-Mssallem et al., 2013)

Date fruit is a rich source of nutrients with high nutritional value. Dates contain a high percentage of carbohydrate (total sugars, 44-88%), protein (2.3-5.6%), fat (0.2-9.3%), essential salts and minerals, vitamins, and an elevated proportion of dietary fiber (6.4-11.5%) (El Hadrami & El Hadrami, 2009). Dates are good source of vitamin B complex, such as thiamine (B_1), riboflavin (B_2), niacin (B_3), pantothenic (B_5), pyridoxine (B_6), and folate (B_9) (Chao & Krueger, 2007; Al-Harrasi et al., 2014). The

nutritional composition may vary, depending on soil type, agronomic practices, cultivar type and fruit maturation stage (Al-Farsi et al., 2007; Amira et al., 2012).

Date flesh is a readily available source of energy due to the presence of high content of reducing sugars, such as 70-85% fresh weight is glucose and fructose (Al-Farsi & Lee, 2008; Rastegar et al., 2012). The presence of non-enzymatic antioxidants (phenolic, flavonoid, and ascorbic acid) and enzymatic antioxidants (catalase, peroxidase, and superoxide dismutase), compounds enhance the free radical scavenging activity and therapeutic effects of dates (Biglari et al., 2009; Mohamed et al., 2014).

Among all the dietary components, polyphenolics account for the broad range of biological effects of dates such as antimutagenic and antioxidant properties (Duthie et al., 2003; Vayalil, 2002). The dietary fibers in dates also modulate the immune system and plays a major role in prevention of cardiovascular diseases (Frankel et al., 1993). Due to the presence of phenolics, dates have anti-viral, anti-bacterial and anti-fungal properties which inhibits chronic inflammations (Kaul & Khanduja, 1998). Studies showed that both the aqueous and ethanolic extracts of dates were effective in improving the severity of gastric ulceration (Al-Qarawi et al., 2003). Hydroxycinnamates and flavonoids including tannins present in dates are famous for their beneficial effects on human health as well as against cancer and cardiovascular diseases (Biglari et al., 2009).

1.3 Date Palm Cultivars

Date palm cultivars are divided into three groups, namely soft, semi-dry and dry depending on the flesh consistency and moisture content at fully-ripen stage (Yahia &

Kader, 2011). During ripening, sucrose is converted into invert or reducing sugars (glucose and fructose) in soft cultivars with a moisture content of >30%. Cultivars such as *Badrayah, Bartamoda, Deglet Beida, Horra, Sakoty*, and *Thoory* are the dry date cultivars with <20% moisture. Cultivars with 20–30% moisture are the semi dry-date cultivars which includes *Amry, Dayri, Deglet Nour, Khalas, Sewy*, and *Zahidi*. In addition to the reducing sugars, both dry and semi-dry dates retain a good amount of sucrose on full ripening (Chao & Krueger, 2007).

Based on fruit characteristics, size and sugar content, other classifications can be found within the same group. The major soft cultivars are *Hillawi, Abada, Amhat, Barhi, Bentaisha, Halawy, Hayani, Honey, Khadrawy*, and *Medjhool*. Date palm cultivars and their characteristics are shown in Table 1.

Table 1: Date palm cultivars and their characteristics

Variety	Colour	Characteristics
Amari	Dark brown	Soft, sweet, medium size fruit, eaten as dried
Barhi	Amber to red-brown	Soft fruit, broadly ovate to round shape, medium thick smooth and translucent skin, sweet, delicious, and luscious taste, best for consumption at the *khalal* stage
Deglet Nour	Dark brown	Oblong-ovate shape, medium thick skin, soft semi-dry fruit with a unique taste
Fard	Dark brown	Semi-dry sweet fruit, pungent shape of thick cylindrical, skin medium thick skin
Hadrawi	Dark brown	Dry dates. sweet and fleshy
Hallawy	Golden brown fruit	Soft, sweet, caramel-like, translucent, oblong shape with rounded apex, thin skin
Hayani	Black and shiny	Soft fruit with less sweet taste, oblong shape
Kabkab	Dark brown to black	Soft fruit with long shape
Khadrawi	Red to brown	Soft fruit, caramel taste, elliptical to ovate shape, medium thick and tender skin

Table 1: Date palm cultivars and their characteristics (continued)

Variety	Colour	Characteristics
Khalas	Amber to red-brown	Delicious , oblong-oval shape with thin skin, tender fruit
Khasab	Red to brown-black	Thick fruit with oval shape, tough separating skin
Lulu	Dark amber	Soft and sweet fruit, oblong-oval shape, thick fruit with less fibrous flesh
Mazafati	Dark brown to black	Soft and fleshy fruit, cylinder shape with desirable taste
Medjool	Light brown to dark Brown	Soft, sweet, and large fruit with an attractive appearance
Piarom	Dark brown to black	Semi-dry and fleshy, long, and thin shape, one of the most expensive and desirable in the world
Rabbi	Red to dark brown	Semi-dry and fleshy, long, and thin shape
Zahidi,	Yellow to brown dry	Semi-dry with smooth consistency, oblong-ovate shape, thick skin, and firm fruit,

(Sources: Ghnimi et al., 2017; Karizaki, 2017)

1.4 Barhi Cultivar

Barhi is a mid-season cultivar extensively cultivated in the Gulf region and there is a growing demand for the cultivar in the international markets (Glasner et al., 1999). Due to low contents of soluble tannins, fruit ripen rapidly, and it is marketed and consumed at the full yellow (*Khalal*) stage as a crispy apple-like fruit (Botes & Zaid, 1999). *Barhi* dates reach the *Khalal* stage during the extremely hot dry summer (early to mid-July) and fruit become softer and sweeter. In this stage, this cultivar is less

astringent than other cultivars that are harvested when they are fully ripen. However, once ripened, this cultivar has a short shelf life (Hong et al., 2006).

In order to market the harvested bisir fruit with minimal physiological and physical disorders it is necessary to control fruit ripening following harvest and maintain a prolong shelf life. Consumers prefer *Barhi* cultivar because of its sweetness, crunchiness, mild hardness, and bright yellow colour. One of the challenges in storage and marketing of fresh *Barhi* dates is its short harvesting season (about 3 weeks). To achieve high season market prices, growers tend to harvest fruit before or as soon as horticultural ripening is reached (Glasner et al., 1999).

The huge production of fresh yellow *Barhi* dates at season causes a vast amount of losses for farmers and thus leads to low prices, which results in a sharp decrease of its economic value. The small fruit size of *Barhi* dates is another limiting factor that influences its marketing. Thus, it would be beneficial to improve quality characters and to prolong the *Khalal* stage of this cultivar in order to expand their shelf life and marketing ability (Al-Qurashi & Awad, 2011). Recently research has been focussed to find substitutes that promote shelf life of fruits due to increased concern among consumers about food safety and harmful health effects of chemical residues.

These alternative methods can maintain the marketable quality of fruits throughout the prolonged storage period. In order to extend shelf life of date fruits, cold storage at full mature stage is of great importance (Abd Elwahab et al., 2019). In the major date producing countries, cold storage of date fruits has received more attention in recent years. Date industries usually store dates at 3°C up to one year (Ismail et al., 2008; Al-Yahayai & Al-Kharusi, 2012).

1.5 Use of Natural Elicitors

Calcium is a key plant nutrient that has a significant role in stabilizing cellular membranes, reduces softening and senescence of fruits (Barker & Pilbeam, 2015). It is also considered as the most important mineral element which determines fruit quality (El-Badawy, 2012). Pre-harvest application of calcium increases calcium content of the cell wall and delay senescence in fruits resulting in firmer and higher fruit quality (Serrano et al., 2004).

Salicylic acid is an endogenous plant produced phenolic compound with high potential to delay ripening, enhancing quality and controlling postharvest losses of fruits and vegetables. It is involved in the regulation of physiological processes and disease resistance mechanisms (AOAC, 1994). Salicylic acid preserves firmness reduces weight loss, colour progress, and disease incidence in plums, strawberry, peach, and pears fruits for up to five weeks without decay (Khadiga, 1993).

Chitosan is an N-acetylated derivative of the polysaccharide chitin. It is a natural polymer with a polycationic nature, which is widely used in agriculture as soil modifier, coating films, fungicide, and elicitor (Deepmala et al., 2014). Pre-harvest spraying with chitosan is extremely reasonable and has a positive effect on fruit quality attributes (Reddy et al., 2000). Romanazzi (2010) Chitosan induces resistance responses of host tissues, and it inhibits the growth of decay-causing fungi. Chitosan films are widely applied on the surface of treated fruits and also have the potential to extend the storage life of many fruits, such as peach, Japanese pear, kiwifruit, strawberry, and sweet cherry (Du et al., 1998).

The active amino and hydroxyl groups of chitosan tend to react with the carbonyl group of salicylic acid. Hence, introducing salicylic acid into the backbone chain of

chitosan can induce formation of a chitosan-g-salicylic acid (CTS-g-SA) conjugate, which has excellent antibacterial effects and water solubility (He et al., 2011). Recently, Zhang et al. (2015) reported that continuous release of salicylic acid by a CTS-g-SA coating treatment induced higher chilling-resistance, reduced weight loss, and increased activities of antioxidant enzymes in cucumber fruit.

Little research has been reported about the effects of chitosan in combination with calcium chloride and/or salicylic acid on fruit quality, physicochemical content, and storage conditions of the ”*Barhi*” fruits. Recently, Ahmed et al. (2021a, 2021b) reported that fruit quality, bioactive compounds, and storage life of date palm (*Phoenix dactylifera* L., cv. Barhi) were improved by the application of natural elicitors such as chitosan (1%), calcium chloride (3%) and salicylic acid (2 mM).

Atia et al. (2020) found that postharvest dipping of *Khalal* “*Barhi*” dates in aqueous calcium chloride and salicylic acid decreased weight loss and fruit decay, increased total soluble solids, maintained colour and texture during cold and controlled atmosphere storage. Postharvest treatments of *Khalal* and *Barhi* dates with $CaCl_2$ and salicylic acid at different ripening levels during cold storage reduced the percentage of weight loss and decay in the fruits, while total soluble solids (TSS) increased (Atia et al., 2018).

1.6 Research Issues

1.6.1 Objectives

i) Provide information and guidance on application of natural elicitors to improve quality production and long-term storage of date fruits.

ii) Highlight significance of cold storage of date fruits in sustaining marketable quality.

iii) Shed light on potential impact of influential environmental conditions on field management and cultivars activities of date fruits.

1.6.2 Aim

The primary aim of this study is to explore the synergistic effects of chitosan in combination with calcium chloride and/or salicylic on fruit quality, physicochemical content, and storage quality of *Barhi* fruits.

