EFFECTS OF SONICATION DURATION ON THE PHYSICOCHEMICAL PROPERTIES, BIOACTIVE CHARACTERS, AND THE SHELF-STABILITY OF NONI JUICE STORED AT DIFFERENT TEMPERATURES

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By

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ABSTRACT

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Choo Yi Xuan

Morinda citrifolia is a plant belonging to the *Rubiaceae* family, widely known as noni. Noni fruit is rich in antioxidant and has been used to treat remedies such as skin allergies, warts, hemorrhoids, diabetes, etc. Those health benefits are associated by the presence of bioactive compounds in noni fruit. Noni fruit is commonly produced into fruit juice through fermentation or freshly extracted using mechanical apparatus. The fresh noni juice is perishable. It must store under refrigerated condition to preserve the flavour and extend shelf-life, otherwise, it must be consumed immediately. Prior to consumption, either fresh or fermented noni juice is usually pasteurised to kill harmful microbes in addition to prolong the shelf-life. However, the heating process might negatively affect the flavour and nutritional values of noni juice. Sonication has emerged as a promising green technology in the food industry to overcome the limitation of pasteurisation. Besides, sonication has been perceived to fulfill the Food and Drug Administration requirement of a 5-log reduction in relevant microorganisms in fruits and vegetable products. Hence, this study aimed to investigate the effects of sonication duration on the physicochemical properties,

bioactive characters, and the shelf-stability of noni juice. In brief, noni juice was sonicated at different duration intervals (20, 40, and 60 min) under constant frequency and temperature of 37 kHz and 30°C, respectively. Pasteurisation was adopted as positive control, while fresh noni juice served as negative control. Results showed that S60 significantly improved ($p < 0.05$) and retained the highest amounts of bioactive compounds (TPC and TFC), phenolic compounds (scopoletin, rutin, vanillic acid, and quercetin), organic acids (ascorbic, malic, fumaric, and citric acids), and antioxidant capacity (FRAP and TEAC) of noni juice compared to the fresh noni juice. Therefore, S60 noni juice was selected for 56 days of shelf-stability study at room temperature (25°C) and refrigerated temperature (4°C) together with the fresh and pasteurised noni juices. The microbial load (aerobic mesophilic bacteria, yeast, and mold counts) of noni juice samples during 56 days of storage were also evaluated. Results demonstrated that S60 retained higher phenolics, organic acids, and antioxidant capacity of noni juice than fresh and pasteurised samples after 56 days of storage at both temperatures. Refrigerated temperature showed better preservation of noni juice nutrients than room temperature storage. No yeast and mold growth observed throughout the storage study. Irrespective of the storage temperatures, the total aerobic mesophilic bacteria count (<2 log CFU/mL) of noni juice samples stored for 56 days fell within the satisfactory level of microbiological standard for ready-to-eat food (<4 log CFU/mL). Overall, this study highlights the feasibility of using sonication processing to enhance the quality of noni juice.

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APPROVAL SHEET

This dissertation entitled "**EFFECTS OF SONICATION DURATION ON THE PHYSICOCHEMICAL PROPERTIES, BIOACTIVE CHARACTERS, AND THE SHELF-STABILITY OF NONI JUICE STORED AT DIFFERENT TEMPERATURES"** was prepared by **CHOO YI XUAN** and submitted as partial fulfillment of the requirements for the degree of Master of Science at Universiti Tunku Abdul Rahman.

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SUBMISSION OF DISSERTATION

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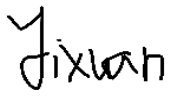

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DECLARATION

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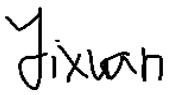

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LIST OF ABBREVIATIONS

a^*	Greenness/Redness
ABTS	2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid
AMB	Aerobic mesophilic bacteria
ANOVA	One-way analysis of variance
AOAC	Association of Official Agricultural Chemists
AR grade	Analytical research grade
b^*	Yellowness/Blueness
BI	Browning index
*C**	Chroma
CE	Catechin
CFU	Colony forming unit
DAD	Diode array detector
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenyl-1-picryl-hydrazyl-hydrate
FDA	Food and Drug Administration
Fe^{2+}	Ferrous iron
FRAP	Ferric reducing antioxidant power assay
FRE	Fresh noni juice
FW	Fresh weight
g	Gram
GAE	Gallic acid equivalents
*h**	Hue
HCl	Hydrochloric acid
HPLC	High-performance liquid chromatography
HSD	Honest Significant Difference

HTST	High temperature short time
kg	Kilogram
kHz	Kilohertz
L	Litre
L^*	Lightness
LOD	Limit of detection
LOQ	Limit of quantitation
M	Molarity (mol/L)
mg	Milligram
min	Minute(s)
mL	Mililitre
mM	Millimolar
MPa	MegaPascal
mPa.s	Millipascal second
NA	Not available
ND	Not detected
NG	No growth
nm	Nanometer
PCA	Plate count agar
PDA	Potato dextrose agar
POD	Peroxidase
POS	Pasteurised noni juice
PPO	Polyphenol oxidase
R^2	Coefficient of determination
RE	Rutin
RP-HPLC	Reverse phase high-performance liquid chromatography
rpm	Revolutions per minute

SD	Standard deviation
S20	Noni juice sonicated for 20 min
S40	Noni juice sonicated for 40 min
S60	Noni juice sonicated for 60 min
TAC	Total anthocyanins content
TC	Total carotenoids content
TE	Trolox
TEAC	Trolox equivalent antioxidant capacity
TFC	Total flavonoids content
TPC	Total phenolics content
TPTZ	2,4,6-tripyridyl-s-triazine
USA	United States of America
UV-Vis	Ultraviolet visible
v:v	Volume to volume ratio
W/cm^2	Watt per square centimetre
w/w	Weight by weight
YM	Yeast and mold
*β*CE	*β*-carotene
Δ*E*	Total colour difference
µg	Microgram
µL	Microlitre
µM	Micromolar (µmol/L)
µm	Micrometer

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Morinda citrifolia (noni) is a plant that belongs to the *Rubiaceae* family. It is native to Southeast Asia and widespread throughout the Caribbean, Mexico, South America, Eastern Polynesia as well as in India (Almeida, de Oliveira and Hotza, 2019). The ripe fruit is translucent-greyish, oval-shaped, with small reddish-brown buds fused to the outer layer and weighs about 50 to 300 g. It is soft, fleshy and contains mostly 90% of water (Chan-Blanco, et al., 2006; Almeida, de Oliveira and Hotza, 2019). Noni fruit is commonly produced into fruit juice, powder and/or concentrate. Additionally, noni fruit can be produced into snacks, supplements as well as tea that is produced from dried noni fruit and leaf (Nelson and Elevitch, 2006).

Noni fruit is claimed to exert numerous therapeutic activities due to presence of bioactive compounds. It was reported that ripened noni fruit is rich in phenolic compounds such as scopoletin, rutin, vanillic acid, and quercetin. Other than that, organic acids such as, malic, fumaric, citric, ascorbic, malonic, benzoic, tartaric, octanoic, and hexanoic acids are also present in the fruit (Pino, Márquez and Castro, 2009; Dussossoy, et al., 2011;Singh, 2012; Bittová, et al., 2015; Wall, et al., 2015).

In 1996, noni juice was listed as a wellness drink owing to its potent medicinal effects (Abou Assi, et al., 2017). Wellness drink can be defined as a functional beverage which contains numerous bioactive compounds such as phenolic acids, flavonoids, ascorbic acid, and tocopherol that promotes health benefits. The existence of bioactive compounds enables functional beverages to possess functional and nutraceutical activities such as antiaging, anti-inflammation, antioxidant, anticancer, antihypertensive, anti-obesity, etc (Islam and Kabir, 2019). In addition, the noni juice also has been recognised by European Union as a novel safe food product (Chan-Blanco, et al., 2006).

The demand of fruit juice has been marked up in the past few years. Consumers are leaning towards healthy lifestyles and thus health-promoting food products like fresh fruit juices or concentrates have gained popularity (Bhat, et al., 2011). According to the Centre for Promotion of Imports Ministry of Foreign Affairs (2021), a sudden surge in fruit juice demand was observed in 2020 and 2021, which is during the Covid-19 pandemic. This ultimately increased the fruit juice sales by 24% in Europe and more than 40% of increment in some other regions (Tetra Pak, 2020). Commercial noni juice is usually pasteurised to extend the shelf-life and elimination of pathogenic microorganism such as *E. coli*, *Salmonella*, and toxins produced by fungus. This is because unpasteurised noni juice is susceptible to contamination during the postharvest handling which includes containers, equipment, air, animals, and humans. Thus, pasteurisation is required to ensure consumer safety in addition to prolong the shelf-life of noni juice (Nelson, 2006). However, pasteurisation may

compromise the nutrients and may cause flavour loss in the noni juice (Brown, 2012; Rabie, et al., 2014).

To date, many processing methods have been proposed to produce fruit juices with higher qualities, improved shelf-life, and microbially safe. These include sonication, pulsed electric field applications, microwave heating, ohmic heating, and high-pressure processing (Vilas-Boas, et al., 2022). Among these, sonication, has been recognised as an emerging green technology in the food industry due to low emission of greenhouse gas, utilises low energy, and has minimal environmental impacts (Bhat, et al., 2011; Putnik, et al., 2020). It also has been perceived to fulfil the Food and Drug Administration (FDA) requirement of achieving a 5-log reduction in relevant microorganisms in fruits and vegetable products, in addition to retain and enhance the flavour and nutritional value of products (Chemat, Zill-E-Huma and Khan, 2011; Zinoviadou, et al., 2015; Dolas, Saravanan and Kaur, 2019). Additionally, previous studies have demonstrated that sonication is not only effective in inactivating microbial activity, but it also improves antioxidant capacity, bioactive compounds such as total phenolics content (TPC), total flavonoids content (TFC) as well as organic acids such as ascorbic acid of kasturi lime, grapefruit, apple, and purple cactus pear fruit juice (Bhat, et al., 2011; Aadil, et al., 2013; Abid, et al., 2013, Zafra-Rojas, et al., 2013).

All the positive effects are due to cavitation induced during sonication. Cavitation is the formation of bubbles that leads to cell wall destruction, improves the permeability of cell membrane and thinning of cell membrane,

causes local high temperature and pressure and free radical production, thereby inactivating microorganism and promotes the release of phenolic particles from the cell wall (Fan, Wu and Chen, 2021). Numerous studies showed that sonicated fruit juice improves retention of nutritional content during storage. For instance, sonicated orange juice and sweet lime juice exhibited higher TPC and organic acids level for up to 10 weeks storage at 4°C than pasteurised and control juice samples (Khandpur and Gogate, 2015). Besides, sonicated grapefruit juice also retained higher antioxidant capacity after 28 days of storage at 4°C than non-sonicated grapefruit juice (Aadil, et al., 2015).

1.2 Problem Statement

Nowadays, health and wellbeing has become a major concern to the masses. Healthy and nutritious beverages have become the first choice rather than artificial flavoured drinks. Fruit juices have been gaining popularity owing to the high nutritional value and the associated health benefits (Rajauria and Tiwari, 2018). The associated health benefits of noni juice are attributable by the presence of bioactive compounds such as rutin, scopoletin, quercetin, ascorbic acid which exhibit neuroprotective effect, analgesic, anti-inflammatory and antioxidant, respectively. In addition, both rutin and scopoletin also showed antidepressant activity (Pandy, et al., 2014; Abou Assi, et al., 2017). Noni juice comes in either fermented or freshly squeezed using mechanical aparatus. The fresh noni juice is perishable. It must store under refrigerated condition to preserve the flavour and extend shelf-life, otherwise, it must be consumed immediately. On the other hand, up to now there is lacking standardisation in noni juice processing. As such, the quality of noni juice and their associated health benefits might be negatively affected (Motshakeri and Ghazali, 2015).

The only common practise in producing fermented and fresh noni juice is pasteurisation. Prior to consumption, both fermented and fresh noni juice is pasteurised to extend shelf-life and inactivation of pathogenic microorganism to ensure safety consumption. However, high temperature during pasteurisation might deteriorate the bioactive compounds, compromising the nutritional content and quality of noni juice (Rajauria and Tiwari, 2018). To date, sonication has been proposed to be an alternative to pasteurisation in the fruit juice

processing. Sonication has been reported to extend shelf-life and reduce microbial load of fruit juice meanwhile improve the nutritional content by its cavitation effects (Tiwari, et al., 2009; Zinoviadou, et al., 2015).

According to Nadeem, et al. (2018), the nutrients of sonicated fruit juice reduced in lower amounts as compared to non-sonicated and chemically preserved juices after 3 months of storage. It was reported that the TPC (286 mg GAE/100 mL) and TFC (196 CE/100 mL) of sonicated carrot and grapefruit juice blend was higher than the untreated juice blend (238 mg GAE/100 mL and 117 CE/100 mL, respectively) (Nadeem, et al., 2018). All these studies indicate the benefits of sonication treatment on improving and retention of the nutrients of fruit juice. However, only a few or no studies regarding the effect of sonication duration on the physicochemical properties, microbial load, and the shelf-stability of noni juice has been conducted.

1.3 Objectives

The main objective of this research is to investigate the effects of sonication duration on the physicochemical properties, bioactive characters, and the shelf-stability of noni juice. The specific objectives of this study are listed as follows:

- To determine the physicochemical properties, bioactive compounds, phenolic compounds, organic acids, and antioxidant capacity of noni juice produced by different sonication durations (0, 20, 40, and 60 min).

- To compare the effect of sonication and pasteurisation on the quality of noni juice.

- To determine the microbial load of sonicated noni juice stored for 56 days under different temperatures (4℃ and 25℃).

1.4 Significance of Research

The bioactive compounds of noni juice must be preserved as it contributes to the health benefits of noni juice and enabling them to be recognised as wellness drink. Also, inconsistent in noni juice processing would affects the quality of noni juice. Although noni juice is usually pasteurised to ensure safe consumption and extend their shelf-life but pasteurisation could destroy the heat-sensitive nutrients and bioactive compounds, resulting in depletion of nutritional content. Hence, sonication was proposed to be an alternative fruit juice processing method to overcome the limitations of pasteurisation; specifically, to preserve those heat-labile nutrients, bioactive compounds, and enhance the overall nutritional content of fruit juice by its cavitation effect. Therefore, the data generated from this study could provide an insight for the fruit juice processing industry in the production of high-quality fruit juice with higher nutrients using sonication. Moreover, this study proposed an alternative noni juice extraction method by using juice extractor which shortens the processing duration as compared to traditional fermentation. Further, this study presented that the perishable fresh noni juice has promising shelf-life and is microbiologically stable with high nutritional content after sonication treatment.

CHAPTER 2

LITERATURE REVIEW

2.1 Characteristics of *Morinda citrifolia* (Noni) and Its Fruit

Morinda citrifolia is a plant that belongs to the *Rubiaceae* family. It is commonly known as noni, 'mengkudu', 'Indian mulberry', 'painkiller bush' and 'cheese fruit'. Noni plant typically starts bearing fruit after 9 to 12 months after planting (Abou Assi, et al., 2017). The fruit production from noni plant is year-round. Noni is known as a resilient plant due to its capability to survive under severe weather and soil conditions, high altitudes environment (up to 215 m above the sea), able to grow within the forests and coastal areas (Almeida, de Oliveira and Hotza, 2019).

Noni fruit is oval, measuring up to 10 cm in length and up to 8 cm in width, with a weight ranging from 50 to 300 g (Almeida, de Oliveira and Hotza, 2019). There are a total of five ripening stages of noni fruit, as depicted in Figure 2.1. The Stage 1 unripe fruit is hard and dark green in colour, which transits to yellowish-white or translucent-greyish when ripe (Stage 5). The fruit surface is covered with numerous small reddish-brown buds, each containing four seeds (Motshakeri and Ghazali, 2015).

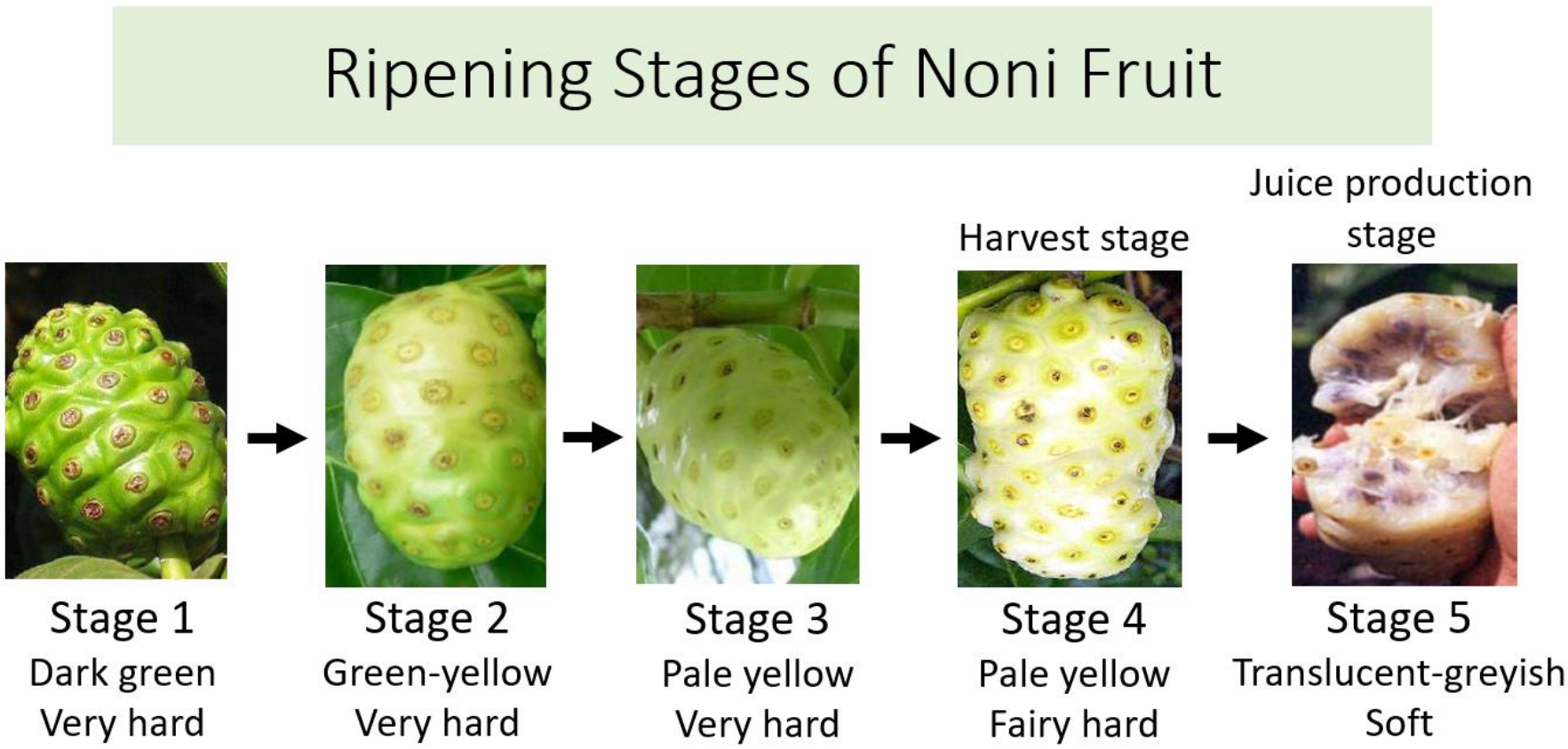

Figure 2.1: Ripening stages of noni fruit (Motshakeri and Ghazali, 2015).

The ripe fruit is soft, fleshy, and juicy. It will continue to ripen naturally, regardless of the maturation stage at which it is harvested (Almeida, de Oliveira and Hotza, 2019). Usually, the fruit is harvested and transported at Stage 4, known as the hard white stage. After harvesting, the fruit is left to ripen at room temperature for a few days until it becomes translucent-greyish and soft (Chan-Blanco, et al., 2006). Stage 5 is considered the optimal stage for industrial processing, especially for juice production, due to its high juice extraction rate of about 50% (w/w). For instance, 78,100 kg of noni fruit per hectare of plantation can yield approximately 35,000 L of juice (Chan-Blanco, et al., 2006; Nelson and Elevitch, 2006). Furthermore, ripe noni fruit is more nutritious than fruit at other ripening stages (Motshakeri and Ghazali, 2015). This is because ripe noni fruit puree contains an average of 1.13 mg/g of vitamin C, which acts as a potent antioxidant against free radicals and prevent biomembranes from lipid peroxidation damage (West, Deng and Jensen, 2011; Pandy, et al., 2020). Additionally, vitamin C content in 100 g of noni puree provides 251% of the recommended daily requirement for adults (West, Deng and Jensen, 2011).

2.2 Biochemical Composition of Noni Fruit

The ripe noni fruit is primarily composed of 90% water and 10% dry matter, which includes soluble solids (fructose, glucose, cellulose, and sucrose), protein, dietary fibers, minerals (calcium, sulfur, magnesium, potassium, phosphorus, sodium, and selenium), and amino acids (valine, methionine, phenylalanine, tryptophan, lysine, isoleucine, aspartic acid, glutamic acid, tyrosine, arginine, leucine, threonine, histidine, etc.) (Chunhieng, Hay and Montet, 2005; Golden and Lindsay, 2012; Motshakeri and Ghazali, 2015). Besides, several studies reported that ripe noni fruit exhibit higher level of bioactive compounds than the unripe fruit. For instance, total phenolics content of ripe fruit ranged from 3053.20-3647 µg GAE/g FW while the total phenolics content of the unripe fruit ranged from 1426.50-3022.80 µg GAE/g FW (Lewis Lujan, et al., 2014). Higher level of ascorbic acid was also reported in ripe noni fruit (53.19-182 mg/100g FW) than the unripe fruit (76.24-100 mg/100g FW) (Iloki Assanga, et al., 2013; Ruhomally, et al., 2016). The main phenolic compounds that are detected in the noni fruit include scopoletin (45.76-65.16 µg/g FW), rutin (72.98-81.34 µg/g FW), quercetin (0.29 mg/100 g FW) and vanillic acid (0.26 mg/100 g FW) (Dussossoy, et al., 2011). Other bioactive compounds such as iridoids (Choi, et al., 2021), alkaloids (xeronine) (Pandy, et al., 2020), and lignans (Inada, et al., 2017) also have been reported to be found in noni fruit.

Furthermore, the fruit is also rich in organic acids such as, malic acid, fumaric acid, citric acid, benzoic acid, tartaric acid, malonic acid, octanoic acid, hexanoic acid and as well as vitamins, particularly ascorbic acid and provitamin A (Farine, et al., 1996;Pino, Márquez and Castro, 2009; Singh, 2012; Bittová, et al., 2015; Wall, et al., 2015; Nascimento, et al., 2018). The presence of organic acids contributes to the low pH of noni fruit (pH < 4.9) (Lewis Luján, et al., 2014). Additionally, the hexanoic and octanoic acids are responsible for the pungent smell and soapy taste of the ripe noni fruit (Wei, et al., 2003). The pungent smell is owing to the hydrolysis of hexanoic and octanoic acids, turning them volatile at the ripen stage (Chunhieng, Hay and Montet, 2005). The sulphur-containing compounds such as dimethyl trisulfide, dimethyl disulfide, and 3-(methylthio)-1-propanol in noni fruit could also contribute to the pungent smell (Chunhieng, Hay and Montet, 2005; Pino, et al., 2010). Besides, the presence of ketone such as 2-heptanone was found to contribute to the cheese-like odour of noni fruit (Wang, et al., 2021). Table 2.1 summarised the biochemical composition that are present in noni fruit.

Table 2.1: Summary of biochemical composition in noni fruit.

Biochemical composition	Examples
Dry matter	Soluble solids, protein, dietary fibers, minerals, amino acids
Bioactive compounds	Phenolic compounds, iridoids, alkaloids, lignans
Organic acids	Malic acid, fumaric acid, citric acid, benzoic acid, tartaric acid, malonic acid, octanoic acid, hexanoic acid, ascorbic acid
Vitamin	Provitamin A
Sulphur-containing compounds	Dimethyl trisulfide, dimethyl disulfide, and 3-(methylthio)-1-propanol
Ketone	2-heptanone

2.2.1 Bioactive Compounds

Bioactive compounds are substances that exhibit biological activity and may promote beneficial health effects on organisms and/or induce toxic effects, depending on its native, bioavailability and the ingested dose of the substance (Guaadaoui, et al., 2014). These compounds are categorised into several groups based on their chemical structure and functions, such as phenolic compounds, alkaloids, and terpenes, as shown in Table 2.2.

Table 2.2: General classifications of bioactive compounds.

Bioactive compounds			References
Polyphenols/ Phenolic compounds	Flavonoids	Flavones: apigenin, tangeretin, baicalein, luteolin, acacetin	Panche, Diwan and Chandra, 2016; Semwal, et al., 2019
		Flavonols: quercetin, rutin, kaempferol	
		Isoflavones: genistin, glycitein, genistin	
		Flavanones: hesperetin, naringenin	
		Anthocyanins: cyanidin, malvidin, peonidin	
		Chalcones: arbutin, phloretin, chalconaringenin	
	Tannins	Condensed tannins: proanthocyanidins	Zhao, Wu and Wang, 2015
		Hydrolysable tannins: taragallotannins, caffetannins	
	Stilbenes	Resveratrol	
	Phenolic acids	Hydroxybenzoic acids: protocatechuic acids, vanillic acids, syringic acid, gentisic acid, salicylic acid, *p*-hydroxybenzoic acid, gallic acid	Vincente, et al., 2014
		Hydroxycinnamic acids: *p*-coumaric, ferulic, caffeic and sinapic acids	
Terpenes (isoprenoids)	Monoterpenes: thymol, limonene, eucalyptol, iridoids		Inada, et al., 2017; Segneanu, et al., 2017; Cyberlipid, 2021
	Diterpenes: abietic acid, gibberellin, retinol, cafestol		
	Triterpenes: squalen, lanosterol		
	Tetraterpenes: carotenoids, lycopen		
	Sesquiterpenes: valerenic acids, abscisic acid, farnesol, parthenolide		
	Sesterterpenes: ophiobolin A, gascardic acid, haslene		
Alkaloids	Caffeine, ephedrine, yohimbine, nicotine, quinine,		Segneanu, et al., 2017

2.2.2 Phenolic Compounds of Noni Fruit

Phenolic compounds are secondary metabolites found in plant, characterised by an aromatic ring attached to one or more hydroxyl groups. They can range from basic phenolic molecules to complex polymers with high molecular weights (Lin, et al., 2016). These compounds can be classified into several groups, including phenolic acids, flavonoids, stilbenes, and tannins (Kumar, Ahmad and Zaidi, 2019). In the case of noni fruit, the main phenolic compounds identified are scopoletin, rutin, quercetin, and vanillic acid (Dussossoy, et al., 2011).

