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EFFECT OF NATURAL ELICITORS AND COLD STORAGEPERIOD ON QUALITY IMPROVEMENT OF UAE DATE PALM FRUIT (PHOENIX DACTYLIFERA L. CV. KHESAB)

Shamsa Salem Nasser Abdulla Alblooshi

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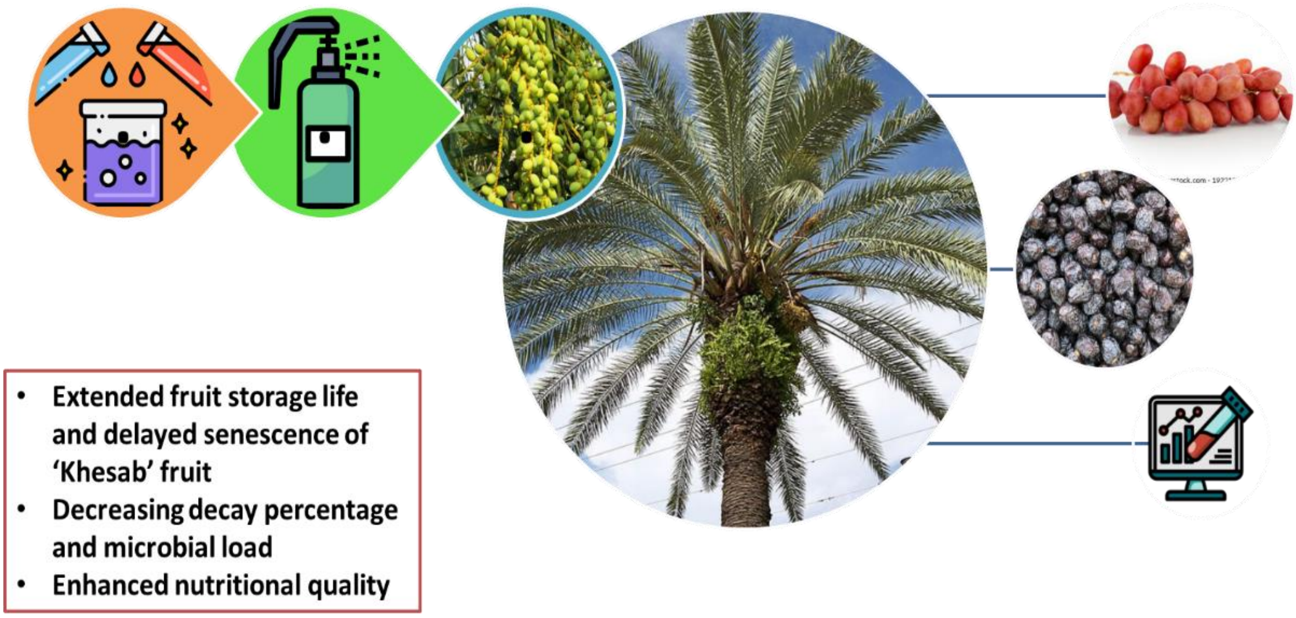

April 2022

United Arab Emirates University

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Shamsa Salem Nasser Abdulla Alblooshi

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

Under the Supervision of Dr. Zeinab Ahmed

April 2022

Declaration of Original Work

I, Shamsa Salem Nasser Abdulla Alblooshi, the undersigned, a graduate student at the United Arab Emirates University (UAEU), and the author of this dissertation entitled "*The Effect of Natural Elicitors and Cold Storage Period on Quality Improvement of UAE Date Palm Fruits (Phoenix dactylifera L., cv. Khesab*)" hereby, solemnly declare that this dissertation is the original research work that has been done and prepared by me under the supervision of Dr. Zeinab Ahmed, in the College of Agriculture and Veterinary Medicine at UAEU. This work has not previously been presented or published or 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 Any materials borrowed from other sources (whether published or unpublished) and relied upon or included in my dissertation have been appropriately cited and acknowledged by appropriate academic conventions.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 dissertation.

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Abstract

Date palm (*Phoenix dactylifera* L.) is one of the most important fruit trees grown in arid and semi-arid regions. Despite the immense capabilities of date palm, it is still a challenge to maintain fruit quality, marketability and shelf life. The objective of this study was to investigate the synergistic effect of a preharvest spray application of the natural elicitor chitosan (Ch) 1% alone and in combination with salicylic acid (SA) 2 mM and calcium chloride (Ca) 3%; (Ch, SA, Ca, Ch+Ca, Ch+ SA, Ch+ SA +Ca) on the quality parameters, shelf life and bioactive compound content of date fruit of the cultivar 'Khasab' during a cold storage period of 60 days. The results showed that all treatments significantly delayed fruit senescence/decomposition compared to the control. Fruits treated with Ch+ SA, followed by Ch and Ch+ SA +Ca had the least weight loss, colour change and decay after 60 days of storage. Fruit treated with Ch+Ca, SA, and Ca had significantly lower total soluble solids values and the highest phenolic, tannin, and flavonoid contents compared with control fruit. Antioxidant activities were observed in all treatments, with a significantly higher effect in Ch+ SA +Ca and Ch+ SA compared to the control. Our results show a synergistic effect of combining triggers to extend the shelf life of date fruit during cold storage by maintaining quality and reducing senescence/deterioration, and we recommend this as a promising strategy.

Keywords: Date Fruit, Natural Elicitors, Synergistic Effect, Salicylic Acid, Chitosan, Storage Quality, Shelf-Life, Marketability.

Title and Abstract (in Arabic)

تأثير بعض المحفزات الطبيعية والتخزين المبرد على تحسين جودة ثمار نخيل التمر صنف الخصاب في دولة الإمارات

الملخص

يعتبر نخيل التمر من اهم أشجار الفاكهة التي تنمو في المناطق الجافه وشبه الجافه. على الرغم من الإمكانيات الهائلة للنخيل، إلا أن الحفاظ على جودة الثمار الطازجة وقابليتها للتسويق وفترة صلاحيتها لا يزال يمثل تحديًا كبيرا. لذلك كان الهدف من هذه الدراسة تقييم التأثير التارزي لمعاملات الرش قبل الحصاد بمركب الكايتوسان الطبيعي 1٪ (Ch) بمفرده أو بالخلط مع حمض الساليسيليك 2 ملي مول (SA) وكلوريد الكالسيوم (Ca) 3٪؛ أو خلط الثلاث ث مركبات معا (Ch + SA + Ca)، وذلك من خلال دراسة معايير جودة ومدة التخزين ومحتوى المركبات النشطة بيولوجيًا لثمار التمر من صنف "الخصاب" أثناء التخزين البارد لمدة 60 يومًا. أظهرت النتائج أن جميع المعاملات تؤخر بشكل معنوي شيخوخة الثمار وتدهورها مقارنة بالثمار الغير معاملة (الكونترول). الثمار المعاملة بالكايتوسان مع حمض الساليسيليك تليها الثمار المعالجة بالكايتوسان فقط ثم الفاكهة المعاملة بالكايتوسان مع حمض الساليسيليك وكلوريد الكالسيوم أظهرت انخفاض في فقد الوزن وتغير في اللون وأقل تدهور حتى بعد 60 يوم من التخزين البارد. بينما أظهرت الثمار المعاملة بالكايتوسان مع كلوريد الكالسيوم وكذلك المعاملة بحمض الساليسليك فقط أو بكلوريد الكالسيوم فقط أظهرت بشكل ملحوظ أقل تغير في المواد الصلبة الذائبة الكلية، وأعلى محتوى إجمالي للمركبات الفينولية والتانينات والفلافونيدات مقارنة بالثمار الغير معاملة. أما بالنسبة الى النشاط المضاد للأكسدة وجد أن جميع المعاملات أظهرت نشاط لمضادات الأكسدة بالمقارنة بالكنترول. التأثير الأكبر كان ملحوظا على الثمار التي تم معاملتها بالكايتوسان وحمض الساليسيليك فقط والثمار التي تمت معاملتها بالكايتوسان مع حمض الساليسيلسك وكلوريد الكالسيوم. أظهرت نتائج هذه الدراسة دليلًا على أن المحفزات الطبيعية لها تأثير تآزري في إطالة عمر ثمار التمر أثناء التخزين البارد من خلال المحافظة على جودتها وتأخير الشيخوخة وتقليل التدهور. توضح النتائج أن استخدام المحفزات الطبيعية في تحسين جودة الثمار له نتائج واعدة وهي استراتيجية يوصى بها للاستخدام تجاريا على أصناف التمر الأخرى أو ثمار الفواكه الأخرى.

مفاهيم البحث الرئيسية: فاكهة التمر، المستخلصات الطبيعية، التأثير التارزي، حمض الساليسيليك، الكايتوسان، جودة التخزين، مدة الصلاحية، القابلية للتسويق

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Dedication

To my country the United Arab Emirates, my young self that did not expect me to go this far, my parents, my siblings God's greatest blessing, my teachers, instructors, and professors. Thank you for believing in me even when I could not. You made me who I am, and I am forever grateful for that.

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Chapter 1: Introduction

1.1 Date Palm

Date palm (*Phoenix dactylifera* L.) is one of the most important fruit trees grown in arid and semi-arid regions. The palm family (*Arecaceae*) is comprised of 183 genera and over 2,400 species. Five major palm species are domesticated fully and are grown as economic species, date palm (*Phoenix dactylifera*) is one of them. Date palm is a diploid (2n=36), perennial and monocotyledonous (Haider et al., 2013). The tree thrives in the hot and dry regions of Middle Eastern and North African countries and is highly valued as a 'blessed tree' known since ancient times. It is an essential part of the cultural heritage, traditional books, and poems of Arabian countries. The date palm substantially contributes to the economic development of these countries (Gantait et al., 2018; Mattar et al., 2021).

Despite the changes throughout the years, dates continue to play an essential role in the region (Ahmed et al., 1995). The date palm has intangible value in desert climates including creating shade and microclimatic conditions suitable for the growth of other crop plants along with functioning as windbreaks and stabilizing earthen irrigation works (Johnson, 2012). In other areas that have different climates including the Americas, southern Europe, Asia, Africa and Oceania, date palm is grown as an ornamental plant (Dransfield et al., 2005).

1.2 Date Fruit

Date palm tree usually at the age of 5 years produces 400–600 kg of date fruit per tree in a year and the fruiting continues up to 60 years. The fruit are arranged on spikelets bearing 20-60 individual dates, and several such spikelets are attached to a central stalk to form a bunch, each tree has between 5 to 30 brunches (Ahmed et al., 1995). Date fruit can be sold fresh or dried, and they can be stored well under low-temperature conditions (Johnson, 2012). The consistency of the fruit varies from soft to dry and the colour varies from yellow to black or from red\dark pink to black depending on the type of the cultivar. Each fruit is a one-seeded berry consisting of a fleshy mesocarp covered by a thin epicarp and the seed is ventrally grooved with a hard endosperm made of a cellulose deposit and usually oblong with a small embryo.

The characteristics of the date fruit and seed can vary depending on the environmental conditions, field management and the cultivar itself. (Ahmed et al., 1995; Zaid & de Wet, 2002; Al-Yahyai & Kharusi, 2012, Ahmed et al., 2022a). When it comes to date fruit production, the top ten producing countries around the world are Egypt, Saudi Arabia, Iran, Algeria, Iraq, Pakistan, Sudan, Oman, Tunisia and the United Arab Emirates, as shown in Figure 1.