1.6.3 Questions

The proposed research questions guide the investigation of the research problem and validate the research questions regarding effects on natural elicitors on date fruit quality production in the UAE agricultural context. These research questions are:

i) RQ1: Does pre-harvest spraying with chitosan affect ripening and maturity of *Barhi* date?

ii) RQ2: Is there a significant difference between applying only chitosan and combination chitosan elicitors?

Chapter 2: Literature Review

The extension of fruit life is an important aim to be attained, many storage techniques have been developed to extent the marketing distances and holding periods for commodities after harvest. One method of extending post-harvest shelf life of fruits is the use of treatment with natural elicitors (Baldwin et al., 2011). Elicitors, can mimic the action of the signalling molecules, activate defence responses and triggering and mediate long-lasting systemic acquired resistance (SAR), that protects against a wide range of pathogens, which causes fruit decay (Chen et al., 2018).

2.1 Chitosan as Natural Elicitor

During the past several decades, the biodegradable nature of natural compounds derived from animal and plants have interested plant pathologist. Among them, chitosan has received increased attention due to its fungicidal effects and elicitation of defense mechanisms in plant tissues (Terry & Joyce, 2004). It is a natural polymer with a polycationic nature, which has numerous applications in agriculture (e.g., as soil modifier, films, fungicide, elicitor) and agroindustry, as well as in cosmetics, biomedicine, environmental protection, wastewater management (Deepmala et al., 2014). Many studies have shown the high potential of chitosan for preserving fresh fruits and vegetables (Rhoades & Roller, 2000). Figure 3 illustrates the chemical structure of chitosan as an N-acetylated derivative of the polysaccharide chitin.

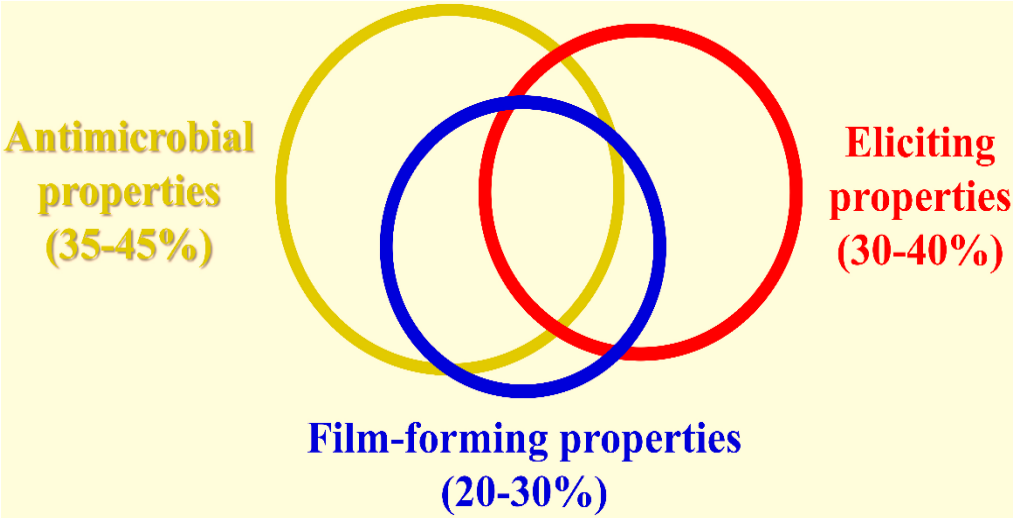


Figure 3: Chitosan's antimicrobial, eliciting, and film-forming properties (Source: Romanazzi et al., 2018)

2.2 Preharvest Application of Chitosan

Pre-harvest spraying with chitosan is highly feasible and has a beneficial effect on fruit quality attributes (Reddy et al., 2000). Preharvest application of chitosan to kiwi fruit have decreased the weight loss and delayed the changes in physicochemical properties that include firmness, soluble solids content, titratable acidity, total sugars, total acids, total phenols, and total lignin (Kumarihami et al., 2021). The preharvest foliar application of 3% chitosan with post-harvest coating of *Aloe Vera* gel extended the storage life of table grapes up to 15 days by reducing decay index, malondialdehyde content, weight loss and polyphenol oxidase (Nia et al., 2021).

Pre-harvest application of chitosan is especially useful for improving quality and antioxidant capacity of strawberry (He et al., 2018). The treatment of chitosan in combination with calcium chloride (Ca-chitosan) at preharvest stage in kiwi fruit resulted in the enhancement of fruit quality and postharvest life (Kim et al., 2018). Shen and Yang (2017) developed chitosan-g-salicylic acid (CTS-g-SA) for preharvest treatment of grapes, CTS-g-SA treatment exhibited enhanced activities of

phenylalanine ammonia lyase, chitinase, and β-1, 3- glucanase, while also promoting accumulation of phenolic compounds and greater resistance to *Botrytis cinerea* decay.

The preharvest treatment of strawberry with chitosan, laminarin, extracts of *Abies* spp., *Polygonum* spp., *Saccharomyces* spp., calcium and benzothiadiazole provided ~30% reduction in postharvest decay against gray mould and Rhizopus rot, without affecting fruit colour and firmness (Feliziani & Romanazzi, 2015). Previous studies also have shown that chitosan reduces decay incidence, mainly caused by *Botrytis cinerea* in tomato (El Ghaouth et al., 1992), decreases microbial count and act as elicitors (Kibar & Sabir, 2018).

In another experiment, chitosan significantly reduced the disease lesion size on tomato fruits when the tomato plants were pre-treated with 1.0% or 2.5% (w/v) COS solution 10 days before being inoculated with *Colletotrichum20C* DFGHJKL;sp. (Munoz et al., 2009). Preharvest chitosan treatments with other applications for storage decay of temperate fruit is shown in Table 2.

Table 2: Preharvest chitosan treatments of various fruits

Fruit	Decay agent	Combination with chitosan	Reference
Mango	Anthracnose (*Colletotrichum gloeosporioides*)	Spermidine	(Jongsri et al., 2017)
	Anthracnose (*Colletotrichum gloeosporioides*)	Lactoperoxidase system incorporated chitosan films	(N'Guettia et al., 2014)
	Anthracnose	*Mentha piperita* L. essential oil	(De Oliveira et al., 2017)
Pomegranate	*Penicillium spp., Pilidiella granati*	Lemongrass film	(Munhuweyi et al., 2017)
Citrus	Green mould (*Penicillium digitatum*)	*Bacillus subtilis*	(Waewthongrak et al., 2014)
	Anthracnose (*Colletotrichum gloeosporioides*)	*Pichia membranifaciens*	(Zhou et al., 2016)
Avocado	Anthracnose (*Colletotrichum gloeosporioides*)	Thyme oil	(Bill et al., 2014)
Tomato	*Alternaria alternate Aspergillus niger*, *Rhizopus stolonifera*	Methyl jasmonate essential oil from *Origanum vulgare* L.	(Chen et al., 2014; Baretto et al., 2016)

(Source: Romanazzi., et al. 2018)

2.3 Postharvest Application of Chitosan and their Derivatives

Coating of blueberries with chitosan, silicon and titanium dioxides results in a gradual increase in polyphenoloxidase and peroxidase enzyme activities, change in acidity,

anthocyanin and minimized the growth of mesophilic aerobic, yeasts, and moulds populations. (Li et al., 2021). Chitosan coating in combination with *Aloe vera* gel suppressed diseases and maintained the natural properties of mango fruit during postharvest storage and extended the storage life of mango fruit (Shah & Hashmi, 2020).

Chitosan composite film (CCF) containing chitosan, dextrin, ferulic acid and calcium effectively reduced the incidence of soft rot in kiwi fruit caused by fungi *B. dothidea* and *Phomopsis* sp. The CCF film increased the content of resistance compounds, activity of defence enzymes, enhanced the yield, quality and prolonged the shelf life of kiwifruit (Zhang et al., 2020). Khatri et al. (2020) recently studied the efficiency of *Aloe vera* gel and chitosan coatings in prolonging the postharvest- life of tomatoes. The composite coating of chitosan and *Aloe vera* gel treatment delayed the ripening process and extended the shelf life of tomatoes up to 42 days (Khatri et al., 2020). Nguyen and Nguyen (2021) investigated the influence of calcium chloride with nano-chitosan coating on the quality of strawberry during postharvest storage. The study concluded that chitosan coating with calcium chloride reduced weight loss, maintained L-ascorbic acid, total anthocyanin, antioxidants, retarded malondialdehyde production and no bitterness detected in the treated strawberries on 15 days of storage at 4°C.

Chitosan treatment has been reported to be more effective in postharvest physiology of fruit crops, when combined with other additives, including salicylic acid (Huang et al., 2017), potassium silicate (Mohamed et al., 2017), calcium chloride (Kim et al., 2018), gum Arabic (Khaliq et al., 2016), *lactoperoxidase* (Cissé et al., 2015), and antagonistic yeast (Meng & Tian, 2009). Apple fruits Cv. Lady William coated with

1.0% chitosan after harvesting maintains the quality attributes up to 80 days during storage at 18±2ºC and 56 ± 2% RH (Zeb et al., 2020).

Saki et al. (2019) reported that fig fruits coated with chitosan and thymol essential oil had less fungal decay incidence, lower weight loss and respiration rate. The coated fruits exhibited shelf-life extension with higher firmness, total soluble solids, and anthocyanin content after 20 days storage at 6°C. A nanocomposite coating of chitosan and MMT (a natural clay) have been developed, and applied on tangerine fruits (Xu et al., 2018). The results showed that the addition of 1% (w/w) MMT reduced the water sensitivity and improved the oxygen barrier properties of chitosan films significantly, and thus provided longer postharvest-life for tangerine fruits (Xu et al., 2018).

Pre-treatment of guava fruits with chitosan and calcium chloride alone and in combination significantly delayed decline in physiological loss of weight, enhanced total soluble solids firmness, acidity, ascorbic acid, sugars, phenols, and total antioxidant activity during storage (Chawla et al., 2018). Mango fruits coated with chitosan, potassium silicate and calcium chloride effectively increased fruit storability and shelf life in comparison to individual treatments and untreated mango fruits (Mohamed et al., 2017).

According to Romanazzi et al. (2018), the authors reported that chitosan mixed with ethanol, wax, and similar types of organic materials, improved the protecting effect on grapes from gray mould compared to the application of chitosan alone. Gayed et al. (2017) reported that 2% $CaCl_2$+1% chitosan was most effective in minimizing weight loss (%) and decay (%), as well as in maintaining maximum firmness and lengthening shelf life of peach fruits. Chitosan integrated with plant derivatives like plant extracts and essential oils, organic salts and acids, and antagonistic microorganisms, including

yeast and bacteria, are effective for reducing postharvest fungal rots (Bautista-Baños et al., 2017).

