EFFECTS OF SEAWEED-BASED BIO-STIMULANT KELPAK® ON CHILLING INJURY INCIDENCE IN TOMATO (*Solanum lycopersicum* L.) FRUIT DURING POSTHARVEST COLD STORAGE

by

MUSARURWA CALVIN KUDAKWASHE

[redacted]

FULL DISSERTATION

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SUPERVISOR : PROF TP MAFEO
CO-SUPERVISOR : DR TK SATEKGE

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DECLARATION

I, Musarurwa Calvin Kudakwashe, [redacted] declare that the full dissertation hereby submitted to the University of Limpopo, for the degree of Master of Agriculture Management in Plant Production, has not been previously submitted by me for a degree at this or any other institution; that it is my work in design and execution, and that all material contained herein are duly acknowledged.

03/04/2025

Signature Date

DEDICATION

I dedicate this study to my family.

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CHAPTER 1
GENERAL INTRODUCTION

1.1. Background

Tomato (*Solanum lycopersicum L.*) is a widely grown crop with significant nutritional, economic, and industrial value. Originating from South America, tomatoes are now integral to global diets due to their versatility and health benefits (Calvo *et al*., 2014). Rich in bioactive compounds like lycopene, carotenoids, phenolics, and vitamins C and E, tomatoes are recognized for possessing antioxidant properties and having a potential to reduce the risks of long-term illnesses such as cancer and heart-related disorders (Sharma *et al*., 2016). However, tomatoes are highly perishable and sensitive to low temperatures, <10°C, cold storage.

Chilling injury poses a considerable physiological challenge in managing horticultural crops after harvest, particularly those sensitive to low temperatures, such as tomatoes (*Solanum lycopersicum*). It results from oxidative stress and disruption of cellular homeostasis when crops are stored at temperatures below their optimal range but above the freezing point (Ruelland and Collin, 2012). This leads to symptoms such as pitting of the skin, discoloration, internal breakdown, and loss of flavour and nutritional quality, which negatively impact marketability and consumer acceptance (Wang *et al*., 2012). Consequently, mitigating chilling injury is critical to maintaining quality and extending the shelf life of tomatoes in refrigerated conditions.

Biostimulants derived from seaweed, like Kelpak® which comes from *Ecklonia maxima*, have demonstrated potential in enhancing crop resilience to both biotic and abiotic stressors, like the stress from low temperatures. Kelpak® is rich in phytohormones such as auxins, cytokinins, gibberellins, and abscisic acid, which are vital for facilitating plant development, growth, and responses to stress (Stirk *et al*., 2014). These hormones affect cellular functions, encompassing antioxidant enzyme activity, membrane stability, and the control of genes that respond to stress, which are crucial in mitigating chilling-induced oxidative damage (Rouphael and Colla, 2020). Studies have shown the effectiveness of Kelpak® in improving the physiological and biochemical characteristics of tomatoes in the period following harvest. The use of

Kelpak® has been associated with an increased production of antioxidants, including carotenoids and phenolic compounds, which neutralize reactive oxygen species (ROS) produced during chilling stress (Fan *et al*., 2011). For instance, phenolic compounds play a role in maintaining the structural integrity of cell walls and membranes, thereby reducing electrolyte leakage and preserving fruit firmness (Cheynier, 2012). Furthermore, the presence of auxins and cytokinins in Kelpak® has been associated with delayed senescence and extended shelf life in various horticultural products (Rouphael and Colla, 2020).

Despite the demonstrated benefits of seaweed biostimulants, the precise mechanisms through which Kelpak® confers resistance to chilling injury remain underexplored. Questions about the optimal concentration and timing of application, as well as interactions between its phytohormonal constituents and plant metabolic pathways, require further investigation. This study aims to evaluate the impact of Kelpak® on the occurrence of chilling injury in tomato fruit shelf life. By addressing critical gaps in understanding its mechanisms of action, the findings may contribute to improved post-harvest management practices, improving the quality and marketability of tomatoes.

1.2. Problem statement

During postharvest cold storage of tomatoes, fruit shelf-life is extended; however after two weeks, low temperature stress induces chilling injury, which diminishes the quality and consumer acceptability of many crops (Albornoz *et al*., 2019, Zhao *et al*., 2009). Various methods such as oxalic acids (Li *et al*., 2016), intermittent warming (Biswas *et al*., 2012) and heat shock (Luengwilai *et al*., 2012) have been used to mitigate chilling injury in tomato. However, information on the cheap and eco-friendly seaweed-based bio-stimulant is limited, even though it has been successfully used in reducing various stresses (Elkelish *et al*., 2020). Some of the stresses include fungal infections, water shortage and imbalance of temperature like extreme cold or hot in horticultural plants like tomato and bell pepper (Siddiq *et al*., 2021). Hence, the present study proposes to use seaweed-based bio-stimulant Kelpak® to reduce chilling injury incidence induced by cold stress on tomato fruit during cold postharvest storage.

1.3. Rationale

Tomatoes are tropical and climacteric vegetable crop with economic and nutritive relevance in South Africa (SA). Nutritionally, tomato fruit is rich in vitamins and antioxidants. This indicates that tomato is an important nutritional fruit that can alleviate poverty and malnutrition in the world. An average family in South Africa eats between five to ten tomato fruit per week (DAFF, 2019). Therefore, tomatoes are a very strategic fruit in reducing poverty and malnutrition in South Africa.

Tomatoes, being climacteric fruit crop, are very perishable and their shelf-life is diminished at room temperature. The shelf life of tomatoes, particularly mature green tomatoes, can be prolonged by cold temperatures. Nonetheless, as a tropical crop, tomatoes are quite vulnerable to damage from low temperatures when below 13 °C, particularly if kept for extended periods (Albornoz *et al*., 2019; Zhao *et al*., 2009). This chilling injury diminishes the quality of the tomatoes and their acceptance in the market. Therefore, cheap, eco-friendly and effective techniques that control chilling injury are needed to extend tomato fruit shelf-life using cold storage technology. Seaweed-based bio-stimulants have effectively reduced abiotic stresses in tomato fruit during preharvest (Carillo *et al*., 2020; Di Stasio *et al*., 2018; Francesca *et al*., 2020). Limited work has been carried out on seaweed-based bio-stimulants effect on postharvest storage stresses, including cold stress. However, studies found that phytohormones such as brassinosteroids and abscisic acid augment cold stress adaption in tomato fields (Elkelish *et al*., 2020), bell pepper (Wang *et al.*, 2012) and zucchini fruit (Zuo *et al*., 2021). Therefore, since seaweed-based bio-stimulants contain brassinosteroids and abscisic acid (Stirk *et al*., 2014), it is hypothesised that their postharvest application would reduce cold storage-related chilling damage in tomatoes.

1.4. Purpose of the study

1.4.1. Aim

Reduction of chilling injury incidence in tomato fruit during postharvest cold storage using seaweed-based bio-stimulant Kelpak®.

1.4.2. Objectives

i. To determine the effect of seaweed-based bio-stimulant Kelpak® on chilling injury incidence and physicochemical properties of tomato fruit stored at chilling temperature.
ii. To determine whether seaweed-based bio-stimulant Kelpak® has an effect on chilling injury and polyphenol properties in tomato fruit during postharvest cold storage.

1.4.3. Null hypotheses

i. Seaweed-based bio-stimulant Kelpak® has no effect on chilling injury incidence and physicochemical properties of tomato fruit stored at chilling temperature.
ii. Seaweed-based bio-stimulant Kelpak® has no effect on chilling injury and polyphenol properties in tomato fruit during postharvest cold storage.

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CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

Currently, an increase in agricultural productivity in the world is of high significance due to the rapid increase of human population. However, because of diverse biotic and abiotic stress factors, limitations are imposed on, high produce yield (Shabir *et al*., 2016). World estimates shows that general food production should improve by 70% by 2050 to be able to feed an additional estimated 2.3 billion people (Tilman *et al*., 2011). The estimation may not be possible to achieve given that chilling stress is an issue in the agricultural production sector. Agricultural produce, specifically horticultural produce experience chilling injury which is a situation resulting from the presence of chilling stress (Tilman *et al*., 2011).

Chilling stress which is brought about by the disturbance of normal plant physiological operations processes, has been recognized as a unique environmental impact on plant physiology for over 70 years (Kratsch and Wise, 2000). On the series of events that lead to the occurrence of chilling stress in plants, the first organelle to experience massive impact are the chloroplasts. The distortion and swelling of thylakoids follow and the appearance of a peripheral reticulum as well while starch granules disappear (Kratsch and Wise, 2000). This stress is likely to occur in all vegetables, plants and fruit crops during pre-harvest and postharvest cold storage.

In the case of cold storage, a temperatures, less than 10°C, triggers chilling stress which leads to chilling damage (Wang, 2012), however, refrigeration has been utilised to preserve (extend shelf life) for horticultural crops (Abbasali *et al*., 2019). Most horticultural produce are susceptible to chilling injury, which is triggered by environmental factors like temperature, light, and relative humidity as well as the product's origin, the genetics of the cultivar, its stage of maturation, the type of tissue metabolites, and its surroundings. (Wang, 2012).

Primary hindrance to severe economic losses initiated by post-harvest losses is this physiological issue (Biswas *et al*., 2014). Stress has the potential to modify plant hormone levels, like how abscisic acid does (Khan *et al*., 2017). Most plants are significantly retarded in their ability to reproduce when they are chilled (Mahajan and Tuteja, 2005). Biochemical strategies are amongst the strategies developed by plants to overcome chilling stress.

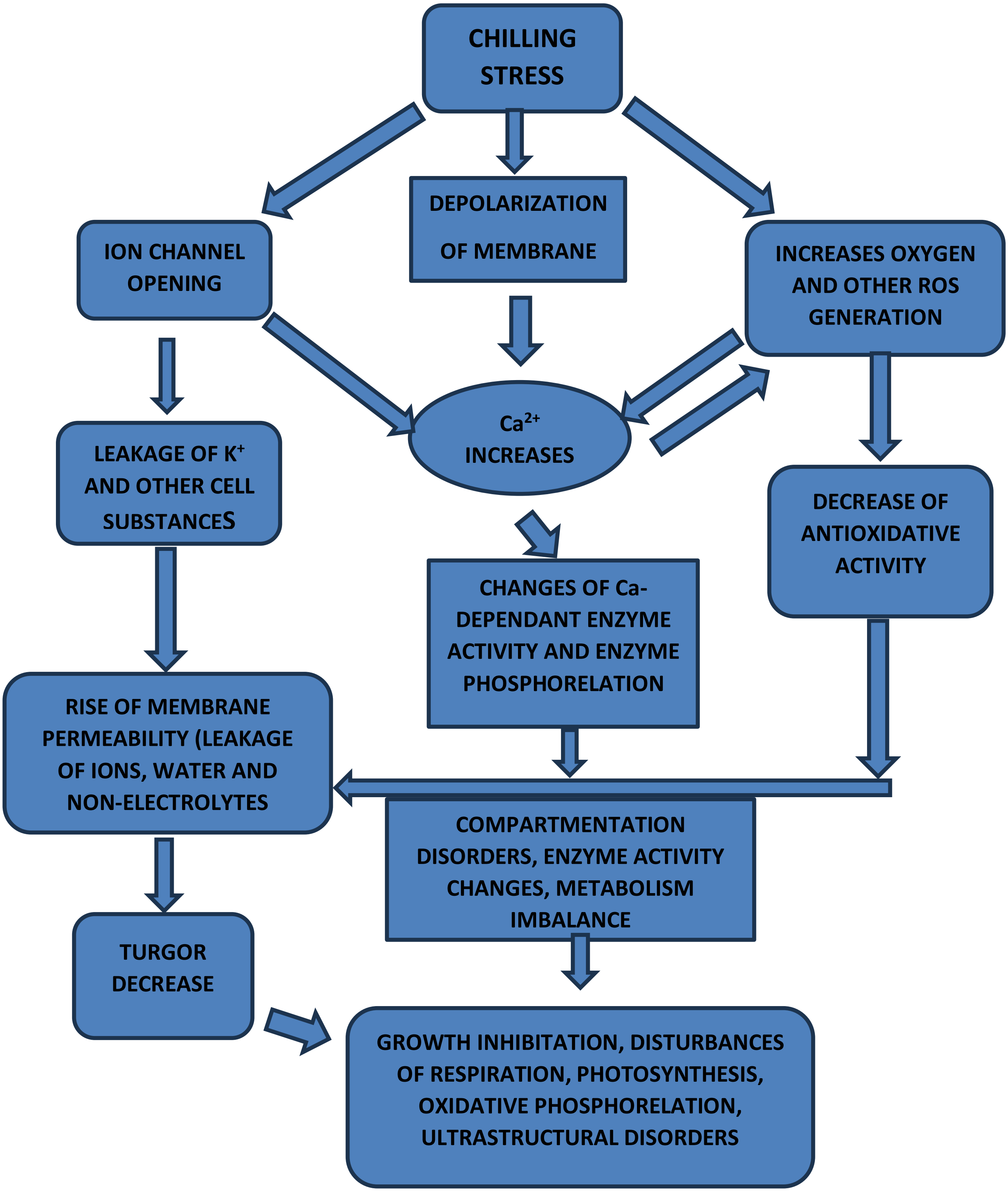

Figure 2.1: The illustration summarizes chilling damage because of physiological and biological events cascading within plant cells.

2.2 Chilling stress in horticultural products-an overview

Chilling stress is a condition that occurs on horticultural produce that are subjected to lower temperatures due to the occurrence of a physiological phenomenon known as oxidative stress (Ruelland and Collin, 2012). Cold temperatures cause chilling stress by disrupting cellular homeostasis, leading to reduced membrane fluidity and impaired functionality of membrane-bound enzymes, which compromise metabolic processes (Lyons, 1973). Low temperatures boost the generation of reactive oxygen species (ROS), resulting in oxidative stress that harms proteins, lipids, and nucleic acids (Saltveit, 2001). Plants have a variation in their tolerance to chilling mostly which occurs in temperature which ranges from 0 – 15 ^{o}C. Whether a plant is sensitive to chilling at that temperature or resistant to it depends on the severity of these modifications and its capacity to sustain them (Wang, 1982). When horticultural produce is subjected to temperatures considerably above the freezing point, chilling damage develops (Rai *et al*., 2022).

When faced with chilling stress, plants undergo a range of physiological and biochemical alterations. The enzyme systems that generally detoxify Reactive Oxygen Species (ROS) will be less efficient stress (Ruelland and Collin, 2012). In plants, symptoms of chilling stress are visible. Plants show certain changes in their normal physiological appearance become prominent. In developing plants, the emergence of seedlings and the initiation of leaves are diminished, along with irregular root cell division and elongation. The occurrence of these events will lead to wilting of the plant due to lack of enough water and nutrients, and dysfunction of photosynthesis (Wu *et al*., 2022). In fruits, different physiological and biochemical changes can be observed (Wang, 2012), with skin pitting, which is brought on by cells breaking just beneath the skin's surface being the most typical indication of chilling injury (Wang, 2012). Other symptoms of Chilling stress in fruits include discoloration, internal breakdown, failure to ripen, loss of flavour, development of odours and decay (Wang. 2009). These indicators of chilling injury have a negative effect affecting the taste, aroma, and visual appeal of tomatoes, thereby reducing their market value and consumer demand (Biswas *et al*., 2014).