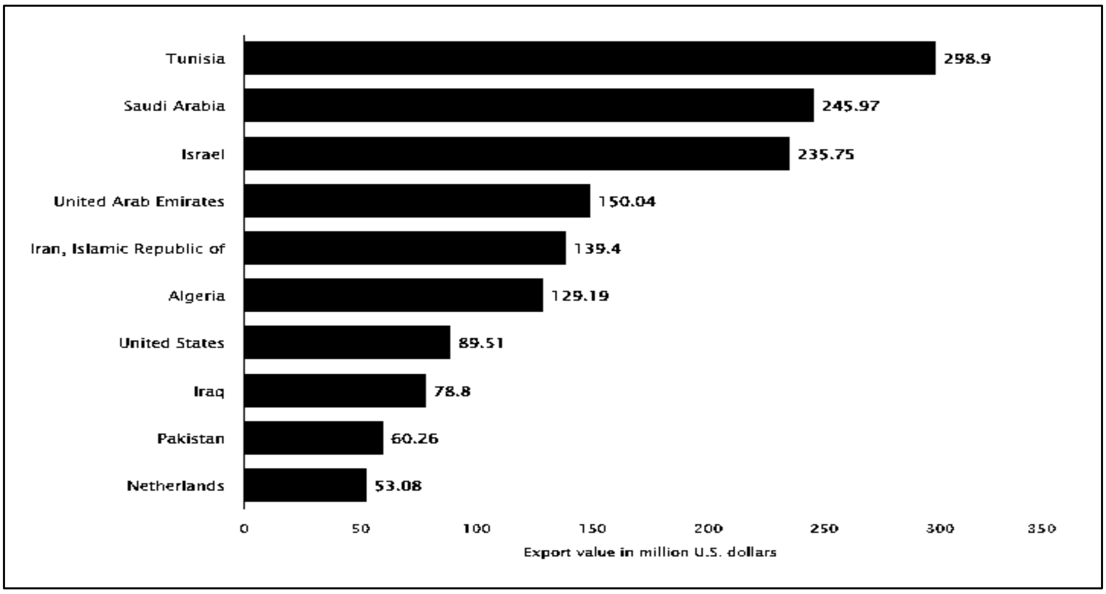

Figure 1: Major date producing countries in the world (FAO, 2020)

On the other hand, top ten exporting countries around the world are Tunisia, Saudi Arabia, UAE, Iran, Algeria, the USA, Iraq, Pakistan, and Netherlands, as shown Figure 2.

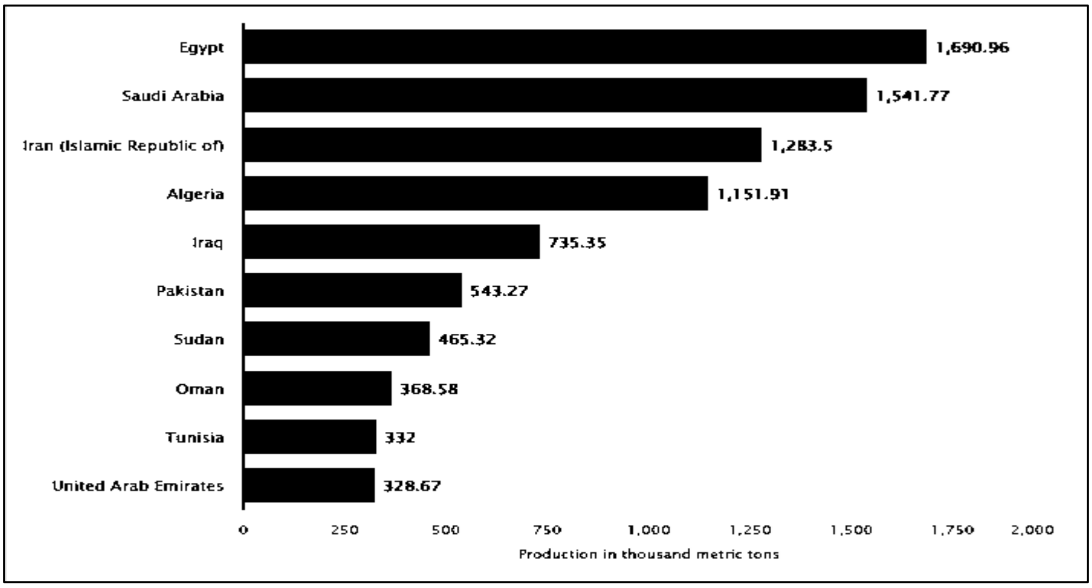

Figure 2: Major date fruit exporter in the world (FAO, 2020)

1.2.1 Fruit growth, development and maturity stages

The maturity of the date fruit varies depending on the cultivar, some of them mature early in the season and the others do not mature until the end of the season. the mature fruit can vary widely in its organoleptic, physical, and chemical characteristics (Ahmed et al., 1995). yet, all the edible fruits go through the same five stages which under the Arabic terminology, Hababouk, Kimri, Bisir or Khalal, Rutab and Tamer in that particular order (Awad et al., 2011a). These stages characterize the cell division, immature green (cell elongation), mature hard full coloured, soft brown, and firm raisin-like fruit, respectively (Awad et al., 2011b).

In general, the harvesting and marketability of the date fruit depend on the cultivar in the context of the level of astringency, physiological conditions, and public demands, for this reason, they are delivered to the market at three stages which are: Bisr (full size, crunchy, coloured and mostly edible), Rutab (the fruit apex becomes soft and brown, most cultivars consumed fresh at this stage) and Tamer (hazel to dark brown, wrinkled, raisin-like dry fruit) (Mohamed et al., 2014).

After fertilisation, the *Hababouk* stage comes, and it is the stage where the loss of two unfertilized carpels occurs. The colour of the date fruit in this stage is creamy to light green and the fruit is not edible yet. Following the *Hababouk* stage, the Kimri stage comes, which is the green stage which lasts about 9 weeks depending on cultivar and location. The date fruit in the Kimri stage is having high water content. The third stage is Bisir which is also known as Khalal, it lasts about 4 to 5 weeks. The fruit attains physiological maturity with a slight decrease in size, weight, and starch content at Khalal stage (Al-Mssallem et al., 2013). In the Bisir stage the colour of the date fruit change from green to either yellow, pink, or red, or yellow spotted with red depending on the cultivar.

The fourth stage is Rutab, and it is the stage where the date fruit starts to accumulate reducing sugars and losing weight. At this stage, the fruit becomes softer, and the colour turns to light brown. The fruit progressively loses water (30–45%), and starch is converted to sugars during the Khalal and Rutab stages (Figure 3). The fifth and the last stage of the date fruit maturation is Tamer. In the Tamer stage, the date fruit is very sweet, its colour is dark brown, and its texture is soft with very low moisture content. The very low moisture content of the fruit in the Tamer stage leads to it is ideal for long-term storage to be consumed during off-season (Kader and Hussein, 2009).

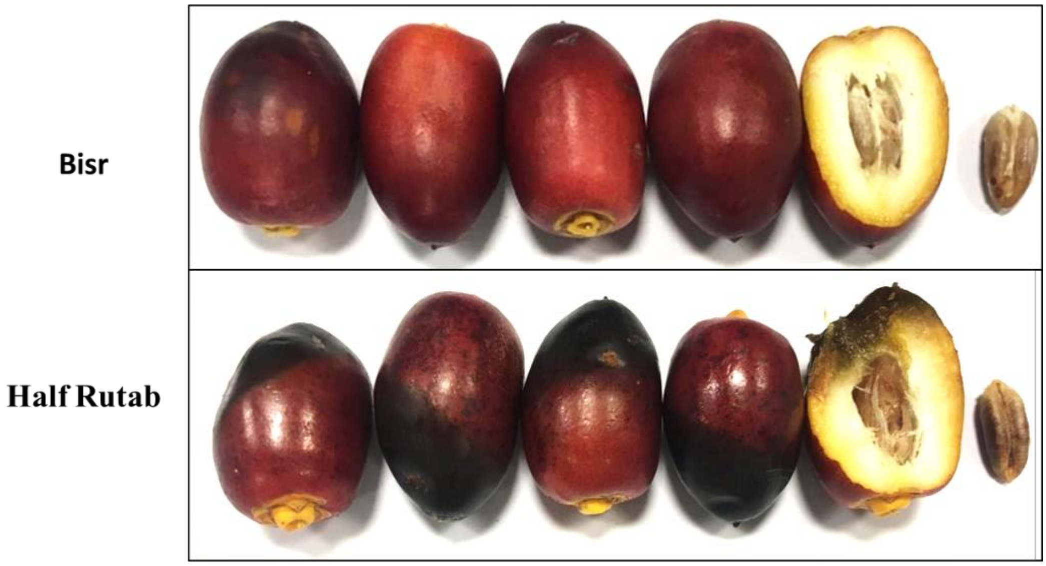

Figure 3: The Bisr and half Rutab fruit maturity stages of Khesab date fruit

1.2.2 Nutritional value and health benefits of dates

Apart from the agricultural and commercial importance of the date fruit in the Arabic Gulf region, the fruit has high nutritional value including dietary fibres (6.4-11.5%), carbohydrates (total sugars, 44-88%), proteins (2.3-5.6%), fat (0.2-9.3%), vitamins, minerals, phenolic, tannins, and antioxidants compounds (El Hadrami and El Hadrami, 2009; Vayalil, 2013; Al-Alawi et al., 2017; Al-Mssallem et al., 2020; Bentrad and Hamida, 2020). A study in the UAE showed that the date is an important source of potassium for regular consumers, and it stated that it provides the Arab world with a remarkable dietary component (Ahmed et al., 1995). In addition, date fruits are a good source of vitamin B complex, such as thiamine (B1), riboflavin (B2), niacin (B3), pantothenic (B5), pyridoxine (B6), and folate (B9) (Chao and Krueger, 2007; Al-Harrasi et al., 2014). Depending on many factors including the soil type, agronomic practices, cultivar type and fruit maturation stage, the nutritional composition can differ (Al-Farsi et al., 2007; Amira et al., 2012).

Date flesh is a readily available source of energy, and this is because of the availability of high content of reducing sugars such as glucose and fructose (70-85% fresh weight) (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., 2008; Mohamed et al., 2014). Studies on date fruit showed that they can be beneficial when they are being consumed, where the polyphenolics account for the broad range of biological effects of dates among the other dietary components that the fruit include. This biological effect includes antimutagenic and antioxidant properties (Vayalil, 2002; Duthie et al., 2003).

The dietary fibres in dates also modulate the immune system and play a major role in the prevention of cardiovascular diseases (Frankel et al., 1993). Dates have anti-viral, anti-bacterial and anti-fungal properties which inhibit chronic inflammations, and this is because of the presence of phenolics (Kaul & Khanduja, 1998). The aqueous and ethanolic extracts of dates are effective in improving the severity of gastric ulceration (Al-Qarawi et al., 2003). Other human health benefits come from the hydroxycinnamates and flavonoids including tannins which are famous for being beneficial against cancer and cardiovascular diseases (Biglari et al., 2008).

1.3 Date Palm Cultivars

Date palm holds the distinction of having the greatest number of named cultivars based on the fruit characteristics among the other types of palms. Date fruit overwhelmingly represent the economic value of the tree (Johnson, 2012). Depending on the flesh consistency and moisture content at the fully-ripen stage, date palm cultivars can be divided into three groups which are namely soft, semi-dry and dry (Yahia & Kader, 2011). The major soft cultivars are *Hillawi, Abada, Amhat, Barhi, Bentaisha, Halawy, Hayani, Honey, Khadrawy*, and *Medjhool*.

During ripening, sucrose is converted into invert or reducing sugars (glucose and fructose) in soft cultivars with a moisture content greater than 30%. Cultivars such as *Badrayah, Bartamoda, Deglet Beida, Horra, Sakoty*, and *Thoory* are dry date cultivars with <20% moisture. Cultivars with 20–30% moisture are the semi dry-date cultivars which include *Amry, Dayri, Deglet Nour, Khalas, Sewy,* and *Zahidi*. In addition to reducing sugars, both dry and semi-dry dates retain a good amount of sucrose on full ripening (Chao and Krueger, 2007). Date palm cultivars are different in fruit characteristics, size and sugar content and this leads to more classifications that can be found within the same group.

1.3.1 Khesab cultivar

Khesab cv. is considered a late-maturity type of date palm cultivar. it has a high productivity character, and it can be consumed in the Rutab and tamer maturity stage. The colour of Khesab cv. varies depending on the stage of maturity. In the Bisir stage, the colour is red, brownish-red in the Rutab stage and dark brown when it is in the Tamer stage. The shape of the fruit is oval and oblong, the ratio of the weight of the fleshy part to the seed is large (Ahmed et al., 2021a). The flowering of Khesab cv. is at the end of February and the harvesting process take a place in the middle of August. Khesab cv. is resistant to salinity and moisture, it is also slightly frost-resistant which means that it can be stored in low-temperature conditions.