Strawberry fruits coated with 1% and 2% chitosan solution and stored at 2°C for nine days showed reduced water loss and delayed the qualitative changes in colour, titratable acidity, and ascorbic acid content. (Petriccione et al., 2015). Post-harvest treatment of chitosan has been studied for efficacy in inhibiting decay and extending shelf life of perishable produces such as plum (Kumar et al., 2017), peach (Elbarbary & Mostafa, 2014), fresh cut melon (Poverenov et al., 2014), strawberry (Wang & Gao, 2013) and cucumber (Ben-Shalom et al., 2003).

Chitosan integrated with essential oil such as lime, thyme, bergamot, clove, and cinnamon reduced the appearance of fungus *B. cinerea, C. gloeosporioides, P. digitatum and Phythophthora drehsleri*, and yeasts and moulds on strawberries, figs, avocado, cucumber, mandarin, and grapes (Mohammadi et al., 2015). Coatings of chitosan combined with oleic and acetic acid reduced disease symptoms for 10 days and decreases decay caused by fungi and bacteria in strawberry and prickly pear (Ochoa-Velasco & Guerrero-Beltrán, 2014).

Velickova et al. (2013) found that chitosan-beeswax coatings decreased the senescence and weight loss of strawberries, modified the respiration rates, and slowed down metabolism by the retention of the colour and the texture of the fruit. 1% chitosan combined with 3 % ammonium carbonate solutions and 2.5% calcium reduced the severity of anthracnose and incidence in papaya during 14 days of storage (Al-Eryani-Raqeeb, et al., 2009). Strawberry ‘Camarosa’ coated with 1.5 % chitosan and 0.5 % calcium gluconate showed no visible symptoms of disease caused by moulds during the whole storage period at 10ºC (Hernández-Muñoz et al., 2008).

2.4 Chitosan Treatment with Calcium and Salicylic Acid

Fresh pistachio treated with chitosan and salicylic acid enhanced the quality of fruit in refrigerated storage with prolonged shelf life. The fruit were lighter, redder, and more yellow with highest sensory scores for colour, texture, and overall acceptance with reduced growth of bacteria and fungi (Molamohammadi et al., 2020). Treatment with preharvest calcium spray and post-harvest chitosan coating extended the storage time, reduced fruit decay rate at the end of storage and improved the quality of Chinese dwarf cherry for up to 30 days (Guo et al., 2020).

Chitosan coating containing calcium chloride showed lower weight loss, improved appearance, reduced browning and enhanced ascorbic acid content in fresh cut apples (Liu et al., 2016). Edible coating of chitosan combined with calcium chloride extends the shelf-life of fresh-cut honeydew melon by maintaining the integrity of soluble pectin (Chong et al., 2015).

Postharvest treatment of pomegranate with salicyloyl chitosan exhibited lower weight loss, respiration rate and ethylene production associated with higher firmness, total soluble solids, and titratable acidity as sensory quality (Sayyari et al., 2016). Vyas et al. (2016) reported that treatment of salicylic acid with calcium chloride and sodium benzoate were found to be effective in enhancing antioxidants, ascorbic acid, and total anthocyanins in phalsa fruit (*Grewia asiatica* L.). The treatment with chitosan in combination with other treatments is reviewed in Table 3.

Table 3: Treatment of vaious fruits with chitosan combination

Fruit	Physiological change	Combination with chitosan	Reference
Apple	Total phenolic, flavonoids, antioxidants, pigments, weight loss	Olive waste extract	(Khalifa et al., 2017)
Sweet cherry	Malondialdehyde content and antioxidant enzymes	----	(Pasquariello et al., 2015)
Mango	Peroxidase (POD) and polyphenol oxidase (PPO) gene expression		(Gutierrez-Martinez et al., 2018)
Kiwi fruit	Induced gene expression and increased enzymatic activity of catalase, superoxide dismutase and ascorbate peroxidase		(Zheng et al., 2017)
Peach	Malondialdehyde content.	Gamma ray	(Elbarbary & Mostafa, 2014)
	Total soluble solids, weight loss, ascorbic acid content.	Silver and zinc oxide	
	Colour and fruit firmness.	nanoparticles	(Kaur et al., 2017; Cindi et al., 2015)
		Polyethylene terephthalate punnets containing thyme oil	
Plum	Fruit firmness, weight loss, total soluble solids, total phenolic content, and titratable acidity.	Calcium chloride	(Gayed et al., 2017)
	Respiration rate, fruit colour, polygalacturonase, superoxide dismutase, peroxidase, catalase, polyphenol oxidase.	Ascorbic acid	(Liu et al., 2014)

Table 3: Treatment of vaious fruits with chitosan combination (Continued)

Fruit	Physiological change	Combination with chitosan	Reference
Strawberry	Weight loss, titratable acidity, pH, total soluble solids, total phenols.	Carboxymethyl cellulose	(Gol et al., 2013)
	Weight loss.		(Sangsuwan et al., 2016)
	pH and soluble solids content.	Lavander and thyme essential oil	(Duran et al., 2016)
	Weight losses, total soluble solids, and titratable acidity.	Natamycin, nisin, pomegranate, grape seed extract and olive waste extract	Khalifa et al., (2016)
Plum	Fruit firmness, weight loss, total soluble solids, total phenolic content, and titratable acidity.	Calcium chloride	(Gayed et al., 2017)
	Respiration rate, fruit colour, polygalacturonase, superoxide dismutase, peroxidase, catalase, polyphenol oxidase.	Ascorbic acid	(Liu et al., 2014)
Citrus	Fruit firmness, weight loss, total soluble solids. Peroxidase and pheny-lalanine ammonialyase	Carboxymethyl cellulose Cyclic lipopeptide antibiotics from Bacillus subtilis	(Arnon et al., 2014; Waewthongrak et al., 2015)

Table 3: Treatment of vaious fruits with chitosan combination (Continued)

Fruit	Physiological change	Combination with chitosan	Reference
Table grapes	Phenylalanine ammonia lyase, chitinase, and β-1, 3-glucanase, phenolic compounds.	Salicylic acid	(Shen and Yang, 2017)
	Total phenols, flavonoids and ascorbic acid content, activities of peroxidase.	Menta essential oil	(Al-Qurashi & Awad, 2015)
	Firmness, titratable acidity, soluble solids, colour, weight loss.		(Guerra et al., 2016)
Pear	Total phenolic and flavonoid contents, superoxide dismutase, peroxidase and catalase activities, total antioxidant activity.	Calcium chloride	(Kou et al., 2014a)
	Malic acid-metabolizing enzymes and related genes expression.	Calcium chloride	(Kou et al., 2014b)

(Source. Romanazzi., et al. 2018)

2.5 Strategies for the Preharvest/Postharvest Treatment of Date Fruits

Postharvest treatment with edible coatings (gelatine, chitosan, guar gum and their combinations) led to extend the shelf life and also influenced the organoleptic characteristics of *Barhi* date fruits (Abu-Shama et al., 2020). Edible coating with soy protein or gelatine at 6, 9 and 12% as a carrier of thyme oil maintained the quality of "Barhi" dates fruits and extended the storage period at 5°C with 85-90% relative humidity for 4 weeks (Yousef et al., 2020).

The postharvest treatment of *Barhi* date palm fruit with chitosan - Propolis extract and chitosan with *Aloe Vera* gel maintained the quality of dates during cold storage at 0°C which results in less weight loss, highest firmness and slow down compositional changes of total phenols, total sugars, and total tannins (Abd Elwahab et al., 2019). "*Barhi*" date fruit can retain its physiochemical properties during cold storage and ambient condition by treating with postharvest edible coating of calcium alginate at 3% (Samra et al., 2019).

El-Dengawy et al. (2018) reported that postharvest treatment of chitosan and sodium carbonate improved the fruit firmness, total sugars and tannins content and decreases microbial load in *Hayani* date palm fruits during cold storage. Gibberlic acid and salicylic acid postharvest treatment in the controlled atmosphere storage was better in conserving the quality of *Barhi* dates at the *Khalal* maturity stage and delaying ripening process (Atia et al., 2018). The combined application of chitosan and Locust Bean Gum in combination with different citrus essential oils inhibited *Aspergillus flavus in vitro* in artificially infected dates for a storage period of 12 days with the absence of off-odour and off-flavour (Aloui et al., 2014).

Chapter 3: Materials and Method

3.1 Plant Material and Sampling

During the 2020, 2021 seasons, six date palm trees (*Phoenix dactylifera* L., cv. Barhi) were selected in the experimental farm of the College of Food and Agriculture located in Al Foah region, Al Ain, UAE, located in the co-ordinate latitude and longitude of 24.2191° N and 55.7146° E. Trees were pruned to keep leaf to bunch ratio at 8: 1 and the number of female spathes per palm was adjusted to 8. The design of the experiment was a Complete Randomized Design with 6 palms (replicate) of *Barhi* cultivar receiving 7 different treatments (one treatment for each bunch). Each treatment was sprayed during three stages of development (5 and 15 weeks from pollination, and two weeks before harvest), with different elicitors: chitosan, SA, and $CaCl_2$ alone and in combinations.

Date palm trees receiving deionized water were served as control. Date palm bunches were harvested at full mature stage (Bisr stage); the whole fruit should be yellow, and the yellowish green area should not exceed 10% on the bunch. Immediately after harvest, fruit were transported to the lab, cleaned, and sorted to have uniform fruit (full yellow fruit at similar degree of development) to be analyzed. 100 fruits of each treatment were randomly collected for initial physical, physiochemical, phytochemical, and bioactive properties analysis at harvest time. For storage, a group of 500 fruit from each treatment were collected and stored in perforated plastic bags (100 fruit per bag) at 2°C and 90-92 % relative humidity for a period of 60 days. Every 15 days, one bag of fruit was randomly withdrawn for analysis.

The ripening/decaying fruits were counted, and then fruits were immediately sliced and blinded. Three samples (10g each) of mixed tissue were utilized for phytochemical analysis, and ten grams were used to produce TSS juice. Another sample of 25 fruit was separately stored in perforated plastic bags for colour and fruit weight loss measurement every two weeks. For microbiological analysis, samples of 25 fruit were aseptically collected in sterile bags and kept under the above conditions. Table 4 shows harvest spry treatments of *Barhi* date fruit with different elicitors.

