VALIDATION OF GASEOUS OZONE (O3) CONCENTRATION AND ITS HALF-LIVES IN CITRUS FRUIT JUICES

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PROJECT APPROVAL SHEET

This project entitled "**Validation of Gaseous Ozone (O_3) Concentration and Its Half-Lives in Citrus Fruit Juices**" prepared and submitted by Nur Farahin Binti Mohd Nor Wir in partial fulfillment of the requirement for the Bachelor of Engineering (Process and Food) is hereby accepted and approved.

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ABSTRACT

Ozone has been broadly utilized and known as powerful disinfectant for application in food industry. Non-thermal treatment is a system which permits processing of food below the temperature used throughout thermal pasteurizations, thus, in this way flavours, fundamental nutrients and vitamins experience negligible or no change during processing. Furthermore, there are no unsafe residuals that need to be removed after ozonation as ozone decomposes quickly. Therefore, the purpose for this study is to validate gaseous ozone (O_3) concentration in various citrus fruit juice using iodine method and to investigate the correlations of ozone half-life in various citrus fruit juice with various physiochemical and quality characteristic. For this study, orange, lime, and lemon juice were chosen as samples. Based on the result, it was found that juices with high pH (2.4) high total soluble solid (TSS) (11.0), and high vitamin C (27.6 mg/100 mL), have the lowest ozone (O_3) half-lives. In conclusion, ozone has a short half-life in juices with high pH, total soluble solid (TSS), and vitamin C. For turbidity and total phenolic content (TPC), relations towards O_3 half-life did not show a significant result. This conclude that, ozone has short half-life and decay rapidly right after treatment.

ABSTRAK

Ozone telah meluas digunakan dan dikenali sebagai pembasmi kuman yang kuat untuk aplikasi dalam industri makanan. Rawatan tanpa haba adalah satu sistem yang membenarkan pemprosesan makanan di bawah suhu yang digunakan di seluruh pasteurisasi haba, dengan itu, dalam hal ini rasa, nutrien asas dan vitamin mengalami tiada perubahan semasa pemprosesan. Tambahan pula, tiada sisa yang tidak selamat yang perlu dikeluarkan selepas pengozonan sebagai ozon terurai dengan cepat. Oleh itu, tujuan kajian ini adalah untuk mengesahkan ozon gas (O_3) kepekatan dalam pelbagai jus buah-buahan sitrus menggunakan kaedah iodin dan untuk menyiasat korelasi ozon separuh hayat dalam pelbagai jus buah-buahan sitrus dengan pelbagai ciri fisiokimia dan kualiti. Untuk kajian ini, oren, limau, dan jus lemon telah dipilih sebagai sampel. Berdasarkan keputusan itu, telah didapati bahawa jus dengan pH yang tinggi (2.4), tinggi jumlah larut pepejal (TSS) (11.0), dan vitamin C yang tinggi (27.6 mg/100 mL), mempunyai ozon rendah (O_3) separuh hayat. Kesimpulannya, ozon mempunyai separuh hayat yang pendek dalam jus dengan pH yang tinggi, jumlah larut pepejal (TSS), dan vitamin C. Untuk kekeruhan dan kandungan jumlah fenol (TPC), hubungan ke arah O_3 separuh hayat tidak menunjukkan hasil yang ketara. Ini membuat kesimpulan bahawa, ozon mempunyai separuh hayat yang pendek dan kerosakan cepat selepas rawatan.

