Cape Peninsula University of Technology

Impact of activated water treatments and packaging systems on physiological responses, phytonutrients and overall quality of minimally processed Swiss chard (*Beta vulgaris* L.)

by

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DECLARATION

I, **Ayanda Gift Masilela**, declare that the contents of this thesis represent my own unaided work, and that the thesis/dissertation has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

31/10/2025

Signed **Date**

ABSTRACT

Swiss chard is highly susceptible to postharvest handling practices that compromise its physicochemical quality and significantly reduce its shelf life, despite its richness in essential nutrients and health promoting compounds. Similar to other fresh-cut leafy vegetables (FCLVs), Swiss chard is prone to oxidative stress, microbial contamination, and physiological degradation during storage. To mitigate these challenges, postharvest sanitisation treatments, particularly those that contain oxidising and chlorine-based agents have been widely applied to preserve the nutritional value and overall quality of both whole and FCLVs. However, growing scientific evidence regarding the environmental persistence and health risks associated with synthetic chemical sanitisers have driven a global transition toward safer, eco-friendly alternatives. Among these, non-thermal activated water treatments, such as micro-nano bubble (MNB) technologies, have gained attention for their antimicrobial efficacy and broad applications across agricultural and food sectors.

The aim of this study was to investigate the impact of MNB water technology and packaging systems on the overall quality, physiological responses, phytonutrients and microbial quality of minimally processed Swiss chard. MNB systems, namely air-generated MNB water and ozone micro-nanobubble (O_3-MNBs), were evaluated for their effectiveness in improving post-harvest quality and storage stability of Swiss chard (cv. Fordhook Giant). Two experiments were conducted under identical storage conditions (5 °C) with samples packaged in BOPP films. In the first experiment, whole Swiss chard was treated for 10-min with air-MNB and compared against conventional sodium hypochlorite (NaOCl) and tap water (TW) over an 8-day storage period. In the second experiment, fresh-cut Swiss chard was treated with O_3-MNBs and tap water for 5, 10, and 15 min, followed by storage for 15 days.

The potential of air-MNBs as an alternative for conventional chlorine-based washing methods in leafy vegetables was investigated. The study examined the physiological responses, as well as the physicochemical and microbiological qualities, of packaged whole Swiss chard leaves. Results showed that air-MNB treatment significantly preserved chlorophyll contents, minimised moisture loss, and maintained leaf colouration better than both NaOCl and TW ($p \leq 0.05$). Additionally, air-MNBs achieved a comparable ≤ 1-log reduction in aerobic mesophilic bacteria counts to that of NaOCl. At the end of storage duration, treatment with air-MNBs resulted in better visual quality compared to the control samples.

To further elucidate the influence of activated water systems on the postharvest quality of minimally processed leafy vegetables, O_3-MNB water was applied to fresh-cut Swiss chard (cv. Fordhook Giant).

Fresh-cut Swiss chard pre-treated with O_3-MNBs exhibited significantly lower weight loss compared to those pre-treated with tap water treatments ($p \leq 0.05$). In addition, O_3-MNBs preserved leaf colour, and suppressed ethylene production relative to tap water. All the O_3-MNB treatments resulted in a decrease of total soluble solids and titratable acidity, however they were better retained in control tap water pre-treated samples ($p \leq 0.05$). Notably, O_3-MNB-treated samples stimulated an early storage increase in phenolic content and induced the formation of new volatile compounds such as 1-Pentanol, associated with improved sensory attributes. Microbial analyses revealed that O_3-MNB treatments significantly reduced the populations of aerobic mesophilic bacteria, yeasts and moulds, with the 10-min exposure achieving the greatest microbial inhibition throughout storage and resulted in ≈ 1.6 Log reduction in total aerobic mesophilic bacteria and ≤ 1 Log reduction for yeast and moulds.

Collectively, these findings underscore the potential of micro-nanobubble technology as an innovative, environmentally friendly postharvest intervention. By providing strong antimicrobial activity without the environmental and toxicological drawbacks of synthetic chemicals, MNB treatments effectively mitigate physiological deterioration while maintaining the sensory and nutritional integrity of leafy vegetables.

Key words: antimicrobial, chlorophyll, leafy vegetables, micro-nano bubbles (MNBs), phenolic content, volatile organic compounds

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DEDICATION

To my precious daughter, **Reoneetswe Ubenathi Moletsane**, the light that inspires me every day and all those dear to me, this journey would not have been possible without your enduring love, support, and faith in me.

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GLOSSARY

1-MCP	1-Methylcyclopropene
ABA	Abscisic acid
ANOVA	Analysis of variance
ARC	Agricultural Research Council
BOPP	Bi-axially oriented polypropylene
CO_2	Carbon dioxide
EW	Electrolysed water
C_2H_4	Ethylene
FCLVs	Fresh cut leafy vegetables
FCVs	Fresh-cut vegetables
GC–MS	Gas chromatography-mass spectrometry
GLVs	Green leafy vegetables
GRAS	Generally Recognised as safe
HCl	Hydrochloric acid
MAP	Modified atmosphere packaging
MDA	Malondialdehyde
$MgCO_3$	Magnesium carbonate
$MgCl_2$	Magnesium chloride
$MgSO_4$	Magnesium sulphate
MNBs	Micro-nanobubbles
$NaBH_4$	Sodium borohydride
NaOH	Sodium hydroxide
NaOCl	Sodium hypochlorite
NF	Nano fibrillated
O_2	Oxygen
O_3	Ozone
ORP	Oxidation-reduction potential
PAW	Plasma activated water
PCA	Plate count agar
PDA	Potato dextrose agar
POD	Peroxidase
PP	Polypropylene
PPO	Polyphenol oxidase
KCl	Potassium chloride
RONS	Reactive oxygen and nitrogen species
ROS	Reactive oxygen species
TA	Titratable acidity
TAMB	Total aerobic mesophilic bacteria
TPC	Total phenolic concentration
TSS	Total soluble solids
EPA	The Environmental Protection Agency
USDA	United States Department of Agriculture
USFDA	United States Food and Drug Administration
VOCs	Volatile organic compounds
WHO	World Health Organization

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CHAPTER 1
GENERAL INTRODUCTION

1.1 Introduction

In response to consumer demands for freshness, convenience, high nutrition, and flavour, the fresh cut vegetable (FCV) market has grown rapidly in recent years (Afzaal *et al*., 2021). Although FCVs are important for their nutritional values and convenience, their high moisture content and phytochemical properties affect their microbiological shelf life and overall quality (Santos *et al*., 2014). During minimal processing of FCVs, mechanical cutting and peeling cause the release of cellular contents at the wound site, which encourages the growth of pathogenic and spoilage microorganisms (De Corato, 2020). Furthermore, FCVs pose a significant challenge due to their active metabolism and high physiological postharvest activities, which result in ripening and senescence. When improperly processed, they may also carry pathogens that can cause diseases (Gombas *et al*., 2017).

Fresh cut vegetables represent the fastest-growing category of convenient foods (Kakade *et al*., 2015). Within this category, fresh cut leafy vegetables (FCLVs) have gained considerable popularity. Swiss chard (*Beta vulgaris* L.) is a biennial herbaceous leafy vegetable crop closely related to beetroot. Due to its nutritional profile, which includes carbohydrates, low fat content, ash, moisture and proteins, Swiss chard has become economically important among leafy green vegetables (LGVs) (Miceli and Miceli, 2014; Ivanović *et al*., 2019; Sindesi *et al*., 2023). Swiss chard is rich in dietary fibre and contains beneficial phytonutrients such as flavonoids and carotenoids (Bulgari *et al*., 2017). Due to its substantial antioxidant content, Swiss chard is associated with anti-cancerous properties and the reduction of blood glucose levels in humans (Yang *et al*., 2014; Ivanović *et al*., 2019). It is also packed with remarkable levels of vitamin A, B, C and E, along with minerals such as iron, phosphorus and calcium (Pyo *et al*., 2004).

Furthermore, it contains significant levels of zinc, beta-carotene, lutein, alpha-lipoic acid and quercetin. Antioxidants such as alpha-lipoic acid are associated with increased insulin sensitivity and lower levels of glucose (Yang *et al*., 2014; Ivanović *et al*., 2019), while flavonoids such as syringic acid assist in preventing diabetes. Swiss chard is consumed in many regions globally, including South Africa, due to its excellent nutritional value and inexpensive cost of production (Gao *et al*., 2009). Swiss chard has become increasingly popular in South Africa, where it is used on salads, consumed with main meals and even enjoyed as juice (Matlala, 2017). It is one of the most cultivated leafy vegetables that play a huge role in alleviating food security and malnutrition (Dumani *et al*., 2022). The major production provinces of Swiss chard in South Africa are KwaZulu-Natal, Mpumalanga, Western Cape,

Gauteng, Eastern Cape and Limpopo (Statistics South Africa, 2016). Though Swiss chard has been successfully grown in South Africa, there is no available information in terms of export market of the vegetable since its production is not commercially important (Motseki, 2008).

During postharvest, fresh or fresh-cut Swiss chard is washed with water in order to promote healing of cut wounds and delay the physiological processes leading to senescence (Meireles *et al*., 2016). Moreover, sanitisers such as chlorine are added in the water during industrial washing to help reduce microbial load on the surface of the Swiss chard (Meireles *et al*., 2016; Botondi *et al*., 2021). However, the role of chlorine and its toxic by-products have raised public concern resulting in additional regulatory barriers for its use (Nyamende *et al*., 2022). This has led to an increased interest in research into alternative control measures such as the use of activated water treatments.

Micro-nanobubbles (MNBs) are ultrafine bubbles which are less than 100 µm in diameter and have remarkable physical and chemical properties (Jia *et al*., 2023). These gas-liquid phase treatments are both highly effective and environmentally friendly and are utilised across diverse fields including agriculture, aquaculture, medicine, water ecosystem restoration as well as sewage and wastewater treatment (Zhang *et al*., 2020). MNBs exhibit distinct physical, chemical, and physiological characteristics, such as small volume, large specific surface area, slow rising speed (long stay time), high gas dissolution rate, high gas-liquid mass transfer efficiency, production of free radicals and high interface potential, of which these properties offer great potential for wide-ranging applications (Jia *et al*., 2023).

1.2 Statement of research problem

Fresh-cut leafy vegetables are hampered by rapid oxidation, the risk of accelerated microbial growth, and physiological deterioration during storage. When Swiss chard is minimally processed, it is exposed to unit operations that include peeling, cutting, shredding, sanitising, and packaging, which usually damages the vegetable tissues (Mulaosmanovic *et al*., 2021). This tissue damage promotes yellowing, decreased nutritional value, loss of texture, translucency, exudation, and the development of off-flavours and odours, all of which contribute to enhanced susceptibility to microbial spoilage, ultimately reducing shelf life (Mulaosmanovic *et al*., 2021). The demand for nutrient-dense and microbiologically safe vegetables has also risen due to the increased incidence of foodborne illness outbreaks (Jung *et al*., 2014). These outbreaks have contributed to the emergence of bactericide- and fungicide-resistant foodborne pathogens (Maffei *et al*., 2016), further complicating the preservation of fresh cut produce. Current postharvest treatments often rely on chemical fungicides, which have exacerbated the emergence of resistant pathogen populations. Consequently, there is a growing negative public perception regarding the use of chemical fungicides.

Additionally, chlorinated sanitisers, such as sodium hypochlorite at concentrations between 50 to 200 mg L^{-1}, are commonly recommended for FCLVs. However, sodium hypochlorite can react with organic matter to form carcinogenic by-products (Nyamende *et al*., 2022). Furthermore, the fruit and vegetable industry has expressed concerns about the additional regulatory challenges, safety limitations, and restrictions associated with the use of chlorine in its current form. In response to these challenges, alternative non-thermal phytosanitary measures, such as MNB water treatment, have gained attention. MNB technology offers the potential to enhance the efficacy of phytosanitary treatments while preserving the nutritional and sensory quality of fresh-cut produce (Shi *et al*., 2023; Malahlela *et al*., 2024b). Furthermore, they have antimicrobial properties, which can help reduce the growth of microorganisms on FCVs (Pongprasert *et al*., 2018).

For instance, Ikeura *et al*. (2011) reported that MNBs were effective in removing residual pesticides and reducing bacterial counts on fresh lettuce (*Lactuca sativa*). Similarly, Pongprasert *et al*. (2016) found that washing fresh-cut course lettuce with MNBs reduced polyphenol oxidase (PPO) activity. In addition, fresh-cut lettuce showed less browning due to the inhibitory effects of the browning enzyme and substrate. It was further found in a study by Pongprasert *et al*. (2018) that washing shredded cabbage (*Brassica oleracea*) with MNBs successfully inhibited PPO activity. Furthermore, the authors reported that the inhibitory effect on the browning enzyme and substrate resulted in lower browning symptoms of shredded cabbage during storage duration.

In a study conducted by Shi *et al*. (2023) MNBs were effective in preserving quality and shelf life of parsley (*Petroselinum crispum*). The results demonstrated that MNBs treatment significantly preserved the sensory quality of parsley, leading to reduced weight loss, respiration rate, ethylene production, and malondialdehyde (MDA) levels. Furthermore, treated parsley exhibited greater firmness and higher levels of vitamin C and chlorophyll content compared to untreated samples. Given these findings, it is of particular interest to evaluate the effects of MNB water treatment in combination with packaging systems on the physiological responses, phytonutrient content, and overall quality of minimally processed Swiss chard during storage.

1.3 Aim and objectives

1.3.1 Aim

The primary aim of this study was to investigate the impact of MNB water technology and packaging systems on the overall quality, physiological responses, phytonutrients and microbial quality of minimally processed Swiss chard.

1.3.2 Objectives

To achieve the aim, the study focused on the following objectives:

- To evaluate the influence of air-MNBs on physicochemical attributes and microbiological stability of packaged whole Swiss chard during storage (Chapter 3).
- To assess the effects of ozone-MNBs (O_3-MNBs) on the physiological responses, physicochemical parameters, phytochemical content and volatile organic profile of fresh-cut Swiss chard (Chapter 4).

1.4 Ethical statement

Ethical clearance with reference: 222348453/10/2024 was obtained from the Faculty of Applied Sciences at the Cape Peninsula University of Technology.

1.5 Thesis outline

The thesis consists of five chapters, each focusing on a definite aspect of the research. Chapter one presents the general introduction, outlining the background, research problem, as well as the aims and objectives of the study. Chapter two provides an extensive literature review on nutritional quality of Swiss chard, postharvest deterioration and handling of leafy vegetables, application of activated water treatments in minimally processed leafy vegetables, and challenges in the advancement of activated water treatments. Chapter three evaluates the decontamination efficacy of MNBs on the microbiological quality and shelf life of fresh Swiss chard. Chapter four assesses the impact of ozone-MNBs on physiological responses, physicochemical parameters, phytochemical properties, and volatile organic compounds of minimally processed Swiss chard. Chapter Five presents the general summary of findings, conclusions drawn from the research, and recommendations for future studies and practical applications.

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CHAPTER 2
LITERATURE REVIEW

COMPARATIVE PERSPECTIVE ON NUTRITIONAL VALUE, POSTHARVEST DISORDERS, HANDLING AND POTENTIAL ADVANCEMENTS OF ACTIVATED WATER TREATMENTS AS SANITISERS FOR SWISS CHARD AND RELATED LEAFY VEGETABLES

ABSTRACT

This review synthesised recent advancements in postharvest technologies and sustainable practices for leafy vegetables, with a focus on Swiss chard. It highlighted the nutritional and health benefits of Swiss chard, including its rich content of vitamins, minerals, and antioxidants. The review discussed the challenges of postharvest deterioration due to physiological and microbial factors, emphasising the importance of innovative preservation methods such as micro-nanobubbles, plasma-activated water, electrolysed water and modified atmosphere packaging. The review found that these eco-friendly approaches have shown promise in reducing microbial load, extending shelf life and maintaining quality while minimising pesticide residues. Prospects and challenges in the advancement of activated water treatments are highlighted including regulations, complexities and upscaling for commercial adaptation.

KEYWORDS: Antioxidants**,** eco-friendly, food safety, nutrition, regulations, shelf-life

2.1 Introduction

Over the past few decades, the global production and consumption of leafy vegetables have steadily increased, with China, the United States, and India emerging as the top three producers (Aramrueang *et al*., 2019). In South Africa, leafy vegetables are cultivated both commercially and at subsistence level, particularly in provinces such as the Western Cape, KwaZulu-Natal, Mpumalanga, Gauteng, Eastern Cape, Limpopo, and North West (Statistics South Africa, 2016). These vegetables form a key component of a balanced diet due to their high nutritional value. They are rich in phytonutrients including lutein, zeaxanthin, beta-carotene, vitamin C, folate, and antioxidants, which contribute to the prevention of cancer, cardiovascular disease, neural tube defects, and age-related eye disorders (Natesh *et al*., 2017; Sreenivasa, 2017; Kumar *et al*., 2020). Their high fibre content and low carbohydrate also promote healthy digestion and help regulate blood sugar levels (Chalise *et al*., 2024). Given their perishable nature and reliance on local production, fresh green leafy vegetables (GLVs) are primarily acquired locally due to storage and long-distance transportation challenges (Knez *et al*., 2024).

2.1.1 Classification and production of Swiss chard in South Africa

Swiss chard (*Beta vulgaris* L.) is one of the widely cultivated leafy vegetables. Belonging to the beetroot family, it is known for its high yield potential and nutritional density, especially in South Africa where it is commonly and wrongfully referred to as spinach (Rathour *et al*., 2024). It has thicker leaves and yields more than genuine spinach (D'Imperio *et al*., 2019; Rathour *et al*., 2024). Swiss chard is taxonomically categorised in the order Caryophyllales and either the Chenopodiaceae family (according to the Cronquist system) or the Amaranthaceae family (according to the Angiosperm Phylogeny Group (APG) system) (Almeida *et al*., 2025). The species is divided into three subspecies and is made up of two groups depending on the cultivation conditions, for example, cultivated varieties and wild varieties (Nikan and Manayi, 2019). Table beet (Conditiva group), Swiss chard (Flavescens group), chard or spinach beet (Cicla group), sugar beet (Altissima group), and fodder beet (Crassa group) are among the cultivated edible species while the sea beets (*Beta vulgaris* L. subsp. maritima), the ancestral form of all cultivated variations, represent the wild species (maritime beets) (Almeida *et al*., 2025).

Swiss chard is widely grown across several provinces, mirroring the overall distribution of leafy vegetable production in the country. According to Ndolowana (2015), the Western Cape, KwaZulu-Natal, Mpumalanga, Gauteng, Eastern Cape, Limpopo, and North West are the main provinces that produce Swiss chard. Its adaptability to a range of climatic conditions, including tolerance to mild frost and optimal growth temperatures of 7–24°C, makes it suitable for year-round cultivation (Rathour *et al*., 2024). Furthermore, it is well adapted to long days and hot conditions (Maboko and Plooy, 2013). Due to its brief growing season prior to the first harvest, it serves as a suitable preceding crop and subsequent crop, in crop rotation systems (Cammerino *et al*., 2025).

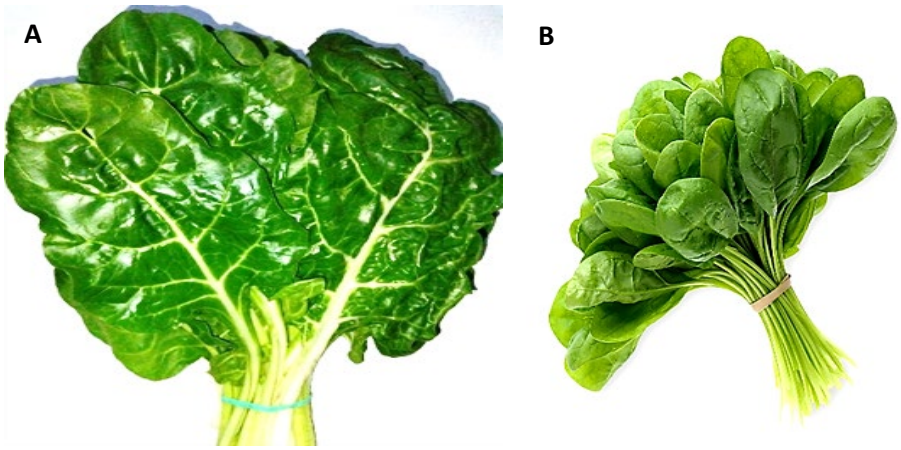

Figure 2.1: Illustration of morphological differences between (A) Swiss chard and (B) Spinach (Photos taken by AG Masilela).

Table 2.1: Key differences between Swiss chard and spinach.

Feature	Swiss chard (*Beta vulgaris*)	Spinach (*Spinacia oleracea*)
Family	Beet *(Chenopodiaceae)* (Cammerino *et al*., 2025)	Amaranth *(Amaranthaceae)* (Moorthi, 2025)
Common name	Known as chard or leaf beetroot, however, often called "spinach" in South Africa (Jakhro *et al*., 2017)	Common spinach, called "true spinach" in South Africa (Rathour *et al*., 2024)
Leaf texture	Thick and broad leaves with colourful or white stalks (Almeida *et al*., 2025)	Flat leaves with green stems (Moorthi, 2025)
Climate	It thrives in temperatures ranging from 7 to 24°C, however it can tolerate heat and mild frost (Rathour *et al*., 2024)	It prefers cooler regions; autumn and winter are the ideal growing seasons (Abolghasemi *et al*., 2024)
Yield potential	Higher yield of 30 tons and more per hectare; vigorous growth (KZN Department of Agriculture, 2001)	Lower yield 10-12 tons per hectare; slower growth (D'Imperio *et al*.,2019; Rathour *et al*.,2024)
Nutritional value	High in iron, magnesium, manganese, and vitamins A, C, E, and K (Sindesi *et al*., 2021)	Higher in Vitamin A; also rich in Vitamins C, E, K, iron, calcium (Moorthi, 2025)
Common cultivars	Fordhook giant, Lucullus (Rathour *et al*., 2024)	Matador, Viroflay, Nobel (Abolghasemi *et al*., 2024)

2.1.2 Nutritional and health benefits of Swiss chard

Considering its high-water content (>92.6%), sugar level (1.1g), vitamin content (C =18 mg, A =306 µm, and K =327 µm), mineral content (ash =1.26%), and (2.1) dietary fibre content (Table 2.2), Swiss chard is one of the most nutritious foods in a healthy diet. It is an excellent source of vitamins A, K, C, magnesium, and manganese (Sindesi *et al*., 2021). It contains 13 individual antioxidants, and these compounds assist blood sugar management, enhance insulin sensitivity, and have anti-inflammatory, and immunomodulatory, properties that may lower the risk of cardiovascular disease particularly when alpha-lipoic acid is present (Gamba *et al*., 2020). Swiss chard is also a good source of magnesium, potassium and iron and has a high fiber content that helps with digestion (Knežević *et a*l., 2014; Bulgari *et al*., 2017).

Table 2.2: Average nutrient content in Swiss chard per 100 g (Adapted from USDA, 2018).

Name	Amount	Unit
Water	92.6	g
Energy	20/84	kcl/Kj
Protein	1.88	g
Total lipid (fat)	0.08	g
Ash	1.26	g
Carbohydrates	4.13	g
Fibre	2.1	g
Total sugars	1.1	g
Calcium	58	mg
Magnesium	86	mg
Phosphorus	33	mg
Potassium	549	mg
Sodium	179	mg
Zinc	0.33	mg
Copper	0.163	mg
Manganese	0.334	mg
Selenium	0.9	µm
Vitamin C	18	mg
Vitamin K	327	µm
Vitamin A	306	µm
Thiamin	0.034	mg
Riboflavin	0.086	mg
Niacin	0.36	mg
Pantothenic acid	0.163	mg
Vitamin B-6	0.085	mg

2.2 Post-harvest deterioration in leafy vegetables

Although the main focus of this study is Swiss chard, current literature lacks data on post-harvest diseases specific to this leafy vegetable. Therefore, the present review incorporates research on similar leafy vegetables, including spinach (*Spinacia oleracea*), lettuce (*Lactuca sativa*), brassicas such cabbage (*Brassica oleracea*) and kale (*Brassica oleracea* var sabellica), as well as various herbs. This approach helps to put the physiological vulnerabilities of Swiss chard into perspective. Additionally, these vegetables are handled and stored similarly, and they also share similar anatomical characteristics such as a high moisture content and a large surface area (Mapholo *et al*., 2016).

Despite their rich nutritional profile, GLVs are among the most perishable horticultural commodities due to their high moisture content (75 to 95% by fresh weight) and pronounced sensitivity to environmental fluctuations (Butnariu and Butu, 2015). Under optimal refrigerated conditions (0–5°C), their shelf life, typically ranges from 3 to 14 days. However, even under such conditions post-harvest deterioration remains a significant challenge primarily due to physiological and microbial susceptibility. Improper post-harvest handling, inadequate cooling, and suboptimal storage conditions often result in rapid quality decline, leading to significant economic losses and diminished nutritional value (Adewoyin, 2023).

In underdeveloped countries, up to 40% of perishable crops including GLVs are lost before they can be marketed (FAO, 2023). These losses amount to billions of dollars in economic losses every year, particularly in sub-Saharan Africa, where post-harvest inefficiencies have a direct influence on national food security, farmer income, and market stability (FAO, 2023). In fact, Seshadri *et al*. (2024) emphasises that reducing post-harvest losses in fresh produce supply chains directly supports both economic stability and the accomplishment of the Sustainable Development Goals (SDGs). Post-harvest quality loss in leafy vegetables is driven by physiological and pathological factors and therefore leafy vegetable spoilage can be classified into two categories, i.e., physiological disorders and pathological disorders.

2.2.1 Physiological disorders

Post-harvest physiological disorders in leafy vegetables are defined as internal or external degradative processes not caused by infectious agents, but rather triggered by environmental stress, mechanical injury, or metabolic imbalances (Watkins, 2017; Yahia and Fonseca, 2020). These disorders usually manifest as visible symptoms of localised cell death such as wilting, chlorosis, and discolouration (Watkins, 2017). Physiological disorders arise from factors such as extreme temperature, mechanical

injury during harvesting or handling, nutritional or nutrient deficiencies, or environmental stress pre- or post-harvest (Masarirambi *et al*., 2011; Yahia and Fonseca, 2020). These disorders significantly compromise both visual and nutritional quality of leafy green vegetables that lead to post-harvest losses.

2.2.1.1 Water loss and wilting

A major factor in the deterioration of leafy vegetables is water loss, which causes a loss of nutritional value, tissue flaccidity, decreased turgor pressure, loss of crispness, and shrivelling of leaves (Patel *et al*., 2021; Yang *et al*., 2024). Excessive moisture loss following harvest is the main cause of water loss and wilting in leafy vegetables, which lowers cell turgor and lowers overall quality (Mampholo *et al*., 2016; Yang *et al*., 2025). Furthermore, harvesting practices also contribute to moisture loss; more moisture is lost when the skin barrier is compromised by mechanical damage such as bruising or abrasion (Shezi *et al*., 2024). Moreover, external variables including temperature, relative humidity, and the composition of the atmosphere surrounding the plant tissue also contributes to moisture loss which causes accelerated wilting and a reduction in the amount of soluble protein, ascorbic acid, as well as chlorophyll (Shezi *et al*., 2024). Therefore, leafy vegetables are especially vulnerable to moisture loss due to their high surface-area-to-volume ratio (Mampholo *et al*., 2016).