Table 4: Harvest spry treatments of *Barhi* date with different elicitors

Treatment	**Chemical**	**Application**
Control	Water	Water
Ch	Chitosan	1 %
Ca	Calcium chloride	2 Mm
SA	Salicylic acid	3 %
Ch + SA	Chitosan + Salicylic acid	1:1, *v/v*
Ch + Ca	Chitosan + Calcium chloride	1:1, *v/v*
Ch + Ca + SA	Chitosan + Calcium chloride + Salicylic acid	1:1:1, *v/v/v*

3.2 Physiochemical Analysis

3.2.1 Fruit characteristics

At harvest, fruit weight and dimensions were recorded according to Rastegar et al. (2012). During storage, the fruit weight loss was recorded for each treatment at both stages (*Bisr* and *Rutab*) once every two weeks and reported as a percentage of weight loss against the original weight before the cold storage, utilizing the following equation:

$$\text{Fruit weight loss \%} = \frac{\text{Initial weight} - \text{Weight at specific interval}}{\text{Initial weight}} \text{x}100$$

3.2.2 Fruit ripening and decay percentage

The fruit were visually observed for ripening evolution (fully Rutab) and decay every other week till complete spoilage of fruit occurred during storage for two months. The ripening and decay percentages were calculated by using the following equation (Kumar et al., 2013).

$$\text{Decay or ripe fruit (\%)} = \frac{\text{Number of ripe or number of decay fruit}}{\text{Total number of fruits}} \text{ x } 100$$

3.2.3 Total soluble solids (TSS)

Ten grams of mashed fruit was combined with 10 mL of distilled water. The blended juice was filtered, and the clear juice was utilized. The TSS as a percentage value was determined in the juice using a digital refractometer (DR 6000, A. Kruss Optronic GmbH, Hamburg, Germany).

3.2.4 Fruit surface colour

Hunter Lab colourimeter (Hunter Lab Inc., Reston, VA, USA) was used to assess the surface colour of the fruit. The colour values of the fruit were represented as L* (brightness), a* (blue/yellow), and b* (red/green). These parameters were then used to determine the total colour difference (ΔE^*), chroma (C^*), and hue angle (h^o), as follows:

$$\Delta E = [(L^* - L^*0) + (a^* - a^*0) + (b^* - b^*0)]^{1/2}$$

$$C^* = (a^{*2} + b^{*2})^{1/2}$$

$$h^\circ = 180^\circ + \arctan\ (b^*/a^*)$$

where *a*0*, *b*0*, and *L*0* values represent control fruit (Maskan, 2001).

3.2.5 Determination of total microbial load

The microbiological examinations were carried out in the microbiological food laboratory. Total bacterial count as well as yeast and mould counts were determined for treated and untreated date fruit samples at harvest (day 0) and at the end of storage time (day 60). One gram of fruit tissue was aseptically mixed with nine milliliters of sterile peptone water under sterile conditions and used for serial dilutions of samples. The pour plate technique was utilized for preparing the plate count agar (PCA) and potato dextrose agar (PDA). PCA plates were incubated at 37°C for 48 h, while PDA plates were incubated at 27°C for 5 d. At the end of the incubation time, the number of colonies was recorded as Log_{10} colony-forming unit per g of fresh weight (Log_{10} CFU g^{-1}).

3.3. Phytochemical Analysis

3.3.1. Extraction of bioactive compounds

Extraction of phenolic compounds was achieved by homogenizing 2 g of fresh fruit samples in 20 mL of 80% methanol using a water bath shaker (150 rpm) at 45°C for 24 h. Then, the samples were filtered using Whatman #1 filter paper, and the supernatant (date extract) was utilized for further analysis.

3.3.2 Total phenolic content (TPC)

Date extract (100 µL each) was added into tubes, followed by 50 µL of Folin Ciocalteu reagent and vortexed. All the tubes were incubated at room temperature for 2 min. Two mL of NaOH (6%) was added to each tube. Absorbance was measured at 750 nm using a spectrophotometer. The total phenolic content of samples was determined and

expressed as milligram gallic acid equivalents (GAE) per 100 g of fresh weight (mg 100 g^{-1} GAE) utilizing the standard curve obtained by measuring the absorbance of known concentrations of gallic acid (Velioglu et al., 1998).

3.3.3 Total flavonoid content (TFC)

Total flavonoid content (TFC) was determined as described by Kim et al. (2003), with some modifications. First, 75 µL of $NaNO_2$ (5%) was added to 250 µL of date extract in a test tube and kept for 5 min in the dark. Then, 75 µL of $AlCl_3$ (10%) was added, and the mixture was vortexed and held in the dark for 6 min. Next, 500 µL of NaOH (1 M) was added and mixed by vertexing. The volume was brought to 2.5 mL using distilled water. The absorbance was measured at 510 nm with a spectrophotometer (Shimadzu, Kyoto, Japan). The obtained results were reported as mg of catechin per 100 g of fresh weight (mg 100 g^{-1} CE).

3.3.4 Antioxidant activities

Antioxidant activity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) radical scavenging activity assays,

3.3.5 DPPH radical scavenging activity

The DPPH radical scavenging activity was determined as defined by Wu et al. (2003) with slight modifications. The 1.5 mL samples with concentrations ranging from 0.5 to 10 mg L^{-1} were mixed with 1.5 mL of 0.15 mM 2,2-diphenyl-1-picryl hydrazyl (DPPH) in 95 % ethanol. The mixture was allowed to stand at room temperature, in the dark, for 30 min. Using a spectrophotometer, the absorbance of the mixture was measured at 517 nm. A sample blank for each concentration was made in the same

way, except that ethanol was utilized in place of DPPH solution. IC50 values (mg mL^{-1}) were calculated for each sample.

3.3.6 ABTS radical scavenging activity

The ABTS radical scavenging activity was determined according to Arnao et al., (2001). The stock solutions were ABTS solution (7.4 mM) and potassium persulphate solution (2.6 mM). The working solution was made by combining equal parts of the two stock solutions and enabling them to react for 12 h at ambient temperature in the dark. The solution was further diluted by combining 1 mL of ABTS solution with 50 mL of methanol, yielding an absorbance of 1.1 ± 0.02 units at 734 nm as measured with a spectrophotometer. For each assay, a fresh ABTS solution was made.

Samples (150 µL) with concentrations ranging from 0.5 to 10 mg mL^{-1} were combined with 2850 µL of ABTS solution and left at room temperature for 2 hours in the dark. The absorbance was then measured at 734 nm. A sample blank for each concentration was made in the same way, except that methanol was utilized in place of ABTS solution. A Trolox standard curve ranging from 50 to 600 µM was generated. The activity was recorded as milligram of Trolox equivalents (TE) per 100 g fresh weight basis (mg 100 g^{-1} TE).

Chapter 4: Data Analysis and Results Discussion

The data from the completely randomized design with six replicates were subjected to statistical analysis using analysis of variance (ANOVA) in the SAS statistical software (SAS Institute Inc., 2000, Cary, NC, USA). Least significant differences (LSD) at level $P \leq 0.05$ were utilized to compare means between treatments at harvest or within a storage time interval. Correlation coefficient between the main biochemical and physical characteristics was also done by SAS.

4.1 Physical Quality Characteristics of Fruit at Harvest

Treated *Barhi* fruit exhibited significant variations in physical characteristics when affected by treatments with preharvest elicitors, The average length, width, and weight were significantly ($p \leq 0.05$) greater in SA-, Ch + Ca-, and Ch + SA-treated fruits compared to controls and other treatments. These enhancements in fruit dimensions could be attributable to the impact of SA in conjunction with other elicitors in preserving cellular integrity and boosting the strength of carbohydrate sink, improving the size and weight of the fruit (Kassem et al., 2010).

Moreover, Mohamed et al. (2014) reported that preharvest spray treatment with SA significantly improved *Barhi* fruit's length, width, and weight relative to control fruit. Fresh fruit's physical quality parameters are critical since they directly reflect the physical impact of the used elicitors. The current results reveal that using SA and Ch alone or in conjunction with Ca and Ch has significant effects on the *Barhi* fruit's physical qualities. Table 4 shows the average length, width, and weight of *Barhi* fruit at harvest. Table 3.1 shows the average length, width, and weight of *Barhi* fruit at harvest.

Table 5: Average length, width, and weight of Barhi fruit at harvest

Treatment	Fruit Weight (g)	Fruit Width (mm)	Fruit Length (mm)
Control	9.44 ± 0.91 [b]	30.56 ±1.02 [bc]	22.60 ± 0.73 [a]
Ch	9.73 ± 0.46 [ab]	30.49 ± 0.81 [c]	22.01 ± 0.56 [ab]
Ca	8.91 ± 0.65 [b]	29.69 ± 1.11 [c]	21.86 ± 0.85 [b]
SA	10.46 ± 0.81 [a]	31.58 ± 1.2 [b]	23.13 ± 1.02 [a]
Ch + SA	10.03 ± 0.32 [a]	34.59 ± 0.92 [a]	23.84 ± 0.99 [a]
Ch + Ca	10.61 ± 0.80 [a]	32.11 ± 1.30 [b]	23.55 ± 0.89 [a]
Ch + SA + Ca	8.46 ± 0.22 [b]	29.19 ± 0.90 [c]	20.81 ± 1.03 [b]

Note: Values are the mean (n = 25) ± SE. Means with the same letter(s) in the same column are not significantly different at $p \leq 0.05$ using the LSD test. Ch: chitosan; SA: salicylic acid; Ca: calcium chloride.

4.2 Fruit Weight Loss During Storage

According to the generated results, the use of elicitors had a significant impact on *Barhi* fruit's weight. During storage, weight loss occurred steadily in all fruits, to various degrees. Significant variations ($p \leq 0.05$) in weight loss were observed across treatments when compared to the controls (see Figure 1). At day 60, the smallest weight loss was observed in Ch-treated fruit, followed by Ch + SA, Ch + SA + Ca, SA, Ch + Ca, Ca, and control, with, 9.4, 10.4, 10.8, 10.8, 12.0, 13.1, and 14.1%, respectively, as shown in Figure 4.

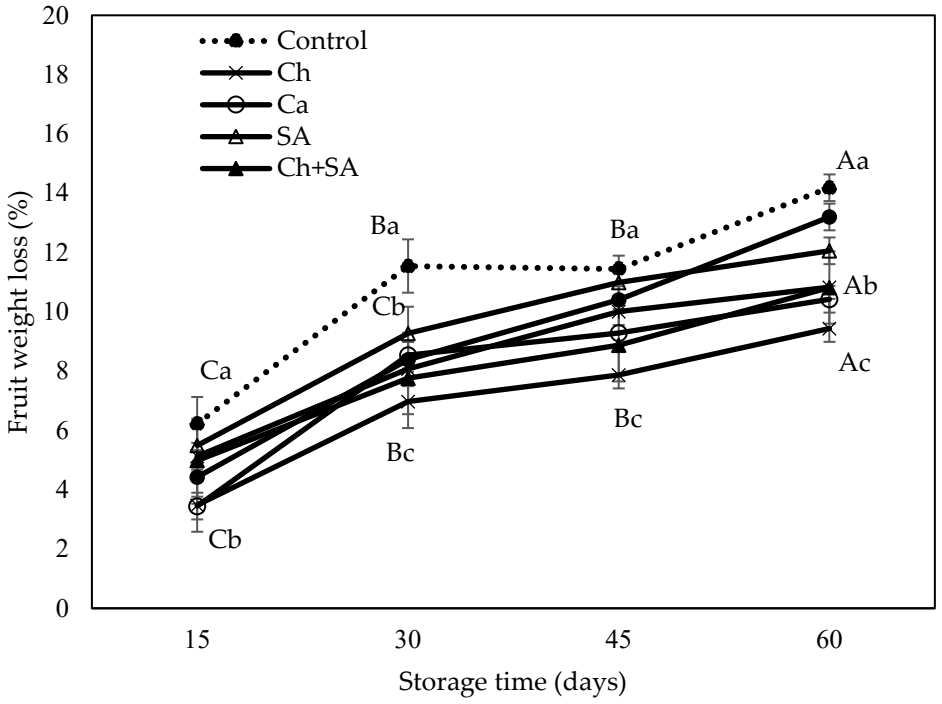


Figure 4: Percentage of weight loss in *Barh* in 60 days under cold storage

Note: values are the mean (n = 25) ± SE. The LSD test at $p \leq 0.05$ was used to compare the means between treatments. Means with the same letter(s) between treatments within storage time intervals (small letter) and at different time intervals (capital letter) are not significantly different. Ch: chitosan; SA: salicylic acid; Ca: calcium chloride.