Khesab cv. is a quite common cultivar in the UAE that is preferred to be consumed at the half or full Rutab stages (Figure 3). However, upon harvesting, the fruit starts losing its firmness, colour, taste, and overall physiology which eventually lowers its market value (Al-Qurashi & Awad, 2011). These changes are outcomes of the postharvest activity of various ripening related enzymes and hormones that soften the fruit tissue, decrease the firmness and actual integrity of the fruit, and ultimately lead to the fruit's senescence (Abu-Shama et al., 2020). Therefore, slowing down the ripening process and senescence is critical in terms of commercial advantages (Mohamed et al., 2014). It is important to hold the date fruits in cold storage at a fully mature stage to extend their shelf life of it (Abd Elwahab et al., 2019 Ahmed et al., 2021a). In recent years, cold storage of date fruits has received more attention in the major date producing countries. Date industries usually store dates at 3°C for up to a year (Ismail et al., 2008; Al-Yahayai & Al-Kharusi, 2012).

1.4 Use of Natural Elicitors

The application of natural elicitors is used to enhance physiological adaptations and accelerate the plant's c defence system (Ahmed and Palta 2010; Baenas, 2014; Moreno-Escamilla et al., 2018). Many different studies on pre and postharvest applications have gained success in different fruit crops by using eco-friendly elicitors such as chitosan (Romanazzi et al., 2013; Shen & Yang, 2017; Rahman et al., 2018; Romanazzi et al., 2018; Abu-Shama et al., 2020), salicylic acid (Zhang et al., 2003;

Sayyari et al., 2009; Luo et al., 2011; Mohamed et al., 2014; Chen et al., 2020; Gomes et al., 2021), and calcium chloride (Irfan et al., 2013; Sohail et al., 2015; Atia et al., 2018), retarding tissue softening, delaying ripening, enhancing quality, extending shelf life, and preventing fruit deterioration.

Chitosan is one of the most recognized natural elicitors known for its antimicrobial, eliciting, and film-forming characteristics which is widely used in agriculture as a soil modifier, coating films, fungicide and elicitor (Romanazzi et al., 2013; Katiyar et al., 2014; Moreno-Escamilla et al., 2018; Rahman et al., 2018; Romanazzi et al., 2018, Ahmed et al., 2021a). It is a partially deacetylated derivative obtained from the chitin of crustacean shell wastes which is considered the second most abundant and important natural biopolymer in the world after cellulose (Hassan & Chang, 2017). Chitosan can control plant diseases and it induce resistance in the host plants and enhance biodiversity in the rhizosphere (Hassan & Chang, 2017). Pre- and post-harvest studies in different fruit crops have shown the positive effects of chitosan on the quality and the shelf and storage life of fruit and vegetables (Romanazzi et al.,2013; Shen & Yang, 2017; Romanazzi et al., 2018; Rahman et al., 2018, Ahmed et al., 2021b).

Salicylic acid (SA) is a simple phenolic phytohormone with various roles in plant growth and developmental processes. It also shows great potential for preventing the deterioration of fruits and vegetables postharvest (Shen & Yang, 2017, Ahmed et al., 2022b; Khalil et al., 2022). Salicylic acid can dramatically enhance disease resistance in mango fruit. In addition to that, studies stated that pre-harvest and post-harvest treatments of salicylic acid improved the quality and shelf life of table grapes (Pila et al., 2010). Salicylic acid preserves firmness reduces weight loss, colour progress, and disease incidence in plums, strawberries, peach and pears fruits for up to five weeks without decay (Khadiga, 1993).

Calcium chloride is another natural elicitor that has a well-known role in the physiology of plant tissue. It is also considered the most important mineral element which determines fruit quality (El Badawy, 2012). The use of calcium chloride after the fruit has been harvested maintains cell turgor, membrane integrity, tissue firmness, and delays membrane lipid catabolism (Atia et al., 2018). Calcium improves the rigidity of cell walls and obstructs enzymes such as polygalacturonate from reaching their active sites, thereby retarding tissue softening and delaying ripening. The use of calcium chloride after the fruit has been harvested maintains cell turgor, membrane integrity, tissue firmness and delays membrane lipid catabolism thus extending the storage life of fresh fruits (Pila et al., 2010).

These elicitors exert their effects by employing different mechanisms such as triggering the synthesis of phytochemicals, enhancing the production of particular antioxidant enzymes, and reducing ethylene production in vegetables and fruit (Ruiz-García & Gómez-Plaza, 2013; Baenas et al., 2014; Romanazzi et al., 2018). 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 the formation of a chitosan-g-salicylic acid (CTS-g-SA) conjugate, which has excellent antibacterial effects and water solubility (He et al., 2011).

1.5 Date palm Fruits in the UAE

In the UAE, the 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 the crop being farmed in various parts of the nation, especially in Al Ain and Abu Dhabi emirates. 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). Khesab cv. can be consumed at Rutab and tamer stage. Khesab cv. has a slow ripening which means that it has late maturity (Ahmed et al., 2021a). Therefore, slowing down the ripening process and retarding fruit senescence is critical for enhancing the marketability and storage life when it is not the season where it is consumed. To the best of our knowledge, there are no reports on the synergistic effects of different natural elicitors on Khesab cv. fruit particularly as preharvest spray treatments.

1.6 Research Objectives

(a) To explore the synergistic effects of natural elicitors such as chitosan, calcium chloride and salicylic acid as preharvest spray treatments on fruit quality and marketability of Khesab cv. variety fruits.

(b) Study of the effect of chitosan in combination with calcium chloride and/or salicylic acid on the shelf life and physicochemical constituents of Khesab cv. fruit.

(c) Investigation of the effects of chitosan in combination with calcium chloride and/or salicylic acid on the storability of fruit of the variety Khesa during two months of cold storage.

Chapter 2: Literature Review

2.1 Introduction

In general, date palm and all crops can get affected by diseases that can affect the fruits and the shelf life of the crops. The use of chemical pesticides and treatments helps preserve the quality of the fruit and extend shelf life. However, this method has been facing some challenges due to the phytotoxicity of this chemical preservative, development of antimicrobial resistance, and food safety concerns regarding the effects of chemical residues on human health and the environment (Shen & Yang, 2017). Therefore, safer, more effective, economical, and convenient storage methods are needed to continue production without affecting human health and the environment (Hassani et al., 2004). The main aim is to extend the shelf life of the fruit, and one of the methods to achieve this goal is using natural elicitors (Baldwin et al., 2011). Elicitors can mimic the action of the signalling molecules, activate defence responses and trigger and mediate long-lasting systemic acquired resistance (SAR), which protects against a wide range of pathogens that causes fruit decay (Baenas, 2014; Chen et al., 2018; Moreno-Escamilla et al., 2018).

2.2 Chitosan as Natural Elicitors

In recent decades, the biodegradability of natural compounds from animals and plants has attracted the interest of plant pathologists. Many studies have been conducted to delay the postharvest quality changes of date fruits. An alternative approach is to use natural triggers to enhance physiological adaptations and accelerate the plant defence system rather than different approaches such as cold storage, modified atmosphere packaging (Alsawmahi et al., 2018), and controlled atmosphere storage (CA) (Baloch et at., 2006; Baenas et al., 2014; Alhamdan et al., 2015; Aleid & Saikhan, 2017; Alsawmahi et al., 2018; Moreno-Escamilla et al., 2018). Chitosan has received increasing attention due to its fungicidal action and triggering of defence mechanisms in plant tissues (Terry & Joyce, 2004). Chitosan is an N-acetylated derivative of the polysaccharide chitin. It is a natural polymer with a polycationic nature. Chitosan has numerous agricultural applications (e.g., soil conditioner, film, fungicide, trigger), agroindustry, cosmetics, environmental protection, biomedicine, and wastewater management) (Katiyar et al., 2014). Many studies have shown the high potential of chitosan for preserving fresh fruits and vegetables (Rhoades & Roller, 2000).

2.2.1 Preharvest application of chitosan

Several studies on pre-and postharvest applications have shown proven success with various fruit crops using environmentally friendly triggers, such as chitosan (e.g., Romanazzi et al., 2013; Shen & Yang, 2017; Romanazzi et al., 2018; Rahman et al., 2018; Abu-Shama et al., 2020), and salicylic acid (Zhang et al., 2003; Sayyari et al., 2009; Luo et al., 2011; Mohamed et al., 2014; Chen et al., 2020; Gomes et al., 2021), and calcium chloride (Irfan et al., 2013; Sohail et al., 2015; Atia et al., 2018). These elicitors lead to retarding tissue softening, delaying ripening, enhancing quality, extending shelf life, and preventing fruit deterioration. In addition, pre-harvest spraying with chitosan is highly functional and has a beneficial effect on fruit quality attributes (Reddy et al., 2000).

The effect of chitosan on controlling plant diseases and pathogenic microorganisms stated that for achieving the goal of sustainable agriculture, chitosan would become a popular plant protectant (Hassan & Chang, 2017). The effects of natural elicitors on the quality of lettuce showed that the application of chitosan exerted a fungistatic effect that reduced the yeast and mould population throughout storage and decreased the activities of PPO and POD, enzymes related to browning processes. Microscopical observations indicate that chitosan directly affects the morphology of the chitosan-treated microorganism, reflecting its fungistatic or fungicidal potential. Other studies strongly suggest that chitosan induces a series of defence reactions correlated with enzymatic activities (e.g., Banos et al., 2006).

Preharvest application of chitosan onto kiwi fruit has 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 a postharvest 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 beneficial for improving strawberries' quality and antioxidant capacity (He et al., 2018). The treatment of chitosan in combination with calcium chloride (Ca-chitosan) at the preharvest stage in kiwi fruit resulted in enhancing fruit quality and postharvest life (Kim, 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 the accumulation of phenolic compounds and more excellent resistance to Botrytis cinerea decay. The preharvest treatment of strawberries with chitosan, laminarin, extracts of Abies spp., Polygonum spp., Saccharomyces spp., calcium and benzothiadiazole provided ~ a 30% reduction in postharvest decay against grey mould and Rhizopus rot, without affecting fruit colour and firmness (Feliziani et al., 2015). Previous studies have shown that chitosan reduces decay incidence, mainly caused by Botrytis cinerea in tomatoes (El Ghaouth et al., 1992), decreases microbial count, and elicits (Kibar & Sabir, 2018). In another experiment, chitosan significantly reduced the disease lesion size on tomato fruit when the tomato plants were pre-treated with 1.0% or 2.5% (w/v) COS solution ten days before being inoculated with Colletotrichum sp. (Munoz et al., 2009).

2.2.2 Postharvest applications of chitosan and their derivatives

Increasing polyphenol oxidase and peroxidase enzyme activities, change in acidity, anthocyanin and minimising the growth of mesophilic aerobic yeasts. Moulds populations can be achieved when blueberries are coated with chitosan, silicon and titanium dioxides (Li et al., 2021). Treating mangos by coating them with chitosan in combination with aloe vera gel leads to suppressed diseases, maintains mango fruit's natural properties during postharvest storage, and extends 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. In addition, the CCF film increased the content of resistance compounds, and the activity of defence enzymes enhanced the yield and quality and prolonged the shelf life of kiwifruit (Zhang et al., 2020). Furthermore, Khatri et al. (2020) indicated that the efficiency of aloe vera gel and chitosan coatings in prolonging the postharvest- the life of tomatoes showed that 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) reported the influence of calcium chloride with nano chitosan coating on the quality of strawberries during postharvest storage resulted 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 d of storage at 4°C. Studies reported that chitosan could be more effective in the postharvest physiology of fruit crops when it is combined with other additives, including salicylic acid (Huang et al., 2017), potassium silicate (Mohamed et al., 2017), calcium chloride (Kim, 2018), gum Arabic (Khaliq et al., 2016), lactoperoxidase (Cissé et al., 2015), and antagonistic yeast (Meng & Tian, 2009). After harvesting coating with 1.0%, chitosan showed that the quality attributes of apple fruits could be maintained for up to 80 days during storage at 18±2°C and 56 ± 2% RH (Zeb et al., 2020).