Scopoletin is a coumarin that has been perceived as biomarker for quality control and pharmacokinetic study of noni products (Pandy, et al., 2014). It exists in two aromatic rings binding with a hydroxyl group, a methoxy group and one oxo group as shown in Figure 2.2. Scopoletin exhibits various bioactivity including antioxidant, antimicrobial, anti-inflammatory, anti-carcinogenic activities, and neuroprotective effect that are essential for human health (Antika, et al., 2022). Anti-depressant effect also has been demonstrated by scopoletin in several animal model studies (Capra, et al., 2010; Luo, et al., 2020).

The rutin and quercetin are flavonoids (Figure 2.2). Flavonoids are the main bioactive compounds in fruits and are usually distributed into six subgroups as presented in Table 2.1. They exist in glycosides (with sugar molecule) and aglycones (without sugar molecule) and the level of glycosylation

determine their antioxidant potency. Frequently, myricetin and quercetin (aglycones) are more active than rutin (glycosides) (Haminiuk, et al., 2012). Numerous biological activities involving antiallergenic, antiviral, anti-inflammatory, and vasodilating actions are found in flavonoids. They are strong antioxidant and widely applicable for treatment of inflammatory disorders (David, Arulmoli and Parasuraman, 2016).

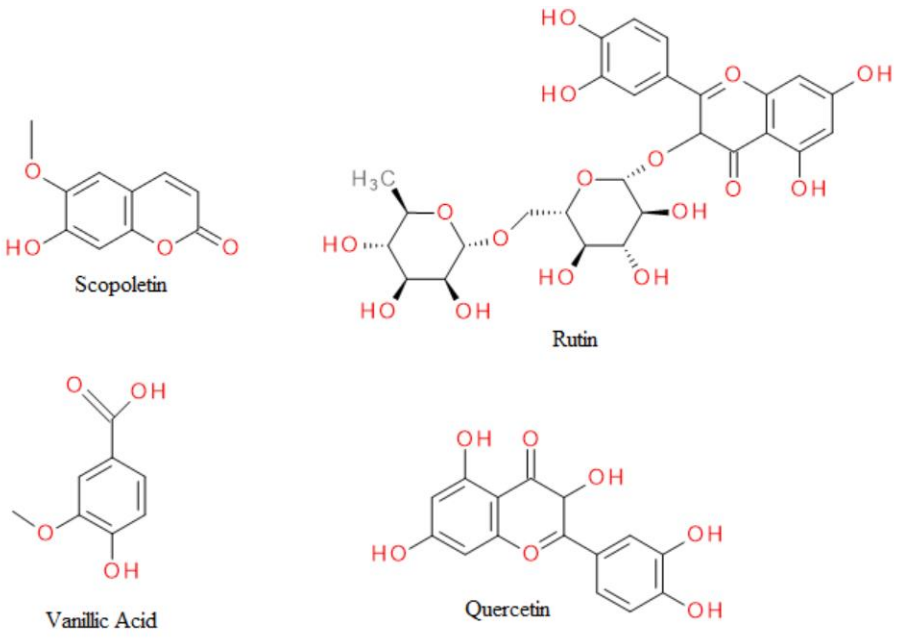

Figure 2.2: The chemical structures of main phenolic compounds in noni fruit.

Phenolic acid is distributed into hydroxybenzoic and hydroxycinnamic acids (Table 2.1). In contrast to other phenolic compounds, both hydroxycinnamic and hydroxybenzoic acids are acidic due to presence of carboxylic group (-COOH) in its structure, as demonstrated by the vanillic acid in Figure 2.2. Vanillic acid is a hydroxybenzoic acid that is widely utilised in food manufacturing industry as antioxidant (prevent browning), preservative, and flavouring agent (Ali, Al-Abbasy and Younis, 2021). Vanillic acid also manifests board spectrum of bioactivity on obesity, neurodegenerative, cancer, diabetes, cardiovascular, and hepatic diseases (Kaur, et al., 2022).

The composition of phenolic compounds highly depends on ripening stages, parts of plants, harvesting seasons, and geographical factor (Abou Assi, et al., 2017; Almeida, de Oliveira and Hotza, 2019). Previous studies have reported the abundant presence of phenolic compounds such as anthraquinones in leaves, bark, and root of noni plant (Potterat and Hamburger, 2007; Almeida, de Oliveira and Hotza, 2019), whereas fresh or fermented noni juice was found to contain very low or no anthraquinones (Sina, et al., 2021). On the other hand, the levels of phenolic compounds, namely scopoletin, rutin, and catechin, exhibited variation in different harvesting seasons. For instance, Lewis Luján, et al. (2014) demonstrated relatively low concentrations of scopoletin and catechin during May-June, with values of 26.91 μg/g FW and 33.94 μg/g, respectively as compared to rutin (60.35 μg/g FW), meanwhile the average values of scopoletin, catechin, and rutin during February-March and November seasons were comparable. Study from Lin, et al. (2014) also observed variation in the scopoletin and rutin of noni fruit harvested at different seasons. In brief, both

scopoletin and rutin levels were found to be higher in June-November than March-August due to drought and temperature stress, which further trigger production of more phenolic compounds to overcome the stress.

2.2.3 Organic Acids of Noni Fruit

Organic acids are naturally occurring soluble compounds found in fruits, commonly used to assess fruit juice quality, including aspects such as colour, shelf-life, and ripeness; thus, organic acids serve as an index to gauge consumer acceptability (Ma, et al., 2018). The main organic acids found in noni fruit are ascorbic acid (24 to 158 mg/100 g), malic acid (3.28 mg/kg), citric acid (0.16 to 2.48 mg/g), fumaric acid (1.03 mg/kg), benzoic acid (0.08 to 8.6 mg/L), malonic acid (1.46 mg/kg), and tartaric acid (1.40 mg/mL) (Farine, et al., 1996; Pino, Márquez and Castro, 2009; Singh, 2012; Bittová, et al., 2015; Wall, et al., 2015).

The levels of organic acids are influenced by various pre- and post-harvest factors, such as plant management methods, temperature, light exposure, fertilisation, water supply, maturation stages, irradiation, and storage conditions (Vallarino and Osorio, 2018). As reported by Ruhomally, et al. (2016), ripe noni fruit contained a higher ascorbic acid level (76.24 mg/100 g) than unripe fruit (53.19 mg/100 g). Additionally, the organic acids levels of noni fruit product such as noni juice might vary depending on the processing methods. Wall, et al. (2015) reported that the fermented noni juice contained higher levels of total organic acids (26.6 mg/mL) than the fresh noni juice (22.59 mg/mL).

Organic acids are claimed to exhibit various biological activities. For example, ascorbic acid is well known for its antioxidant property against free radicals such as reactive nitrogen species and reactive oxygen species as well as reducing oxidative stress (Pehlivan, 2017; Dhalaria, et al., 2020). Furthermore, ascorbic acid also has been reported to possess depigmenting effect (Hwang, et al., 2009; Panich, et al., 2011). The fumaric acid has been applied in treatment for skin disease such as psoriasis in several European countries for over 20 years (Balak, 2014; Kirtschig and Schaefer, 2015; Blair, 2018). Malic and citric acid have also demonstrated cardioprotective effects on myocardial ischemia/reperfusion injury in *in vivo* rat model, potentially due to their anti-inflammatory, antiplatelet aggregation, and direct cardiomyocyte protective effects (Tang et al., 2013). Not only that, citric acid has shown promising kidney stone prevention (Gul and Monga, 2014) and ischemic liver injury protection (Kim, et al., 2019) as well as protection of brain and liver from oxidative damages (Abdel-Salam, et al., 2014).

On the other hand, Lee, Jang and Kim (2021) demonstrated that malonic acid possesses anti-inflammatory effects that could be a potential treatment for neuroinflammatory diseases. Tartaric acid is found to be a potential antihypertensive agent contributed by its capability in balancing blood pressure. Previous literature review claimed that appropriate dosage of benzoic acid could help to improve gut health (Mao, et al., 2019). All the biological activities as stated above indicating that the presence of organic acids could promote noni fruit as a nutritive and healthy food product.

Other than exhibiting biological activities, organic acids are widely applied as acidulants, flavour enhancers, preservatives, and antioxidant in the food industry. The common acidulants such as citric, malic, fumaric, and tartaric acids are used to increase the acidity of foods as well as flavour enhancers (Gurtler and Mai, 2014). Ascorbic acid plays the role as antioxidant in the food industry to reduce and retard oxidation, preserve the quality and nutrients of food products (Lourenço, Moldão-Martins and Alves, 2019). Benzoic acid and its salt (sodium benzoate) is used as preservative to prevent bacteria, yeast, and mold growth in food products (Joye, 2019). In this case, those organic acids present in noni fruit might play the role as antioxidant, antimicrobial agent as well as providing flavour to the noni fruit.

2.3 Health Benefits of Noni Fruit

Noni juice is gaining global popularity due to the recognised health benefits. An epidemiological study by Westendorf and Mettlich (2009) based on consumption of noni juice (Tahitian Noni® juice) by more than 1000 Europeans suggested that noni juice can help with digestion, immunological function, sleep, and gum help. Polynesian have been using noni fruit to treat numerous illness/discomforts, including skin allergies, warts, hemorrhoids, osteoarthritis, rheumatism, joint problems, diabetes, burns, etc (West, et al., 2018). In the USA, noni juice extracts are recognised as a dietary supplement owing to its antifungal, antiparasitic, and analgesic properties. In addition, people also used it to relief illness like cough, skin infections, urinary tract infections, tuberculosis, etc. by consuming the fresh or fermented juice (Sina, et al., 2021).

In noni fruit, its antioxidant activity is attributed to the presence of various bioactive compounds such as coumarin, flavonoids, phenolic acids, iridoids, ascorbic acid, and tocopherol (Pandy, et al., 2020). The *in vitro* study of (Wang, et al., 2002) showed that noni juice exhibited 2.8, 1.4, and 1.1 times higher antioxidant activity against superoxide anion free radicals and lipid hydroperoxide compared to vitamin C, pycnogenol, and grape seed, respectively. Other pharmacological activities of noni including anti-inflammatory (Dussossoy, et al., 2011); antimicrobial (Meza-Gutiérrez, et al., 2022); antidepressant (Deng and West, 2011; Narasingam, et al., 2017); anti-drug addiction (Narasingam, Pandy and Mohamed, 2016; Pandy, et al., 2018) and anti-alcohol dependence (Khan and Pandy, 2020), anti-diabetic (Nerurkar, et al.,

2012), anti-dyslipidemia (Shoeb, et al., 2016), anticancer effects (Chanthira Kumar, et al., 2022) also have been reported in previous studies.

2.4 Noni Plants and Its Byproducts

The translucent-greyish noni fruit (Stage 5) are commonly used to produce juice either fermented or fresh one (Figure 2.3). Fermented noni juice is usually produced through drip extraction. The noni fruit is stored in a closed jar for 2-8 weeks and the seepage of juice will be periodically drained off for consumption purposes (Nelson and Elevitch, 2006). Fresh noni juice is extracted using mechanical apparatus (Yashaswini, et al., 2014). The ripe fruits have an unpleasant cheesy odour (Potterat and Hamburger, 2007). Therefore, they are often flavoured with other fruit juices such as grape or blackberry juice, before being commercialised to overcome the astringent and cheesy odour (Chan-Blanco, et al., 2006).

Figure 2.3: Fresh (left) and fermented (right) noni juices (Nelson and Elevitch, 2006).

The noni fruit and leaf can be processed into powder or concentrate, making them versatile ingredients for a range of cosmetic products. These products include soap, hand cream, and shampoo (Nelson and Elevitch, 2006). In Malaysia, there is an extensive selection of noni-based products. These products include noni extract powder, fermented juice, capsules, tea, coffee, coffee-based collagen beverage, balm and even toothpaste as shown in Figure 2.4. With such a diverse range of noni-infused options, consumers can easily incorporate the beneficial properties of noni fruit and leaf into their daily beauty, personal care routines and as well as the diet.

Figure 2.4: Examples of noni derived products (Bionutricia Manufacturing, 2023; Sureco Sure Return, 2023). Note: (a) noni coffee; (b) noni balm; (c) coffee-based collagen beverage; (d) fermented noni juice; (d) noni toothpaste; (e) noni extract powder.

2.5 Storage Stability of Noni Juice

Commercialised noni juice is usually fermented due to its perishable nature. The fermentation process of noni juice occurs naturally and it is difficult to stop once the fruit is overripe. Fermentation could prolong the shelf-life of fruit juice due to low pH of the final products. Hence, the fermented noni juice with pH 3.1-3.5 can be stored at room temperature for at least a year or longer in tightly sealed container (Nelson and Elevitch, 2006). In spite of the acidic nature of fermented noni juice, pasteurisation is needed to ensure safety of consumption (Brown, 2012). This is because noni fruit is susceptible to contamination during harvesting and processing (Nelson and Elevitch, 2006).

On the other hand, fresh noni juice that is extracted using mechanical apparatus such as hydraulic juice press or juice extractor is unfermentable, it is usually consume immediately, or stored under refrigeration/frozen condition to extend a longer life-span and maintain its organoleptic properties (Nelson and Elevitch, 2006; Rajauria and Tiwari, 2018). This is because fresh fruit juice is usually perishable due to high moisture content, especially the freshly squeezed juice that has not pasteurised (Ashurst, 2016). Kaddumukasa, et al (2017) demonstrated that unpasteurised fresh juices stored at room temperature (24°C) can be safely consumed within 1 day, meanwhile the refrigerated (4°C) one can last to a maximum of 2 days (Kaddumukasa, et al., 2017). Other literature study reported that unpasteurised fresh juice has a life-span of up to 7 days when it is stored, distributed, and displayed under refrigerated (2-5°C) conditions (Ashurst, 2016).

2.5.1 Storage Temperature

Storage temperature is one of the significant factors that affect the nutritional values and shelf-stability of fruit juice. A research study by Yang, et al. (2007a) on the effects of different storage temperatures on the radical scavenging activity of fresh noni juice were conducted. Results promoted that fresh noni juice stored at frozen temperature (-18°C) reduced the radical scavenging activity the least by 15%, refrigerated temperature (4°C) reduced by 55%, while room temperature (24°C) reduced the greatest by 90%, after 3 months of storage. It is noteworthy that frozen and refrigerated temperatures exerted better retention of antioxidants in noni juice than that of room temperature.

Temperature around 40°F to 140°F (4.44°C to 60°C) is a danger zone, as this range of temperature allow rapid microbial growth (USDA, 2017). This phenomenon will result in deterioration and spoilage of fruit juice, thereby shortening the storage stability of fruit juice. Therefore, it is recommended to store fresh food products such as fruit juice at refrigerated temperatures to prevent spoilage caused by microorganisms and toxin production, as well as to prolong the shelf-life (Kaddumukasa, et al., 2017).

2.6 Microbial Load of Noni Juice

The microbial load of noni juice studied in the recent research is ranging from <1 to 4 log CFU/mL as tabulated in Table 2.3. It was reported that the variation in microbial load of noni juice is depending on the maturity stages of fruit, postharvest handling methods, and fermentation scale (Khadka, et al., 2017; Amadi and Nwala, 2021).

Table 2.3: Total bacteria and yeast and mold count (log CFU/mL) of noni juice.

Fresh Noni Juice		**Fermented Noni Juice**		**Reference**
Bacteria	Yeast and mold	Bacteria	Yeast and mold	
3	1	NA	NA	Chan-Blanco, et al., 2005
<1	NA	<4	NA	Wall, et al., 2015
<1	<1	NA	NA	Adjou, et al., 2020
4	3	4	2	Amadi and Nwala, 2021

Note: NA = Not available.

The microbiological risk of noni juice also has been studied. Results revealed that noni juice does not exhibit direct microbiological risk for consumer as the bacteria and yeast and mold count of noni juice were below 1 log CFU/mL (Table 2.2) (Adjou, et al., 2020). Although noni juice has shown promising antimicrobial characteristic against foodborne pathogen like *E.coli* O157:H7 (Meza-Gutierrez, et al., 2022), lacking in standardisation in the noni juice processing can cause microbial contamination, safety issues and may further expose to health hazards. Inconsistency in processing will also affect the noni juice quality (Motshakeri and Ghazali, 2015). Thus, proper handling and

processing of noni juice are important to control the growth of spoilage microorganism and its quality. Study by Wall, et al. (2015) demonstrated that there are no detectable bacteria in pasteurised noni juice as compared to fresh noni juice (<1 log CFU/mL), indicating the importance of proper processing of noni juice for safe consumption.

2.7 Fruit Juice Treatments

2.7.1 Traditional Technique

Fruit juice is considered as a low-risk food due to its low pH property. However, it is still susceptible to contamination by several ways: vegetative microorganism, soil, air, dust, insect pest, animals, human, equipment, and water (Dewanti-Hariyadi, 2014). Thus, fruit juice is usually preserved by thermal treatments to ensure safe consumption in addition to extend shelf-life (Deak, 2014). Thermal treatments are classified into sterilisation and pasteurisation (Table 2.2). Exposure to high temperature can generate stress and causes lipid phase transitions and protein confirmation alterations, which ultimately results in cell death (Petruzzi, et al., 2017). Moreover, enzymes such as polyphenol oxidase (PPO) and peroxidase (POD) are responsible in browning and degradation of polyphenols, which further results in discoloration and reduction of antioxidant activity. Hence, inactivation of these enzymes is essential in preserving the nutritional quality of fruit juice (Petruzzi, et al., 2017).

The major difference between sterilisation and pasteurisation: sterilisation is usually applied for the low acidic (pH > 4.5) fruit juice such as mango, banana, watermelon, coconut, longan, and jackfruit juices. The low acidic fruit juice is susceptible to *Clostridium botulinum* which is the spore-forming, heat-resistant, and toxin-producing bacteria (Catherine M.G.C. Renard and Jean Francois Maingonnat, 2012). Hence, sterilisation targets to the complete destroy of microorganism including vegetative, pathogenic, toxin-

forming, and spore-forming microorganism (Chiozzi, Agriopoulou and Varzakas, 2022). In contrast to sterilisation, pasteurisation targets to destroy vegetative pathogenic and non-pathogenic microorganism (Chiozzi, Agriopoulou and Varzakas, 2022) in highly acidic (pH < 4.5) fruit juice, namely, orange, apple, lemon, grape, strawberry, pineapple, and berries juices (Ağçam, Akyildiz and Dündar, 2018). Pasteurisation is sufficient to preserve the highly acidic fruit juice as growth or presence of spore-forming bacteria is not the risk factor. High acidic fruit juice also contains enzymes such as catalase, PPO and POD that will cause browning and nutrients deterioration. Some of these enzymes, mainly POD exhibits higher heat resistance than spoilage microorganism. Thus, pasteurisation is aiming to inactive the enzymes and non-spore forming spoilage microorganism (Ağçam, Akyildiz and Dündar, 2018). Generally, either pasteurisation or sterilisation, both are the thermal treatments that share the identical purpose which is to decrease microbial load of fruit juice, ensuring the safe consumption, healthy, quality, and extended shelf-life of fruit juice.

Pasteurisation is commonly used in fruit juice treatments and numerous studies have shown promising microbial reduction in the pear juice (Saeeduddin, et al., 2015), litchi juice blend with aloe-vera (Swami Hulle and Srinivasa Rao, 2016), strawberry juice (Tomadoni, et al., 2017), watermelon juice (Wang, et al., 2018; Mandha, et al., 2023), and mango juices (Mandha, et al., 2023), in comparison to the respective fresh juice samples. The significant reduction of browning enzymes activity (PPO and POD) also observed in red grapefruit juice (Gao, et al., 2015), Pear juice (Saeeduddin, et al., 2015), litchi juice blend with

aloe-vera (Swami Hulle and Srinivasa Rao, 2016), and watermelon juice (Wang, et al., 2018; Mandha, et al., 2023), and mango juices (Mandha, et al., 2023).

Pasteurisation is the most cost-effective processing method to ensure microbial safety and enzymes inactivation; however, its negative impact on the nutritional and quality of fruit juice have been reported in previous studies. A study by Saeeduddin, et al. (2015) demonstrated pasteurised pear juice contained significantly lower ascorbic acids, TPC, TFC, and antioxidant capacity that that of fresh pear juice. Furthermore, despite the non-detectable microbial load and complete inactivation of POD in pasteurised red grapefruit juice, Gao, et al. (2015) reported that pasteurisation resulted in significant loss of ascorbic acid, TPC, DPPH, and FRAP, as well as significant alteration of colour attributes (L^*, a^*, b^*) and increased in browning degree were also observed in pasteurised samples in as compared to fresh sample. Pasteurisation would accelerate the degradation of heat-labile bioactive compounds, thereby compromising the nutrients in the fruit juice (Lagnika, et al., 2017), in addition to negatively affect the colour of fruit juice (Gao, et al., 2015).

Previous study also demonstrated that fresh cape gooseberry juice contained higher amounts of ascorbic acid (37.7 mg/100 g) than thermally pasteurised juice (30.20 mg/100 g) after 21 days of storage at 4°C. In addition, greater loss in TPC and DPPH of pasteurised cape gooseberry juice was observed in comparison to the fresh sample (Rabie, et al., 2014). Besides, Escudero-López, et al. (2016) reported a 42% and 24.6% loss in ascorbic acid and flavanones level, respectively, in pasteurised fermented orange juice as

compared to unpasteurised fermented orange juice. The details regarding the effects of pasteurisation on different fruit juices are tabulated in Table 2.4.

Table 2.4: Application of pasteurisation on fruit juices processing.

Fruit juice	Pasteurisation Conditions	Key Findings	Reference
Cape gooseberry juice	-90°C/10 min -Storage study: 21 days, 4°C	-Pasteurisation significantly decreased TC and retained lower TC after storage. -Greater loss in TPC observed in pasteurised sample than fresh sample. -A decrease of DPPH by 39% for the pasteurised sample during storage. -Pasteurised sample retained lower ascorbic acid than fresh samples after immediate pasteurisation and at the end of storage study. -pH, titratable acidity, total soluble solids did not significantly change after immediate pasteurisation as compared to fresh sample, but they significantly changed during 21 days of storage. -Pasteurisation change the colour attributes (L^*, a^*, b^*, h^*, ΔE). -Viscosity increased after pasteurisation and retained higher value than that of fresh sample after storage.	Rabie, et al., 2014
Red grapefruit juice (*C. grandis* L.)	110°C/8.6 s	-Complete inactivation of POD after pasteurisation as compared to fresh sample. -Not detectable total plate count and yeast and mold count as compared to fresh sample. -Significant loss of TPC and ascorbic acid after pasteurisation as compared to fresh sample. -Decreased of DPPH and FRAP in pasteurised sample was observed as compared to fresh sample. -Pasteurisation significantly change the colour attributes and browning degree (L^*, a^*, b^*).	Gao, et al., 2015

Table 2.4: (Continued.)

Fruit juice	Pasteurisation Conditions	Key Findings	Reference
Pear juice	65°C/10 min and 95°C/2 min	-Significant reduction of PPO and POD at both pasteurisation conditions. -Significantly lower total plate count and yeast and mold at 65°C/10 min and non-detectable total plate count and yeast and mold at 95°C/2 min. -Significant reduction of ascorbic acid, TPC, TFC, and antioxidant capacity at both pasteurisation conditions. -Total soluble solids, pH, and titratable acidity were unaffected in all samples.	Saeeduddin, et al., 2015
Fermented orange juice	85°C/30 s	-Significant decreased in ascorbic acid by 42% and total flavonones by 24.6% after pasteurisation as compared to unpasteurised sample. -Major carotenoids pigments significantly decreased by 44.9% to 51.6% after pasteurisation as compared to unpasteurised sample. -Significant decreased in antioxidant capacity after pasteurisation as compared to unpasteurised sample.	Escudero-López, et al., 2016
Litchi juice blend with aloe-vera	95°C/10 min	-Aerobic mesophiles and yeast and mold below 1 log CFU/mL detection limit. -PPO and POD reduced for 79% and 78%, respectively. -Significant decreased in ascorbic acid level to 31%.	Swami Hulle and Srinivasa Rao, 2016

Table 2.4: (Continued.)

Fruit juice	Pasteurisation Conditions	Key Findings	Reference
Strawberry juice	-90°C/60 s -Storage study: 10 days, 5°C	-Non-detectable mesophilic bacteria, psychrophilic bacteria, and yeast and mold in pasteurised sample as compared to fresh sample. -Pasteurisation retained the lowest mesophilic and psychrophilic bacteria count while yeast and mold remain undetectable after the storage as compared to fresh sample. -DPPH decreased drastically at the end of storage, retaining the lowest DPPH than fresh sample. -TPC was higher in pasteurised sample than fresh sample at Day 0. -Total soluble solids and titratable acidity of fresh and pasteurised samples were unaffected. -Colour (L^* and h^*) changed after pasteurisation.	Tomadoni, et al., 2017
Watermelon juice	110°C/2 s, 120°C/2 s, 135°C/2 s	-Total microbial colonies significantly decreased after pasteurisation at different temperatures in comparison to fresh sample. -TPC reduced significantly at lower pasteurisation temperature while preserved with increasing pasteurisation temperature. -PPO decreased with increasing pasteurisation temperature. -Significant changed in colour difference (ΔE) at higher pasteurisation temperature (120°C and 135°C). -Soluble solids content was unaffected by pasteurisation. -Pasteurisation did not affect the aroma of watermelon juice as compared to fresh sample.	Wang, et al., 2018

Table 2.4: (Continued.)