The minimal weight loss seen in fruits sprayed with chitosan alone might be due to the film-forming characteristics of chitosan, which greatly reduces the rate of water evaporation from the fruit, as observed with other chitosan-treated fruits (Petriccione et al., 2015a; Petriccione etal., 2015b; Romanazzi et al., 2013). Furthermore, as compared to SA, Ca had significantly ($p \leq 0.05$) smaller weight loss. According to Atia et al. (2020), pre-storage treatment with Ca decreased weight loss in *Barhi* dates better than SA.

In addition, Mohamed et al. (2014) found that preharvest treatment with SA decreased weight loss during storage in treated *Barhi* fruit compared to controls. SA is widely recognized for its ability to minimize chilling damage, decrease ripening, and resist different abiotic and biotic stressors (Ennab et al., 2020); this may suggest that fruit treated with SA has a better physiological state most likely due to a lower respiration rate, which can also be related to enhanced fruit turgidity. Weight loss is a major factor that affects the shelf life and marketability of harvested fruits (Mohamed et al., 2014; Kassem et al., 2010); it occurs as a result of the increase in respiration rate and/or moisture loss from the fruit during storage (Mohamed et al., 2014; Atia et al., 2018; Shiri et al., 2013).

According to the findings of this study, the weight loss reduction observed in fruit treated with Ch alone, or in conjunction with Ca and SA, signifies that the treated fruit had better physiological conditions, most likely as a result of a reduction in transpiration and respiration rates, as well as the regulating influence of these elicitors on the ripening process. In addition, the capacity of Ch to form a film, which creates a barrier to gas exchanges, reduces respiration, and decrease fruit weight loss (Romanazzi et al., 2018).

4.3 Total Soluble Solids (TSS)

The results of TSS concentration at harvest (day 0) and during cold storage at 2 °C for 60 days are presented in Figure 3.2. Significant differences ($p \leq 0.05$) were observed between different treatments in relation to TSS concentration. In all fruits, the concentration of TSS increased steadily with ripening throughout the storage time. At harvest, and after 60 days of cold storage, the control fruit showed the greatest TSS concentration (from 30 to 42%), followed by Ch-treated fruit (from 30 to 41%). The

lowest TSS concentration was observed with Ca-treated fruit (from 26% to 36%); this result might be attributable to the high Ca (3%) concentration in this treatment. Likewise, Ch + Ca-, Ch + SA + Ca-, and Ch +

SA-treated fruit showed significantly ($p \leq 0.05$) lower concentrations of TSS relative to the control fruit after 15, 30, 45, and 60 days of storage. These data are compatible with the weight reduction results and are supported by the positive correlation. Similarly, Kassem et al. (2010) reported comparable TSS concentrations in SA-treated *Barhi* fruit. However, Mohamed et al. (2014) found no significant difference ($p \leq 0.05$) in TSS concentrations between the control and SA-treated *Barhi* fruit. Figure 5 shows effects of preharvest treatments with natural elicitors on *Barhi* fruit's total soluble solids during cold storage for two months.

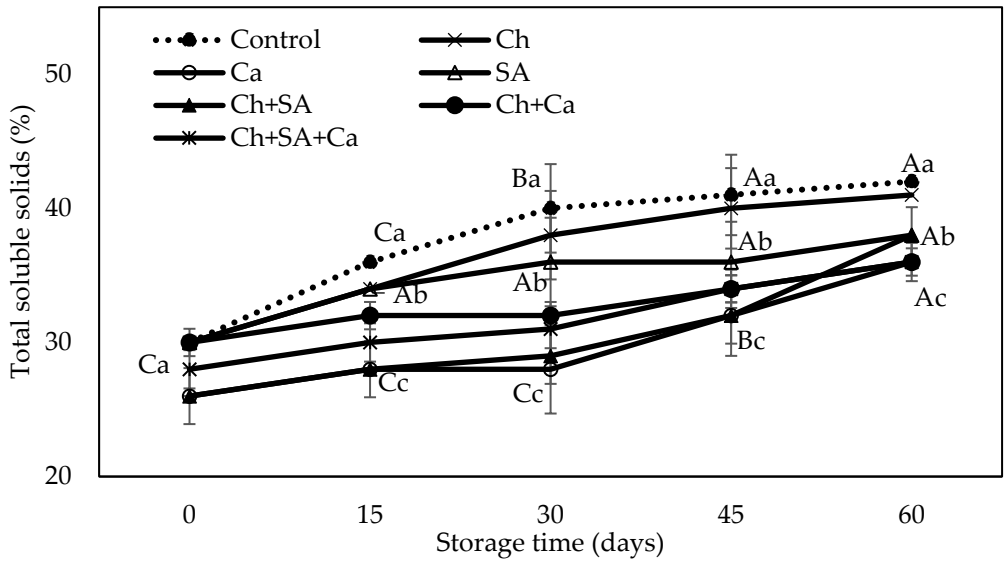


Figure 5: Effects of treatments with natural elicitors on preharvest *Barhi*

Note: Values are the mean (n = 3) ± SE. Means with the same letter(s) between treatments within storage time intervals (small letter) and at different time intervals (capital letter) are not significantly different. Ch: chitosan; SA: salicylic acid; Ca: calcium chloride.

Generally, the formation of smaller sugars by the breakdown of larger polysaccharide molecules via enzymatic actions and loss of moisture throughout storage are accountable for the increased TSS during storage (Mohamed et al., 2014; Hazbavi et al., 2015). Additionally, the efficacy of elicitors has been reported to be controlled by the response of the fruit tissues, which decreases as the fruit ripens (Romanazzi et al., 2018). As per our results, Ca alone or in conjunction with Ch and SA delays polysaccharide breakdown, leading to lower TSS levels in treated *Barhi* fruit and, hence, a slower rate of maturation and ripening, while maintaining the fruit's quality.

4.4 Fruit Colour Characteristics

Significant colour parameters namely, L^*, h^o, ΔE, and C^*—were determined in order to detect colour changes in the fruit throughout the cold storage (Figure 3.3). Overall, colour parameters were significantly ($p \leq 0.05$) affected by elicitor treatments. Additionally, colour values steadily declined in all fruits with the increase in storage time, with the exception of Ca-treated fruit, which showed the lowest colour change values. In all fruits, L^* values decreased as storage progressed, indicating a substantial decline in fruit lightness. At day 60, the lowest decrease in L^* was detected in fruit treated with Ca, followed by Ch + SA and Ch + Ca + SA, compared to other treatments and controls. When compared to other treatments, including controls, the same treatments showed smaller ΔE values, with Ch + Ca displaying the greatest ΔE.

Likewise, by the end of the storage period, Ca-treated fruit had the highest C* values, followed by Ch + SA- and Ch + Ca + SA-treated fruits, whereas Ch + Ca showed the opposite pattern. The latter treatment contains calcium which could also be responsible for delaying ripening progress and change in colour as well as chitosan, which forms a thin film that delays gas exchanges and slows the metabolism. The L^*, h^o, and C^*

values show positive correlation with rising TSS, while ΔE shows the opposite. The observed delay in the colour change in fruits treated with Ca could be due to influence of Ca on ethylene activity, which could unmask chlorophyll pigment and lower the respiration rate of the fruit, thus slowing the colour change (Irfan et al., 2013). Accordingly, SA- and Ch-treated pistachio fruit showed better colour values relative to controls (Molamohammadi et al., 2020). Figure 6 (A-D) shows effects of preharvest treatments with natural elicitors on *Barhi* fruit's colour during cold storage for two months

(A)

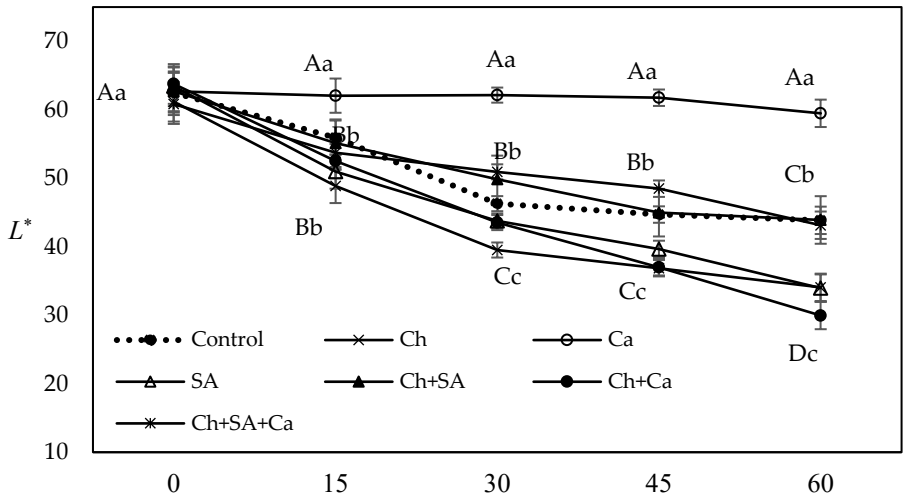
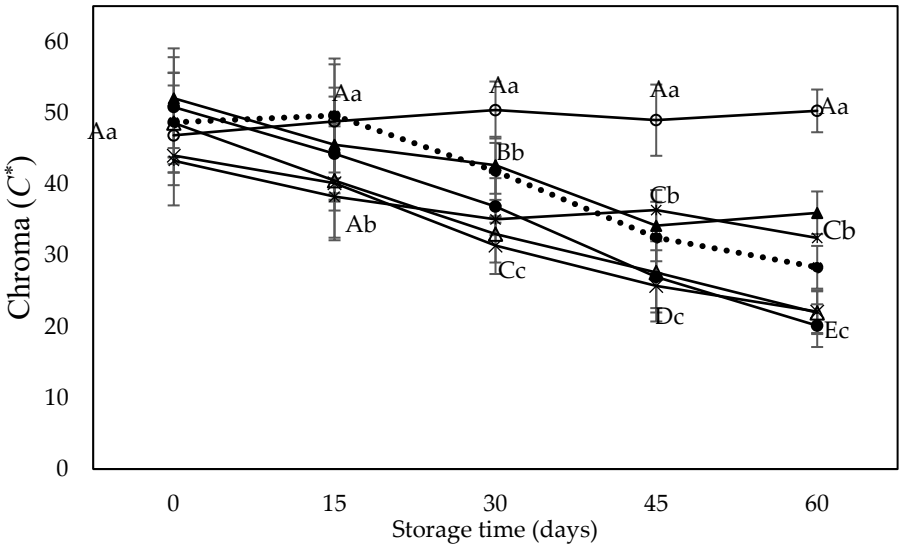


Figure 6 : Preharvest treatment effects with natural elicitors on *Barhi*'s colour during cold storage (A-D)

Note: Values are the mean (n= 25) ± SE. The LSD test at $p \leq 0.05$ was used to compare the means between treatments. Means with the same letter(s) between treatments within storage time intervals (small letter) and at different time intervals (capital letter) are not significantly different. Ch: chitosan; SA: salicylic acid; Ca: calcium chloride.

