UNIVERSITI PUTRA MALAYSIA

EFFECTS OF AQUEOUS OZONE PASTEURIZATION ON S. typhimurium INACTIVATION AND QUALITATIVE ATTRIBUTES OF CHOKANAN MANGO (Mangifera indica L.) FRUIT JUICE

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By

NUR AMIRA BINTI MOHD SUPIAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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The juice and beverages industry shows rapid growth from year to year in line with the rise of fruits consumption. The consumers tend to add fresh fruit juice to supplement their healthy lifestyle and these choices include nutrient-rich mango fruit of Chokanan variety. Nonetheless, the risk of cross-contamination of fresh fruits are increasing as the import volume from other countries are also amplifying. The risk may exacerbate if the hygiene practice of the fruit juice handlers is abysmal. Secondary preservation methods to produce safe fruit juice is imperative as *S. typhimurium* contamination of mango juice could potentially cause severe harm to consumers since this pathogen can attach and infiltrate the mango fruit. Even though the thermal pasteurization method has been proven effective towards microorganism inactivation, the treatment can affect the nutritional quality of mango juices (MJ). Thus, the juice industry and academia are actively investigating alternative pasteurization technologies and ozone is one of the novel technologies attracting their interests because it does not leave any undesirable residue in the treated food product in addition to its ability to destroy harmful pathogens. However, it is challenging to predict ozone's antimicrobial behaviour in fruit juices as it depends not only on the ozone processing parameters (processing time and ozone dose) but also on the treated juice composition. Hence, this study was done to evaluate the efficiency of ozone as a substitute pasteurization method to thermal treatment towards *S. typhimurium* inactivation and retention of physicochemical and antioxidant properties of various concentration ratios of Chokanan mango juice diluted with distilled water (DW); 100MJ:0DW, 75MJ:25DW, and 50MJ:50DW. The above-mentioned mango juice samples were then treated to ozone treatment with ozone doses of 0.33, 0.67, 1.00, and 1.33 mg/mL for 10, 20, 30, and 40 min. The

results from this study have shown that the efficacy of ozone pasteurization is significantly ($p < 0.05$) affected by mango juice concentration ratios; where it can be seen that the inactivation rates for 5-log reduction of *S. typhimurium* was observed at 36.39 min, 1.20 mg/mL (for sample 100MJ:0DW), 28.38 min, 0.95 mg/mL (for sample 75MJ:25DW) and 24.82 min, 0.82 mg/mL (for sample 50MJ:50DW). In addition to that, the inactivation of *S. typhimurium* in MJ samples has successfully achieved the 5-log reduction within 40 min (at ozone dose of 1.33 mg/mL) and 30 min (at ozone dose of 0.33 mg/mL) and these results fitted well to the Weibull model. This study also offers some insight into the effects of ozone on the quality attribute of MJ samples. The results showed significant changes ($p < 0.05$) in the pH and total soluble solids (TSS) value of MJ samples. Ozone was found to be effective in lowering the pectin methylesterase (PME) activity ($p < 0.05$) from de-esterifying the pectin molecules and increased ($p < 0.05$) the DPPH activity of MJ samples, thereby increasing the juice's quality. However, a significant decrease ($p < 0.05$) was seen in the total color difference (*TCD*), ascorbic acid, and total phenolic content (TPC) on ozone-treated MJ samples. Therefore, to further support physicochemical analysis findings, colour kinetic was studied to predict mango fruit juice's quality degradation due to ozone treatment. The first-order reaction model was shown to best fit the color L^*, b^*, and *TCD* values. A significant difference ($p < 0.05$) can be observed between the degradation rate constant (k-value) for all MJ samples, which implies that the k-value is not only affected by ozone dose but also by juice's matrix. The 50MJ:50DW sample also shows the lowest percentage changes in color (b^*) value and k_{TCD} degradation corresponds to juice composition. In summary, the results of this study proved that aqueous ozone can potentially be used as pasteurization method in juice processing; where it can reduce *S. typhimurium* by 5-log reduction and managed to minimize the nutrient content changes in MJ samples. Nevertheless, it is imperative to examine the juice composition as a crucial intrinsic parameter in order to gauge the efficacy of juice preservation perusing ozone technology.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KESAN PASTEURISASI OZON BERAIR TERHADAP PENYAHAKTIFAN *S. typhimurium* DAN SIFAT KUALITI JUS BUAH MANGGA CHOKANAN (*Mangifera indica* L.)