Fruit juice	Pasteurisation Conditions	Key Findings	Reference
Watermelon and mango juices	80°C/1 min, 2.5 min, 5 min, 10 min, 15 min	-Reduction of total plate counts and yeast and mold count to below 1 log CFU/mL detection limit at different pasteurisation durations. -Significantly reduced POD and PPO with increasing duration. -Decreased ascorbic acid for 27% in mango juice, while undetectable in watermelon juice with increasing pasteurisation duration. -Colour of mango juice was unaffected. -Significant change in colour of watermelon juice with increasing pasteurisation duration. -Titratable acidity, pH and total soluble solids of watermelon and mango juices were unaffected by different pasteurisation. -TPC, DPPH and ABTS of watermelon and mango juices were unaffected.	Mandha, et al., 2023

2.7.2 Sonication

Recently, sonication has been acknowledged as an alternative to conventional thermal treatment in the food industry, especially in fruit juice processing (Tiwari, et al., 2009; Zinoviadou, et al., 2015). It is also widely applied in other industries as a surface disinfectant for medical equipment, jewellery, and aircraft (Kentish and Feng, 2014). Sonication is classified into high and low energy. Low energy sonication usually ranges at frequency above 100 kHz and intensity at <1 W/cm^2. This low energy sonication is used for non-invasive food process control and characterisation of physicochemical properties of food. It is also used for surface sterilisation, emulsification, drying, and freezing including meat tenderisation. Alternatively, high energy sonication ranges at frequency between 18 to 100 kHz and intensity at >1 W/cm^2. The high energy sonication is usually applied for enzymes/proteins extraction, enzyme inactivation, liquid food degassing as well as induce nucleation for crystallisation (Knorr, et al., 2004). Sonication can work alone or incorporated with mild heat treatment (thermosonication), combined with pressure (<600 MPa) (manosonication) or combination of sonication, heat treatment and pressure (manothermosonication) to enhance the overall performance (Zinoviadou, et al., 2015).

Sonication induces cavitation which is a phenomenon where bubbles are formed and collapse, which further results in cell wall/structure disruption and dispersion of bound particles (Margean, et al., 2020). During cavitation process, sound waves compress and stretch liquid molecules. Negative pressure is generated during the stretching of liquid molecules. When the negative pressure exceeds the force between the liquid molecules, the liquid molecules decompose and form a cavity. The cavity then grows and forms bubbles, where the bubbles continue to expand and collapse, generating a local high pressure (50MPa) and temperature (5500K) (Fan, Wu and Chen, 2021).

There are two phases of cavitation: stable and transient phases, as presented in Figure 2.5. During stable cavitation, the equilibrium-sized bubbles oscillate in a regular pattern for multiple acoustic cycles, forming microstreaming phenomenon. This generates shear force and cause cell wall disruption. In transient cavitation, the bubbles formed are unstable and they collapse vigorously in a short time, changing the local temperature and pressure. This results in cell wall destructions and triggers the release of biomolecules from the cells (Dolas, Saravanan and Kaur, 2019; Fan, Wu and Chen, 2021), as shown in Figure 2.6.

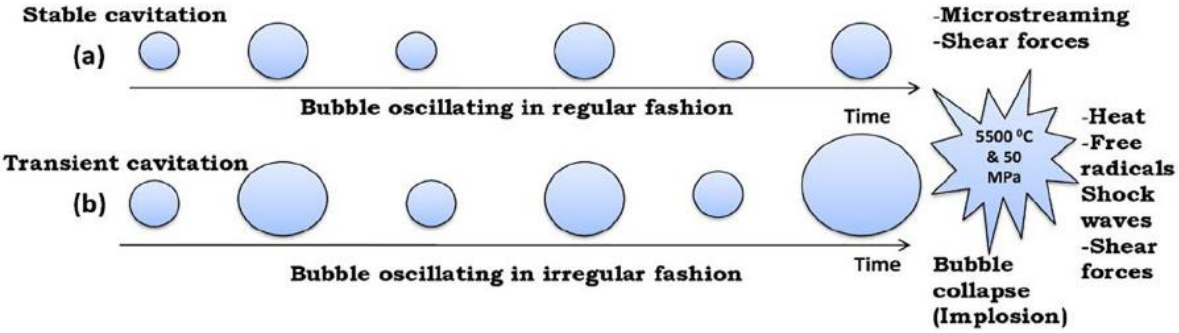

Figure 2.5: The phases of cavitation. Note: a) Stable cavitation b) Transient cavitation (Dolas, Saravanan and Kaur, 2019).

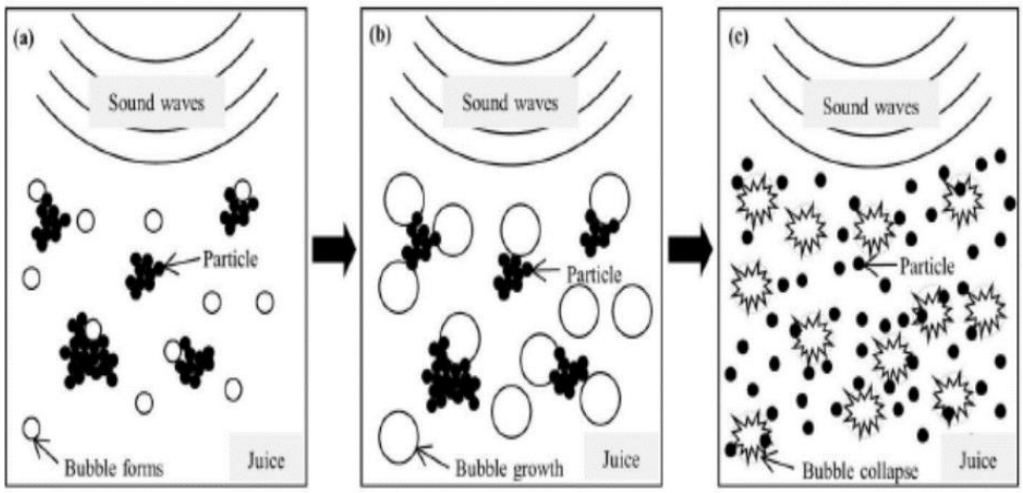

Figure 2.6: Cavitation phenomenon in fruit juice (Rani, et al., 2020). Note: a) bubbles formation; b) bubbles growth to the maximum; c) bubbles collapse, causing particle dispersion and cell disruption.

The cavitation effects cause chemical, thermal, and mechanical changes in the medium. To illustrate, during sonication, water molecule breakdown into free radicals (H^+ and OH^-). Later, the hydrogen atoms and hydroxyl radicals form hydrogen peroxide (H_2O_2), inducing chemical changes in the aqueous medium. In terms of thermal changes, heat is generated when a part of the ultrasonic energy is absorbed. This thermal effect enables sonication to be applied in thawing, drying and sterilisation processes. Furthermore, mechanical changes, such as cell wall disruption and enzyme inactivation, are mainly attributed to the mechanical shocks during sonication (Dolas, Saravanan and Kaur, 2019).

Sonication has been recognised as a potential alternative processing method to conventional pasteurisation for extending the shelf-life by inactivating microorganism and improving the nutritional values of fruit juice. This is because the cavitation effect of sonication promotes the release of free phenolics and organic compounds from the cell walls, as well as the bound phenolic compounds from colloidal particles, thereby increasing the nutritional value of fruit juice (Margean, et al., 2020). Additionally, the cell wall rupture, formation of free radicals and hydrogen peroxide during cavitation play important role in microbial inactivation (Figure 2.7). Therefore, sonication has been perceived to fulfil the Food and Drug Administration (FDA) requirement of a 5-log reduction in relevant microorganisms in fruits and vegetable products (Dolas, Saravanan and Kaur, 2019).

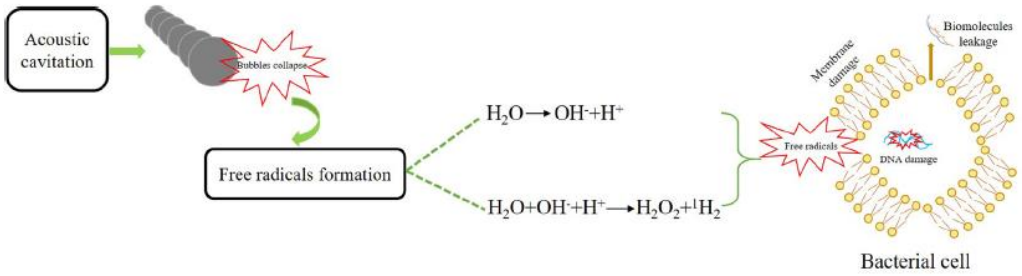

Figure 2.7: Microbial inactivation of free radicals by cavitation (Fan, Wu and Chen, 2021).

Numbers of studies have been done to evaluate the effect of sonication on the quality of different fruit juices. Generally, the results demonstrated significant improvement in the bioactive compounds as well as antioxidant capacity of grapefruit juice, purple cactus pear juice, maoberry fruit juice, strawberry juice, apple-grape juice blend, pomelo juice, and red pitaya fruit juice after the sonication treatment as compared to the fresh and/or thermal pasteurised samples (Aadil, et al., 2015; del Socorro Cruz-Cansino, et al., 2015; Chaikham, Prangthip and Seesuriyachan, 2016; Gani, et al., 2016; Bhat and Goh, 2017; Yildiz and Aadil, 2020; Aadil, et al., 2020; Basumatary, et al., 2020; Tan, et al., 2023).

Besides, the reduction of browning enzymes activity, such as PPO and POD, has also been demonstrated in sonicated maoberry fruit juice in comparison to the fresh and thermal pasteurised juices (Chaikham, Prangthip and Seesuriyachan, 2016) and sonicated strawberry juice in comparison to the fresh juice (Bhat and Goh, 2017). In addition, microbial load reduction has been observed in sonicated grapefruit juice (Aadil, et al., 2015), maoberry fruit juice

(Chaikham, Prangthip and Seesuriyachan, 2016), purple cactus pear juice (del Socorro Cruz-Cansino, et al., 2015), pomelo juice (Basumatary, et al., 2020), strawberry juice (Yildiz and Aadil, 2020). Moreover, sonication enhances retention of the nutritional content in fruit juices such as purple cactus pear juice, strawberry juice, and carrot-grape juice blend, during storage in comparison to control samples (del Socorro Cruz-Cansino, et al., 2015; Gani, et al., 2016; Nadeem, et al., 2018; Yildiz and Aadil, 2020). The details regarding the effects of sonication on different fruit juices are tabulated in Table 2.5.

Table 2.5: Application of sonication treatment on fruit juices processing.

Fruit Juice	Sonication Conditions	Key Findings	Reference
Grapefruit juice	Ultrasonic bath -28 kHz, amplitude 70% -Sonication: 0 (fresh), 30, 60, 90 min -Temperature: 20°C	-Increased in TC, lycopene, sugar contents (sucrose, glucose, and fructose) and phenolic compounds (phloridzin, epicatechin, caffeic acid, and chlorogenic acid) ($p < 0.05$), with greatest values observed in 90 min of sonication compared to fresh sample. -Total plate count and yeast and mold count decreased after 30, 60, 90 min of sonication ($p < 0.05$), with lowest microbial load observed in sonicated grapefruit juice for 90 min.	Aadil, et al., 2015
Purple cactus pear juice (*Opuntia ficus indica*)	Ultrasonic probe -20 kHz, 1500 W power, amplitude 80%, 13 mm probe -Thermosonication: 0 (fresh), 15, 25 min -Pasteurisation: 70°C, 30 min -Storage: 4°C, 28 days	-Thermosonicated samples exhibited lower total plate count compared to fresh and pasteurised sample. -Enterobacteriae was undetected in thermosonicated sample but detected in fresh and pasteurised samples. -TPC increased after thermosonication and pasteurisation as compared to fresh sample ($p < 0.05$). -Ascorbic acid increased after thermosonication and treated juice at 80% 25 min retained highest value during storage. -ABTS and DPPH increased after thermosonication and retained higher values than fresh and pasteurised samples ($p < 0.05$) during storage. -pH, total soluble solids, titratable acidity, browning index were unaffected by thermosonication. -Thermosonication resulted in significant changes in viscosity and colour attributes (L^*, a^*, b^*, h^*, C^*, ΔE).	del Socorro Cruz-Cansino, et al., 2015

Table 2.5: (Continued.)

Fruit Juice	Sonication Conditions	Key Findings	Reference
Maoberry fruit juice (*Antidesma bunius* L.)	Ultrasonic probe -20 kHz, amplitudes 20-80% -Sonication time: 30 min/amplitude -Thermal treatment: 75°C, 30 min	-Non-detectable total plate count, yeast and mold, fecal coliforms in sonicated (80% of amplitude) compared to fresh sample ($p < 0.05$). -Pasteurisation decreased ascorbic acid, antioxidant capacity (DPPH inhibition, FRAP) ($p < 0.05$) compared to fresh sample. -Sonication at 80% of amplitude decreased PPO ($p < 0.05$) compared to fresh and pasteurised samples. -Comparable POD decreased of sonicated (80% of amplitude) sample compared to pasteurised sample. -Sonication at 60% and 80% of amplitudes increased TPC ($p < 0.05$) compared to fresh juice sample. -Total soluble solids, pH, viscosity, total anthocyanins were unaffected by sonication and pasteurisation. -Sonication and pasteurisation changes colour attributes (L^*, a^*, b^*, C^*, ΔE).	Chaikham, Prangthip and Seesuriyachan, 2016

Table 2.5: (Continued.)

Fruit Juice	Sonication Conditions	Key Findings	Reference
Strawberry juice (*Chandler*, cultivar)	Ultrasonic bath -33 kHz, 60 W power -Sonication: 0 (fresh), 10, 20, 30, 40, 60 min -Temperature: 25°C -Storage: 4°C, 15 days	-Greatest ABTS, DPPH and TPC values were observed after 40 min of sonication ($p < 0.05$) compared to fresh and other sonicated samples. -Sonication for 40 min retained highest ABTS, DPPH and TPC values during storage ($p < 0.05$). -Ascorbic acid increased after sonication for 20 min and constantly decreased during 30, 40, 60 min of sonication ($p < 0.05$). -Sonication for 30 min retained highest ascorbic acid level throughout the storage. -Lowest bacterial and yeast and mold count in 60 min of sonication compared to fresh and other sonicated samples ($p < 0.05$). -Sonication for 40 min retained lowest bacterial and yeast and mold count during storage ($p < 0.05$). -Sonication resulted in significant changes in colour (L^*, a^*, b^*, ΔE), pH, titratable acidity and total soluble solids of strawberry juice. -All sonicated samples retained the colour of strawberry samples during storage as compared to fresh sample, except sonicated samples for 60 min.	Gani, et al., 2016

Table 2.5: (Continued.)

Fruit Juice	Sonication Conditions	Key Findings	Reference
Strawberry juice (*Fragaria x anannassa* Duch.)	Ultrasonic bath -25 kHz, 70% power -Sonication: 0 (fresh), 15, 30 min -Temperature: 20°C	-Polyphenol oxidase activity reduced after 30 min of sonication ($p < 0.05$) -Sonication for 30 min increased TPC, ascorbic acid, total anthocyanins, inhibition of DPPH ($p < 0.05$). -No significant changes for pH, titratable acidity, total soluble solids, turbidity, water activity and colour attributes ($L^*a^*b^*$) in sonicated and fresh strawberry juices. -Viscosity decreased while cloud value increased after sonication for 15 and 30 min ($p < 0.05$).	Bhat and Goh, 2017
Carrot-grape juice blend	Ultrasonic probe -20 kHz, 0.5 in. probe -70% amplitude (525 W power) -Sonication: 0 (fresh), 2, 4, 6 min -Temperature: 15°C -Chemical preservation: 1% potassium-meta bisulphite -Storage study: 90 days, 4°C	-TPC, TFC, total antioxidant activity, reducing power and DPPH were increased after sonication (2, 4, 6 min), with greatest values observed in 6 min of sonication. -Sonication retained higher TPC, TFC, total antioxidant activity, reducing power and DPPH levels compared to fresh and chemically preserved juice blend during storage. -Sonication did not affect pH, but increased total soluble solids at 6 min of sonication.	Nadeem, et al., 2018

Table 2.5: (Continued.)

Fruit Juice	Sonication Conditions	Key Findings	Reference
Apple-grape juice blend	Ultrasonic probe -25 kHz, 70% amplitude -Sonication: 0 (fresh), 5, 10 min -Thermosonication: 40°C (5 and 10 min); 50°C (5 and 10 min); -Thermal treatment (blanching): 100°C, 4 min -Pasteurisation: 72°C, 15 s	-TPC, TFC and total flavonols increased in all sonicated (5 and 10 min) and thermosonicated samples (40°C and 50°C) compared to fresh apple-grape juice blend ($p < 0.05$). -Blanching resulted in lowest amount of TPC, TFC and total flavonols ($p < 0.05$). -Sonication resulted in higher total antioxidant capacity compared to blanching, pasteurisation, and thermosonication. -Sonication for 10 min resulted in highest phenolics level compared to fresh, blanching, pasteurisation, and thermosonication ($p < 0.05$). -Blanching, pasteurisation, sonication, and thermosonication treatments significantly changed the viscosity, turbidity, soluble solids content, and color attributes ($L^*a^*b^*$) ($p < 0.05$). -pH and titratable acidity were unaffected by all treatments.	Aadil, et al., 2020

Table 2.5: (Continued.)

Fruit Juice	Sonication Conditions	Key Findings	Reference
Pomelo juice (*Citrus maxima*)	Ultrasonic bath -Thermosonication: 33 kHz at 30, 40, and 50°C, respectively -Thermosonication: 44 kHz at 30, 40, and 50°C, respectively -Time: 0 (fresh), 15, 30, 45, 60 min -Pasteurisation: 90°C, 60 s	-Thermosonication at 44kHz achieved higher DPPH, TPC, TFC compared to fresh, pasteurised and thermosonicated (33 kHz) samples. -Pasteurisation resulted in lower DPPH compared to fresh sample. -Pasteurisation resulted in lower ascorbic acid level compared to fresh and thermosonication. -Thermosonication reduced total plate count, yeast, and mold counts for approximately 2 log CFU/mL compared to fresh ($p < 0.05$). -Thermosonication and pasteurisation changed the pH, titratable acidity, cloudiness, and browning index of pomelo juice compared to control. -Total soluble solids was unaffected by thermosonication and pasteurisation.	Basumatary, et al., 2020

Table 2.5: (Continued.)

Fruit Juice	Sonication Conditions	Key Findings	Reference
Strawberry juice	-Ultrasonic probe -20 kHz, probe diameter 12.5 mm -Sonication: 0 (fresh), 5, 10, 15 min -Pasteurisation: 72°C, 15 s -Storage study: 14 days, room temperature	-A 5-log reduction of *E. coli* O157:H7 was achieved by sonication for 5 min (5.04 log CFU/mL), 10 min (5.36 log CFU/mL) and 15 min (6.08 log CFU/mL). -Higher retention of colour attributes (*L*a*b**) in sonicated strawberry juice compared to pasteurised sample. -Antioxidant capacity and TPC increased with increasing sonication duration. -Sonication for 10 and 15 min retained higher ascorbic acid content compared to fresh and pasteurised strawberry juice during storage. -Sonication for 15 min retained higher antioxidant capacity and TPC compared to fresh and pasteurised samples during storage.	Yildiz and Aadil, 2020
Red pitaya fruit juice (*Hylocereus polyrhizus*)	Ultrasonic bath -37 kHz, 600 W -Sonication: 0 (fresh), 20, 40, 60 min -Temperature: 30°C	-Sonication for 60 min increased citric and succinic acids ($p < 0.05$). -TPC, TAC and DPPH increased for 24%, 44% and 41%, respectively after 60 min of sonication. -Sonication did not affect total soluble solids, titratable acidity, and pH of red pitaya juice. -Viscosity of red pitaya juice increased after 20 min of sonication ($p < 0.05$). -Sonication changed *L**, *a**, *b**, *C**, ΔE, *h**, and browning index ($p < 0.05$) as compared to fresh red pitaya juice.	Tan, et al., 2023

2.7.3 Other Fruit Juice Treatments

Other than sonication, there are also several emerging alternative fruit juice treatments namely microwave heating, ohmic heating, high-pressure processing, and pulse-electric field. The microwave heating and ohmic heating are non-conventional thermal treatments alternative to traditional thermal treatments (pasteurisation and sterilisation). In contrast to traditional thermal treatments, microwave heating utilised electromagnetic waves to heat up food from its outer surface to its interior. The heating process is via vibration of polar molecules like water as the heat transfer medium. Figure 2.8 illustrates the schematic diagram of laboratory scale microwave heating. The heating degree on food products is depending on its electrical conductivity (Marlena Pielak, Ewa Czarniecka-Skubina and Ingrida Kraujutiene, 2022). Fruit juice is high in water content, it absorbs energy rapidly and reach the desired temperature in short time, thereby minimising the deterioration of the bioactive compounds present.

In fruit juice processing, a microwave functions at the frequency of 0.3 to 300 GHz (Vilas-Boas, et al., 2022). Generally, a house-hold microwave usually functions at 2.45 GHz, while industry-based microwave usually functions at 0.915 GHz or 2.45 GHz. It was claimed that the lower the frequency, the higher the heat penetration (Guo, et al., 2017). On the other hand, power, temperature, and time are also the important parameters that will affect the final fruit juice quality (Martins, et al., 2021). In the previous study, microwave heating has shown to be a promising alternative to traditional pasteurisation as

it promoted lower browning index and significant increase in the ascorbic acid, total phenolics, carotenoids, and higher antioxidant activity than the thermal pasteurised and fresh orange juice-milk beverages (Martins, et al., 2021). Microwave heating at 120 s also significantly increased the ascorbic acid level, antioxidant activity, and reduced the aerobic mesophilic and yeast and mold count of persimmon juice in comparison to the untreated persimmon juice (Lalou, Ordoudi and Mantzouridou, 2021).

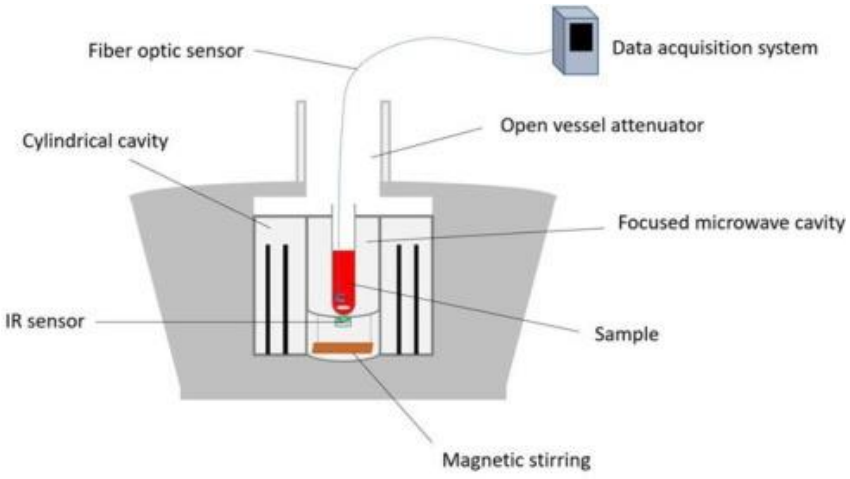

Figure 2.8: Schematic diagram of laboratory scale microwave heating (Martins, et al., 2021).

Ohmic heating is another non-conventional thermal processing approach that utilised electrical current to heat up liquid or solid food products placed between two electrodes (Figure 2.9). The food products will act as electrical resistance and subsequently generate heat in its interior (López-Pedrouso, et al., 2019). Thereby, ohmic heating produces fast and even heating without degrading the nutritional and organoleptic properties of food because the food is heated

internally, which is an advantage in comparison to microwave heating (Vilas-Boas, et al., 2022). This approach is highly effective for fruit juice as it contains high amounts of water and ionic salts to provide electrical resistance to generate heat inside the juice. The heating process is highly depending on the electrical conductivity of food product, current, voltage and temperature used (López-Pedrouso, 2019).

It was reported that ohmic heating alone is insufficient to inactivate pathogenic microorganism, but incorporation of heat would help to increase its effectiveness in inactivating the pathogenic microorganism Park and Kang (2013) observed that apple juice treated with ohmic heating at 58°C for 30 s could achieved higher reduction levels of *E. coli* O157:H7, *S. typhimurium*, and *L. monocytogenes*, by 4.00-, 4.63-, and 1.11-log reductions, respectively in comparison to traditional heating under identical conditions that only achieved 1.58-, 1.42-, and 0.41-log reductions, respectively. Recent study has demonstrated the capability of ohmic heating in significantly increasing the phytochemicals (ascorbic acids, antioxidant capacity, TFC, and TPC) and effectively inactivate the PPO of mango juice as compared to fresh and conventional heated samples. The study also illustrated that ohmic heating achieved comparable results with conventional heating in inactivation of total microbial load of mango juice (Tarek Gamal Abedelmaksoud, et al., 2022).

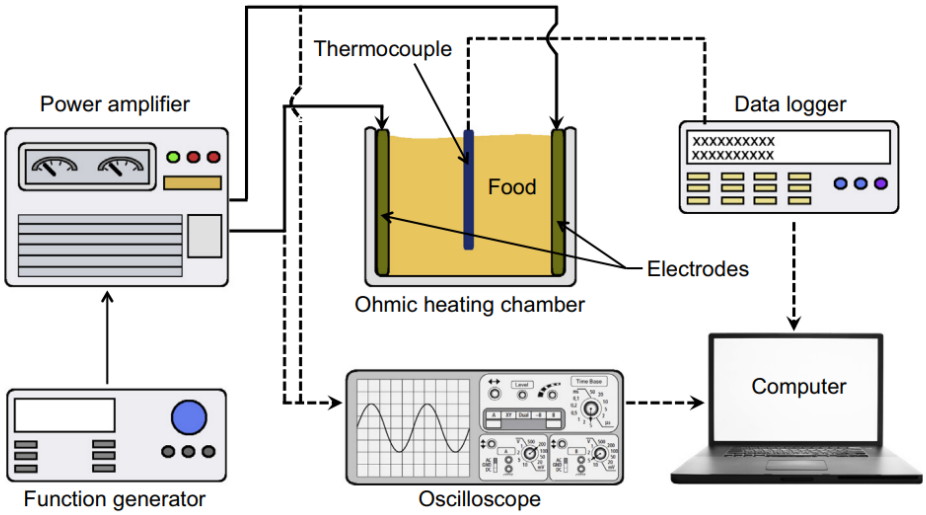

Figure 2.9 Schematic diagram of ohmic heating set up (Koubaa, et al., 2019).