2.3 Effects of *Ecklonia maxima* on chilling stress overview

The kelp *E. maxima,* a brown kelp species, is native to the cold temperate waters of South Africa and Namibia, thriving in nutrient-rich, wave-exposed coastal regions (Anderson *et al*., 2006). It is widely recognized for its role in marine ecosystems and for its bioactive components used in agricultural and pharmaceutical applications. It is used to make the seaweed extract Kelpak®, which is declared as a biostimulant for use in horticulture (Stirk *et al*., 2013). It triggers a variety of positive effects, including more notable resistance to biotic and abiotic stressors (Stirk *et al*., 2013). Its positive effect is attributed to cytokinins, auxins, brassinosteroids, gibberellins, abscisic acid and polyamines (Stirk *et al*., 2013). Papenfus *et al*., (2012) stipulated that Kelpak® consists of several cytokinins, including free bases, O-glucoside subordinates, and aromatic cytokinins, as well as auxins, four amino acid conjugates, three other conjugates and polyamines.

Phytohormones are essential for a variety of plant defensive responses to biotic and abiotic stressors (Abbadi *et al.,* 2015). Alongside their part in defense signalling, they moreover control different physiological, development, and formative forms in plants. Due to their unique balances within the exercises of these qualities, phytohormones are included in almost all cellular metabolic forms. These signalling defensive reactions are the results of the interaction of various qualities (Abbadi *et al.,* 2015).

2.4 Phytohormones in *E. maxima* extract: effect on chilling stress

Gibberellins

Various stresses, including responses to cold stress in plants, are found to be mediated by the phytohormone gibberellin (GA). This hormone controls essential biological processes that contribute to the growth and development of plants (Zhu et al., 2016), such as germination, expansion of leaves, elongation of stems, as well as flower and fruit development (Sponsel et al., 2004). It is widely recognized that GAs, along with other phytohormones, interact during numerous developmental processes and responses to stimuli. Increasing research highlights the importance of gibberellins in responding to and adapting to abiotic stress (Sponsel *et al*., 2004).

In research conducted by Zhu *et al*., (2016), where gibberellic acid treatment was done on tomato fruit, when compared to fruit maintained at ambient temperature, chilled fruit had reduced endogenous gibberellic acid (GA3) levels, and the decrease in active GA levels was linked to the down-regulation of the GA biosynthesis genes GA20ox1 and GA3ox1. In pink cherry tomato fruit, external application of GA3 greatly alleviated CI symptoms. However, the detailed molecular processes behind GA-fruit resistance to cold stress and the effects of external GA3 on GA signalling and metabolism are still unclear (Zhu *et al*., 2016).

The transcription factors known as C-repeat/dehydration-response element-binding factors (CBFs) have in the past been demonstrated to be crucial for the cold response mechanism in higher plants (Oakenfull *et al*., 2013). Three CBF genes (CBF1, CBF2, and CBF3) make up the CBF locus in tomatoes, one of which, CBF1, is triggered by cold conditions (Zhang *et al*., 2004). In tomato fruit, CBF1 transcripts demonstrated a negative connection with the CI index and a positive correlation with cold tolerance (Zhao *et al*., 2009). Tomato plants with increased expression of Arabidopsis CBF1 exhibited significantly greater resistance to cold temperatures compared to wild-type plants.

Consequently, it was hypothesized by Zhu *et al*., (2016) that transcription factors like CBF1 may be partially responsible for GA's ability to increase the freezing tolerance of tomato fruit. After obtaining results from this study, it could be explained that a thorough understanding of the mechanisms through which GA controls the tolerance of fruit to cold stress over extended periods of storage was brought about and suggested potential directions for developing efficient postharvest chilling injury management strategies for fruit sensitive to cold (Zhu *et al*., 2016).

Abscisic acid (ABA)

Abscisic acid (ABA) occurs naturally in plants (Chen *et al*., 2019). It plays several roles in controlling how plants grow, develop, and react to environmental challenges. It is the most common studied phytohormone due to its responsive and distinctive role in plants adapting to abiotic stresses and is commonly known as the stress hormone

(Wani *et al.,* 2015). Low temperature is one environmental stress that abscisic acid helps plants better withstand (Verslues and Zhu, 2005) and drought as well (Ma *et al*., 2008). Also, ABA functions as a negative growth regulator in favourable conditions, preventing seed germination and subsequent growth after germination (Kim, 2007).

Abscisic acid is highly influential in various physiological processes, development stages, protein and lipid synthesis, stomatal opening (Mahajan and Tuteja, 2005). Its major role is seen in abscission of plant leaves (Wani *et al*., 2015). According to Ikegami *et al*. (2009), during periods of water scarcity, ABA transitions from leaves to roots and only gathers in leaves when both leaves and roots experience distinct sources of limiting water. Zhang *et al*., (2018) confirmed that the synthesis of ABA is done in the leaves, and it is transported to the rest of the organs. Passive diffusion of ABAH via the plasma membrane is possible, and when the cytoplasm's alkalinity increases due to osmotic stressors, the diffusion of ABA decreases significantly. Therefore, it can be concluded that one crucial aspect of ABA's function in the plant's comprehensive stress reactions is its transport across cells, tissues, and organs (Chen *et al*., 2019).

Kulaeva and Prokoptseva, (2004), carried out an investigation of ABA signal transduction mechanisms using *Arabidops* mutants with reduced, absent or increased sensitivity to abscisic acid. They utilised one of the most popular experimental models for studies of ABA action, which is the stomatal guard cells. Reactive oxygen species (ROS) for transduction of ABA signal are also involved in this experimental model. It was discovered that ABA in these cells increases ROS which stimulates processes resulting in stomatal closure and opening, regulating transpiration (Kulaeva and Prokoptseva, 2004).

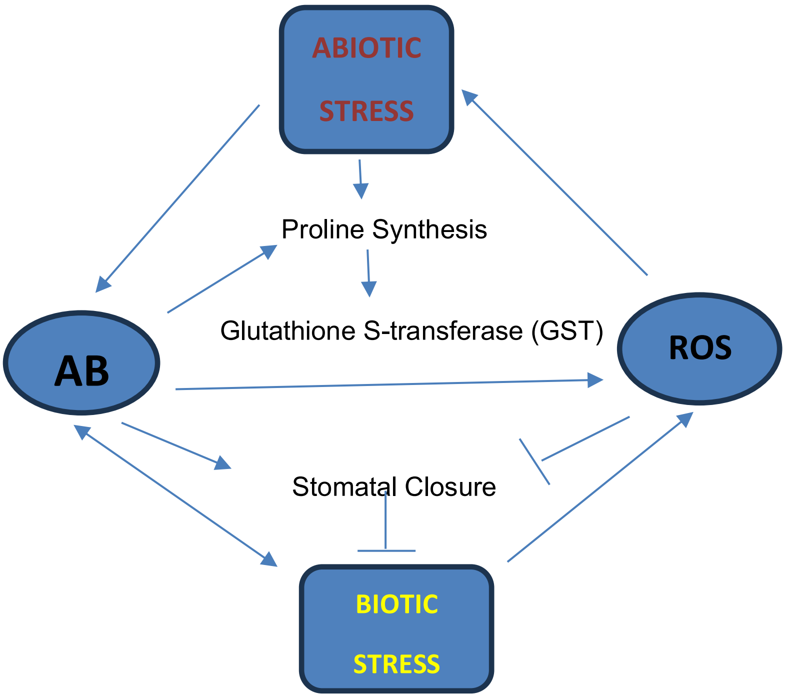

Figure 2.2: An illustration of how ABA signalling takes place according to Li *et al* (2022).

Apart from causing stomata to open, ABA influences the transcription of numerous genes. It activates 382 genes in Arabidopsis, including AIL1, RAB18, EM6, among others. These are referred to as traditional ABA-responsive indicator genes, known for safeguarding plants against dehydration damage (Chen *et al*., 2019). Additionally, as illustrated in the above figure, ABA stimulates the expression of OsGST4, resulting in the production of glutathione S-transferase (GST) which enhances ROS clearance activity and consequently suppresses stomatal closure. This activity leads to the enhancement of salt tolerance of rice and other plants (Li *et al*., 2022).

Castro-Cegri *et al.,* (2023), contacted a study to evaluate the possible impacts of applying ABA externally to Zucchini fruits immediately post-harvest to improve quality during cold storage. The research indicated that ABA treatment enhanced the fruit's antioxidant capacity by upregulating ascorbate and phenylpropanoid pathways. Additionally, the fruits in the control group experienced more weight loss than those treated with ABA. Furthermore, following extended storage after the cold period, Zucchini fruits treated with ABA demonstrated significantly reduced chilling injury compared to the control. (Castro-Cegri *et al.,* 2023).

Thus, the enhancement of antioxidant mechanisms induced by ABA played a key role in diminishing chilling injury in zucchini fruits. It can be inferred that during postharvest cold storage, the external application of ABA boosts the chilling tolerance of zucchini fruits. Moreover, the research highlighted ABA as an important phytohormone in defending against cold stress, and this insight could be used to enhance and maintain the quality of horticultural products after harvest.

Brassinosteroids

Brassinosteroids, a newly recognized group of polyhydroxy steroidal plant hormones, show considerable promise in enhancing plant growth and development (Wani *et al*., 2015). They have an important role in alleviating the impact of different non-living stress factors on plants, such as high temperatures, chilling stress, among others (Wani *et al*., 2015). According to Vardhini *et al*., (2006), recent research demonstrated the enormous potential of brassinosteroids and related substances in adjusting the components of the antioxidant defense system to respond to and mitigate the oxidative burst caused by abiotic stress. Brassinosteroids are classified as PGRs (Plant Growth Regulators) and trigger a range of physiological responses. They affect the growth of stems and roots, initiate flowering, develop flowers and fruits, and promote cell division and elongation, as well as seed production (Stirk, 2004). Moreover, they protect plants from biotic threats (pathogens) and (oxidative stress, water deficiency, salinity, nutrient deficiencies, extreme temperatures, toxic metals, and herbicides); (Bajguz and Hayat 2009). For instance, rice produced under salt stress and maize grown under water stress both benefit from exogenous application of brassinosteroids (Bajguz and Hayat 2009). Brassinosteroids, like other PGRs, are effective in minimal amounts (Zaharah *et al*. 2012).

Auxins

Despite over a hundred years of investigation, the mechanisms of auxin production, movement, and signalling remain ambiguous (Ke *et al*., 2015). As the most adaptable phytohormone, IAA (indole-3-acetic acid) plays an essential role in coordinating both growth under stress conditions and normal growth and development. (Kazan, 2013). Single-celled green algae with auxin synthesis, signalling, and transport provide compelling evidence of the evolutionary significance of auxin during plants' adaption to varied terrestrial environments. Despite recent advancements in the comprehension of how auxin influences the growth of plants, our understanding of the function as a restrictor remains limited (Kazan, 2013). Auxin is recognized as a crucial element in defense mechanisms as it controls multiple genes and facilitates interplay between responses to non-living and living stress factors (Wani *et al*., 2015). Palni *(*1983), outlined that from a certain experiment they carried out, it was discovered that auxin concentration is inversely related to cytokinin stability within the plant. Higher levels of auxin will result in instability (lower levels) of cytokinin in the plant. Lower levels of auxin will lead to higher levels of cytokinin (Palni *et al.,* 1988).

Polyamines

Natural polycations called polyamines are present in all living things, which are aliphatic nitrogenous compounds that have two or more essential amino groups that are water-soluble, low molecular weight polycationic. The types of amines they include vary from diamine putrescine, triamine spermidine, to tetraamine spermine. (Takahashi and Kakehi, 2010). These have a wide range in living organisms and are concentrated in high levels in cells that reproduce successfully. Translation, RNA modification, protein blending, and protein-tweaking activities are just a few of the major types that polyamines are capable of (Takahashi and Kakehi, 2010).

As a result of their effects on cell division and separation, blooming, development, advancement, and natural product maturation, polyamines in plants are crucial for executing numerous functions, such as growth and development (Khan *et al.,* 2008). These polyamines are described as having an administrative impact on increasing the plants' productivity (Gharib and Ahmed, 2005). When cell division is occurring more often during the onset of embryogenesis, putrescine plays an active role then spermidine and spermine are implicated in promoting additional embryo development and seed germination (Rakesh, 2021). They are recognized as anti-senescent experts and powerful for delaying softening in some natural items since they help with cell layer judgment (Khan *et al*., 2007). Directly scavenging free radicals is what polyamines do. (Velikova *et al.,* 2000).

Cytokinins

Cytokinins are considered as the best controllers for controlling plant growth and improvement and are crucial for various aspects of plant growth (Kang, 2012 and Nishiyama, 2011). Endogenous cytokinin levels can alter due to abiotic stresses like salinity and drought (O'Brien, 2013, Kang, 2012). Focus is on the critical association between mutant and transgenic cells and tissues with changed cytokinin, metabolic chemistry, or recognition systems and a few features, such as greater effectiveness and enhanced resilience to stress (Zalabak *et al*., 2013).

Even though cytokinin reactions in plants have frequently been studied through their application from the outside, it is also known that stressful situations can boost their endogenous levels through absorption and improved synthesis. (Pospilová, 2003). Benzylaminopurine (BAP) (Cronauer and Krikorian, 1984), isopentenyladenine (2iP) (Dore Swamy *et al*., 1983), zeatin (ZN) (Vuylsteke and De Langhe, 1985), and kinetin (KN) (Cronauer and Krikorian, 1984) are cytokinins that have been often employed. In plant tissue culture, cytokinins encourage cell proliferation and division. By including a cytokinin in the proliferation medium, growth is induced in the micro-propagation of temperate fruit trees. Numerous studies have shown the types of cytokinins that are suited for each species as well as their concentrations (Hutchinson and Zimmerman, 1987).