2.2.1.2 Leaf senescence and chlorosis

The predominant post-harvest issue affecting leafy vegetables is leaf senescence, which can be recognized by wilting and yellowing of the leaves (Tan *et al*., 2018). Post-harvest leaf senescence in leafy vegetables is a complex process influenced by various environmental and internal factors such as energy deficit, reactive oxygen species (ROS), nutrition, developmental stage, different stressors, and phytohormones (Tan *et al*., 2021). Ethylene accelerates leaf senescence, while cytokinins like 6-benzylaminopurine retard it (Zhang *et al*., 2011). The expression of senescence-associated genes, such as Gene 12, pheophytinase, pheophorbide a oxygenase, and red chlorophyll catabolite reductase, are closely linked to chlorophyll degradation (Zhang *et al*., 2011). Furthermore, transcription factors such as WRKY65 regulate senescence by directly activating senescence-associated genes (Fan *et al*., 2017). Abscisic acid (ABA) plays a crucial role in post-harvest senescence, with increasing ABA levels observed during storage at room temperature (Miret *et al*., 2018). Exogenous ABA application can suppress senescence and alter the transcriptional regulation of various metabolic pathways (Miret *et al*., 2018).

2.2.1.3 Discolouration

One of the primary challenges during harvesting, transportation, storage, and processing is the enzyme reactions that cause fruits and vegetables to change colour (Singh *et al*., 2018). Leafy vegetable discolouration represents a complex postharvest challenge influenced by multiple factors, with enzymatic browning primarily mediated by polyphenol oxidase (PPO) and peroxidase (POD), identified as principal underlying mechanisms (Hunter *et al*., 2017; Singh and Singh 2018; Wang *et al*., 2023). The PPO enzyme catalyses the oxidative reactions of phenolic compounds in the presence of oxygen to produce a variety of polymerised products (Shezi *et al*., 2024). This oxidation of phenolic compounds leads to production of quinones and the synthesis of melanin results in a change of colour (Singh and Singh, 2018). Furthermore, POD, a thermostable enzyme belonging to the category of oxidases that use hydrogen peroxide (H_2O_2) as a catalyst, oxidises phenolic compounds (Singh and Singh, 2018) therefore leading to the accelerated colour changes of leaf tissues.

Numerous vegetables, including leafy vegetables, have been found to exhibit browning following a mechanical or physiological injury sustained during harvest or storage (Singh and Singh, 2018). Furthermore, the development of discolouration is also influenced by cultivation and post-harvest handling processes (De Jaegere *et al*., 2022). As a result, brown pigments (melanin-like compounds) are created when polyphenols and enzymes interact due to cell damage (Paudel *et al*., 2020). Additionally, temperature and humidity are environmental factors which accelerate the rate of discolouration (De Jaegere *et al*., 2022). There are several different kinds of discolouration, such as reddening, yellowing, and browning, and each has its own unique biochemical pathway (Wang *et al*.,2023). Therefore, developing efficient control strategies to preserve quality and reduce losses in the food supply chain, an understanding of enzymatic as well as non-enzymatic mechanisms of discolouration is required (Wang *et al*., 2023).

2.2.2 Pathological disorders

Post-harvest pathological disorders refer to detrimental changes in harvested crops induced by biotic agents, particularly microorganisms, which compromise the physiological, biochemical, and structural integrity of the produce, thereby reducing its quality, shelf life, and marketability (Singh and Sharma, 2018). Leafy vegetables are particularly susceptible to a range of microbial infections, with bacterial and fungal pathogens being the most common contributors to postharvest deterioration (Golding *et al*., 2019). *Bacillus* spp., fluorescent *Pseudomonas* spp., *Enterobacter cloacae* and Erwinia spp. are the causative agent of soft rot, which frequently occurs during post-harvest storage, especially in leafy vegetables kept at warm temperatures or when heat builds up within storage containers (Abu-Obeid,

2019). Soft rot is distinguished by the slimy decay of infected tissue and an unpleasant, foul odour (Acedo, 2010). Additionally post-harvest losses from bacterial soft rot have been estimated to fluctuate from 15 to 30 percent of the harvested crop (Abu-Obeid, 2019). The use of antimicrobial treatments such hypochlorite, formaldehyde solution, and antibiotics, as well as better management methods, reducing wound infections, and breeding resistant cultivars, are the main methods for controlling soft rot (Bhat *et al*., 2012). However, Yi *et al*. (2021) highlights the continuing lack of effective and safe measures to prevent post-harvest soft rot in vegetables.

2.3 Post-harvest handling of fresh leafy vegetables

Post-harvest handling fresh leafy vegetables is crucial for maintaining quality and reducing losses. Due to their high surface-area-to-volume ratio, these vegetables are susceptible to quick deterioration, which can result in moisture loss and compromised quality (Mampholo *et al*., 2016). Leafy vegetables are important for nutrition and food security, but because of poor handling, storage, and transportation conditions, they face up to 50% post-harvest losses (Gogo *et al*., 2016). According to Budhathoki *et al*. (2022) efficient post-harvest handling techniques include harvest maturity, cleaning and sorting, grading, pre-cooling, packaging, appropriate storage, and transportation. Therefore, enhancing and investigating more suitable post-harvest handling practices for leafy vegetables is required to minimise losses and ensuring food security (Gogo *et al*., 2016).

2.3.1 Harvest maturity

According to Quamruzzaman *et al*. (2022) it is crucial to harvest produce at the appropriate maturation stage. Harvesting leafy vegetables at the wrong maturity stage can lead to quality issues during storage, including higher rates of decay, discoloration (browning), and reduced processing yield (Budhathoki *et al*., 2022). Furthermore, the nutritional content of leafy vegetables may be adversely affected by improper maturity stage upon harvest (Maseko *et al*., 2019). Leafy vegetables are susceptible to quick moisture loss after harvest, which can result in physical and biochemical alterations that degrade quality (Mampholo *et al*., 2016).

2.3.2 Pre-cooling

Pre-cooling is the process of removing field heat from recently harvested perishable produce in order to reduce deterioration and metabolism before storage or transportation (Elansari *et al*., 2019). It is essential to pre-cool produce after harvest not only for preservation of quality, however for increased shelf life, reduction of post-harvest losses, adherence to rules, marketability, and food safety more especially for leafy vegetables (Subedi *et al*., 2022; Muskan *et al*., 2024). Various techniques employ

different materials to eliminate field heat (Garrido et al., 2015; Subedi et al., 2022). For instance, passive evaporative cooling made up of zero energy brick coolers or evaporative charcoal coolers effectively maintained leafy Amarant's (*Amaranthus* spp.) quality by preserving lower temperatures and higher relative humidity than ambient conditions (Ambuko *et al*., 2017).

Vacuum cooling which is a rapid cooling method that uses pressure reduction to induce moisture evaporation, therefore removing heat efficiently (Zhu *et al*., 2018; Alabi, 2024), extended the shelf-life of spinach (*Spinacia oleracea*) by minimising weight loss, protecting membrane integrity, and retaining chlorophyll content (Xie *et al*., 2013). Furthermore, Wang *et al*. (2022a) investigated forced air cooling, a method that accelerates cooling up to eight times faster than conventional techniques by using fans or blowers. Their findings revealed that loading lettuce (*Lactuca sativa*) pallets in batches rather than loading them simultaneously, enhances cooling efficiency, reduces energy consumption, and minimises overall cooling duration.

Hydro-cooling method involves immersing (in ice) or spraying freshly harvested fruits and vegetables with cold water to quickly cool them (Wang and Zhang, 2020; Alabi, 2024). Hydro-cooling at 0 °C and 4°C, followed by cold storage, improved quality and shelf life of butter lettuce (*Lactuca sativa*) (França *et al*., 2015) and arugula (*Eruca vesicaria* subsp. sativa) (Moreira et al., 2019). Furthermore, magnetic field hydro-cooling offered enhanced postharvest benefit by applying weak magnetic fields (396.8 mT), which effectively lowered microbial load and maintained cellular microstructure in jute mallow (*Corchorus olitorius*) and bitter leaf (*Vernonia amygdalina*) more efficiently than conventional cooling methods (Alabi and Obateru, 2022; Alabi *et al*., 2023). Pre-cooling is a crucial post-harvest procedure for maintaining physiological responses and marketability of leafy vegetables. It decreases microbial growth, prolongs shelf-life, and mitigates metabolic activity by efficiently eliminating field heat, which lowers post-harvest losses and improves food safety as shown by studies.

2.3.3 Washing and sanitation

Following the removal of field heat from harvested leafy vegetables, washing or sanitizing is a crucial next step. In addition to reducing pesticide residues and microbiological contamination, this process is essential for maintaining product quality. There are several treatments that have proven effectiveness in this regard. Different washing techniques can effectively reduce bacterial counts, including those of foodborne pathogens such as *Salmonella* and *Listeria* (Pezzuto *et al*., 2016) and reduce pesticide residues (Kwon *et al*., 2013). For instance, washing or sanitising with chlorine in the form of sodium hypochlorite (NaOCl), and oxidising sanitisers such as food grade hydrogen peroxide (Pezzuto et al., 2016) have proven to be effective at reducing microbial loads (Yang *et al*., 2022). In a

study by Pezzuto *et al*. (2016) following artificial contamination of raw rocket (*Eruca sativa*), the efficacy of six distinct washing techniques, with or without sanitisers (peracetic acid and percitric acid, sodium bicarbonate, NaOCl), and vinegar were evaluated. The authors found that the only technique that was able to significantly reduce *Salmonella* counts by 2 Log reduction was washing with NaOCl (200 mg/L). All the investigated washing techniques were successful in the case of *Listeria monocytogenes,* the best results were obtained with a solution of peracetic and percitric acids and 200 mg/L NaOCl solution reaching 1.5 and 2 Log reductions, respectively (Pezzuto *et al*., 2016).

Yang *et al*. (2022) examined the efficiency of several washing techniques in lowering pesticide residues in five different green leafy vegetable, including ssamchoo (*Brassica lee* ssp. namai cv. Ssamchoo), spinach (*Spinacia oleracea*), perilla leaves (*Perilla frutescens*), lettuce (*Lactuca sativa*), and crown daisy (*Glebionis coronaria*). Contaminated leafy vegetables were exposed to nine treatments, namely washing with alkaline electrolyzed water with pH of 9.3, calcium oxide, sodium bicarbonate, running tap water at 170mL/s, detergent, tap water washing-stagnant water, ultrasonic cleaning, vinegar water washing, as well as boiling for 5 minutes and blanching for 30s. Yang *et al*. (2022) found that the effectiveness of decontamination methods varied, with overall microbial reduction ranging from 43.7% to 77.0%, while reductions across the five tested leafy vegetables ranged from 40.6% to 67.4%. Among the methods evaluated, running water achieved the highest reduction (77.0 ± 18.0%), followed by boiling (59.5 ± 31.2%), whereas detergent treatment resulted in the lowest reduction (43.7 ± 14.5%).

There is, however, a gradual but consistent reduction in the usage of synthetic chemicals and are being restricted in many nations due to scientific concerns about their detrimental effects on human health and environmental permanence (Nyamende *et al*., 2022). For instance, chlorine reacts with organic matter which produces carcinogenic by-products leading to possible health risks and inability of the agent to effectively inactivate microorganisms (Joshi, 2016; Nyamende *et al*., 2022). Additionally, although hydrogen peroxide has antibacterial properties, its intense oxidising properties are also capable of damaging produce through oxidative damage, which could adversely affect texture or shelf life if not adequately managed (Alexandre *et al*., 2012). These drawbacks highlight the necessity for safer, more reliable, and environmentally friendly substitutes of synthetic sanitisers in postharvest handling of fresh produce.

2.3.4 Packaging

Different packaging systems have been utilized for different leafy vegetables because packaging materials play a crucial role in the quality and shelf life of leafy vegetables. Packaging keeps the produce isolated from the outside environment and contributes to sterile conditions, or at least less exposure to contaminants and pathogens (Jildeh *et al*., 2021). Greater emphasis has been placed on modified atmosphere packaging (MAP) compared to other packaging technologies, due to its potential to extend shelf life and maintain postharvest quality (Caleb *et al*., 2013). MAP extends the shelf life of fresh produce by altering the composition of the surrounding gaseous environment (Caleb *et al*., 2016; McMillin, 2020). By altering the amounts of oxygen (O_2) and carbon dioxide (CO_2), respiring produce is sealed in polymeric film packages, and the overall goal is to achieve low O_2 and high CO_2 concentrations to reduce metabolism and decay (Kundana *et al*., 2022). The postharvest quality of fresh and fresh-cut fruits and vegetables packaged in modified atmosphere systems is strongly affected by their physiological processes, especially respiration and transpiration (Caleb *et al*., 2012).

With an emphasis on the amount of chlorophyll and anthocyanins, Lee and Chandra (2018) investigated how different packaging techniques affected the quality, shelf life, and biochemical characteristics of leaf lettuce (*Lactuca sativa*) stored at 10 °C for 16 days. There were four different types of packaging material used namely, perforated polypropylene film with a piercing density of 1.5 holes/cm² resulting in 1320 holes of 1 mm diameter (PPP-1320-hole), perforated polypropylene film with 4 holes of 6.5 mm diameter (PPP-4-hole), non-perforated polypropylene film and non-perforated polypropylene film with anti-fogging properties. Results showed that the most effective films for maintaining chlorophyll levels, colour quality, and overall visual quality during storage were non-perforated polypropylene films, especially those with anti-fogging qualities (Lee and Chandra, 2018).

Furthermore, use of several packaging films was investigated by Agustin- Salazar *et al*. (2024) with a special emphasis on biopolymers and how well they preserve the quality and shelf life of baby spinach (*Spinacia oleracea*). Two packaging films namely: nano fibrillated cellulose (NF) and standard polypropylene (PP) films were used to package baby spinach and stored for 15 days at 5 ± 1 °C. In comparison to the PP film, the authors found that NF film, composed of bio-based compostable cellulose, preserved higher quality attributes and gas composition O2 and CO2 levels for baby spinach (Agustin-Salazar *et al*., 2024). Studies have shown that MAP is crucial in maintaining the quality of leafy vegetables postharvest by adjusting gas levels to suppress respiration and deterioration (Lee and Chandra, 2018; Agustin-Salazar *et al*., 2024). Overall, customed-made packaging systems significantly influence physiological responses, microbial safety and marketability of fresh produce.

2.3.5 Storage

The postharvest physiological responses of fresh and processed leafy vegetables are influenced by their storage conditions (Sahu *et al*., 2024). Cold storage techniques, such as refrigeration and zero energy cool chambers are commonly used to reduce the post-harvest losses and extend the shelf-life of leafy vegetables (Garande *et al*., 2019). In a study by Garande *et al*. (2019) the quality and shelf life of minimally processed leafy vegetables, including fenugreek (*Trigonella foenum-graecum*), coriander (*Coriandrum sativum*), spinach (*Spinacia oleracea*), pokala (*Amaranthus blitum*), and rajgira (*Amaranthus cruentus*), were examined in relation to different storage settings, such as room temperature, zero energy cool chamber and refrigerated storage. The authors found that compared to zero energy cool chamber and room temperature, refrigerated storage prolonged shelf life by up to 8 days by significantly preserving moisture, total minerals, ascorbic acid, and chlorophyll, while room temperature storage caused quality degradation and resulted in increased physiological weight loss, yellowing, decay, and microbial counts.

Comparing the two methods, refrigerated storage prolongs shelf life by decreasing moisture loss, microbial development, and metabolic activities (Kumar *et al*., 2014) as compared to zero energy cool chambers, however, it is more expensive (Sahu *et al*., 2024). Moreover, zero energy cool chambers, which are constructed from natural materials, offer a temperature that is lower than room temperature which increases the rate of deterioration, resulting in nutritional degradation, colour changes, texture loss, and flavour degradation (Sahu *et al*., 2024). Storing fresh and FCVs, including leafy vegetables, between 0 and 5 °C is advised since this lowers respiration rate, enzyme activity, and microbial development while increasing beneficial chemicals (Oliveira *et al*., 2015).

2.3.6 Transportation

Inadequate transportation infrastructure and poor logistics management limit the proper preservation of GLV, particularly in developing countries (Elik *et al*., 2019). According to Rattanawong and Ongkunaruk (2018), about 30–40% of fresh produce is lost during transportation. Effective temperature control is essential during transportation, as insufficient cooling can lead to quality degradation. Moreover, monitoring temperature and humidity is recommended to ensure suitable conditions for transported GLV (Matthews, 2014). Elik *et al*. (2019) also emphasised key transport practices such as careful loading and unloading, minimising transition times and protecting fresh vegetables from physical damage. Sairi *et al*. (2021) investigated three transportation methods namely evaporative-cooled truck, canvas truck, and cold truck on choy sum (*Brassica parachinensis*) for the course of a five-hour trip. Among all the treatments evaluated, choy sum transported in an

evaporative-cooled truck exhibited the lowest percentage of weight loss relative to its fresh weight. Additionally, the authors observed that using an evaporative-cooled truck-maintained choy sum quality for up to two weeks when stored at 5°C (Sairi *et al*., 2021). These findings underscore the importance of adopting optimised transport systems to safeguard GLVs freshness and reduce supply chain losses.

2.4 Prospects for activated water treatments on the quality of green leafy vegetables

The high susceptibility of green leafy vegetables to microbial contamination reduces their quality and shelf life, and the use of traditional sanitising methods such as chlorine washing is restricted due to environmental and potential health risks. Activated water (AW) treatments such as micro-nano bubbles (MNBs), plasma-activated water (PAW) and electrolysed water (EW) are increasingly being utilized for their sustainability and effective alternative to chemical disinfectant in the post-harvest sector (Nyamende *et al*., 2023; Malahlela *et al*., 2024a, b). These waters are physically or chemically modified to enhance their antimicrobial efficacy which makes them suitable for improving food safety and extend shelf life of fresh produce (Malahlela *et al*., 2024a, b). As a non-thermal technique, AW treatments demonstrated to inactivate microorganisms, while maintaining quality and nutritional value (Thirumdas *et al*., 2018; Malahlela *et al*., 2024a). Additionally, they have potential to reduce pesticides residues (Pal and Kioka, 2025).

2.4.1 Micro-nano bubbles

Microbubbles (MBs) and nanobubbles (NBs) are both small gaseous entities that have diameters of 10–50 µm (MBs) and less 200 nm (NBs) respectively (Jia *et al*., 2023; Zhang *et al*., 2023). When these gaseous matrices, consisting of either single or mixed gases, are distributed throughout a solution or solid-liquid interface they are known as micro-nano bubbles (MNBs) (Zhang *et al*., 2023). MNBs have gained significant attention for application such as water treatment (Liu and Tang, 2019; Zhang *et al*., 2020), food industry (Phan *et al*., 2020; Zhang *et al*., 2023), and in agriculture (English, 2022; Shan *et al*., 2023; Malahlela *et al*., 2024b). Moreover, MNBs widely used in medicine (Zhang *et al*., 2021a; Kancheva *et al*., 2023), and groundwater remediation (Li *et al*., 2014; Haris *et al*., 2020). Their application is widely distributed due to their unique physicochemical properties (Table 2.3), such as a large specific surface area, small size, high zeta potential, extended residence durations in water, hydroxyl radicals and a high oxygen transfer efficiency (Haris *et al*., 2020; Zhang *et al*., 2020; Zhao *et al*., 2025).

Table 2.3: Summary of the physicochemical properties of micro-nano bubbles.

Physicochemical properties	Description	References
Large surface area	A bubble's size and degree of dispersion in a liquid are described by its specific surface area, which is the ratio of its total surface area to the volume of the gas it contains.	Zhao *et al*. (2025)
Small size and extended duration in water	The small size of MNBs slows down their rising velocity, making it longer for them to quickly float to the surface, which results in a comparatively long residence time in the water.	Zhang *et al*. (2020)
Zeta potential	The zeta potential shows how strongly adjacent, similarly charged particles in dispersion repel one another. When molecules and particles are sufficiently small, their zeta potential will show stability, meaning that the solution or dispersion will not coalesce.	Prakash *et al*. (2014)
Hydroxyl radicals	The removal of chemicals is accelerated by hydroxyl radicals, which are strong oxidants that react rapidly with a range of dissolved molecules.	John *et al*. (2022)
Gas mass transfer efficiency	The mass transfer rate of a gas depends on the mass transfer area of gas–liquid phases, which is significantly higher for MNBs. As a result, the gas dissolution rate in water can reach the supersaturated state, increasing the efficiency of gas–liquid mass transfer.	Sakr *et al*. (2021)

2.4.1.1 Physicochemical properties micro-nano bubbles

Micro-nano bubbles' small diameter and large specific surface area enhances gas-liquid mass transfer and enable the generation of reactive oxygen species (ROS), which is crucial for oxidation and microbial inactivation (Liu and Tang, 2019; Jin *et al*., 2022; Malahlela *et al*., 2024b). Moreover, MNBs exhibit slow buoyancy and extended duration in water that leads to prolonged interactions with contaminates (Wang *et al*., 2019; Zhang *et al*., 2020). Another key feature of MNBs is their high interfacial zeta potential, which is comparable to that of colloid particles (Zhang *et al*., 2023). This high potential arises from the accumulation ions on the bubble surface and counter ions forming on the inner surface (Zhang *et al*., 2020). Zeta potential is a crucial physical property that determines how strongly bubbles and particles are attracted to or repelled by electrostatic forces (Prakash *et al*., 2014), therefore elevated zeta potential increases the repulsive electrostatic forces to stop the bubbles from coalescing thus increasing their stability (Ushikubo *et al*., 2010; Kamble *et al*., 2022).

Upon collapse, MNBs generate high energy (Chaurasia *et al*., 2023) and free radicals such as, hydroxyl radicals, which are powerful oxidants that react quickly with a variety of dissolved substances to increase the rate at which compounds are removed (John *et al*., 2022). These properties make MNBs effective in enhancing mass transfer in gas-liquid processes, a crucial rate-limiting element that restricts system performance and production in several agricultural and environmental systems (Marcelino *et al*., 2023). Another important aspect for mass transfer efficiency is volume and diameter of the gas in the liquid that determines the specific surface area of the gas and liquid, which in turn determines the mass transfer efficiency of the gas (Wang *et al*., 2024b).

2.4.1.2 Generation methods

Micro-nano bubbles can be generated physically or chemically (Table 2.4), using several approaches to produce stable gas-filled cavities at the micro or nanoscale. Mechanical methods usually involve pressurizing gas and liquid mixtures to form bubbles, whereas chemical methods use chemical or electrolytic reactions to produce MNBs (Wang *et al*., 2024b). Unlike mechanical systems, chemical methods rely heavily on specific catalysts and chemical reagents (Wang *et al*., 2024b). Different sectors require different bubble characteristics, which explains the range of generation techniques. For instance, medical imaging requires consistent, stable nanobubbles (Khan *et al*., 2020; Zhao *et al*., 2025), wastewater treatment benefits from high bubble density and reactivity (Zhao *et al*., 2025), while agricultural industry may place a higher priority on large-scale, cost-effective generation (Zhao *et al*., 2025). In practical applications, MNBs are frequently generated using mechanical methods because they are safe and easy to operate (Wang *et al*., 2024b).

Table 2.4: Overview of the methods used for generating micro-nano bubbles.

Method type	Technique	Principle	References
Physical methods	Mechanical stirring	Shear forces generated by rapid agitation causes gas to spilt into small bubbles.	Zhao *et al*. (2025)
	Pressurised gas dissolution	Bubbles are generated when gas dissolves under pressure and is then released.	Zhai *et al*. (2024)
	Cavitation	Bubbles are generated in fluid flow by rapid changes in pressure.	Favvas *et al.* (2021)
	Membrane filtration	Bubbles are generated by forcing gas into a liquid across nanoporous membranes.	Malahlela *et al*. (2024b)
Chemical methods	Electrolysis	Voltage is applied to the electrode, water molecules separate into H_2 and O_2 gasses, generating bubbles.	Selihin and Tay (2022)
	Chemical reaction	Chemical reagents are added to the solution, causing it to strongly react and produce MNBs.	Wang *et al*. (2024b)

Among the physical techniques, mechanical stirring is the simplest and most cost-effective, producing bubbles through shear forces and turbulence created by agitation (Wang and Wang, 2023). Although straightforward, this method often requires surfactants to stabilize bubbles and suffers from limited control over bubble size and stability (Zhao *et al*., 2025). A more controlled approach is pressurised gas dissolution (Figure 2.2A), where gas is dissolved under high pressure and released through a nozzle, forming uniform MNBs (Zhao *et al*., 2025). While effective, this technique demands high energy input and complex equipment (Zhai *et al*., 2024). Another widely studied method is cavitation, which can be induced hydrodynamically or acoustically (Mondal *et al*., 2021; Seridou and Kalogerakis, 2021; Chaurasia *et al*., 2023). Hydrodynamic cavitation, triggered by rapid pressure changes in fluid flow, is energy-efficient and scalable, whereas acoustic cavitation uses ultrasonic waves to generate oscillating bubbles but is less practical for industrial use due to cost and technical expertise requirements (Jia *et al*., 2023; Sakr *et al*., 2021). Finally, membrane filtration (Figure 2.2B), introduces gas into liquid through nanoporous membranes, producing stable bubbles suitable for biomedical and food applications, though high operating costs and pore blockage limit its large-scale utility (Favvas *et al*., 2021).

Chemical methods, in contrast, generate bubbles through electrolysis (Figure 2.2C) or direct chemical reactions. Electrolysis involves applying voltage to electrodes submerged in water, splitting molecules into hydrogen and oxygen gases that form bubbles (Selihin and Tay, 2022; (Kuo, *et al*., 2022; Chaurasia *et al*., 2023). Despite its ability to produce reactive species, this method is constrained by high energy consumption and electrode wear (Liu and Tang, 2019). The chemical reaction approach(Figure 2.2D), which has considerable potential for the synthesis of functional materials (Jia *et al*., 2023) can also be used to generate MNBs. However, because of the constrained reaction conditions and reactants, this technique can only be used in specific conditions, which limits the kinds of bubbles that can form (Jia *et al.,* 2023; Wang *et al*., 2024b). For instance, *Xu et al*. (2023) induced active MNBs chemically, using sodium borohydride (NaBH4), water and sodium hydroxide (NaOH) as reagents. NaBH4 undergoes hydrolysis in water, releasing hydrogen gas. To stabilize this reaction, NaOH is added in the solution. While a minor fraction of the generated hydrogen dissolves in the solution, the majority remains suspended as bubbles (Xu *et al*., 2023).