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

Using chitosan and calcium chloride individually and in combination as a pre-treatment of guava fruit leads to a significant delay decline in physiological loss of weight, enhanced total soluble solids firmness, acidity, and ascorbic acid, sugars, phenols and total antioxidant activity during storage (Chawla et al., 2018). In addition, chitosan, potassium silicate, and calcium chloride coating on mangos showed an increase in fruit storability and shelf life compared to the mangos treated individually or not been coated from the beginning (Mohamed et al., 2017).

Romanazzi et al. (2018) reported that using the treatment of chitosan mixed with ethanol, wax, and similar types of organic materials improved the protecting effect on grapes from grey mould compared to the application of using chitosan alone. Furthermore, Gayed et al. (2017) reported that 2% CaCl2+1% chitosan was most effective in minimising weight loss (%) and decay (%), as well as in maintaining maximum firmness and lengthening the shelf life of peach fruits. Furthermore, when using chitosan integrated with plant derivatives like plant extracts and essential oils, organic salts and acids, and antagonistic microorganisms, including yeast and bacteria, results show that it effectively reduces 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). Using chitosan as a postharvest treatment has been studied for efficacy in inhibiting decay and extending the 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). The use of chitosan integrated with essential oil including lime, thyme, bergamot, clove and cinnamon leads to a reduction of the appearance of fungus *B. cinerea*, *C. gloeosporioides, P. digitatum* and *Phytophthora drehsleri*, and yeasts and moulds on strawberries, figs, avocado, cucumber, mandarin and grapes (Mohammadi et al., 2015).

Coatings, the combination of chitosan with oleic and acetic acid, reduced disease symptoms for ten days. They decreased decay caused by fungi and bacteria in strawberries and prickly pears (Ochoa-Velasco & Guerrero-Beltrán, 2014, Xylia et al., 2021). Velickova et al. (2013) reported that chitosan-beeswax coatings decreased the senescence and weight loss of strawberries, modified the respiration rates and slowed down metabolism by retaining the colour and the texture of the fruit. Combining 1% chitosan with 3% ammonium carbonate solutions and 2.5% calcium reduces the severity of anthracnose and incidence in papaya during 14 days of storage (Al-Eryani-Raqeeb et al., 2009). Coating strawberry 'Camarosa' with 1.5% chitosan and 0.5% calcium gluconate showed no visible symptoms of disease caused by moulds during the whole storage period at 10°C (Hernández-Muñoz et al., 2008).

2.2.3 Treatments with chitosan and combination of calcium chloride and salicylic acid

Chitosan and salicylic acid treatment on fresh pistachio enhanced fruit quality in refrigerated storage with prolonged shelf life. The fruit was lighter, redder, and more yellow with the highest sensory scores for colour, texture, and overall acceptance with reduced growth of bacteria and fungi (Molamohammadi et al., 2020, Ahmed et al., 2021b). Preharvest treatments of calcium spray with the postharvest treatments of chitosan coating on the Chinese dwarf cherry resulted in an extension in the storage time and reduced fruit decay rate at the end of storage. In addition, they improved the quality of the fruit for up to 30 days (Guo et al., 2020). Treating the fresh-cut apples with chitosan combined with calcium chloride showed lower weight loss, improved appearance reduced browning and enhanced ascorbic acid content (Liu et al., 2016).

The integrity of soluble pectin is shown to be maintained in the fresh-cut honeydew melon when using the edible coating of chitosan combined with calcium chloride that extends the shelf-life of the fruit (Ahmed and Palta 2011, Chong et al., 2015). Furthermore, postharvest treatment of pomegranate with salicylic chitosan exhibited lower weight loss, respiration rate and ethylene production associated with higher firmness, total soluble solids, and titrable acidity as sensory quality (Sayyari et al., 2016). In addition, Vyas et al. (2016) reported that salicylic acid treatment combined with calcium chloride and sodium benzoate effectively enhanced antioxidants, ascorbic acid, and total anthocyanins in phalsa fruit (*Grewia asiatica* L.). More treatments of chitosan in combination with other treatments is reviewed in Table 1.

Table 1: Physiological changes in fresh fruits after chitosan treatment and with other elicitors

Fruit	Physiological change	Combination with chitosan	Reference
Apple	• Total phenolic, flavonoids, antioxidants, pigments, weight loss	Olive waste extract	Khalifa et al., 2017
Peach	• Malondialdehyde content • Total soluble solids, weight loss, ascorbic acid content • Colour and fruit firmness	Gamma-ray Silver and zinc oxide nanoparticles Polyethylene terephthalate punnets containing thyme oil	Elbarbary & Mostafa, 2014. Kaur et al., 2017; Cindi et al., 2015
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 1: Physiological changes in fresh fruits after chitosan treatment and with other elicitors (Continued)

Fruit	Physiological change	Combination with chitosan	Reference
Sweet cherry	• Malondialdehyde content and antioxidant enzymes	-	Pasquariello et al., 2015
Citrus	• Fruit firmness, weight loss, total soluble solids • Peroxidase and phenylalanine ammonia-lyase	Carboxymethyl cellulose Cyclic lipopeptide antibiotics from Bacillus subtilis	Arnon et al., 2014 Waewthongrak et al., 2015
Mango	• Peroxidase (POD) and polyphenol oxidase (PPO) gene expression		Gutierrez-Martinez et al., 2017
Kiwi fruit	• Induced gene expression and increased enzymatic activity of catalase, superoxide dismutase and ascorbate peroxidase		Zheng et al., 2017

Table 1: Physiological changes in fresh fruits after chitosan treatment and with other elicitors (Continued)

Fruit	Physiological change	Combination with chitosan	Reference
Strawberry	• Weight loss, titratable acidity, pH, total soluble solids, total phenols • Weight loss • pH and soluble solids content • Weight losses, total soluble solids and titratable acidity	Carboxymethyl cellulose Lavander and thyme essential oil Natamycin, nisin, pomegranate, grape seed extract Olive waste extract	Gol et al., 2013 Sangsuwan et al., 2016 Duran et al., 2016 Khalifa et al., 2016
Grapes	• Phenylalanine ammonia-lyase, chitinase, and β-1, 3-glucanase, phenolic compounds • Total phenols, flavonoids and ascorbic acid content, activities of peroxidase • Firmness, titratable acidity, soluble solids, colour, weight loss	Salicylic acid Menta essential oil	Shen and Yang, 2017 Al-Qurashi and Mohamed, 2015 Guerra et al., 2016

Table 1: Physiological changes in fresh fruits after chitosan treatment and with other elicitors (Continued)

Fruit	Physiological change	Combination with chitosan	Reference
Pear	• Total phenolic and flavonoid contents, superoxide dismutase, peroxidase and catalase activities, total antioxidant activity	Calcium chloride	Kou et al., 2014a
	• Malic acid-metabolising enzymes and related genes expression	Calcium chloride	Kou et al., 2014b

(Source. Romanazzi., et al. 2018)

2.3 Current Strategies for the Preharvest/Post-Harvest Treatment of Date Fruit

According to Lin et al. (2021), the various stages describe the cell division, juvenile green, the experienced hard full shaded, the delicate brown, and the firm raisin-like organic product. As stated by Khesab cv. It is an exceptionally regular cultivated product in the region of the UAE, and it is consumed at the Rutab stage, which is half the delicate stage of the product. After collecting, the natural product begins losing its solidness, shading, taste, and generally speaking physiology, which finally brings down the commonly estimated quality of the fruits. These changes are results of the post-harvest action of different ageing-related chemicals and chemicals that mellow the natural product tissue, decline the immovability and genuine uprightness of the organic product, and also, at last, lead to the natural product's senescence.

According to Hinkaew (Hinkaew et al., 2020), salicylic acid is considered a phenolic factor that increases the ability to prevent disease and postpones the ripening stage of the fruit. At the same time, calcium is also considered an essential element that delays the maturity of the fruits. Therefore, the application of these harmless chemicals can increase the longevity of the fruit, which is the pre- and post-harvesting scenario of the dates. According to Kumarihami et al. (2020), during the pre-cultivating stage pre- and post-harvesting stages, the application of the solution (Ch) 1% with salicylic acid (SA) and calcium chloride (Ca) (the solution can be in the combination of Ch, SA, Ca, Ch+Ca, Ch+SA, Ch+SA+Ca) can increase the quality of the products. According to Elmenofy (2021), natural and eco-friendly components like chitosan, calcium chloride, and salicylic solution can increase the tissue softening of the dates, visibly delaying the ripening stage and restoring quality, extending the shelf life of the dates and preserving them properly.

According to Badawy (2019). as chitosan, calcium chloride is considered a natural solution that strengthens the tissues of the trees and fruits. It is a natural solution applied in pre- and post-harvest situations to Khesab cv. Dates and increase its prospects of taste and quality. At the same time, calcium is considered the element of tissue softening and an active agent that delays the ripening stage of the Khesab cv. dates. Abdel-Hamid (2020) states that the solution of chitosan, calcium chloride, and salicylic solution can increase the quality of the Khesab cv. dates which tend to lose their taste and quality if it is ripened enough. It is essential to increase the self-life and market life of the fruits to increase their economic value. These solutions can provide natural and eco-friendly support to plant-based harvesting and increase the self-life and quality of the fruits. Thus, in the pre-and post-harvesting situation, the farmers implement these solutions to incorporate various benefits and sustain the quality of the dates to increase their market prospects.

Chapter 3: Materials and methods

3.1 Plant Material and Sampling

During the 2020 season, uniform date palm trees (*Phoenix dactylifera* L., cv. Khesab) were randomly 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^{o}N and 55.7146^{o}E. In addition, there were eight female spathes per palm tree, and the trees were pruned for an 8:1 leaf to bunch ratio. The design of the experiment was a randomized complete block design with six palms (replicate) from each 'Khasab' palm receiving seven different treatments (one treatment for each bunch).

Three stages of development (5 and 15 weeks from pollination and two weeks before harvest) were selected for spraying treatments with different elicitors: chitosan, SA, and CaCl2 alone and in combinations (7 treatments): Control (Water), Ch, SA, Ca, Ch+Ca, Ch+SA, Ch+SA+Ca with adding 0.2 of between 20 which help in reducing the tension of the water and create a soap texture that helps the treatments in holding onto the date fruit. The control date palm trees were sprayed with only deionized water for control. The harvesting of bunches was done at the commercial stage (when roughly about 50% of dates have reached the Rutab stage (half Bisr).

After washing, the fruit was carefully separated according to their maturity level (Bisr and 50% Rutab (so-called Rutab). Then Bisr fruit was used for further analysis. Randomly, 100 fruit were collected from each treatment for initial physicochemical, phytochemical, bioactive properties, and microbial analysis at harvest time (day 0) (Awad & Al-Qurashi, 2012). Next, another batch of 500 fruit from each treatment was collected and stored in punched plastic bags (100 fruit in each bag) and stored at 2°C and 90–92% relative humidity (RH) for 60 days. For 15 days, a bag was withdrawn randomly for analysis for each time interval. According to Hinkaew (Hinkaew et al., 2020), salicylic acid is considered a phenolic., as shown in Table 2.

Table 2: Preharvest spry treatments of Khesab cv. date fruit with different elicitors

Treatment	**Chemicals**	**Application**
Ch	Chitosan	1%
Ca	Calcium chloride	3%
SA	Salicylic acid	2 mM
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

Fruit dimension studies were conducted after harvest according to Raste-gar et al. (2012). Fruit weight loss during storage was recorded every two weeks for each treatment and expressed as percent weight loss from the original weight before cold storage, using the following equation:

$$\textit{Fruit weight loss}\ (\%) = \frac{\textit{Initial weight} - \textit{Weight at specific interval}}{\textit{Initial weight}} \times 100$$

3.2.2 Fruit ripening and decay percentage

A visual evaluation of fruit was done to record the ripening burst (fully *Rutab*) and spoilage every other week until a complete decay of fruit occurred during storage for 60 days. The ripening and decay percentages were calculated using the following equation (Kumar et al., 2013):

$$\textit{Decay or ripe fruit}\ (\%) = \frac{\textit{Number of ripe or number of decay fruit}}{\textit{Total fruit number}} \times 100$$

3.2.3 Total soluble solids (TSS)

Ten grams of pitted date fruit were pureed and mixed with 10 mL of distilled water in a blender to form a slurry, which was filtered to obtain the clear juice. The Brix value was determined in the juice using a digital refractometer (DR 6000, A. Kruss Optronic GmbH, Hamburg, Germany).