High-pressure processing (Figure 2.10) is the used of high pressure (100-800 MPa, max 1000 MPa) evenly and rapidly in the sample through water as the pressure transmission medium to improve mass transfer rate, solvent permeability in cells, and secondary metabolites diffusion (Vilas-Boas, et al., 2022). The pressure, holding time, and temperature are the variables that affecting the efficiency on achieving FDA requirement of a 5-log reduction in microbial load fruit juices, bacterial spores, and enzymes inactivation. The nutrients and sensory properties of fruit juice can be well-preserved after high-pressure processing because the pressure used has minimal effect on the low molecular weight compounds that are present in the fruit juice (Chiozzi, Agriopoulou and Varzakas, 2022).

Study by Hu, Wang and Chen (2020) demonstrated that jaboticaba juice treated by high pressure processing promoted highest overall sensory acceptance, scoring 6.02-6.61 out of 10 at different intensity as compared to thermal pasteurised and fresh samples that scored 3.86 and 4.23, respectively. High-pressurised jaboticaba juice was microbiologically safe throughout 28 days of storage at 7°C with zero detection on aerobic plate counts, coliforms, psychrotrophs, and yeast and mold and preserved higher antioxidant capacities, TPC, TFC, and monomeric anthocyanin content, as well as lower browning degrees than the thermal pasteurised sample (Hu, Wang, and Chen, 2020). As reported by Chang, et al. (2017), the high-pressurised white grape juice at higher pressure significantly decreased the PPO and POD to 51.2% and 52.1%, respectively as compared to fresh sample that contained 100% activity of each enzyme. Upon 20 days of storage at 4°C, both PPO and POD activity of high-pressurised white grape juice retained below 50% in comparison to the fresh sample that retained above 8-% of PPO and 77.9% of POD activity.

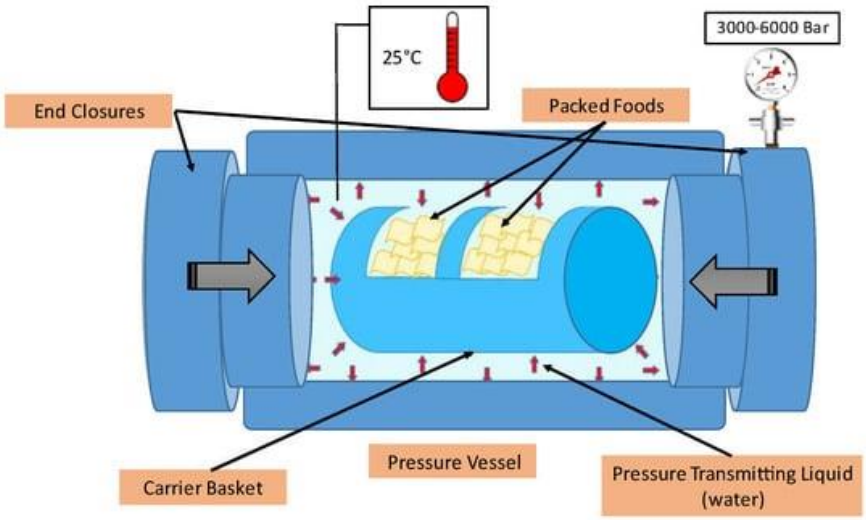

Figure 2.10: Schematic diagram of high-pressure processing vessel (Chiozzi, Agriopoulou and Varzakas, 2022).

Pulsed electric field apply short pulses of high electric fields in extremely short duration (micro- to milliseconds) and high voltage ranged from 10-80 kV/cm to induce electroporation for inactivation of microorganisms (Vilas-Boas, et al., 2022). The electroporation induced can also assists in enzymes inactivation and release of cell components like bioactive compounds due to the loss of cell membrane's integrity (Koubaa, et al., 2018). As illustrated in Figure 2.11, the food sample is placed and being processed between two electrodes. This approach is highly suitable for liquid foods without bubbles such as fruit juice, dairy and alcoholic beverages due to the presence of charges molecules that can transfer electric charges formed during the processing. Temperature, time, electric field strength, pulse frequency, and energy input are the important factors that affect the efficiency of pulsed electric field (Putnik, et al., 2020).

Similar to the other processing methods as mentioned pulsed electric field provides positive outcome on the nutritional and sensory properties on food product due to the low temperature and short processing time used (Vilas-Boas, et al., 2022). Based on the findings by (Kantala, et al., 2022), pulsed electric field achieved comparable 5-log reduction of *S. aureus* and *E. coli* with thermal pasteurised orange juice and retained higher quantity of ascorbic acids, sugar, and mineral than the thermal pasteurisation without compromising the physicochemical properties (pH, colour, viscosity, and total soluble solids) of orange juice. Pulsed electric filed processed strawberry juice also showed significantly improve in TPC and radical scavenging activity as compared to thermal pasteurisation, in addition to decreased the mesophilic aerobic and yeast and mold counts significantly below 2 log CFU/mL (Yildiz, et al., 2020).

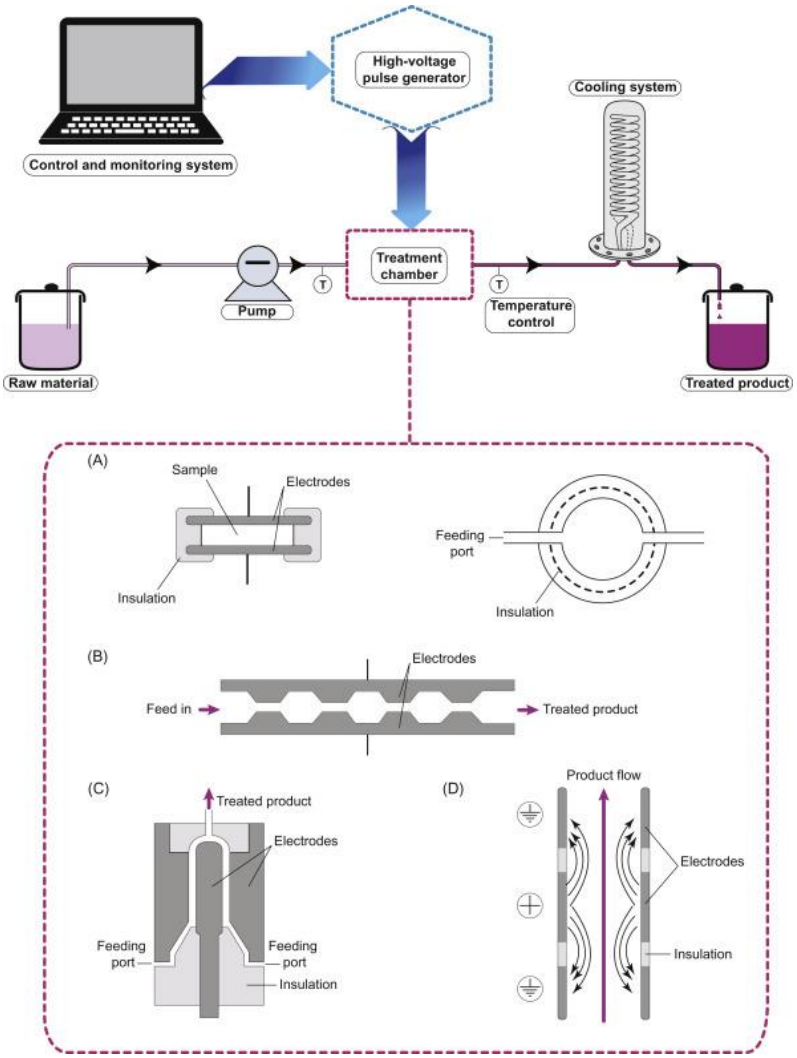

Figure 2.11: Schematic diagram of pulsed electric filed system (Koubaa, et al., 2018). Note: (A) Static chamber; (B) side view of a basic continuous design; (C) coaxial chamber; and (D) colinear chamber.

In general, these processing methods are aiming to produce microbiologically safe, free from browning enzymes, extended shelf-life, and high quality in terms of nutritional content and organoleptic properties of fruit juice, and most importantly friendly to the environment. The main advantages of these alternative approaches to thermal treatment: 1) the used of low temperature and 2) short duration to heat up the fruit juice (Martins, et al., 2021; Vilas-Boas, et al., 2022); thereby leading to maximum retention or improvement

of the nutrients including TPC, TFC, and antioxidant capacity (Martins, et al., 2021; Yildiz, et al., 2020; Kantala, et al., 2022), high sensory acceptance (Hu, Wang, and Chen, 2020) meanwhile achieving significant microbial load reduction (Park and Kang, 2013; Yildiz, et al., 2020; Lalou, Ordoudi and Mantzouridou, 2021; Kantala, et al., 2022) and browning enzymes inactivation (Chang, et al., 2017; Tarek Gamal Abedelmaksoud, et al., 2022). However, high initial capital cost for the equipment like high-pressure processing and pulsed electric field can be a major drawback in implementing this technology in the industry (Putnik, et al., 2020). Besides, the impact on the final quality of fruit juice is highly affected by the matrix and composition of fruit (Petruzzi, et al., 2017; Koubaa, et al., 2018). The implementation of one of these technologies in the food industry should be thoroughly evaluated in order to optimise all the associated parameters because all processing technologies have benefits and drawbacks. Table 2.6 summarised the alternative fruit juice treatments and their respective advantages and disadvantages.

Table 2.6: Summary of alternative fruit juice treatments used in the industry.

Methods	Mechanism and Application	Advantages	Disadvantages	Reference
Microwave heating	-Use electromagnetic waves at frequency of 0.3-300 GHz and water as the heat transfer medium. -Power, temperature, frequency, and time are the parameters that affect its efficiency. -Suitable for fruit juice or food products with high water content.	-Able to preserve bioactive compounds in fruit juice due to high water content, energy absorb quickly, minimising the deterioration of bioactive compounds. -Reduce thermal gradient and rapid heating of fruit juice. -Shorter processing time and utilise lesser energy to produce fruit juice with high nutritional quality. -Cost effective.	-Industrial application is limited due to lack of information and knowledge on the chemical impact in food matrix. -Require the uses of temperature. -May cause uneven heating distribution, especially in solid or semi-solid food products.	Guo, et al., 2017; Martins, et al., 2021; Lalou, Ordoudi, and Mantzouridou, 2021; Marlena Pielak, Ewa Czarniecka-Skubina and Ingrida Kraujutiene, 2022

Table 2.6: (Continued.)

Methods	Mechanism and Application	Advantages	Disadvantages	References
Ohmic heating	-Heating of food using electric current. -Electrical conductivity of food sample, current, voltage, and temperature are the parameters that affect its efficiency. -Suitable for fruit juice as it contains high amounts of water and ionic salts that provide electrical resistance to generate heat inside the juice.	-Rapid and uniform heating because the heat is generated internally. -Minimise thermal damage and nutritional losses. -Increase the lethality against microorganism. -Lower capital cost. -Shorter treatment time. -Enviromentally friendly since 90% of electrical energy is transformed into heat.	-Require application of temperature to achieve lethal effect on pathogenic microorganism as the electric field strength of ohmic heating alone is insufficient to inactivate pathogenic microorganism.	Petruzzi, et al., 2017; López-Pedrouso, 2019; Vilas-Boas, et al., 2022

Table 2.6: (Continued.)

Methods	Mechanism and Application	Advantages	Disadvantages	References
High-pressure processing	-Use of high pressure (100-1000 MPa) and at temperature ranging from -20-60°C. -Pressure, holding time, and temperature are the parameters that affect its efficiency. -Suitable for solid, liquid, packed, or unpacked food. -Widely applied in fruits, vegetables, juices, beverages, dairy products, meat products, seafood, shellfish, and ready-to-eat meals.	-Provide safe products. -Shorter processing time. -Maximum retention of fresh-like flavour and taste due to the lower temperatures used. -Environment-friendly as it requires only electric energy and does not generate new by-products.	-Batch operation mode. -High initial capital for the equipment.	Putnik, et al., 2020; Chiozzi, Agriopoulou and Varzakas, 2022; Vilas-Boas, et al., 2022

Table 2.6: (Continued.)

Methods	Mechanism and Application	Advantages	Disadvantages	References
Pulsed electric field	-Use of short-pulses of high electric fields with a duration of micro-to milliseconds and intensity of 10-80 kV/cm. -Temperature, time, electric field strength, pulse frequency, and energy input are the parameters that affect its efficiency. -Suitable for liquid food without bubbles, eg: fruit juices, dairy, and alcoholic beverages.	-Low energy consumption -Does not generate waste -Preserve nutritional and sensory properties due to short time and low temperature	-Not effective in inactivation of spores, require to combine with other processing methods to achieve desirable results. -High initial capital for the equipment.	Putnik, et al., 2020; Vilas-Boas, et al., 2022

CHAPTER 3

MATERIALS AND METHODS

3.1 Chemicals and Reagents

The chemicals/reagents used in this project are tabulated in Table 3.1.

Table 3.1: Chemicals/reagents and their respective manufacturers list.

Chemicals/Reagents	**Manufacturers**
Phenolphthalein indicator	Systerm®
β-carotene, 99%	Alfa Aesar
Glacial acetic acid	Emsure®
Hydrochloric acid, 37%	Emsure®
Methanol, 99.8% (AR grade)	Synerlab
Methanol, 99% (HPLC grade)	LiChrosolv®
Ethanol, 95% (AR grade)	Sime Scientific
Scopoletin, ≥98%	ChemFaces
Rutin, 97+%	Acros Organics
Quercetin dihydrate, 99%	Sisco Research Laboratories Pvt. Ltd
Vanillic acid, 98%	Alfa Aesar
Caffeic acid, ≥98%	Sigma-Aldrich
Organic acids kit (analytical standard)	Merck
Monopotassium phosphate	Emsure®
o-phosphoric acid, 85% (AR grade)	RCI Labscan Limited
Sodium nitrite	Quality Reagent Chemical™
Aluminium chloride	Merck
Sodium hydroxide (pellet)	R&M Chemicals
Sodium carbonate	HiMedia Laboratories Pvt. Ltd
Gallic acid	Sisco Research Laboratories Pvt. Ltd.
Folin-Ciocalteu reagent	R&M Chemicals
TPTZ	Glentham Life Sciences
Ferric chloride hexahydrate	Nacalai Tesque
Ferrous sulfate (anhydrous)	R&M Chemicals
Sodium acetate (anhydrous)	Emsure®
Trolox, 97%	Acros Organics
ABTS (Diammonium salt)	Nacalai Tesque
Potassium peroxodisulfate	Sigma-Aldrich
Ascorbic acid	Systerm®
Potato dextrose agar	HiMedia Laboratories Pvt. Ltd
Plate count agar	Liofilchem
Tartaric acid	Systerm®
Soyatone BactoBio for Bacteriology	Sisco Research Laboratories Pvt. Ltd.

3.2 Noni Juice Preparation

Hard white stage (Stage 4) noni fruit was harvested in October 2021 from Sureco Sure Return Farm, Perak Malaysia. Figure 3.1 shows harvested hard white stage noni fruit while Figure 3.2 shows different maturity stages of noni fruit. The fruits were washed with tap water and sanitised with a bleach solution (1 teaspoon of bleach per 4 L of distilled water) based on previous studies by Yang, et al. (2007) and Barraza-Elenes, et al. (2019). The surface of the fruit was dried with absorbent tissue paper before being kept at room temperature (25°C ± 2°C) for 1-2 days to ripen, as characterised by translucent-greyish colour and soft texture (Barraza-Elenes, et al., 2019). A stainless-steel knife was used to cut the fruit into halves, and the seeds were manually separated. The pulp and peel were extracted using a commercial juice processor (NBL-C501SS, Nippon, Selangor, Malaysia). The noni juice was filtered (Figure 3.4) through a sterile muslin cloth and then equally divided into these five groups (Figure 3.5): pasteurisation (POS), fresh (FRE), sonication for 20 min (S20), sonication for 40 min (S40) and sonication for 60 min (S60). Each group had triplicates.

Figure 3.1: Hard white stage of noni fruit harvested from Sureco Sure Return Farm.

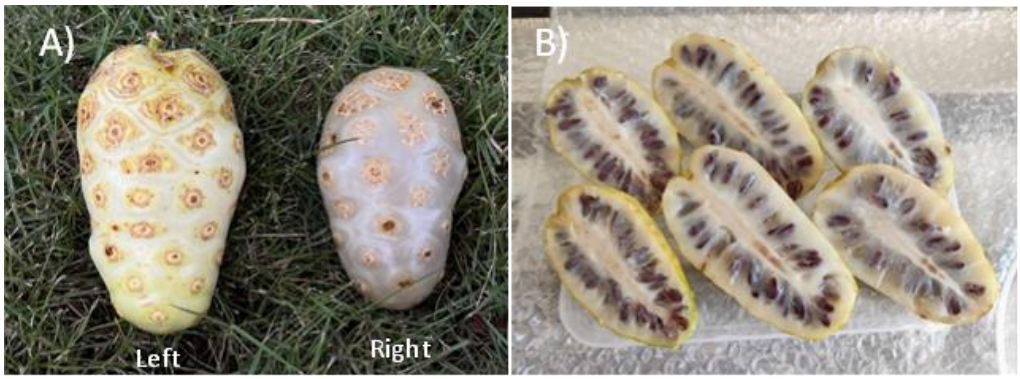

Figure 3.2: Different maturity stages of noni fruit. A) Left: Hard white stage. A) Right: Ripe translucent-greyish fruit. B) Longitudinal section of noni fruit.

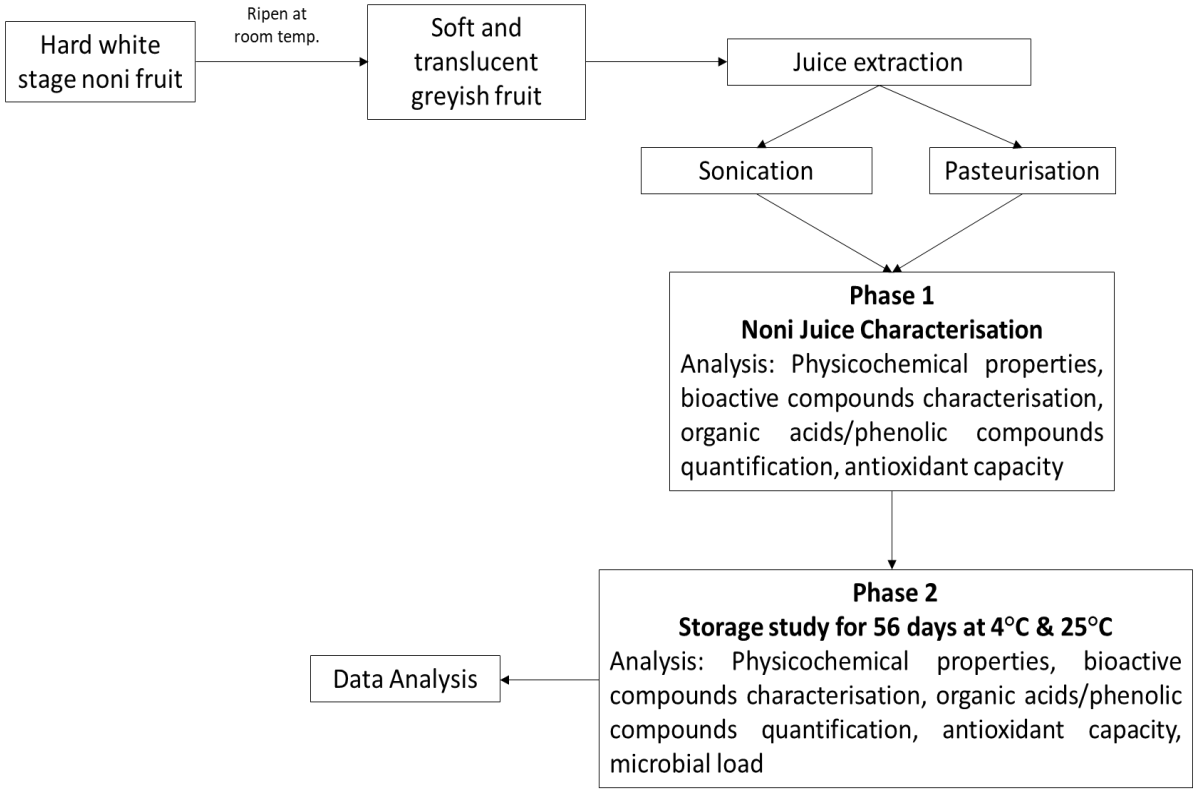

Figure 3.3: Experimental design.

Figure 3.4: Noni juice during juice extraction.

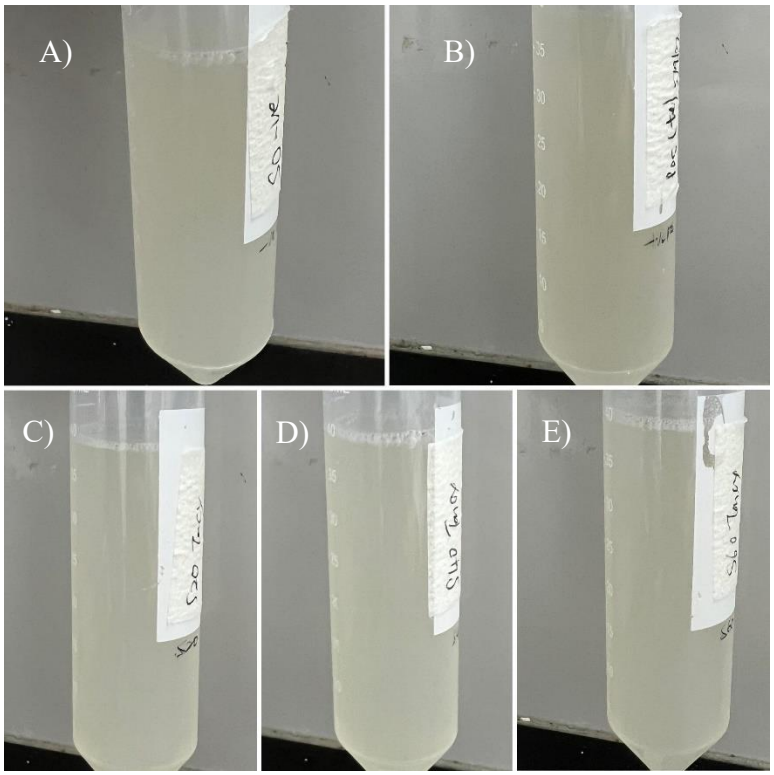

Figure 3.5: Noni juice samples packed in bottles. A) Fresh noni juice; B) Pasteurised noni juice; C) Noni juice sonicated for 20 min; D) Noni juice sonicated for 40 min; E) Noni juice sonicated for 60 min.

3.3 Sonication and Pasteurisation Treatments

The noni juice was sonicated using an ultrasonic bath (Elmasonic EASY, Baden-Wurttemberg, Germany; 37 kHz ultrasonic frequency, 600 W ultrasonic output power, 11.8″ × 9.4″ × 5.9″ (L × W × H) internal dimensions) at different time intervals (20, 40 and 60 min) as presented in Figure 3.6 under a constant temperature of 30°C and a frequency of 37 kHz (Bhat, et al., 2011). The sonication temperature (30°C) was maintained by water circulation and monitored using the thermostat on the ultrasonic bath. The use of mild temperature during the sonication process is an optimal approach to improve the phytochemical compounds extraction meanwhile minimise the degradation of these thermolabile compounds (Tan, et al., 2017; Moraes, et al., 2022). Thus, present study applied a mild sonication temperature of 30°C. The sonication process was carried out in a dark environment to avoid light interference. Pasteurisation was conducted by heating the noni juice under temperature of 90°C for 1 min (Farhadi Chitgar, et al., 2016) to serve as positive control. All juice samples were stored in bottles wrapped with aluminium foil at -20°C until further use within 15 days. Fresh noni juice served as the negative control in this study.

Figure 3.6: Sonication of noni juice using ultrasonic bath.

3.4 pH, Titratable Acidity, Total Soluble Solids, and Colour

The pH noni juice was measured using a digital pH meter (Eutech pH 700, Waltham, MA, USA). The titratable acidity was determined using the AOAC 942.15 standard procedure (AOAC, 2007). The total soluble solids were measured using a handheld refractometer (Atago PAL-3, Tokyo, Japan) at room temperature. The colour attributes were measured using a colorimeter (Konica Minolta CM-600d, Osaka, Japan). Results were expressed as L^*, a^* and b^*. L^* measures lightness from 0 (black) to 100 (white). Positive a^* indicates red and negative a^* indicates green. Positive b^* indicates yellow and negative b^* indicates blue. Chroma (C^*), hue (h^*), browning index (BI) and total colour difference (ΔE) were calculated based on the following equations (Tan, et al., 2023):

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

$$h^* = 180 + \tan^{-1}\left(\frac{b^*}{a^*}\right), \text{when } a^* < 0$$

$$h^* = \tan^{-1}\left(\frac{b^*}{a^*}\right), \text{when } a^* > 0$$

$$\text{BI} = \frac{100\ (\text{Z} - 0.31)}{0.172}, \text{where Z} = \frac{(\text{a}^* + 1.75\text{L}^*)}{(5.645\text{L}^* + \text{a}^* - 0.3012b^*)}$$

$$\Delta E = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2}$$

3.5 Sample Preparation for Characterisation of Bioactive Compounds and Antioxidant Capacity

Exactly 1 mL of noni juice was mixed with 5 mL of 60% methanol and centrifuged at 10,000 rpm for 15 min at room temperature. The supernatant layer (extract) was collected for the determination of bioactive compounds (TPC, TFC, TC) and antioxidant capacity (FRAP and TEAC) (Guerrouj, et al., 2016). The 60% methanol is applied in the present study because it is commonly used in extraction of bioactive compounds with high concentration as presented in the previous studies by Proestos and Komaitis (2006), Mussatto, et al. (2011) Guerrouj, et al. (2016) and Zhou, et al. (2019).