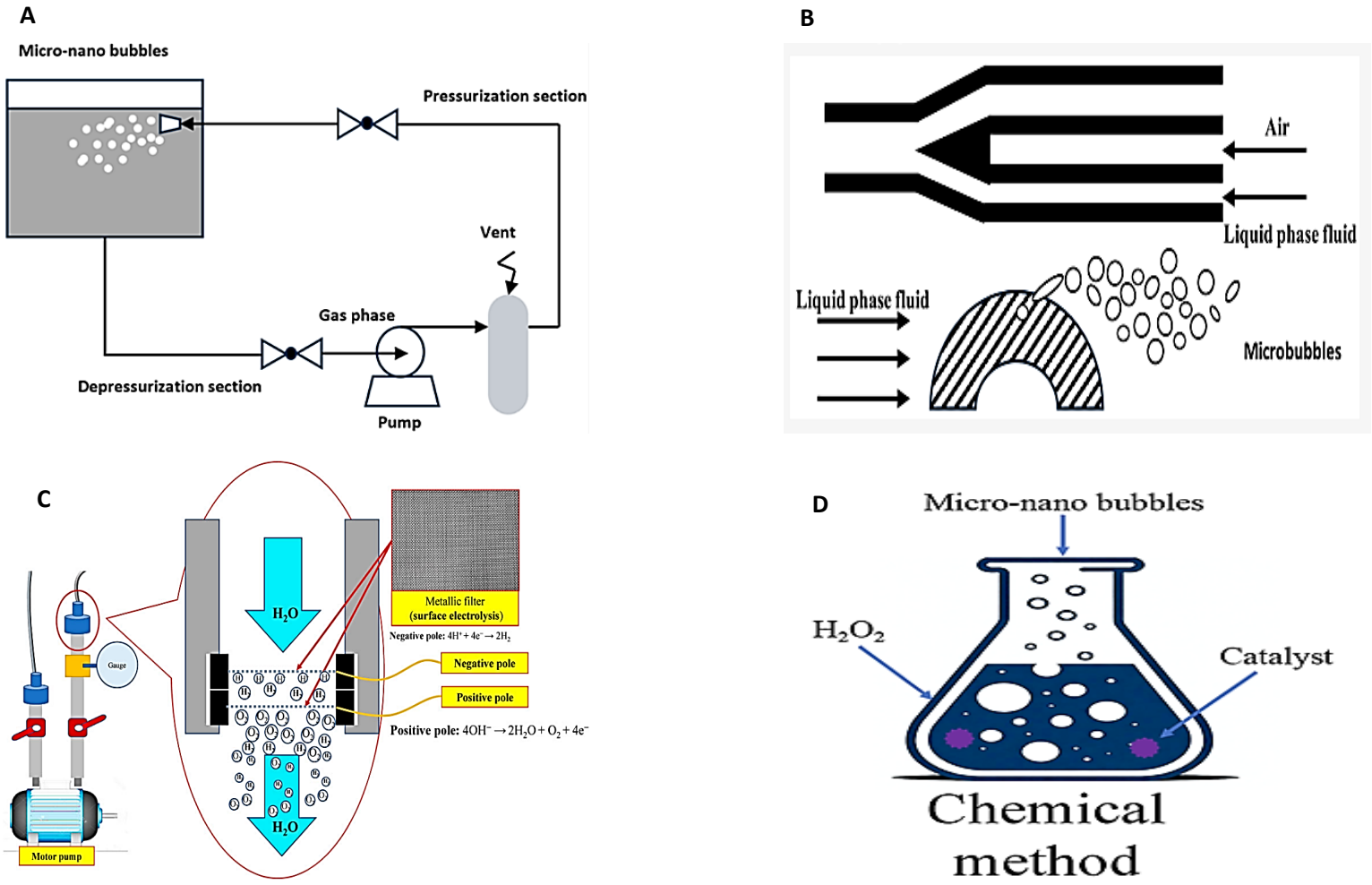

Figure 2.2: Schematic diagram of physical and chemical methods of generating micro-nano bubbles: (A) Pressurised gas dissolution (Adapted from Zhao *et al*., 2025); (B) Membrane filtration (Adapted from Zhao *et al*., 2025); (C) Electrochemical (Adapted from Kuo et al., 2022); and (D) Chemical method (Adapted from You *et al*., 2025).

2.4.1.3 Factors affecting efficiency of micro-nano bubbles

There are various factors as shown in (Table 2.5) that affects the efficiency of MNBs. For instance, MNBs functionality is influenced by bubble size. Smaller bubbles have a larger surface area to volume ratio, which ensures gas dissolution and mass transfer (Nguyen *et al*., 2024; Zhao *et al*., 2025). Moreover, reactions such as pollutant degradation or oxygenation, take place more swiftly and efficiently when mass transfer is improved (Nguyen *et al*., 2024; Zhao *et al*., 2025). In aeration systems for example, optimal gas transfer is influenced by both bubble sizes and water depth, with ideal sizes reported as 42–194 µm for air and 127–470 µm for oxygen (Fan *et al*., 2023). Furthermore, NBs bigger than 200 nm, showed greater efficiency in ultrasound-mediated gene delivery applications (Kida *et al*., 2022).

Various gases undergo different chemical reactions and dissolve in water to differing degrees, which might change the ionic environment surrounding particles (Tamboli and Munaf Tade, 2025). Consequently, this alters the zeta potential by influencing the electrical double layer and particle surface charges (Zhao *et al*., 2025). According to Li *et al*. (2014), high absolute zeta potential causes bubbles to electrostatically resist one another, which minimises coalescence. This increases the system's stability and sustains more reactive microbubbles, which increases its overall effectiveness (Li *et al*., 2014; Zhao *et al*., 2025). Stable bubbles preserve their structure and improve the effectiveness of processes such as flotation and aeration by preventing premature collapse or coalescing (Zhao *et al*., 2025). Moreover, systems that combine MBs and NBs tend to outperform those using either one alone, due to their complementary interaction and enhanced bubble stability (Choi *et al*., 2023).

A variety of gases, such as oxygen, ozone (O_3), carbon dioxide (CO_2), nitrogen, 1- methylcyclopropene (1-MCP) and ethylene (C_2H_4) can be combined with MNB water to form different types of active MNBs (Ushikubo *et al*., 2010; Grzegorczyk-Frańczak *et al*., 2021). These gases have an impact on oxidation potential, solubility, and reactivity, which influences the efficiency of a given application (Sairam *et al*., 2019; Grzegorczyk-Frańczak *et al*., 2021). Ozone for instance, has high oxidation potential, making it more effective for disinfection or degradation of pollutants, therefore choosing the right gas ensures optimal chemical interaction in specific applications (Sairam *et al*., 2019).

Destabilising agents promote rapid collapse which reduces the interaction duration and may reduce efficiency, whereas stabilising surfactants provide protective layers surrounding bubbles, increasing their lifespan and efficacy in capturing or reacting with particles (Zhao *et al*., 2025). In aqueous solutions, the interaction of anionic surfactants and nanobubbles can reduce surface tension, potentially producing new surface-modifying agents for a range of sectors (Anastasios *et al.,* 2022). However, surfactants can affect the size, stability, and production of MNBs; in general, larger concentrations increase zeta potential and decrease bubble size (Li *et al.,* 2014)

Table 2.5: Factors influencing the efficiency of micro-nano bubbles.

Factor	Effect on efficiency	Description	References
Bubble size	The smaller the bubbles the higher the efficiency	Higher surface area-to-volume ratios in smaller bubbles facilitates gas dissolution and mass transfer.	Nguyen *et al*. (2024); Zhao *et al*. (2025)
Bubble stability	The interaction time increases with the stability of the bubbles	Stable bubbles enhance retention and reactivity by preventing collapse and coalescence.	Zhao *et al*. (2025)
Zeta potential	The higher the absolute zeta potential the better the stability of bubbles	Through electrostatic repulsion, a high absolute zeta potential prevents coalescence, thus improving bubble stability.	Li *et al*. (2014); Zhao *et al*. (2025)
Gas type	Oxygen, ozone and carbon dioxide behave differently in terms of dissolution and chemical activity	Different gases affect reactivity, solubility and oxidation potential, influencing application-specific efficiency.	Sairam *et al*. (2019)
Surfactants	Bubbles may be stabilised or destabilised by surfactants	Certain additives prolong the duration of bubbles, while others cause them to collapse quickly, therefore affecting attachment or removal efficiency.	Zhao *et al*. (2025)

2.4.1.4 Impact of MNBs on post-harvest quality of leafy vegetables

MNBs can improve the post-harvest quality of leafy vegetables by reducing microbial load, increasing shelf life, and enhancing appearance. Pongprasert *et al*. (2014) examined the impact of ozone micro-bubbles (O_3-MBs) (0.5 ppm, for 5 min at ambient temperature) on fresh cut lettuce (*Lactuca sativa*) stored at 4°C for 6 days. A 5-minute treatment at ambient temperature significantly reduced yeasts and molds, total bacterial counts and coliforms by up to 2 Log CFU/g-^{1}. Furthermore, the authors observed that PPO activity was suppressed by O_3-MBs washing for up to 6 days of storage at 4 °C, which is associated with a lower quinone level during storage.

Ushida *et al*. (2017) treated Chinese cabbage (cultivar not specified) with MNBs (110 nm) combined with electrolyzed water (50 mg/L effective chlorine) and sodium hypochlorite (50 ppm) for 3 minutes at room temperature (21 ± 2 °C). These treatments reduced viable bacterial count by 1.8 and 2.4 CFU/g^{-1} respectively, as compared to 0.17 log CFU/g reduction from water washing. Furthermore, Pongprasert *et al*. (2018), demonstrated that treating shredded organic red cabbage with 0.5 mg. L $^{-1}$ O_3-MBs for 5 min at a room temperature resulted in a 1-2 log CFU/g reduction in yeast, mould, coliform, and total bacteria. The treatment further lowered the PPO activity of shredded cabbage after 6 days of storage at 4 °C. Wang *et al*. (2020) reported that treatment of spinach with 4 mg. L $^{-1}$ O_3-MNBs for 5 min maintained the post-harvest quality and extended the shelf-life at 20 °C for 8 days. This was attributed to the inhibition of respiration and C_2H_4 production that slowed down nutrient consumption and senescence.

More recently, Shi *et al*. (2023) treated parsley with 2.5 mg. L $^{-1}$ O_3-MNB for 10 min and stored at 20° C for 5 days. The treated samples maintained sensory quality attributes, higher levels of firmness, vitamin C, and chlorophyll content and decreased weight loss, respiration rate, C_2H_4 production, and MDA levels compared to untreated parsley. MNBs offer a sustainable and effective substitute for traditional sanitisers such as sodium hypochlorite, along with the benefits of maintaining nutritional quality and overall visual appearance of fresh and fresh-cut produce. They could be widely used in the fresh-cut processing and postharvest handling sectors due to their adaptability to various leafy vegetables and treatment conditions.

2.4.1.5 Pesticide residue removal

Pesticides are essential to global food security because of their ability to increase agricultural yields and decrease pest-related losses (Hassan Mhya and Mohammed, 2025). However, their application may leave residues on leafy vegetables, and these derivatives could result in detrimental effects on human health such as cell dysplasia, neurological damage, carcinogenicity, and reproductive

damage (Giang *et al*., 2022; Tripathy *et al*., 2022). Therefore, consuming fresh or processed vegetables that contain residual pesticides is harmful to humans which is why an efficient removal technique is necessary (Yang *et al*., 2022). Ikeura *et al.* (2011) evaluated the efficacy of O_3-MB (>1ppm dissolved O_3 concentration) for the removal of pesticide residues from lettuce (*Lactuca sativa*). Their study demonstrated that treating lettuce with 1.0-2.0 ppm O_3-MB significantly reduced the high concentration of fenitrothion (FT) residual (>200 ppm) to below 100 ppm in 5-10 min.

Similarly, Zhang *et al.* (2021b) utilized O_3-MNBs (41.4 µm) in conjunction with a depressurising generator set at 0.5 MPa to eliminate pesticides from baby cabbage (*Brassica oleracea*). After a 15-min treatment with O_3-MNBs, the residual removal efficiencies for phoxim and chlorothalonil were 67.7% and 59.4%, respectively. These values were approximately 2- 4 times higher than those achieved by standard water washing, which resulted in removal rates of only 30.7% for phoxim and 20.2% for chlorothalonil respectively. For fresh vegetables, it has been hypothesised that incorporating O_3 with MNBs may enhance the quantity of O_3 dissolved in water to sustain an efficient concentration through a constant bubble supply, thus ensuring effect postharvest handling impacts (Shan *et al*., 2023).

Moreover, in comparison to gaseous O_3 and macro bubbles, MNBs provide an enhanced way of O_3 solubilisation and stabilisation (Zhang *et al.*, 2021a). Compared to other types of ozone and disinfectants, previous research found that using O_3-MNBs to wash vegetables decreased leaf damage (Zhang *et al*., 2021a). The pesticide-removal capabilities of various O_3-MNBs compositions have also been observed to vary (Li *et al*., 2021). Therefore, introduction of O_3-MNBs as a novel technology has led to an increase in research in postharvest technology.

2.4.2 Other activated water treatments

2.4.2.1 Plasma activated water

Plasma activated water (PAW) is a promising alternative to conventional sanitisers in the fresh produce sector (Vaka *et al*., 2019). It is generated by exposing water to plasma (Figure 2.3) which generates reactive oxygen and nitrogen species (RONS) responsible for its antimicrobial activities (Gao *et al*., 2022; Gülenç *et al*., 2024). PAW can be generated using various atmospheric cold plasma discharge modes. These include direct plasma discharges within liquids, gas-phase discharges positioned above liquid surfaces, and multiphase systems such as bubble-mediated or contact sprays (Sharma *et al*., 2021; Xiang *et al*., 2022). A range of devices are utilized to facilitate these discharges, including corona discharge, dielectric barrier discharge, gliding arc plasma, radio frequency discharge, and microwave-induced plasma systems (Chen *et al*., 2019; Gao *et al*., 2022; Malahlela *et al*., 2024a).

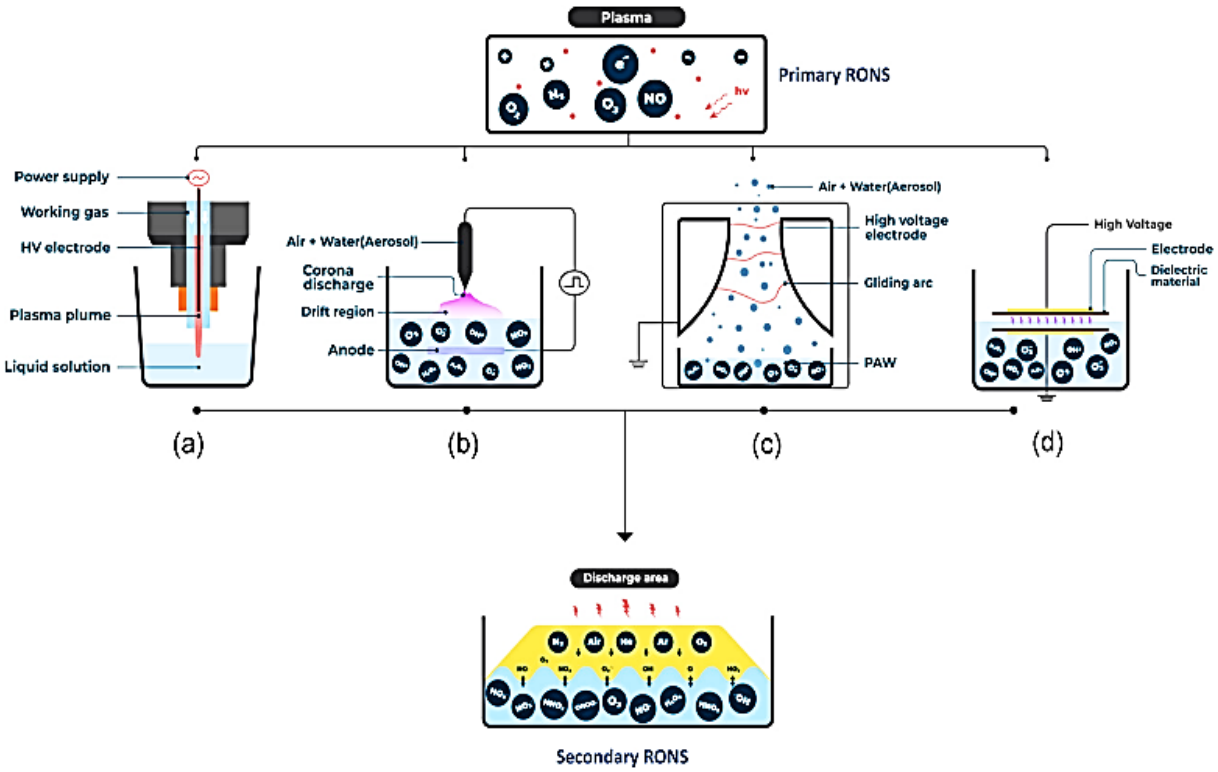

Figure 2.3: Schematic illustration of plasma subjected to different plasma discharge devices (Adapted from Malahlela *et al*., 2024a).

Plasma activated water has become a viable non-thermal technology for improving fresh produce's shelf life and microbial safety, especially for leafy vegetables. With reductions ranging from 1.5 to 6 Log CFU/g^{-1}, recent studies (Table 2.6) have shown that PAW is effective in significantly lowering microbial populations on spinach (*Spinacia oleracea*), kale (*Brassica oleracea* var *sabellica*), rocket leaves (*Eruca sativa*), kimchi cabbage (*Brassica campestris* ssp*. Pekinensis*), and lettuce (*Lactuca sativa*), including *E. coli* amongst other spoilage bacteria. In addition to microbial inactivation, PAW treatments have demonstrated minimal or no effect on firmness and colour, as well as maintaining chlorophyll content and delaying senescence (Vaka *et al.* 2019; Laurita *et al*., 2021; Chamorro *et al*., 2023). Additionally, metabolomic analyses indicate that PAW may improve antioxidant capacity and alter metabolic pathways, which would improve nutritional quality and prolong shelf life (Rangel-Huerta *et al*., 2021).

Table 2.6: Summary of plasma activated water treatments reported in literature on the decontamination efficacy and quality of leafy vegetables.

Leafy vegetable	Treatments	Storage conditions	Findings	References
Baby spinach (*Spinacia oleracea*)	PAW treatment (11 kV for 20 min)	Stored at 4 °C for 8 days	Showed potential for disinfection, colour retention and shelf-life extension.	Vaka *et al.* (2019)
Kimchi cabbage (*Brassica campestris* ssp. *Pekinensis*)	PAW (120-PAW at 1:10 w/v ratio, 10 min, under agitation)	-	Greater microbial inactivation and reduced peroxidase activity without quality loss.	Choi *et al*. (2019)
Rocket leaves (*Eruca sativa*)	PAW treatment (2–5 min, 2–20 min range)	-	Reduction in spoilage bacteria of 1.7-3 Log CFU/g^{-1} and no alteration of nutritional and quality value.	Laurita *et al*. (2021)
Spinach (*Spinacia oleracea)*	Metabolomic analysis using various treatments including PAW	Stored for 8 days in a refrigerator	Increased antioxidant capacity, resilience to stress, and delayed senescence through changes in the metabolism of carbohydrates.	Rangel-Huerta *et al*. (2021)
Kale (*Brassica oleracea* var *sabellica*)	8 minutes treatment by PAW generated by 5 min plasma discharge	Stored at 4 °C for 12 days	PAW treatment reduced populations of *E. coli* on kale by roughly 1.55 Log CFU/ g^{-1}.	Wang *et al.* (2022)

Spinach and Kale	45-min PAW treatment	Stored at 4 °C for 24 hrs.	There was a 6-log reduction of *E. Coli* from spinach and kale after PAW treatment moreover there was no significant difference ($P > 0.05$) in the brightness values of both spinach and kale.	Perinban *et al.* (2022)
Fresh-cut lettuce (*Lactuca sativa*)	1-5 min PAW treatment	Stored at 4 °C for 7 days	Reduced microbial contamination and maintained quality over 7 days of storage.	Chamorro *et al*. (2023)

2.4.2.1.1 Efficacy and impact of plasma activated water on the quality of leafy vegetables

There has been extensive research on the effect of PAW on the quality of leafy vegetables and inactivation efficacy of microbes. For instance, Vaka *et al*. (2019) assessed the impact of PAW treatment (11 kV for 20 min) on baby spinach (*Spinacia oleracea*) stored at 4 °C for 8 days. Colour parameters *L**(lightness), *a** (green-red), and *b** (blue-yellow) showed no visible differences between treated and untreated samples, indicating that PAW preserved a fresh-like appearance and maintained colour stability, whereas water rinsed samples demonstrated higher *b** values (increased yellowness). Wang *et al*. (2022) evaluated the impact of PAW on *E. coli* reduction on kale (*Brassica oleracea* var *sabellica*) stored at 4 °C for 12 days. Their findings showed that the population of *E. coli* on kale decreased by approximately 1.55 Log CFU/g^{-1} following the administration of 8 min treatment by PAW generated by 5 min plasma discharge.

Similarly, Perinban *et al*. (2022) reported a 6-log reduction of *E. coli* from spinach (*Spinacia oleracea*) and kale (*Brassica oleracea* var *sabellica*) after a 45-min PAW treatment. However, the authors further stated that the study also revealed a change in certain nutritional attributes of the vegetables. The chlorophyll content in kale decreased from 323.6 ± 0.52 mg/g to 214.5 ± 0.09 mg/g, while spinach exhibited a reduction from 99.3 ± 0.15 mg/g to 85.5 ± 0.05 mg/g. In another study, Laurita *et al*. (2021) compared the efficacy of PAW (2-20 min) untreated water and hypochlorite solution on rocket leaves (*Eruca sativa*), against endogenous spoilage bacteria. A PAW treatment of 2 to 20 min resulted in a 1.7-3 Log reduction in total mesophilic and *Psychrotrophic* bacteria and *Enterobacteriaceae*, without altering the qualitative and nutritional attributes.

Additionally, Choi *et al*. (2019) reported that treating shredded salted kimchi cabbage (*Brassica campestris* ssp. *Pekinensis*) with PAW (120-PAW at a ratio of 1:10 (w/v), 10 min, under agitation) reduced mesophilic aerobic bacteria, lactic acid bacteria, yeast and moulds, and coliforms by 2.0, 2.2, 1.8, and 0.9 log CFU/g^{-1}, respectively. Rangel-Huerta *et al*. (2021) conducted a metabolomic analysis of spinach leaves (*Spinacia oleracea*) subjected to various treatments, including PAW, to assess potential alterations in the metabolite composition following 8 days of refrigeration. The study revealed that PAW treatment significantly enhanced the antioxidant capacity and stress resistance of the spinach leaves. Furthermore, PAW was shown to improve food quality and extend shelf life by modulating carbohydrate-related metabolic pathways, thereby delaying the onset of senescence (Rangel-Huerta *et al*., 2021).

Chamorro *et al*. (2023) investigated effect of the PAW (1 and 5 min, in 1 L) on the quality and preservation of fresh-cut lettuce (*Lactuca sativa*) stored at 4 °C for 7 days. The authors found that PAW treatments significantly delayed chlorophyll degradation from day 3 of storage, while no noticeable changes were observed in firmness. Moreover, microbiological analysis showed that PAW treated

lettuce had the highest reduction of 1.45 Log CFU/g^{-1} in, psychrotrophs, after three days of storage (Chamorro *et al*., 2023).

The potential of PAW as an effective and environmentally friendly method for improving the quality and safety of leafy crops is highlighted in the above-mentioned studies. PAW has continuously shown significant microbial reductions, including those of *E. coli*, mesophilic, and psychrotrophic bacteria, without altering texture or visual appearance of respective leafy vegetables. Moreover, PAW promoted antioxidant capacity, delayed senescence, and extended shelf life of leafy vegetables. These results present PAW as an ideal option to replace conventional sanitizers, providing fresh produce preservation that is effective and delicate.

2.4.2.2 Electrolysed water

Electrolysed water (EW) is a cutting-edge technique that reduces microbial contamination, lowers pesticide residues, and enhances the quality of produce postharvest (Lu *et al.,* 2022). It is one of the most practical substitutes for hazardous sanitizing agents due to its antimicrobial effects, cost of production and easy application on fresh produce. EW is a viable option for a variety of industries, such as food industry (Lu *et al.*, 2022), animal industry (Zheng *et al.*, 2016) and medicine (Yan *et al.*, 2021; Chen and Wang, 2022) due to its adaptability and environmentally favourable qualities. There are three fundamental parameters that are considered to have a direct impact on EW's sanitizing effectiveness which are its oxidation-reduction potential (ORP), pH, and accessible chlorine concentration (Nyamende *et al*., 2022, 2023). The primary mechanism for EW's bactericidal activity is the production of chlorine compounds (hypochlorous acid, chlorine gas and hypochlorite ion) through a series of reactions in the electrolysis system (Lu *et al.*, 2020; Zhao *et al.*, 2021). These chlorine compounds attack the cell wall, cell membrane, cellular ribosome, enzyme, ribonucleic acid and other components to demonstrate their antibacterial ability (Liao *et al.*, 2017).

EW can be produced simply by electrolysing distilled water and a diluted salt solution (Lu *et al*., 2022; Nyamende *et al*., 2023), meaning it is extremely inexpensive to produce it and, its on-site production eliminates transportation and storage concerns (Nyamende *et al*., 2023). Considering that it is a non-thermal treatment, it does not negatively impact the sensory qualities of produce, including colour, flavour, texture, and odour (Rahman *et al*., 2016). EW has no negative impact on the environment and human health since it turns back into regular water when diluted with tap water or reverse osmosis (Rebezov *et al*., 2022). In contrast of all the benefits provided by electrolysed water, it has its own limitations. For instance, extended storage or high temperatures may lead EW to lose its antibacterial qualities because it will self-decompose and lose chlorine (Lu *et al*., 2022). Additionally, organic matter such as protein can react with free chlorine to diminish the amount of chlorine available for disinfection, which will reduce the effectiveness of EW (Nyamende et al., 2022, 2023). Moreover, there

is a possibility of corrosiveness, hand skin irritation, and phytotoxicity because of the acidic nature of EW consisting high ORP and high free chlorine content (Zheng *et al*., 2016).

EW is generated by the electrolysis of a diluted salt solution (Figure 2.4), such as sodium chloride (NaCl), potassium chloride (KCl) or magnesium chloride ($MgCl_2$) in an electrolysis chamber separated by a membrane (Nyamende *et al*, 2022, 2023). Following electrolysis, the anode side produces acidic electrolysed water (AEW) with a pH of 2.0 to 3.5, a high ORP > 1000 mV, and an available chlorine concentration of 10–90 mg L^{-1}, while the cathode side produces an alkaline electrolysed solution with a pH of 11–13 and an ORP of -795 to -900 mV (Nyamende *et al.,* 2023). Other kinds of EW can be produced by modifying the configuration of the electrolytic cell, adjusting reaction conditions, and varying the composition of the input solution (Nyamende *et al*., 2023). These variations result in neutral EW (pH 6.5–7.5) or slightly acidic EW (pH 5.0–6.5) (Nyamende *et al*., 2023).

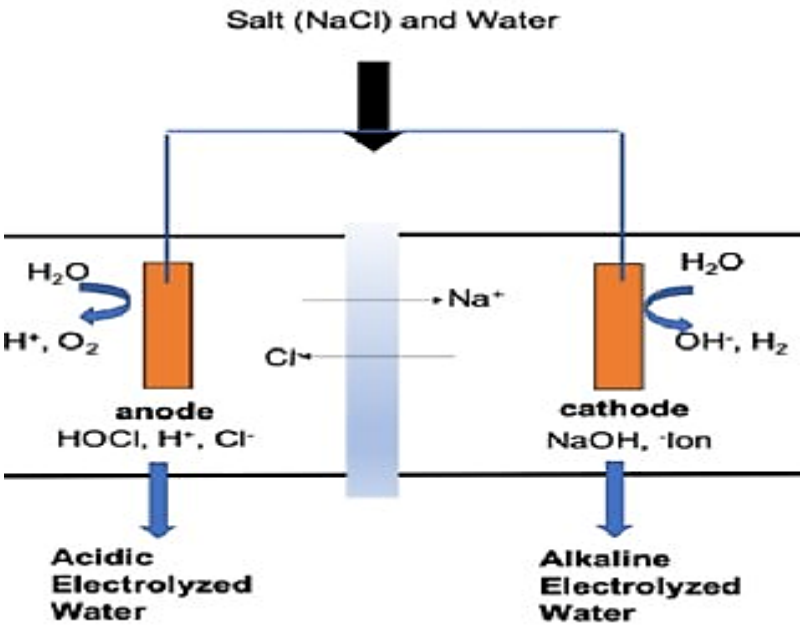

Figure 2.4: Schematic diagram of electrolysed water (Adapted from He, 2021).

EW has been thoroughly studied as a leafy vegetable postharvest sanitiser. Recent research has demonstrated that EW enhances the quality of postharvest leafy vegetables in addition to eliminating microbes and pesticide residues from it (Lu *et al.*, 2022). EW can successfully lower the microbial load on green leafy vegetables such as spinach (Ogunniyi *et al.,* 2021) and lettuce (Cap *et al.,* 2020) and reduce pesticide residues on lettuce (Cap *et al*., 2020). Even though EW's antibacterial properties are influenced by pH, available chlorine concentration and ORP, (Nyamende *et al*., 2023) it should be noted that is effectiveness might be affected by factors such vegetable surface roughness, contact time, electrode material, and electrolysis time.