Oleh

NUR AMIRA BINTI MOHD SUPIAN

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Industri jus dan minuman menunjukkan pertumbuhan pesat dari tahun ke tahun sejajar dengan peningkatan pengambilan buah-buahan. Pengguna cenderung menambah jus buah segar untuk menokok gaya hidup sihat mereka dan pilihan ini termasuklah buah mangga dari jenis Chokanan yang kaya dengan nutrien. Namun begitu, risiko pencemaran silang dari buah segar semakin meningkat apabila jumlah import dari negara lain juga semakin bertambah. Risiko boleh bertambah jika amalan kebersihan pengendali jus buah adalah buruk. Dengan itu, kaedah pengawetan sekunder untuk menghasilkan jus buah yang selamat adalah mustahak kerana pencemaran jus mangga oleh *S. typhimurium* berpotensi menyebabkan kemudaratan kepada pengguna lantaran patogen ini dapat melekat pada permukaan dan menyusup masuk ke dalam buah mangga. Walaupun kaedah pasteurisasi terma telah terbukti berkesan terhadap penyahaktifan mikroorganisma, rawatan tersebut dapat merosotkan kualiti nutrien jus mangga (MJ). Oleh itu, industri jus dan ahli akademik sedang aktif meneliti teknologi pasteurisasi alternatif dan ozon adalah salah satu teknologi baru yang menarik minat mereka kerana ia tidak meninggalkan sisa yang tidak diingini dalam produk makanan yang dirawat selain kemampuannya untuk memusnahkan patogen merbahaya. Walau bagaimanapun, adalah sukar untuk meramalkan tingkah laku antimikrobial ozon di dalam jus buah kerana ia tidak hanya bergantung pada parameter pemprosesan ozon (masa pemprosesan dan dos ozon) tetapi juga pada komposisi jus yang dirawat. Oleh itu, kajian ini dijalankan untuk menilai kecekapan ozon sebagai kaedah pasteurisasi menggantikan rawatan terma terhadap penyahaktifan *S. typhimurium* dan pengekalan sifat fizikokimia dan antioksidan pelbagai nisbah kepekatan jus mangga Chokanan yang dilarutkan dengan air suling (DW); 100MJ: 0DW, 75MJ: 25DW, dan 50MJ: 50DW. Sampel jus mangga yang disebutkan di atas kemudian dirawat dengan rawatan ozon dengan dos ozon sebanyak 0.33, 0.67, 1.00, dan 1.33 mg / mL selama 10, 20, 30, dan 40 minit. Hasil dari kajian ini menunjukkan bahawa keberkesanan pasteurisasi ozon adalah dipengaruhi secara signifikan

($p < 0.05$) oleh nisbah kepekatan jus mangga. Di mana dapat dilihat bahawa kadar penyahaktifan untuk mengurangkan 5-log *S. typhimurium* telah diperhatikan pada kadar 36.39 min, 1.20 mg/mL (untuk sampel 100MJ: 0DW), 28.38 min, 0.95 mg/mL (untuk sampel 75MJ: 25DW) dan 24.82 min, 0.82 mg/mL (untuk sampel 50MJ:50DW). Selain itu, penyahaktifan *S. Typhimurium* dalam sampel MJ juga berjaya mencapai pengurangan 5-log dalam masa 40 minit (pada dos ozon 1.33 mg/mL) dan 30 minit (pada dos ozon 0.33 mg / mL) dan keputusan ini adalah berpadanan dengan model Weibull. Kajian ini juga memberikan beberapa gambaran mengenai kesan ozon terhadap sifat kualiti sampel MJ. Hasil kajian telah menunjukkan perubahan yang ketara ($p < 0.05$) pada nilai pH dan jumlah pepejal larut (TSS) sampel MJ. Ozon didapati berkesan dalam menurunkan aktiviti pectin metilesterase (PME) ($p < 0.05$) daripada membebaskan ikatan molekul pektin dan meningkatkan ($p < 0.05$) aktiviti DPPH pada sampel MJ, dengan secara langsung meningkatkan kualiti jus. Walau bagaimanapun, penurunan yang ketara ($p < 0.05$) dapat dilihat pada jumlah perubahan warna (*TCD*), asid askorbik, dan jumlah kandungan fenolik (TPC) pada sampel MJ yang dirawat oleh ozon. Oleh itu, untuk lebih menyokong penemuan analisis fizikokimia, kinetik warna telah dikaji untuk meramalkan kemerosotan kualiti jus buah mangga akibat rawatan ozon. Model reaksi tertib pertama ditunjukkan paling sepadan dengan nilai warna L^*, b^*, dan *TCD*. Perbezaan yang ketara ($p < 0.05$) dapat diperhatikan di antara pemalar kadar degradasi (nilai-*k*) untuk semua sampel MJ, yang menunjukkan bahawa nilai-*k* tidak hanya dipengaruhi oleh dos ozon tetapi juga oleh matrik jus. Sampel juga 50MJ: 50DW menunjukkan perubahan nilai peratusan terendah untuk nilai warna (b^*) dan kemerosotan k_{TCD} yang berkadaran dengan komposisi jus. Secara ringkasnya, hasil kajian ini membuktikan bahawa rawatan ozon berair berpotensi digunakan sebagai kaedah pasteurisasi dalam pemprosesan jus; di mana ia dapat mengurangkan *S. typhimurium* sebanyak 5-log pengurangan dan dapat mengawal perubahan kandungan nutrien yang minimum dalam sampel MJ. Walau bagaimanapun, adalah sangat mustahak untuk mengkaji komposisi jus sebagai parameter intrinsik yang sangat penting untuk mengukur keberkesanan pengawetan jus yang menggunakan teknologi ozon.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