3.2.4 Fruit surface colour

The surface colour of fruits was determined by using a Hunter Lab colourimeter (Hunter Lab Inc., Reston, VA, USA).

The values were represented as L* (brightness), a* (blue/yellow), and b* (red/green) (Ferrer et al., 2005). These values were further used to calculate the Hue angle ((h^o), chroma (C*), and total color difference (ΔE*) as follows; ho = 180◦ + arctan (b*/a*); (C*) = (a*2 + b*2)1/2.

$\Delta E = [(L^*-L^*0) + (a^*-a^*0) + (b^*-b^*0)]1/2$ (Maskan, 2001), where L*0, a*0, and b*0 values were from control fruit at harvest time (day zero) (Feliziani et al., 2015).

3.2.5 Determination of total microbial load

The total fungal/mould and bacterial count on fruit samples were determined upon harvest (day 0) and at the end of storage (60 days). One gram of mashed fruit tissue was vortexed vigorously in 9 mL autoclaved buffered peptone water under sterile conditions and serially diluted to prepare several different dilutions. Employing the pour plating technique, a 1 mL sample from each dilution was plated with plate count agar (PCA) which was incubated at 37°C for 48 h and potato dextrose agar (PDA) incubated at 27°C for 5 d. The number of colonies was recorded at the end of incubation time and calculations were performed using Log10 colony-forming unit per g of fresh weight (Log10 CFU g^{-1}) (Xylia et al., 2021)

3.2.6 Extraction of bioactive compounds

The extraction of phenolic compounds was performed via homogenizing two grams of fresh fruit samples in 20 mL of 80% methanol with a water bath shaker at 150 rpm for 24 h, 45°C. Whatman no.1 filter paper was used to filter the slurry. The filtrate (date extract) was used for further analysis (Ahmed et al., 2021a).

3.3 Phytochemical Analysis

3.3.1 Total Phenolic Content (TPC)

Following (Velioglu et al., 1998, Ahmed et al., 2021c), total phenolic content was estimated with some modifications. First, date extract (100 µl each) was added to clean and dry test tubes, followed by 50 µl Folin Ciocalteu reagent, and then vortexed. Then, all tubes were incubated at room temperature for 2 min. Then, 2 mL of NaOH (6%) was added to each tube and incubated in the dark for 45 minutes. Finally, absorbance was measured at 750 nm using a UV spectrophotometer. Results were expressed as milligrams of gallic acid equivalents (GAE) per 100 g fresh weight (mg 100 g-1 GAE) according to the standard curve obtained by measuring the absorbance of known gallic acid concentrations.

3.3.2 Total Tannin Content (TTC)

The amount of tannin in the date extracts was determined using the colourimetric method described by Bentebba et al. (2020), with some modifications. First, 1 mL of a 4% vanillin solution prepared in absolute ethanol and 0.2 mL of HCl (37%) was added to 0.4 mL of extract or catechin as a standard. The mixture was then shaken and allowed to react in the dark at room temperature for 15 min before absorbance was measured at 500 nm using a spectrophotometer. Total tannin content was expressed as milligrams of catechin equivalents per 100 g fresh weight (mg 100 g-1 CE) (Ahmed et al., 2021c).

3.3.3 Total Flavonoids Content (TFC)

According to Kim et al. (Kim et al., 2003), total flavonoids were determined with some modifications. First, 75 µL NaNO2 (5%) was added to the data extract (250 µL) in a test tube and vortexed, followed by 5 min incubation in the dark. Next, 75 µL of AlCl3 (10%) was added; the mixture was vortexed and kept in the dark for 6 min, followed by 500 µL of NaOH (1 M), then the volume was made up to 2.5 mL with distilled water. Then, the absorbance was measured using a spectrophotometer (Shimadzu, Kyoto, Japan) at a wavelength of 510 nm. Results were expressed as milligrams of catechin per 100 g fresh weight (mg 100 g-1 CE).

3.4 Antioxidant Activities

The evaluation of the antioxidant activities was based on the method developed by Abd Elwahab et al. (2019).

3.4.1 DPPH radical scavenging activity

DPPH radical scavenging activity was determined according to Ahmed et al. (2022b) method with slight modifications. The 1.5 mL samples with concentrations ranging from 0.5 mg to 10 mg L-1 were mixed with 1.5 mL of 0.15 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) in 95% ethanol. After incubation for 30 min at room temperature in the dark, the values were measured at 517 nm using a spectrophotometer. The blank sample was prepared similarly for each concentration, except that ethanol was used instead of DPPH solution. IC50 values (mg mL-1) were calculated for each sample.

3.4.2 ABTS radical scavenging activity

The activity of the ABTS radical scavenger was determined as follows. Stock solutions of ABTS (7.4 mM) and potassium persulfate (2.6 mM) were prepared and stored in a refrigerator (4°C). Equal parts of the two stocks were mixed and allowed to react for 12 hours at room temperature in the dark. Before performing the assay, the solution was further diluted by mixing 1 mL of the ABTS solution with 50 mL of methanol, which gave an absorbance of 1.1 ± 0.02 units at 734 nm, as measured by a spectrophotometer. A new ABTS solution was prepared for each assay.

Then, 150 µL of the sample with concentrations ranging from 0.5 mg to 10 mg mL-1 was mixed with 2850 µL of ABTS solution and left in the dark at room temperature for two hours. The absorbance was then measured at 734 nm using a UV spectrophotometer. The blank was prepared in the same manner for each concentration, except that methanol was used instead of ABTS solution. A Trolox standard curve was prepared in the range of 50 µM to 600 µM. Activity was expressed as milligrams of Trolox equivalents (TE) per 100 g fresh weight (mg 100g-1 TE).

3.5 Statistical Analysis

Analysis of variance (one-way ANOVA) was employed for the completely randomized design experiment with six replicates using 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 each time interval for each analysis. The correlation coefficient between the main biochemical and physical characteristics was calculated by SAS.

Chapter 4: Results and Discussion

4.1 Physical Quality Characteristics of Fruit at Harvest

The average weight, width and length of fruit of cultivar Khesab at harvest are shown in Table 3 - significant differences ($p < 0.05$) in physical characteristics of treated cultivar Khesab. Fruit was affected by the preharvest elicitor treatments: the average weight, width and length of Khesab cv. Fruit was significantly ($p < 0.05$) higher in the SA and Ch+ SA treatments than in the control and the other treatments. These improvements in fruit physical characteristics could be due to the influence of SA in combination with Chin, which maintains cell integrity and increases firmness and improves fruit weight and size (Kassem et al., 2010). Similarly, Mohamed et al. (2014) described that preharvest spray application of SA significantly increased the weight, width, and length of date fruits compared to control fruits. The physical characteristics of the fresh fruit are of critical importance as they directly indicate the physical effects of the applied elicitors. The above results show that the application of SA alone or in combination with Ch affected the physical properties of fresh fruits of Khesab cultivar.

4.2 Fruit Weight Loss during Storage

Weight loss is a significant factor that reduces postharvest fruit shelf life and marketability (Kassem et al., 2010; Mohamed et al., 2014). The results showed that the fruit weight of cultivar Khesab was significantly ($p < 0.05$) affected by the application of triggers. All fruits experienced continuous weight loss during storage, which varied among treatments. However, significant differences in weight loss were evident between the treatments and the control. After 60 days of storage, the least weight loss was observed in fruits treated with Ch+ SA, followed by SA, Ch+Ca, Ca, Ch with 12.64, 14.74, 15.06, 15.76, 16.57%, respectively. The weight loss during storage may be due to an increase in respiration rate and/or overall moisture loss from the fruit (Shiri et al., 2013; Mohamed et al., 2014; Atia et al., 2018). Figure 4 shows the per cent weight loss that occurred during cold storage at 20°C and 90-92% RH for 60 days in the harvested fruits.

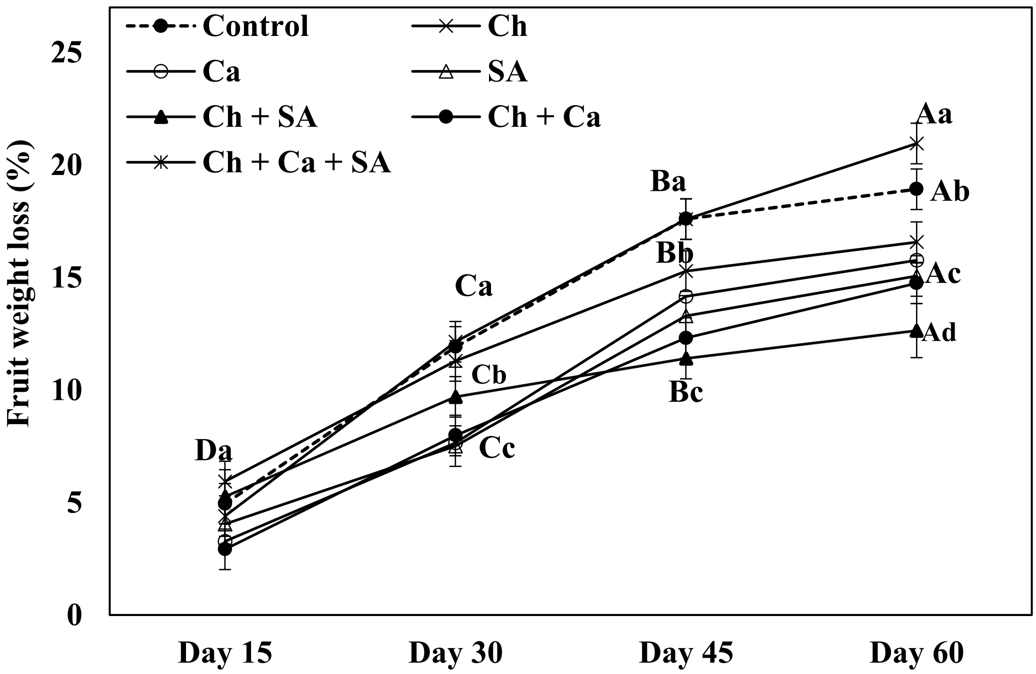

Figure 4: Effect of different preharvest treatments on weight loss of Khesab cv. fruit during cold storage

Note: Values are the mean (n = 25) ± SE. Means with different letters between treatments (small letters) at a different time interval (capital letters) are significantly different at $p < 0.05$ using the LSD test. Ch: chitosan; Ca: calcium chloride; SA: salicylic acid.

Table 3 shows the potential effects of different preharvest treatments on Khesab cv. fruit physical characteristics at harvest.

Table 3: potential effects of different preharvest treatments on Khesab cv.

	Fruit Weight (g)	Fruit Length (mm)	Fruit Width (mm)
Control	10.2 ± 0.11b	35.0 ± 0.38b	23.5 ± 0.24b
Ch	10.9 ± 0.22b	35.2 ± 0.36b	24.1 ± 0.27ab
Ca	10.0 ± 0.22b	34.7 ± 0.38b	23.9 ± 0.23b
SA	11.8 ± 0.25a	36.7 ± 0.40a	24.2 ± 0.25ab
Ch + SA	12.4 ± 0.46a	37.2 ± 0.48 a	25.6 ± 0.32a
Ch + Ca	10.8 ± 0.39b	34.9 ± 0.51 b	24.2 ± 0.34ab
Ch + Ca + SA	10.6 ± 0.16b	35.2 ± 0.33b	24.2 ± 0.21ab

Note: Values are the mean (n = 25) ± SE. Means with different letters between treatments (small letters) at a different time interval (capital letters) are significantly different at $p < 0.05$ using the LSD test. Ch: chitosan; Ca: calcium chloride; SA: salicylic acid.

The low weight reduction observed in fruit sprayed with chitosan alone might be due to the film-forming properties which significantly decreased the evaporation rate of water from the fruit, as noted in the case of other fruit treated with chitosan (Petriccione et al., 2015; Petriccione et al., 2015; Romanazzi et al., 2018, Maan et al., 2021). Also, Ca and SA were found to have significantly lower weight loss compared to control in constant to Atia et al. (Atia et al., 2020) reported that pre-storage treatment with Ca and SA reduced weight loss in 'Barhi' dates at *Khalal* stage. Also, Kassem et al. (2010)

and Mohamed et al. (2014) found that the preharvest application of SA reduced weight loss during storage in treated Khesab cv. fruit compared to the control. SA is well known to reduce chilling injuries, inhibit ripening, and act against various abiotic and biotic factors (Ennab et al., 2020).