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LIST OF ABBREVIATIONS

EPRI	Electric Power Research Institute
FQSD	Food Safety and Quality Division
GMP	Good Manufacturing Practice
GRAS	Generally Recognized as Safe
HACCP	Hazard Analysis and Critical Control Point
HSB	Hoigné-Staehelin-Bader
HLT	Half-Life Time
GTP	Gordon-Tomiyasu-Fukutomi
TPC	Total Phenolic Content
TSS	Total Soluble Solid
UV	Ultraviolet
USFDA	United States Food and Drug Administration

CHAPTER 1

INTRODUCTION

1.1 An Overview of Ozone

Ozone has been broadly utilized and known as powerful disinfectant for application in food industry. Ozone is formed when three atoms of oxygen bond together and defined as high oxidation potential which conveys on bactericidal and viricidal properties (Burleson et al., 1975; Horvath et al., 1985; Kim et al., 1999). Ozone is also called powerful broad-spectrum antimicrobial specialist against parasites, microbes, infections, protozoa, bacterial, and growth spores (Khadre et al., 2001).

Ozone is a pale blue gas with an impactful smell and solid oxidizer (Muthukumaparappan et al., 2000). Ozone is induced by the reaction of free oxygen radicals with diatomic oxygen to form triatomic oxygen molecules. A single oxygen molecule will rapidly combine with another available O_2 to form very reactive ozone O_3 using high energy input. Furthermore, initiated of free radical oxygen to generate the ozone is carried out using ultraviolet (UV) radiation and corona discharge methods. Ozone can also be created by chemical, thermal, chemo atomic and electrolytic technique (Kim et al., 1999). Ozone, unstable gas will decay into hydroxyl ($\bullet OH^-$), hydroperoxy ($\bullet HO_2$), and superoxide radicals (O_2^-).

Through modernization, consumer's perception for food quality and safety have changed. Modern consumer interest for tasty, healthier, organic, natural, and fresh-like nourishment produces in a naturally friendlier way with economical techniques and little carbon footprints (Koutchma et al., 2009, Shah, 2015). Therefore, it triggers the advancement of food preservation system which includes different non-thermal preservation as the technique is believed to maintain product quality and freshness.

Non-thermal treatment is a system which permits processing of food below the temperature used throughout thermal pasteurizations, thus, in this way flavours, fundamental nutrients and vitamins experience negligible or no change during processing (Cànova et al., 2009).

Ozonation may be a standout amongst those non-thermal food preservation systems that uses ozone as disinfectant with kill destructive microorganisms. Ozone has gained interest in issue of food safety as ozone is 1.5 times stronger over chlorine and is powerful, considerably wider spectrum of microorganisms (Xu, 1999; Patil and Bourke, 2012). Besides, there are no unsafe residuals that need to be removed after ozonation as ozone decomposes rapidly. (Solomon et al., 1998).

In 2001, United States Food and Drug Administration (USFDA) has allowed that ozone in the gaseous and aqueous stage as an antimicrobial agent for treatment, storage, and processing of food (Khadre et al., 2001). Moreover, ozone has been viewed as Generally Recognized as Safe (GRAS) status by USFDA in 1997, guaranteeing its utilize as a disinfectant. As a result, ozone as a disinfectant for food has encourages the food industry to explore its many possibility benefits. For addition, these rules starting with USFDA have triggered the researchers to apply gaseous ozone as an alternative processing and preservation system for several of fruit juices. Ozone treatment has been effective over eliminating or lessening microbial growth in different of fruit juices including apple cider, orange juice, strawberry juice and apple juice (Choi et al., 2012; Patil et al., 2009; Tiwari, 2012; William et al., 2004). Thus, it is no doubt that ozone treatment has been acknowledge for a quite a while as another alternative for pasteurization of fruit juices.

Food Act 1983 and Food Regulations 1985 are the Malaysia's food enactment exists to guarantee the quality and safety of food. These regulations are established to screen and monitor public against health hazard and fraud in preparation, handling, processing, packaging, storage, transportation, bargain and consumption of food. Currently, in Malaysia, there is no public enactment released particularly focused on alternative preservation techniques for liquid food. Nevertheless, Malaysia's Food Policy is required to follow USFDA regulations as guided by Food Safety and Quality Division (FQSD), shaped under the Food Act 1983 and Food Regulations 1985, specifying all food producers are to comply with USFDA regulation concerning to food preparing. Thus, Hazard Analysis and Critical Control Point (HACCP) framework has turn into a significant safety procedure for food producers in Malaysia.