3.6 Characterisation of Bioactive Compounds

3.6.1 Total Phenolics Content (TPC)

The TPC assay was carried out according to the method Dars, et al. (2019). Briefly, 100 µL of juice extract was mixed with 400 µL of sterile ultrapure water and 500 µL of Folin-Ciocalteu reagent (1:10, v:v) in a falcon tube. After incubating in dark for 5 min at room temperature, 1000 µL of 7.5% sodium carbonate solution was added into the mixture and further incubated in the dark for 30 min at room temperature. A spectrophotometer (DLAB Scientific SP-V1000, Beijing, China) was used to measure the absorbance at 765 nm against a blank. The TPC was determined using a gallic acid standard curve, and the results were expressed as mg gallic acid equivalents (GAE)/100 mL of juice.

3.6.2 Total Flavonoids Content (TFC)

The TFC assay was carried out according to the method as described by Abid, et al. (2013) with minor modifications. Initially, 500 µL of juice extract, 250 µL of sterile ultrapure water, and 150 µL of 5% sodium nitrite solution were mixed and incubated for 6 min at room temperature. The mixture was then combined with 300 µL of 10% aluminium chloride solution. After 5 min, 1000 µL of 1 M sodium hydroxide solution was added to the mixture. The absorbance was measured at 510 nm against a blank using a spectrophotometer (DLAB Scientific SP-V1000, Beijing, China). The TFC was determined using a rutin standard curve, and the results were expressed as mg rutin equivalents (RE)/100 mL of juice.

3.6.3 Total Carotenoids Content (TC)

The TC was determined according to the method of Tan, et al. (2018) with modifications. A calibration curve in the range of 0-2.5 µg/mL was initially constructed by dissolving *β*-carotene in methanol. Exactly 250 µL juice extract was mixed with 500 µL methanol in a falcon tube and vortexed. The absorbance was measured at 440 nm against a blank using a spectrophotometer (DLAB Scientific SP-V1000, Beijing, China). The total carotenoid content of the sample was expressed as mg *β*-carotene equivalents (*β*CE)/100 mL juice.

3.7 Antioxidant Capacity Analysis

3.7.1 Ferric Reducing Antioxidant Power (FRAP)

The FRAP assay was carried out using method described by Benzie and Devaki (2018). FRAP working reagent was freshly prepared by combining 25 mL of 0.3 M acetate buffer (pH 3.6), 2.5 mL of 10 mM TPTZ solution in 40 mM hydrochloric acid, and 2.5 mL of 20 mM ferric chloride hexahydrate solution. The FRAP reagent (1.5 mL) was added to 50 µL of extract and incubated at 37ºC for 10 min. The absorbance was measured against a blank at 593 nm using a spectrophotometer (DLAB Scientific SP-V1000, Beijing, China). A ferrous sulfate standard curve was constructed. The results were expressed as µM ferrous iron equivalents (Fe^{2+})/kg of juice.

3.7.2 Trolox Equivalent Antioxidant Capacity (TEAC)

The TEAC was carried out according to the method of Tan, et al. (2018). An ABTS radical cation stock solution was prepared by mixing 7 mM of ABTS powder with 2.55 mM potassium peroxodisulfate in 10 mL of deionised water in the dark for 16 hours at room temperature. The working solution was prepared by diluting the stock solution with absolute ethanol to an absorbance of 0.70 ± 0.05 at 734 nm. The juice extract (100 µL) was mixed 1000 µL of ABTS working solution and incubated at room temperature in the dark for 6 min. The absorbance of mixture was measured at 734 nm against a blank using a spectrophotometer (DLAB Scientific SP-V1000, Beijing, China). A Trolox

calibration curve was prepared. The TEAC of samples were expressed as mM of Trolox equivalents (TE)/kg of juice.

3.8 Sample Preparation for Phenolic Compounds and Organic Acids Quantification

Exactly 5 mL of noni juice was centrifuged at 10,000 rpm for 15 min at room temperature. The supernatant was filtered through a 0.22 μm syringe filter, degassed at 25°C for 5 min, and used for phenolic compounds and organic acids quantification.

3.9 Phenolic Compounds Quantification

The quantification of phenolic compounds in noni juice was performed according to (Saikia, Mahnot and Mahanta, 2015). A high-performance liquid chromatograph (HPLC) (Shimadzu LC-10AD, Kyoto, Japan) equipped with UV-Vis detector (Shimadzu SPD-20A, Kyoto, Japan) set at 325 nm was used. Exactly 20 μL of the filtered sample was injected into a LiChrospher RP-18 column (125 mm × 4 mm, with a particle size of 5 μm; Merck, Darmstadt, Germany). The mobile phase consisted of acidified ultrapure water (pH 3.2 adjusted with glacial acetic acid) (mobile phase A) and methanol (mobile phase B). The gradient elution parameters were as follow: 20% B (0–8 min), 35% B (9–12 min), 55% B (13–16 min), 70% B (17– 20 min), 80% B (21–30 min), and 90% B (31–34 min), followed by column washing with 35% B (35–39 min) and a final elution with 20% B (40–45 min) to ensure the column pressure returns to its initial reading before injecting the next sample. The column temperature was kept at 30°C and the elution was performed at a flow rate of 0.5 mL/min. Identification and quantification of the phenolic compounds were based on the external standards of vanillic acid, rutin, quercetin, and scopoletin.

3.10 Organic Acids Quantification

The quantification of organic acids in noni juice was conducted according to the method of Scherer, et al. (2012). An HPLC (Shimadzu LC-10AD, Kyoto, Japan) equipped with a UV-Vis detector (Shimadzu SPD-20A, Kyoto, Japan) set at 210 nm was used. Exactly 20 μL of the filtered sample was injected into a LiChrospher RP-18 column (125 mm × 4 mm, with a particle size of 5 μm; Merck, Darmstadt, Germany). The temperature of the column oven was set at 30°C. The mobile phase was 0.01 mol/L monopotassium phosphate buffer solution (pH 2.60 adjusted with *o*-phosphoric acid) with isocratic elution at a flow rate of 0.5 mL/min. Identification and quantification of the organic acids were based on the external standards of an organic acid kit.

3.11 Microbial Load Analysis

Noni juice was serially diluted with sterile 0.1% peptone water and plated into microbiological media. Aerobic mesophilic bacteria count was examined using PCA, and yeast and mold counts were examined using PDA combined with 10% tartaric acid. The PCA plate was incubated at 37ºC for 1 day, whereas the PDA plate was incubated at 25ºC for 5 days (Bhat, et al., 2011). Results were expressed as log colony forming units (CFU) per mL of juice.

3.12 Storage Study

Among the sonicated samples, only the best sonicated juice sample which is the S60 was selected for storage study. The selection was based on how well the sonicated samples retained or enhanced the bioactive compounds (TPC and TFC), phenolic compounds, organic acids, antioxidant capacity as well as the physicochemical properties. As shown in Table 4.3, 4.4, 4.6 and 4.8, S60 significantly improved the TPC, TFC, phenolic compounds, organic acids as well as the antioxidant capacity of noni juice.

Pasteurised (POS), fresh (FRE) and sonicated noni juice (S60) were stored at refrigerated temperature (4℃ ± 2℃) and room temperature (25℃ ± 2℃) for 56 days. Microbial load tests were conducted every 14 days while pH, titratable acidity, total soluble solids, colour, antioxidant capacity, TPC, TFC, phenolic compounds, and organic acids were determined after 56 days.

3.13 Statistical Analysis

All analyses were performed in triplicate. Statistical analysis was done using IBM SPSS Statistics 26.0 software. One-way analysis of variance (ANOVA) and Tukey's Honest Significant Difference (HSD) were used to compare the difference among means between sonication and pasteurisation. Paired sample *t*-test was used to compare the difference among means before and after storage study. The significant level was set at $p < 0.05$.

CHAPTER 4

RESULTS

4.1 Effect of Sonication Duration on the Quality Attributes of Noni Juice

4.1.1 Physicochemical Properties of Noni Juice at Different Sonication Duration

The results on the effect of sonication duration on the pH, titratable acidity, and total soluble solids of noni juices are tabulated in Table 4.1. As shown, no significant differences were observed in the pH, titratable acidity, and total soluble solids among the pasteurised, fresh and all sonicated (S20, S40, and S60) noni juices.

Table 4.1: The pH, titratable acidity, and total soluble solids of noni juice samples at different sonication duration.

Sample	pH	Titratable Acidity (%)	Total Soluble Solids (°Brix)
POS	3.91 ± 0.01^{a}	0.18 ± 0.02^{a}	1.37 ± 0.06^{a}
FRE	3.90 ± 0.01^{a}	0.17 ± 0.01^{a}	1.33 ± 0.06^{a}
S20	3.91 ± 0.01^{a}	0.17 ± 0.01^{a}	1.30 ± 0.00^{a}
S40	3.91 ± 0.01^{a}	0.17 ± 0.01^{a}	1.30 ± 0.00^{a}
S60	3.91 ± 0.01^{a}	0.17 ± 0.01^{a}	1.33 ± 0.06^{a}

Values are expressed as mean ± SD (n = 3). Different superscript letters in the same column indicate significant difference at $p < 0.05$. POS = pasteurised noni juice; FRE = fresh noni juice; S20 = noni juice sonicated for 20 min; S40 = noni juice sonicated for 40 min; S60 = noni juice sonicated for 60 min.

The colour attributes of noni juices at different sonication duration are presented in Table 4.2. Sonication (S20, S40, and S60) significantly increased the L^* values and decreased b^* values compared to the fresh noni juice. The negative a^* values did not change significantly in all noni juice samples. A significant decrease was observed in the C^* values of all sonicated noni juices (C^* = 3.47-4.60) compared to fresh noni juice (C^* = 6.49). The h^* values of noni juice (h^* = 93.94-95.75) was unaffected by sonication and pasteurisation. There were no significant differences in L^*, a^*, and b^* values between pasteurised and fresh noni juices. Pasteurisation did not significantly decrease the browning index of noni juice, while sonication resulted in lower browning index for 0.29 to 0.49, compared to the fresh sample. The ΔE of pasteurised noni juice (ΔE = 1.16) was lower than the S20, S40, and S60 noni juices (ΔE = 5.20-6.11).

Table 4.2: The colour attributes of noni juice samples at different sonication duration.

Sample	L^*	a^*	b^*	Hue (h^*)	Chroma (C^*)	Browning Index (BI)	Total Colour Difference (ΔE)
POS	47.66 ± 0.32^{a}	-0.41 ± 0.03^{a}	5.46 ± 0.03^{bc}	94.33 ± 0.34^{a}	5.48 ± 0.03^{bc}	0.49 ± 0.06^{ab}	1.16 ± 0.85^{a}
FRE	47.55 ± 0.47^{a}	-0.44 ± 0.03^{a}	6.47 ± 0.75^{c}	93.94 ± 0.73^{a}	6.49 ± 0.74^{c}	0.66 ± 0.19^{b}	Reference
S20	52.92 ± 0.31^{b}	-0.39 ± 0.06^{a}	4.30 ± 0.36^{ab}	95.21 ± 0.31^{a}	4.32 ± 0.37^{ab}	0.26 ± 0.02^{a}	5.84 ± 0.19^{b}
S40	52.35 ± 0.53^{b}	-0.35 ± 0.11^{a}	4.59 ± 0.28^{ab}	94.37 ± 1.54^{a}	4.60 ± 0.27^{ab}	0.37 ± 0.19^{ab}	5.20 ± 0.61^{b}
S60	52.71 ± 0.47^{b}	-0.35 ± 0.06^{a}	3.45 ± 0.70^{a}	95.75 ± 0.16^{a}	3.47 ± 0.71^{a}	0.17 ± 0.04^{a}	6.11 ± 0.44^{b}

Values are expressed as mean ± SD (n = 3). Different superscript letters in the same column indicate significant difference at $p < 0.05$. POS = pasteurised noni juice; FRE = fresh noni juice; S20 = noni juice sonicated for 20 min; S40 = noni juice sonicated for 40 min; S60 = noni juice sonicated for 60 min. Reference serves as control for total colour difference. L^* = lightness; $+a^*$ = redness; $-a^*$ = greenness; $+b^*$ = yellowness; $-b^*$ = blueness. ΔE measures the colour difference between treated samples and control. C^* = saturation of samples. h^* = visual colour difference with reference to grey colour under same lightness.

4.1.2 Total Phenolics, Total Flavonoids, and Total Carotenoids Content of Noni Juice at Different Sonication Duration

The results on the effect of sonication duration on total phenolics content (TPC), total flavonoids content (TFC), and total carotenoids content (TC) of noni juices are shown in Table 4.3. The TPC of noni juice varied between 2.62 to 3.19 mg GAE/100 mL. Specifically, S60 noni juice (3.19 mg GAE/100 mL) exhibited a significantly greater TPC value than the pasteurised, fresh, S20, and S40 samples (2.62-2.93 mg GAE/100 mL). However, no significant difference was observed in the TPC between the S20, S40, and the fresh noni juice.

The TFC of noni juice ranged between 1.01 to 1.48 mg RE/100 mL. The TFC of S60 noni juice (1.48 mg RE/100 mL) was statistically higher ($p < 0.05$) than fresh noni juice (1.01 mg RE/100 mL). There was no significant difference observed between the TFC of pasteurised and fresh noni juices. On the other hand, no significant difference in TC was observed among all the noni juices. The obtained TC values were also relatively low, ranging 0.1 to 0.2 mg βCE /100 mL.

Table 4.3: Total phenolics, total flavonoids, and total carotenoids content of noni juice samples at different sonication duration.

Sample	TPC (mg GAE/100 mL)	TFC (mg RE/100 mL)	TC (mg *β*CE/100 mL)
POS	2.62 ± 0.05^{a}	1.14 ± 0.17^{ab}	0.01 ± 0.00^{a}
FRE	2.93 ± 0.07^{b}	1.01 ± 0.19^{a}	0.02 ± 0.00^{a}
S20	2.88 ± 0.08^{b}	1.20 ± 0.17^{ab}	0.01 ± 0.00^{a}
S40	2.93 ± 0.05^{b}	1.40 ± 0.06^{ab}	0.01 ± 0.00^{a}
S60	3.19 ± 0.06^{c}	1.48 ± 0.11^{b}	0.02 ± 0.00^{a}

Values are expressed as mean ± SD (n = 3). Different superscript letters in the same column indicate significant difference at $p < 0.05$. POS = pasteurised noni juice; FRE = fresh noni juice; S20 = noni juice sonicated for 20 min; S40 = noni juice sonicated for 40 min; S60 = noni juice sonicated for 60 min.

4.1.3 Phenolic and Organic Acids Profile of Noni Juice at Different Sonication Duration

The phenolic compounds in noni juice were determined via high-performance liquid chromatography (HPLC). Figure 4.1 presents the chromatogram of phenolic compounds standard and Figure 4.2 shows the chromatogram of phenolic compounds detected in noni juice. The detected phenolic compounds were scopoletin, rutin, and vanillic acid while quercetin was not detected in all noni juices. Table 4.4 shows the concentration of phenolic compounds that were detected in noni juice. The retention time, linear equation, limit of detection (LOD), and limit of quantitation (LOQ) of phenolic compounds standard are tabulated in Table 4.5.

The S60 noni juice was found to possess the highest concentration ($p < 0.05$) of scopoletin (1.47 mg/100 mL), rutin (4.02 mg/100 mL), and vanillic acid (12.17 mg/100 mL) compared to fresh (scopoletin–0.96 mg/100 mL; rutin–2.75 mg/100 mL; vanillic acid–9.02 mg/100 mL) and pasteurised noni juices (scopoletin–0.83 mg/100 mL; rutin–2.22 mg/100 mL; vanillic acid–7.95 mg/100 mL). There was no significant difference observed between the scopoletin, rutin, and vanillic acid levels of fresh, S20, and S40 noni juices. Vanillic acid was found to be the highest concentration of phenolic compounds in noni juice, followed by rutin and scopoletin.

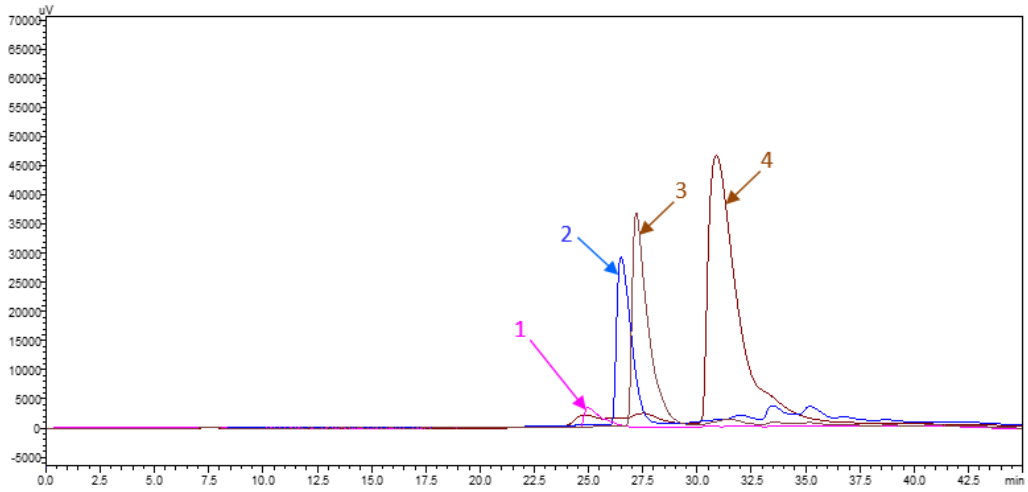

Figure 4.1: Phenolic compounds standard chromatogram. Numbers indicate the peaks of analytes: 1 = Vanillic acid; 2 = Scopoletin; 3 = Rutin; 4 = Quercetin.

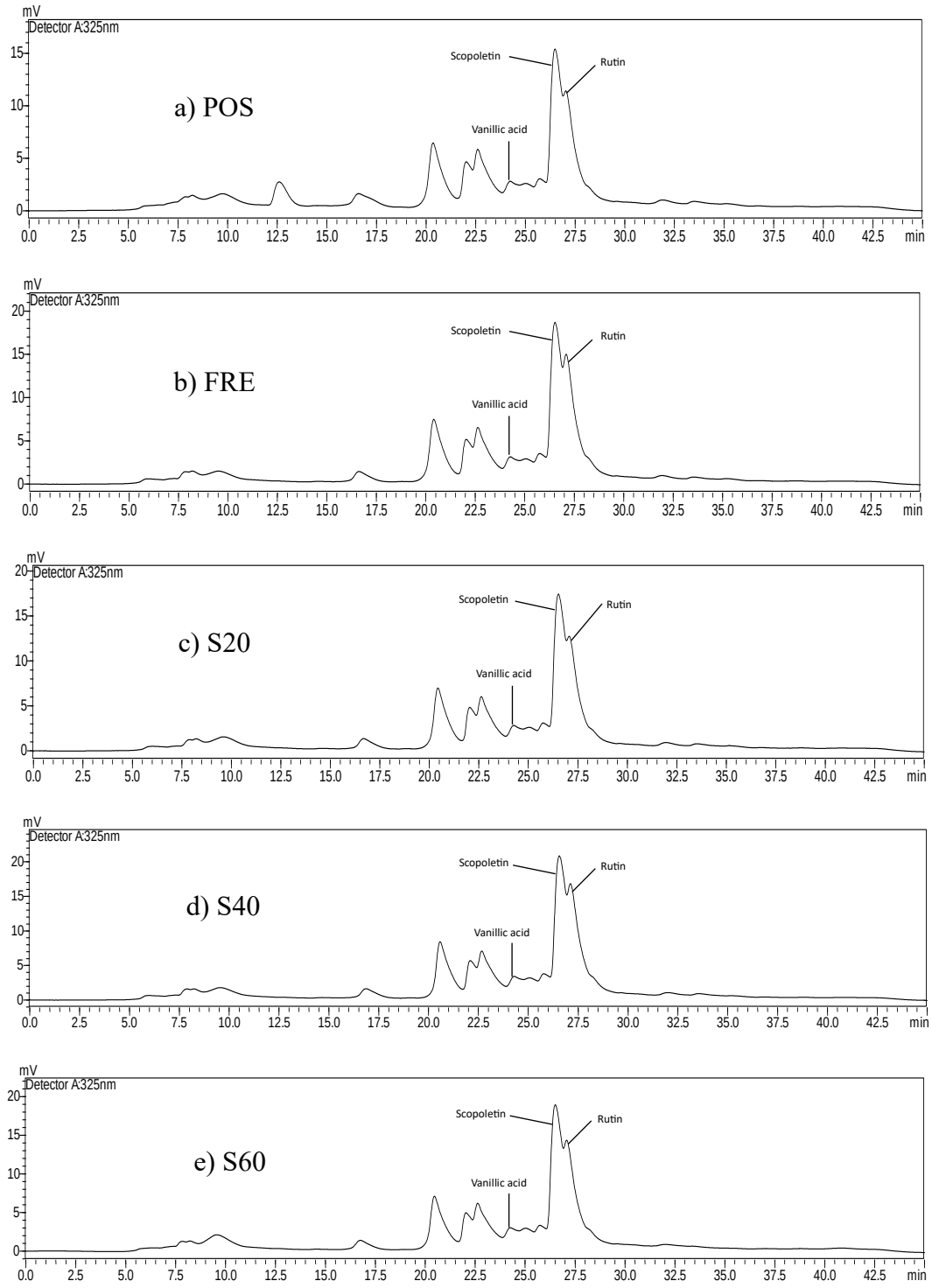

Figure 4.2: HPLC chromatogram of phenolic compounds detected in noni juice. Note: POS = pasteurised noni juice; FRE = fresh noni juice; S20 = noni juice sonicated for 20 min; S40 = noni juice sonicated for 40 min; S60 = noni juice sonicated for 60 min.

Table 4.4: Phenolic compounds detected in noni juice (mg/100 mL) at different sonication duration.

Sample	Phenolic Compounds			
	Scopoletin	Rutin	Vanillic Acid	Quercetin
POS	0.83 ± 0.06^{a}	2.22 ± 0.25^{a}	7.95 ± 1.37^{a}	ND
FRE	0.96 ± 0.01^{a}	2.75 ± 0.02^{a}	9.02 ± 0.25^{ab}	ND
S20	0.98 ± 0.01^{a}	2.64 ± 0.02^{a}	9.52 ± 1.10^{abc}	ND
S40	1.00 ± 0.01^{a}	2.92 ± 0.03^{a}	11.74 ± 1.02^{bc}	ND
S60	1.47 ± 0.23^{b}	4.02 ± 0.84^{b}	12.17 ± 1.18^{c}	ND

Values are expressed as mean ± SD (n = 3). Different superscript letters in the same column indicate significant difference at $p < 0.05$. POS = pasteurised noni juice; FRE = fresh noni juice; S20 = noni juice sonicated for 20 min; S40 = noni juice sonicated for 40 min; S60 = noni juice sonicated for 60 min. ND = Not detected.

Table 4.5: Retention time, linear equation, LOD, and LOQ of phenolic compounds standard.

Phenolic Compounds	Retention Time (min)	Linear Equation	R^2	LOD (mg/ 100 mL)	LOQ (mg/100 mL)
Scopoletin	26.5	y = 68494x - 53867	0.9934	0.29	0.88
Rutin	27.2	y = 24069x - 97311	0.9987	0.32	0.97
Vanillic Acid	24.7	y = 923.26x + 6885.4	0.9990	0.78	2.36

R^2 = Coefficient of determination; LOD = limit of detection; LOQ = limit of quantitation.

Figure 4.3 shows the chromatogram of the organic acids standard while Figure 4.4 shows the chromatogram of the organic acids profile of noni juice. Ascorbic acid, malic acid, fumaric acid, and citric acid were detected in noni juice whereas malonic acid, tartaric acid, and benzoic acid were not detected (refer Figure 4.4). The organic acids profile of noni juice is tabulated in Table 4.6 and the retention time, linear equation, LOD, and LOQ of the organic acids standard are presented in Table 4.7.

Noni juice that underwent 60 min of sonication (S60) was found to contain significantly higher concentrations of organic acids (ascorbic acid – 31.55 mg/100 mL; malic acid – 89.31 mg/100 mL; citric acid – 4.78 mg/100 mL), when compared to fresh noni juice (ascorbic acid – 26.93 mg/100 mL; malic acid – 76.43 mg/100 mL; citric acid – 1.50 mg/100 mL). Sonication for 40 min (S40) also significantly improved the levels of citric, fumaric, and ascorbic acids in comparison to fresh noni juice. Pasteurised noni juice exhibited the lowest concentrations of ascorbic acid (17.15 mg/100 mL), malic acid (57.54 mg/100 mL), fumaric acid (0.35 mg/100 mL), and citric acid (0.90 mg/100 mL) when compared to sonicated (S20, S40, and S60) and fresh noni juices. Generally, the amount of citric acid and fumaric acid were found to be the low in noni juice whereas malic acid was the highest concentration of organic acids detected in noni juice.

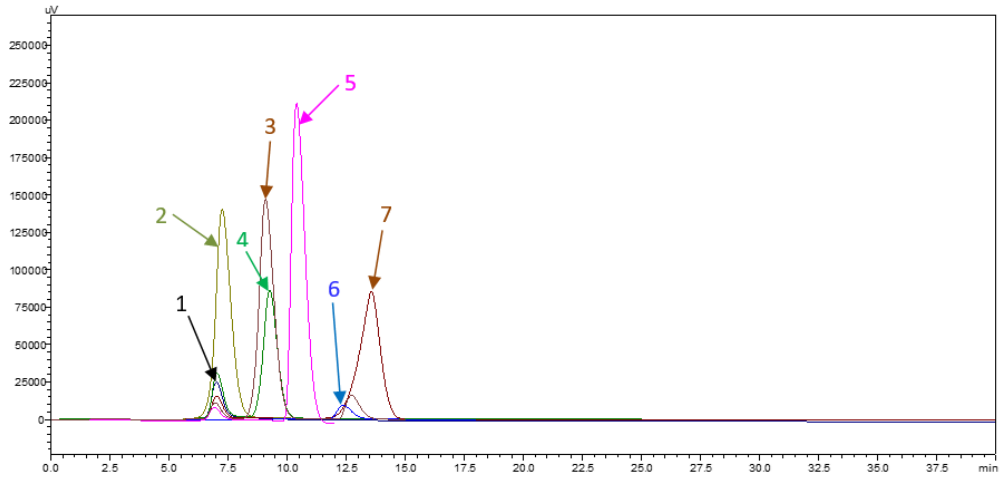

Figure 4.3: Organic acids standard chromatogram. Numbers indicate the peaks of analytes: 1 = Benzoic acid; 2 = Tartaric acid; 3 = Malic acid; 4 = Malonic acid; 5 = Ascorbic acid; 6 = Fumaric acid; 7 = Citric acid.