AOAC	Association of Official Analytical Chemists
CAGR	Compound annual growth rate
CDC	Center for Disease Control and Prevention
CPJ	Cold-pressed juice
DOA	Department of Agriculture Malaysia
DPPH	2,2-diphenyl-1-picrylhydrazyl
DW	Distilled water
FAMA	Federal Agricultural Marketing Authority
FDA	Food and Drug Administration
GPM	Gallon per minute
ISO	International Organization for Standardization
MJ	Mango juice
NACMCF	National Advisory Committee on Microbiological Criteria for Food
NCTC	National Collection of Type Cultures
PEMANDU	Performance Management and Delivery Unit
USFDA	United States Food and Drug Administration
WHO	World Health Organization

LIST OF NOMENCLATURES

D, δ	Association of Official Analytical Chemists
k	Inactivation rate (min^{-1})
l	Shoulder length (min)
p	Scale and shape of the inactivation curve
R^2	Coefficient determination
RSME	Root square mean error
s	Time (seconds)
SSE	Sum of square error
t	Time (min)
z	Number of degrees (Celsius or Fahrenheit) required to change D-value by one factor of ten (℃)

CHAPTER 1

INTRODUCTION

1.1 Research Background

The fruit juice market is increasing globally as consumer's growing health issues have arisen. In 2020, the global fruit juice market gained about 46.8 billion litres and was expected to grow at a compound annual growth rate (CAGR) of 2.1% in the forecast period of 2021-2026 to reach 53 billion litres (Expert Market Research, 2021). The market statistics show that fruit juices are a well-accepted and preferred drink for all age groups, as it contains many essential nutrients beneficial to human health. This includes mango juice as a popular fruit juice among consumers (Expert Market Research, 2021). Mango is rich in vitamins, calcium, iron, potassium, zinc (López-Cobo *et al*., 2017), and mango's peculiar characteristics contain many bioactive compounds in addition to the accompanying carotenoid pigment (Ribeiro & Schieber, 2010). In Malaysia, mango var. Chokanan has tremendous commercial value and high demand due to its distinctive taste, pleasant aroma, and palatable color. It is oblong-shaped with tapered tips, firm, fibrous texture, and vibrant yellowish-orangey flesh color (Santhirasegaram *et al*., 2015b).

Malaysia's production and hectarage of mango in all states were 15,307 mt and 6,373 Ha (DOA, 2019). The value of mango exports from Malaysia to various countries in the Asia Pacific, Middle East, and the European Union is about USD 5.2 million (International Trade Centre, 2017). In line with the government's effort to boost gross national income (GNI), twelve National Key Economic Areas (NKEAS), which include the agricultural sector, were launched by the Performance Management and Delivery Unit (PEMANDU) agency in September 2010 (PEMANDU, 2010). In addition, the Economic Planning Unit Agency under the Prime Minister Department of Malaysia has prepared the National Agro-food Policy (DAN) 2011-2020, specifically for the agro-food industry (FAMA, 2011). One of this policy's main ideas is to increase agricultural modernization by research and development (R & D) technology and strengthening fruit industry production. The Ministry of Agriculture (MOA) Malaysia expects the projected increases in area productivity of 12.9 tonnes per hectare, including mango fruit, which will increase fruit production to 2.6 million tonnes by 2020 (FAMA, 2011). In further realizing this policy, diversifying mango fruit production, exportation, and processing into valuable food products are highly encouraged. The gross income for specifically mango var. Chokanan was reported to be RM 536,200 (191,500 kg) in 2018 (DOA, 2019). In addition, mango (either Chokanan or other variety) ranks at six (49.2%) in weekly fruit consumption among Malaysian adults (Othman *et al*., 2013). Mango var. Chokanan is well-known in Malaysia, and it is frequently selected to be processed into juice because of its sweet taste and availability throughout the year (Abdullah, 2018).