This suggests the positive physiological condition of fruit treated with SA presumably owing to decreased respiration rate that can also be associated with the improved fruit turgidity. In dates, typically, the weight of the fruit increases as the maturation advance and at its peak at the *Khalal* stage, then promptly decreases as ripening progresses (Alqarni & Bazzi, 2019). Based on the above results, the weight loss reduction observed in fruit treated with Ch alone or in combinations with SA and Ca, indicates the good physiological conditions of the treated fruit, most likely as a result of the reduced respiration and transpiration rates, and the regulating effects of these elicitors on the ripening process.

4.3 Total Soluble Solids (TSS) during cold storage

Overall, the concentration of TSS gradually increased in all fruits as ripening progressed during cold storage. At harvest and after 60 days of cold storage, control fruits had the highest TSS concentration (from 32 to 42%), followed by Ch+ SA treated fruits (Feliziani et al., 2015). Ca treated fruits exhibited the lowest TSS concentration (from 28% to 38%). Similarly, Ch+Ca and SA treated fruits had significantly ($p < 0.05$) lower levels of TSS compared to control fruits after 15, 30, 45 and 60 days of cold storage (Figure 5). It has been reported that elicitor efficacy is determined by fruit tissue reactivity, which decreases as ripening progresses (Romanazzi et al., 2018). In this study, the observed TSS concentrations were similar to those described by Kassem et al. (2010) in SA treated date fruit. However, Mohamed et al. (2014) reported no significant difference between SA treated date fruits and the control fruits in terms of TSS content.

The results of Ca treatment might be due to the high Ca concentration (3%) in the treatment. The usual increase in TSS during cold storage could be due to the degradation of large polysaccharide molecules into smaller sugars by enzymatic activities and water loss (Mohamed et al., 2014; Hazbavi et al., 2015). The TSS concentrations showed significant differences ($p < 0.05$) between the different treatments, as shown in Figure 5. These results are consistent with the weight loss results shown in Figure 4. According to our findings, the degradation of polysaccharides was slowed down by Ca alone or in combination with Ch and SA, resulting in lower TSS concentrations in the treated fruits and, consequently, in a lower ripening rate.

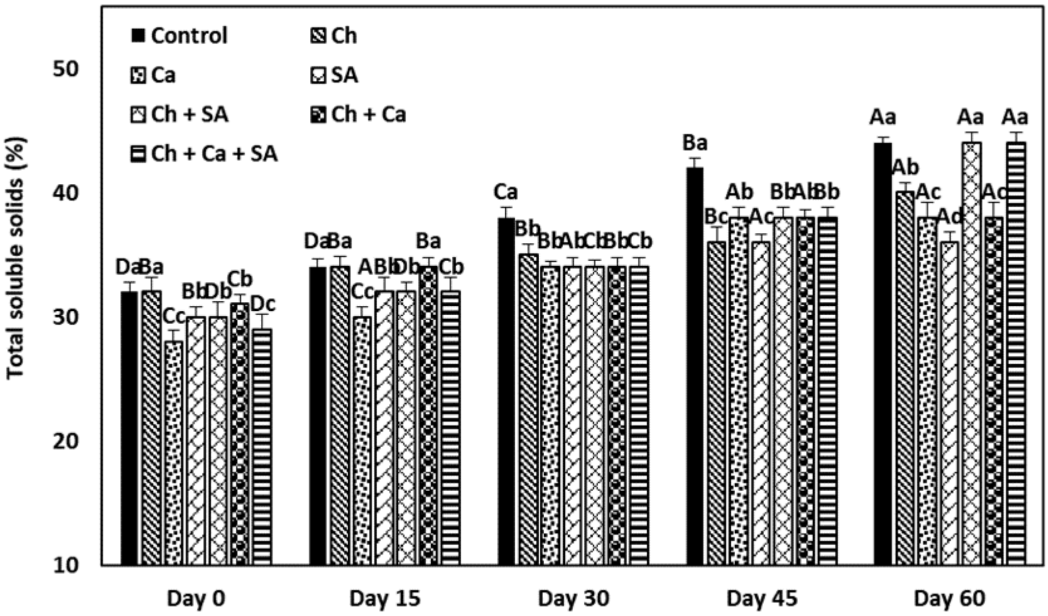

Figure 5: Effect of different preharvest treatments of Khesab cv. fruit during cold storage

Note: values are the mean (n = 25) ± SE. Means with different letters between treatments (small letters) at a different time interval (capital letters) are significantly different at $p < 0.05$ using the LSD test. Ch: chitosan; Ca: calcium chloride; SA: salicylic acid.

4.4 Fruit Ripening

The results of fruit ripening during cold storage at 2°C for 60 days are shown in Figure 6. The different treatments significantly delayed fruit ripening during the storage period ($p < 0.05$). In general, the rutab content gradually increased during cold storage in all treatments. At the end of storage period, the highest rutab content was observed in control fruits (90.3%), compared to SA, Ch+ SA, Ch+Ca, Ch, Ch+ SA +Ca and Ca treated fruits with a range of 79-83%. Ch+ SA +Ca treatment alone significantly delayed normal ripening of Bisr fruit compared to the other treatments in combination with Ca. These results are consistent with those of TSS in Figure 5, where fruit treated with Ca, Ch+Ca, Ch+ SA +Ca, and SA had lower TSS content than the control and the other treatments. Moreover, fruit maturity (Rutab %) was positively correlated with the increase in TSS and weight loss (Table 4).

These observations may also indicate that the elicitors delay the ripening of Khesab cv. fruit during cold storage. It is well known that calcium administration slows down the respiration rate and delays fruit ripening (Irfan et al., 2013; Sohail et al., 2015; Atia et al., 2018). Similarly, preharvest application of SA delayed date fruit ripening by three weeks compared to control fruits (Kassem et al., 2010). In addition, pre- and postharvest treatments with SA were reported to improve the quality and shelf life of many fruits and prevent their spoilage (Pila et al., 2010; Shen & Yang, 2017; Atia et al., 2018). In addition, fruit ripening can be slowed by the film-forming ability of Ch, creating a barrier to gas exchange and reduced respiration. It should be noted that less mature fruits are less susceptible to postharvest rot (Romanazzi et al., 2018). Figure 6 illustrates various preharvest treatments on Khesab cv. In ripening stage “*Rutab*”.

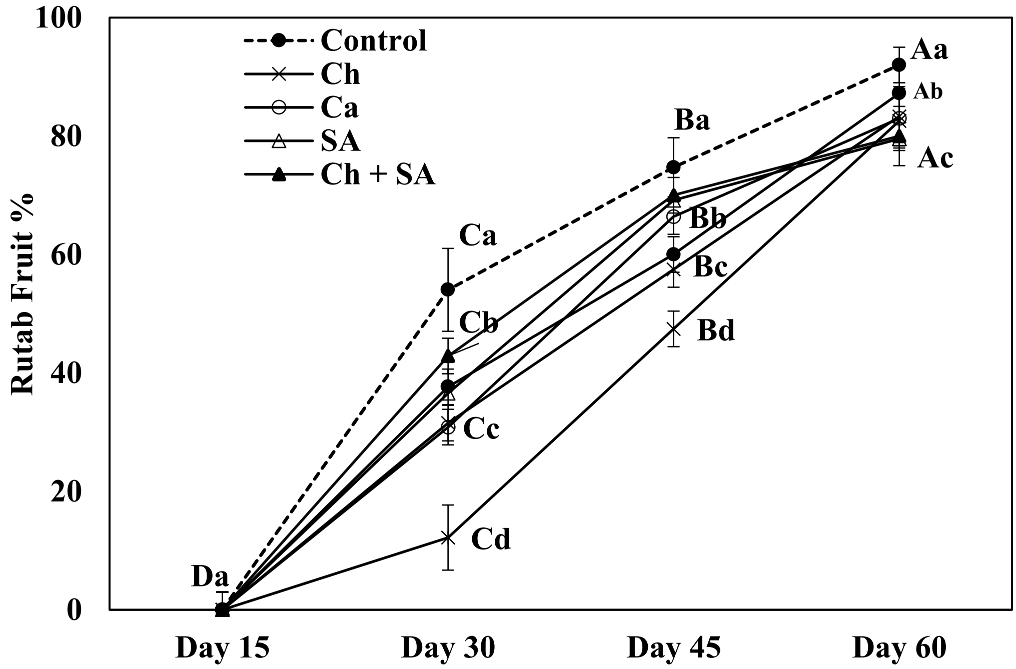

Figure 6: Effect of different preharvest treatments on Khesab cv. fruit ripening during cold storage

Note: Values are the mean (n = 25) ± SE. Means with different letters between treatments (small letters) at a different time interval (capital letters) are significantly different at $p < 0.05$ using the LSD test. Ch: chitosan; Ca: calcium chloride; SA: salicylic acid.

Fruit ripening is a natural phenomenon that leads to membrane impermeability and gradual senescence of fruit tissue due to oxidation of essential membrane components (Awad et al., 2011b). Ethylene is the main hormone responsible for the processes of ripening and senescence (Kassem et al., 2010). It is possible that the sprayed elicitors can control the production or action of ethylene, thereby slowing down fruit ripening (delaying the onset of rutab). Our results showed the retarding effect of Ch, Ca and SA in different combinations on fruit ripening compared to the control. Nevertheless, date ripening is not an asynchronous process for all date fruits in the same cluster, as there may be fruits at different stages of ripening in the same cluster at any given time. Bisr fruit that received the same treatment could enter the rutab stage later than control fruit in the same cluster. As a result, different significant biological variations between individual fruits within the same cluster may be responsible for certain contradictory results (Mohamed et al., 2014).

4.5 Fruit Decay During Storage

The results of the percentage of fruit deterioration during cold storage at 2°C for 60 days are shown in Figure 4. It was found that the different preharvest spray treatments had a significant effect on fruit decay during cold storage ($p < 0.05$). Fruit rotted progressively during storage, except for fruit treated with Ch and Ch+ SA, where no rot was observed up to 45 days (Figure 4). Among all treatments, the control samples had the highest percentage of fruit rot (about 55.6%) after 60 days of storage, while Ch+ SA +Ca, Ch and Ch+ SA had the lowest values (11.6, 25.9-25%) compared to the other treatments (Figure 5). Similarly, SA was reported to be effective in reducing berry drop and rot in grapes at concentrations of 1 and 2 mmol L-1 (Gomes et al., 2021). In addition, SA and Ca alone or in combination increased fruit hardness and delayed softening, resulting in less rot in strawberry fruit (Shafiee et al., 2010).

Equally, elicitors such as SA, Ca and oxalic acid were reported to induce a defence response and reduce decay in other fruits (Tian et al., 2006; Wang et al., 2011; Shen & Shen Yang, 2017). The results of the present study show that the use of Chin in combination with Ca and SA could protect Khesab cv. fruit from decay during cold storage, probably by triggering defence mechanisms in the fruit and maintaining the firmness of the exocarp. Figure 7 illustrates the potentia effect of different preharvest treatments on decay of Khesab cv. fruit during cold storage. Figure 8 shows the possible effect of various preharvest treatments on total contents of Khesab cv. fruit.