2.4.2.2.1 Impact of EW on quality and pesticide residue removal of leafy vegetables

The effectiveness of disinfecting four different types of vegetables lettuce (*Lactuca sativa*), endive (*Cichorium endivia*), perilla (*Perilla frutescens*), and kale leaves (*Brassica oleracea* var *sabellica)*) with slightly acidic EW (30 ppm of effective chlorine at 20 ± 1°C, ORP of 562 ± 23 mV, pH 6.4) was assessed by Park *et al*. (2017). It was discovered by the authors that washing leafy vegetables in SAEW for 10 minutes could lower the total number of microorganisms by roughly 2 Log CFU/ g^{-1}. Similarly, the effectiveness of a NEW with a pH of 7.0, ACC of 56 mg/L, and ORP of 857 mV in comparison to peroxyacetic acid-based sanitiser in lowering the overall microbial load and inoculated populations of *Salmonella Enteritidis*, *Escherichia coli*, and *Listeria innocua* on baby spinach leaves (*Spinacia oleracea*) over a 10 day storage period was examined by Ogunniyi *et al*. (2023). The authors found that the treatment reduced the microbial load by 0.5 Log CFU/g^{-1}. In addition, the treatment had no effect on the overall quality of spinach leaves during storage (Ogunniyi *et al*., 2023).

Elimination of pesticides by EW on leafy vegetables was studied by Hao *et al*. (2011). Fresh spinach (*Spinacia oleracea*) was treated electrolysed water reducing (ER) water (pH of 11.6, ORP of 860 mV) and the electrolysed oxidizing (EO) water (pH 2.3, ACC of 70 ppm, ORP of 1170 mV) for 30 minutes. Researchers found that both the treatments could both efficiently decrease the pesticide residues of acephate by 74% (EO) and 86% (ER), omethoate by 62% (EO) and 75% (ER), and dimethyl dichloroviny phosphate (DDVP) by 59% (EO) and 46% (ER). Furthermore, the same results for DDVP- contaminated cabbage (*Brassica oleracea*), and leek (*Allium porrum*) revealed similar degradation efficacies to those of spinach (Hao *et al*., 2011). Similarly, the effects of treating fresh-cut cabbage (*Brassica oleracea*), with alkaline and acidic electrolysed water (AlEW, AcEW) on the elimination of pesticides (phorate, chlorpyrifos, lambda-cyhalothrin, cyfluthrin, procymidone, and chlorothalonil) and texture quality were examined by Liu *et al*. (2021). The authors observed that pesticide residues in the three fresh-cut cabbage were considerably reduced by EW, and the treated samples showed no substantial textural degradation.

The results from these studies provided a strong proof that different types of EW are an effective and sustainable way to reduce pesticide residues and decontaminate microorganisms on leafy vegetables. Furthermore, these findings support the beneficial effect of EW as a practical, environmentally friendly substitute for traditional sanitizers in maintaining food safety and increasing shelf life, especially in fresh-cut and ready-to-eat vegetable applications, without affecting the produce's visual or nutritional value.

2.5.2 Complexities and upscaling

The activated waters presented in this review such as PAW, EW and MNBs have merged as a promising alternative to conventional chemical disinfectants for application in agriculture and postharvest treatments. This is due to their strong antimicrobial properties, environmental friendliness, and the ability to maintain quality and enhance shelf life of fresh produce (Zhao *et al*., 2020). However, despite their potential, a significant challenge remains with regards to upscaling and commercial adaptation (Foster *et al*., 2018). For PAW, the complexity of its RONS chemistry hinder its adoption, as does the difficulty in designing and running large-scale plasma reactors (Sharma *et al*., 2021). Moreover, the interaction of RONS with food constituents and their potential effects on nutritional and sensory qualities remains poorly understood (Wang and Salvi, 2021).

Similarly, while EW demonstrated broad spectrum of antimicrobial and degradation of pesticide residues, the lack of clarity surrounding its mode of action, variability in treatment effectiveness across different pesticides and high equipment cost affects its scalability (Wang *et al.,* 2022b; Lu *et al.,* 2022; Nyamende *et al.,* 2023). On the other hand, MNB technology offers additional advantages such as improved agricultural practices (Arablousabet and Povilaitis, 2024), microbial decontamination and quality retention of fresh produce (Shan *et al.,* 2023; Malahlela *et al.,* 2024a). However, upscaling is limited by challenges in bubble size standardisation, inconsistent production method and lack of transparency in commercially available MNB generators (Seridou and Kalogerakis, 2021; Zhang *et al.*, 2023; Babu and Amamcharla, 2023). Moreover, most research remains at lab or small pilot scale, and a comprehensive cost-benefit analysis is needed to assess industrial feasibility (Khan *et al.,* 2020; Marcelino *et al.,* 2023). In addition, continued research is essential to address these barriers and unlock large-scale application (Seridou and Kalogerakis, 2021).

2.6 Conclusion

Leafy vegetables, such as Swiss chard, serve as an essential source of nutrients, particularly in rural communities. Their affordability, year-round availability, and nutrient density make them important for addressing dietary deficiencies. These vegetables are rich in essential nutrients, including phenolic compounds, lutein, folic acid, and vitamins A and C, all of which play key roles in physiological processes such as energy metabolism, immune function, bone health, and vision. Despite their nutritional benefits, leafy vegetables are highly susceptible to microbial contamination, which significantly reduces their postharvest quality and shelf life. In this context, activated water treatments such as ozone-microbubble technology and plasma activated water, as well as electrolysed water,

offer promising solutions for enhancing the postharvest management of fresh leafy produce. These innovative treatments have demonstrated the potential to reduce microbial loads, preserve quality, and minimise environmental impact. Consequently, their integration into the supply chain could contribute to a more sustainable, efficient, and safer food system.

Although several postharvest handling practices have been investigated in leafy vegetables broadly, Swiss chard specific studies remain limited. Key research gaps persist. There is insufficient data on Swiss chard's unique postharvest physiology and disease susceptibility compared to other leafy vegetables. Current reliance on synthetic sanitisers such as chlorine raises health and environmental concerns, yet eco-friendly alternatives remain underexplored. Additionally, packaging systems tailored to Swiss chard's respiration and moisture dynamics are lacking, while genetic resistance to postharvest diseases has received minimal attention. Localised handling strategies suitable for resource-constrained environments in sub-Saharan Africa are urgently needed, alongside long-term studies on storage and transportation under diverse climatic and infrastructural conditions. Addressing these gaps is essential to reduce losses, safeguard nutritional quality, and strengthen food security.

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CHAPTER 3

EFFECTS OF AIR MICRO-NANOBUBBLE WATER TREATMENT ON PHYSIOLOGICAL RESPONSES, PHYSICOCHEMICAL, AND MICROBIOLOGICAL QUALITIES OF PACKED WHOLE SWISS CHARD (*BETA VULGARIS* L. CV. FORDHOOK GIANT)

Abstract

In this study, the effectiveness of air-generated micro-nanobubbles (MNBs) water as an alternative to traditional chlorine-based washing for leafy vegetables was evaluated. Freshly harvested Swiss chard (*Beta vulgaris* L cv. Fordhook Giant) was washed for 10 min using either sodium hypochlorite (NaOCl, 100 mg L^{-1}; standard industry practice), air MNBs or tap water (control). All samples were packaged using bi-axially oriented polypropylene (BOPP) film and stored at 5 °C for 8 days. Air-MNB pre-treatments significantly preserved chlorophyll content while effectively reducing weight loss and preserving leaf colour compared to NaOCl and tap water. Furthermore, air-MNBs maintained the best visual quality, however had a comparable 1-log reduction efficacy against aerobic mesophilic bacteria with NaOCl pre-treated samples. These findings therefore position air-MNBs as a viable eco-friendly alternative to NaOCl for leafy vegetables, though further research could optimise its antimicrobial efficacy while preserving visual and biochemical quality.

Keywords: Cold storage, modified packaging atmosphere, post-harvest sanitation, spoilage, sodium hypochlorite, visual quality,

3.1 Introduction

Swiss chard (*Beta vulgaris*) is a nutrient-rich leafy vegetable, rich in essential minerals such as calcium, magnesium, potassium, and iron, as well as bioactive phenolic compounds (Čeryová *et al*., 2025). Despite its nutritional value, it is highly susceptible to rapid post-harvest deterioration driven by microbial growth and decay, which poses significant challenges for the fresh produce industry (Shezi *et al*., 2024). In order to maintain the quality of green leafy vegetables, proper and suitable preservation and storage techniques are not only very important but also a big challenge for this emerging sector. Modified atmosphere packaging (MAP) is a crucial technology for preserving the quality of freshly processed green leafy vegetables by creating a controlled atmosphere within the packaging (Carnelossi *et al.*, 2012; Haque *et al.*, 2021). The main mechanism of MAP is the reduction of oxygen (O_2) levels and increase of carbon dioxide (CO_2) concentration inside the package. This altered gas environment suppresses the respiratory activity of the vegetables, slowing their metabolic

rate, which in turn delays spoilage processes such as enzymatic browning, microbial growth and product decay (Carnelossi *et al.*, 2012; Haque *et al.*, 2021).

Conventional sanitisation techniques which are effective in controlling microbial growth and spoilage, commonly employ chlorine-based sanitisers such as sodium hypochlorite (NaOCl). Typically, NaOCl is applied at concentrations ranging from 50 to 200 mg L^{-1} (Klintham *et al.,* 2017). However, repeated use of these solutions may facilitate the development of microbial resistance by promoting resistant genes among microorganisms (Li *et al*., 2023). In addition to producing disinfection by-products, chlorine-based sanitisers can cause adverse impacts in humans such as respiratory difficulties as well as skin irritations (Deng *et al.,* 2019; Fattahi-Zaim *et al.,* 2025). This has raised public concern, resulting in additional regulatory measures for its use (Nyamende *et al.,* 2022). Consequently, the drive to find safer and more effective alternatives to chlorine-based sanitisers is growing, as both research and regulatory trends highlight the limitations of chlorine-based sanitation practices.

Micro-nanobubble (MNB) technology is a new concept that offers a potential solution as a decontaminant in fresh produce. MNB involves the generation of ultrafine gas bubbles in water, typically ranging from 1-100 µm (microbubbles) in diameter, which exhibits unique physicochemical properties such as prolonged stability, high gas dissolution efficiency, and oxidative capabilities (Malahlela *et al.,* 2024; Xiao *et al.,* 2025). Recent research shows that the highly energetic state of micro-nanobubbles (MNBs) in the liquid phase enables gases such as oxygen (O_2) and ozone (O_3) to dissolve at concentrations above their standard saturation point (Malahlela *et al.,* 2024). Furthermore, when MNBs collapse, they can generate reactive species that have powerful effects in disinfection and microbial inactivation processes, making MNB technology particularly promising for food safety applications (Malahlela *et al.,* 2024).

A limited number of studies have assessed the efficacy of MNB technology on quality and safety of leafy vegetables. For instance, Klintham *et al.* (2017) showed that combining MNBs with NaOCl and acidic electrolyzed water (AEW) for 5 min reduced *Escherichia coli* and *Salmonella Typhimurium* by 2-3 log on sweet basil and Thai mint, respectively. In another study, Xueqing *et al.* (2020) found that treating spinach with 4 mg/L ozone MNBs for 5 min, then storing for (20 ± 1) °C for 8 days, slowed aging and reduced respiration and ethylene production. Additionally, by delaying loss of vitamin C and chlorophyll content, MNB application enhanced sensory and nutritional quality of spinach (Xueqing *et al.,* 2020). Similarly, Chen *et al.* (2023) reported over 2 log reductions in *Salmonella Typhimurium* and *E. coli* populations after 3 min of O_3-MNB exposure and also found reduced pesticide residues on Napa cabbage. Moreover, the quality of parsley treated with various concentrations of O_3-MNBs was

examined during storage at 20 °C for 5 days (Shi *et al.,* 2023). The authors discovered that parsley exposed to 2.5 mg·L^{-1} O_3-MNBs for 10 min effectively preserved its sensory quality while maintaining higher levels of firmness, vitamin C, and chlorophyll content compared to untreated controls. More recently, da Silva *et al.* (2025) showed O_2 and O_3 MNBs applied for 5 -10 min on lettuce resulted in up to a 1.59 log reduction in aerobic mesophilic bacteria after 6 days at 5 °C storage.

Notably, antimicrobial studies using MNBs have combined them with agents such as electrolysed water and chlorine, yet none have examined the impact of air-MNBs on fresh Swiss chard. Based on current literature, the individual and combined effects of MNBs and MAP in Swiss chard preservation remain underexplored. This study therefore aimed to quantitatively assess their combined effect on (a) physiological and phytochemical attributes and (b) microbial load of fresh Swiss chard stored at 5 °C for 8 days.

3.2 Materials and methods

3.2.1 Plant material

Fresh organically grown Swiss chard (*Beta vulgaris* L. cv. Fordhook Giant) was obtained at commercial maturity from Kraaifontein Shelf Storage, Kraaifontein, Western Cape, South Africa. The harvested produce was transported in cool conditions (4°C) to the Postharvest and Agro-Processing Pilot plant at the Agricultural Research Council (ARC), Stellenbosch, South Africa. Upon arrival, the samples were sorted for uniformity in size, colour, and overall appearance. Only healthy Swiss chard leaves were selected and stored at 5°C and 95% RH.

3.2.2 Preparation of micro-nano bubbles

Micro-nano bubbles were generated using a Mk4- NanoBubbler, Fine bubble technologies Co., Ltd., South Africa. An air cylinder was connected to an MNB generator that creates air-MNB by producing ultra-fine/nano bubbles with an average diameter of 76.4 nm and 2.22 X 10^{10} NBs/mL of water. Following production, the hydrogen ion concentration, pH and redox potential of air-MNBs were measured using a HI 98121 pH-oxidation reduction potential (ORP) meter (Hanna Instruments, Cape Town, South Africa). The MNBs were composed of pH of 7.30 ± 0.10 and ORP: -268 mV ± 4.00. Food-grade sodium hypochlorite (NaOCl, 11.5% M/V) solution procured from Protea Chemicals in Sandton, South Africa was used in this study. The NaOCl's available chlorine content (ACC) was 100 mg L^{-1}, with ORP: -489 mV ± 2.65, and pH of 12.2 ± 0.92.

3.2.3 Swiss chard preparation and treatment

Swiss chard (cv. Fordhook Giant) was prepared by removing outer, damaged, and yellowed leaves, along with stems, to ensure uniformity in size and colour. After the initial sorting, the samples were randomly divided into three pre-treatment batches (i.e., air-micro-nanobubble (MNB), sodium hypochlorite (NaOCl), and tap water (TW). The pre-treatments were applied to each batch for 10 min and allowed to air dry at room temperature (20 °C). Thereafter, approximately 100 g of the pre-treated chards were packed in bi-axially oriented polypropylene film (BOPP) with OTR rate of 8.5×10^{-12} mol m^{-2} s^{-1} Pa^{-1} at 23 °C and 0% RH and film thickness of 25 µm a per respective pre-treatment. The films were manually heat sealed using an Impulse Foot Heat Sealer (KS-F 600, Vala Impulse sealer, South Africa). To ensure consistency, sealing parameters were rigorously standardized: a power setting of 6 (≈120 °C), impulse time of 1.2 s, and a 2 s cooling under full foot pressure. Treatment description and their abbreviations are listed in Table 3.1. All samples were then stored at 5°C and 90 ± 2% RH for 8 days. Packed samples were taken out in triplicate on days 0, 2, 4, 6 and 8 for further analysis.

Table 3.1: Name and description of pre-treatments used in this study.

Treatment(s)					Treatment(s) Abbreviation
Active Compound	**Concentration (mg L^{-1})**	**pH**	**ORP**	**Dipping duration (min)**	
Sodium hypochlorite	100	11.2	489 mV	10	NaOCl-BOPP
Tap water	-	7.11	273 mV	10	TW-BOPP
Air-micro nano-bubble water	-	7.30	268 mV	10	MNB-BOPP

NaOCl-BOPP = Sodium hypochlorite standard industry practice applied for 10 min; TW-BOPP = Tap water control treatment, applied for 10 min; MNB-BOPP = Air micro-nano bubble water treatment applied at 10 min, respectively; ORP = Oxidation reduction potential.

3.2.4 Physiological response

3.2.4.1 In-package atmosphere

To account for the physiological activity of Swiss chard leaves, the study followed a method by Belay *et al.* (2018) that is based on mass balance principles. With the use of mass balance equations 4 and 5 that monitor the dynamic exchange of in-package gases (O_2 and CO_2), this method predicts the transient behaviour of oxygen Y_{O_2} and carbon dioxide Y_{CO_2} .

$$\frac{\partial Y_{O_2}}{\partial_t} = \frac{\frac{A_P P_{O_2} P_{atm}}{L}\left[Y_{O_{2i}} - Y_{O_{2f}}\right]}{V} - W \quad (1)$$

$$\frac{\partial Y_{CO_2}}{\partial_t} = \frac{\frac{A_P P_{O_2} P_{atm}}{L}\left[Y_{CO_2 f} - Y_{CO_{2i}}\right]}{V} - W \quad (2)$$

where $\frac{\partial Y_{O_2}}{\partial_t}$ and $\frac{\partial Y_{CO_2}}{\partial_t}$ represent the variation in O_2 and CO_2 concentrations over time, P_{O_2} is permeability to O_2, P_{atm} is atmospheric pressure, A_P is surface area of the package, L is thickness of the packaging film, W is weight of the chard leaves and V is free volume. The initial O_2 and CO_2 concentrations are represented by $Y_{O_{2i}}$ and $Y_{CO_{2i}}$ and the final O_2 and CO_2 concentrations are represented by $Y_{O_{2f}}$ and $Y_{CO_{2f}}$ respectively.

3.4.2.2 Weight loss

Weight loss was measured using a slightly modified method by Sahu *et al.* (2024). An electronic balance (FTA20, Güss, South Africa) was used to weigh individual packages of chard leaves at the beginning of the experiment (initial weight) and each subsequent sampling day during storage. The weight loss of the packed Swiss chard was calculated using the average weight loss per pre-treatment on each sampling day. The percentage weight loss was calculated by averaging the weight difference between the initial and post-storage measurements using Eq. (3):

$$WL = \frac{Wo - Wf}{Wo} \times 100 \quad (3)$$

where WL is the weight loss (%); W_o is the initial (g) and W_f is the final weight (g) of packaged Swiss chard.

3.2.5 Physicochemical properties

3.2.5.1 Colour

'Fordhook Giant' Swiss chard leaves were measured for colour changes following a method by Adedeji *et al.* (2025) using a digital chroma-meter (CR 400/410 Konica Minolta Sensing Inc., Japan) based on the Commission International del'Eclairage (CIE) colour system. Before every measurement, the chroma-meter was colour-calibrated, and measurements were obtained from the opposite side of each chard leave using three leaves per treatment (n=18). The following were measured: *L** indicating lightness and *b** indicating yellow (+)/blue (−). The *h*° and chroma (*C**) angles were also calculated using Eq. (4) and Eq. (5).

$$Hue\ (h^o) = (b^*/a^*) \quad (4)$$

$C^* = \sqrt{a^{*2} + b^{*2}}$

3.2.6 Chlorophyll content

Chlorophyll content determination of Swiss chard was conducted following ESS Method 150.1 (1991), with slight modifications. Swiss chard leaves samples (1 cm^2) were collected on days 0, 2, 4, 6 and 8 and each sample was ground with 0.2 mL of $MgCO_3$. The homogenate was transferred into 10 mL of 90% aqueous acetone and kept in a dark. Aliquots were then adjusted to a final volume of 13 mL with 90% acetone, vortexed and stored in the dark at 4 °C overnight. Extracts were clarified by centrifugation at 500 rpm for 20 minutes. The absorbance of the supernatant was measured using a nano-photometer (N60, Implen, Germany) at absorbance: 750, 665, 663, 645, and 630 nm, using 90% acetone as a blank. Chlorophyll contents (*a* and *b*) were calculated using Eq. (6) and Eq. (7), by Tang *et al*. (2023), while for total chlorophyll, Eq. (8) by Tang *et al*. (2022), was followed.

$$Chlorophyll\ a = (11.64\ A_{663} - 2.16A_{645} + 0.10A_{630})v/IV \quad (6)$$

$$Chlorophyll\ b = (20.97\ A_{645} - 3.94A_{663} - 3.66A_{630})v/IV \quad (7)$$

$$Total\ Chlorophyll = (12.25\ A_{663} - 2.79A_{645}) + (21.5A_{645} - 5.1A_{663}) \quad (8)$$

where A_{663} is the absorption value at 663 nm and A_{645} is absorption value at 645 nm

3.2.7 Microbiological analysis

Using the total plate count method, samples were examined for yeasts and moulds, and total aerobic mesophilic bacteria (TAMB) (Nyamende *et al*., 2022). To quantify the microbial load on the leaf's surface, 2 g of a leaf sample was placed in an aseptic physiological saline (PS) solution and gently vortexed continuously for 30 min. Precisely 1.0 mL of diluents were mixed with 9.0 mL of PS to create threefold dilutions. Thereafter, 1.0 mL of each dilution was added to the media (PDA for yeast and moulds and PCA for bacteria) to enumerate the microbial load. For yeasts and moulds, plates were incubated for 3-5 days at 28°C, while for TAMB, plates were incubated for 2 days at 37°C. Colony forming units (CFU g^{-1}) per g of surface leaf were calculated and converted to Log CFU g^{-1}.

3.2.8 Statistical analysis

The design of experiment followed a two-factor completely randomised design with sampling at regular intervals. A Two-way analysis of variance (ANOVA) was conducted using Statistica Software (version 13; StatSoft Inc., TIBCO Software Inc., USA) to evaluate the effects of treatment and storage duration on the measured attributes of 'Fordhook Giant' Swiss chard. Where significant differences were detected, Duncan's multiple range test was applied to determine significant differences between

mean values ($p \leq 0.05$). Each treatment was performed in triplicate, and the mean and standard deviation of the results were reported.

3.3 Results and discussion

3.3.1 Gas composition

Figure 3.1 shows the in-package gas composition (O_2 and CO_2) of fresh 'Fordhook Giant' Swiss chard during 8 days storage at 5 °C. Treatment conditions and storage duration had a significant influence on fresh 'Fordhook Giant' Swiss chard during storage ($p < 0.05$) (Figure 3.1A, B). Throughout the storage duration, MNB-treated samples maintained low CO_2 concentrations (<3%) and high O_2 concentrations (>20%). This stability aligns with studies showing that the combination of pre-treatment and MAP can delay senescence by suppressing oxidative metabolism (Toivonen & Brummell, 2008). Furthermore, the limited CO_2 rise under MNB pre-treatment implies that the product was well preserved, with minimal signs of deterioration throughout 8 days storage. Contrastingly, NaOCl pre-treated samples exhibited sporadic atmospheric shifts with CO_2 fluctuating from an initial 0.8% to just above 1.1 % at the end of 8 days storage (Figure 3.1B). Furthermore, O_2 levels varied between (<9.8%) and (>20.4%) respectively.

According to Agustin-Salazar *et al.* (2024), such variations in gas composition indicate that NaOCl treatments may induce physiological stress, preventing equilibrium in the packaging atmosphere even after extended storage. This non-equilibrium can foster the development of anaerobic fermentation and off odours, posing a risk to product quality. Moreover, TW pre-treated samples followed an intermediate trend with O_2 levels gradually declining and CO_2 gently rising, mirroring typical storage induced respiration patterns (Figure 3.1A, B). Such substantial atmospheric fluctuations can signal incomplete sterilisation or physiological injuries, leading to increased spoilage risk (Carnelossi *et al.*, 2012). Therefore, the results obtained from the current study demonstrate that MNB pre-treatment, in combination with BOPP packaging effectively stabilised the in-packaging atmosphere of 'Fordhook Giant' Swiss chard during storage.

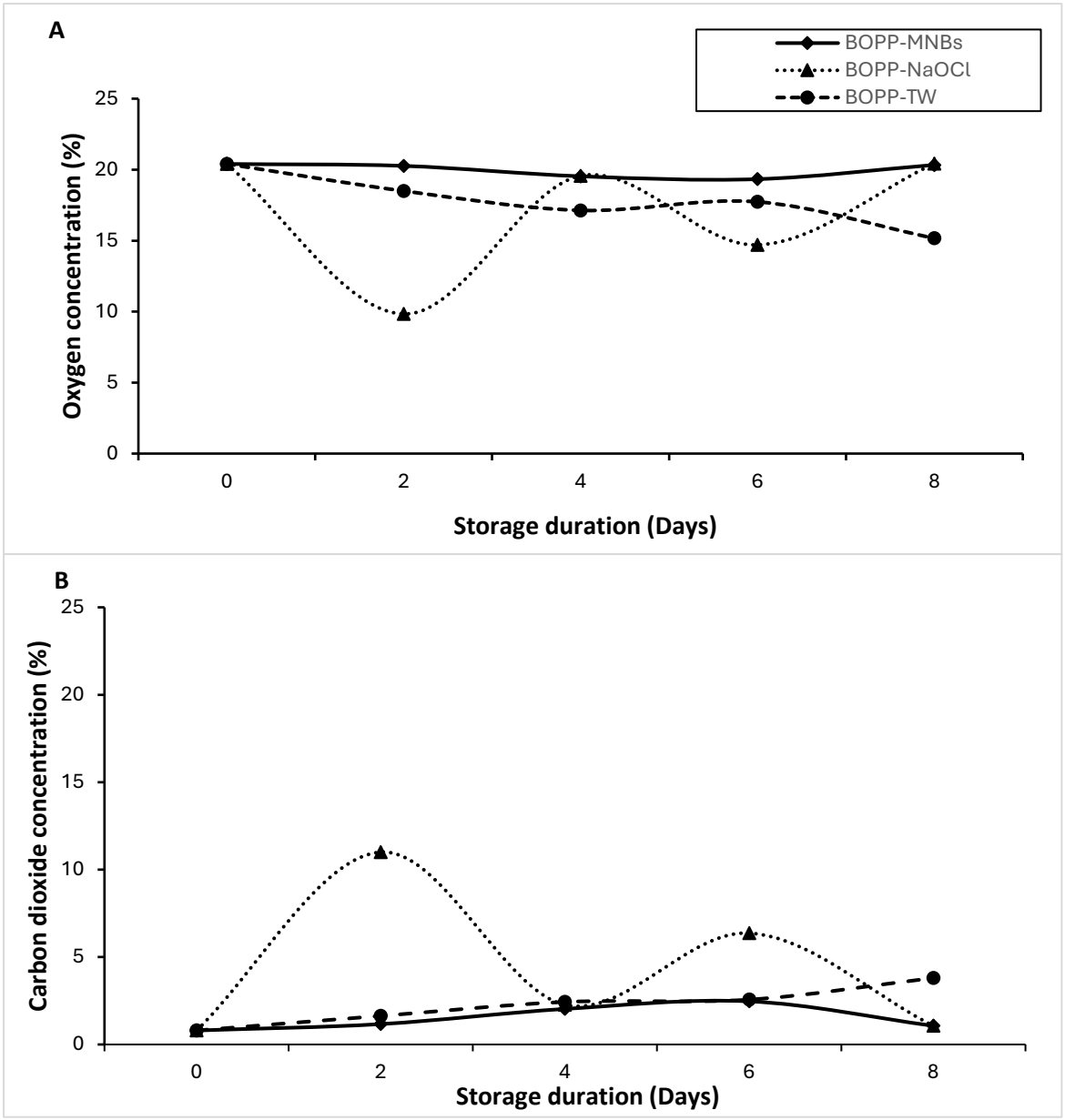

Figure 3.1: Effect of pre-treatments and MAP on **(A)** O_2 concentration and **(B)** CO_2 concentration of packaged Swiss chard leaves (cv. Fordhook Giant) pre-treated with air-MNB, NaOCl and TW, and stored at 5 °C for 8 days. Mean value (n=3). Pre-treatment abbreviations (MNB-BOPP, NaOCl-BOPP and TW-BOPP) are listed in Table 3.1.