Production of mango fruit juice currently employs grinding mills with sharp knives rooted on rotating shafts to crush the fruits and centrifugation machine or enzymatic liquefaction for juice viscosity (Mushtaq *et al*., 2018; Reyes-De-Corcuera *et al*., 2014; Sharma *et al*., 2014). Until recently, various studies have been conducted based on demand for least processed juice products to minimize the impact of the juice extraction process. This involves cold-pressed techniques that have the ability to achieve these attributes. Mushtaq et al. (2018) pointed out that mechanical processing used to extract or squeeze the juices can influence fruit juice's nutritional and antioxidant quality. A cold-pressed juicer uses a squeezing method rather than grinding the fruits (Kim *et al*., 2017; Khor, 2015). The juice produced by this method is known as cold-pressed juice (CPJ) (Hatab *et al*., 2016) and has been widely popular among individuals seeking juice products with retained nutritional qualities as fresh fruits. Several studies have revealed that a cold-pressed juicer is an ideal method in producing juice with the ability to either maintain or even increase the antioxidant capacity and the juices' yield in purple prickly pear, tomato, and grape juice (Gouws *et al*., 2019; Kim *et al*., 2017; Kim *et al*., 2015).

The rapid growth of cold-pressed juice in the national or global market depended on the higher juice yield after extraction with minimum nutritional quality losses (Biancaniello *et al*., 2018; Koutchma *et al*., 2016). After extraction, fruit juices may be sold directly with their original content (100% fruit juice), reconstituted with water, or mixed with other fruit juices. Fruit juices diluted with water are not offering high nutritional quality as 100% fruit juice; but can fulfill the juice market's availability and consumers' demand for drink products (Kuo *et al*., 2008). Furthermore, the pathogen such as *S. typhimurium* has been recorded by USFDA (2003) as a microorganism frequently discovered in the juice industry and is a health risk to the human body. *S. typhimurium* is a Gram-negative bacteria (Shahbaz *et al*., 2018) and very susceptible to heat. The close contact with wildlife, contaminated irrigation water, untreated manure, and inadequate hygiene methods all have the potential to expose fresh fruit or juice to *S. typhimurium* infection (Iwu and Okoh, 2019). Mukhopadhyay & Ramaswamy (2012) also indicate that the absence of a natural skin barrier may lead to *S. Typhimurium* contamination in fresh fruit. Thus, an ideal method is needed to inactivate the *S. typhimurium* in mango juice (MJ) while preserving its nutritional consistency. For these purposes, non-thermal pasteurization technology, ozone, has been proposed in this study.

Ozone is the allotropic form of obtuse-angled oxygen and naturally presents as a colorless gas, and the odor smell close to "clean air after a thunderstorm" (Oner & Demirci, 2016). It is also known as a powerful oxidizing agent (+2.07 eV) (Asokapandian *et al*., 2018), effective as bactericidal towards microbiological activity pertinent in fruit juice. The United States Food and Drug Administration (USFDA) has acknowledged that ozone as Generally Regarded as Safe (GRAS) in the gaseous and aqueous state as an antibacterial agent for treatment, storage, and processing of foods (FDA, 2001). In the juice industry, the juice processor must obey the 5-log reduction performance standard requirement to treat the fruit juice, and ozone has been proven to inactivate pertinent microorganisms such as *S. typhimurium* effectively. Furthermore, ozone has been the research subject since the 1990s as one alternative treatment for food processing. Previous researches include the ozone-treated acai, apple, melon, probiotic orange juice, peach, and sugarcane juices (Bernardi *et al*., 2019; Diao *et al*., 2019; Abhilasha & Pal 2018; Fundo *et al*., 2018; Jaramillo-Sánchez *et al*., 2018; Oliveira *et al*., 2018; Rodrigues *et al*., 2017; Almeida *et al*., 2015; Garcia-Loredo *et al*., 2015; Song *et al*., 2015; Sung *et al*., 2014; Torlak, 2014; Choi *et al*., 2012). Ozone provides environmentally-friendly antimicrobial agents where the absence of residual effect of ozone makes it highly suitable for fruit juices processing.