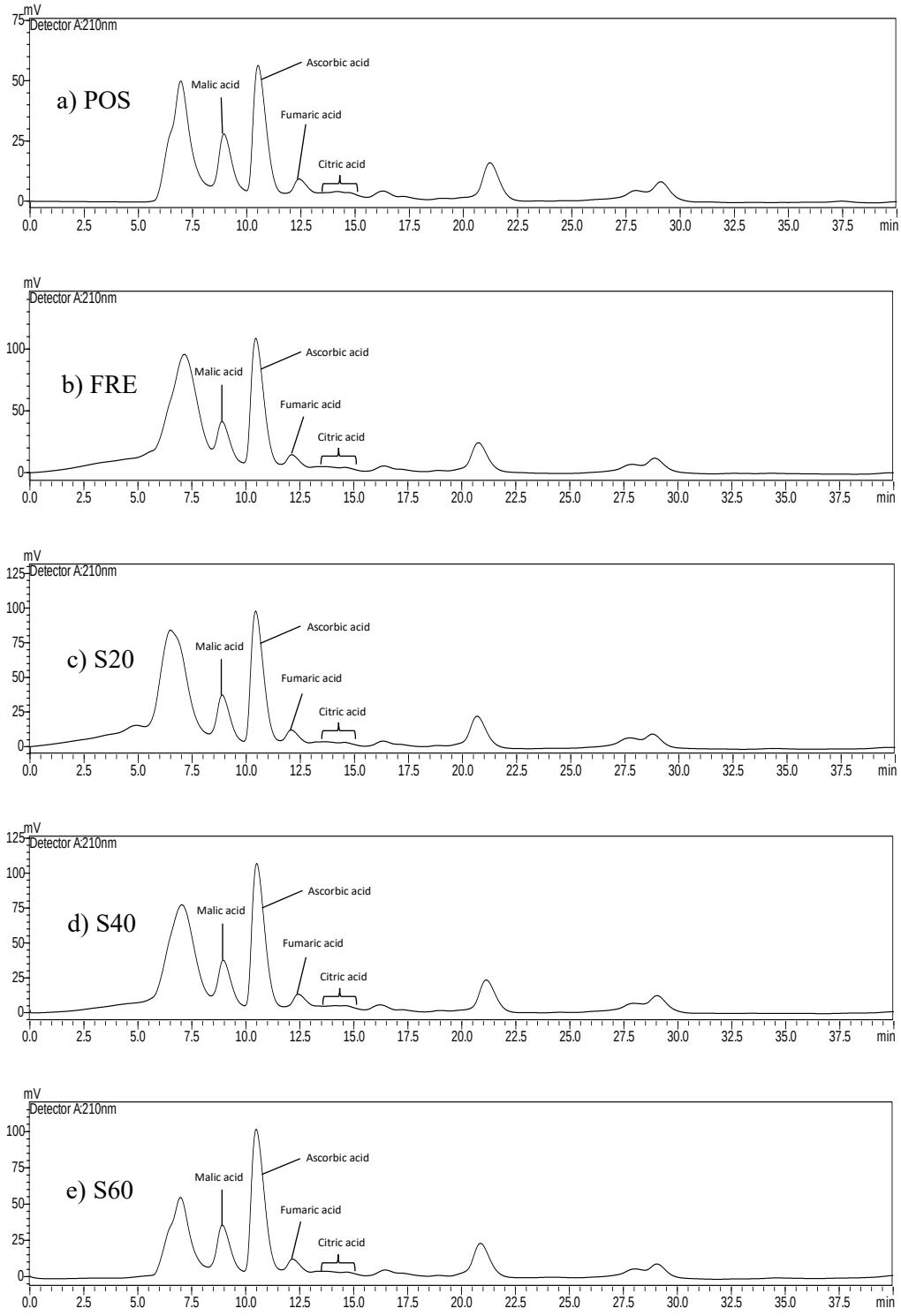

Figure 4.4: HPLC chromatogram of organic acids detected in noni juice. Note: POS = pasteurised noni juice; FRE = fresh noni juice; S20 = noni juice sonicated for 20 min; S40 = noni juice sonicated for 40 min; S60 = noni juice sonicated for 60 min.

Table 4.6: Organic acids detected in noni juice (mg/100 mL) at different sonication duration.

Sample	Organic Acids			
	Ascorbic Acid	Malic Acid	Fumaric Acid	Citric Acid
POS	17.15 ± 0.20^{a}	57.54 ± 3.97^{a}	0.35 ± 0.05^{a}	0.90 ± 0.13^{a}
FRE	26.93 ± 1.17^{b}	76.43 ± 2.72^{b}	0.39 ± 0.05^{ab}	1.50 ± 0.07^{b}
S20	28.01 ± 1.50^{bc}	84.34 ± 6.52^{bc}	0.41 ± 0.02^{ab}	1.86 ± 0.12^{c}
S40	30.21 ± 0.71^{cd}	86.58 ± 4.43^{bc}	0.51 ± 0.02^{c}	2.24 ± 0.18^{d}
S60	31.55 ± 1.43^{d}	89.31 ± 5.36^{c}	0.45 ± 0.04^{bc}	4.78 ± 0.06^{e}

Values are expressed as mean ± SD (n = 3). Different superscript letters in the same column indicate significant difference at $p < 0.05$. POS = pasteurised noni juice; FRE = fresh noni juice; S20 = noni juice sonicated for 20 min; S40 = noni juice sonicated for 40 min; S60 = noni juice sonicated for 60 min. ND = Not detected.

Table 4.7: Retention time, linear equation, LOD, and LOQ of organic acids standard.

Organic Acids	Retention Time (min)	Linear Equation	R^2	LOD (mg/100 mL)	LOQ (mg/100 mL)
Ascorbic Acid	10.3	y = 16812x - 328929	0.9873	5.92	17.93
Malic Acid	8.7	y = 2414.2x - 108372	0.9955	15.77	47.79
Fumaric Acid	12.3	y = 152605x + 87467	0.9904	0.09	0.28
Citric Acid	13.5	y = 3268.6x – 4487.2	0.9996	0.15	0.46

R^2 = Coefficient of determination; LOD = limit of detection; LOQ = limit of quantitation.

4.1.4 Antioxidant Capacity of Noni Juice at Different Sonication Duration

The effect of sonication duration on the antioxidant capacity of noni juice, as measured by FRAP and TEAC assays, is presented in Table 4.8. Based on the FRAP results, the S60 noni juice exhibited the highest FRAP (59.63 μM Fe^{2+}/kg), which was significantly higher than other noni juice samples. In contrast, pasteurised noni juice showed the lowest FRAP value (29.92 μM Fe^{2+}/kg) among all samples.

Regarding the TEAC results, the S40 (19.39 mM TE/kg) and S60 (19.65 mM TE/kg) noni juices showed significantly higher values compared to the S20 (18.32 mM TE/kg), fresh (17.65 mM TE/kg), and pasteurised (15.52 mM TE/kg) noni juices. Generally, the TEAC value of pasteurised noni juice was the lowest among all samples.

Table 4.8: Antioxidant capacity of noni juice samples at different sonication duration.

Sample	FRAP (μM Fe^{2+}/kg)	TEAC (mM TE/kg)
POS	29.92 ± 2.57^{a}	15.52 ± 0.31^{a}
FRE	37.90 ± 4.69^{ab}	17.65 ± 0.26^{b}
S20	38.73 ± 5.23^{ab}	18.32 ± 0.27^{b}
S40	45.38 ± 0.72^{b}	19.39 ± 0.26^{c}
S60	59.63 ± 1.88^{c}	19.65 ± 0.30^{c}

Values are expressed as mean ± SD (n = 3). Different superscript letters in the same column indicate significant difference at $p < 0.05$. POS = pasteurised noni juice; FRE = fresh noni juice; S20 = noni juice sonicated for 20 min; S40 = noni juice sonicated for 40 min; S60 = noni juice sonicated for 60 min.

4.2 Storage Study of Noni Juice at Different Temperatures for 56 Days

4.2.1 Physicochemical Properties of Noni Juice During Storage

The results of pH, titratable acidity, and total soluble solids of noni juice during 56 days of storage at 4°C and 25°C are tabulated in Table 4.9. At day 56, a significant decrease in pH was observed in all noni juices at both storage temperatures, with the pH dropping from approximately 3.90 to 3.70. The noni juice stored at 25°C exhibited a lower pH (ranging 3.70 to 3.71) compared to the noni juice stored at 4°C (ranging 3.76 to 3.77).

The titratable acidity of noni juice did not show significant changes during the storage period. In term of the total soluble solids, the pasteurised (1.37 °Brix to 1.60 °Brix), fresh (1.33 °Brix to 1.60 °Brix), and S60 (1.33 °Brix to 1.60 °Brix) noni juices stored at 4°C showed a significant increase. In contrast, the pasteurised (1.37 °Brix to 1.40 °Brix), fresh (1.33 °Brix to 1.40 °Brix), and S60 (1.33 °Brix to 1.40 °Brix) noni juices stored at 25°C showed a non-significant increase.

Table 4.9: The pH, titratable acidity, and total soluble solids of noni juice samples stored at 4°C and 25°C for 56 days.

Sample	Storage Duration	pH	Titratable Acidity (%)	Total Soluble Solid (°Brix)
4ºC - POS	**Day 0**	3.91 ± 0.01*	0.18 ± 0.02	1.37 ± 0.06*
	Day 56	3.77 ± 0.02*	0.20 ± 0.01	1.60 ± 0.00*
4ºC - FRE	**Day 0**	3.90 ± 0.01*	0.17 ± 0.01	1.33 ± 0.06*
	Day 56	3.76 ± 0.01*	0.19 ± 0.02	1.60 ± 0.00*
4ºC - S60	**Day 0**	3.91 ± 0.01*	0.17 ± 0.01	1.33 ± 0.06*
	Day 56	3.76 ± 0.01*	0.17 ± 0.01	1.60 ± 0.00*
25ºC - POS	**Day 0**	3.91 ± 0.01*	0.18 ± 0.02	1.37 ± 0.06
	Day 56	3.70 ± 0.01*	0.18 ± 0.02	1.40 ± 0.00
25ºC - FRE	**Day 0**	3.90 ± 0.01*	0.17 ± 0.01	1.33 ± 0.06
	Day 56	3.71 ± 0.01*	0.18 ± 0.02	1.40 ± 0.00
25ºC – S60	**Day 0**	3.91 ± 0.01*	0.17 ± 0.01	1.33 ± 0.06
	Day 56	3.71 ± 0.01*	0.18 ± 0.01	1.40 ± 0.00

Values are expressed as mean ± SD (n = 3). ***** Indicates statistically significant difference at $p < 0.05$ during paired sample *t*-test between Day 0 and Day 56. POS = pasteurised noni juice; FRE = fresh noni juice; S60 = noni juice sonicated for 60 min.

The results of the colour attributes (L^*, a^*, and b^*) of noni juice during storage at 4°C and 25°C for 56 days are presented in Table 4.10a. At day 56, the a^* value of all noni juices increased, especially in fresh and S60 noni juices stored at 25°C, going from -0.44 to 0.41 and -0.35 to 0.30, respectively. The L^* and b^* values also showed an overall increase.

Table 4.10a: The colour attributes (L^*, a^*, b^*) of noni juice samples stored at 4°C and 25°C for 56 days.

Samples	Storage Duration	L^*	a^*	b^*
4ºC - POS	**Day 0**	47.66 ± 0.32	-0.41 ± 0.03	5.46 ± 0.03
	Day 56	53.14 ± 2.18	-0.10 ± 0.12	5.71 ± 0.28
4ºC - FRE	**Day 0**	47.55 ± 0.47*	-0.44 ± 0.03	6.47 ± 0.75*
	Day 56	54.40 ± 1.23*	-0.14 ± 0.20	4.58 ± 0.91*
4ºC - S60	**Day 0**	52.71 ± 0.47	-0.35 ± 0.06*	3.45 ± 0.70
	Day 56	52.19 ± 0.46	-0.16 ± 0.06*	5.02 ± 0.57
25ºC - POS	**Day 0**	47.66 ± 0.32*	-0.41 ± 0.03*	5.46 ± 0.03
	Day 56	51.39 ± 0.62*	-0.11 ± 0.06*	6.44 ± 1.72
25ºC - FRE	**Day 0**	47.55 ± 0.47	-0.44 ± 0.03*	6.47 ± 0.75
	Day 56	50.17 ± 1.36	0.41 ± 0.16*	9.05 ± 1.38
25ºC – S60	**Day 0**	52.71 ± 0.47	-0.35 ± 0.06*	3.45 ± 0.70*
	Day 56	50.57 ± 1.03	0.30 ± 0.03*	8.48 ± 0.16*

Values are expressed as mean ± SD (n = 3). * Indicates statistically significant difference at $p < 0.05$ during paired sample *t*-test between Day 0 and Day 56. POS = pasteurised noni juice; FRE = fresh noni juice; S60 = noni juice sonicated for 60 min. L^* = lightness; $+a^*$ = redness; $-a^*$ = greenness; $+b^*$ = yellowness; $-b^*$ = blueness.

The values of h^*, C^*, ΔE, and browning index of noni juice during 56 days of storage are shown in Table 4.10b. Overall, the h^* of all noni juices decreased significantly, while the pasteurised and fresh noni juices stored at 4°C showed a non-significant decrease. The C^* value also showed an increase after the storage study in overall, with higher C^* value observed in the noni juice samples during storage at 25°C than the 4°C. Likewise, the saturation of noni juice samples during storage at 25°C was higher than that of samples stored at 4°C, as shown in Figure 4.5.

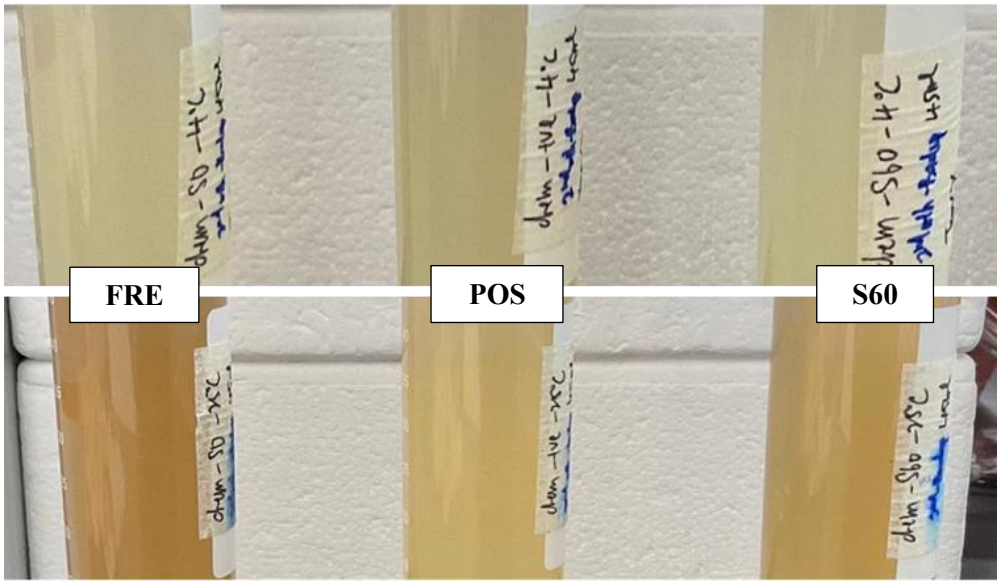

Figure 4.5: The noni juice samples after 56 days of storage at different temperatures. Note: Top: Refrigerated storage (4°C); Bottom: Room temperature storage (25°C). FRE: fresh noni juice; POS: pasteurised noni juice; S60: noni juice sonicated for 60 min.

Throughout 56 days of storage period, the pasteurised, fresh, and S60 noni juice samples stored at a refrigerated temperature (4°C) did not exhibit significant changes in the browning index. The browning index of pasteurised noni juice during storage at 25°C (BI = 1.06) did not show a significantly increase compare to its initial value (BI = 0.49). However, the browning index of the fresh noni juice (increasing from 0.66 to 2.32) and S60 (increasing from 0.17 to 2.06) significantly increase during storage at 25°C. Generally, storing the noni juice at 25°C resulted in a greater browning index (BI = 1.06-2.32) than storing at 4°C (BI = 0.64-0.91). On the other hand, the pasteurised (ΔE = 2.51) and S60 (ΔE =2.27) noni juices stored at 4°C exhibited moderate colour difference as compared to fresh noni juice. The S60 noni juice stored at 25°C (ΔE = 1.66) exhibited moderate colour difference meanwhile pasteurised noni juice stored at 25°C (ΔE = 3.06) showed major colour difference. *newly added (5/11/23)

Table 4.10b: The colour attributes (h^*, C^*, BI, ΔE) of noni juice samples stored at 4°C and 25°C for 56 days.

Sample	Storage Duration	Hue (h^*)	Chroma (C^*)	Browning Index (BI)	Total Colour Difference (ΔE)
4ºC - POS	**Day 0**	94.33 ± 0.34	5.48 ± 0.03	0.49 ± 0.06	1.16 ± 0.85
	Day 56	91.01 ± 1.20	5.71 ± 0.29	0.91 ± 0.16	2.51 ± 1.55
4ºC - FRE	**Day 0**	93.94 ± 0.73	6.49 ± 0.74*	0.66 ± 0.19	Reference
	Day 56	91.80 ± 2.65	4.59 ± 0.91*	0.64 ± 0.31	Reference
4ºC - S60	**Day 0**	95.75 ± 0.16*	3.47 ± 0.71	0.17 ± 0.04	6.11 ± 0.44*
	Day 56	91.85 ± 0.85*	5.03 ± 0.57	0.72 ± 0.18	2.27 ± 1.27*
25ºC - POS	**Day 0**	94.33 ± 0.34*	5.48 ± 0.03	0.49 ± 0.06	1.16 ± 0.85
	Day 56	90.98 ± 0.28*	6.44 ± 1.72	1.06 ± 0.25	3.06 ± 2.81
25ºC - FRE	**Day 0**	93.94 ± 0.73*	6.49 ± 0.74	0.66 ± 0.19*	Reference
	Day 56	87.45 ± 0.65*	9.06 ± 1.38	2.32 ± 0.56*	Reference
25ºC - S60	**Day 0**	95.75 ± 0.16*	3.47 ± 0.71*	0.17 ± 0.04*	6.11 ± 0.44*
	Day 56	87.95 ± 0.22*	8.48 ± 0.16*	2.06 ± 0.01*	1.66 ± 0.60*

Values are expressed as mean ± SD (n = 3). * Indicates statistically significant difference at $p < 0.05$ during paired sample *t*-test between Day 0 and Day 56. POS = pasteurised noni juice; FRE = fresh noni juice; S60 = noni juice sonicated for 60 min. h^* = visual colour difference with reference to grey colour under same lightness. C^* = saturation of samples. ΔE measures the colour difference between treated samples and control. Reference serves as control for total colour difference.

4.2.2 Total Phenolics and Total Flavonoids Content of Noni Juice During Storage

The TPC and TFC of noni juice stored at 4°C and 25°C for 56 days are tabulated in Table 4.11. The data showed that the decrease in TPC of fresh and S60 noni juices stored at 4°C was 8.5% and 7.5%, respectively, which was not statistically significant. In contrast, the TPC of the pasteurised noni juice decreased by 20% significantly after 56 days of storage at 4°C. The pasteurised, fresh, and S60 noni juices storing at 25°C demonstrated a significant decrease in TPC at day 56.

No significant decrement in TFC was observed in all noni juice samples stored at 4°C, as well as in the pasteurised and fresh noni juices stored at 25°C. However, the TFC of S60 noni juice stored at 25°C significantly decreased by 33% after 56 days of storage.

Table 4.11: Total phenolics and total flavonoids content of noni juice samples stored at 4°C and 25°C for 56 days.

Sample	Storage Duration	TPC (mg GAE/100 mL)	TFC (mg RE/100 mL)
4ºC - POS	**Day 0**	2.62 ± 0.05*	1.14 ± 0.17
	Day56	2.08 ± 0.12*	1.22 ± 0.40
4ºC - FRE	**Day 0**	2.93 ± 0.07	1.01 ± 0.19
	Day56	2.68 ± 0.06	0.99 ± 0.09
4ºC - S60	**Day 0**	3.19 ± 0.06	1.48 ± 0.11
	Day56	2.95 ± 0.19	1.28 ± 0.20
25ºC - POS	**Day 0**	2.62 ± 0.05*	1.14 ± 0.17
	Day56	1.11 ± 0.05*	1.48 ± 0.16
25ºC - FRE	**Day 0**	2.93 ± 0.07*	1.01 ± 0.19
	Day56	1.32 ± 0.02*	0.84 ± 0.14
25ºC - S60	**Day 0**	3.19 ± 0.06*	1.48 ± 0.11*
	Day56	1.65 ± 0.03*	0.99 ± 0.15*

Values are expressed as mean ± SD (n = 3). * Indicates statistically significant difference at $p < 0.05$ during paired sample *t*-test between Day 0 and Day 56. POS = pasteurised noni juice; FRE = fresh noni juice; S60 = noni juice sonicated for 60 min.

4.2.3 Phenolic and Organic Acids Profile of Noni Juice During Storage

The phenolic profile of noni juice stored at 4°C and 25°C for 56 days are shown in Table 4.12. The scopoletin content of noni juice stored at 4°C decreased significantly in pasteurised (0.50 mg/100 mL) and fresh (0.63 mg/100 mL) noni juices. A non-significant decrease was observed in S60 (0.90 mg/100 mL) at the end of the storage period. Conversely, the scopoletin content of pasteurised (1.26 mg/100 mL) and fresh (1.42 mg/100 mL) noni juices stored at 25°C showed a significant increase, while S60 noni juice (1.67 mg/100 mL) exhibited a non-significant increase at day 56.

The levels of rutin in all noni juices decreased significantly at day 56 as compared to the initial values (2.22-4.02 mg/100 mL). It was observed that pasteurised noni juice retained a higher rutin level (0.75-1.10 mg/100 mL) than the fresh and S60 noni juices during storage at both temperatures.

The vanillic acid content of all noni juices storing at 4°C decreased significantly from the range of 7.95-12.17 mg/100 mL to 1.80-7.48 mg/100 mL. A significant decrease was also observed in the vanillic acid content of fresh and S60 noni juices storing at 25°C, while pasteurised noni juice demonstrated a non-significant decrease after 56 days of storage.

Table 4.12: Phenolic compounds detected in noni juice samples (mg/100 mL) stored at 4°C and 25°C for 56 days.

Sample	Storage Duration	Scopoletin	Rutin	Vanillic Acid
4ºC - POS	**Day 0**	0.83 ± 0.06*	2.22 ± 0.25*	7.95 ± 1.37*
	Day 56	0.50 ± 0.02*	0.75 ± 0.01*	1.80 ± 0.28*
4ºC - FRE	**Day 0**	0.96 ± 0.01*	2.75 ± 0.02*	9.02 ± 0.25*
	Day 56	0.63 ± 0.03*	ND*	6.96 ± 0.45*
4ºC - S60	**Day 0**	1.47 ± 0.23	4.02 ± 0.84*	12.17 ± 1.18*
	Day 56	0.90 ± 0.02	ND*	7.48 ± 0.37*
25ºC - POS	**Day 0**	0.83 ± 0.06*	2.22 ± 0.25*	7.95 ± 1.37
	Day 56	1.26 ± 0.01*	1.10 ± 0.10*	7.76 ± 0.32
25ºC - FRE	**Day 0**	0.96 ± 0.01*	2.75 ± 0.02*	9.02 ± 0.25*
	Day 56	1.42 ± 0.02*	ND*	ND*
25ºC – S60	**Day 0**	1.47 ± 0.23	4.02 ± 0.84*	12.17 ± 1.18*
	Day 56	1.67 ± 0.01	ND*	ND*

Values are expressed as mean ± SD (n = 3). * Indicates statistically significant difference at $p < 0.05$ during paired sample *t*-test between Day 0 and Day 56. ND = Not detected; POS = pasteurised noni juice; FRE = fresh noni juice; S60 = noni juice sonicated for 60 min.

The results of organic acids of noni juice during 56 days of storage at 4°C and 25°C are tabulated in Table 4.13. The concentration of ascorbic acid, malic acid, fumaric acid, and citric acid ranged from 2.90 to 17.33 mg/100 mL, 34.91 to 64.79 mg/100 mL, 0.09 to 0.16 mg/100 mL, and 16.25 to 38.09 mg/100 mL, respectively, at day 56. It was observed that the concentration of ascorbic acid, malic acid, and fumaric acid decreased significantly in all noni juices, while there was a significant increase in citric acid at day 56.

Table 4.13: Organic acids detected in noni juice samples (mg/100 mL) stored at 4°C and 25°C for 56 days.