3.3.2 Changes in weight Loss

In the present study, the weight loss of 'Fordhook Giant' Swiss chard significantly increased ($p < 0.05$) throughout the storage duration. On day 2, weight loss (%) was 5.39, 6.48 and 10.32 % for MNB, NaOCl and TW treated and packed Swiss Chards (Figure 3.2), respectively. By the end of storage (day 8), NaOCl samples showed the highest weight loss (38.89 %), followed by TW (29.72 %) while MNB treatment maintained the lowest weight loss (17.34 %) (Figure 3.2). The pronounced weight loss in NaOCl treated samples could be due to its disruptive effects on the cell wall and cell membranes as well as its impact on enzymatic activity, which increases cell transpiration and respiration during storage (Jalali *et al*. 2021). Moreover, the available chlorine content of NaOCl could cause tissue damage and tainting in fresh produce (Sun *et al.*, 2012).

In contrast, MNB treated samples consistently exhibited the lowest weight loss of the Swiss chard throughout the storage duration compared to both TW and NaOCl treated samples. The observed delay of weight loss in MNB treated samples could be attributed to the inhibition of polyphenol oxidase (PPO) activity, which lowers respiration (Zhu *et al.,* 2018). These findings are consistent with Shi *et al.* (2023), who reported that parsley samples treated by ozone micro-nano bubble water for 10 minutes, exhibited lower weight loss, compared to control. Conversely, the behaviour of NaOCl contrast with Zudaire *et al.* (2018), who reported better weight loss of fresh-cut calçots (*Allium cepa*) treated with NaOCl (100 mg L^{-1}) and stored for 15 days at 4 °C.

This variation may be due to the difference in commodity type, and cell structure. Weight loss in leafy greens is generally attributed to cell transpiration and respiration (Zhang *et al.* 2023). Additionally, factors such as, stomata opening, surface wounds, storage temperature, relative humidity, and air velocity can contribute to weight loss in leafy green (Wanakamol *et al.,* 2022). The overall results of this study indicate that MNB treatment minimized weight loss in Swiss chard, which could be due to reduced respiration and transpiration rates. The treatment helped maintain cellular hydration and structural integrity by reducing metabolic activity and water vapour loss (Shezi *et al.,* 2024), which decreased overall weight loss during storage.

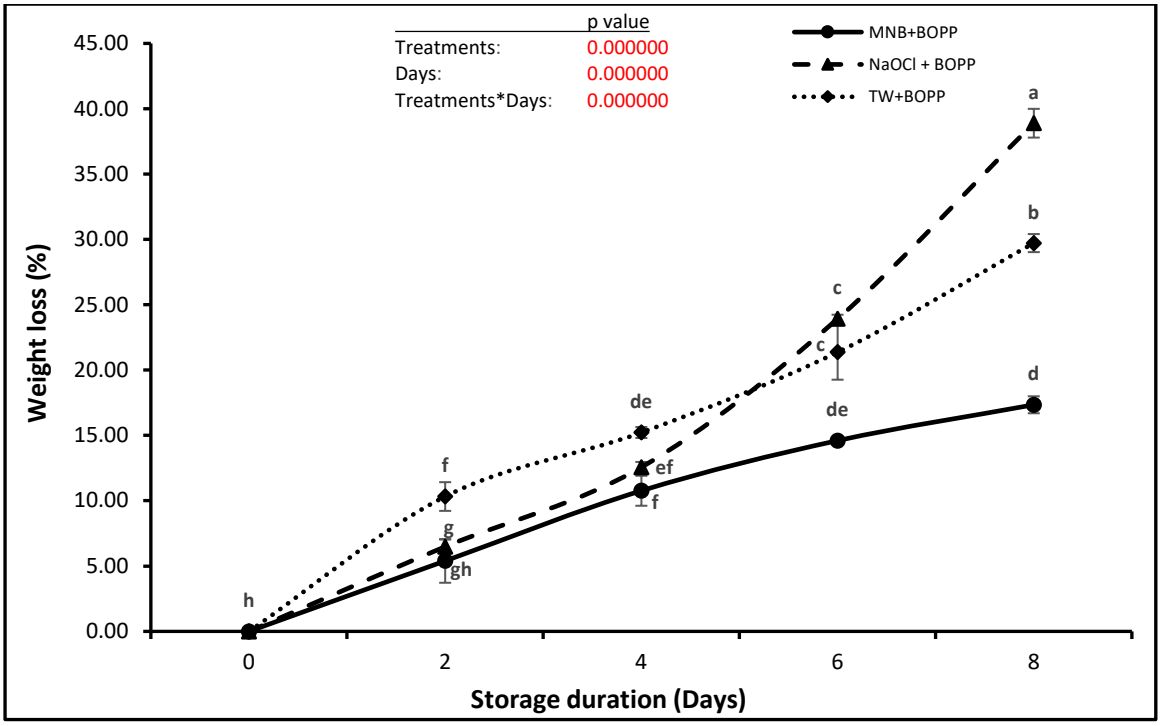

Figure 3.2: Effect of pre-treatment and MAP on weight loss of packaged Swiss chard leaves (cv. Fordhook Giant) pre-treated with air-MNBs NaOCl and TW and stored at 5 °C for 8 days. Mean value (n=3) with standard deviation. Means with the same letters are not significantly different (p≤0.05). Pre-treatment abbreviations (MNB-BOPP, NaOCl-BOPP and TW-BOPP) are listed in Table 3.1.

3.3.3 Colour

Colour is a vital sensory attribute of fresh produce, playing a key role in quality assessment and influencing consumer purchasing decisions (Farcuh *et al*., 2020). In our study colour parameters were significantly influenced ($p < 0.05$) by the treatment conditions (MNBs, NaOCl, TW) and storage duration, as shown in Table 3.2, reflecting differences in senescence progression and potential quality changes in chard leaves. The interaction of treatment condition and storage duration ($p = 0.0027$), and treatments ($p = 0.0029$) were found to have a significant effect on the *L** values of fresh chard leaves (Table 3.2). The *L** values of MNBs were significantly lower than those of NaOCl and TW ($P < 0.05$), throughout the storage duration suggesting that air-MNBs preserved a greener appearance of the leaves (Figure 3.3). By the end of storage duration, the treatments resulted in 38.65 for MNBs, 42.49 for NaOCl and 41.85 for TW respectively.

Additionally, the lightness value in all the treatments increased over time, however a notable change was observed for NaOCl treated samples, indicating a possible cellular damage and chlorophyll degradation (Khan *et al.,* 2022). This observed trend agrees with a study by Xueqing *et al.* (2020) who reported lower *L** values on MNB-treated Spinach as compared to the control group. Chlorophyll

degradation reduces green pigmentation, thus increasing lightness through lower absorption and higher reflectance, explaining the elevated *L* values in the control group (Merzlyak *et al.,* 1999; Khan *et al.,* 2022).

Further analysis revealed that treatment conditions and storage duration had a significant (p= 0.000008) impact on *b** values, which relate to chard leave yellowness. This could be due to the unmasking of yellow carotenoids previously obscured by chlorophyll, causing a rise in *b* (yellowness)* values (Sun *et al.,* 2018). This shift is particularly evident in senescing or stressed leaves, where chlorophyll catabolism exposes underlying pigments, altering perceived colour (Kuai *et al*., 2018). Notably, throughout storage duration NaOCl treatment condition had the highest *L** and *b** when compared with TW and MNB pre-treatments. Furthermore, by day 8 the treatment further resulted in skin damage, darkening, and bleaching of the chard leaves (Figure 3.3). This observation suggests that the chard leaves were sensitive to NaOCl pre-treatment, whereas MNB and TW pre-treatments did not induce any visible damage by the of storage duration (Figure 3.3).

The initial hue angle 120.9° indicated a dominant green colouration, characteristic of chlorophyll-rich tissue. Over the storage period, all samples exhibited an increase in hue angles. After two days of storage, MNB treated samples showed a significantly high hue angle of 124.74° suggesting a temporary retention of greenness, likely due to MNB pre-treatment slowing chlorophyll breakdown (Hu *et al.,* 2024). This was followed by TW treated samples, which reached a hue angle of 123.03°. However, by day 8, the hue angles of both MNB and TW-treated samples declined to 122.77° and 122.45°, respectively. In contrast, NaOCl samples maintained relatively stable hue angles between 121 and 122° (Table 3.2). These findings align with those reported by da Silva *et al.* (2025), who observed a similar trend in lettuce pre-treated with ozone and oxygen MNBs for 5 and 10 min, as well as chlorine for 15 min.

Colour intensity (chroma) in both TW and NaOCl treated samples declined over the storage period, decreasing from a baseline of 27.60 % to 25.68 % and 23.02 % respectively, which reflects a fading of the green colour. Additionally, the chroma value for MNB-treated samples increased on days 2 and 4, however on day 6 it decreased. By the end of storage duration MNB treated samples best retained chroma ending up with a value of 29.41 %. This matches findings that chroma decreases with chlorophyll degradation however may rebound if other pigments accumulate (Khan *et al.,* 2022).

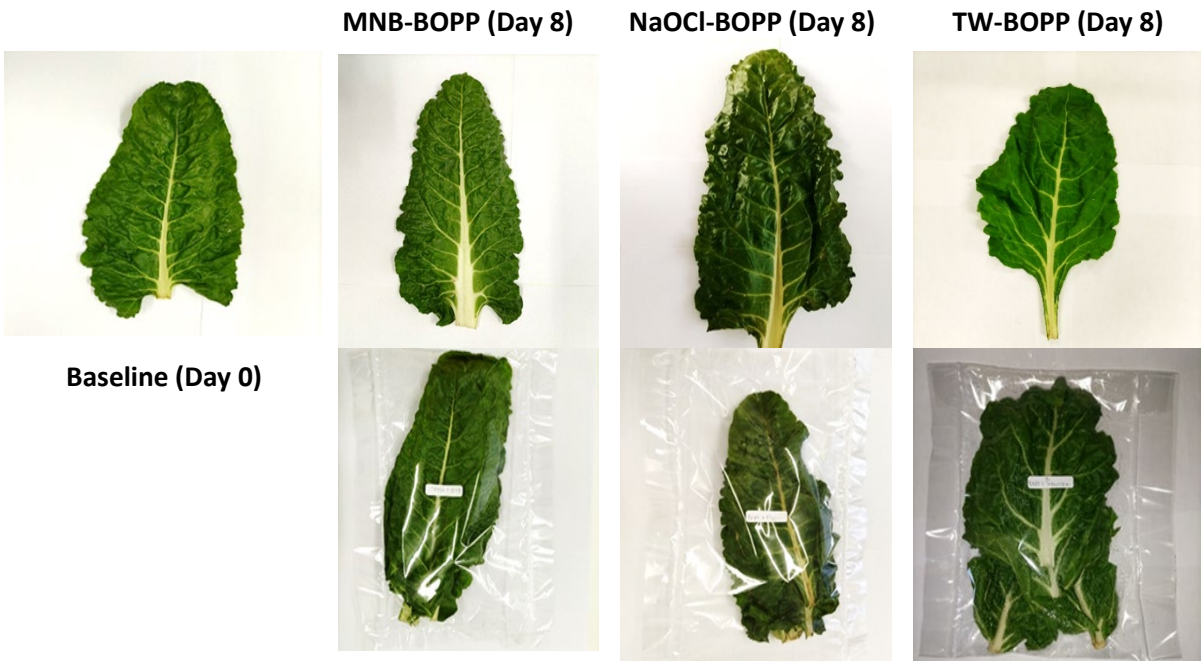

Figure 3.3: Visual documentation packaged Swiss chard leaves (cv. Fordhook Giant) pre-treated with air-MNBs, NaOCl and TW, and stored at 5°C for 8 days. Pre-treatment abbreviations (MNB-BOPP, NaOCl-BOPP, and TW-BOPP) are listed in Table 3.1 respectively.

Table 3.2: Effect of pre-treatments and MAP on colour attributes of fresh Swiss chard cv. 'Fordhook Giant' stored at 5 °C for 8 days.

			Pre-treatments and MAP			ANOVA	*P*- value
Parameters	**Storage days**		**MNB-BOPP**	**NaOCl-BOPP**	**TW-BOPP**		
Lightness	0	36.43 ± 2.71^{CD}				***Treatment (T)***	0.002902
	2		36.65 ± 4.95^{CD}	43.81 ± 5.50^{A}	37.73 ± 5.48^{CD}	***Storage duration (S)***	0.053225
	4		37.25 ± 2.59^{CD}	44.20 ± 5.84^{A}	40.70 ± 2.14^{A}	***T × S***	0.002692
	6		37.39 ± 1.50^{CD}	44.05 ± 3.36^{A}	41.86 ± 3.21^{AB}		
	8		38.65 ± 4.95^{BCD}	42.49 ± 1.04^{A}	41.85 ± 1.39^{AB}		
b	0	22.69 ± 1.80^{BC}				***Treatment (T)***	0.000000
	2		19.90 ± 1.61^{DEFG}	21.60 ± 1.51^{CDE}	21.62 ± 2.78^{CDE}	***Storage duration (S)***	0.003302
	4		19.43 ± 3.25^{EFG}	24.88 ± 2.17^{AB}	22.32 ± 1.94^{CD}	***T × S***	0.000008
	6		18.85 ± 1.66^{FG}	26.08 ± 3.14^{AB}	19.80 ± 3.79^{DEFG}		
	8		20.40 ± 1.32^{DEF}	26.73 ± 3.20^{A}	17.72 ± 1.66^{G}		
Hue	0	120.90 ± 1.32^{D}				***Treatment (T)***	0.000000
	2		124.74 ± 2.85^{A}	121.74 ± 1.45^{CD}	123.03 ± 1.73^{ABC}	***Storage duration (S)***	0.003804
	4		124.11 ± 2.53^{AB}	121.51 ± 1.45^{CD}	122.89 ± 2.44^{ABCD}	***T × S***	0.000004
	6		121.15 ± 2.06^{CD}	121.11 ± 1.36^{BCD}	122.48 ± 2.33^{BCD}		
	8		122.77 ± 1.46^{ABCD}	122.54 ± 1.95^{BCD}	122.45 ± 2.06^{BCD}		
Chroma	0	27.60 ± 1.79^{AB}				***Treatment (T)***	0.117483
	2		30.64 ± 3.32^{A}	23.73 ± 1.71^{DEFG}	21.56 ± 1.54^{G}	***Storage duration (S)***	0.002914
	4		31.39 ± 3.88^{A}	24.30 ± 1.02^{DEF}	23.85 ± 3.95^{DEFG}	***T × S***	0.175169
	6		25.42 ± 1.34^{CDE}	22.36 ± 1.83^{FG}	26.07 ± 1.92^{CD}		
	8		29.41 ± 2.30^{AB}	23.02 ± 3.56^{EFG}	25.68 ± 2.90^{CDE}		

3.3.4 Changes in chlorophyll content

Post-harvest treatment and storage duration showed a significant difference ($p < 0.05$) in Swiss chard chlorophyll constituents, (a, b, and total chlorophyll), respectively (Figure 3.4). All chlorophyll constituents followed a similar declining trend, throughout the storage duration, for all the assessed pre-treatments, with an initial concentration of 16.96, 23.83 and 25.35 g mL^{-1}, for chlorophyll a, chlorophyll b and total chlorophyll, respectively. For the different treatment conditions, a significant decline was noted in NaOCl throughout the storage duration, reaching the lowest chlorophyll constituents of 2.27, 3.26 and 1.33 g mL^{-1} for chlorophyll a and b, as well as total chlorophyll by the end of storage (day 8), respectively. The NaOCl treatment condition showed a decline in all chlorophyll constituents (Figure 3.4A, B, C) throughout the storage duration (day 8). The significant degradation of the chlorophyll constituents illustrated by the NaOCl pre-treatment, may have been caused by the intensity of chlorine present in the treatment, which is known for the disruption of cell structures (Li *et al,* 2023).

Throughout the storage duration, MNB treatments also led to a decline in all chlorophyll components, resulting in final concentrations of 6.95, 8.78, and 6.15 $g \cdot mL^{-1}$ for chlorophyll a, chlorophyll b, and total chlorophyll, respectively, by day 8. However, MNB pre-treatment demonstrated better chlorophyll retention than NaOCl treatments. The observed retention of chlorophyll constituents from MNB pre-treatment could have resulted from the collapsing of air molecules within the bubbles, which produces free radicals (Malahlela *et al.,* 2024). These radicals have the ability to bind reactive compounds into inert forms, which contributes greatly to preventing oxidative stress in the commodity (Shi *et al.,* 2023).

The overall behaviour of chlorophyll content for Swiss chard pre-treated with NaOCl, in relation to TW is in contrary with the observation by Gao *et al.* (2017). The authors observed greater retainment of chlorophyll content on coriander leaves treated with sodium hypochlorite, compared to samples treated with tap water and stored at 4 °C for 10 days. Conversely, Xiao *et al.* (2014) reported a similar trend in chlorophyll content of radish microgreens that were un-treated, washed with tap water, 50mg/L free chlorine and 100mg/L free chlorine and stored at 1 °C for 28 days. Chlorophyll is a crucial pigment in leafy vegetable, and plays a vital role during photosynthesis, also offering protection against photooxidation (Liu *et al.,* 2025).

Chlorophyll degradation can lead to leafy vegetable losing their green colour, and experience darkening of the leaves caused by senescence (Zhu *et al.,* 2018), which is supported by the colour parameters observed from the present study. Furthermore, the degradation of chlorophyll may also result from membrane damage, and the reaction of chlorophyll with chlorophyllase (Wanakamol *et al.,* 2022). Overall, Swiss chard samples pre-treated with MNBs exhibited relatively better retention of chlorophyll constituents throughout the 8 days storage at 5 °C, exhibiting less disruption of cell structures and preventing oxidative stress**.**

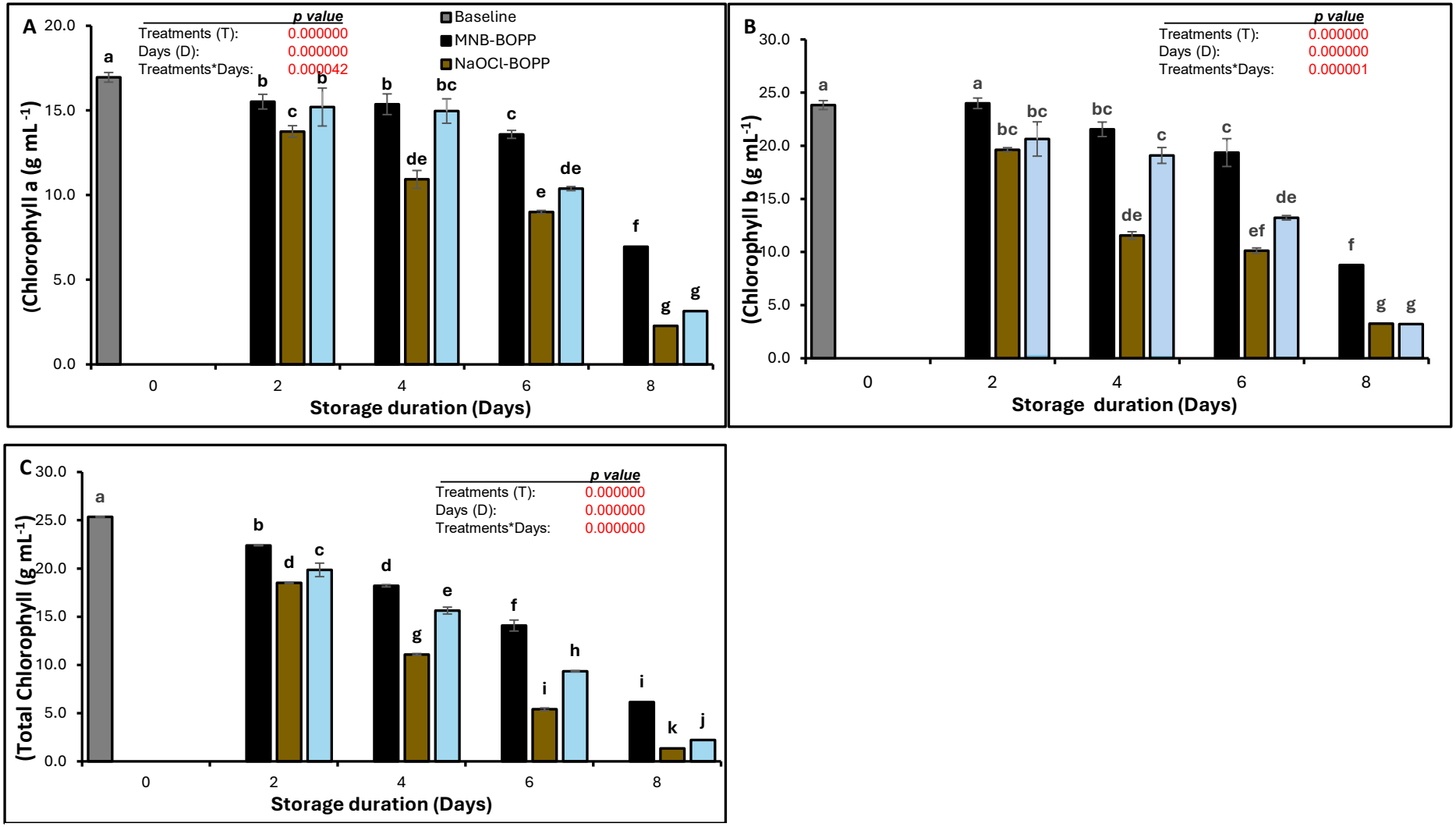

Figure 3.4: Effect of pre-treatment and MAP on (A) chlorophyll a; (B) chlorophyll b and (C) total chlorophyll of packaged Swiss chard leaves (cv. Fordhook Giant) pre-treated with air-MNBs NaOCl and TW and stored at 5 °C for 8 days. Mean value (n=3) with standard deviation. Means with the same letters are not significantly different (p≤0.05). Pre-treatment abbreviations (MNB-BOPP, NaOCl-BOPP and TW-BOPP) are listed in Table 3.1.

3.3.5 Microbiological findings

The initial microbial loads of aerobic mesophilic bacteria and yeasts and moulds on fresh cv. 'Fordhook Giant' Swiss chard were 4.05 Log CFU g^{-1} and 3.95 Log CFU g^{-1}, respectively. After 8 days storage, the highest microbial growth was observed for TW-BOPP (3.85 Log CFU g^{-1}) for aerobic mesophilic bacteria and NaOCl-BOPP (3.79 Log CFU g^{-1}) for yeast and moulds compared to other treatments (Figure 4A, B). Furthermore, there was no significant interaction (p = 0.873705) between treatment condition and storage duration for aerobic mesophilic bacteria (Figure 3.5A). Compared with NaOCl and TW pre-treatments, MNBs had a stronger ability to reduce microbial growth, however, no significant differences were observed among NaOCl pre-treated samples on day 4. At the end of storage (day 8), both MNB and NaOCl treatments resulted in a 0.53 Log reduction in aerobic mesophilic bacteria relative to initial load (Figure 3.5A).

Supporting literature confirms these trends in a variety of produce. For instance, NaOCl and AEW-based MNBs demonstrated microbial reductions of ≥2 Log on sweet basil and Thai mint respectively, comparable to sodium hypochlorite alone (Klintham *et al*., 2018). Furthermore, the authors reported that following treatments, all of the leafy vegetables appeared to be fresh and green, emphasising that the MBs-assisted sanitisation did not compromise the visual quality or freshness of both the sweet basil and Thai mint. Additionally, Zhang and Tikekar (2021), found that following a 5-min treatment of MBs combined with 100 mg/L NaOCl treatment resulted in bacterial reductions of 3.3, 0.8, and 1.0 log CFU/g on grape tomatoes, blueberries, and baby spinach, respectively. However, these reductions were not significantly different from those obtained using NaOCl alone ($P > 0.05$).

Ci *et al.* (2025) emphasised that MNBs produce reactive oxygen species, such as hydroxyl radicals, which disrupt bacterial cell membranes and facilitates microbial inactivation. This technique provides a disinfecting method that is both efficient and eco-friendly without leaving chemical residues common in hypochlorite treatments. While NaOCl is a widely used sanitiser, its application may induce higher oxidative stress and increase respiration rates, accelerating senescence as observed in this study. Moreover, chlorinated water treatments are constrained by drawbacks such as interactions with organic matter, chemical residues and environmental impact concerns (Fraisse *et al*., 2011).

Tap water exhibited negligible antimicrobial effects, consistent with produce-washing studies by Van Haute *et al.* (2013) and Romanovski *et al.* (2020) reinforcing its role as a negative control. Furthermore, the exponential CO_2 rise in NaOCl treated samples after Day 2 suggests microbial proliferation due to the yeasts being capable to grow in the presence of high CO_2 as well as the anoxic conditions which led to fermentation at the end of storage (Caleb *et al.,* 2016; Guadalupe-Daqui *et al.,* 2023). All

treatment conditions used in this study were effective in keeping aerobic mesophilic bacteria and yeast and moulds below the maximum permissible limit of 7 log CFU mL^{-1} and 5 log CFU mL^{-1} after 8 days according to the FCD Act 54 of 1979 in South Africa (Figure 3.5A, B). These findings corroborate similar outcomes in green leafy vegetable studies where MNB treatments provide effective microbial control along with quality retention and longer shelf life, positioning MNB as a promising sustainable alternative to traditional chemical sanitisers.

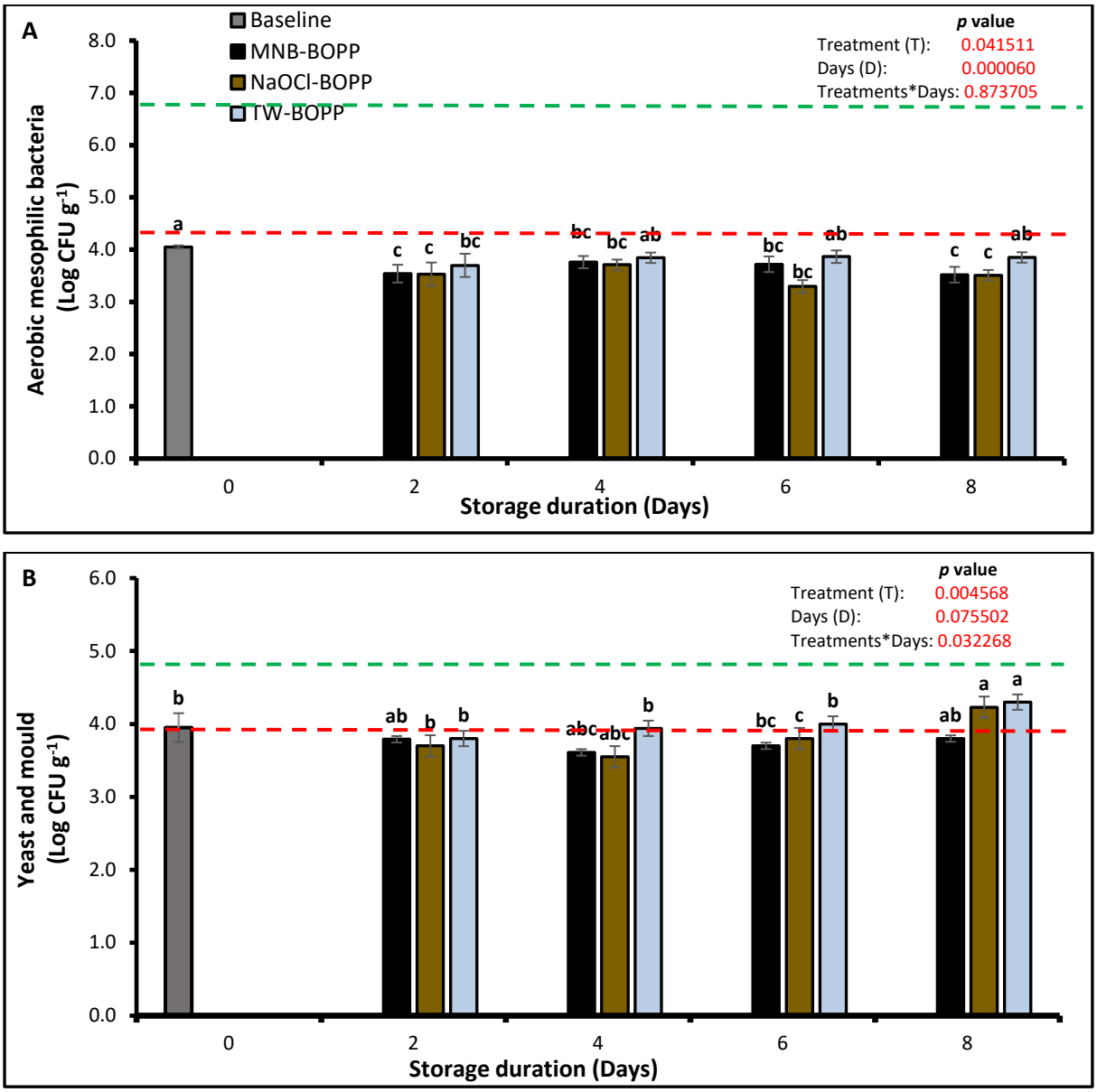

Figure 3.5: Effect of pre-treatment and MAP on **(A)** aerobic mesophilic bacteria and **(B)** yeast and mould counts of packaged Swiss chard leaves (cv. Fordhook Giant) pre-treated with air-MNB, NaOCl and TW, and stored at 5°C for 8 days. Mean value (n = 3) with standard deviation. Continuous red dashed line indicates baseline measurement. Continuous green dashed line indicates the maximum allowable aerobic mesophilic bacterial count (7 log CFU mL^{-1}) and yeast and mould (5 log CFU mL^{-1}). Pre-treatment abbreviations (MNB-BOPP, NaOCl-BOPP, and TW-BOPP) are listed in Table 3.1.