(B)



(C)

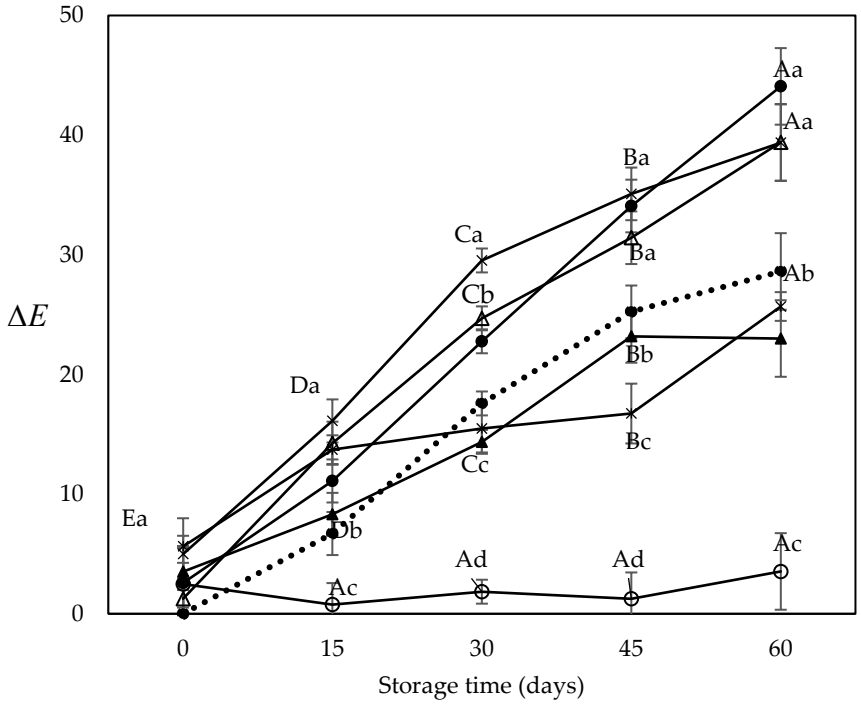


Figure 7 : Preharvest treatment effects with natural elicitors on *Barhi*'s colour during cold storage (A-D) (continued)

(D)

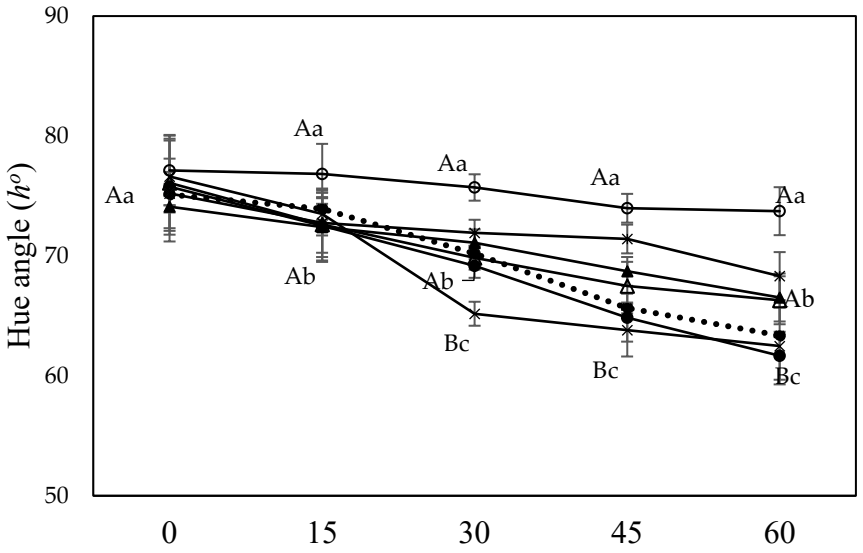


Figure 8 : Preharvest treatment effects with natural elicitors on *Barhi*'s colour during cold storage (A-D) (continued)

Colour is an important visual characteristic of food especially fresh fruit and it can affect product appeal and acceptance (Fernández-vázquez et al., 2011). As a result, the influence of treatments on the colour of fresh *Barhi* fruit was considered in the current study. Damaging colour reactions particularly enzymatic ones are a naturally occurring phenomenon produced mostly by the activities of polyphenol oxidase and peroxidase throughout the ripening and storage of fruit, and have been associated with a decrease in fruit market value (Awad et al., 2011a; Mortazavi et al., 2015). These reactions have been attributed to the oxidation of phenolic constituents and the formation of dark brown pigments in date fruits (Awad et al., 2011a). Given the current results, it is likely that applying Ca alone or in conjunction with SA and Ch delayed colour change reactions in fresh "Barhi" fruit to different degrees.

4.5 Total Phenolic Content (TPC) of Fruit at Harvest and during Storage

The type of elicitor used significantly ($p \leq 0.05$) impacted the total phenol content (TPC) at harvest and during the period of cold storage. Overall, TPC progressively declined in all fruits with the increase in storage time. Ca-treated fruit showed the greatest TPC content, which gradually declined from 445.7 mg 100 g^{-1} GAE at day 0 to 260.66 mg 100 g^{-1} GAE at day 60, followed by Ch + SA + Ca (from 458.3 to 148.57 mg 100 g^{-1} GAE), in comparison with other treatments, including controls.

Several studies, however, have revealed differences in TPC concentrations between different date cultivars (50–400 mg 100 g^{-1}) (Al-Qurashi & Awad, 2011; Awad et al., 2011a, 2011b; Mohamed et al., 2014) which could be due to cultivar differences and/or environmental variables. In general, in different date cultivars, the concentration of phenols decreases over time from the early stages of growth to maturity and ripening (Awad et al., 2011a). Figure 7 shows the TPC content in *Barhi* fruit at harvest and during the course of cold storage time.

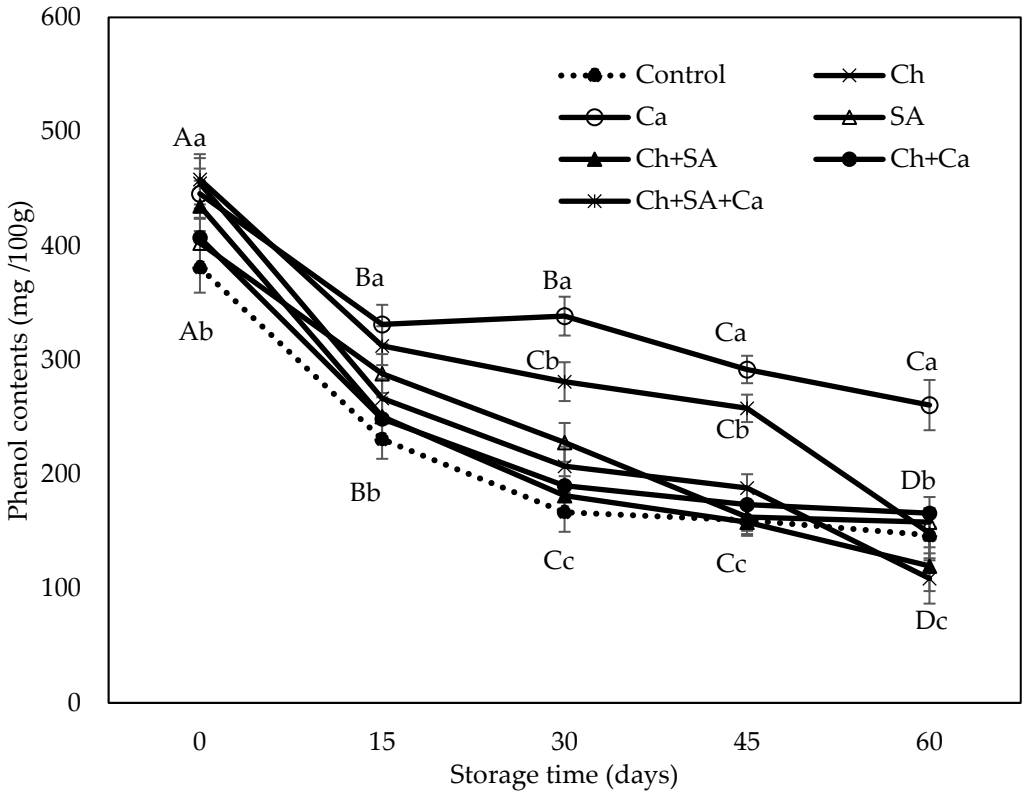


Figure 9: Preharvest application effects of natural elicitors on TPC at harvest in cold storage

Note: Values are the mean (n = 3) ± SE. The LSD test at $p \leq 0.05$ was used to compare the means between treatments. Means with the same letter(s) between treatments within storage time intervals (small letter) and at different time intervals (capital letter) are not significantly different. Ch: chitosan; SA: salicylic acid; Ca: calcium chloride.

Because of its capacity to stimulate antioxidant systems and heat shock proteins, SA has previously been demonstrated to reduce chilling damage in peach fruit during cold storage (Wang et al., 2006). The use of elicitors i.e., chitosan, Ca, and SA has been shown to increase the content of polyphenolic compounds in fruits and, thus, improve their quality (Ruiz-García & Gómez-Plaza, 2013).

The present study reveals that the continuous decline observed in TPC during storage (see Figure 7) is positively correlated with the decrease in color parameters such as *L**,

h^o, and C^* while the opposite is true of ΔE (see Table 6 and Figure 6 (A-D)), in all fruits, at various levels. According to these findings, preharvest Ch + SA + Ca treatment enhances TPC levels, improving the quality and shelf life of *Barhi* fruit. Table 5 shows Pearson's correlation coefficients between some physical and biochemical properties of *Barhi* fruit regarding Total Phenolic Content (TPC) and Total Soluble Solids (TSS).