1.2 Problem statement

The frequency of fresh fruit outbreaks increases as there are increments in cross-country shipping and/or importing products from other countries. Contamination of fruit juice with spoilage and pathogen bacteria can potentially cause harm to consumers. Fresh produce, including fruits, has been recognized as a common source for *Salmonella* since the bacteria can attach and internalize in fruit (Kilonzo-Nthenge and Mukuna, 2018). The *S. typhimurium* outbreaks linked to fresh fruits have occurred in 2012 and 2010 at multistate locations in the United States (CDC, 2012; CDC, 2010). The outbreaks were caused by Cantaloupe melon and frozen Mamey fruit pulp consumption, respectively, with 228 and 9 infected individuals. In 2005, a multistate outbreak of *S. typhimurium* infection linked with unpasteurized orange juice was reported in the United States involved 152 cases (Jain *et al*., 2009).

A foodborne illness caused by *Salmonella sp.* infection is called *Salmonellosis*. Most people infected with *Salmonella sp.* will have diarrhea, fever, stomach cramps, and required hospitalization if severe. Meanwhile, Typhoid fever is a foodborne illness caused by *Salmonella* serotype Typhi bacteria (CDC, 2018). In Malaysia, the exact number of *Salmonellosis* incidences associated with fresh fruits remains unknown as a chain of events must be addressed before it is brought to the authority. The only *Salmonella* cases in fruit juice were recorded locally in a research study by Diana et al. (2012). The study had stated that the prevalence and concentration of *S. typhimurium* in apple juice was 30% and 9.20 MPN/g, meanwhile for orange juice was 10% and 6.10 MPN/g and in starfruit juice at 10% and 3.00 MPN/g, sold by fruit juice vendors and hawker stall in Serdang and various places in Klang Valley. It was proven that fruits could be a reservoir for *Salmonella*. Hence, more prevalence studies on *Salmonella* in fresh produce and its by-products are needed to produce food safety and quality.

Consequently, the juice industry is encouraged to implement the proper pasteurization and preservation technique in its processing line to avoid food safety issues. This includes conventional heating methods used widely in the fruit juice processing industry to pasteurize and preserve fruit juice products. The low-temperature long-time (LTLT) and high-temperature short-time (HTST) heating methods are conventionally applied as a preservation method in fruit juice to achieve the minimum 5-log reduction of the microbial pathogen (USFDA, 2001). The conventional heat pasteurizing method on mango juice utilizes a temperature of 90 ± 5℃ and time up to 60 seconds (Jolayemi, 2019; Abedelmaksoud *et al*., 2018; Santhirasegaram *et al*., 2015a). Although the thermal treatment has been proven its effectiveness towards the inactivation of microbial growth, the treatment affected the nutritional quality of fruit juices, including bioaccessibility of carotenoids leading to the degradation of beneficial phenolic contents (Aschoff *et al*., 2015).

Furthermore, the usage of preservatives, either natural or chemical, could avoid fruit juice spoilage caused by microbes. Chemical preservatives such as sodium benzoate, potassium sorbate, and natural preservatives include bacteriocins and organic acids commonly used to inactivate microbial growth and subsequently prolong fruit juice's shelf life (Pandey & Negi, 2018). However, the excessive presence of these preservatives has been reported to have high risks to human health (Walker & Philips, 2018).