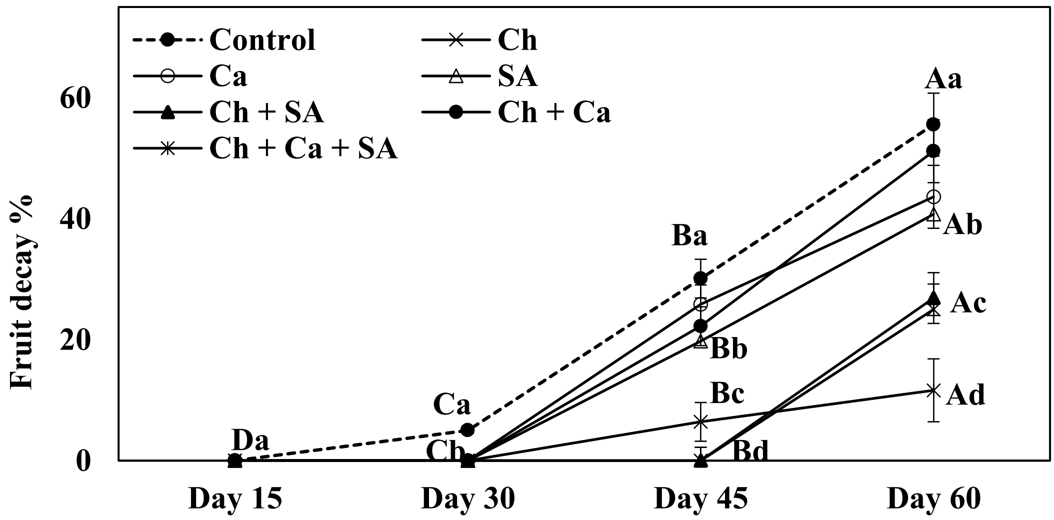

Figure 7: Effect of different preharvest treatments on Khesab cv. fruit decay during cold storage

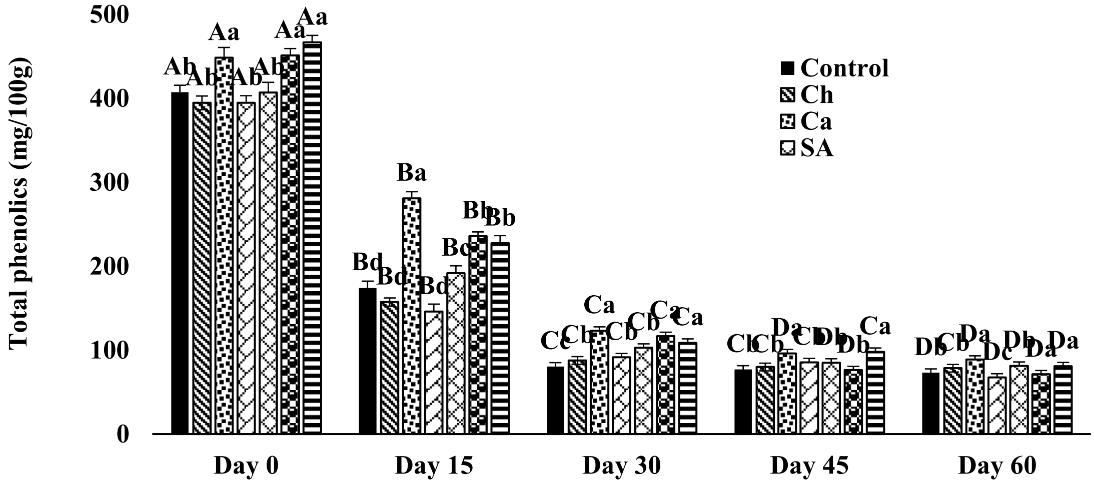

Figure 8: Effect of different preharvest treatments on total phenol content of Khesab cv. fruit during cold storage

Note: Values are the mean (n = 3) ± SE. Means with different letters between treatments (small letters) at a different time interval (capital letters) are significantly different at $p < 0.05$ using the LSD test. Ch: chitosan; Ca: calcium chloride; SA: salicylic acid.

4.6 Colour Attributes

Colour is an essential visual attribute of food, especially fresh fruit, which directly affects the attractiveness and acceptance of the product by consumers (FernÁndez-Vázquez et al., 2011). Therefore, the effect of treatments on colour differences was considered when evaluating the quality of fresh fruit of the Khesab variety. Important colour parameters, i.e., ΔE, C*, L*, and h^0, were determined to follow fruit colour changes during cold storage after elicitor treatments and are presented in Table 4. In general, the colour parameters were significantly ($p < 0.05$) affected by the Elicitor application. In addition, colour values gradually decreased with increasing storage time for all fruits. For example, the l* values of all fruits decreased as storage progressed, with a significant decrease in fruit brightness from 37 to 21 after 60 days. After 30 days, significant differences were found between treatments, as Ch and Ch+ SA showed the least decrease in L*, C*, and h^0.

After 60 days, the lowest decrease in L* was observed in fruit treated with Ca, followed by SA, Ch and Ch+ SA (Table 4). The same treatments also had lower ΔE values than other treatments, including the control. Similarly, C* was highest in Ch+Ca at the end of the storage period, followed by Ca, Ch, control, Ch+Ca+ SA, Ch+ SA, and SA treated fruits. The L*, C* and h o values were positively correlated with the increase TSS and ripening, (as shown in Table 5), while ΔE was the opposite. The observed delay in colour change of fruits treated with Ca, SA and Ch could be due to the interruption of ethylene action, the unmasking of chlorophyll pigment and the reduction of fruit respiration rate, leading to a slowdown in colour change (Irfan et al., 2013). Similarly, pistachio fruits treated with Ch and SA maintained their colour quality compared to the control (Molamohammadi et al., 2020).

Colour deterioration reactions (browning of date fruits), especially enzymatic reactions, are a naturally occurring phenomenon mainly triggered by the action of peroxidase and polyphenol oxidase during fruit ripening and storage and are associated with a loss of fruit market value (Awad et al., 2011b; Mortazavi et al., 2015). These responses are due to oxidation of phenolic compounds and development of dark brown pigments in date fruits (Awad et al., 2011a). In the present study, the steady decrease in TPC during storage (Figure 7) was positively correlated with the decrease in colour parameters, i.e., h^0, C*, L* and negatively correlated with ΔE, to varying degrees in all fruits (Table 4). Thus, based on the present results, it is possible that the application of Ca alone or in combination with Ch and SA delayed the responses to colour change to different degrees and, accordingly, preserved the colour of the fruits.

4.7 Total Phenolic Content of Fruit at Harvest and During Storage

The TPC content of Khesab cv. fruits at harvest and during cold storage is shown in Figure 8. The type of elicitor administered had a significant effect on TPC content at harvest and during cold storage. In general, TPC content decreased with increasing storage time for all fruits. Similarly, phenolic content decreased at different time points from early development stage to maturity and ripening (Awad et al., 2011a). In the present study, Ch+Ca+ SA treated fruits had the highest TPC concentration, which progressively decreased from 486.8mg 100g-1 GAE at day 0 to 66.9mg 100g-1 GAE at day 60, followed by Ca (from 448.5 to 82.8) and Ch (394.6 to 68.5mg 100g-1 GAE) compared to the control and the other treatments.

However, TPC concentration was found to vary (50-400mg 100 g-1) among different date cultivars in several studies (Al-Qurashi & Awad, 2011; Awad et al., 2011b; Mohamed et al., 2014), which could be due to cultivar differences and/or environmental factors. In other fruits, SA has been shown to reduce cold exposure injury in peaches for the duration of cold storage (Wang et al., 2006) due to its ability to induce antioxidant systems and heat shock proteins (Wang et al., 2006). It has been reported that the application of triggers such as chitosan, SA and Ca increases the content of polyphenols in fruits, improving their quality (Ruiz-García & Gómez-Plaza, 2013, Khalil et al., 2022).

In the present study, Ch+ SA +Ca treatment showed low rotting rate during cold storage (Figure 8) and had the lowest rutab content after Ca treatment (Figure 6). In addition, TPC showed a negative correlation with weight loss, rutab content, and decay rate (Table 4), indicating an essential role in date fruit ripening/senescence. Based on these results, preharvest application of Ch, Ch+Ca and Ch+ SA +Ca improved TPC content, which improved fruit quality and shelf life of Khesab cultivar.

Table 4: Effect of different preharvest treatments on Khesab cv. fruit colour attributes during cold storage

	*L**							Hue Angle						
	Control	**Ch**	**Ca**	**SA**	**Ch + SA**	**Ch + Ca**	**Ch + Ca + SA**	**Control**	**Ch**	**Ca**	**SA**	**Ch + SA**	**Ch + Ca**	**Ch + Ca + SA**
Day 0	33.1± 0.44b	33.1± 0.36b	32.6± 0.58b	31.2± 0.52b	33.44 ± 0.83b	37.63 ± 0.67a	37.72 ± 0.86a	27.65 ± 0.34a	26.03 ± 0.36b	24.95 ± 0.38c	23.87 ± 0.37c	27.29 ± 0.47a	24.23 ± 0.32c	27.42 ± 0.41a
Day 15	27.4± 0.54b	29.5± 38a	29.8± 0.64a	27.9 ± 0.51b	29.68 ± 0.54a	29.66 ± 0.70a	30.85 ± 0.91a	28.88 ± 0.46b	30.28 ± 0.45a	26.88 ± 0.31d	25.39 ± 0.33d	27.85 ± 0.39c	26.12 ± 0.46d	29.94 ± 0.49a
Day 30	25.2 ± 0.59b	26.7± 0.64b	28.4± 0.85a	26.0± 0.91b	28.50 ± 0.78a	27.08 ± 0.94a	27.43 ± 0.89a	38.08 ± 0.51a	35.93 ± 0.53b	29.99 ± 0.45d	28.80 ± 0.44d	34.17 ± 0.84c	34.50 ± 0.91c	38.74 ± 0.81a
Day 45	22.0± 0.32c	23.4± 0.43b	25.2± 0.69a	22.9± 0.47bc	23.21 ± 0.60b	24.64 ± 0.48a	24.08 ± 0.44a	38.00 ± 0.53b	38.30 ± 0.67b	35.89 ± 0.74c	34.42 ± 0.68c	37.46 ± 0.73b	38.34 ± 0.94b	40.61 ± 0.90a
Day 60	21.2± 0.52b	21.7± 0.43b	23.7 ± 0.88a	21.22 ± 0.44b	21.71 ± 0.36b	23.30 ± 0.67a	22.22 ± 0.41b	41.91 ± 0.46a	41.15 ± 0.66a	37.08 ± 0.62c	36.85 ± 0.41c	39.39 ± 0.45b	39.39 ± 0.82b	42.17 ± 0.44a

	Chroma							**Δ*E***						
	Control	**Ch**	**Ca**	**SA**	**Ch + SA**	**Ch + Ca**	**Ch + Ca + SA**	**Control**	**Ch**	**Ca**	**SA**	**Ch + SA**	**Ch + Ca**	**Ch + Ca + SA**
Day 0	30.67 ± 0.47bc	31.77 ± 0.82b	29.94 ± 0.39c	28.75 ± 0.38c	32.88 ± 0.76b	32.57 ± 0.56b	34.32 ± 0.714a	0e	1.41 ± 0.06d	1.67 ± 0.04d	3.35 ± 0.13b	2.25 ± 0.07c	5.25 ± 0.10a	5.87 ± 0.16a
Day 15	27.36 ± 0.56c	29.77 ± 0.46b	29.35 ± 0.44b	28.09 ± 0.41bc	32.09 ± 0.54a	27.55 ± 0.42c	28.55 ± 0.43b	6.67 ± 0.14a	3.98 ± 0.08bc	3.53 ± 0.08c	5.85 ± 0.15b	3.71 ± 0.09c	4.71 ± 0.08b	3.30 ± 0.05c
Day 30	10.41 ± 0.23d	12.86 ± 0.21c	15.63 ± 0.24b	13.08 ± 0.25bc	18.39 ± 0.23a	11.77 ± 0.18c	14.40 ± 0.22b	21.98 ± 0.36a	19.14 ± 0.33b	15.79 ± 0.15c	18.96 ± 0.31b	13.39 ± 0.23d	19.96 ± 0.34b	17.69 ± 0.24b
Day 45	6.57 ± 0.12c	7.45 ± 0.11b	8.70 ± 0.13a	6.10 ± 0.11c	7.32 ± 0.12b	5.83 ± 0.14c	8.91 ± 0.12a	26.53 ± 034a	25.33 ± 0.46a	23.46 ± 0.37b	26.65 ± 0.41a	25.48 ± 0.53a	26.40 ± 0.43a	23.89 ± 0.35b
Day 60	5.15 ± 0.14a	5.70 ± 0.9a	5.75 ± 0.11a	3.69 ± 0.08b	4.59 ± 0.10a	4.91 ± 0.05a	4.97 ± 0.09a	28.15 ± 0.57a	27.49 ± 0.72b	26.63 ± 0.51b	29.48 ± 0.64a	28.47 ± 0.69a	28.76 ± 0.47a	27.72 ± 0.42ab

Note: Values are the mean ($n = 25$) ± SE. Means with different letters in the same row for each colour index are significantly different at $p > 0.05$ using LSD test. Ch: chitosan; Ca: calcium chloride; SA: salicylic acid.