Table 6: Pearson's correlation coefficients of physical/biochemical file of *Barhi*

Traits	TSS	TPC	Rutab %	Decay %	Weight Loss %	*L**	Δ*E*	*C**
TPC	−0.72 ***							
Rutab %	0.72 ***	−0.93 ***						
Decay %	0.60 ***	−0.44 ***	0.54 ***					
Weight % loss	0.51 ***	−0.79 ***	0.68 ***	0.44 ***				
L*	−0.67 ***	0.85 ***	−0.91 ***	−0.39 ***	−0.60 ***			
Δ*E*	0.66 ***	−0.80 ***	0.89 ***	0.46 ***	0.57 ***	−0.98 ***		
C*	−0.61 ***	0.72 ***	−0.83 ***	−0.48 ***	−0.53 ***	0.94 ***	−0.96 ***	
***h*°**	**−0.69 *** **	**0.82 *** **	**−0.88 *** **	**−0.53 *** **	**−0.62 *** **	**0.93 *** **	**−0.92 *** **	**0.90 *** **

(***) significant at $p \leq 0.001$.

4.6 Total Flavonoids, Tannins, and Antioxidant Activity at Harvest

At harvest, the effects of applied elicitors on total flavonoid content (TFC), total tannin content (TTC), and antioxidant activity were investigated (Table 3.3). Significant variations ($p \leq 0.05$) between different treatments in TFC, TTC, and antioxidant

activity were observed to be affected by the applied elicitors. In comparison to all treatments, Ca-treated fruit had the highest TTC and TFC, with 136 mg 100 g^{-1} CE and 113 mg 100 g^{-1} CE, respectively, followed by CH + SA (130 and 105 mg 100 g^{-1} CE, respectively), CH (126 and 105 mg 100 g^{-1} CE, respectively), and CH + SA + Ca (120 and 107 mg 100 g^{-1} CE, respectively), whereas CH + Ca-treated fruit had lower TTC and TFC (114 and 89 mg 100 g CE, respectively), but was not significantly different compared to controls. Based on this finding, the CH + Ca treatment had some influence on TTC and TFC in treated fruit, meriting further investigation.

Regarding antioxidant activity, CH + SA + Ca-treated fruit had the greatest antioxidant activity as measured by ABTS (682 mg 100 g^{-1} TE) and DPPH (IC_{50} = 2.1 mg mL^{-1}) radical scavenging activity assays (Table 3.3). The lower IC_{50} numbers in the DPPH radical scavenging activity assay measurements indicated a 50% reduction in the extract concentration required to scavenge the DPPH radical, signifying an increase in antioxidant activity. Comparable results were reported by Mohamed et al. (2014), who observed that preharvest spraying with SA significantly ($p \leq 0.05$) improved DPPH scavenging activity in “Barhi” fruit relative to control fruit.

Preharvest spraying of chitosan enhanced phenolic compounds and flavonoids up to 2.6-fold in strawberries compared to controls (Rahman et al., 2018). Furthermore, it was reported that the use of SA increased the levels of antioxidant compounds in table grapes (Gomes et al., 2021). Table 7 shows the potential effects of preharvest application of natural elicitors on phytochemical contents and antioxidant activity of *Barhi* fruit at harvest.

Table 7: Application effects of natural elicitors on phytochemical contents and antioxidant activity of *Barhi*

Treatments	Tannin (mg 100 g^{-1} CE)	Flavonoids (mg 100 g^{-1} CE)	ABTS (mg 100 g^{-1} TE)	IC_{50} (mg mL^{-1})
Control	108.73 ± 5.92 ^{d}	88.32 ± 1.32 ^{c}	338.45 ± 2.72 ^{f}	3.60 ± 0.34 ^{b}
Ch	126.55 ± 6.81 ^{b}	105.91 ± 2.28 ^{b}	427.63 ± 3.11 ^{e}	3.26 ± 0.54 ^{d}
Ca	136.17 ± 5.23 ^{a}	113.81 ± 1.05 ^{a}	638.61 ± 3.98 ^{b}	3.00 ± 0.21 ^{d}
SA	118.63 ± 6.41 ^{cd}	84 .52 ± 1.18 ^{d}	610.26 ± 2.89 ^{c}	3.66 ± 0.12 ^{b}
Ch + SA	130.06 ± 5.67 ^{a}	105.92 ± 1.76 ^{b}	633.85 ± 3.05 ^{bc}	3.77 ± 0.32 ^{a}
Ch + Ca	114.27 ± 6.31 ^{d}	89.11 ± 1.92 ^{c}	537.64 ± 4.21 ^{d}	3.49 ± 0.21 ^{c}
Ch + SA + Ca	120.25 ± 4.87 ^{c}	107.13 ± 2.31 ^{b}	682.05 ± 4.96 ^{a}	2.10 ± 0.40 ^{e}

Note: Values are the mean (n = 3) ± SE. Means with the same letter(s) in the same column are not significantly different at $p \leq 0.05$ using the LSD test. Ch: chitosan; SA: salicylic acid; Ca: calcium chloride.

These results in Table 7 are consistent with the small *Rutab* percentage (low ripening) and 0% decay noticed with the CH + SA + Ca treatment (see Figures 7 and Figure 8). In general, the concentrations of antioxidants in dates decrease as they move from the early developmental stage (Bisr) to the ripening stages as an example of *Rutab* and *Tamer* types (Awad et al., 2011b). These findings are in line with those obtained for TPC at harvest, (see Figure 7), suggesting that phenolic compounds play a critical role in the antioxidant capacity of "Barhi" fruit. In addition, a positive connection between antioxidant compound concentrations and antioxidant capability has been reported in five date cultivars, including the *Barhi* date (Awad et al., 2011b).

As a climacteric fruit, oxidative stress in date fruit is thought to be responsible for the decrease in antioxidant components (see Figure 7) during ripening, as a result of the decline in free radical scavenging capability (Mohamed et al., 2014). The formation of extra reactive oxygen species (ROS) such as superoxide and H_2O_2 may emerge and be implicated in the ripening and senescence of the date fruit, as reported with other fruits (Ferrer et al., 2005). According to these findings, applying Ch + SA + Ca before harvest boosted the amount of antioxidant compounds, which improved quality and shelf life, most likely by reducing the abundance of damaging radicals.

4.7 Fruit Ripening

As compared to the controls, all treatments significantly ($p \leq 0.05$) delayed fruit ripening during cold storage. Rutab percentage increased steadily during the cold storage period in all treatments, to different degrees (see Figure 8). By the end of the storage period, the greatest Rutab percentage was observed in the control fruit, followed by SA-, Ch + SA-, Ch + Ca-, Ch-, Ch + SA + Ca-, and Ca-treated fruit, with 90.3, 76.3, 73.1, 71.7, 61.0, 49.0, and 14.7%, respectively.

These results are consistent with the TSS results depicted in Figure 6, where the same treatments showed lower TSS content compared to other treatments, including controls. Additionally, the Rutab percentage showed a positive correlation with the increase in weight loss and TSS (see Table 6 (A-D)). Figure 8 shows the potential effects of preharvest application of natural elicitors on *Barhi* fruit's ripening percentage during cold storage at 2°C for two months.

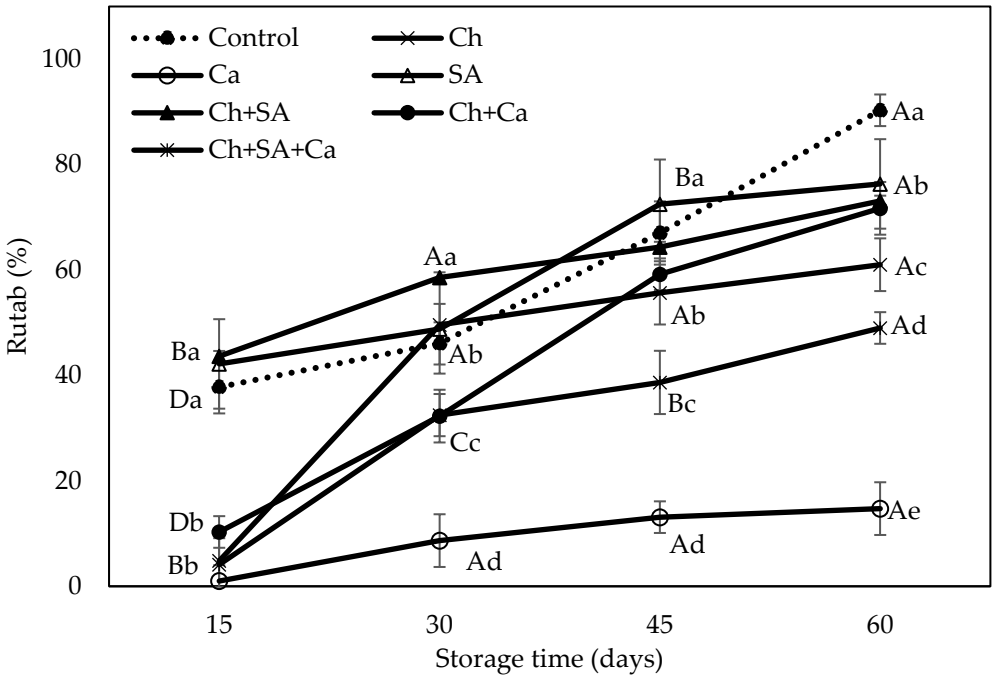


Figure 10: Preharvest application effects of natural elicitors on *Barhi's* ripening percentage during cold storage

Note: values are the mean (n = 25) ± SE. The LSD test at $p \leq 0.05$ was used to compare the means between treatments. Means with the same letter(s) between treatments within storage time intervals (small letter) and at different time intervals (capital letter) are not significantly different. Ch: chitosan; SA: salicylic acid; Ca: calcium chloride.

These findings might be regarded as evidence of elicitors decreasing "Barhi" fruit's ripening during cold storage. Preharvest treatment with SA also slowed the ripening of "Barhi" fruit by three weeks as compared to control fruit (Kassem et al., 2010). In addition, the capacity of Ch to form a film, which creates a barrier to gas exchanges and reduces respiration, can also delay fruit ripening. It is worth noting that a less ripe fruit is less likely to be exposed to postharvest rot (Romanazzi et al., 2018). Moreover, calcium treatment is well recognized for slowing respiration and delaying ripening in fruits (Irfan et al., 2013; Sohail et al., 2015; Atia et al., 2018).

The present results reveal the delaying impact of Ch, SA, and Ca in various combinations on fruit maturation and ripening in comparison to controls. Fruit ripening is a biological process that causes membrane not permeability and, ultimately, senescence as a result of membrane components' oxidation (Awad et al., 2011; Ahmed & Palta, 2015). Ethylene is believed to play a major role in ripening and senescence processes (Molamohammadi et al., 2020; Ahmed & Palta, 2016); as a result, it is possible that sprayed elicitors reduce fruit ripening by controlling ethylene production or activity (decline of Rutab occurrence in date fruit).