Table 2.1 Bio-stimulant concentrations and their effects on different plants

Treatment	Concentration	Key findings	References
24-epibrassinolide (EBR)	-10.60nM	-Stimulated the activity of superoxide dismutase enzyme in tomato leaves.	(Mazorra, 2002)
	-0.01 mg dm-3	-increased the levels of both free and bound ABA for the entire storage period (on average by about 80%), decreased sprouting by 36–38 days, boosted ethylene production following 1 and 7 days of storage by nearly 300 and 150%, and more.	(Korableva, 2002)
Polyhydroxylated spirostanic analogue of brassinosteroids (MH5)	-2.12nM	-Enzyme activity was greater in tomato leaves	(Mazorra, 2002)
(Auxins) IAA and Dicamba	0.5 mgl	-Improved plant regeneration and somatic embryogenesis from seven spring and winter wheat genotypes	

Cytokinin - Benzylaminopurine (BAP)	24.8 mM	-Increased shoots from 2.8 shoots to 3.5 shoots for banana Cultivar Kiburi	(Chory, 1994)
Polyamines (Spermine, Putrescine)	- 1 mM/L	Influence on *Pinus sylvestris* callus development with two distinct media, Krogstrup (K) and Murashige Skoog (M) In K media, polyamines showed poor callus growth, - Spermine had an inhibitory effect. - Putrescine reduced the activities of Arginine decarboxylase (ADC) and Ornithine decarboxylase (ODC). In M medium, positive responses were observed, - Putrescine enhanced dry weight by twofold when compared to K medium and increased ADC and ODC activities. - In both mediums increased ethylene production observed	(Rakesh *et al*., 2021)
Putrescine, Spermidine and Spermine	1.5mM	-In *Pinus virginiana*, Putrescine, Spermidine and Spermine showed 19.4%, 18.9% and 1.4% recovery rates respectively. -There was also an increase in the activity of antioxidant enzymes. However, the combination of these polyamines showed lower recovery rate when compared to their individual performance.	(Tang *et al*., 2004)

Putrescine, Spermidine and Spermine	40µM Spm	High shooting efficiency observed in Banana plant. -Stimulated several shoots, resulting in 46.4 shoots per node. in *Withania somnifera*	(Rakesh, 2021)
	20mg/L Spd	-82% shoot formation observed in Cucumis sativa	(Zhu and Chen, 2005)
Abscisic acid (ABA)	1µmol/L, 5µmol/L & 10µmol/L	*Solanum photeinocarpum* -In the Mining ecotype, in comparison to the control (0µmol/L) -increased root biomass by 12.82%, shoot biomass by 2.44% -increased root biomass by 27.18%, shoot biomass by 12.49% -increased root biomass by 53.83%, shoot biomass by 18.07%	(Wang *et al.,* 2016)
	1µmol/L, 5µmol/L & 10µmol/L	In the Farmland ecotype, in comparison to the control (0µmol/L) -increased root biomass by 6.01%, shoot biomass by 3.80% -increased root biomass by 8.11%, shoot biomass by 7.00% -increased root biomass by 12.31%, shoot biomass by 10.80%	(Wang et al., 2016)
Gibberellin (GA3)	2 mM/L	-In pink cherry tomato fruit, exogenous treatment of GA3 dramatically reduced CI symptoms.	(Zhu *et al*., 2016)

(GA4+7)	0.5 mM/L	-Successfully promoted fruit set and enhance fruit size in cucumber, pear, and apple. - accumulated more sugar and less organic acids than pollinated fruit.	(Zhu *et al*., 2016).

2.5. Conclusion

Abiotic and biotic stressors constrain agricultural productivity, increasingly important due to population growth and the necessity for food security. Chilling stress is a major issue for horticultural crops, particularly in cold storage after harvesting. This physiological challenge leads to biochemical and structural irregularities by disrupting plant cellular functions. Fruits such as tomatoes, showing signs like skin pitting, discoloration, and internal collapse, experience significantly reduced marketability and nutritional quality. Developing effective strategies to mitigate chilling stress is essential in tackling the financial losses from postharvest decay.

Seaweed extracts are highly effective biostimulants for enhancing resistance to chilling stress, particularly Kelpak® derived from *E maxima*. The extract's rich content of plant hormones like gibberellins, cytokinins, auxins, and abscisic acid, play a vital role in controlling plant growth and stress responses. Studies indicate that Kelpak® can boost antioxidant defences, delay aging, and maintain cellular structure during postharvest storage. For instance, brassinosteroids and polyamines reduce oxidative stress and aid in cellular repair, while gibberellins and abscisic acid significantly enhance cold tolerance by boosting antioxidant activity and regulating genes responsive to stress. Despite encouraging outcomes, there is a lack of detailed understanding of the exact molecular mechanisms through which biostimulants like Kelpak® affect plant physiological processes. Research aimed at the signalling pathways these phytohormones activate could provide more precise approaches for alleviating chilling stress. Additionally, determining optimal application levels and investigating synergistic interactions with other biostimulants or storage methods could further strengthen postharvest durability. Filling these knowledge gaps will be critical in enhancing the quality, longevity, and market appeal of horticultural products, promoting sustainable agricultural practices and ensuring global food security.

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CHAPTER 3

THE EFFECT OF BIOSTIMULANT KELPAK® ON CHILLING INJURY AND PHYSCOCHEMICAL PROPERTIES OF TOMATO FRUIT

3.1 Introduction

Tomatoes (*Solanum lycopersicum*) are a popular fruit appreciated for their abundant nutritional value, offering significant health benefits (Carrillo-Lopez and Yahia, 2012). They contain a small number of calories and rich in vitamins such as vitamin C and K, antioxidants such as lycopene, and essential nutrients like potassium and dietary fiber, which support overall health, immune function, and gut wellness (Silva *et al*., 2018; Willcox, Catignani, and Lazarus, 2003; Slavin, 2005). Processed tomato products, such as tomato sauce, enhance lycopene bioavailability, further amplifying their contribution to lowering the risk of long-term illnesses, such as cardiovascular disease and some types of cancer (Rao and Rao, 2007; Shi and Maguer, 2000).

During transportation and storage, tomatoes are often kept at cooler temperatures to decrease respiration, slow ripening, and limit the growth of spoilage microorganisms. This practice helps maintain their quality, prolongs shelf life, and decreases post-harvest losses (Saltveit, 2005). However, these low temperatures can lead to cold damage. Chilling injury (CI) in tomatoes is a condition related to the functioning of biological systems that arises when the fruit is exposed to temperatures below its ideal storage range, generally between 10°C and 12°C (Saltveit, 2002). As tropical fruits, tomatoes are especially vulnerable to chilling injury due to their origins in warmer regions (Wang, 2010). Low temperature exposure causes negative effects, such as surface pitting, inconsistent ripening, increased decay susceptibility, and diminished flavour quality (Wang, 2010).

To prevent chilling injury in tomatoes, it is crucial to implement effective postharvest management strategies. Storing tomatoes at temperatures just above the chilling threshold (12°C–15°C) helps avoid cold damage while prolonging their shelf life (Saltveit, 2002). Additionally, controlled atmosphere storage, which adjusts levels of oxygen and carbon dioxide, has been effective in minimizing chilling symptoms by reducing the rate of respiration and lowering reactive oxygen species production

(Wang, 2010). Moreover, research has explored using chemical treatments such as calcium, salicylic acid, and methyl jasmonate to strengthen membrane stability and boost antioxidant defences in tomatoes, thus alleviating chilling effects (Lurie and Sabehat, 1997). However, these methods often encounter issues like being environmentally unfriendly and not cost-effective. As a result, there is an opportunity to develop new, more sustainable and economical techniques, such as the biostimulant seaweed extract Kelpak®.

Biostimulants are either natural or synthetic materials that boost growth, yield, and stress tolerance, when applied to plants and soil (Du Jardin, 2015). They facilitate physiological processes instead of directly supplying nutrients by improving the capacity of the plant to take in and make use of nutrients already available in the soil (Du Jardin, 2015, Rouphael and Colla 2020). Kelpak®, a commercially available biostimulant derived from the brown seaweed (*Ecklonia maxima*) is abundant in phytohormones like auxins and cytokinins (Khan *et al*., 2009). It has demonstrated effectiveness in enhancing stress resistance in various crops, including tomatoes, by enhancing antioxidant activity, cell membrane stability, and metabolic functions. Research suggests that Kelpak® can alleviate the effects of chilling injury by enhancing antioxidant defense mechanisms, which lessen oxidative harm caused by reactive oxygen species (ROS) due to low temperatures (Khan *et al*., 2009, Du Jardin, 2015). Despite its potential to decrease tomato chilling injury, there is limited knowledge on the impact of different Kelpak® concentrations on reducing chilling injury, thus the study focuses on minimizing the incidence of cold damage in tomatoes after harvest during shelf life storage using the seaweed-based biostimulant Kelpak®. These compounds have attracted interest in horticulture and agriculture for their ability to enhance plant resilience to environmental stresses, including chilling injury (Du Jardin, 2015).

3.2. Methodology and Analytical Procedures

Experimental site and Plant materials

Mature green tomato fruit were purchased from the commercial market in Polokwane, Limpopo Province. Subsequently moved to the postharvest laboratory at the Agricultural Research Council – Tropical and Subtropical Crops (ARC-TSC) in Mbombela, Mpumalanga Province South Africa for treatment application, cold storage and analysis.

Treatments, experimental design and procedures

Fruits chosen for the experiment were consistent in size, color, and free of imperfections. 10g of fungicide Dithane M.45 per 5L of water was prepared for fruit sanitation. Fruit was dipped in the fungicide for 3 minutes and was left to air dry at ambient temperature. After air-drying, fruit were grouped into four groups. Each group was dipped in biostimulant Kelpak® at 0 (control), 0.2%, 0.4% and 0.6% concentrations respectively. Fruits were left to airdry at ambient temperature. Subsequently, kept at 10 °C for a period of 20 days. Following the cold storage phase, the fruit was stored at ambient temperature (±25 ºC) to simulate retail conditions (Nunes *et al*., 2006; Luengwilai *et al*., 2014). The experiment comprised of four treatments and each treatment was replicated 3 times. Each treatment had 60 fruit. Then 18 fruit per treatment were used to measure non-destructive assays such as chilling injury, mass loss, firmness, and peel colour on each sampling day. The study utilized a 4 by 4 factorial, set up within a completely randomized design (CRD). Kelpak® concentrations at 4 levels (0, 0.2%, 0.4% and 0.6%) and shelf-life duration at 4 levels (0, 2, 4 and 6 days) were considered factors.

3.3. Data Collection

Determination of chilling injury index

Chilling injury incidence was measured utilizing a 4-point rating scale (Li *et al*., 2016) and the index calculated according to equation 1 below:

$$Chilling\ injury\ index = \sum \frac{[(chilling\ injury\ level)\ X\ (number\ of\ fruit\ at\ the\ CI\ level)]}{(total\ number\ of\ fruit)} X100 \qquad (1)$$

Determination of mass loss

Mass of fruit was measured before and after cold storage. Each fruit was placed on the weighing balance (SCALTEC SBA 61) and the readings were recorded. The difference of the two mass values was calculated and expressed as a percentage according to equation 2 below:

$$Mass\ loss\ (\%) = \frac{(Initial\ mass - Final\ mass)\ X\ 100}{Initial\ mass} \tag{2}$$

Determination of firmness

The firmness of the fruit was assessed utilizing a portable densimeter (Model: 53524, Bareiss, Oberdischingen, Germany) featuring a 5 mm tip. The densimeter was held and pressed against two equidistant points in the equatorial regions of the fruit (top and bottom part of the fruit. The measurements were taken and recorded in Newtons (N).

Determination of BrimA Index and TSS:TA ratio(maturity index)

The BrimA index was determined following the methodology of Jordan *et al* (2001) using the following eduation 3 :

BrimA = TSS - k (TA) (3)

where k = (3), a constant that represents the high sensitivity of the tongue to titratable acidity in comparison to total soluble solids (Jordan *et al*., 2001).

The TSS:TA ratio was calculated utilizing the formula from (Jordan *et al*., 2001);

TSS:TA ratio (maturity index) = TSS/TA (4)

Determination of peel colour

Peel colour was measured using a portable hand calorimeter (Lovibond LC 100 Tintometer Group) expressing CIELAB colour space; b* and chroma. The device automatically computed the color parameters, including L* value (indicating lightness or brightness), chroma (C*), a* (representing redness or greenness), b* (signifying yellowness or blueness), and hue angle, with all measurements being recorded.

Determination of Total Soluble Solids (TSS), Titratable Acidity (TA) and pH

These parameters were measured by an automatic titrating machine. Six plastic containers were rinsed and set up. 50ml of distilled water was added in each of the containers. 10ml of fruit juice from each treatment was added in each of the containers. The containers were put on a flash automatic titrator (LASEC STEROGLASS AUTOSAMPLER 24), which does titration as well as measuring pH automatically. Values were recorded for TSS, TA and pH for each replication and treatment

3.4. Data analysis

The data gathered was subjected to a variance analysis (ANOVA) to identify significant differences amongst the treatments using GenStat 23rd edition software (VSN International, UK). Means for the significant variables were distinguished utilizing Duncan's Multiple Range Test with a significance level of 5%.

3.5. RESULTS AND DISCUSSIONS

Effect of kelpak® concentration on chilling Injury Index of tomato fruits

Chilling injury is a physiological condition affecting tomato fruit and other tropical and subtropical plants when they experience low, but not freezing temperatures, typically below 10-13°C. Application of kelpak® concentrations on tomatoes did not significantly ($p>0.05$) reduce chilling injury incidence. As observed in Figure 3.5.1, Kelpak® treatments did not effectively alleviate chilling injury. Chilling injury index increased as the storage period increases. However, it is observed that 0.4% concentration had lower chilling injury incidence compared to other concentrations. Therefore, the results suggest that it is the most effective at minimizing chilling injury in tomatoes. This suggests that 0.4% concentration of Kelpak® provides optimal activation of protective mechanisms, potentially such as antioxidant activity and membrane stabilization, which are vital in mitigating chilling injury (du Plooy *et al*., 2010). Lower concentrations of Kelpak® may be less effective at preventing chilling injury, as they might not offer sufficient phytohormonal or biochemical support to fully activate the plant's stress-response mechanisms (Bulgari *et al*., 2019). To achieve optimal protection, it is necessary to use an adequate concentration to fully utilize Kelpak® treatment’s mode of action, ensuring strong antioxidant activity, membrane stability, and effective stress signalling (Hayat *et al*., 2010). Different tomato genotypes may exhibit varying sensitivity to biostimulants due to genetic differences in hormone response pathways. Some genotypes might not react as efficiently to Kelpak® components, limiting its effectiveness in alleviating chilling injury (Bulgari *et al*., 2019). For example, certain tomato varieties have been identified as less responsive to external applications of auxins or cytokinins (Hayat *et al*., 2010).

Also, Kelpak® might not had any significant effect on the chilling index of tomatoes because of the developmental stage at which the fruit was stored at. Numerous research has shown that the severity of chilling injury in horticultural commodities lessens as the maturity level increases. For instance, tomatoes ripened at the green stage demonstrated chilling injury symptoms at 12 °C, whereas those ripened at the red stage displayed symptoms only when the temperature dropped below 7 °C. (Zhang *et al*., 2021). A similar study by Zhao *et al* (2009), showed that mangoes picked during the green-ripened stage exhibited a greater degree of CI compared to those gathered during the yellow-ripened stage at 2 °C after 12 days of room temperature storage.