Over the past few years, industry and food academia have explored novel alternative technology to destroy harmful pathogens while improving or maintaining the fruit juice's quality and shelf life. The Generally Recognized as Safe (GRAS) status by FDA (Food and Drug Administration) (FDA, 2001) makes ozone to be one of the promising non-thermal technology used for pasteurization in the food processing industry. Jermann et al. (2015) reported that ozone has the potential to be used in the drinks and beverages industry, and it has been categorized as a potential technology for commercialization in the next five years. Ozone does not involve a heating process but exploits its oxidation potential (+2.07eV) to pasteurize and preserve fruit juices. Despite that, it is challenging to predict ozone behavior in juices as it depends not only on the ozone processing parameter (time and concentration) but also on the juice matrix or composition. Several studies have proven the microbial reductions in ozone-treated fruit juices, including cantaloupe melon, peach, apple, and orange juice (Sroy *et al*., 2019; Garcia Loredo *et al*., 2015; Torlak, 2014, Patil *et al*. 2010b). Ozone treatment had reduced the *L. innocua* population to 5.3 log reductions at ozone concentration 7.7 g/L after 6 minutes of ozone exposure in Cantaloupe melon juice (pH and TSS: 6.35 and 10.65 ^{o}Brix) (Sroy *et*., 2019). However, the majority of previous researches mainly focused on the microbial reduction perusing ozone processing parameters, and studies are limited on the intrinsic parameters of the juice. Prabha et al. (2015) reported that ozone molecules' reaction in fruit juice could be disturbed by the presence of organic or inorganic matter in juice. The solid content of the juice contributes to the limitation of ozone's synergistic towards its bactericidal effect. Choi et al. (2012) review that at an ozone concentration of 0.10 and 0.90 g/hr in 36 ^{o}Brix of apple juice solid content, it needed 6 to 10 min treatment time to achieve 5.0 log reduction while in 18 ^{o}Brix of solid juice content, it took only 6 min. Furthermore, certain microorganisms are able to survive the acidic condition of the juice, including Gram-negative bacteria, *S. typhimurium* (Pandey and Negi 2018). An acid-loving microorganism such as *Salmonella* can adapt and survive for a few days or even weeks in an acidic environment formed by organic acids of the fruit juices

Therefore, it is vital to study the influence of juice composition on aqueous ozone efficacy for antimicrobial activity and its effect on the quality attribute of fruit juices. Specifically, the effects of juice ratio (different pH and TSS) and ozone dose toward mango juices will be studied in-depth.

1.3 Research objectives

This research evaluates the efficiency of ozone treatment towards microbiological inactivation and its effect on physicochemical properties, antioxidant contents, and antioxidant activities of mango (*Mangifera indica* L.) fruit juice (MJ). The specific objectives of this study are:

1) To determine and model the inactivation kinetics of *S. typhimurium* of ozone-treated mango (*Mangifera indica* L.) fruit juice.

2) To study and correlate the effect of ozone treatment on the physicochemical properties (pH, TSS, turbidity, PME activity, and color), antioxidant contents (TPC, AA), and antioxidant activities (DPPH) of mango (*Mangifera indica* L.) fruit juice.

3) To investigate the kinetic modeling of color degradation of ozone-treated mango (*Mangifera indica* L.) fruit juice as a means to predict the effect of ozone treatment on the antioxidant contents of MJ.

1.4 Scope of research

The selected sample was limited to a fully ripe yellow color of mango fruit (var. Chokanan) purchased from the local market in Serdang, Selangor. The samples were prepared according to the ratio (mango juice (MJ): distilled water (DW)) of 100MJ:0DW, 75MJ:25DW, and 50MJ:50DW and store at the temperature of 4 ± 1 ℃ prior to ozone treatment. *S. typhimurium* species NCTC 12023 was used in this study as it is the most frequent serovars that caused the foodborne outbreak to human health (Herrero-Fresno & Olsen *et al*., 2018; Abdul-Mutalib *et al*., 2015). Even though it was reported that *S. typhimurium* is primarily found in the poultry product, Kilonzo-Nthenge and Mukana (2018) indicate that this pathogen can attach and internalize in fresh fruit. In this study, the untreated mango juices undergo treatment with ozone parameters at a treatment time of 10, 20, 30, and 40 min. The limitation was fixed by the ozone generator model (Model GL-3189, China) with the maximum power and frequency output of 20 W and 50 Hz, respectively. The ozone dose at the specific treatment time of 10, 20, 30, and 40 min was calculated based on Oxidation Technology's formula (Oxidation Technology, 2017), which gave out 0.33, 0.67, 1.00, and 1.33 mg/mL. Meanwhile, the physicochemical, antioxidant content and antioxidant activity analysis were done based on the previous research method; pH, total soluble solids (TPC) (AOAC, 1996), turbidity (Shah, 2015), PME activity (Agcam *et al*., 2014), color (Aguilar *et al*., 2018), ascorbic acid (AOAC, 2007), total phenolic content (TPC), and DPPH (Guan *et al*., 2016). Furthermore, for the microbial study, the method was adopted from the standard EN ISO 6579-1:2017 (ISO, 2017) and research by Sung et al. (2014). The chosen agar and media were explicitly chosen for enumeration of *S. typhimurium* (refer to Chapter 3) for a positive outcome.