Date fruit maturation, however, is not an asynchronous phenomenon in all date fruits in the same bunch, since fruit at varying stages of differentiation can be found in the same bunch at any given time. Bisr fruits that received the same treatment, for example, may reach the Rutab stage at different periods in the same bunch. Thus, there are several significant biological differences between individual fruits (Mohamed et al., 2014). As a result, substantial inherent biological variations between individual fruits within the same bunch may be responsible for certain discrepancies.

4.8 Fruit Decay

All preharvest spray treatments significantly ($p \leq 0.05$) decreased the occurrence of fruit decay during cold storage compared to controls, to different degrees. The decay began after 28 days of cold storage and progressed throughout the storage period with the exception of the Ch + Ca and Ch + Ca + SA treatments, which showed 0% decay after 60 days of cold storage. Among all the treatments, and after 60 days of storage, the control treatment showed the highest decay percentage, with 58%, followed by the SA, Ch, Ca, and Ch + SA treatments with 35, 31, 14, and 7% decay, respectively. Figure 9 shows the percentage of fruit decay during 60 days of cold storage.

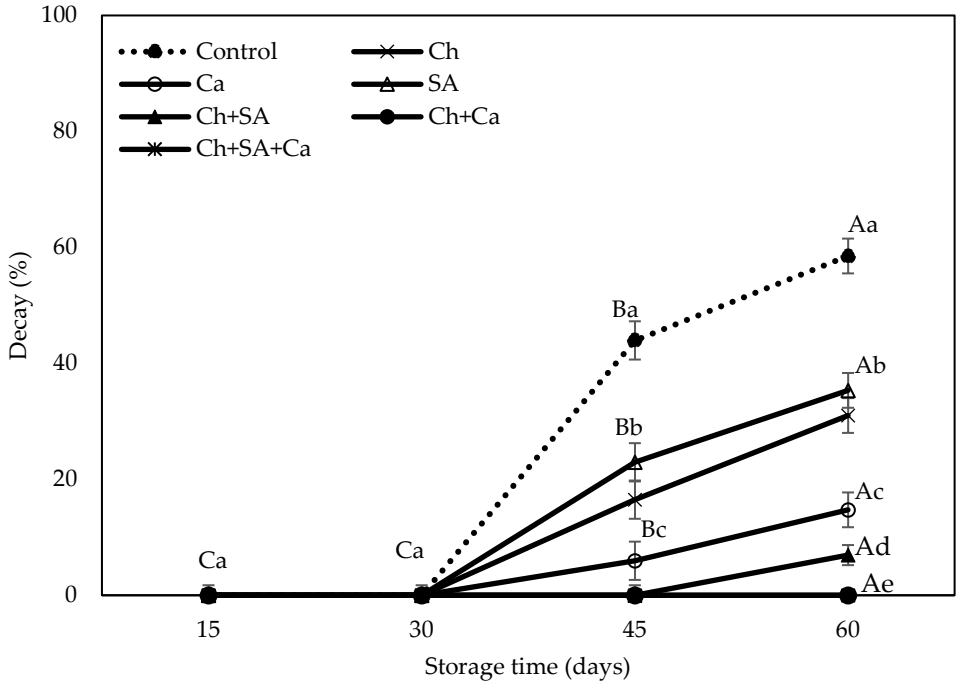


Figure 11: Effects of preharvest application of natural elicitors on *Barhi* decay percentage in cold storage

Note: Values are the mean (n= 25) ± SE. The LSD test at $p \leq 0.05$ was used to compare the means between treatments. Means with the same letter(s) between treatments within storage time intervals (small letter) and at different time intervals (capital letter) are not significantly different. Ch: chitosan; SA: salicylic acid; Ca: calcium chloride.

The results might be attributed to the protective effect of the applied elicitors. Similarly, Gomes et al. (2021) found that SA at concentrations of 1 and 2 mmol L^{-1} was effective in decreasing grapes' berry decay. Ca and AS, alone or in conjunction, delayed strawberry fruit' softening, resulting in less decay (Shafiei et al., 2010). The use of elicitors such as SA, Ca, and oxalic acid has been shown to stimulate defensive response and reduce rotting in other fruits (Shen & Yang, 2017; Wang et al., 2011; Tian et al., 2006). In the present study, the Ch + SA + Ca-treated fruit underwent no decay throughout the cold storage period (Figure 6), while the lowest Rutab percentage was found after Ca treatment (see Figure 8).

Additionally, there was a negative correlation between TPC and weight loss, decay, and Rutab%, indicating that TPC plays an essential role in the ripening and senescence of date fruit. The results of this study show that applying Ch in conjunction with Ca and SA could protect *Barhi* fruit from decay for a long period during cold storage, most likely via activating the fruit's defence mechanisms, resistance responses of fruit tissue, and/or inhibition of the development of decay-causing pathogens (Romanazzi et al., 2018). The high TPC in the fruit as a result of the slow ripening following Ch + SA treatment could be related to the reduced respiration rate, weight loss, and decay incidence, as well as the positive influence on TSS (Shen & Yang, 2017).

4.9 Microbiological Quality of Fruit

Overall, fungal/mould counts (FMCs) and total bacterial counts (TBCs) exhibited significant differences ($p \leq 0.05$) at harvest and at the end of the storage period, showing the impact of elicitor treatments. When compared to controls and other treatments, "Barhi" fruits treated with Ch, SA, Ch + Ca, and Ch + Ca + SA showed lower TBCs at day 0, while FMCs in Ch- and Ch + Ca + SA-treated fruits were significantly ($p \leq 0.05$) lower than in other treatments, including controls. At day 60, Ch + Ca + SA-treated fruits, followed by Ch + AS- and Ch-treated fruits, had lower TBCs relative to controls and other treatments, while the lowest FMCs were observed in the Ch and Ch + Ca treatments. Figure 10 shows the microbial counts (Log_{10} CFU g^{-1}) on "Barhi" fruit samples at harvest and by the end of the cold storage time.

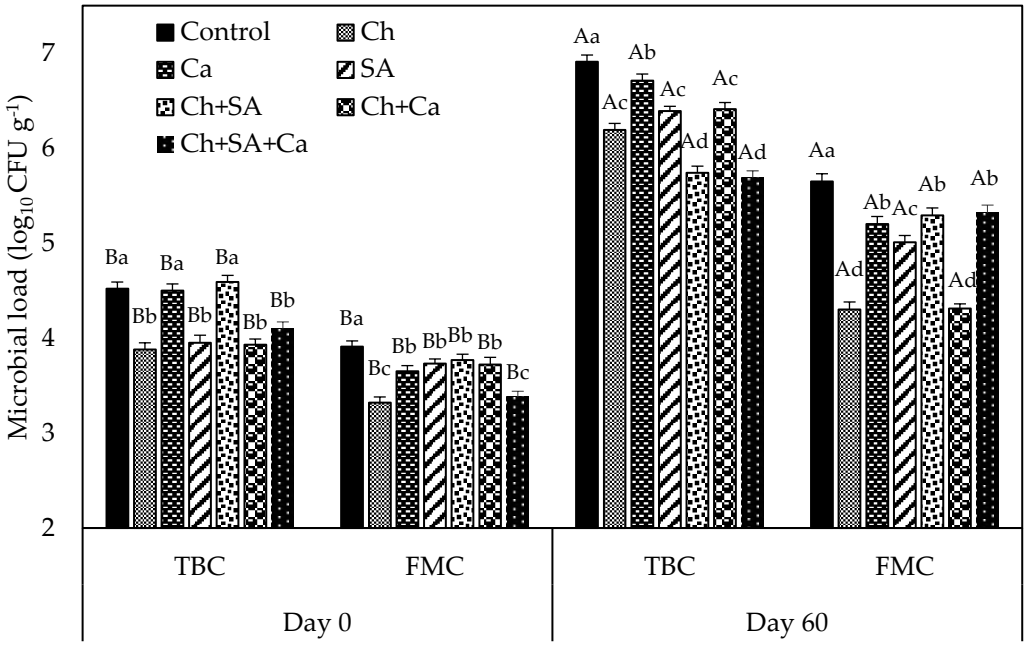


Figure 12: Preharvest application effects of natural elicitors on microbial counts on *Barhi* in cold storage

Note: Values are the mean (n = 3) ± SE. The LSD test at $p \leq 0.05$ was used to compare the means between treatments. Means with the same letter(s) between treatments within storage time intervals (small letter) and at different time intervals (capital letter) are not significantly different. Ch: chitosan; SA: salicylic acid; Ca: calcium chloride.

Among the various elicitors, SA showed the best results in inducing defensive reactions and minimizing decay in pear fruits (Tian et al., 2006). Chitosan has also been proven to boost plants' defenses against bacteria and fungi, among other pathogens (Romanazzi et al., 2018). Furthermore, either alone or in combination, the Ch and SA treatments significantly decreased the growth of fungi and bacteria in pistachio fruits (Molamohammadi et al., 2020). According to the current findings, Ch + SA + Ca had a greater antimicrobial impact than the other treatments, including controls. The same treatment had the lowest percentage of decay, as well as the greatest antioxidant activity, which could explain these microbial results (see Figure 6 and

Table 6). Accordingly, increasing phenols and antioxidant activity via the application of various elicitors, separately or in combination, may trigger defensive mechanism(s) that lead to disease resistance and an increase in “Barhi” fruit’s shelf life, as has been observed in other fruits (Romanazzi et al., 2018; Wang et al., 2011; Sathiyabama et al., 2014).

Chapter 5: Conclusion

The results of this study show that using different elicitor combinations enhances the biochemical properties of fresh *Barhi* fruit during maturity and storage. Comparing the combinations of the elicitor treatments, Ch + Ca- and Ch + Ca + SA-treated fruit showed no decay even after 60 days of storage. The Ca treatment, followed by Ch + Ca + SA, exhibited the greatest TPC and postponed the fruit ripening during cold storage. When compared to other treatments, Ch + SA showed the highest antibacterial and antioxidant activities, followed by the Ch + SA + Ca and Ch treatments. Based on these observations, it is possible that the delayed ripening and reduced fruit decay were due to the impact of the sprayed elicitors on the ripening process and pathogens attack, thereby decreasing *Barhi* fruit's senescence and shelf-life loss.

Accordingly, the Ch + SA + Ca and Ch + SA combinations of elicitors may be recommended for large-scale application. Furthermore, this eco-friendly approach using combinations of natural elicitors could be a more effective, reasonable, and sustainable way to improve *Barhi* date fruit's quality. Additionally, since no immediate-type allergic reactions to chitosan-based treatments have been reported, it is unlikely that these treatments would affect consumers' preferences towards treated-*Barhi* fruit.

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