3.4 Conclusion

This study investigated the effects of air-micro-nanobubble (MNB), sodium hypochlorite (NaOCl) and tap water (TW) pre-treatments on the overall quality of fresh Swish chard packed in BOPP and stored at 5 °C for 8 days. Air-MNB-BOPP significantly reduced weight loss while maintaining chlorophyll levels and leaf colour attributes. Furthermore, there was no significant difference in decontamination efficacy of air-MNB and NaOCl in aerobic mesophilic bacteria at the end of storage. However, air-MNB were more effective in reducing yeast and mould counts compared to the other pre-treatments. These findings highlight air-MNB-BOPP as a promising method for extending the shelf life of Swiss chard by mitigating physiological deterioration when compared to NaOCl and TW pre-treatments. Therefore, further research that integrates proteomics and targeted enzymatic assays could substantially advance understanding of Swiss chard's biochemical responses to MNBs.

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CHAPTER 4

EFFECTS OF OZONE MICRO-NANOBUBBLE WATER TREATMENTS ON PHYSIOLOGICAL RESPONSES, PHYSICOCHEMICAL ATTRIBUTES AND PHYTOCHEMICAL COMPOSITION OF FRESH-CUT SWISS CHARD (*BETA VULGARIS* L. CV. FORDHOOK GIANT)

Swiss chard (*Beta vulgaris* L.) undergoes rapid postharvest quality deterioration due to high metabolic activity, tissue damage, moisture loss and microbial proliferation. This study evaluated the effects of ozone micro-nanobubble (O_3-MNB) water treatments on the physiological responses, physicochemical attributes, phytochemical composition, volatile organic compounds, microbial load, and overall postharvest quality of minimally processed Swiss chard (*Beta vulgaris* L. cv. Fordhook Giant) during 15 days of storage at 5 °C. O_3-MNB treatments effectively reduced weight loss, preserved leaf colour, and inhibited ethylene production and respiration compared to tap water controls. While total soluble solids and titratable acidity decreased across all treatments, they were better retained in control samples. Total phenolic compounds increased significantly in O_3-MNB-treated samples during early storage, suggesting an induced antioxidant response. Moreover, microbial loads of aerobic mesophilic bacteria, yeasts, and moulds were significantly reduced by O_3-MNB treatments, with the 10-min exposure providing the greatest microbial inhibition throughout storage. These findings demonstrated that O_3-MNB water treatment is a promising and sustainable sanitisation technology capable of extending shelf life and maintaining the quality of minimally processed leafy vegetables by reducing microbial growth and physiological deterioration while preserving nutritional and sensory attributes.

Keywords: minimally processed, oxidative stress, reactive oxygen species, sensory quality, weight loss

4.1 Introduction

Fresh-cut leafy vegetables (FCLVs) have gained increasing popularity due to their convenience, high nutritional value and contribution to healthy diets (Raffo and Paoletti, 2022). Among these, Swiss chard (*Beta vulgaris* L.) is particularly valued for its abundance of essential mineral, vitamins and antioxidant compounds. However, Swiss chard, like other FCLVs exhibit high metabolic activity and complex cellular structures, which make it highly susceptible to rapid physiological deterioration during postharvest handling and storage (Grzegorzewska *et al*., 2024). In addition, the processing steps involved in producing fresh-cut Swiss chard, such as cutting, causes tissue injury that disrupts natural protective barrier and increases the risk of microbial contamination (Fufa, 2021; Shezi *et al*.,

2024). Pathogenic microorganisms such as, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella* species can be introduced through contaminated water wash, processing equipment's or contact surfaces (Petri *et al*., 2015; Doukaki *et al*., 2024).

To mitigate these risks, chlorine-based sanitisers have been widely used due to their ability to efficiently eliminate microorganisms, and its cost effectiveness accounts for its success in commercial applications (Fattahi-Zaim *et al*., 2025). Chlorine based sanitisers are applied at concentrations ranging 50 to 200 mg L^{-1} for effective disinfection (Nyamende *et al*., 2022). However, chlorine is widely criticised because it generates potentially harmful by-products that could negatively impact human health and the environment (Belay *et al*., 2021). These disinfection by-products (DBPs) include: Trihalomethanes, Chlorite and Chlorate, Haloacetic acids, Haloacetaldehydes, Haloacetonitriles and Haloketones (Parveen *et al*., 2022; Fattahi-Zaim *et al*., 2025). Moreover, there are concerns about the growing regulatory constraints, safety protocols and barriers associated with using chlorine in its current form (Nyamende *et al*. 2022). Recently, alternative sanitisation methods, including micro-nanobubbles (MNBs) technologies has been emerging as effective interventions for reducing microbial load while maintaining the quality of whole and fresh-cut fruits and vegetables (FCFVs) (Botondi *et al*., 2021; Gonçalves-Magalhães *et al*., 2025).

Among these, ozone micro-nano bubbles (O_3-MNBs) have received particular attention as a novel disinfection approach (Shi *et al*., 2023). When ozone is applied through submersion in water, its antibacterial properties are enhanced, particularly when combined with microbubbles in the 1 to 100 µm range (and especially below 50 µm) (Gonçalves-Magalhães *et al*., 2025). In addition, the high dissolution efficiency and collapse dynamics of MNBs facilitate the breakdown of ozone into reactive oxygen species. This process generates hydroxyl radicals (·OH), thereby amplifying the oxidative disinfection potential of the treatment (Pal and Kioka, 2025). Recent studies have demonstrated the effectiveness of O_3-MNB treatments in preserving postharvest quality various leafy vegetables.

For instance, in a study by Xueqing *et al*. (2020), spinach treated with 4 mg/L O_3-MNBs and distilled water (control) for 5 minutes and stored at 20 ± 1 °C for 8 days, demonstrated significant enhancement of the physiological parameters. The treatment preserved membrane integrity, reduced malondialdehyde (MDA) accumulation, minimized cellular damage, inhibited respiration and ethylene production, and ultimately delayed senescence while preserving nutrient content. Similarly, Shi *et al*. (2023) demonstrated that parsley treated with 2.5 mg·L^{-1} O_3-MNBs and distilled water (control) for 10 minutes and stored for 5 days 20 ± 1 °C, retained better sensory quality and exhibited enhanced level of antioxidant compounds and antioxidant enzyme activity. More recently, da Silva *et al*. (2025)

investigated the effects of several sanitisation treatments on lettuce, including exposure to O_3-MNBs for (5 and 10 minutes), a 15-min chlorine wash and untreated control stored at 5 °C with 87% RH for 6 days. The study found that the 10-min O_3-MNBs reduced total aerobic mesophilic (TAMB) by 1.59 log CFU g^{-1} from the initial value of 6.37 log CFU g^{-1}. Furthermore, by the end of storage duration the 5-min O_3-MNBs resulted in a log reduction of 0.56 CFU g^{-1} for yeast and moulds from a baseline of 4.68 log CFU g^{-1}.

Despite the increased interest in MNB technology for postharvest application, its potential in preserving the quality of fresh cut Swiss chard remains largely unexplored. To date, no study has investigated its effects on the physiological responses, phytonutrient retention, volatile organic compounds (VOCs), and microbial quality of Swiss chard during storage. Therefore, this study aimed to evaluate the effects of O_3-MNBs on physiological responses, phytonutrients, VOCs and microbial load in fresh cut packed Swiss chard during 15 days of storage at 5°C.

4.2 Materials and Methods

4.2.1 Plant material

Fresh Swiss chard (*Beta vulgaris*), (cv. Fordhook Giant), was purchased at commercial maturity from Agri-Hub, located at the Cape Peninsula University of Technology, Wellington Campus, Western Cape, South Africa. The produce was transported under refrigerated conditions (4 °C) to the Postharvest and Agro-Processing Pilot Plant at the Agricultural Research Council (ARC), Infrutec-Nietvoorbij, Stellenbosch. Upon arrival, the chard was sorted to ensure uniformity in size, colour, and overall appearance. Only visually healthy leaves were selected and subsequently stored at 5°C with 95% RH. All handling utensils were thoroughly sterilised with 70% ethanol before use.

4.2.2 Ozone micro-nano bubble water preparation

O_3-MNBs were generated by connecting an ozone gas cylinder to an MNB generator. Ozone gas was introduced into fresh tap water, which was continuously recirculated with a pump for 2h. The Mk4-Nanobubbler system (MK4, Fine bubble technologies Co., Ltd., South Africa) facilitated ozone infusion by drawing gas through its suction tube into the circulating tap water, resulting in O_3-MNB water. Following generation, the pH and oxidation-reduction potential (ORP) of both O_3-MNB water and un-treated tap water were measured using an HI 98121 pH-ORP meter (Hanna Instruments, Cape Town, South Africa).

4.2.3 Swiss chard preparation and treatment

Prior to treatment, any damaged or yellow leaves and stems were removed from the Swiss chard. The remaining leaves were then cut into uniform samples (4cm x 4cm) using a sterilised knife. The samples were then randomly divided into two pre-treatment groups, the O_3-MNB and tap water (TW) groups as control. The O_3-MNB batch was treated for 5 (MNB1), 10 (MBN2), and 15 min (MNB3), while TW batch was treated for 5 min, after which all samples were air-dried at room temperature (20 °C). Following drying, fresh-cut leaves (150 g) were manually packaged in bi-axially oriented polypropylene film (BOPP) with OTR rate of 8.5×10^{-12} mol m^{-2} s^{-1} Pa^{-1} at 23 °C and 0% RH and film thickness of 25 µm and heat sealed with a packaging machine (KS-F 600, Vala Impulse sealer, South Africa) for 2 sec. Samples were then stored at 5 °C and 9 ± 2% RH for 15 days. Sampling was done in triplicate on days 0, 3, 6, 9, 12, and 15 for quality evaluation.

Table 4.1: Summary of treatments and abbreviations used in the study.

Treatment medium	pH	ORP (mV)	Dipping duration (min)	Abbreviation
Tap water (control)	7.80	656	5	TW
Ozone micro-nano bubble water	8.22	677	5	MNB1
Ozone micro-nano bubble water	8.22	677	10	MNB2
Ozone micro-nano bubble water	8.22	677	15	MNB3

TW = Tap water control treatment, applied for 5 min; MNB1–MNB3 = Ozone micro-nano bubble water (O_3-MNB) treatments differing only in dipping duration (5, 10, and 15 min, respectively); ORP = Oxidation–reduction potential.

4.2.4 Physicochemical properties

4.2.4.1 Determination of weight loss

Weight loss was determined following the method by Oliveira *et al*. (2016). Fresh cut, packaged Swiss chard leaves (n = 12) were weighed using an electronic balance (FTA20, Güss, South Africa) with an accuracy of ± 0.01 g. The overall weight loss was calculated as the difference between the initial weight and final weights during storage. The average weight loss was calculated using Eq. (1):

$$WL = \frac{Wo - Wf}{Wo} \times 100 \quad (1)$$

where, WL is the weight loss (%); W_o is the initial (g) and W_f is the final weight (g) of minimally processed packaged chard leaves.

4.2.4.2 Determination of leaf colour

The colour of Swiss chard leaves was measured using a handheld digital CR 400/410 Chroma Meter (Konica Minolta Sensing Inc., Tokyo, Japan) based on the Commission International del'Eclairage (CIE) colour system Islam *et al*. (2019). Prior to measurement, the instrument was calibrated against a white tile. The individual colour parameter changes were monitored and reported as L*, a*, and b* respectively. Measurements were taken at the opposite sides of three leaves per treatment (n = 18). The h° and the chroma (C*) angles were also calculated using Eq. (2) and Eq. (3).

$$Hue\ (h^o) = \ tan^{-1}\frac{b^*}{a^*} \quad (2)$$

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (3)$$

4.2.4.3 Measurement of total soluble solids (TSS) and titratable acidity (TA)

TSS and TA were measured following the method by Karim and Yusof (2021). On each sampling day, juice samples from Swiss chard leaves were obtained using a Braun 4294 J700 juice extractor. A calibrated pocket-sized PAL-1 refractometer (Atago) was used to measure TSS, which was represented as °Brix. Titratable acidity (TA, malic acid) was measured using a Crison Titromatic 1S/2B autotitrator (Crison Instruments). Juice samples (about 53.7 mL) were titrated with NaOH (0.333 N) to achieve a pH of 8.2. TA was expressed as g 100 mL^{-1}.

4.2.5 Physiological responses

4.2.5.1 Gas composition evaluation

The concentrations of carbon dioxide (CO_2) and oxygen (O_2) within the package headspace were measured using a headspace gas analyser (Dansensor CheckPoint 3, PBI, Denmark), on each sampling day. This was achieved by inserting a gas-tight needle through a septum positioned on the surface of the package. To measure the exchange of gases within the package the mass balance equations 4 and 5 were applied (Belay *et al*., 2018).

$$\frac{\partial Y_{O_2}}{\partial_t} = \frac{\frac{A_P P_{O_2} P_{atm}}{L}\left[Y_{O_{2i}} - Y_{O_{2f}}\right]}{V} - W \quad (4)$$

$$\frac{\partial Y_{CO_2}}{\partial_t} = \frac{\frac{A_P P_{O_2} P_{atm}}{L}\left[Y_{CO_2 f} - Y_{CO_{2i}}\right]}{V} - W \quad (5)$$

where, temporal changes in oxygen (O_2) and carbon dioxide (CO_2) concentrations are represented by $\frac{\partial Y_{O_2}}{\partial t}$ and $\frac{\partial Y_{CO_2}}{\partial t}$. P_{O_2} represents permeability to O_2, P_{atm} is the atmospheric pressure, A_P is the surface area of the package, thickness of the packaging film is represented by L and W is weight of the chard leaves while the free volume within the package is represented by V. Initial concentrations of oxygen and carbon dioxide are denoted by $Y_{O_2 i}$ and$Y_{CO_2 i}$, while their final concentrations are indicated by $Y_{O_2 f}$and$Y_{CO_2 f}$, respectively.

4.2.5.2 Ethylene determination

The ethylene concentration was determined using an ICA 56-ethylene analyser (International Control Analyser) at regular intervals. The amount of ethylene produced per unit mass of the packaged chard leaves per unit time (µL/kg h) was used to determine the ethylene production rate.

4.2.6 Phytochemical properties

4.2.6.1 Chlorophyll content measurement

Chlorophyll content of minimally processed Swiss chard was determined following the ESS method 150. 1 (1991). Each sampling day, a 1 cm^2 portion of chard leaves was used for extraction. Samples were ground with 0.2 mL of $MgCO_3$ to prevent degradation, and the homogenate was mixed with 10 mL of 90% aqueous acetone. Aliquots were put into centrifuge tubes, filled with acetone solution to a volume of 13 mL, vortexed, and then placed back in the dark box to be stored at 4 °C for the night. On the following day, centrifugation at 500 rpm for 20 minutes was used to clarify the extract, and a nano-photometer (N60, Implen, Germany) was used to measure absorbance at 663, 645 and 630nm using aqueous acetone as a blank. Eqs. (5) and (6) by Tang *et al*. (2023) were used when calculating the chlorophyll contents (a and b), whereas Eq. (7) by Tang *et al*. (2022) was used to calculate the total chlorophyll.

$$Chlorophyll\ a = (11.64\ A_{663} - 2.16A_{645} + 0.10A_{630})v/IV \quad (6)$$

$$Chlorophyll\ b = (20.97\ A_{645} - 3.94A_{663} - 3.66A_{630})v/IV \quad (7)$$

$$Total\ Chlorophyll = (12.25\ A_{663} - 2.79A_{645}) + (21.5A_{645} - 5.1A_{663}) \quad (8)$$

where, A_{663} is the absorption value at 663 nm and A_{645} is absorption value at 645 nm

4.2.6.2 Total phenolic compounds (TPC) quantification

The total phenolic content (TPC) was quantified using the Folin–Ciocalteu (FoC) assay, following a slightly modified method by Tolcha and Oanh (2024). Extracts were combined with the FoC reagent and sodium carbonate solution in a shake incubator (Solab, SL 222, Piracicaba, Brazil). The mixture was centrifuged at 5000 rpm for 10 min at 25 °C. The reaction was allowed to proceed in the dark at approximately 20 °C for 2h. Absorbance was measured at 765 nm using a microplate spectrophotometer (Čeryová *et al*., 2025). TPC was calculated from a gallic acid standard curve and expressed as mg GAE L^{-1}.

4.2.7 Volatile organic compounds (VOCs) determination

Volatile organic compounds (VOCs) were analysed following the method by Belay *et al*. (2025). Samples were thawed overnight at room temperature, and approximately 5 mL of Swiss chard juice was placed into 20 mL solid-phase micro-extraction (SPME) vials. To inhibit enzymatic degradation and facilitate the movement of volatiles into the headspace, Swiss chard aliquots were mixed in equal parts with 30% NaCl. Using SPME, the aroma volatiles were captured and extracted from the vial headspaces. After allowing the vials to equilibrate for 5 min at 50°C in the CTC autosampler incubator, a 50/30 µm coated fibre with divinylbenzene, carboxylase, and polydimethylsiloxane were exposed to the headspace for 10 min at 50°C. Following extraction, the gas chromatography-mass spectrometry (GC-MS) injection port was used to desorb the volatile compounds from the fibre coating for 10 min. The injection temperature was maintained at 250°C.

Volatile compounds were separated using an Agilent 6890 N gas chromatograph (Agilent, Palo Alto, CA) coupled to an Agilent 5975 mass spectrometer. The GC-MS system was equipped with an Rtx®-5Sil MS column (30 m × 0.25 mm × 0.5 µm). Helium was used as the carrier gas at a rate of 1 mL min^{-1}. The oven temperature was programmed from 40°C (held for 6 min) to 260°C at 8°C min^{-1}, with a final hold for 3 min. The ion source and quadrupole temperature were maintained at 230°C and 150°C, respectively, and the transfer line was set at 280°C. Mass spectrometry data were acquired in full scan mode. Compounds were identified by comparing their retention index (RI) and retention time (RT) to those listed with the National Institute of Standards and Technology (NIST v.05, Gaithersburg, MD, USA).

4.2.8 Microbial load determination

Total viable aerobic mesophilic bacteria and yeasts and moulds were measured using a slightly modified method defined by Degaga *et al*. (2022). Following submerging in a sterile saline solution, the chard leaves were gently vortexed for 30 min. Subsequently, serial dilutions of the resulting suspension were prepared using saline solution. Aliquots of 1 mL from each dilution (10^{-1}–10^{-3}) were pipetted and spread onto a pre-solidified standard plate count agar (PCA) media for bacteria and potato dextrose agar (PDA) for yeasts and moulds. The PCA plates were incubated for 2 days at 37 °C while PDA plates were incubated at 28 °C for 3 to 5 days. Following incubation, colony enumeration was conducted and the total number of colony-forming units (CFU), was expressed as Log CFU/cm^{-2}.

4.2.9 Statistical analysis

The experiment followed a two-factor completely randomised design (CRD), with randomised sampling at consistent intervals. A two-way ANOVA was carried out with Statistica Software (version 13; StatSoft Inc., TIBCO Software Inc., USA) to analyse the impact of treatment and storage duration. Where significant differences were observed, Duncan's multiple range test was applied for pairwise comparisons between mean values at a significance threshold of $p \leq 0.05$. All treatments were conducted in triplicate, and results were presented as means with corresponding standard deviations.

4.3 Results and discussion

4.3.1 Weight loss

Both MNB-treated and control samples of fresh-cut Swiss chard showed progressive weight loss over the storage duration, however, the weight loss in the control group was significantly higher (Figure 4. 1). The highest weight loss observed in both treated and control groups could be due to the tissue damage and consecutive effects due to the cutting during processing. Swiss chard under control samples (washed with tap water) lost 15.50% of their weight by day 6 of storage as compared to 9.08% MNB1, 11.07% MNB2 and 12.90% MNB3 of O_3-MNBs samples, respectively. Furthermore, by the end of storage (day 15), TW samples showed the highest weight loss (32.82%), followed by MNB3 (30.12 %), MNB2 (23.71%) and MNB1 with the lowest weight loss (21.95 %) (Figure 4.1). The significant weight loss observed in TW samples could be due to the absence of preservation chemicals, which often assist in lowering transpiration and inhibiting metabolic processes (Quansah *et al*., 2022).

Additionally, the variation in weight loss among O_3-MNBs pre-treatments indicates an interaction between beneficial effects and possible ozone-induced stress. For instance, short exposure (5-10 min) of O_3-MNBs significantly reduced oxidative stress without causing tissue damage on the delicate leaves (Liu *et al*., 2021; Shi *et al*., 2023). This resulted in better water retention leading to minimal weight loss. However prolonged exposure (15 min) may have compromised epidermal cells or induced mild oxidative stress, resulting in increased permeability and transpiration, hence enhancing water loss (da Silva *et al*., 2025; Sitoe *et al*., 2025).

The results obtained for both the O_3-MNBs and the TW (control) treatments during the storage period are consistent with findings from previous studies. Shi *et al*. (2023) reported that O_3-MNB treatment reduced weight loss in parsley when compared to distilled water control samples. In contrast, Liu *et al*. (2020) observed minimal weight loss in fresh-cut cabbage pre-treated with distilled water for 5 minutes, compared to samples treated with 1.4 m/L aqueous ozone for 1, 5 or 10 min and stored at 4 ± 1°C with 90% RH for 12 days. However, the authors reported that the difference was not statistically significant. MNBs have high surface area and reactive properties which are known for improving water retention and lowering microbial spoilage (Pal and Kioka, 2025). Furthermore, in this study, O_3-MNBs treatments mitigated weight loss compared to TW washing by inducing antimicrobial effects which delay transpiration rate and decay (Liu *et al*., 2021; da Silva *et al*., 2025).

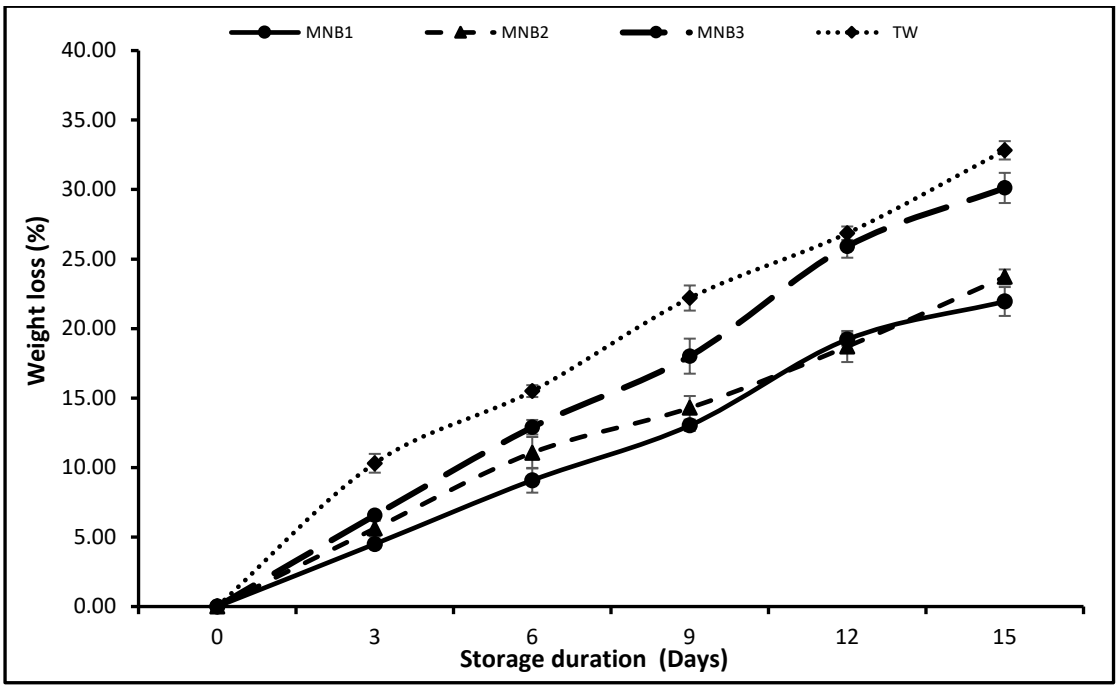

Figure 4.1: Effect of ozone micro-nanobubble water (O_3-MNB) and tap water (TW) treatments on the weight loss of packed fresh cut Swiss chard (cv. Fordhook Giant) during storage at 5 °C for 15 days. Values are means ± standard deviation (n = 3). Treatment abbreviations (MNB1, MNB2, MNB3, and TW) are defined in Table 4.1.

4.3.2 Swiss chard colour

Table 4.2 presents the effects of MNB and control treatment on the instrumental colour parameters of Swiss chard. The results showed that the *L** values fluctuated across all treatments as compared to the initial day 0 value (39.83 ± 4.35). Significantly highest *L** value (46.22 ± 2.94) was observed for MNB3 on day 15 and based on colorimeter measurements, these results suggest that the 15-min O_3-MNB treatment may have partially bleached the Swiss chard leaves (Hou *et al*., 2022). The control samples TW demonstrated higher retention of *L** value (40.47 ± 5.27), followed by 5 min of O_3-MNB treatment (41.02 ± 3.13) suggesting enhanced preservation of lightness and appearance. A similar observation was reported by Ikeura *et al*. (2013), where there were no significant differences in the $L*$ value of either the red or green persimmon leaves treated with O_3-MNBs and dechlorinated water (control) for 15 min.

Comparing the results, TW pre-treated samples exhibited the most stable *a** values throughout the storage period and reached at −13.33 ± 0.80 by day 15 (Table 4.2). This minimal fluctuation indicated that rinsing with TW could be sufficient to maintain green colour in the short term. Both 5- and 10-min MNB treatments followed a similar trend, with the *a** values on day 3 reached at −15.26 ± 1.43 and at −15.25 ± 1.22 for 5 min and for 10 min, respectively. By day 15, a slight decline was observed and the *a** values reached at −14.13 ± 1.03 and at −14.46 ± 0.70 for MNB1 and MNB2, respectively. MNB2 showed slightly better retention of green colour than MNB1, possibly due to more effective microbial suppression (Klintham *et al*., 2017). Additionally, MNB3 initially followed the same greening trend however ended with the highest *a** value −12.86, indicating the loss of green colouration, consistent with its corresponding *L** values.