Sample	Storage Duration	Ascorbic Acid	Malic Acid	Fumaric Acid	Citric Acid
4ºC – POS	**Day 0**	17.15 ± 0.20*	57.54 ± 3.97*	0.35 ± 0.05*	0.90 ± 0.13*
	Day 56	2.91 ± 0.15*	44.61 ± 0.70*	0.09 ± 0.09*	18.25 ± 0.31*
4ºC – FRE	**Day 0**	26.93 ± 1.17*	76.43 ± 2.72*	0.39 ± 0.05*	1.50 ± 0.07*
	Day 56	12.01 ± 0.71*	62.27 ± 0.92*	0.11 ± 0.01*	24.53 ± 0.50*
4ºC – S60	**Day 0**	31.55 ± 1.43*	89.31 ± 5.36*	0.45 ± 0.04*	4.78 ± 0.06*
	Day 56	17.33 ± 0.71*	64.79 ± 0.91*	0.12 ± 0.02*	32.83 ± 0.92*
25ºC – POS	**Day 0**	17.15 ± 0.20*	57.54 ± 3.97*	0.35 ± 0.05*	0.90 ± 0.13*
	Day 56	2.92 ± 0.10*	34.91 ± 1.47*	0.09 ± 0.01*	16.25 ± 0.45*
25ºC – FRE	**Day 0**	26.93 ± 1.17*	76.43 ± 2.72*	0.39 ± 0.05*	1.50 ± 0.07*
	Day 56	2.90 ± 0.02*	40.86 ± 1.12*	0.16 ± 0.01*	29.16 ± 0.81*
25ºC – S60	**Day 0**	31.55 ± 1.43*	89.31 ± 5.36*	0.45 ± 0.04*	4.78 ± 0.06*
	Day 56	3.22 ± 0.06*	41.79 ± 1.20*	0.13 ± 0.02*	38.09 ± 0.63*

Values are expressed as mean ± SD (n = 3). * Indicates statistically significant difference at $p < 0.05$ during paired sample *t*-test between Day 0 and Day 56. POS = pasteurised noni juice; FRE = fresh noni juice; S60 = noni juice sonicated for 60 min.

4.2.4 Antioxidant Capacity of Noni Juice During Storage

The antioxidant capacity of noni juice during the storage study is presented in Table 4.14. Based on the results, a significant decrease in FRAP was observed in pasteurised noni juice stored at 4°C (from 29.92 to 20.52 μM Fe^{2+}/kg), S60 noni juice stored at 4°C (from 59.63 to 41.58 μM Fe^{2+}/kg), pasteurised noni juice stored at 25°C (from 29.92 to 14.62 μM Fe^{2+}/kg), fresh noni juice stored at 25°C (from 37.90 to 16.66 μM Fe^{2+}/kg), and S60 noni juice stored at 25°C (from 59.63 to 15.61 μM Fe^{2+}/kg). A non-significant decrease in FRAP was observed in fresh noni juice stored at 4°C (from 37.90 to 34.81 μM Fe^{2+}/kg). Generally, the noni juice stored at 25°C resulted in a greater loss in FRAP (15.3-44.02 μM Fe^{2+}/kg) than the noni juice stored at 4°C (3.09-18.05 μM Fe^{2+}/kg).

The TEAC of pasteurised noni juice stored at 4°C (from 15.52 to 9.72 mM TE/kg), fresh noni juice stored at 4°C (from 17.65 to 13.56 mM TE/kg), S60 noni juice stored at 4°C (from 19.65 to 15.50 mM TE/kg), pasteurised noni juice stored at 25°C (from 15.52 to 3.57 mM TE/kg), fresh noni juice stored at 25°C (from 17.65 to 7.65 mM TE/kg), and S60 noni juice stored at 25°C (from 19.65 to 8.80 mM TE/kg) were significantly decrease after 56 days of storage. Generally, the noni juice stored at 25°C demonstrated a greater loss in TEAC (10-11.95 mM TE/kg) compared to the noni juice stored at 4°C (4.09-5.80 mM TE/kg).

Table 4.14: Antioxidant capacity of noni juice samples stored at 4°C and 25°C for 56 days.

Sample	Storage Duration	FRAP (μM Fe^{2+}/kg)	TEAC (mM TE/kg)
4ºC - POS	**Day 0**	29.92 ± 2.57*	15.52 ± 0.31*
	Day56	20.52 ± 0.85*	9.72 ± 0.43*
4ºC - FRE	**Day 0**	37.90 ± 4.69	17.65 ± 0.26*
	Day56	34.81 ± 0.09	13.56 ± 0.44*
4ºC - S60	**Day 0**	59.63 ± 1.88*	19.65 ± 0.30*
	Day56	41.58 ± 0.65*	15.50 ± 0.26*
25ºC - POS	**Day 0**	29.92 ± 2.57*	15.52 ± 0.31*
	Day56	14.62 ± 1.78*	3.57 ± 0.53*
25ºC - FRE	**Day 0**	37.90 ± 4.69*	17.65 ± 0.26*
	Day56	16.66 ± 1.43*	7.65 ± 0.92*
25ºC - S60	**Day 0**	59.63 ± 1.88*	19.65 ± 0.30*
	Day56	15.61 ± 0.52*	8.80 ± 0.57*

Values are expressed as mean ± SD (n = 3). ***** Indicates statistically significant difference at $p < 0.05$ during paired sample *t*-test between Day 0 and Day 56. POS = pasteurised noni juice; FRE = fresh noni juice; S60 = noni juice sonicated for 60 min.

4.2.5 Microbial Load of Noni Juice During Storage

The microbial load, including aerobic mesophilic bacteria, yeast, and mold counts of noni juice during 56 days of storage at 4°C and 25°C are tabulated in Table 4.15. Figure 4.6 and Figure 4.7 present the microbial load of noni juice before and after 56 days of storage at different temperatures, respectively. No yeast and mold growth were detected during 56 days of storage, regardless of the storage temperatures. The aerobic mesophilic count of the noni juice remained consistently below 2 log CFU/mL throughout the entire storage duration. Statistical analysis revealed no significant difference in the aerobic mesophilic count between fresh and S60 noni juices stored at 4°C and 25°C. The pasteurised noni juice stored at both 4°C and 25°C maintained a level <1 log CFU/mL throughout the 56 days of storage. In addition, it was observed that fresh and S60 noni juices stored at 25°C exhibited a significantly lower total plate count on day 56 compared to samples stored at 4°C.

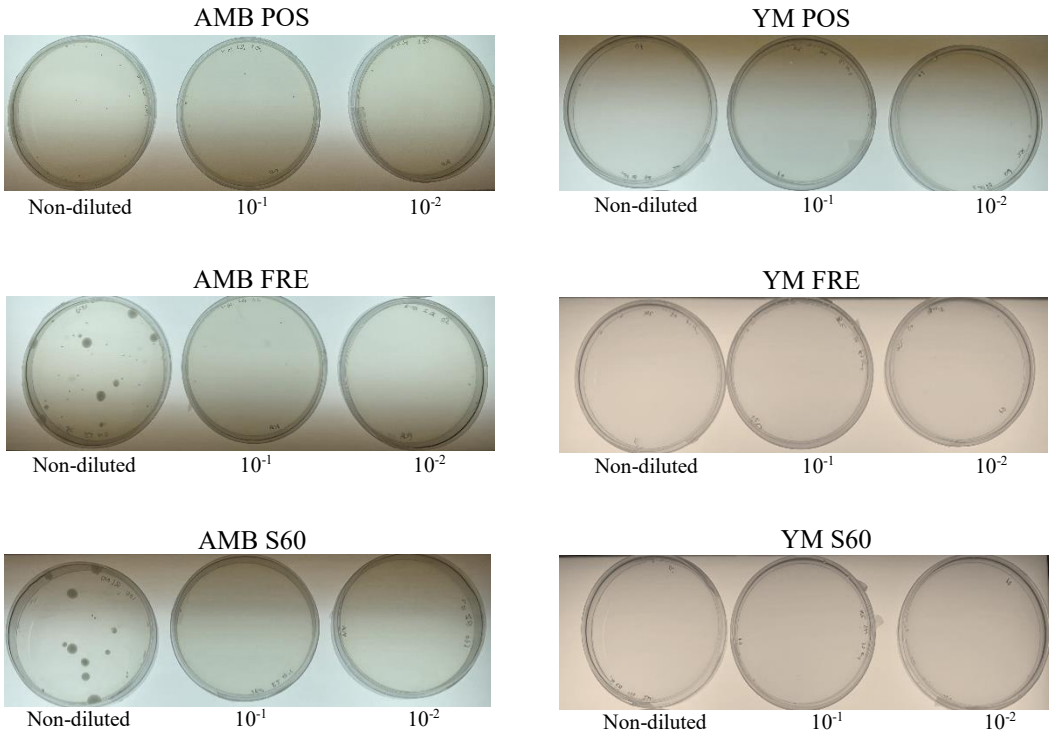

Figure 4.6: Microbial load of noni juice before 56 days of storage. Note: POS: Pasteurised noni juice; FRE: fresh noni juice; S60: noni juice sonicated for 60 min; AMB: aerobic mesophilic bacteria; YM: yeast and mold; Dilution factor: non-diluted > 10^{-1} > 10^{-2}.

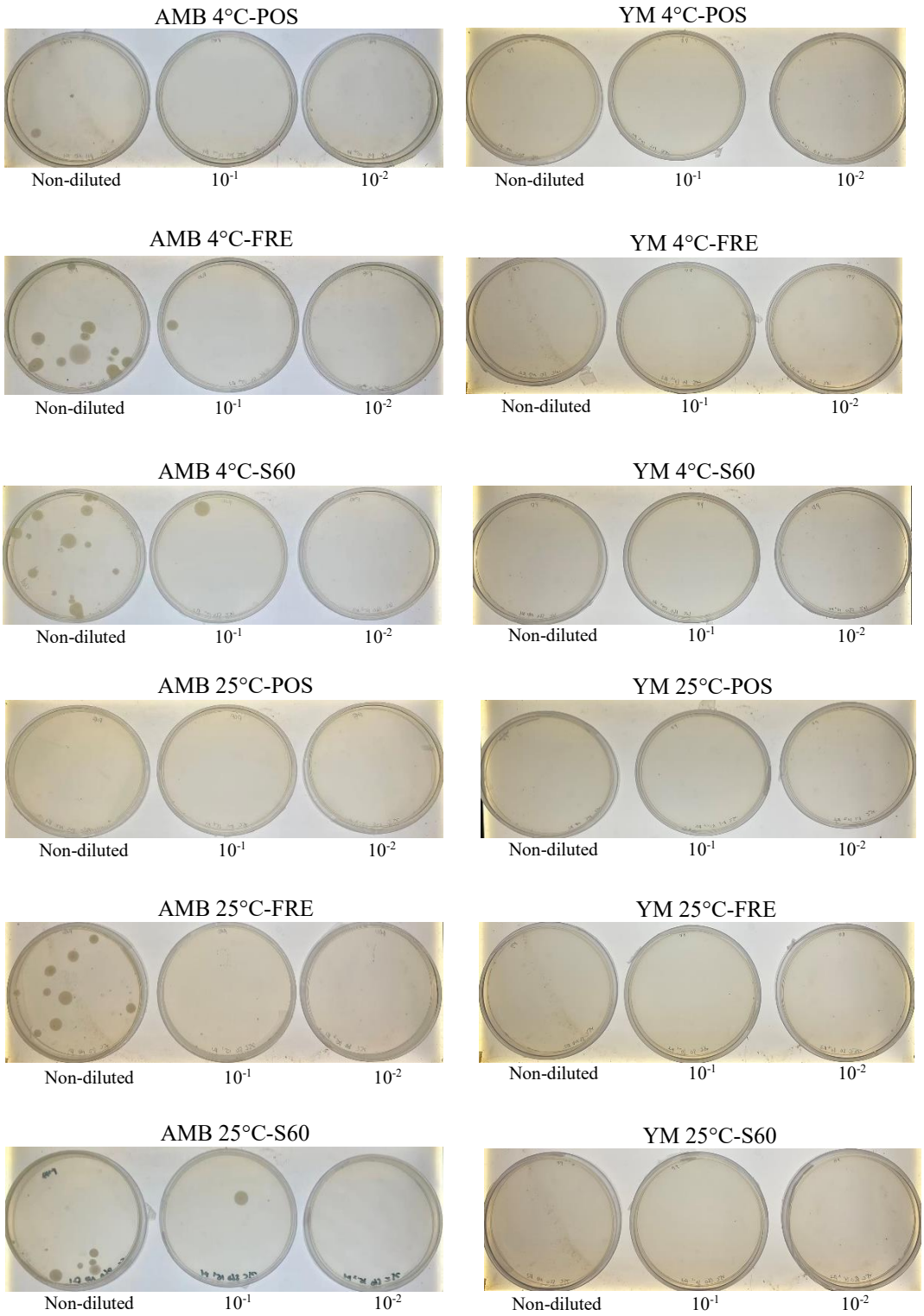

Figure 4.7: Microbial load of noni juice after 56 days of storage at 4°C and 25°C. Note: POS: pasteurised noni juice; FRE: fresh noni juice; S60: noni juice sonicated for 60 min; AMB: aerobic mesophilic bacteria; YM: yeast and mold; Dilution factor: non-diluted > 10^{-1} > 10^{-2}.

Table 4.15: The microbial load (log CFU/mL) of noni juice samples stored at 4°C and 25°C for 56 days.

Sample	Day 0		Day 14		Day 28		Day 42		Day 56	
	AMB	**YM**	**AMB**	**YM**	**AMB**	**YM**	**AMB**	**YM**	**AMB**	**YM**
4ºC - POS	$<1.00^{aA}$	NG	$<1.00^{aA}$	NG	$<1.00^{aA}$	NG	$<1.00^{aA}$	NG	$<1.00^{aA}$	NG
4ºC - FRE	1.71 ± 0.11^{bA}	NG	1.70 ± 0.03^{bA}	NG	1.70 ± 0.02^{bA}	NG	1.77 ± 0.06^{cA}	NG	1.84 ± 0.14^{cA}	NG
4ºC - S60	1.83 ± 0.00^{bAB}	NG	1.88 ± 0.04^{bB}	NG	1.75 ± 0.05^{bA}	NG	1.76 ± 0.02^{cA}	NG	1.78 ± 0.14^{cAB}	NG
25ºC - POS	$<1.00^{aB}$	NG	$<1.00^{aAB}$	NG	$<1.00^{aAB}$	NG	$<1.00^{bA}$	NG	$<1.00^{aAB}$	NG
25ºC - FRE	1.71 ± 0.11^{bA}	NG	1.66 ± 0.05^{bA}	NG	1.67 ± 0.09^{bA}	NG	1.60 ± 0.04^{cA}	NG	1.51 ± 0.03^{bA}	NG
25ºC - S60	1.83 ± 0.00^{bC}	NG	1.68 ± 0.03^{bB}	NG	1.70 ± 0.07^{bBC}	NG	1.58 ± 0.01^{cAB}	NG	1.48 ± 0.00^{bA}	NG

Values are expressed as mean ± SD (n = 2). Superscript in different small letter indicates significant difference ($p < 0.05$) among samples, whereas different big letter indicates significant difference ($p < 0.05$) between storage duration. AMB = aerobic mesophilic bacteria; YM =yeast and mold; NG = no growth; CFU = colony forming unit; POS = pasteurised noni juice; FRE = fresh noni juice; S60 = noni juice sonicated for 60 min.

CHAPTER 5

DISCUSSION

5.1 Effect of Sonication Duration on the Quality Attributes of Noni Juice

5.1.1 Physicochemical Properties of Noni Juice at Different Sonication Duration

The pH measures the hydrogen ion concentration while the titratable acidity measures the total acid concentration in food (Sadler and Murphy, 2010). Total soluble solids measure soluble organic material, sugar content, and soluble proteins of fruit juice (Bhat and Goh, 2017; Kusumiyati, et al., 2020). As described by Alam, et al. (2023) pH, titratable acidity, and total soluble solids of the juice are corresponding to its sensory quality. Thus, changes in these three parameters would negatively affect the sensory quality of fruit juice. Results showed that the pH, titratable acidity, and total soluble solid were unaffected by both sonication and pasteurisation. Similar results were also observed in mango juice (Santhirasegaram, Razali and Somasundram, 2013), barberry juice (Farhadi Chitgar, et al., 2016), and gooseberry juice (Ordóñez-Santos, Martínez-Girón and Arias-Jaramillo, 2017). This scenario might be due to the energy level of sonication and pasteurisation applied to the juice samples did not affect the molecular structures of high molecular weight that associated with these physicochemical properties juice (Ordóñez-Santos, Martínez-Girón and Arias-Jaramillo, 2017). It is noteworthy that the parameter of both sonication and

pasteurisation in the present study did not affect the physicochemical properties of noni juice meanwhile preserving its sensory quality.

Fruit juice with pH lower than 4.6 is considered to be highly acidic (Bhat and Goh, 2017). In the present study, pH of noni fruit juice was found to be comparable to that of fermented noni juice, with a pH of 3.70 (Barraza-Elenes, et al., 2019), suggesting the acidic nature of noni juice. Additionally, literature data reported that low pH of noni juice was attributed to the high level of ascorbic acid (Nowak, et al., 2018). The total soluble solid of noni juice (1.30-1.37°Brix) in the present study was found to be lower than that of enzyme-treated hydraulically pressed noni juice (5.80 °Brix) (Dussossoy, et al., 2011), possibly due to the release of additional sugars and organic acids after the tissue breakdown by the enzymes. Instead of being sweet, noni pulp is bitter or astringent (Motshakeri and Ghazali, 2015). Thus, noni juice exhibits low total soluble solids values as shown in the present study in comparison to other fruit juice that taste sweet, which includes the apple (11.5 °Brix), banana (22.0 °Brix), pear (12.0 °Brix), orange (11.8 °Brix), and pomegranate (16.0 °Brix) juices (Clemens, et al., 2015).

The colour of fruit juice serves as the basis for consumers to judge the overall product quality. As shown in Table 4.2, S20, S40, and S60 noni juice samples resulted in significant differences in lightness (L^*), yellowness ($+b^*$), and chroma (C^*) values as compared with fresh juice. These results are in line with the findings by Santhirasegaram, Razali and Somasundram (2013), who reported that sonicated mango juice showed an increased L^* value and a decreased $+b^*$ value. The noni juice colour turned less saturated, as indicated by the C^* values after sonication. This result is also observed in the findings by Tan, et al. (2023), whereby sonication decreased the C^* values of red pitaya juice. It was claimed that colour degradation in sonicated juice samples might be attributed to the oxidation reactions as a result of interaction of free radicals formed during sonication (Dias, et al., 2015). Colour change in fruit juice has been reported as a result of cavitation during sonication (Tiwari, et al., 2008). The h^* values obtained indicate noni juice is in greenish-yellow colour as presented in Figure 3.4. The h^* of noni juice was unaffected by the sonication and pasteurisation. Results showed that the three chromaticity coordinates (L^*, a^*, and b^* values) was unaffected by pasteurisation which in contrast to a study on barberry juice by Farhadi Chitgar, et al. (2016). According to Manzoor, et al. (2020), no difference in L^* and a^* values was found between pasteurised and fresh sugarcane juice, but the b^* value decreased after pasteurisation. Thus, it can be concluded that all three chromaticity coordinates in juice are greatly affected by the food processing conditions and the composition of fruit cultivars (Lee and Coates, 1999; Manzoor, et al., 2020).

The browning index determines the presence of enzymatic or non-enzymatic browning reactions during juice processing by measuring the purity of the brown colour. Results demonstrated that sonication resulted in lower browning index of noni juice for 0.29 to 0.49. This might be due to inactivation of browning enzymes such as polyphenol oxidase and peroxidase, contributed by the cavitation effect during sonication (Dias, et al., 2015; Wang, et al., 2019). The polyphenol oxidase and peroxidase are the major enzyme that involved in browning of fruits and vegetables. Browning causes deterioration of food quality, safety, and nutritional value (Hamdan, et al., 2022). Thus, inactivation of browning enzymes could be beneficial in retaining the flavour and nutritional properties of fruit juice.

The total colour difference (ΔE) is a useful measure to determine the differences in perceivable colour. The difference in colour could be classified as very distinct ($\Delta E > 3$), distinct ($1.5 < \Delta E < 3$) and small difference ($\Delta E < 1.5$) (Manzoor, et al., 2020). According to Table 4.2, all sonicated noni juices, namely, S20, S40, and S60 resulted in a very distinct colour variation (ΔE = 5.20-6.11) as compared with the fresh noni juice, possibly as a result of ultrasound-induced hydroxylation of the phenolic aromatic ring, which, in turn, changes the visible spectrum area (Manzoor, et al., 2020). In accordance with the findings of Manzoor, et al. (2020), pasteurisation resulted in a small colour difference (ΔE = 1.16) in the juice.

5.1.2 Total Phenolics, Total Flavonoids, and Total Carotenoids Content of Noni Juice at Different Sonication Duration

More than 150 phytochemicals have been detected in noni fruit. Among these, phenolic compounds are the main phytochemicals (Valdés, et al., 2009). Phenolic compounds are one of the main contributors to antioxidant activity in fruit juices. In the present study, the TPC of noni juice ranged between 2.62 to 3.19 mg GAE/100 mL (refer to Table 4.3). There is a large variation in the TPC of noni juice reported in literature, ranging from 3.1 to 210 mg GAE/100 mL (Yang, et al., 2007b; Yang, et al., 2007a; Nugroho, Rahardjo and Jovitatera, 2019). Results showed that S60 significantly yield higher TPC values than that of pasteurised, fresh, S20, and S40 noni juice samples. This agrees with the findings by Bhat, et al. (2011), who reported that the TPC of lime juice significantly improved after 60 min of sonication.

Flavonoids are natural phenolic compounds present in fruits with anti-inflammatory properties. As presented in Table 4.3, the TFC values of noni juice are consistent with the TFC values (0.66–2.48 mg quercetin equivalents/100 mL) reported for fermented noni juice (Yang, et al., 2007b). The present study demonstrated that S60 significantly increased the yield of TFC by 47%, as compared to the fresh sample. Following 60 min of sonication, the increment of TPC and TFC in the noni juice might be related to the liberation of bound phenolics and flavonoids. It might be also attributable to the attachment of non-chemically produced hydroxyl radicals to the aromatic rings of phenolic and flavonoid compounds. It has been reported that the addition of a second hydroxyl

group to the *ortho*- or *para*- positions can improve the antioxidant activity of the phenolic and flavonoid compounds (Bhat, et al., 2011).

Carotenoids are the pigmenting agents that account red, yellow, or orange colour for fruits and vegetables (Gilbert, 2013). They are classified into two basic groups which are the xanthophylls and carotenes. The *β*-carotene is one of the members of carotenes (Saini, Nile and Park, 2015). In this study, the carotenoids content of noni fruit was measured spectrophotometrically using *β*-carotene as a standard. According to Table 4.3, the TC of noni juice was relatively low. This observation is supported by Barraza-Elenes, et al. (2019), who also reported a low level of carotenoid content (1.06 mg *β*CE/100 g) in noni juice. On the other hand, the present study also demonstrated that noni juice has relatively lower amounts of carotenoids compared to apple juice (1.22-1.55 µg/mL FW) (Abid, et al., 2014). *β*-carotene is rich in yellow and orange fruits such as apple, banana, apricot, grapes, pineapple as well as green leafy vegetables (Tang, 2010; Saini, Nile and Park, 2015). The levels of carotenoids in fruits can be predicted by their colour. Thus, it is expected that the translucent-greyish noni fruit contains low level of carotenoids. Additionally, due to the lipophilic nature of carotenoids (González-Peña, et al., 2023), these compounds may not leach out effectively from the fruit during juice extraction, under hydrophilic environment.

5.1.3 Phenolic and Organic Acids Profile of Noni Juice at Different Sonication Duration

Vanillic acid, rutin, and scopoletin were detected in the pasteurised, fresh, and sonicated (S20, S40, S60) noni juices as shown in Table 4.4. In contrast to the findings by Deng, West and Jensen (2010), quercetin was not detected in the noni juice in the present study, possibly due to the use of different juice extraction method. The S60 noni juice yielded significantly higher amount of scopoletin, rutin, and vanillic acid than fresh and pasteurised noni juice. These observations are corresponding to the TPC results as shown in Table 4.3. The individual phenolic compounds can be present in soluble forms in vacuoles or bound to colloidal particles such as pectin, cellulose, hemicellulose, and lignin of the cell wall. During sonication, cavitation triggers the rupture of the cell wall, thereby enhancing the release of bound phenolic compounds from the colloidal particles. In addition, sonication accelerates the release of free phenolic compounds from the ruptured cell wall (Margean, et al., 2020).

Compared to the fresh noni juice, S60 resulted in an increase of 53% of scopoletin, 46% of rutin, and 35% of vanillic acid in the noni juice. Likewise, Abid, et al. (2014) also reported that, individual phenolic compounds in apple juice significantly increased after sonication for 60 min. However, no significant difference was observed between the scopoletin, rutin, and vanillic acid levels of fresh, S20, and S40 noni juices. This might be attributed to incomplete cell wall disruption due to an insufficient sonication; hence, fewer bound phenolics were released.

This study showed that fresh noni juice contained 0.96 mg/100 mL of scopoletin, 2.75 mg/100 mL of rutin, and 9.02 mg/100 mL of vanillic acid as compared with the scopoletin (1.32 mg/100 mL), rutin (4.63 mg/100 mL), and vanillic acid (0.26 mg/100 mL) of enzyme-treated hydraulically pressed noni juice (Dussossoy, et al., 2011). In short, the level of scopoletin and rutin in noni juice were lower than Dussossoy, et al. (2011) but the vanillic acid level obtained in this study was higher. The variation in the phenolic compounds level might be affected by harvesting season, geographical environment factors such as, sunlight, temperature, sunlight, moisture, air, etc (Deng, West and Jensen, 2010).

The four organic acids, namely, malic, ascorbic, citric, and fumaric acids were determined in noni juice (refer to Table 4.6). The present study showed that malic acid as the predominant organic acid in noni juice. Bittová, et al. (2015) also reported malic acid as the main organic compound in commercial noni fruit products, such as juice, powder, and capsules. Malic acid was originally extracted from apple juice and has broad applications in food, pharmaceuticals, plastic production, and metal cleaning. Compared to the fresh sample, S60 significantly increased the yield of malic acid by 17%, whereas pasteurisation significantly decreased the yield of malic acid by 25%. According to Giavoni, et al. (2022), the malic acid of pasteurised orange pulp byproduct was reduced by 18%, from 74.78 mg/100 g to 61.08 mg/100 g.

The present study demonstrated that the amounts of citric acid and fumaric acid in noni juice were consistently low, irrespective of the juice processing method. Similarly, Chunhieng, Hay and Montet (2005) also reported a low amount of citric acid in hydraulically pressed noni juice (3 mg/100 mL). It was reported that fumaric acid only exist in trace amounts and naturally in fruits (Gurtler and Mai, 2014). The citric acid of noni juice was significantly enhanced after 40 (S40) and 60 min (S60) of sonication while the fumaric acid was significantly increased after 60 min. Generally, longer sonication duration would prolong the cavitation effect, resulting in more swelling, fragmentation, pore formation of cell wall, and greater mechanical rupture of intracellular structures, such as plastids. This enhances the release of these organic compounds into the aqueous medium of the beverage, thereby increase their concentrations more drastically than that of bound phenolic compounds (Silva, et al., 2020; Kumar, Srivastav and Sharanagat, 2021).