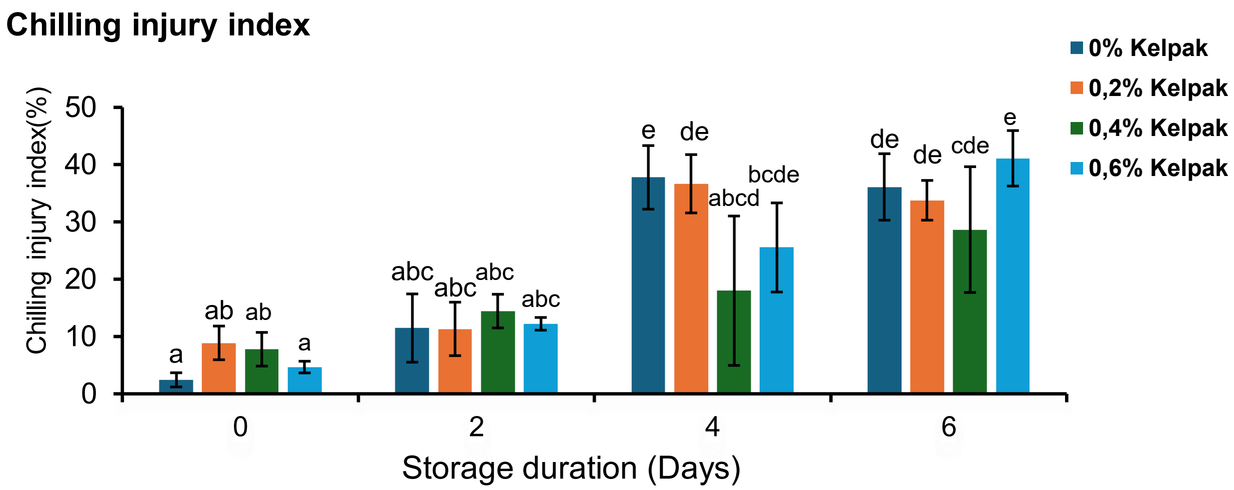

Figure 3.1 Effect of various Kelpak® treatments (0%, 0,2%, 0,4%, and 0,6%) on chilling injury index, over 6 days of ambient storage. Error bars indicate ± SE of means at P ≤ 0.05. Different letters above each bar indicates statistically significant differences among the treatments at a 95% confidence level.

Effect of Kelpak® on mass loss of tomato fruit

Different concentration of Kelpak® had no significant (p>0.05) effect on the mass loss of tomatoes during storage (Figure 3.2). However, there is decrease in mass loss percentage in all the concentrations. Where 0.2% is showing lower mass loss of 73,55 compared to 0, 0.4, 0.6% with a mass loss of 74.06, 76.28 and 73.72g respectively at the end of storage. Mass loss in tomatoes, is a critical indicator of post-harvest quality. The significance of mass loss primarily relates to factors like freshness, marketability, consumer acceptance, and shelf life (Kader, 2002). Higher concentrations of Kelpak® are more effective at activating the pathways involved in stress tolerance, including those related to chilling injury and dehydration stress. When Kelpak® is applied at low concentrations, it may not sufficiently activate these metabolic pathways to provide a protective effect. This limited activation can leave green tomatoes prone to desiccation and mass loss during storage Stirk and Singh (2020). The results of our study contradicted the results from a similar study made by Sharma *et al* (2016) which states that Kelpak® treatments reduce postharvest water loss in tomatoes by strengthening the cell wall and reducing permeability. Sharma and Singh (2020) also found out that rate of mass loss in lettuce that was treated with Kelpak® was reduced which was also contradicted by the findings of our study.

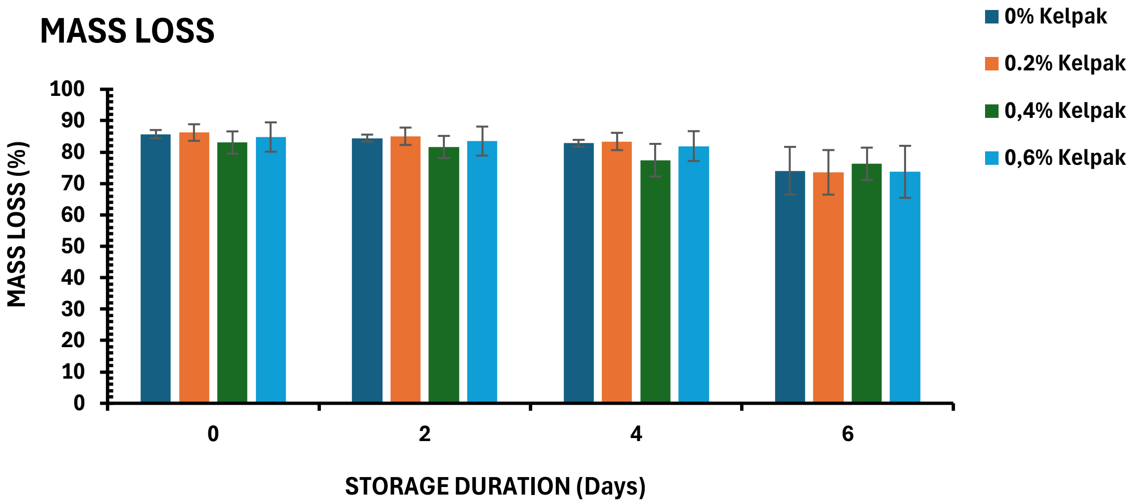

Figure 3.2 Effect of various Kelpak® treatments (0%, 0,2%, 0,4%, and 0,6%) on mass loss, over 6 days of ambient storage. Error bars indicate ± SE of means at P ≤ 0.05. Different letters above each bar indicates statistically significant differences among the treatments at a 95% confidence level.

Effect of Kelpak® on firmness

The firmness of tomato fruit is an indicator of the tomato's texture, maturity, freshness and it is crucial for determining the fruit's ripeness stage and shelf life (Harker *et al*., 1997). Figure 3.3 shows that there was a gradual decline of firmness on the tomato fruit from day 0 until day 6 storage period. Kelpak® concentrations had a significant effect ($p < 0.05$) on the firmness of the tomato fruits. Where, 0.2 and 0.4% had higher firmness of 43.62N And 41.82 N, respectively compared to the control. Therefore, the results suggest that Kelpak® is more effective in retaining firmness in lower concentration. This is because lower concentration of Kelpak® provides enough auxin to support cell wall maintenance, avoiding the excessive enzymatic activity that could degrade cell structure, promoting firmness without over-softening (Craigie, 2011).

Based on research conducted by Masny *et al*. (2004), strawberries were treated with Kelpak® and they showed notable differences in fruit firmness when they were compared to the untreated control groups. Kelpak® successfully retained firmness in similar produce like the strawberries, which supports the findings of our study that Kelpak® effectively retain firmness in horticultural produce particularly tomato fruit. Tomatoes with appropriate firmness are more likely to be perceived as fresh and of higher quality by consumers, while overly soft tomatoes are often associated with over ripeness and shorter shelf life (Giovannoni, 2001).

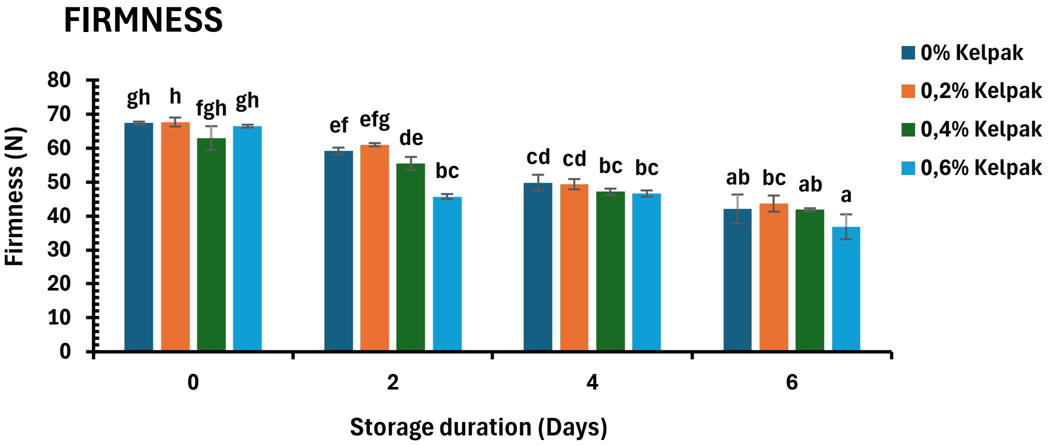

Fig 3.3 Effect of various Kelpak® treatments (0%, 0,2%, 0,4%, and 0,6%) on maturity index, over 6 days of ambient storage. Error bars indicate ± SE of means at P ≤ 0.05. The use of different letters above each bar indicates statistically significant differences among the treatments at a 95% confidence level.

Effect of Kelpak® on Maturity Index

The maturity index represents the balance between total soluble solids and acidity, often used to assess ripeness and post-harvest quality in horticultural products (Yuan *et al*., 2008). Kalpak® had a significant impact ($p < 0.05$) on the maturity index of the fruit. On day 0 maturity index starts at relatively similar across all treatments. However, as the day of storage progresses there is variation in the maturity index of the treatments. Where On day 2, the control shows a slightly higher maturity index than the 0.2% and 0.4% and slightly lower than the 0.6% Kelpak® treatments. The maturity index increases more significantly across all treatments by end of storage duration (day 6), with 0.2% reaching the highest index, followed closely by 0.6% Kelpak® and the control. Interestingly, the 0.4% displayed slightly lower maturity index compared to the control. The maturity index values for all concentrations remain relatively stable on day 4. This phenomenon indicated that Kelpak® treatments may help maintain quality over medium storage durations, likely by reducing respiration rates or slowing down enzymatic activities related to ripening (Yuan et al., 2008).

Research indicates that the use of Kelpak® can have an indirect impact on maturity by enhancing growth and development rates. In crops like leafy greens and tomatoes, the biostimulant aids in boosting overall growth, yield, and quality, which can influence both harvest timing and shelf life after harvest. Nevertheless, its direct impact on attributes typically associated with the maturity index, such as sugar levels, acidity, and firmness, tends to be more variable and reliant on specific contexts. For instance, trials with tomatoes have shown that the application of biostimulants like Kelpak® can optimize fruit size and quality characteristics, indirectly influencing perceived maturity stages (Quamruzzaman *et al.*, 2022; Kelpak, 2023).

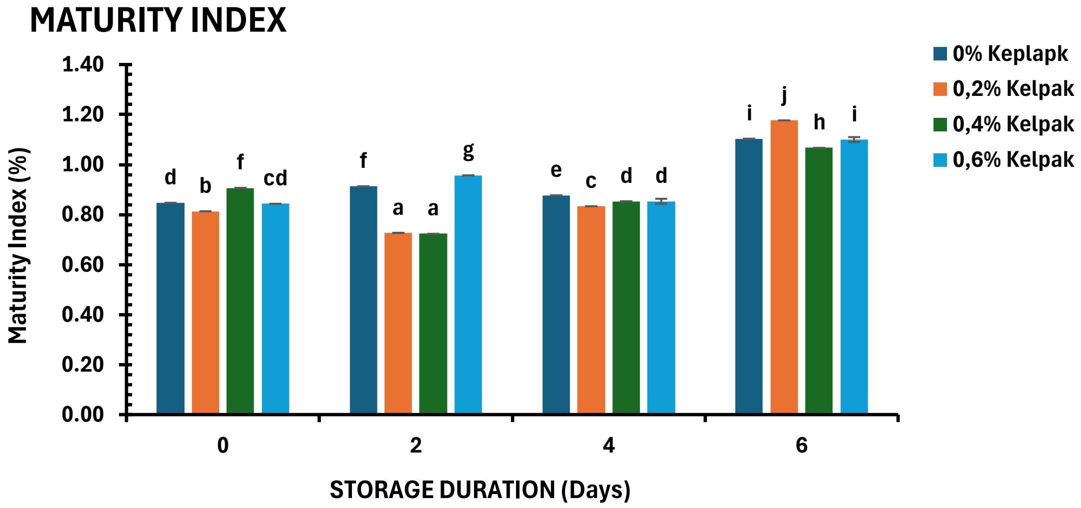

Fig 3.4 Effect of various Kelpak® treatments (0%, 0,2%, 0,4%, and 0,6%) on maturity index, over 6 days of ambient storage. Error bars indicate ± SE of means at $P \leq 0.05$. Different letters above each bar indicates statistically significant differences among the treatments at a 95% confidence level.

Effect of Kelpak® on BrimA Index

Figure 3.5 shows the effect of Kelpak® concentrations on BrimA index of tomato during 6 days of shelf life. The findings show that kelpak significantly ($p<0.05$) increased BrimA index of tomato where 0.2% showed higher BrimA index of 0.68 °brix compared to 0, 0.4, and 0.6% at the end of storage. 0.4% and 0.6% showed significantly lower BrimA index of 0.24 °brix and 0.33 °brix respectively compared to the control with 0.33 °brix. BrimA (Brix minus acidity) is a crucial metric used to assess the sweetness and overall flavour of tomato fruit (Fan *et al.*, 2011). The BrimA index is often used as a measure of fruit sweetness and acidity balance, with higher values generally indicating a sweeter profile. Therefore, the results suggest that Kelpak® concentration above

0.2% results in lower BrimA index of treated tomatoes indicating a decrease in sweetness of tomato, whereas lower concentration increases the sweetness. This effect might be because excessive application of biostimulants can lead to oxidative stress, which may impair enzymatic pathways involved in sugar synthesis and conversion. This can negatively affect the sweetness of the fruit (Fan *et al*., 2011).

The BrimA Index benefits from the seaweed-based biostimulant Kelpak®, which enhances sugar content (TSS) and improves the taste of fruits, often modifying BrimA in a way that is favourable to consumer preferences. Additionally, using Kelpak® (which naturally contains hormones like cytokinins and auxins) raises sugar levels in tomatoes and some commodities like strawberries and grapes (Hassan *et al*., 2016; Masny et al., 2004). In essence, Fig 3.5 suggests that lower concentrations of Kelpak® (specifically 0.2%) might delay the ripening process during storage, as shown by a lower initial BrimA index and a higher value at the end. These results are consistent with studies that show biostimulants such as Kelpak® can improve post-harvest attributes by adjusting ripening speeds and increasing sweetness in certain horticultural crops (Hassan *et al*., 2016; Masny *et al*., 2004).