The *b** values on the other hand, generally increased over time, particularly under MNB3 (26.93 ± 2.33) by day 15, which may reflect natural yellowing of the Swiss chard. Moreover, both MNB1 and TW control samples maintained relatively stable *b** values over the storage duration, suggesting better control over pigment degradation. The higher *b** values in green vegetables can be associated with the degradation of chlorophyll as well as senescence (Gutarowska *et al*., 2023). In contrast to Xueqing *et al*. (2020), the trend of *b** values observed in fresh-cut chard leaves pre-treated with O_3-MNBs differed notably from those treated with TW. The authors found that spinach treated with O_3-MNBs had lower *b** values compared to samples treated with distilled water and stored for 8 days at (20 ± 1) °C. This variation could be attributed to O_3 concentration, exposure time and water properties which can influence the oxidative impact.

By day 15, the *C** was best retained by O_3-MNBs as compared to the TW control samples indicating vibrant colouration. In addition, Hue remained relatively stable across treatments throughout the storage duration. However, MNB3 showed the highest decline of hue angle by day 15, recording a value of 115. 63 ± 2.56 suggesting a noticeable shift in colour. In contrast, MNB1 maintained the highest hue angles (120–122°) throughout the storage duration which aligns with its *L** values. Similar observation was reported by Mersinli *et al.* (2021) where higher doses of ozonated water (2 ppm) applied on spinach resulted in low hue angles after 25 days of storage.

The current study showed that pre-treating of fresh-cut Swiss chard (cv. Fordhook Giant) with O_3-MNBs for 5 or 10 min was more effective than using 5 min TW in maintaining the visual quality of the chard throughout storage (Figure 4.2). Higher hue angles, slower rises in b* and lower *L** values all indicate that these treatments significantly decreased colour alterations. However, exposure time turned out to be a crucial component: longer exposure (15 min) showed adverse reactions, whereas shorter treatments duration (5-10 min) encouraged the retention of green colour. The enhanced colour preservation observed with O_3-MNB treatments can be attributed to their ability to accelerate oxidative degradation of surface-bound contaminants via ROS, as reported by Xueqing *et al*. (2020).

Table 4.2: Physicochemical attributes of fresh cut Swiss chard (Beta vulgaris cv. Fordhook Giant) after ozone micro-nano bubble water (O3-MNB) pre-treatments during at 5°C and 90 ± 2% RH for 15 days.

			Pre-treatments and MAP			
Parameters	**Storage days**		**MNB1**	**MNB2**	**MNB3**	**TW**
Lightness	0	39.83 ± 4.35^{ab}				
	3		39.56 ± 4.05^{ab}	37.28 ± 4.54^{ab}	36.78 ± 2.37^{b}	35.73 ± 4.97^{b}
	6		40.06 ± 2.28^{b}	42.50 ± 5.84^{ab}	39.20 ± 3.35^{b}	39.36 ± 5.31^{b}
	9		35.80 ± 6.21^{b}	39.42 ± 4.43^{a}	39.58 ± 1.45^{a}	40.82 ± 3.05^{a}
	12		41.08 ± 4.93^{ab}	39.24 ± 2.81^{b}	42.15 ±3.25^{ab}	40.68 ± 2.26^{ab}
	15		41.02 ± 3.13^{b}	44.82 ± 2.48^{a}	46.22 ± 2.94^{a}	40.47 ± 5.27^{b}
*a**	0	-13.93 ± 1.21^{a}				
	3		-15.26 ± 1.43^{a}	-15.25 ± 1.22^{a}	-13.70 ± 2.19^{c}	-14.09 ± 1.34^{b}
	6		-13.46 ± 1.03^{b}	-14.37 ± 1.47^{a}	-13.98 ± 1.07^{b}	-14.33 ± 1.08^{a}
	9		-13.09 ± 1.19^{a}	-13.80 ± 1.13^{a}	-13.91 ± 1.06^{a}	-13.38 ± 1.03^{a}
	12		-14.60 ± 1.88^{a}	-13.68 ± 1.01^{b}	-14.51 ± 1.05^{a}	-13.40 ± 0.91^{b}
	15		-14.13 ± 1.03^{a}	-14.46 ± 0.70^{a}	-12.86 ± 0.93^{c}	-13.33 ± 0.80^{b}
*b**	0	20.62 ± 1.25^{bc}				
	3		24.82 ± 3.22^{a}	24.27 ± 3.05^{a}	21.42 ± 2.46^{b}	21.64 ±2.45^{b}
	6		21.06 ± 3.72^{bc}	23.94 ± 3.19^{a}	22.06 ± 2.86^{b}	23.11 ±3.89^{a}
	9		19.74 ± 3.89^{b}	21.33 ± 4.69^{a}	21.10 ± 3.59^{a}	21.09 ±2.18^{a}
	12		23.20 ± 4.23^{a}	21.34 ± 2.06^{b}	23.62± 2.74^{a}	19.76 ± 3.20^{c}
	15		23.89 ± 2.18^{c}	25.64 ± 2.94^{b}	26.93 ± 2.33^{a}	23.67 ± 1.45^{c}
Hue	0	122.89 ± 3.19^{a}				
	3		122.08 ± 2.72^{a}	122.27 ± 2.31^{a}	122.58± 4.83^{a}	123.98 ± 2.09^{a}
	6		122.97 ± 3.71^{a}	121.11 ± 2.20^{a}	122.56± 2.96^{a}	122.11 ± 2.76^{a}
	9		123.98 ± 4.00^{a}	123.48 ± 4.26^{a}	123.77 ± 3.57^{a}	122.50 ± 2.71^{a}
	12		122.45 ± 2.36^{a}	122.74 ± 2.76^{a}	121.73 ± 3.18^{a}	123.79 ±2.09^{a}
	15		120.67 ± 2.04^{a}	119.60 ± 2.55^{a}	115.63 ± 2.56^{b}	119.39 ± 0.93^{a}
Chroma	0	24.17 ± 1.95^{a}				
	3		29.31 ± 3.08^{a}	28.68 ± 3.08^{b}	24.82 ± 1.92^{c}	25.84 ± 2.63^{c}
	6		25.02 ± 3.53^{b}	27.94 ± 3.35^{a}	26.14 ± 2.77^{a}	27.22 ± 3.83^{a}
	9		23.74± 3.74^{a}	25.46 ± 4.47^{a}	25.31± 3.44^{a}	24.99 ± 2.11^{a}
	12		27.43 ± 4.51^{a}	25.37± 1.95^{b}	27.76 ± 2.55^{a}	23.63 ± 3.34^{c}
	15		27.77 ± 2.20^{c}	29.46 ±2.75^{a}	29.87 ± 2.11^{a}	27.16 ± 1.60^{b}
TSS (°Brix)	0	5.90 ± 0.36^{a}				
	3		4.50 ± 0.17^{b}	5.10 ± 0.30^{c}	5.80 ± 0.30^{a}	5.30 ± 0.50^{c}
	6		4.80 ± 0.30^{c}	5.20 ± 0.20^{d}	5.40 ± 0.40^{b}	5.70 ± 0.20^{a}
	9		5.10 ± 0.30^{a}	4.30 ± 0.30^{d}	4.60 ± 0.20^{c}	4.70 ± 0.70^{b}

	12		5.80 ± 0.30^{a}	5.00 ± 0.25^{d}	5.10 ± 0.36^{c}	5.60 ± 0.44^{b}
	15		4.50 ± 1.15^{b}	4.60 ± 0.46^{b}	4.70 ± 0.56^{a}	4.80 ± 0.95^{a}
TA (malic	0	1.05 ± 0.09^{a}				
acid, g 100	3		0.75 ± 0.25^{bc}	0.69 ± 0.04^{a}	0.84 ± 0.04^{a}	0.86 ± 0.06^{a}
mL^{-1})	6		0.77 ± 0.07^{d}	0.85 ± 0.05^{b}	0.83 ± 0.13^{c}	0.93 ± 0.03^{a}
	9		0.68 ± 0.08^{a}	0.63 ± 0.08^{b}	0.64 ± 0.14^{b}	0.67 ± 0.07^{a}
	12		0.85 ± 0.13^{b}	0.84 ± 0.05^{b}	0.77 ± 0.06^{c}	0.93 ± 0.04^{a}
	15		0.84 ± 0.14^{a}	0.79 ± 0.19^{b}	0.69 ± 0.29^{b}	0.87 ± 0.09^{a}

Treatment abbreviations (MNB1 MNB2, MNB3 and TW) are defined in Table 4.1. Values are means ± standard deviation (n = 3). Means with the same letter are not significantly different ($p \leq 0.05$).

Figure 4.2: Visual appearance and colour changes of fresh cut Swiss chard (cv. Fordhook Giant) leaves pre-treated with ozone micro-nanobubble water (O_3-MNB) or tap water (TW), packaged and unpackaged, during storage at 5 °C for 15 days. Treatment abbreviations (MNB1 MNB2, MNB3 and TW) are defined in Table 4.1.

4.3.3 Total soluble solids and titratable acids

Table 4.2 presents the TSS values of treated and control Swiss chard during storage. The TSS values varied throughout the storage duration of the study (Table 4.2) however, it consistently decreased from the initial value of 5.90 ± 0.365 by day 15 across all treatments, suggesting gradual alterations in the physicochemical characteristics throughout storage (Mersinli *et al*., 2021). At the end of storage duration TW pre-treated samples retained the highest °Brix level 4.80 ± 0.95, indicating better preservation of sugars. In contrast, MNB1 showed a reduction to 4.50 ± 0.30, which may be due to metabolic activity of fresh-cut Swiss chard or moisture loss (Mersinli *et al*., 2021). This trend aligns with findings from other studies on O_3-MNBs and ozonated water. Shi *et al*. (2023) reported a higher TSS level on the distilled water control samples as compared to O_3-MNBs treated samples. Similarly, observation of decline in TSS contents of spinach treated with various concentration of ozonated water (0.5, 1 and 2ppm) then stored at 0 °C with 90 ± 5% RH for 25 days was also reported by Mersinli *et al*. (2021). In contradiction to our study, da Silva *et al*. (2025) reported an increase of TSS contents on lettuce treated with 5 and 10 min O_3-MNBs and stored at 5 °C with 80% RH for 6 days. The observed decrease in TSS content observed in this study can be attributed to sugar conversion or elevated respiration rates within the packaging environment (Shi *et al*., 2023).

TA declined initially across all treatments, consistent with expected metabolic shifts of chard leaves during storage (Schvambach *et al*., 2020). TW pre-treated samples retained acidity most effectively (0.87 ± 0.09) by day 15, potentially contributing to flavour preservation. In contrast, the lowest TA content (0.69± 0.29) was recorded in fresh cut Swiss chard treated O_3-MNBs for 15 min. Schvambach *et al*. (2020), also reported a decrease in TA of lettuce leaves packaged in polypropylene film and then stored at 5 °C for 20 days. Furthermore, there was a decline of TA contents of lettuce treated with O_3-MNBs for 5 min for stored 6 days at 5 °C with 80% RH (da Silva *et al*., 2025). This reduction may be attributed to respiration which depletes organic acids, water loss, and the enzymatic degradation of starch into soluble sugars (Zhao *et al*., 2019; Botondi *et al*., 2021), factors commonly linked to a decrease in both TSS and TA concentrations during the storage duration of fresh-cut vegetables (Zhao *et al*., 2019; Botondi *et al*., 2021).

4.3.4 In-package gas composition and ethylene production rate

The O_2 and CO_2 concentrations were not significantly affected by either storage duration or pre-treatments during cold storage (Figure 4.3A, B). O_2 levels remained relatively stable across all treatments, fluctuating only slightly around the concentration of 20%, indicating that none of the treatments altered the internal O_2 environment (Figure 3A). The initial O_2 concentration (20 ± 0.08%) increased slightly to 20.2% in TW samples and 20.03% in MNB2 samples, while it decreased to 19.83% in MNB1 and 19.77% in MNB3 samples on day 3. By Day 9 of storage, all O_3-MNB samples exhibited a reduction in O_2 compared to the TW control. Specifically, MNB1, MNB2, and MNB3 recorded O_2 levels of 19.90%, 19.67%, and 19.80%, respectively, while TW samples maintained a higher concentration of 20.27%. This decline in oxygen among the O_3-MNB suggests a possible increase in respiration which may influence the physiological stability and shelf life of the packaged produce (Glowacz *et al*., 2015). By the end of storage duration, the O_2 concentrations were between 20.30 and 20.60% for all the samples.

The initial CO_2 concentration across all samples was 0.47 ± 0.05%. By Day 3 of storage, a slight increase in CO_2 levels was observed in all treatments. TW and MNB1 samples both reached 0.83%, indicating similar respiration activity. MNB2 showed a more moderate rise to 0.67%, while MNB3 exhibited the highest increase at 0.90%. By Day 9 of storage, MNB1, MNB3, and TW samples each recorded a CO_2 level of 0.43%, indicating a relatively stable respiration rate and minimal physiological stress. In contrast, MNB2 samples exhibited a higher CO_2 concentration of 0.67%. By the end of storage duration both MNB1 and TW samples maintained the lowest CO_2 concentration of 0.23%. The observed decrease in CO_2 concentration can be attributed to lower respiration rate (Medina *et al*., 2012).

In addition, the MNB2 and MNB3 recorded a 0.47% and 0.30% concentration of CO_2 respectively. Overall, the gas composition trends align with passive MAP behaviour, as supported by previous findings (Batziakas *et al*., 2020) and suggest that none of the treatments induced significant atmospheric modification.

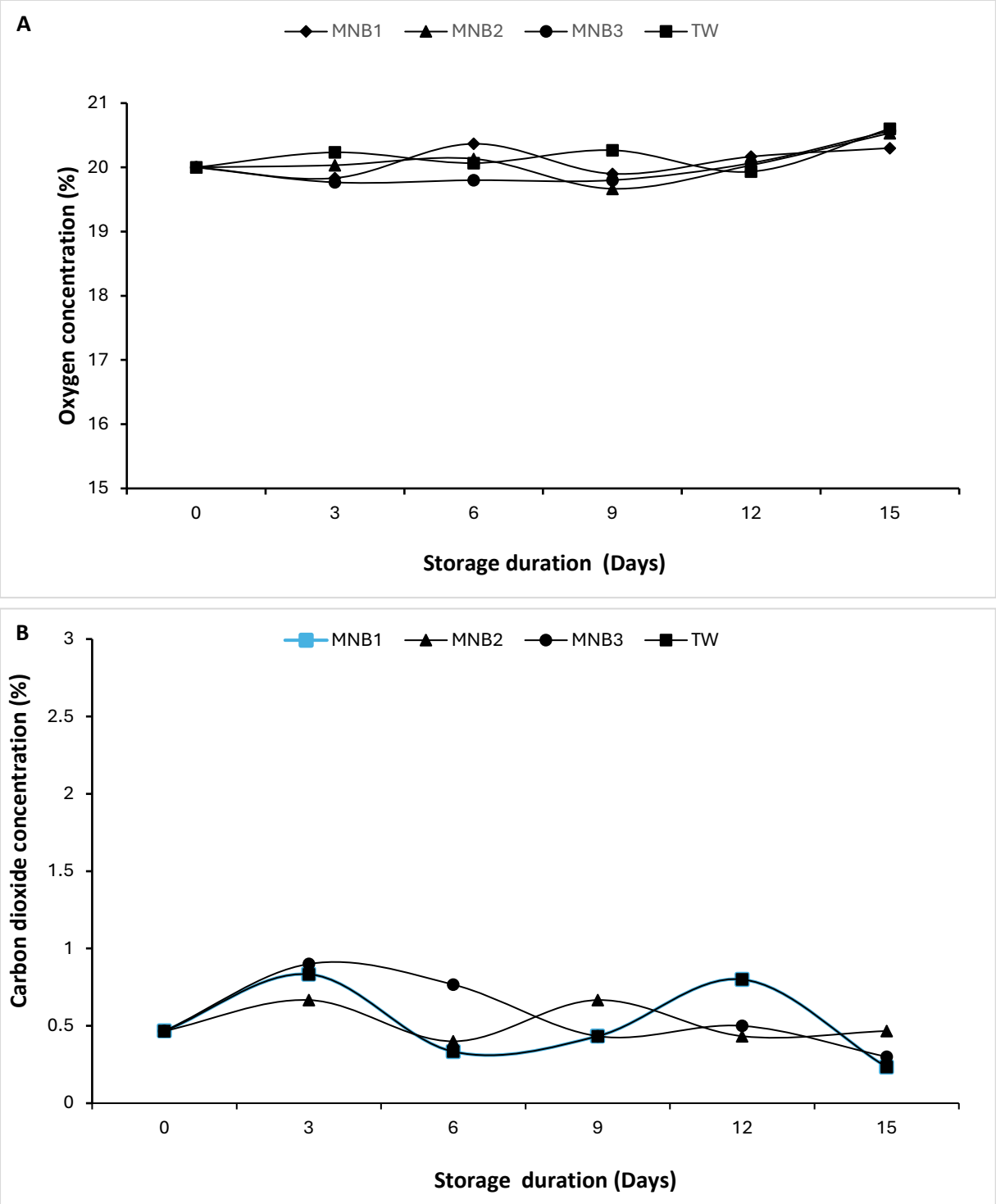

Figure 4.3: Effect of ozone micro-nanobubble (O_3-MNB) and tap water (TW) treatments on (A) O_2 concentration and (B) CO_2 concentration of fresh cut and packaged Swiss chard (cv. Fordhook giant) leaves stored at 5°C for 15 days. Values are means ± standard deviation (n = 3). Treatment abbreviations (MNB1, MNB2, MNB3, and TW) are defined in Table 4.1.

Over a 15-day storage period, the ethylene rates of fresh cut Swiss chard (cv. Fordhook Giant) differed considerably across treatments (MNB1, MNB2, MNB3, and TW) (Figure 4.4). The initial production rate of ethylene for fresh-cut processed Swiss chard was 0.51 µL kg^{-1} h^{-1}. However, there was a significant decline in ethylene production rates between day 3 and 6 of storage. MNB3 had the lowest ethylene production rates 0.05 to 0.10 µL kg^{-1} h^{-1} throughout the storage duration, while control samples increased on day 12 reaching 0.45 µL kg^{-1} h^{-1} and 0.44 µL kg^{-1} h^{-1} by day 15 respectively. By the end of storage duration MNB1 had the lowest ethylene production rates 0.07 µL kg^{-1} h^{-1}. It has been proven that mechanical damage caused by cutting or trimming leafy vegetables during fresh-cut processing increases the production of ethylene in the affected tissues (Koukounaras, 2009). However, it was demonstrated in our study that O_3-MNBs were able to reduce the ethylene production as compared to the control TW treatments.

Consistent with the findings by Xueqing *et al*. (2020) the authors observed that ethylene production in the control group (distilled water) remained consistently higher than in the O_3-MNBs treated spinach, with statistically significant differences evident between days 4 and 6 of storage. Moreover, Shi *et al*. (2023) also observed a lower concentration of ethylene on O_3-MNBs treated parsley. O_3-MNBs have been demonstrated to reduce ethylene levels, a crucial hormone that promotes cell respiration and induces senescence (Shi *et al*., 2023). By inhibiting mechanisms of cellular respiration, O_3-MNBs promote antioxidant stability and decelerate the degradation of cellular structure ultimately extending shelf life furthermore, ozone's strong oxidative properties suppress ethylene biosynthesis by inactivating key enzymes involved in its production (Liu *et al*., 2021).

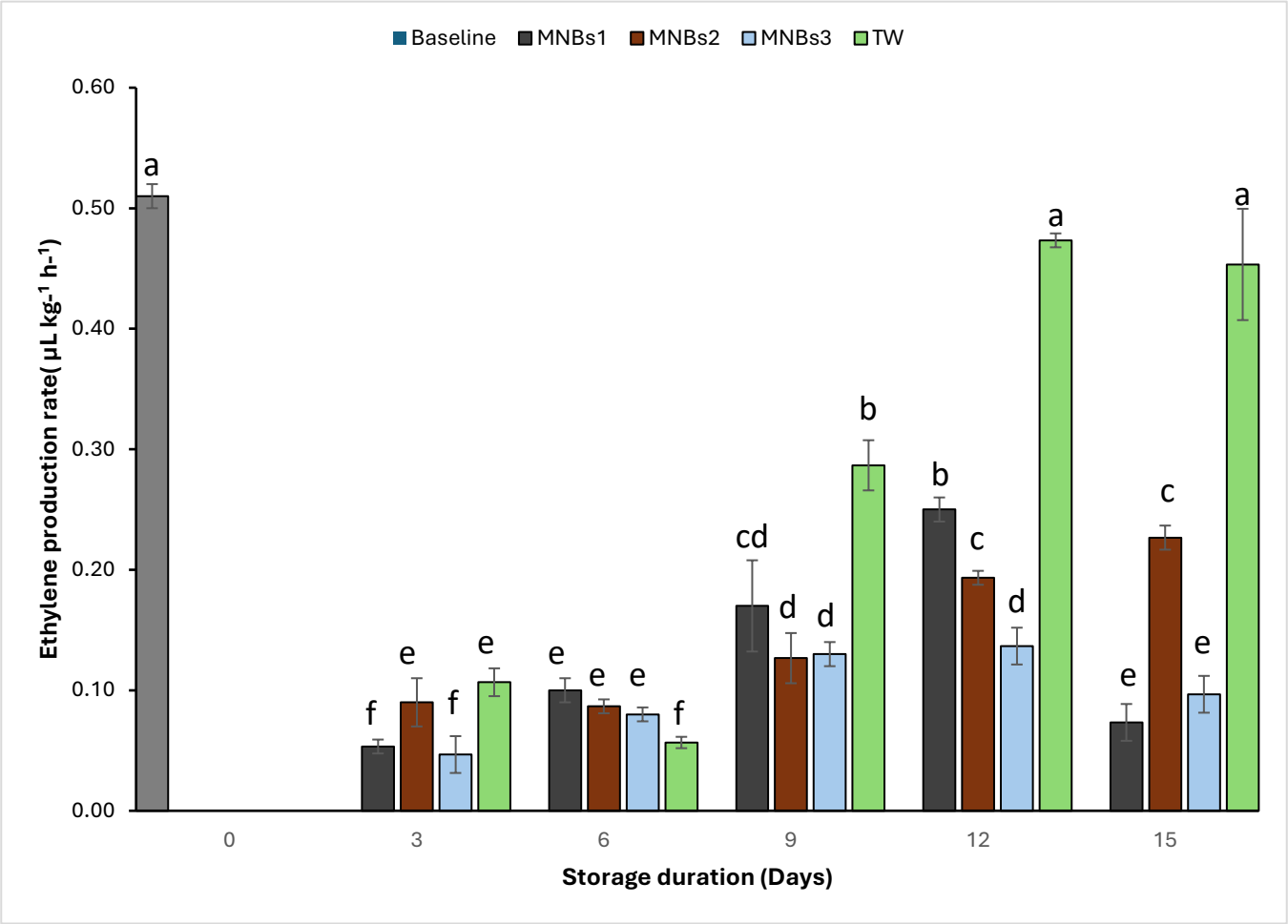

Figure 4.4: Effect of ozone micro-nanobubble (O_3-MNB) and tap water (TW) treatments on ethylene production rate of fresh cut and packaged Swiss chard leaves (cv. Fordhook iant) stored at 5°C for 15 days. Values are means ± standard deviation (n = 3). Means with the same letter are not significantly different ($p \leq 0.05$). Treatment abbreviations (MNB1, MNB2, MNB3 and TW) are defined in Table 4.1.

4.3.5 Chlorophyll content

Postharvest treatment and storage duration had a significant impact on the chlorophyll content of fresh cut Swiss chard (Figure 4.5A, B, C). Across all O_3-MNB pre-treated and TW control samples, chlorophyll levels exhibited a consistent decline over the storage period. Chlorophyll exists in two main forms, both essential for photosynthesis: chlorophyll-b assists in absorbing light energy and transferring it to chlorophyll-a, while chlorophyll-a functions as the primary pigment in the light harvesting process (Zhang *et al*., 2025). Initially, concentrations were recorded at 19.35 ± 1.97 g mL^{-1} for chlorophyll a, 17.88 ± 2.70g mL^{-1} for chlorophyll b, and 21.06 ± 0.98 g mL^{-1} for total chlorophyll. Among the treatment conditions, the 5 min TW control samples showed the most pronounced decline, with final concentrations decreasing to 6.43 ± 0.53 g mL^{-1} and 4.03 ± 0.12 g mL^{-1} for chlorophyll a, chlorophyll b, and 6.09 ± 0.07 g mL^{-1} for total chlorophyll, respectively, the end of storage duration.

Similar observations were reported by Shi *et al*. (2023). The chlorophyll content of parsley treated with O_3-MNBs remained consistently higher than that of the control group throughout the entire storage period. Similarly, Karaca and Velioglu (2020) observed a comparable trend in samples pre-treated with distilled, ozonated, or chlorinated water for 5 min and stored at 5 ± 1 °C with RH of 85 ± 7% for 15 days. Their findings revealed a decline in chlorophyll content, with chlorophyll a decreasing by 30–45% and chlorophyll b by 24–39%, indicating progressive pigment degradation during storage. Chlorophyll degradation is directly linked to the visual quality (Figure 4.2) and senescence of leafy vegetables, as it leads to a noticeable loss of the characteristic green colour (Karaca and Velioglu, 2020).

This link is further evidenced by elevated *a** and *L** colour parameters in the present study, which show enhanced discolouration consistent with chlorophyll loss. Degradation of chlorophyll in green vegetables is the main cause of their distinctive colour loss, which lowers the nutritional content, degrades the quality of the product and ultimately limits consumers' acceptance (Zhang *et al*., 2025). Furthermore, Zhang *et al*. (2023) observed that exposure to light can trigger the production of ROS, enhance oxidation processes, and increase the rate of breakdown of chlorophyll in plant tissues. In our study, all treatments showed a decrease in chlorophyll content, which is indicative of a natural senescence process. However, the degree of this degradation can be reduced by postharvest treatments such as MNBs, storage conditions and correct packaging film. Therefore, this study determined that fresh cut Swiss chard samples treated with O_3-MNBs maintained higher levels of chlorophyll over 15 days of storage at 5 °C, showing potential for prolonging shelf life and enhancing appearance of fresh cut leafy vegetables.