1.5 Thesis structure

The introductory chapter (Chapter 1) briefly reviews ozone technology, the selected fruit sample (mango var. Chokanan), and fruit juice's pathogen (*S. typhimurium*). This chapter also presents the problem statements, the research objectives, scopes, and this study contribution.

Chapter 2 reviews the previous studies in mango fruit juice, the potential market of mango and its product (juices), and their nutritional value. This chapter also presented information on fruit juice processing (mechanical and cold-pressed technology), fruit juice outbreaks, and the regulation applied in the juice industry. The pasteurization method details on the conventional (thermal) and non-thermal (HPP, PEF, ultrasound, and ultraviolet) was briefly described in this chapter. This chapter also discussed the previous works on ozone pasteurization on fruit juice, including its regulations, advantages, and disadvantages of this treatment. Besides that, the kinetic modeling of bacterial inactivation used in fruit juice studies is also listed and discussed in this chapter.

Chapter 3 fully described all aspects needed for experimental research, and a summary of the overall experimental designs is represented in the flow charts. This chapter highlights mango fruit juice preparation (MJ) and comprehensively presented the experimental ozone treatment setup. In addition, the chapter presents the methodologies for microbiological (*S. typhimurium*), physicochemical (pH, TSS, turbidity, PME activity, color), and antioxidant analysis (TPC, DPPH, ascorbic acid), and statistical analysis procedures (General Linear Model, Full Factorial Design, and Pearson Correlation).

Chapter 4 reports and discussed the findings of research on the objectives mentioned previously. The preliminary study observes the effect of different untreated (control) mango juice ratios on physicochemical, antioxidant properties, and microbiological (*S. typhimurium*) population are done and further analyzed. Then, the effects of ozone treatment on physicochemical (pH, TSS, turbidity, PME activity, color), antioxidant properties (TPC, DPPH, ascorbic acid), and microbiological (*S. typhimurium*) inactivation in various treated mango juices are elaborately discussed. The inactivation kinetics of *S. typhimurium* (Log-linear model and Weibull model) and modeling color (Zero-order model and First-order model) are also discussed in this chapter.

A summary of all findings is presented in Chapter 5. This chapter also mentioned the recommendation for future work of ozone pasteurization in fruit juice.

1.6 Contribution of thesis

Ozone is actively being studied on various fruit juices because of its antimicrobial properties and zero toxic by-products. Thus, this research study could contribute further knowledge for the fruit juice industry on the usefulness of ozone as an alternative to a non-thermal pasteurization method. This study focused on the effect of ozone-treated mango juice (MJ) on the inactivation kinetics of *S. typhimurium*, physicochemical properties, antioxidant content, and antioxidant activity. This research data could be used as a reference source by food processors or researchers to design or implement aqueous ozone applications in their industry or field of study, respectively.

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LIST OF PUBLICATIONS

Articles – Published

Shah, N. N. A. K., Ong, M. C., Supian, N. A. M., & Sulaiman, A. (2020). Ultrasound-assisted extraction and gaseous ozone as preservation method for Roselle (*Hibiscus sabdariffa* L.) fruit juice. *Food Research*, *4*(S6), 85–95.

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Articles – Accepted

Supian, N. A. M, Shah, N. N. A. K, Shamsudin, R, Sulaiman, A. Inactivation kinetics of *S. Typhimurium* NCTC 12023 in Chokanan Mango (Mangifera Indica L.) fruit juice by aqueous ozone treatment. *Food Research*, July 2021.

Articles – Submitted and Reviewed

Supian, N. A. M, Shah, N. N. A. K, Shamsudin, R, Sulaiman, A. Effects of aqueous ozone treatment on nutritional attributes of mango (*Mangifera Indica* L.) fruit juice. *International Food Research Journal*, February 2021.

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Shah, N.N.A.K., A. Sulaiman, N.S.M. Sidek, & N.A.M. Supian. (2017). Effects of gaseous ozone on physicochemical characteristics of citrus fruit juices. International Conference on Food Science and Nutrition (ICFSN2017), 25-26 October 2017, Kota Kinabalu, Sabah, Malaysia. (Oral Presenter)

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