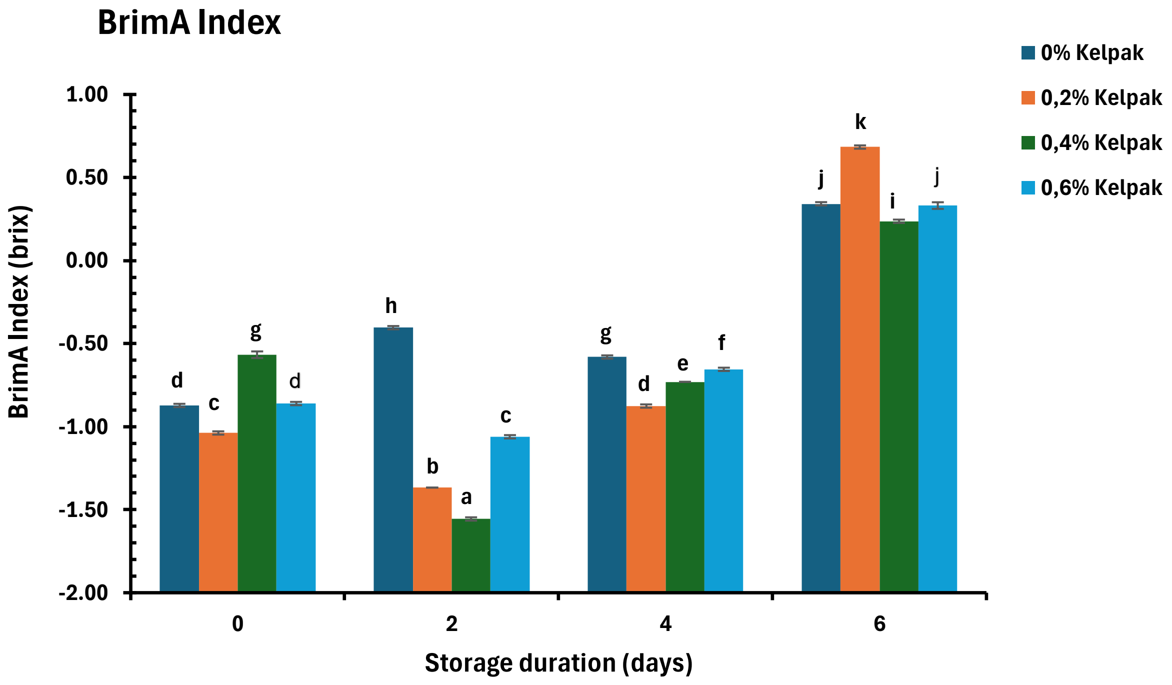

Figure 3.5 Effect of various Kelpak® treatments (0%, 0,2%, 0,4%, and 0,6%) on BrimA index, over 6 days of ambient storage. Error bars indicate ± SE of means at $P \leq 0.05$. Different letters above each bar indicates statistically significant differences among the treatments at a 95% confidence level.

Effect of Kelpak® on colour parameters (L*, b*, C* and h*)

Kelpak® treatments did not significantly impact colour parameters ($p > 0.05$), indicating that neither the colour lightness (L*), yellowness (b*), chroma (C*), nor hue (h*) values showed significant changes based on Kelpak® concentration during ambient temperature storage (Figure 3.5.6). Lightness (L*), across the storage period, lightness values showed minimal variation across treatments, with no distinct trends tied to increasing Kelpak® concentrations. This implies that Kelpak® does not affect the lightness of tomatoes during ambient storage. In terms of yellowness (b*), like lightness, the values of yellowness remain unchanged across different treatments and storage periods, indicating that Kelpak® does not influence the yellow colour of tomatoes.

Research on biostimulants reveals that while they may slow the degradation of pigments, they usually do not significantly affect b* values (Blanco-Díaz *et al*., 2020). For chroma (C*), which indicates colour saturation, there were minor variations without any distinct pattern between tomatoes treated with Kelpak® and those that were not. This implies that Kelpak® neither enhances nor diminishes colour intensity, corroborating the conclusions of Cantwell *et al*. (2008) that colour stability in stored tomatoes is not necessarily affected by certain treatments. Hue (h*), values slightly declined over storage days, which is common as tomatoes ripen and become redder. Nevertheless, no notable distinction was found between various Kelpak treatments, suggesting that Kelpak® does not markedly influence the colour shift associated with ripening. Overall, these findings suggest that Kelpak®, regardless of concentration, does not substantially change the colour parameters of tomatoes during short-term storage. These conclusions are in line with other studies showing that biostimulants have limited effects on fruit colour when used in moderate amounts (Ercolini, 2010).

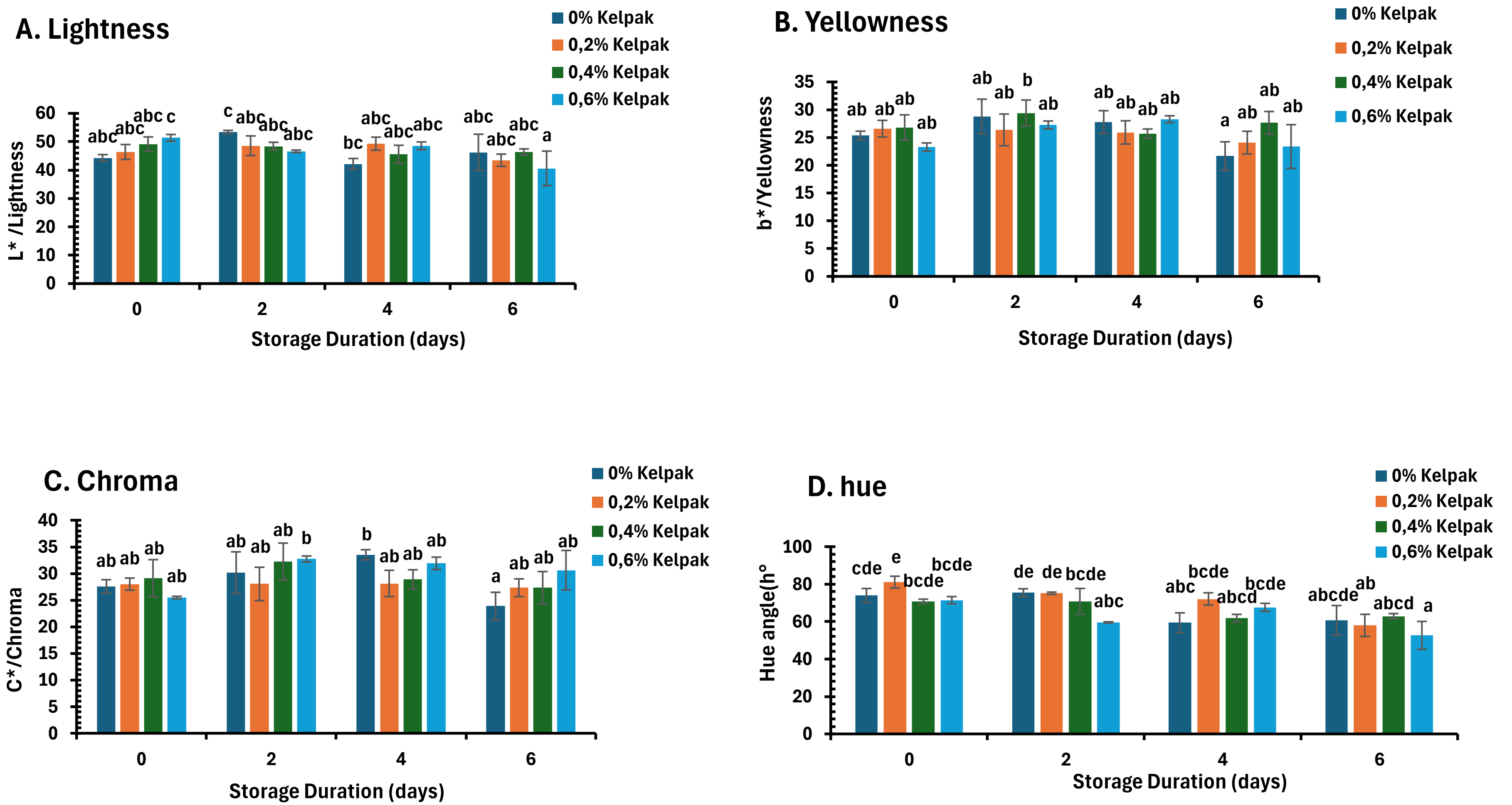

Figure 3.6 Effect of Kelpak® treatments (0%, 0,2%, 0,4%, and 0,6%) on chromatic colour parameter, L*/Lightness (A), b*/Yellowness (B), c*/Chroma (C) and h*/Hue (D) over 6 days of ambient storage. Error bars indicate ± SE of means at $P \leq 0.05$. The use of different letters above each bar indicates statistically significant differences among the treatments at a 95% confidence level.

Effect of Kelpak® on Total Soluble Solids (TSS)

Figure 3.7 shows the effect of kelpak® concentrations on TSS of tomato during 6 days of shelf life. The outcomes show that kelpak® significantly ($p<0.05$) increased TSS of tomato where 0.2% showed higher TSS of 4.83% compared to 0%, 0.4%, and 0.6% at the end of storage. 0%, 0.4% and 0.6% showed significantly lower TSS values of 4.43%, 4.51% and 4.32% respectively. The Total Soluble Solids (TSS) content is a crucial quality parameter in tomatoes, especially for their sensory characteristics such as sweetness, flavour, and overall consumer acceptance. Therefore, the results suggest that kelpak® concentration of 0.2% results in higher TSS of treated tomatoes whereas higher concentration decreases the TSS. This effect might have occurred because excessive application of biostimulants can lead to oxidative stress, which may impair enzymatic pathways involved in sugar synthesis and conversion (Fan *et al*., 2011).

Our findings indicate that Kelpak® initially impacts the soluble solid content, but its influence diminishes as tomatoes continue to ripen and are stored, likely due to biochemical transformations within the fruit. Higher TSS values typically correlate with increased sugar concentration, contributing to the sweetness and taste appeal of tomatoes (Fan *et al*., 2011). Our study highlights that Kelpak®-treated fruit exhibited elevated TSS levels compared to untreated fruit, demonstrating Kelpak®'s effectiveness in sustaining higher TSS levels which also explains the increase in the sweetness (BrimA Index) of the tomato fruit. Ali, Pervez, and Ashraf (2022) discovered that tomatoes treated with Kelpak® exhibited a notable rise in TSS in comparison to the control group. Similarly, cucumbers treated with seaweed extracts, including Kelpak®, demonstrated a higher concentration of TSS (Sharma and Singh 2020).

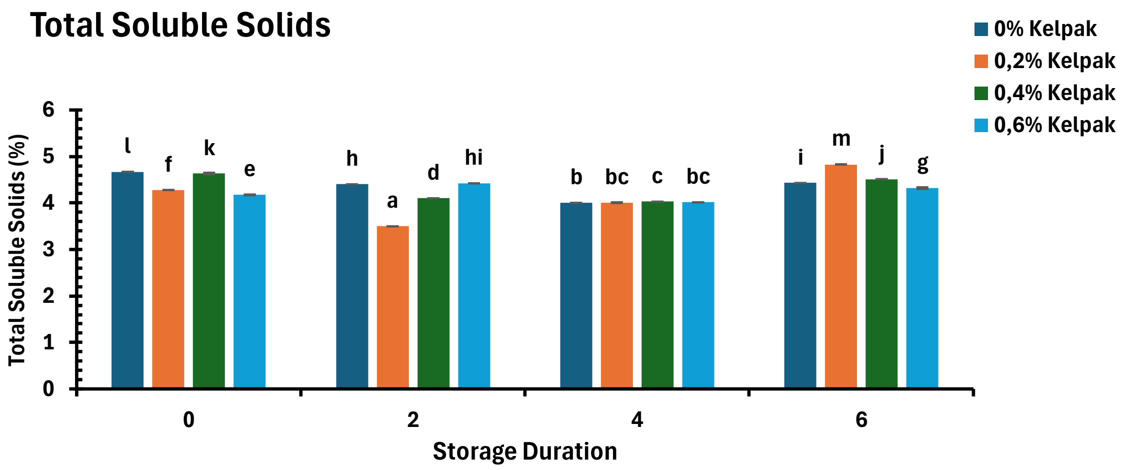

Figure 3.7 Effect of various Kelpak® treatments (0%, 0,2%, 0,4%, and 0,6%) on total soluble solids, over 6 days of ambient storage. Error bars indicate (variability of uncertainty in the TSS) ± SE of means at P ≤ 0.05. Different letters above each bar indicates statistically significant differences among the treatments at a 95% confidence level.

Effect of Kelpak® on Titratable Acidity (TA)

Figure 4.1 shows the effect of Kelpak® concentrations on TA of tomato during 6 days of storage. The results shows that Kelpak® significantly ($p<0.05$) increased TA of tomato where 0.4% showed higher TA of 4.24% compared to 0%, 0.2%, and 0.6% at the end of storage. 0%, 0.2% and 0.6% showed significantly lower TA values of 4%, 4.1% and 3.9% respectively. The Titratable Acidity (TA) content is an essential quality metric in tomatoes, especially for the taste of tomatoes which is determined by the balance between sweetness (due to sugars like glucose and fructose) and acidity (due to organic acids) (Baldwin *et al*., 2000). Higher titratable acidity generally leads to a more sour or tangy flavour, while lower acidity results in a bland taste.

Consumers often prefer tomatoes with a higher acid content, especially for cooking, as this enhances the taste profile (Baldwin *et al*., 2000). From our study it is evident from Figure 3.8, that 0,4% Kelpak® concentration retained higher levels of titratable acidity in the tomato fruit. Kelpak®'s high auxin content delayed the ripening process by diminishing the effect of ethylene, which is responsible for fruit ripening. The slower ripening helps retain higher acid concentrations for a longer period, as organic acids are typically consumed or transformed into sugars in the final phases of ripening (Gomez-Bellot *et al.*, 2013).

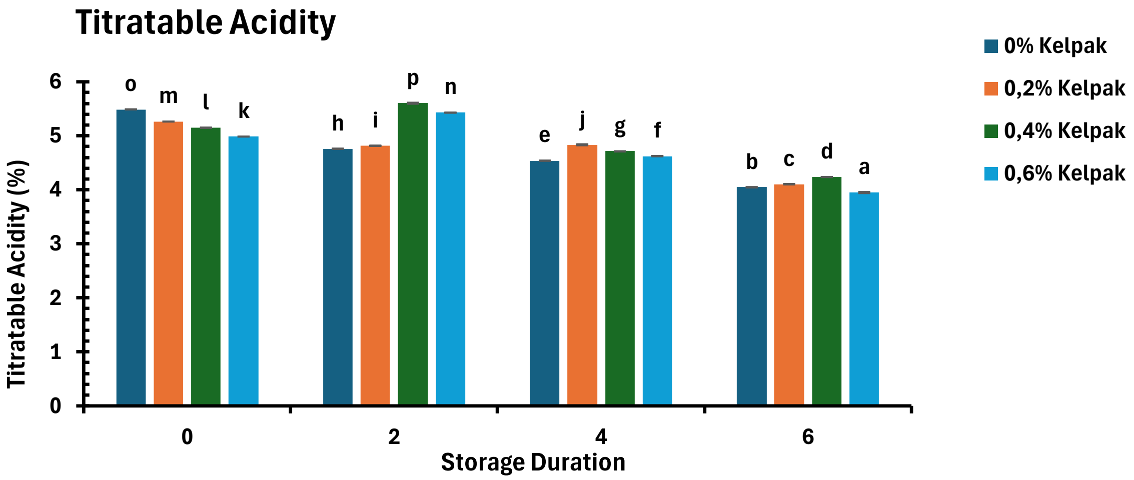

Figure 3.8 Effect of various Kelpak® treatments (0%, 0,2%, 0,4%, and 0,6%) on titratable acidity, over 6 days of ambient storage. Error bars indicate (variability of uncertainty in the TA) ± SE of means at $P \leq 0.05$. Different letters above each bar indicates statistically significant differences among the treatments at a 95% confidence level.