The results of present study showed a significant increase in ascorbic acid of the S40 and S60 noni juices as compared to the fresh noni juice. Similar results were also observed in sonicated lime, grapefruit, and apple juices (Bhat, et al., 2011; Aadil, et al., 2013; Abid, et al., 2013). These studies revealed that the ascorbic acid level of sonicated fruit juices for more than 30 min was enhanced as compared to control samples. The enhancement in ascorbic acid level in noni juice could be due to the mild temperature used for sonication and the elimination of dissolved oxygen by cavitation. Heat and oxygen are the main factors that contribute to ascorbic acid degradation (Silva, et al., 2020). Moreover, pasteurisation decreased the ascorbic acid amount by 36% in noni

juice compared to the fresh sample because the high-heat processing might result in oxidation of ascorbic acid to dehydroascorbic acid.

Low levels of benzoic, tartaric, and malonic acids were reported in literature data (Farine, et al., 1996; Pino, Márquez and Castro, 2009; Wall, et al., 2015). Yet, benzoic, tartaric, and malonic acids were not detected in this study (refer Figure 4.4), possibly due to the use of different detection methods and juice extraction model (Almeida, de Oliveira and Hotza, 2019).

5.1.4 Antioxidant Capacity of Noni Juice at Different Sonication Duration

The antioxidant capacity of noni juice was measured by FRAP and TEAC assays. FRAP is a spectrophotometric method that measures the ability of antioxidants (bioactive compounds) in the juice samples to reduce ferric ions (Fe^{3+})–ligand complex into blue-coloured ferrous ions (Fe^{2+}) complex by electron transfer under acidic medium (pH 3.6). It was observed that the difference in FRAP values between sonication duration intervals (20, 40, 60 min) in the present study are greater in comparison to the TEAC values. This might be due to any substance other than antioxidant present in the noni juice sample with redox potential lower than the redox pair Fe^{3+}/Fe^{2+}, could reduce Fe^{3+}–Fe^{2+} and further contribute to the FRAP value (Gulcin, 2020). It is noteworthy that a single antioxidant assay will only provide a reductive suggestion regarding the antioxidant properties of a sample, hence, multiple assays are required to examine the antioxidant properties of a sample. As such, TEAC is also adopted to validate the antioxidant capacity of noni juice as well. TEAC measures the ability of bioactive compounds in noni juice to inhibit the ABTS radical cation by hydrogen donation. Results showed that TEAC values did not present the similar variation as FRAP, possibly due to the used of wavelength at 734 nm to measure the inhibition of ABTS radical cation. It was reported that wavelength at 734 nm able to diminish any interference from other absorbing compounds and from sample turbidity (Gulcin, 2020).

Despite the variation observed in the FRAP and TEAC, results (Table 4.8) showed that FRAP was significantly enhanced as observed in the S60 noni juice in comparison to the fresh, pasteurised, S20, and S40 noni juices. Both S40

and S60 noni juices resulted in significantly higher TEAC values than the S20, fresh, and pasteurised noni juices. Generally, the results of both assays showed that sonication for 60 min significantly improved the antioxidant capacity of noni juice as compared to the fresh and pasteurised samples. Study by Wang, Vanga and Raghavan (2019), also reported an increase of total antioxidant capacity of sonicated kiwifruit juice which ranged from 53.57 up to 121.88 μmol/100 mL as compared to the non-sonicated kiwifruit juice. The increment in the antioxidant activity of S60 noni juice might be attributed to the increase in bioactive compounds such as phenolics and organic acids (Table 4.4 and 4.6). Other likely reason could be due to inactivation of enzyme like polyphenol oxidases which took part in enzymatic browning (Nadeem, et al., 2018; Wang, Vanga and Raghavan, 2019). During enzymatic browning, the polyphenol oxidases will catalyse phenolics to form undesirable brown pigments, thereby reducing the antioxidant activity of the phenolics and subsequently decreasing the antioxidant capacity of fruit juice. Therefore, inactivation of enzyme like polyphenol oxidases could assists in increasing the antioxidant capacity in fruit juice (Wang, et al., 2019).

5.2 Storage Study of Noni Juice at Different Temperatures for 56 Days

Among the sonicated samples, S60 was selected for a storage study due to its high content of phenolics, organic acids, and antioxidant capacity. During the storage study, the pasteurised, fresh, and S60 noni juices were stored at refrigeration (4°C) and room temperature (25°C) for 56 days to evaluate their shelf-stability. The microbial load of noni juices was evaluated every 14 days during storage. Determination of physicochemical properties, TPC, TFC, phenolics, and organic acid profiles, and antioxidant capacity was conducted before and after 56 days of storage. The TC was not included in the storage study due to its low values (0.1-0.2 mg βCE/100 mL). This result is comparative with the findings by Barraza-Elenes, et al. (2019) and Fontes, et al. (2023) which demonstrated relatively low amounts of carotenoids in noni juice (0.01 mg βCE/100 mL) and noni pulp (0.007 mg βCE/100 mL).

5.2.1 Physicochemical Properties of Noni Juice During Storage

As presented in Table 4.9, the pH of pasteurised, fresh, and S60 noni juices stored decreased significantly after 56 days of storage at 4°C and 25°C, possibly due to increase of citric acid level in noni juice (Table 4.13). It was reported the pH of noni fruit juice is affected by organic acids that are present (Lewis Luján, et al., 2014). The noni juices stored at room temperature (25°C) had slightly lower pH than the samples stored at refrigerated temperature (4°C), which in accordance with the findings by Thirukkumar, Vennila and Kanchana (2018).

The present study showed that the titratable acidity of pasteurised, fresh, and S60 noni juices stored at 4°C and 25°C, did not significantly change following 56 days of storage (refer to Table 4.9). Titratable acidity was found to correspond with organic acids level in fruit juice (Cha, et al., 2019). Wibowo, et al. (2015) observed that the titratable acidity of orange juice remained relatively stable when the content of citric and malic acids remained unchanged during the storage study. In this study, ascorbic, fumaric, and malic acids decreased significantly while citric acid increased significantly by the end of storage (refer to Table 4.13). Further studies are needed to demonstrate the linkage of organic acids and titratable acidity.

The increasing trend observed in total soluble solids of pasteurised, fresh, and S60 noni juice samples during storage at 4°C and 25°C for 56 days (as shown in Table 4.9) is similar to the roselle-fruit juice blends during storage of 6 months at 4°C and 28°C (Mgaya-Kilima, et al., 2014). The possible reasons of increment in total soluble solids during storage study might be due to breakdown of polysaccharide into monosaccharides and oligosaccharides via hydrolysis and increase in concentration of juice due to dehydration (Bhardwaj and Pandey, 2011). It was reported that retention or minor increment in total soluble solids of fruit juice during storage is preferrable to preserved juice quality (Bhardwaj and Pandey 2011). This may be due to removal of water via dehydration and increasing in soluble solids like sugar will reduce the water activity of fruit juice. The loss of water activity will create an osmotic shock and cells plasmolysis, resulting in inactivating the microorganisms, thereby extend the shelf-life of fruit juice (Erkmen and Bozoglu, 2016). Additionally, formation of water-soluble pectin could also increase the total soluble solids of fruit juice during the storage (Rehman, et al., 2014).

According to Table 4.10a, an overall increased in L^* value following 56 days of storage at 4°C and 25°C is in accordance with del Socorro Cruz-Cansino, et al. (2015) which demonstrated an increase of L^* value in purple cactus pear juice during 28 days of storage at 4°C. They claimed that increase of L^* value may be due to partial precipitation of unstable suspended particles (del Socorro Cruz-Cansino, et al., 2015). On the other hand, the storage of noni juice also resulted in increased of b^* and a^* values (refer to Table 4.10a), indicating the browning of noni juice during storage. This could be attributed to enzymatic

browning caused by residual polyphenol oxidase or non-enzymatic browning resulting from ascorbic acid degradation (Selen Burdurlu and Karadeniz, 2003; Zhu, et al., 2019).

The h^* and C^* values (as shown in Table 4.10b) are calculated from the a^* and b^* values. Hence, the decreased in h^* values in overall explained the browning of noni juice (Zhu, et al., 2019), causing colour changes in the noni juice as shown in Figure 4.5. In the present study, the C^* was increased in overall at day 56 (refer to Table 4.10b), indicating increase in saturation due to browning of noni juice after storage. All samples stored at 25°C had higher C^* values than the samples stored at 4°C, which in line with the finding by Martí, Pérez-Vicente and García-Viguera (2001). This scenario indicating browning effect of noni juice was more obvious at storage of 25°C than those stored at 4°C (refer Figure 4.5), which also corresponds with the higher browning index of noni juice stored at 25°C (BI = 1.06-2.32) than 4°C (BI = 0.64-0.91), as presented in Table 4.10b. In short, higher browning index indicates greater browning effect (Wang, et al., 2019). The greater browning effect observed at higher storage temperature (25°C) was possibly due to acceleration of ascorbic acid degradation (Yin, et al., 2022) and increase in the browning enzyme activity by residual polyphenol oxidase (Moon, et al., 2020).

The results, as shown in Table 4.10b, indicated that pasteurised, fresh, and S60 noni juice samples stored at 4°C did not exhibit significant changes in the browning index. The browning index of pasteurised noni juice did not significantly increase during storage at 25°C, possibly due to the partial

inactivation of browning enzyme (polyphenol oxidase) during pasteurisation. Anaya-Esparza, et al. (2017) illustrated that pasteurised soursop nectar had the lowest residual activity of polyphenol oxidase, with only 19% compared to fresh (100%) and sample sonicated at 30°C (34-67.1%). Storage at 25°C resulted in significant increase in the browning index of both fresh (from BI = 0.66 to 2.32) and S60 (from BI = 0.17 to 2.06) noni juices. In accordance with the findings by Muche, Speers and Rupasinghe (2018), grape juices stored at 25°C (67–69%) and 35°C (71–75%) resulted in higher increments in the browning index than compared to samples stored at 5°C (46–48%) for 360 days. It is clearly seen that the browning of fruit juice is temperature dependent.

Among the samples stored at 4°C, the pasteurised (ΔE = 2.51) and S60 (ΔE =2.27) noni juices possessed moderate colour difference as compared to fresh noni juice. The S60 noni juice stored at 25°C (ΔE = 1.66) exhibited moderate colour difference meanwhile pasteurised noni juice stored at 25°C (ΔE = 3.06) resulted in major colour difference. The major colour difference showed by pasteurised noni juice stored at 25°C might be correlated to the weaker browning intensity as compared to the fresh noni juice (refer Figure 4.5). This scenario is also corresponding to the lower browning index of pasteurised noni juice (BI = 1.06) in comparison to fresh noni juice (BI = 2.32), following 56 days of storage (refer to Table 4.10b). On the other hand, the moderate colour different resulted from S60 noni juice might be due to its comparable browning intensity to fresh noni juice.

5.2.2 Total Phenolics and Total Flavonoids Content of Noni Juice During Storage

Generally, TPC of noni juice samples decreased irrespective of storage temperatures (refer to Table 4.11), possibly due to oxidative degradation of phenolics associated with residual peroxidase activity and polymerisation with protein during storage (Odriozola-Serrano, et al., 2009; Yildiz and Aadil, 2020). Irrespective of the storage temperature, the decrease in TPC of S60 noni juice was much lower compared to the fresh and pasteurised noni juices. This might be contributed by release of more phenolic compounds and enhanced antioxidant capacity of noni juice due to the additional of hydroxyl radicals to the aromatic ring of phenolic compounds during sonication (Jiang, et al., 2014; Wang, Vanga and Raghavan, 2019). On the other hand, pasteurised noni juice retained the lowest TPC value among the samples might be due to the used of high temperature (90°C) during pasteurisation. Lagnika, et al. (2017) reported that degradation of phenolic compounds is accelerated at higher temperature like 70°C, resulting in greater loss of TPC.

A previous study showed that sonicated orange juice and sweet lime juice retained higher TPC levels up to 10 weeks of storage at 4°C, outperforming pasteurised and control samples (Khandpur and Gogate, 2015). A similar trend was also observed in a study involving sonicated carrot and grapes juice blend after 90 days of storage at 4°C (Nadeem, et al., 2018). These findings align with the present study, suggesting that sonication is capable in maintaining the integrity of phenolics of fruit juice during storage as compared to the commonly used pasteurisation technique.

The present study also revealed that storing noni juice at 25°C caused a greater reduction in TPC (48.3-57.6%) compared to noni juice samples stored at 4°C (7.5-20%). Likewise, Mohamad Salin, et al. (2022) observed a significant decrease in the phenolic content of watermelon juice stored at 25°C compared to samples stored at refrigerated cold (4°C), refrigerator freeze (-8°C), and freeze (-80°C) conditions. The study claimed that higher storage temperature could cause phenolic degradation due to the unstable structure of these compounds (Mohamad Salin, et al., 2022).

No significant decrement in TFC was observed in fresh and S60 noni juices stored at 4°C, as well as the fresh noni juice stored at 25°C. However, the TFC of S60 noni juice stored at 25°C significantly decreased by 33% after 56 days of storage (refer to Table 4.11), possibly due to residual peroxidase activity, which might not be completely inactivated during sonication (Lu, et al., 2019). The pasteurised noni juice stored at 4°C and 25°C showed non-significant increment in TFC from 1.14 to 1.22 mg RE/100 mL and 1.48 mg RE/100 mL, respectively. Vieira, et al. (2018) found that thermally pasteurised orange juice significantly increased TFC level during storage at 4°C, while other processing method such as high-pressure processing decreased TFC. It is postulated that some unknown compounds, which react with aluminium chloride are formed during storage, thereby leading to an increase in TFC level of fruit juice (Vieira, et al., 2018). Nevertheless, similarly to the TPC, the present study showed that storing noni juice at 25°C reduced greater TFC level by up to 33% compared to those storage at 4°C (up to 13.5%), as demonstrated by the fresh and S60 samples.

5.2.3 Phenolic and Organic Acids Profile of Noni Juice During Storage

The scopoletin levels of noni juice obtained after storage at 4°C and 25°C are comparable to the scopoletin content of fermented noni juice stored at 6°C (1.45 mg/100 mL) and 28°C (1.42 mg/100 mL) for 10 weeks (Sirait and Hutasoit, 2021). In the present study, the scopoletin content of noni juice samples stored at 4°C showed a decreasing trend, whereas the scopoletin content of noni juice samples stored at 25°C increased up to 1.26-1.67 mg/100 mL. This phenomenon might be due to the biosynthesis of scopoletin by microbial cells as secondary metabolite during storage at 25°C in the presence of carbohydrate monomers, such as glucose and catalytic enzymes (He, et al., 2022). Other possible reason could be the conversion of scopoletin from ferulic acid, which is catalysed by coenzyme A ligase and feruloyl coenzyme A 6′-hydroxylase (Yang, et al., 2015; An, Choi and Ahn, 2020). The utilisation of carbohydrate monomers in the biosynthesis of scopoletin could explain the lower increment in total soluble solids of noni juice stored at 25°C (refer to Table 4.9).

Irrespective of the storage temperature, the vanillic acid of all noni juice samples exhibited a decreasing trend after 56 days of storage. A study by Kemsawasd and Chaikham (2021) also reported decreasing trend of vanillic acid in pasteurised, pressurised, and sonicated maoberry juices during refrigerated storage for 30 days. Overall, level of rutin in pasteurised, fresh, and S60 noni juices significantly decreased during storage at both 4°C and 25°C for 56 days. The decrement of vanillic acid and rutin levels may be attributed to oxidative phenolic degradation caused by residual peroxidase activity (Odriozola-Serrano,

et al., 2009). In contrast, Neves, et al. (2021) observed no changes in the rutin level of elderberry juice concentrate during 5 months of refrigerated and room temperature storage. The present study showed that pasteurised noni juice retained higher rutin level (0.75-1.10 mg/100 mL) compared to fresh and S60 noni juices during storage at both 4°C and 25°C. This might be due to lower residual peroxidase activity involved in rutin degradation in the pasteurised sample, as reported by Etzbach, et al. (2019).

The malic acid content of pasteurised, fresh, and S60 noni juices was significantly reduced after 56 days of storage at 4°C and 25°C. Likewise, Igual, et al. (2010) also observed a significant decrease in malic acid content of fresh, conventionally pasteurised, and microwave pasteurised grapefruit juices stored at different temperatures after 60 days of storage. In the present study, fumaric acid content of pasteurised, fresh, and S60 noni juices was significantly decreased following storage at both 4°C and 25°C for 56 days. A similar decreasing trend was also observed in the fumaric acid content of pomegranate juice after 60 days of storage at 4°C as reported by Aarabi, Barzegar and Azizi (2008).

The ascorbic acid content of all noni juice significantly reduced during storage at both 4°C and 25°C. These findings are consistent with previous studies on fresh, pasteurised, sonicated, and combined sonication-UV treated orange and sweet lime juice, which showed a significant reduction in ascorbic acid levels after 10 weeks of storage at 4°C (Khandpur and Gogate, 2015). It has been reported that factors such as oxygen, heat, light, storage temperature, and

duration can induce both aerobic and anaerobic degradation of ascorbic acid during storage (Rashid, et al., 2014). The present results also showed that, irrespective of food processing method, ascorbic acid level in noni juice samples stored at 25°C (2.90-3.22 mg/100 mL) was lower than those stored at 4°C (2.91-17.33 mg/100 mL). This can be attributed to the heat sensitivity of ascorbic acid, as its degradation rate into dehydroascorbic acid increases at higher temperatures, leading to greater loss of ascorbic acid in fruit juice (Nisha, Shinghal and Panditt, 2004).

Contrary to malic, fumaric, and ascorbic acids, citric acid exhibited a significant increase during storage for 56 days at 4°C and 25°C (refer to Table 4.13). In line with the present study, Tembo, Holmes and Marshall (2017), showed that the level of citric acid of baobab juice significantly increased at the end of storage. The study reported that breakdown of carbohydrates or phenolic compounds may happen during the storage of fruit juice, which leading to increase in citric acid at the end of storage (Tembo, Holmes and Marshall, 2017).

Generally, each of the organic acids values in noni juice stored at 4°C were greater than those stored at 25°C, indicating that refrigerated storage (4°C) is more effective in preserving organic acids of fruit juice than storing it at room temperature (25°C). Irrespective of storage temperature, S60 noni juice exhibited the highest values in each organic acids compared to fresh noni juice whereas pasteurised noni juice possessed the lowest values. This study revealed that sonication is capable of enhancing retention of higher amount organic acids than pasteurisation during storage.

Organic acids play a crucial in understanding the organoleptic and microbiological quality of foods. Consequently, changes in the organic acids profile during storage may affect the flavour, colour, and microbial stability of foods (Tembo, Holmes and Marshall, 2017). For instance, a decrease in ascorbic acid content may indicates nutrient loss of juice as ascorbic acid is frequently used as an indicator of the juice's nutritional quality (Khandpur and Gogate, 2015). Additionally, a low fumaric acid amount (0.09-0.16 mg/100 mL) at the end of storage may indicate the absence of microbial spoilage in the juice, because an increase in fumaric acid levels could be attributed to presence of spoilage microorganism capable of synthesising fumaric acid (Gökmen and Acar, 2004). Other than that, changes in the level of organic acids such as fumaric, citric, and malic acids may alter the flavour of juice as they contribute to the flavour of juice product (Igual, et al., 2010).

5.2.4 Antioxidant Capacity of Noni Juice During Storage

Similar to the TPC results (refer to Table 4.11), the S60 noni juice yielded higher FRAP and TEAC values than the fresh and pasteurised samples during 56 days of storage at both 4°C and 25°C as presented in Table 4.14. This is due to the increased extraction efficiency of the antioxidant molecules such as phenolics and flavonoids during sonication (Aadil, et al., 2020). Likewise, del Socorro Cruz-Cansino, et al. (2015) showed that thermosonicated purple cactus pear juice retained a significantly higher antioxidant capacity, as measured by ABTS and DPPH than the fresh and pasteurised samples during storage at 4°C for 28 days. A study by Yildiz and Aadil (2020) also showed that the antioxidant capacity of sonicated strawberry juice, measured by DPPH (1224.35-2027.72 μmol TE/L), was higher than that of the fresh (897.14 μmol TE/L) and pasteurised (911.54 μmol TE/L) strawberry juices during storage at room temperature for 14 days. However, in the present study, S60 noni juice at storage of 25°C exhibited slightly lower FRAP than that fresh sample (refer to Table 4.14). This discrepancy could possibly be attributed to weaker reducing capability of the remaining antioxidants (phenolic compounds) on the ferric ions, resulting in a lower FRAP reading (Gulcin, 2020).

This study demonstrated that storing noni juice at 25°C resulted in a greater reduction in FRAP and TEAC compared to noni juices stored at 4°C. These results were in line with the findings by Jiang, et al. (2014), who reported that black mulberry juice stored at 5°C had a higher DPPH scavenging activity (<22%) compared to storage at 25°C (<18%) and 15°C (<20%) over an 8-day periods. Likewise, noni fruit juice blend with amla squash stored at 4°C (160 mg

TE/100 mL) exhibited higher antioxidant capacity than noni juice blend stored at 32°C (152 mg TE/100 mL) after 6 months of storage (Thirukkumar, Vennila and Kanchana, 2018). The antioxidant capacity corresponds to the total phenolic compounds in the fruits as they are potent antioxidants (Nadeem, et al., 2018). As mentioned earlier, higher storage temperatures can accelerate the degradation of phenolics, thereby reducing the antioxidant capacity.

5.2.5 Microbial Load of Noni Juice During Storage

Food processing plays an important role in inactivating naturally occurring microorganisms that are responsible for foodborne illness. As presented in Table 4.15, regardless of the storage temperatures, no growth of yeast and mold was detected, while the count of total aerobic mesophilic bacteria remained below 4 log CFU/mL. This indicates that noni juice complies with the microbiological standards for ready-to-eat food, as it falls within the satisfactory range (Choo, et al., 2018). The use of noni juice in preserving the quality of fresh-cut mango cubes was demonstrated by Ulloa, et al. (2015). In their study, the presence of antioxidant compounds such as phenolic compounds were used to explain the antimicrobial activity of noni juice towards the mango cubes over 15 days of refrigerated storage at 6°C.

The present study showed no significant difference in the aerobic mesophilic bacteria count between fresh and S60 noni juices after 56 days of storage at both 4°C and 25°C. The antimicrobial properties of noni juice might be associated with its low pH (refer to Table 4.1 and 4.9) because most of the microorganism do not grow or grow very slowly at pH values lower than 4.6 (U.S. Food and Drug Administration, 2001). In addition, it was observed that the fresh and S60 juice samples stored at 25°C had significant lower aerobic mesophilic bacteria count on day 56 compared to the fresh and S60 juice samples stored at 4°C. This difference could be attributed to the lower pH of the noni juice samples stored at 25°C (pH = 3.70-3.71) compared to the pH of noni juices stored at 4°C (pH = 3.76-3.77). As shown, the lower the pH, the lower the

bacterial growth. At low pH, microbial cells may experience cytoplasmic acidification that would cause irreversible nucleic acids and enzymes denaturation, metabolic inhibition as well as collapse of proton gradient in maintaining neutral cytoplasmic pH (Gurtler and Mai, 2014; Lund, et al., 2020). However, low temperature storage such as refrigeration only slow down the enzymatic and microbial changes. Basically, microbial cells are not killed under refrigerated temperature (Erkmen and Bozoglu, 2016). It is noteworthy that pH is deem to be more effective in controlling microbial growth when comparing to storage at refrigerated temperature, as demonstrated in the present study.

Low pH and the presence of antimicrobial agents, such as phenolic compounds, could explain the low microbial load of noni juice, which remained below 2 log CFU/mL throughout the 56 days of storage at 4°C and 25°C. In agreement with Basumatary, et al. (2020), pasteurisation was found to be better than sonication in inactivating microorganisms of the fruit juice. The high temperatures used in the pasteurisation process might destroy organic substances that are essential for the proper functioning of microbes, resulting in cell lysis. Irrespective of the juice processing method, the level of microbial counts in noni juice was within the satisfactory range, indicating that, despite pasteurisation offers better antimicrobial activity than sonication, both techniques managed to produce noni juice that was safe for consumption when stored at 4°C and 25°C.

CHAPTER 6

CONCLUSION

Sonication for 60 min was the best sonication duration as it significantly improved the nutritional content including TPC, TFC, phenolic compounds, organic acids, and antioxidant capacity of noni juice in comparison to 20 min and 40 min of sonication. Storage study showed that sonicated and pasteurised noni juice are shelf-stable after 56 days storage at refrigerated and room temperature, thus both are safe for consumption. However, in terms of nutritional content, sonicated juice is a better choice than pasteurised juice. Refrigerated storage was found to preserve the nutritional content of noni juice better than room temperature storage. It can be concluded that sonication serves as a better alternative to produce noni juice, owing to the higher retention of nutritional content with comparable microbial activity, as compared to conventional pasteurisation. Additionally, the alternative noni juice extraction method using a juice extractor, as proposed in the present study, can be applied in the food industry to reduce noni juice processing time in comparison to traditional fermentation. Further study on higher sonication temperatures and shorter durations are recommended to obtain an optimised noni juice processing condition with a shorter processing time. It is also advisable to conduct a study on a wider range of storage temperatures, such as frozen temperature (-18°C) and higher temperature (37°C), to evaluate the shelf-stability of noni juice. This recommendation stems from the fact that the highest daily air temperature in

Malaysia exceeds 30°C. Additionally, 37°C is frequently used in assessing the storage stability of fruit juice in the previous studies. It is recommended to identify phenolic compounds using other methods, such as gas chromatography mass spectrometry, in order to study additional phenolic compounds in noni juice. Besides, different extraction solvent concentration such as 80% to absolute methanol can be adopted in the future to examine their effects on the concentration of phenolic compounds in noni fruit. Lastly, incorporating fermented noni juice in future studies can be valuable in evaluating the nutritional content of noni juice produced through different processing methods.

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