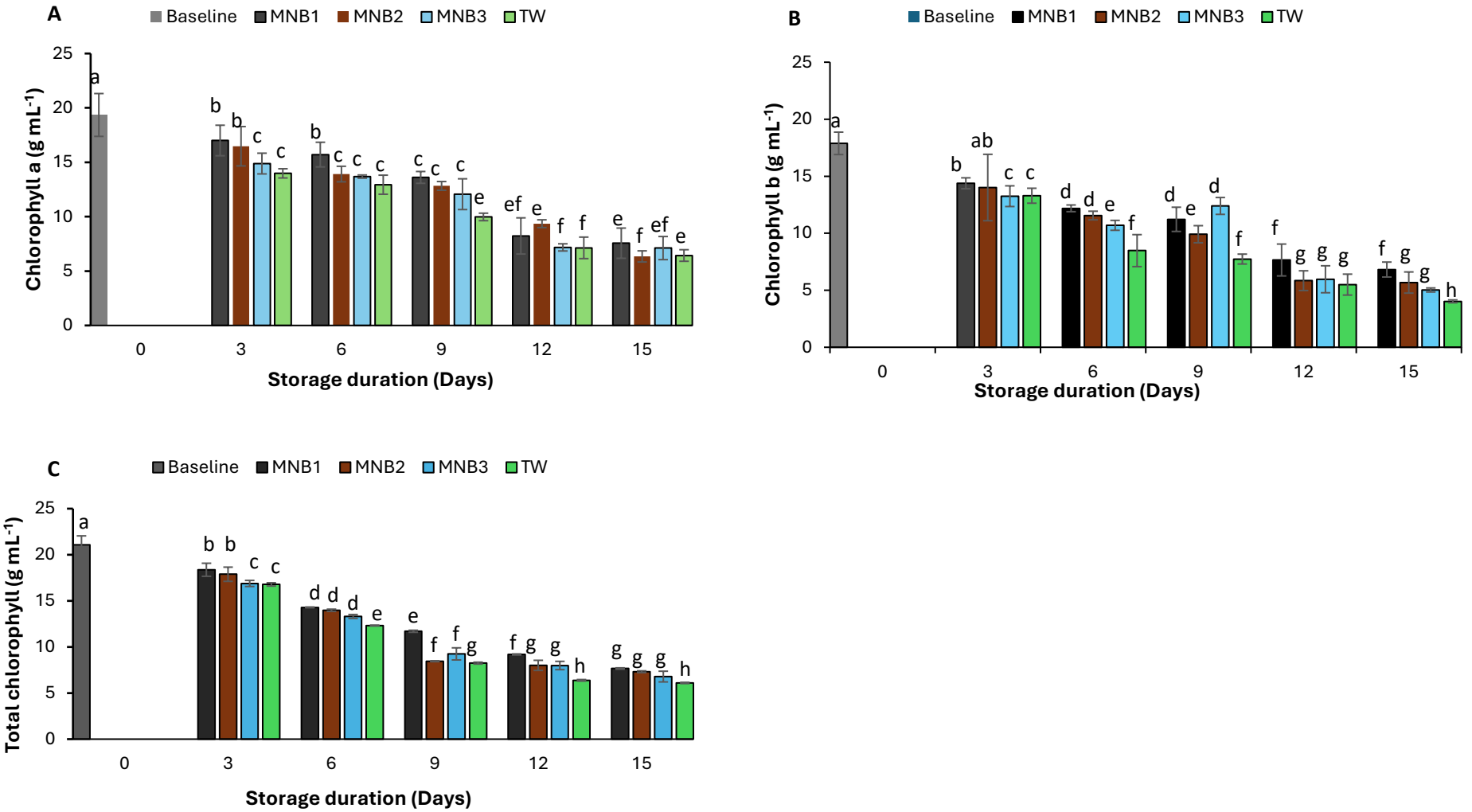

Figure 4.5: Effect of ozone micro-nanobubble water (O_3-MNB) and tap water (TW) treatments on **(A)** chlorophyll a, **(B)** chlorophyll b and **(C)** total chlorophyll content of fresh `cut Swiss chard leaves (cv. Fordhook Giant) stored at 5°C for 15 days. Values are means ± standard deviation (n = 3). Means with the same letter are not significantly different (p≤0.05). Treatment abbreviations (MNB1, MNB2, MNB3, and TW) are defined in Table 4.1.

4.3.6 Total phenolic compounds

TPC of both O_3- MNB treated, and TW treated Swiss chard samples was significantly influenced by the treatments and storage duration. In the TW samples, TPC fluctuated over time, showing a decrease to 15.63 mg GAE L^{-1} by day 3 from the initial baseline of 27 mg GAE L^{-1}. In contrast, all O_3-MNBs pre-treated samples exhibited a significant increase in TPC from day 0 to day 9 (Figure 4.6). The increase of phenolic compounds observed on these days may be attributed to the stimulation of phenylalanine ammonia-lyase (PAL) activities in response to various abiotic stressors (minimal processing, packaging material, temperature, and storage duration) (Shi *et al*., 2023). However, by days 12 and 15, the TW control samples showed higher TPC compared to the O_3-MNB pre-treated samples.

In contrast to our findings, Phornvillay *et al*. (2022) reported that a four-day exposure to hydrogen peroxide micro-nano bubbles (H_2O_2 + MNBs) led to an increase in the total phenolic content of roselle microgreens. However, in agreement with our results, similar findings were reported by Shi *et al*. (2023), where parsley pre-treated with O_3-MNBs exhibited lower phenolic content than the control group by the end of storage. Similarly, Liu *et al*. (2021), reported that treating fresh-cut cabbage with 1.4 m/L aqueous ozone for 1, 5 or 10 min resulted in degradation of phenolic compounds. The degradation of phenolic compounds observed on this study may be attributed to secondary compounds formed through various ozone related chemical reactions (Liu *et al*., 2021).

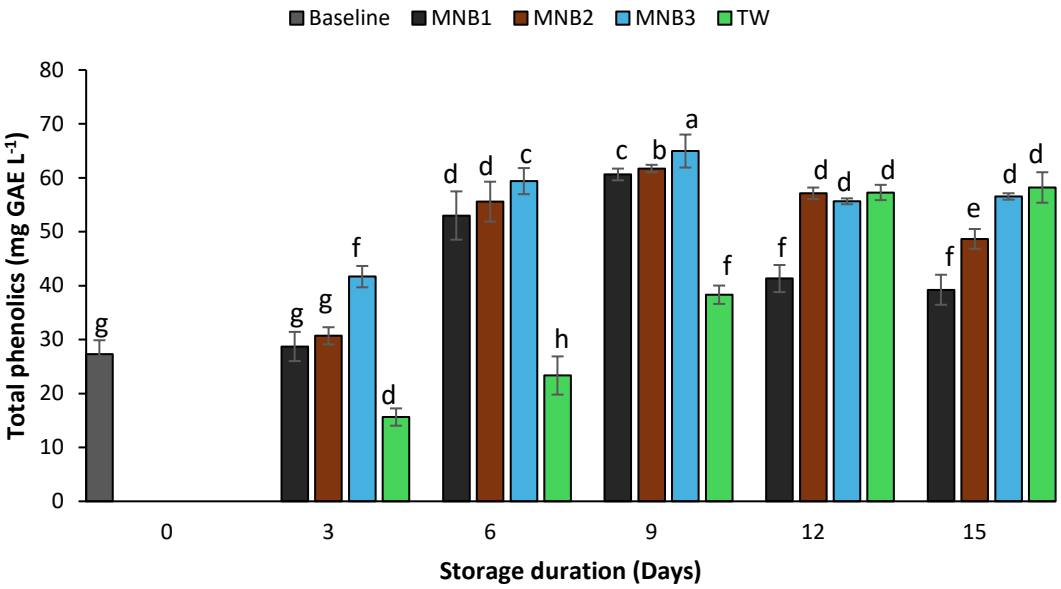

Figure 4.6: Effect of ozone micro-nanobubble water (O_3-MNB) and tap water (TW) treatments on total phenolic concentration of fresh cut Swiss chard leaves (cv. Fordhook Giant) during storage at 5°C for 15 days. Values are means ± standard deviation (n = 3). Means with the same letter are not significantly different ($p \leq 0.05$). Treatment abbreviations (MNB1, MNB2, MNB3, and TW) are defined in Table 4.1.

4.3.7 Volatile organic compounds

The results for fresh cut Swiss chard volatile organic compounds (VOCs) are presented in Table 4.3. In total, 29 compounds including 16 alcohols, 6 ketones, 3 aldehydes, 2 furan derivatives, 1 ester and 1 pyrazine derivative were identified. In the baseline analysis, alcoholic VOCs (cis-2-Penten-1-ol, 1-Hexanol, cis-3-Hexen-1-ol, trans-3-Hexen-1-ol, and 2-Ethylhexanol, aldehydes (hexanal, trans-2-Hexenal, β-Cyclocitral), and ketones (6-Methyl-5-hepten-2-one, Pinocarvone and β-Ionone) VOCs were highly detected. From this analysis, it was evident that alcohols were found to be the most dominant chemical class compared to other VOCs at the beginning of the study. However, after 15 days of storage, treatment with O_3-MNBs led to either minimal or no VOC detection. For instance, MNB1 and MNB2 treatments showed no presence of hexanal and only low levels of trans-2-Hexenal were detected. In contrast, the TW treatment maintained the detection of hexanal and trans-2-Hexenal aldehydes. These compounds were present in the baseline samples, indicating TW treatment had no impact on these VOCs throughout the storage period. The dominant functional groups identified in our study were alcohol and aldehydes.

These findings align with those of Dhakal *et al*. (2021) who reported that alcohols made up 43.10% of the total 90.13% VOCs identified in Swiss chard subjected to various treatments. Additionally, their study also noted the presence of 1-Hexanol across all treatments, which is consistent with our observations. Similarly, Mzoughi *et al*. (2019) identified non-terpene derivatives, specifically straight-chain alcohols and aldehydes as the predominant functional group in wild edible Swiss chard, accounting for 40.6% of the total VOCs. Four compounds, β-Cyclocitral, 1-Hexanol, cis-2-Penten-1-ol, and 6-Methyl-5-hepten-2-one were consistently detected across all samples, including the baseline. In contrast, eight volatiles—1-Penten-3-ol, 1-Pentanol, Linalool, 3-Methyl-4-penten-1-ol, 4-Vinylguaiacol, Acetoin, 3-Pentanone, and 2-Ethylfuran were only found in samples treated with O_3-MNBs. Additionally, β-Ionone was present in both the baseline and 5-min TW-treated samples, however its levels decreased following O_3-MNB treatment during storage.

The emergence of new volatiles in the O_3-MNB-treated samples could be attributed to oxidative stress induced by ozone exposure, which may have triggered lipid peroxidation, enzymatic transformations, or breakdown of cellular components (Li *et al*., 2017). These biochemical changes can lead to the formation of secondary metabolites and novel VOCs, contributing to the altered volatile profile observed in treated Swiss chard (Li *et al*., 2017; Opriş *et al*., 2019). Our results are in contrast with those reported by Shi *et al.* (2023) for O_3-MNB treated parsley. The authors reported that while the production of volatile compounds in parsley shifted during storage, the original volatile profile remained stable in the O_3-MNB treated samples. Volatilomics analysis successfully identified molecular alterations in fresh-cut Swiss chard throughout storage after being treated with O_3-MNB water. These

treatments led to significant shifts in the volatile organic compounds' profile over time. Notably, samples exposed to O_3-MNB exhibited elevated concentrations of alcohols and ketones, which are likely contributors to the characteristic aroma of fresh cut Swiss chard.

Table 4.3: Volatile organic compounds identified by GC-MS in fresh cut Swiss chard (*Beta vulgaris* L. cv. Fordhook Giant) at day 0 and after storage duration at 5°C.

Compounds	CAS	RT	Baseline	TW	MNB1	MNB2	Odour
Aldehydes							
Hexanal	66-25-1	7.03	▪	▪	-	-	Grass, tallow
trans-2-Hexenal	6728-26-3	10.98	▪	▪	▫	▫	Green, leaf
β-Cyclocitral	432-25-7	20.27	▪	▪	▪	▪	Tropical, saffron, herbal,
Alcohols							
1-Penten-3-ol	616-25-1	9.80	-	-	▪	▪	Green
isoamyl alcohol	584-02-1	10.99	-	-	▫	▫	Herbal
1-Pentanol	71-41-0	12.05	-	-	▪	▪	Fermented
cis-2-Penten-1-ol	1576-95-0	13.79	▪	▪	▪	▪	Green
1-Hexanol	111-27-3	14.59	▪	▪	▪	▪	Herbal
cis-3-Hexen-1-ol	928-96-1	15.31	▪	▫	▪	▪	Fresh, green
trans-3-Hexen-1-ol	928-97-2	15.81	▪	▫	▪	▪	Green
6-Methyl-5-hepten-2-ol	1569-60-4	17.07	▫	▫	▫	▫	Sweet, oily, green, coriander
2,6-Dimethyl-oct-7-en-2-ol	18479-58-8	17.23	-	▫	▫	▫	Citrus
2-Ethylhexanol	104-76-7	17.64	▪	▫	▪	▪	Citrus, fresh, floral, oily
Linalool	78-70-6	18.92	▫	▫	▪	▪	Citrus, floral, waxy, lavender
3-Methyl-4-penten-1-ol	51174-44-8	19.06	▫	▫	▪	▪	NF
Fenchyl alcohol	1632-73-1	19.58	-	-	▫	▫	Camphor, pine, woody, dry
Menthol	89-78-1	20.74	▫	▪	▪	▪	Peppermint, cooling, woody
o-Cresol	95-48-7	27.44	-	▫	▫	▫	Musty, phenolic, medicinal
4-Vinylguaiacol	7786-61-0	30.45	-	-	▪	▪	Spicy, powdery, clove,
Ester							
Linalyl acetate	115-95-7	20.67	-	▫	▫	▫	Sweet, green, citrus,
Ketones							
Acetoin	513-86-0	12.97	-	-	▪	▪	Sweet, buttery, creamy
3-Pentanone	96-22-0	4.14	-	-	▪	▪	Ethereal, acetone

2,2,6-Trimethylcyclohexan-1-one	2408-37-9	13.32	▫	▫	▫	▫	Pungent, thujonic, labdanum
6-Methyl-5-hepten-2-one	110-93-0	13.99	▪	▫	▫	▫	Citrus, green, musty
Pinocarvone	30460-92-5	19.19	▪	▪	▪	▪	Balsam, Herbal
β-Ionone	14901-07-6	26.24	▪	▪	▫	▫	Floral, woody, sweet,
Furan derivatives							
2-Ethylfuran	3208-16-0	3.71	▫	▫	▪	▪	Chemical, beany, ethereal
cis-2-(2-Pentenyl) furan	70424-13-4	13.07	▫	▫	▫	▫	NF
Pyrazine derivatives							
2-sec-Butyl-3-Methoxypyrazine	24168-70-5	17.85	-	-	▫	▫	Green, musty, pea green pea

– indicate no detection and ▫/▪ indicate low detection or high detection of the volatile compound, respectively.

CAS=CAS number; RT, retention time; NF=not found

Odour description (https://scentsandflavors.com/database/)

4.3.8 Microbiological analysis

The initial microbial loads of total aerobic mesophilic bacteria (TAMB) and yeasts and moulds on fresh-cut Swiss Chard leaves (cv. Fordhook giant) were 4.04 Log CFU cm^{-2} and 4.18 Log CFU cm^{-2}, respectively (Figure 4.7). Following MNB treatments, the populations of yeast and moulds were substantially reduced by 1 Log CFU/cm^2 (Figure 4.7B). Similarly, the aerobic mesophilic bacteria were significantly reduced by approximately 1.6 Log CFU/cm^2 under MNB treatments (Figure 4.7A). After 3 days of storage the TW pre-treated samples exhibited a substantial increase in mesophilic bacterial counts reaching 4.06 Log CFU cm^{-2}. However, from day 6 to day 15 all samples showed a progressive reduction in mesophilic bacterial counts. Final total counts decreased by 0.46, 1.00 and 0,28 log CFU cm^{-2} in samples treated with O_3-MNBs for 5, 10, and 15 min respectively, compared to a 0,24 log CFU cm^{-2} reduction in the 5 min TW control samples.

There was an observation of microbial growth in yeast and moulds on day 6 with counts reaching 4.19 Log CFU cm^{-2}. Moreover, by the end of storage duration, yeast and mould counts were reduced by 0.64, 1.59, 0.68 log CFU cm^{-2} following treatments with O_3-MNBs for 5, 10, and 15 min respectively. The 5-min TW control samples achieved only a reduction of 0.33 Log CFU cm^{-2}. The microbial load reduction for 10 min treatment with O_3-MNB was consistent throughout the duration storage as a result, O_3-MNB treatment on day 15 maintained the lowest load of aerobic mesophilic bacteria (3.04 Log CFU/cm^{-2}) and yeasts and moulds (2.59 Log CFU/cm^{-2}).

A similar observation was reported by da Silva *et al*. (2025), who investigated the effects of O_3-MNB treatment on lettuce. Their study found that exposing lettuce to 1.60 mg L^{-1} of O_3-MNB for 5 and 10 min, followed by storage at 5 °C and 80% relative humidity for 6 days, significantly reduced TAMB. Specifically, the 5-min treatment resulted in a 0.71 log reduction, while the 10-min exposure achieved a 1.59 log reduction compared to the untreated control. Additionally, Liu *et al*. (2021) reported reductions of 1.2, 1.5, and 1.6 $\log_{10}$ CFU/g in TAMB of fresh cut cabbage pre-treated with aqueous ozone for 1, 5, and 10 min, respectively starting from undetectable baseline (<1 $\log_{10}$ CFU/g), compared with levels observed in the control samples. Moreover, the authors further reported that yeast counts were reduced by approximately 1.1-1.4 $\log_{10}$ CFU/g following 5-and 10-min treatments on day 12 compared to 5-min distilled water control samples. Furthermore, shredded red cabbage treated with 0.5 mg L^{-1} O_3-MBs for 5 min resulted in 1-2 log reduction of bacteria counts, coliform counts and yeast and moulds count compared to control samples (Pongprasert *et al*., 2016). Moreover, Sengun (2013) reported that after being treated with ozonated water (0.5, 1.0, and 1.5 ppm) for 3, 5, and 10 min, the TAMB of the parsley and lettuce samples decreased by 0.40-1.03 log units and 0.48-1.25, from the initial of 6.99 log CFU/g and 5.93 log CFU/g respectively.

In the current study, it was observed that 10 min treatment had an effect on microbial load reduction as compared to other treatments. The ROS produced by ozone-MNBs have strong antibacterial properties and can efficiently lower the microbial load on vegetable surfaces (Pal and Kioka, 2025), by minimizing pathogen-induced respiration brought on by infection or stress (Malahlela *et al*., 2024; 2025). Additionally, the treatment conditions applied in this study successfully maintained TAMB and yeast and moulds count below the maximum allowable limits of 7 log CFU mL^{-1} and 5 log CFU mL^{-1}, after 15 days of storage, in accordance with the Foodstuffs, Cosmetics and Disinfectants (FCD) Act 54 of 1979 (South Africa).

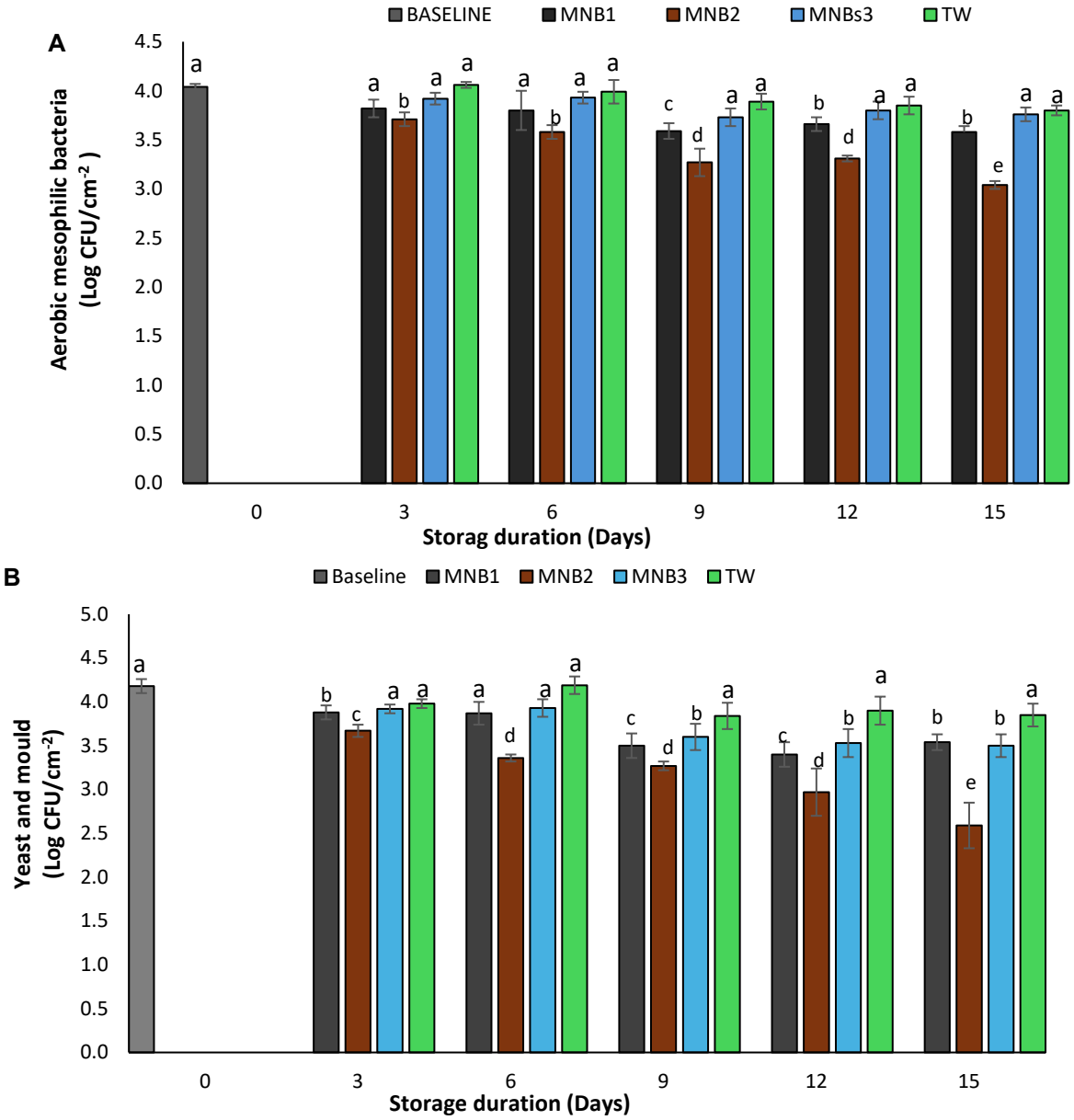

Figure 4.7: Effect of ozone micro-nanobubble water (O_3-MNB) and tap water (TW) on **(A)** aerobic mesophilic bacteria and **(B)** yeast and mould counts of fresh cut Swiss chard stored at 5 °C for 15 days. Values are means ± standard deviation (n=3). Means with the same letter are not significantly different ($p \leq 0.05$). Abbreviations (MNB1, MNB2, MNB3, and TW) are defined in Table 4.1.

4.4 Conclusion

This study investigated the effects of O_3-MNB treatments on the various quality parameters of Swiss chard stored at 5 °C for 15 days. O_3-MNB treatments significantly reduced weight loss and microbial loads, including aerobic mesophilic bacteria, yeasts, and moulds, thereby lowering the risk of spoilage and contamination. O_3-MNB treatments also preserved key quality attributes such as chlorophyll content and leaf colour, reduced ethylene production and changes in package gas composition. Although TSS and TA declined during storage, O_3-MNB application helped maintain these parameters better than the control. Total phenolic compounds initially increased following O_3-MNB exposure. Furthermore, O_3-MNBs resulted in formation of new volatile compounds. Overall, O_3-MNB treatment represents a promising and sustainable postharvest technology for fresh-cut Swiss chard, improving microbial safety while maintaining nutritional and sensory quality, thus supporting extended marketability and enhanced consumer acceptability.

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CHAPTER 5

GENERAL SUMMARY, CONCLUSION AND RECOMMENDATIONS

Fresh-cut vegetables such as Swiss chard are characterised by high perishability due to tissue damage resulting from minimal processing operations, which accelerates oxidation, microbial proliferation, and physiological deterioration during storage. Conventional chemical sanitisers such as sodium hypochlorite are increasingly discouraged owing to environmental and health concerns, highlighting the need for safe and sustainable alternative postharvest treatments. The aim of this study was to determine the impact of activated water treatments micro-nano bubble (MNBs) and packaging systems on physiological responses, phytonutrients, and overall quality of minimally processed Swiss chard (*Beta vulgaris* L. cv. Fordhook Giant) during storage.

The objectives in the study were:

1. To evaluate the influence of air-MNBs on physicochemical attributes and microbiological stability of packaged whole Swiss chard during storage (Chapter 3).

2. To assess the effects of ozone-MNBs (O_3-MNBs) on the physiological responses, physicochemical parameters, phytochemical content and volatile organic profile of fresh-cut Swiss chard (Chapter 4).

The first objective was achieved through evaluating the effects of air-MNBs on the physicochemical and microbial quality attributes of whole Swiss chard. Air-MNB pre-treatments significantly preserved chlorophyll content while effectively reducing weight loss and preserving leaf colour compared to sodium hypochlorite (NaOCl) and tap water. The treatment minimised colour changes evidenced by lower L* values and slower b* increases, furthermore the visual documentation, demonstrated that pre-treatment with air-MNBs resulted in enhanced appearance of whole Swiss chard as compared to NaOCl pre-treatments which resulted in leaf bleaching.

Furthermore, air-MNBs exhibited comparable antimicrobial effects with NaOCl on microbial load reduction of aerobic mesophilic bacteria resulting in a 0.53 Log reduction, respectively. Additionally, air-MNBs consistently maintained the lowest gas composition throughout storage. The results of this study showed that Swiss chard pre-treated with air-MNBs combined BOPP had better preservation impacts on the overall quality. Despite exhibiting comparable < 1-log microbial reduction ($p > 0.05$), air-MNBs performed better than NaOCl in maintaining postharvest quality without causing oxidative damage on whole Swiss chard leaves.

The second objective was successfully accomplished by evaluating the effects of ozone-micro nanobubbles (O_3-MNBs) on physicochemical, physiological and microbial quality attributes as well as volatile organic compounds of fresh-cut Swiss chard. O_3-MNB treatments effectively reduced weight loss preserved leaf colour, and inhibited ethylene production and in package gas composition compared to tap water controls. While total soluble solids and titratable acidity decreased across all treatments, they were better retained in control samples. Total phenolic compounds increased significantly in O_3 MNB-treated samples during early storage, suggesting an induced antioxidant response. Moreover, microbial loads of aerobic mesophilic bacteria, yeasts, and moulds were significantly reduced by O_3-MNB treatments, with the 10-min exposure providing the greatest microbial inhibition throughout storage.

The study further analysed volatile organic compounds of fresh-cut Swiss chard after 15 days of storage. It was evident that alcohols were found to be the most dominant chemical class compared to other VOCs at the beginning of the study. However, after 15 days of storage, treatment with O_3-MNBs led to either minimal or no VOC detection. For instance, MNB1 and MNB2 treatments showed no presence of hexanal and only low levels of trans-2-Hexenal were detected. In contrast, the TW treatment maintained the detection of hexanal and trans-2-Hexenal aldehydes. These compounds were present in the baseline samples, indicating TW treatment had no impact on these VOCs throughout the storage period. Alcohols and aldehydes were the predominant functional groups detected in our analysis.

While post-harvesting handling practices such as washing and sanitisation, packaging and storage have been studied extensively in other leafy vegetables, little evidence exists on their effectiveness for Swiss chard. Additionally, reliance on synthetic sanitisers also raises concerns about food safety and environmental sustainability, yet commercialisation and adaptation of eco-friendly alternative remain insufficiently explored. This study introduced a novel strategy for the postharvest sector to control microbial populations of bacteria, yeast and moulds on whole and fresh-cut leafy green vegetables, offering a sustainable alternative to conventional sanitisation methods. The results from this study highlighted the effectiveness of micro-nanobubbles (MNBs) in maintaining the postharvest freshness of Swiss chard. This approach helps minimise both the quality loss and economic impact during storage, supporting MNBs as a potential eco-friendly preservation technology for leafy vegetables.

Recommendations

- Investigating the impact of repeated uses of the same MNB water on decontamination efficacy and food safety. Understanding whether microbial or organic matter accumulation

affects sanitising performance over multiple cycles is crucial for sustainable operational design.

- Additionally, research should target the optimisation of packaging materials, specifically focusing on permeability characteristics to better regulate moisture levels and gas exchange, thereby improving shelf-life and product quality in fresh-cut leafy vegetables.
- Further exploration into the development and refinement of MNB technology is needed to meet the critical demands of food safety and reduce postharvest losses. This includes standardising bubble size for consistent antimicrobial action, improving generation methods for commercial scalability, and enhancing transparency about the specifications and capabilities of commercially available MNB generators. Such efforts will underpin the reliability and broader adoption of MNBs as eco-friendly disinfection alternatives in fresh produce handling.

Collectively, these recommended research directions will facilitate the translation of micro nanobubble technology from experimental studies to practical, industry-standard applications that maintain produce freshness, enhance microbial safety, and address environmental sustainability goals through optimised resource use and reduced chemical inputs.