Effect of Kelpak® treatments on pH of stored tomato fruit

Application of Kelpak® had a significant ($p < 0.05$) effect on the pH of tomato fruit. At day 0, the pH values are relatively similar across all Kelpak® concentrations, ranging between approximately 3.8 and 4.0 units. The 0.2% Kelpak® treatment has a slightly lower pH than the other concentrations, suggesting an initial acidifying effect. On day 2, the pH increases for all concentrations, with the 0.2% Kelpak® treatment showing the highest increase, reaching around 4.2 units. Other concentrations, such as 0% and 0.6% Kelpak®, exhibit moderate increases in pH, though still lower than the 0.2% treatment. By day 4, the pH decreases again across all treatments. The 0.6% Kelpak® showed the lowest pH value around this time, which could imply a retention of acidity compared to the other treatments. This trend suggests that the pH of the tomato may stabilize or decrease as storage duration increases. On day 6, control (0%) Kelpak® treatment shows a significant rise in pH, reaching approximately 4.3, while the other treatments exhibit lower values. This may indicate that tomatoes without Kelpak®

treatment experience a notable increase in pH (reduction in acidity) over extended storage, possibly due to microbial activity or ripening processes.

Kelpak®, with its auxin and cytokinin content, can postpone the ripening of fruits, thereby preserving a greater number of organic acids and causing slower pH variations. Normally, organic acids break down during ripening, resulting in a pH increase. By postponing the ripening process, Kelpak® can help sustain lower pH levels for an extended duration, as shown in research on strawberries and tomatoes treated with seaweed extracts (Khan *et al.*, 2009). Our study shows that Kelpak® applications, especially at elevated concentrations, tend to keep pH levels higher (indicating lower acidity) throughout the storage duration compared to untreated controls. This implies that Kelpak® may retard the rise in acidity that generally happens during storage. Stress can frequently impair the plant's capability to retain acid levels, causing a pH increase. Based on our results, the phytohormones present in Kelpak® boost stress resilience, uphold acid metabolism, and thus help stabilize the pH in fruits such as tomatoes and cucumbers (Khan *et al.*, 2009).

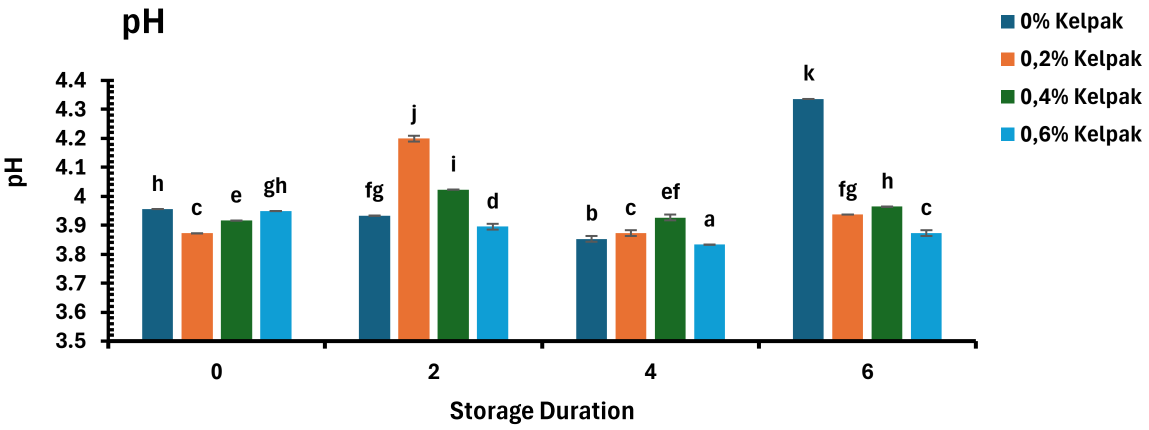

Figure 3.9 Effect of various Kelpak® treatments (0%, 0,2%, 0,4%, and 0,6%) on pH, over 6 days of ambient storage. Error bars indicate (variability of uncertainty in the pH) ± SE of means at $P \leq 0.05$. The use of different letters above each bar indicates statistically significant differences among the treatments at a 95% confidence level.

3.6. Conclusion and recommendations

The use of Kelpak® biostimulant on tomatoes yielded varying effects on several post-harvest quality parameters, such as firmness, maturity index, BrimA index, total soluble solids (TSS), titratable acidity (TA), and pH. However, it did not significantly reduce chilling injury (CI) or weight loss in tomatoes, which are crucial indicators of post-harvest quality. Lower concentrations of Kelpak®, specifically 0.2%, showed promising outcomes in maintaining firmness, TSS, and BrimA index, yet the biostimulant did not effectively alleviate CI across different concentrations. Additionally, Kelpak® treatments had a minimal impact on colour retention (L*, b*, C*, and hue), suggesting it might not be suitable for preserving visual quality during storage. These observations imply that while Kelpak® can enhance certain aspects of tomato quality, its capacity to diminish CI is limited, possibly due to factors such as the maturity stage, storage conditions, and genotype sensitivity to biostimulants. Notably, the 0.2% concentration of Kelpak® proved most effective for improving quality metrics like firmness and BrimA index.

Further research should explore whether adjusting concentration or repeating applications could boost Kelpak®'s efficacy, especially concerning CI and weight loss reduction. Furthermore, since Kelpak® alone does not significantly reduce CI, it may yield better results when used in combination with other post-harvest methods, like controlled atmosphere storage, which may enhance CI reduction. Given that CI severity may depend on ripeness, creating guidelines that factor in both genotype and maturity stage at harvest could improve Kelpak®'s effectiveness.

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CHAPTER 4

THE EFFECTS OF SEAWEED-BASED BIOSTIMULANT KELPAK® ON THE BIOACTIVE COMPOUNDS OF TOMATO FRUIT DURING POSTHARVEST STORAGE.

4.1. Introduction

Tomatoes (*Solanum lycopersicum* L.) are a widely grown crop worldwide, originating from South America, especially in the Andean areas, where native cultures have appreciated them for both culinary and medicinal purposes (Calvo *et al*., 2014a). Over centuries, tomatoes have become a staple in diets worldwide due to their versatility, nutritional benefits, and the potential health advantages they offer. The health benefits associated with tomato consumption are largely attributed to the inclusion of several bioactive compounds, including lycopene, carotenoids, phenolics, and proline, which are known for their antioxidant and anti-inflammatory properties (Sharma *et al*., 2016a). Hence, they are highly valued by farmers, as they contribute to economic stability and food security, especially within small-scale agricultural systems (Ronga *et al*., 2018).

Bioactive compounds such as lycopene serves as a powerful antioxidant, and it has been linked to a reduced risk of long-term illnesses, including cancer and heart-related illnesses (Khan *et al*., 2009). Besides carotenoids, tomatoes also contain phenolic compounds, which offer antioxidant defense by neutralizing free radicals and strengthening cellular integrity (Cheynier, 2012). These compounds act together to boost health by preventing oxidative harm at the cellular level. However, during cold storage these compounds delay senescence by scavenging free radicals and reducing lipid peroxidation, which helps maintain cell membrane integrity and prevents spoilage. Additionally, bioactive compounds modulate the activity of enzymes linked to browning and degradation, ensuring better quality during storage (González-Aguilar *et al*., 2010; Ali *et al*., 2019).

Current study has focused on enhancing the quantities of these bioactive compounds in tomatoes to enhance their health advantages. Research has investigated the application of biostimulants like seaweed extracts to boost lycopene and carotenoid concentrations in tomatoes after harvest (Rouphael and Colla, 2020). Various techniques have been examined to optimize bioactive compounds in tomatoes and other produce after harvest, with the goal of improving their nutritional and antioxidant characteristics. These approaches cover UV light treatments (Costa *et al*., 2006), thermal treatment, hormonal applications (Ding et al., 2002), and the use of elicitors (Bautista-Baños *et al*., 2006) among others. Although progress has been made, a considerable lack of comprehension remains regarding the exact mechanisms through which these approaches increase bioactive compounds. This creates an opportunity to investigate more efficient, cost-effective, and economical alternatives like Kelpak®.

Kelpak® is a natural plant growth enhancer derived from the seaweed *Ecklonia maxima*, a type of brown seaweed located off the coast of South Africa (Calvo *et al*., 2014). This seaweed extract is processed to maintain high levels of growth-promoting substances, mainly auxins and cytokinins, which are pivotal plant hormones that are recognized for their function in stimulating growth and advancement. Kelpak® also includes an array of nutrients, minerals, and other organic substances that aid plant metabolic processes (Spinelli *et al*., 2010). Kelpak® is gaining recognition as an eco-friendly alternative to synthetic chemicals, offering both environmental and economic advantages. Its natural formulation makes it suitable for use in both organic and conventional agriculture, providing farmers with a safe, residue-free means to enhance the quality of their commodities avoiding adverse side effects. Auxins together with cytokinins present in Kelpak® promote root development, boost nutrient absorption, and increase stress resistance, which in turn enhances crop resilience during post-harvest handling and storage (Rouphael and Colla, 2020). Consequently, our study focuses on employing Kelpak® to encourage the production of bioactive compounds in tomatoes during postharvest storage.

4.2. METHODOLOGY AND ANALYTICAL PROCEDURE

Experimental site and Plant materials

Mature green tomato fruit were sourced from the commercial market in Polokwane, Limpopo Province, a region recognized as South Africa’s primary tomato production hub. The choice of this market ensured a consistent supply of high-quality tomatoes with uniform maturity. Subsequently, the tomatoes were transported to the Agricultural Research Council Tropical and Subtropical Crops (ARC-TSC) postharvest laboratory in Mbombela, Mpumalanga Province, South Africa, for treatment application, cold storage, and analysis.

Treatments, experimental design and procedures

Fruits that were even in size, consistent in color, and free of imperfections were chosen for the experiment. 10g of fungicide Dithane M.45 per 5L of water was prepared for fruit sanitation. Fruit were dipped in the fungicide for 3 minutes and was left to air dry at ambient temperature. After air-drying, fruit were grouped equally according to their treatments (Treatment 1, Treatment 2, Treatment 3 and Treatment 4). Each group was dipped in biostimulant Kelpak® at 0 (control)(T1), 0.2%(T2), 0.4%(T3) and 0.6%(T4) concentrations respectively. Fruits were allowed to airdry at room temperature. Subsequently, stored at 10 °C for 20 days. Following the period of cold storage, the fruit were maintained at room temperature (22 - 25 °C) to simulate retail conditions (Nunes *et al*., 2006; Luengwilai *et al*., 2014). The experiment comprised of 4 treatments and each treatment was replicated 3 times. Each treatment had 60 fruits and 42 fruits per treatment were used to collect data for destructive assays throughout the sampling period, with 6 fruits being taken per treatment on each sampling day. These fruits were used to collect fruit juice, which was used to measure TSS, TA, pH and used to analyse bioactive compounds.

The experiment utilized a 4 by 4 factorial set up in a completely randomized design. (CRD). Kelpak® concentrations at 4 levels (0, 0.2%, 0.4% and 0.6%) and shelf-life duration at 4 levels (0, 2, 4 and 6 days) were considered factors.

4.3. Data collection

Total carotenoids and chlorophyll

The amounts of total carotenoids and chlorophyll were assessed using an adapted version of the technique outlined by Francesca *et al.* (2020). In summary, 0.25g of the tomato sample was extracted with 24mL of a 40:60 acetone: hexane mixture (v/v). Subsequently, the solution was centrifuged (Mistral 1000, MSE, USA) at 15,000 rpm for 5 minutes. The collected supernatants were then stored at -20 °C until they were analyzed. The absorbance was measured using a spectrophotometer (Jenway 7305, Bibby Scientific Ltd., UK) at 470, 663 and 645 nm for carotenoids, chlorophylls a and b, respectively. The cumulative quantities of pigments were calculated using formulas advised by Lichtenthaler and Wellburn (1983) as follows:

$$\text{Chlorophyll a} = 11.75\ A_{662} - 2.350\ A_{645} \qquad \text{eq (1)}$$

$$\text{Chlorophyll b} = 18.61\ A_{645} - 3.960\ A_{662} \qquad \text{eq (2)}$$

$$\text{Carotenoids} = 1000\ A_{470} - 2.270\ \text{Chl a} - 81.4\ \text{Chl b}/227 \qquad \text{eq (3)}$$

The findings were presented as milligrams of pigment per gram of fresh mass (mg g-1 FM).

Lycopene

Lycopene was assessed following the procedure of Nkolisa *et al.* (2019), with some alterations. A 100 µL Drummond micro pipettor was used to take a sample of tomato juice. After the sample was drawn into the pipette, the sample was transferred to a 20 – 125mL screw cap tube. In addition, multiple blank samples containing 100 µL of water in place of tomato pulp were prepared. Using a repipetter, 8ml of a blend of hexane, ethanol, and acetone in a 2:1:1 ratio was introduced to the samples. The tube was promptly sealed and mixed using a vortex, then stored away from light. Following a minimum of 10 minutes, 1 ml 1 ml of distilled water was introduced into each sample and mixed again using the vortex. The samples were left undisturbed for 10 minutes to enable the separation of hexane layers and to ensure all air bubbles disappeared. The cuvette was cleaned using the top layer from one of the blank samples. After discarding this, a new blank was used to calibrate the spectrophotometer to zero at 503 nm. The absorbance at 503 nm of the top layers in the lycopene samples was measured. The concentration of lycopene in the hexane extracts was subsequently determined in the following manner:

$$\text{Lycopene (mg/kg)} = (A_{503} \times 537 \times 8 \times 0,\ 55)/\ (0,\ 1 \times 172)$$

$= A_{503} \times 137, 4$ eq (4)

Where, lycopene molecular weight = 537g/mole

Volume of mixed solvent = 8mL

Volume ratio of the upper layer to the mixed solvents = 0, 55

Weight of tomato added = 0, 1

Extinction coefficient for lycopene in hexane = 172 mM^{-1}

Proline

Proline was determined using the acid ninhydrin modified method (Shan *et al*., 2007; Zhao *et al*., 2009). Proline was extracted from tissues using 3% (v/v) sulfosalicylic acid at 100 °C. A test tube was used to combine 2 ml of the sample with 2 ml of ninhydrin reagent and 2 ml of glacial acetic acid, followed by boiling for 1 hour at 100 °C. (To prepare the ninhydrin reagent, 2.5 g of ninhydrin was mixed into a solution comprising 60 ml of glacial acetic acid and 40 ml of 6 mol l - 1 phosphoric acid. After cooling the reaction in an ice bath, 4 ml of toluene was used to extract the mixture and thoroughly agitated using a vortex mixer for 15 to 20 seconds. The reddish phase was transferred to a cuvette, and its absorbance was recorded at 520 nm utilizing a UV/Vis spectrophotometer with toluene as the blank. The concentration of proline was determined by employing a standard curve ranging from 0 to 5 µg ml-1, and the results were normalized to fresh mass (g µg -1 FM).

4.3.4. Malondialdehyde

The MDA was determined calorimetrically as illustrated by Collins *et al*. (2004). For each sample, two replicates consisting of 210 µl of plasma were placed into separate test tubes with 11 µl of 500 mM butylated hydroxytoluene and 5.3 µl of concentrated hydrochloric acid. The tubes were sealed, shaken, and heated to 60°C for 80 minutes before being brought back to room temperature. Afterward, 680 µl of N-methyl-2-phenylindole in acetonitrile was added. The tubes were again shaken and spun in a centrifuge at 13,000 g for 5 minutes. New tubes were set up, into which 660 µl of the clear supernatant and 115 µl of concentrated HCl were transferred. These tubes were sealed, shaken, and incubated again at 45°C for 60 minutes. The samples were then spun in a centrifuge at the same speed of 13,000 g for an additional 5 minutes, and the supernatant was analysed using a spectrophotometer at a wavelength of 575 nm.