Table 5: Pearson's correlation coefficients between some biochemical and physical characteristics of Khesab cv. Fruit

Traits	**TSS**	**TPC**	**Rutab %**	**Decay %**	**Weight Loss %**	***L****	***ΔE***	***Chroma***
TPC	-0.73 ***							
Rutab %	0.74 ***	-0.92 ***						
Decay %	0.61 ***	-0.42 ***	0.56 ***					
Weight loss %	0.52 ***	-0.77 ***	0.65 ***	0.43 ***				
*L**	-0.67 ***	0.85 ***	-0.91 ***	−0.39 ***	−0.60 ***			
ΔE	0.68 ***	-0.81 ***	0.88 ***	0.47 ***	0.55 ***	−0.96 ***		
Chroma	-0.63 ***	0.72 ***	-0.85 ***	−0.49 ***	−0.52 ***	0.93 ***	-0.94 ***	
H°	-0.67 ***	0.81 ***	-0.89 ***	−0.55 ***	−0.63 ***	0.92 ***	-0.90 ***	0.91 ***

(***) significant at level *P* = 0.001

4.8 Total Tannin, Flavonoids, and Antioxidants Concentrations at Harvest

The effect of the different elicitors on total tannin (TTC), flavonoids, and antioxidant activity was determined at harvest (Table 6). TTC, TFC, and antioxidant activity affected by the applied elicitors showed significant differences among the different treatments. Compared with all treatments, fruit treated with Ch+Ca+ SA had the highest TTC and TFC values, 116.5 mg 100 g-1 CE and 91.0 mg 100 g-1 CE, respectively, followed by Ch+Ca (80.0 and 82.7 mg 100 g-1 CE) compared with the control and the other treatments. The lowest levels of these phytochemicals were SA (23.8 and 56.3 mg 100 g-1 CE) and Ch (21 and 73.6 mg 100 g-1 CE). These antioxidant compounds showed higher activity in fruits treated with CH + SA +Ca , as did the radical scavengers ABTS (323.7 mg 100 g-1 TE) and DPPH with the lowest values (IC50 = 1.5 mg mL-1) (Table 6).

The lower IC50 values in the determination of DPPH radical scavenging activities indicate that the extract concentration required to scavenge the DPPH radical decreased by 50%, indicating an improvement in the antioxidant activity of the phenolic compounds found in the fruit. This result is consistent with the low ripening (low rutab content) and decay observed in the same treatment (Figures 6 & 8). In general, the concentration of antioxidants in dates decreases from the early stage (Bisr) to the ripening stage (Awad et al., 2011). These results are in agreement with those found for TPC at harvest (Table 6), highlighting the crucial role of phenolic compounds in the antioxidant capacity of Khesab variety fruits. Moreover, a positive correlation between antioxidant compound content and antioxidant capacity was found in five-date cultivars (Awad et al., 2011a).

In strawberry fruit, preharvest spraying with chitosan increased flavonoids and phenolic compounds up to 2.6-fold compared to the untreated control (Rahman et al., 2018). Application of SA was found to increase antioxidant compounds in table grapes (Gomes et al., 2021). Comparable results were also reported by Mohamed et al. (2014). They found that preharvest spraying with SA significantly increased DPPH scavenging activity at the bisr stage of date fruit compared to control fruit. In date, as a climacteric fruit, oxidative stress during ripening is thought to be responsible for the reduction of antioxidant compounds (Figure 7) at the bisr and/or rutab stages as a result of the decrease in free radical scavenging ability (Mohamed et al., 2014). Therefore, more reactive oxygen species (ROS), including H2O2 and superoxide, may develop and be involved in the ripening/senescence of date fruit and other fruits due to the development of free radicals (Ferrer et al., 2005, Khalil et al., 2022). According to the above results, preharvest application of Ch+ SA +Ca increased the antioxidant compound content and antioxidant activity, which improved the quality and shelf life of Khesab cv. fruits possibly by reducing harmful radicals.

Table 6: Effect of different preharvest treatments on Khesab cv. fruit phytochemical analysis at harvest

	TPC (mg/100 gm)	**TTC (mg/100 gm)**	**TFC (mg/100 g)**	**(DPPH) IC50 (mg/mL)**	**ABTS (mg/g)**
Control	407.4 ± 12.2c	73.9± 0.75c	71.5± 0.34bc	1.7 ± 0.03b	1.9 ± 0.07b
Ch	394.6 ± 8.89c	42.0 ± 0.43e	87.8± 0.42b	3.3 ± 0.10a	1.6 ± 0.11c
Ca	448.5 ± 11.3b	61.9 ± 0.68d	67.9 ± 0.22c	1.7 ± 0.06b	1.5 ± 0.09c
SA	394.9 ± 10.7c	47.8 ± 0.26e	66.4 ± 0.31c	2.1 ± 0.07b	1.3 ± 0.17d
Ch + SA	407.2 ± 13.2c	59.5 ± 0.45d	86.6 ± 0.49b	1.8 ± 0.05ab	1.9 ± 0.18b
Ch + Ca	451.1 ± 13.4b	80.7 ± 0.64b	82.9 ± 0.54b	1.8 ± 0.04ab	1.2 ± 0.12d
Ch + Ca + SA	486.8 ± 12.7a	116.2 ± 0.73a	91.4 ± 0.41a	1.5 ± 0.04b	3.2 ± 0.13a

Note: Values are the mean (n = 3) ± SE for the phytochemical analysis. Means with different letters in the same column are significantly different at $p > 0.05$ using LSD test. TPC = Total Phenolic Content; TTC = Total Tannin Content; TFC = Total flavonoid Content. Ch: chitosan; Ca: calcium chloride; SA: salicylic acid.

4.9 Microbiological Quality of Date Fruit

The microbial load (Log10 CFU g-1) of Khesab cv. samples at harvest and at the end of cold storage is shown in Figure 9. The analysis was performed for only two days, at day 0 and day 60. In general, total bacterial count (TBC) and fungal/mould count

(FMC) at harvest and at the end of storage showed significant variations influenced by the application of triggers. For example, date fruit treated with Ch, SA, Ca, Ch+Ca, Ch+ SA and Ch+Ca+ SA had lower TB counts than the control group at day 0, while FMC counts were significantly lower in fruit treated with Ch, Ca and Ch+Ca+ SA compared to the other treatments and the control group.

After 60 days, SA and Ch-treated fruits showed lower FMC counts, followed by Ch+ SA and Ch+Ca+ SA compared to the control group and the other treatments, while the same treatments showed significantly lower FMC counts than the control group and the other treatments (Figure 9). Consistently, different elicitors such as SA showed the best results in eliciting defence responses and minimising microbial infestation and rot in pear fruit (Tian et al., 2006). In addition, chitosan has been shown to stimulate plant defences against various pathogens, including fungi and bacteria (Romanazzi et al., 2018, Ahmed et al., 2021a). Moreover, treatment with Ch and SA either separately or in combination drastically reduced bacterial and fungal growth in pistachio fruits (Molamohammadi et al., 2020).

Based on the present results, the combination treatment with different elicitors showed better antimicrobial activity than other treatments. Moreover, the same treatment showed the highest antioxidant activity and the lowest percentage of decay, which explains the results of microbial analysis (Irfan et al., 2013). Thus, the increase in antioxidant activity and phenolics by treatment with different elicitors alone or in combination may induce defence mechanisms that lead to resistance to pathogens and prolong fruit shelf life, as has been reported in other fruits (Wang et al., 2011; Sathiyabama et al., 2014; Romanazzi et al., 2018).

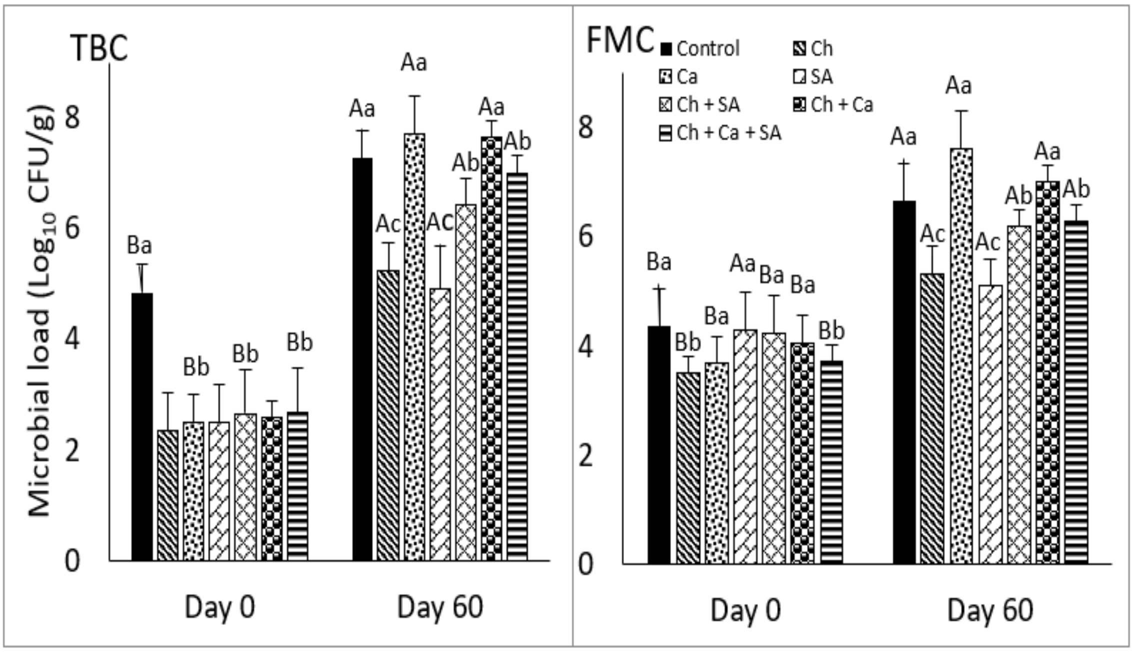

Figure 9: Effect of preharvest treatment on viable microbial load (Log10 CFU/g) of Khesab cv. at day 0 and day 60 of cold storage

Note: Values are the mean (n = 3) ± SE. Means with different letters between treatments (small letters) at a different time interval (capital letters) are significantly different at $p < 0.05$ using the LSD test. TBC = Total Bacterial Count, FMC = Yeast and Mold Count. Ch: chitosan; Ca: calcium chloride; SA: salicylic acid.

Chapter 5: Summary and Conclusions

This study showed that the application of different combinations of elicitors improved the biochemical properties of fresh fruits of Khesab cv. throughout the ripening and storage period. The synergistic effect of elicitors was found when different treatments were combined; Ch+Ca and Ch+Ca+ SA and Ch+ SA treated fruits in harvested Bisr date fruits showed reduced rot and microbial load. At the end of cold storage, Ca followed by Ch+Ca+ SA had the highest TPC concentration as they delayed natural ripening. Moreover, antioxidant activity was highest in Ch+ SA followed by Ch+ SA +Ca and Ch compared to the other treatments.

These results suggest that the delay in ripening and reduction in fruit decay may be due to the regulating effect of the sprayed elicitors on the ripening process and thus delaying senescence of fresh fruit of Khesab cultivar. Based on the results of this experiment, (Ch+ SA and Ch+ SA +Ca) may be considered suitable for large-scale application and should be tested on different dates and other fruits. The use of natural compounds can be an efficient, cost-effective and natural way to improve the quality and marketability of date fruits and protect them from a variety of decaying microbes.

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This thesis is about the research conducted in the field of postharvest quality of date palm. Date fruit is the most important fruit in UAE. The research highlighted the effect of using natural compounds on extending shelf/storage life and delayed senescence, decreasing decay percentage and microbial load of ‘Khesab’ date fruit. These treatments when applied before harvesting were able to enhance fruit nutritional quality during long storage. The results recommended these to be used commercially on other date fruit cultivars or other fruit species to extend the fruit shelf life and improve the quality during storage.

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