Sample concentration was ascertained utilizing a calibration curve based on five standards.

Total phenolics

The Folin-Ciocalteu (FC) colorimetric assay method, as described by Meyers in 2003, was employed to assess the overall phenolic content. A sample consisting of 125 µL of tomato juice was combined with 0.5 mL of distilled water and 125 µL of FC reagent. Following this, 2 mL of Folin-Ciocalteu phenol reagent was added to 0.2 mL of the diluted mixture. This was stirred and left to sit at ambient temperature for 6 minutes. Subsequently, 2 mL of a 7% $NaCO_3$ solution was added, stirred again, and maintained at room temperature in a dark environment for 90 minutes. The absorbance was then recorded at a wavelength of 750 nm with a spectrophotometer, and the overall phenolic content was reported as milligrams of gallic acid equivalents (GAE) per kilogram of fresh weight (FW).

4.4. Data analysis

The collected data was subjected to analysis of variance (ANOVA) to identify notable differences between the treatments, utilizing GenStat 23rd edition software (VSN International, UK). The means of the significant variables were distinguished employing Duncan's Multiple Range Test with a 5% significance level.

4.5. Results and Discussion

Effect of kelpak® treatment on total carotenoid content of tomato fruit

Carotenoids are crucial pigments in tomatoes, providing red and orange colours, and are usually produced as tomatoes mature (Calvo *et al.*, 2014a). Kelpak® treatments had a significant effect ($p < 0.05$) on the overall carotenoid concentration in tomatoes stored for 6 days at ambient temperature after cold storage (Figure 4.1). 0.2%, 0.4% and 0.6% concentration of Kelpak® exhibited the highest carotenoid levels of 11.2, 34.81 and 27.8 mg g-1FM; respectively compared to the control with lowest carotenoid level of 3,41 mg g-1FM. However, amongst the concentrations, highest total carotenoids were observed in the 0.4% Kelpak® by the conclusion of the storage period.

This indicates that a 0.4% Kelpak® concentration could be ideal for stimulating or preserving carotenoid synthesis of tomatoes after cold storage, potentially by activating the biosynthetic pathway, which is consistent with some studies highlighting the role of biostimulants in boosting carotenoid accumulation in tomatoes (Calvo *et al.*, 2014a). This pathway involves key enzymes such as phytoene synthase (PSY), phytoene desaturase (PDS), and lycopene β-cyclase, which convert precursor molecules like geranylgeranyl pyrophosphate into various carotenoids, including lycopene, β-carotene, and lutein. Biostimulants like Kelpak® may enhance the expression or activity of these enzymes, thereby increasing carotenoid production post-cold storage. Specifically, concentrations around 0.4% have been reported as effective for optimizing the balance between growth promotion and metabolic activity without causing phytotoxic effects (Calvo *et al.*, 2014a).

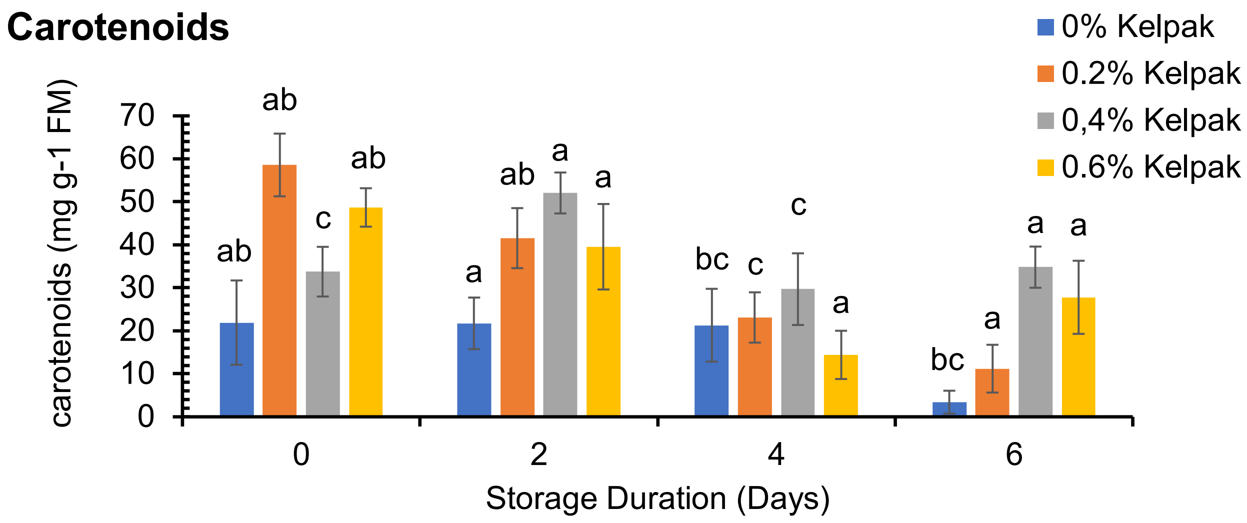

Figure 4.1 Effect of Kelpak® concentrations (0%, 0,2%, 0,4% and 0,6%) on Carotenoids of tomato fruit over 6 days of ambient temperature storage. Error bars indicate ± SE of means at $p<0.05$. Different letters above each bar indicates statistically significant differences among the treatments at a 95% confidence level.

Effect of Kelpak® treatment on chlorophyll content (a and b) of tomato fruit

Chlorophyll is a key pigment in photosynthesis that usually breaks down as tomatoes ripen, leading to a colour transition from green to red (Ronga *et al*., 2018). The application of Kelpak® significantly influenced ($p < 0.05$) the breakdown of both chlorophyll a and chlorophyll b in tomatoes during postharvest storage. In the study, both chlorophyll a and b decreased as the storage duration increases. Compared to the control, 0.2%, 0.4%, and 0.6 % of kelpak® treatment significantly ($p<0.05$) delayed the degradation of chlorophyll. However,0.4% concentration of Kelpak® was more effective compared to the other concentration levels (Figure 4.2 and 4.3). Therefore, the results indicate the Kelpak® delayed the chlorophyll degradation in tomatoes, with 0.4% being the most effective concentration. This trend implies that lower concentrations, such as 0% and 0.2%, lead to faster decreases in chlorophyll b, indicating a lack of sufficient delay in the ripening process. These results support previous reports suggesting that biostimulants can reduce the breakdown of chlorophyll, thereby delaying ripening (Ronga *et al*., 2018).

The process of carotenoid build-up and chlorophyll breakdown are linked in how tomatoes ripen (Calvo *et al*., 2014). Generally, chlorophyll breakdown occurs before carotenoids are produced as tomatoes change colour from green to red. The study indicates that a 0.4% concentration of Kelpak® successfully slows down chlorophyll breakdown (both chlorophyll a and b) while boosting carotenoid production, potentially striking a balance between ripening and preserving shelf life. Kelpak® at an optimal 0.4% concentration appears to activate biochemical pathways that both preserve chlorophyll and enhance carotenoid synthesis, aligning with findings that biostimulants can delay senescence while promoting pigment production (Rouphael and Colla, 2020). Lower concentrations (0% and 0.2%) are insufficient for these effects, whereas higher levels (0.6%) may lead to reduced efficacy, potentially due to overstimulation or stress. Calvo et al. (2014b) also observed similar results, showing that optimal biostimulant concentrations enhance pigment synthesis and stress resilience, while excessive amounts may induce oxidative stress or nutrient imbalances. Conversely, some studies, like those by Khan *et al*. (2009), found that higher biostimulant doses occasionally promoted greater metabolic activity, emphasizing the variability of responses depending on plant species, environmental conditions, and application protocols.

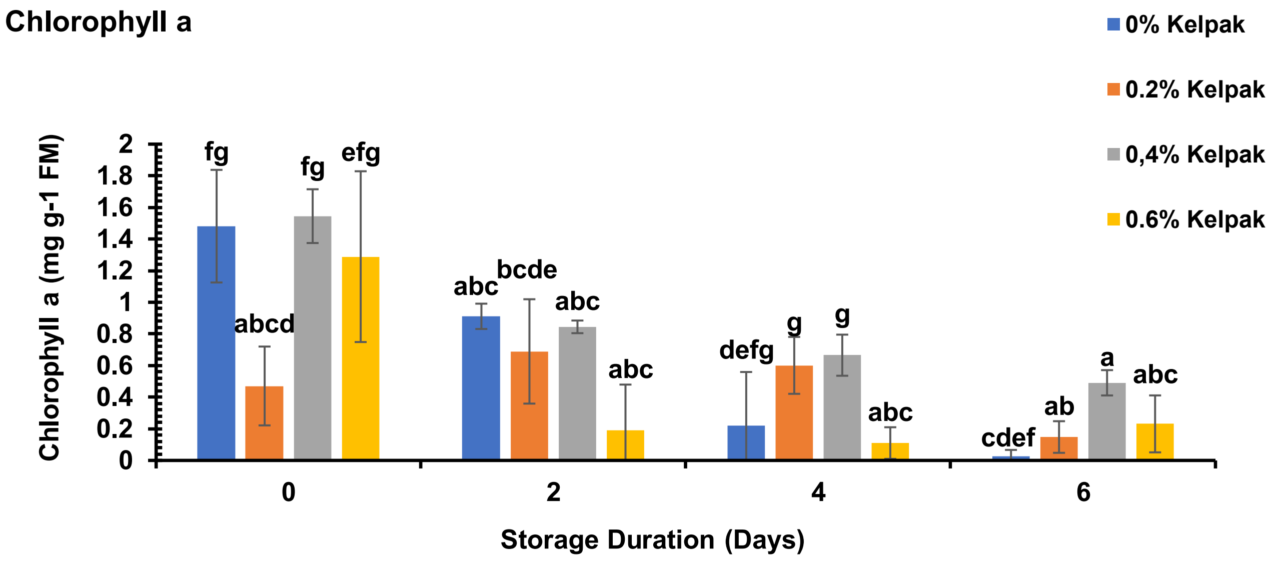

Figure 4.2 Effect of Kelpak® concentrations (0%, 0,2%, 0,4% and 0,6%) on Chlorophyll a of tomato fruit over 6 days of ambient temperature storage. Error bars indicate ± SE

of means at p<0.05. Different letters above each bar indicates statistically significant differences among the treatments at a 95% confidence level.

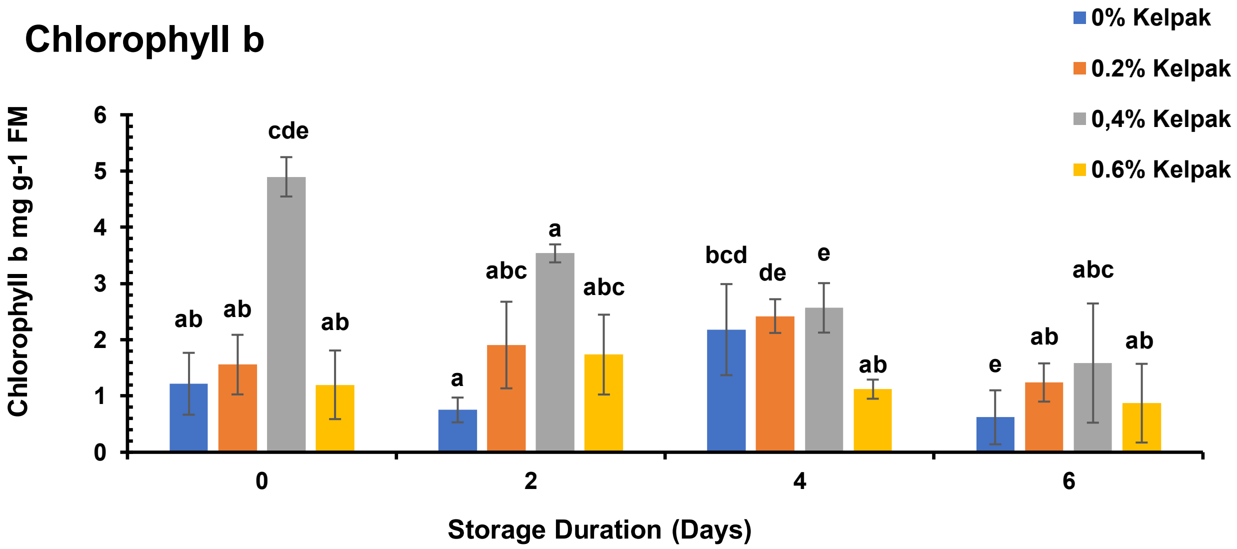

Figure 4.3 Effect of Kelpak® concentrations (0%, 0,2%, 0,4% and 0,6%) on Chlorophyll b of tomato fruit over 6 days of ambient temperature storage. Error bars indicate ± SE of means at p<0.05. Different letters above each bar indicates statistically significant differences among the treatments at a 95% confidence level.

Effect of Kelpak® on lycopene content of tomato fruit

Lycopene is a crucial pigment that influences the colour and antioxidant qualities of the fruit. As shown in Figure 4.4, the content of lycopene presented an increasing trend in both control and kelpak® treated fruit during shelf-life period. Lycopene content significantly (p<0.05) increases progressively across all treatments as storage duration increases. Among the treatments, 0.4% Kelpak® consistently results in the highest lycopene content of 153.44 mg/g FM at the end of storage. In contrast, 0.6% Kelpak® shows lower lycopene accumulation of 64.38 mg/g FW, while 0.2% Kelpak® and the control resulted in moderate increases of 58.02mg/g FW and 104.22mg/g FW, respectively by the conclusion of storage. Therefore, the outcome suggests that 0.4% Kelpak® is the optimal treatment for maximizing lycopene accumulation during storage, while higher (0.6%) or lower (0.2%) concentrations are less effective or even inhibitory. The response difference of Kelpak® treatments could be due to the complex biochemical and physiological interactions between plant growth regulators (PGRs) present in the extract and the metabolic pathways involved in lycopene synthesis (Khan *et al*., 2009). Khan et al. (2009) stated that seaweed extracts like Kelpak can enhance antioxidant activity and pigment accumulation in fruits by inducing metabolic processes that favour secondary metabolite synthesis. Sharma *et al*. (2016b) found that biostimulants contribute to increased lycopene content in tomatoes due to their ability to enhance stress tolerance and antioxidant enzyme activity, which helps to preserve lycopene during postharvest storage.This could be due to physiological stress or an inhibitory impact on lycopene synthesis pathways. The observed effects of Kelpak® on lycopene content align with findings from other studies that indicate the impact of seaweed-based biostimulants on fruit quality.

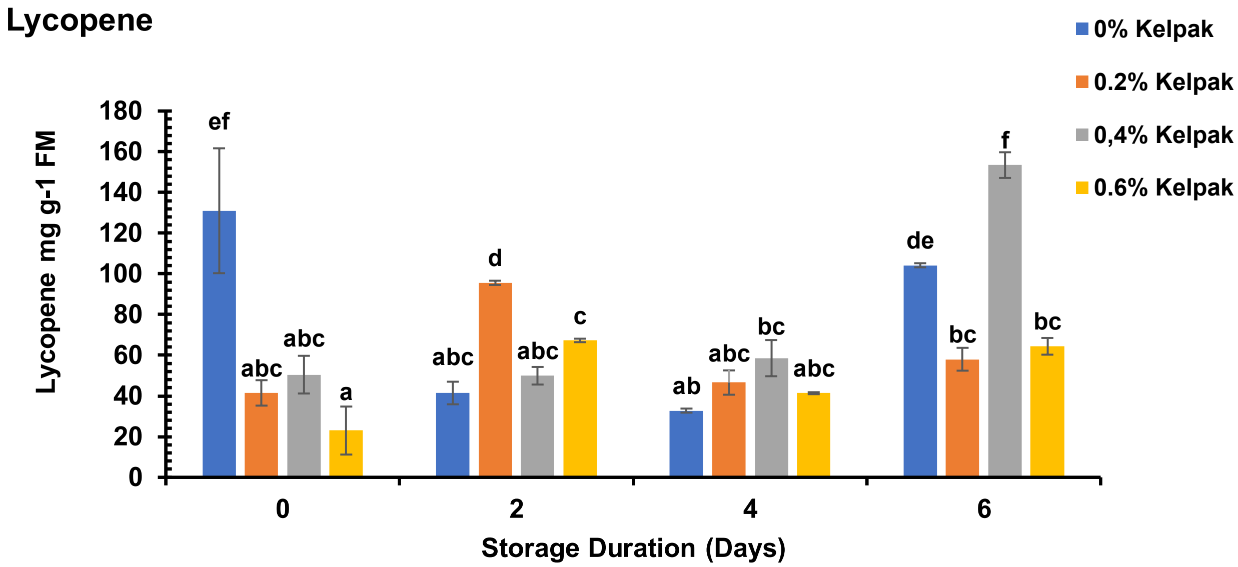

Fig 4.2 Effect of Kelpak® concentrations (0%, 0,2%, 0,4% and 0,6%) on Lycopene of tomato fruit over 6 days of ambient temperature storage. Error bars indicate ± SE of means at $p<0.05$. Different letters above each bar indicates statistically significant differences among the treatments at a 95% confidence level.

Effect of Kelpak® on Proline content

Proline constitutes an amino acid which builds up in plants when they face stress, often helping in osmotic regulation and cellular protection (Khan *et al*., 2009). Kelpak® treatments did not significantly ($p > 0.05$) increase proline levels in tomato during the 6-day post-harvest storage. However, from Figure 4.5 it is evident that the 0.2% Kelpak treatment seems to be the most efficient in boosting proline content in green tomato fruit, particularly on day 2, where it significantly outperforms the other treatments having a value of 0,84mg L.

Kelpak$^{®}$ is known for its high concentrations of auxins and cytokinins, but it does not include direct compounds or mechanisms that encourage the synthesis of proline, a process mainly triggered by stress (Ashraf and Foolad, 2007). Once the fruit is picked, it is removed from the plant's regulatory system, which restricts the physiological pathways responsible for proline production. Kelpak$^{®}$ primarily functions through auxin and cytokinin pathways to promote growth and does not directly trigger stress pathways related to proline synthesis in harvested fruit (Hare and Cress, 1997). Therefore, there is little evidence to support the idea that Kelpak raises proline levels in harvested fruits specifically.

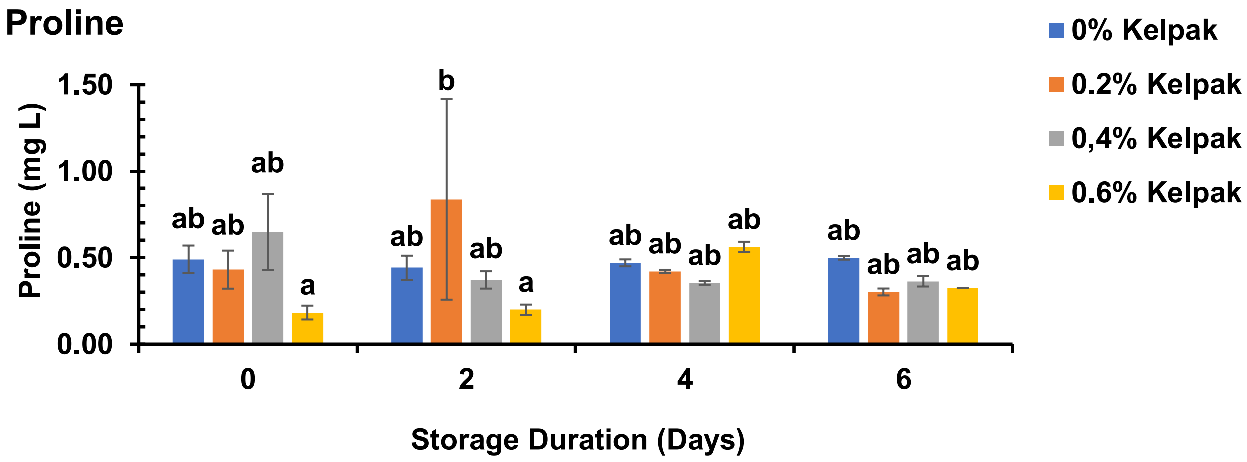

Fig 4.5 Effect of Kelpak$^{®}$ concentrations (0%, 0,2%, 0,4% and 0,6%) on Proline of tomato fruit over 6 days of ambient temperature storage. Error bars indicate ± SE of means at $p<0.05$. Different letters above each bar indicates statistically significant differences among the treatments at a 95% confidence level.

Effect of kelpak$^{®}$ treatment on malondialdehyde content of tomato fruit

In the study, MDA content in tomato fruit was not significantly ($p > 0.05$) affected by the kelpak treatments during six days of storage (Figure 4.6). Malondialdehyde (MDA) serves as a biomarker for lipid peroxidation and is frequently used to indicate oxidative stress in plant tissues. Elevated MDA levels indicate greater lipid damage, which is generally undesirable in stored produce since it can lead to more rapid deterioration (Sharma *et al*., 2014). Although this finding does not achieve statistical significance, the trend implies that the 0.4% concentration might exert a slight antioxidant effect, potentially reducing oxidative stress during storage.

Additionally, our study's results opposed the findings of Kumar *et al*. (2016), who assessed the impact of Kelpak on tomato plants under drought conditions and found a notable decrease in MDA levels in the treated plants. Similarly, Fan *et al*. (2011) discovered that Kelpak®-treated strawberry plants exhibited lower MDA levels in their fruit during postharvest storage. Their study indicated that applying Kelpak® boosted the fruit's antioxidant defense, reducing lipid peroxidation and preserving cell membrane stability. This was especially advantageous for prolonging the storage life and condition of the strawberries while stored.

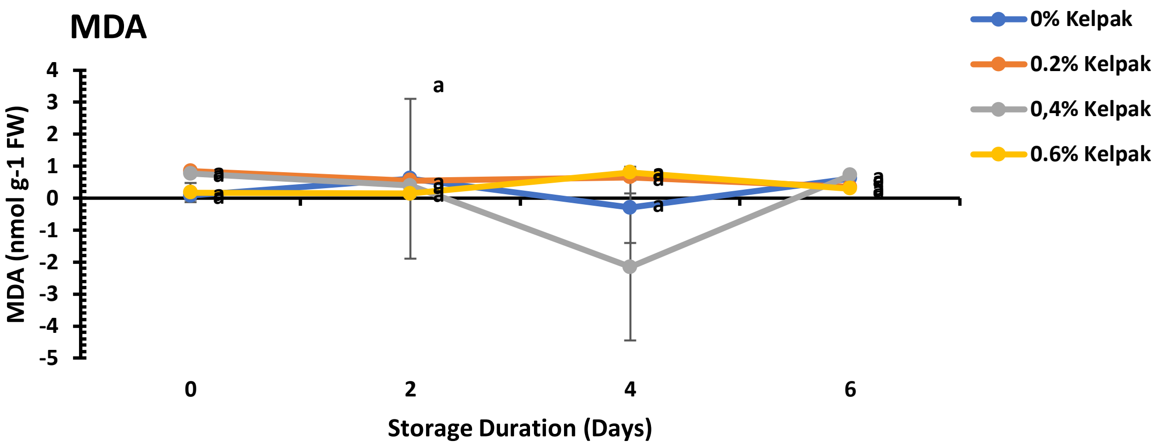

Figure 4.6 Effect of Kelpak® concentrations (0%, 0,2%, 0,4% and 0,6%) on malondialdehyde (MDA) of tomato fruit over 6 days of ambient temperature storage. Error bars indicate ± SE of means at $p<0.05$. Different letters above each bar indicates statistically significant differences among the treatments at a 95% confidence level.

Effect of Kelpak® on phenolic compounds

Change in total phenolic content in tomato fruits are shown in Figure 4.7 both control and Kelpak® treated tomato fruit indicated an increasing trend in the total phenolic content during 6 days of ambient storage. Tomato fruit treated with 0.4% kelpak® and the control showed a significantly($p<0.05$) higher levels (0.06mg/g GAE) of phenolic content compared to the control, 0.2% and 0.6% treatments at the end of storage duration. However, 0.2% had a higher content of 0.04mg/g GAE at the end of storage, followed by 0.6% with 0.03mg/g GAE, when compared to the control (0.02 mg/g GAE).

The 0.4% Kelpak® treatment was the most effective in enhancing or maintaining phenolic content over the storage duration, especially noticeable on days 4 and 6, where it showed statistically higher values than other treatments. This indicates that moderate concentrations (0.2% and 0.4%) of Kelpak® might boost phenolic accumulation or reduce phenolic degradation in green tomato fruit, potentially due to its influence on antioxidant pathways (Craigie, 2011; Fan *et al*., 2011).

Phenolics encompass a broad range of plant compounds known for their antioxidant capabilities, playing essential roles in pathogen defense, UV shielding, and maintaining structural integrity. They enhance plant resilience and offer considerable health benefits in human consumption (Cheynier, 2012). The increase in phenolic content of Kelpak® treated fruits could be due to its capacity to enhance antioxidant defense and secondary metabolite production (Spinelli *et al*., 2010a; Sharma *et al*., 2014). Literature indicates that Kelpak® has been shown to increase the synthesis of phenolic compounds in various horticultural products.

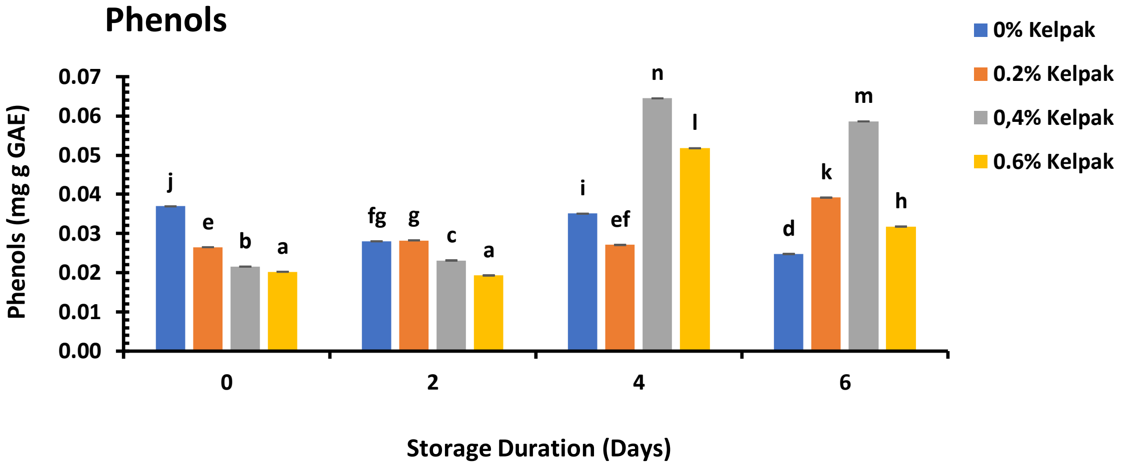

Figure 4.7 Effect of Kelpak® concentrations (0%, 0,2%, 0,4% and 0,6%) on phenolics of tomato fruit over 6 days of ambient temperature storage. Error bars indicate ± SE of means at $p<0.05$. Different letters above each bar indicates statistically significant differences among the treatments at a 95% confidence level.

4.6. Conclusion

The study demonstrated that Kelpak® biostimulant significantly influences pigment retention, lycopene synthesis, and phenolic content in green tomatoes during shelf-life period. This research indicates that observed that fruits treated with 0.4% Kelpak® concentration was the most effective in delaying chlorophyll degradation, promoted carotenoid synthesis and lycopene content. Furthermore, 0.4% Kelpak® treatment promoted higher accumulation of total phenolic compound compared to the other treatments. Whereas, higher (0.6%) and lower (0.2%) concentrations of Kelpak® were less effective, and high concentrations showed potential for adverse effects, likely because of overstimulation or oxidative stress. Therefore, according to the results concentration of 0.4% Kelpak® is recommended for postharvest use to enhance pigment retention and antioxidant content, thereby increasing both the visual attractiveness and nutritional value of tomato fruit. Thereby, increasing consumer acceptance and marketability of tomato fruits.

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Chapter 5

CONCLUSION and RECOMMENDATIONS

5.1. **Conclusion**

The study evaluated the effects of Kelpak®, a seaweed-based biostimulant, on the postharvest quality of tomatoes at shelf life. Although Kelpak® had a limited impact on reducing symptoms of chilling injuries, it maintained quality attributes such as firmness, sweetness and maturity index, at a concentration of 0.4% and partially 0.2%. The study revealed that a concentration of 0.4% was optimal, improving pigment retention, lycopene synthesis, and phenolic content while delaying chlorophyll degradation. Lower (0.2%) and higher (0.6%) concentrations were less effective, with higher concentrations possibly causing adverse effects such as oxidative stress. The study demonstrated that Kelpak® positively impacts tomato fruit quality at shelf life, though its role in directly mitigating chilling injury remains limited. The study provides a foundation for optimizing biostimulant use in postharvest management. These findings highlight the selective benefits of Kelpak® in maintaining tomato quality during storage.

This study highlights the benefits of using seaweed-based biostimulants for tomato producers, stakeholders in the supply chain and consumers. It also, emphasizes eco-friendly and cost-effective methods to improve post-harvest tomato quality, reduce waste and improve marketability. Consumers benefit from better taste and nutrition, while researchers gain insights into biostimulant applications for sustainable agriculture. The study contributes to understanding the role of biostimulants in reducing chilling stress and maintaining post-harvest quality, and it identifies opportunities for future research on biochemical and molecular mechanisms and integration of biostimulants with advanced storage technologies.

5.2. Recommendation

The study suggests conducting additional research to determine the best timing for Kelpak® application, whether before or after harvest because there is limited literature as to whether Kelpak® is more effective in improving the shelf life of tomato fruit when applied pre- or post-harvest. Moreover, to investigate the effects of combining it with other biostimulants or stress-reducing agents to enhance its benefits.