1.2 Problem Statement

Ozone is a potential alternative compound to current strong disinfectant (Isikber and Oztekin 2009; Kells et al. 2001; Sousa et al. 2016). Ozone is attractive because it does not leave any undesirable residue in the food product (Cullen et al. 2010) and ozone can be generated in-situ using ozone generator (Pandiselvam and Thirupathi 2015) at the time of use, eliminating the need to transport and store chemical containers (Mendez et al. 2003). In addition, energy input required for ozone fumigation treatment is much lower than thermal and radiation treatment (Khadre et al., 2001). Since the USFDA has approved ozone as a direct food additive (FDA 2001), thus the right dosage of ozone concentration and treatment time are pertinent. This is because if the dosage of ozone is over the limits, it may cause harms to consumers and if the dosage of ozone is lower, it may insufficient to kill the pathogens. Thus, by knowing the half-life of ozone can help to get suitable dosage for ozone in fruit juice treatment.

At the moment, there is no specific way to measure gaseous ozone (O_3) concentration in fruit juice. Dissolved ozone in fruit juice thus, validation of ozone concentration and its half-lives in various fruit juice is vital, since different fruit juice has different characteristics. Therefore, by validating the amount of dissolved ozone in fruit juice can be determined. Method of validation is an essential part of good measurement practice because valid data can only be produced when the strengths and weaknesses of a method are understood.

On the other hand, ozonation treatment as a mean of pasteurizing is still new and not well-known among the consumers. Past studies have shown good effects as bactericidal and does not destroy the nutritive value of fruit juice as no heat is being used in this treatment. However, consumer’s acceptance on ozone is still in deliberate. Thus, this study focuses on the amount of dissolved ozone (O_3) in the different sample of citrus fruit juices that has different physicochemical and quality characteristics. With that, by determining the half-life of gaseous ozone in fruit juices, it can convince the consumer that residual ozone in treated fruit juice will decrease and dissipate so that the fruit juice is safe to be consumed.

1.3 Objectives

The objectives of this study are:

a) To determine gaseous ozone (O_3) concentration in various citrus fruit juice (orange, lime and lemon) using iodine method.
b) To study the correlations of ozone half-life in various citrus fruit juice (orange, lime and lemon) with different physicochemical and quality characteristics (pH, TSS, vitamin C, turbidity, TPC)

1.4 Scope of Study

This research study focuses on validation of gaseous ozone concentration and its half-life in various citrus fruit juices, which are orange juice, lime juice and lemon juice. The thesis is started with the description and overview of gaseous ozone. Furthermore, the explanation of problem statement, objective of the research and the importance of this research study also be stated in this chapter.

The second chapter reviews on past literatures which are related to sample fruit juices; orange, lime and lemon juice. In addition, food borne illness outbreaks and ozone treatment method, application and its effects and half-life are also discussed in this chapter.

Chapter three explains detailed experimental and method design used for this study. This chapter discusses on the methods used to validate gaseous ozone concentration in citrus fruit juice using iodine method. Besides, the methods to investigate the correlation of ozone of half-life in various fruit juice samples with different physicochemical and quality characteristics will be discussed. Method of statistical analysis will also be elaborated in this chapter.

Meanwhile, chapter four reports the result of the experiment which are done to investigate the objectives mentioned previously. Detailed discussion of results obtained will be presented in this chapter.

Lastly, a brief summary of all findings in previous chapters will be discussed in chapter five. Recommendation for future works are also detailed in the final chapter.

1.5 Contribution of Thesis

This study if proven will contribute to a knowledge gap in regrards ozone preservation technique in liquid food. In food industry, especially in fruit juice production, determination of ozone half-life will ascertain the amount of dissolved ozone in fruit juice. Thus, this can prove that there will be no or minimal gaseous ozone (O_3) is left in fruit juices long after ozone treatment has ended. Furthermore, this study will provide knowledge on ozone pasteurizing efficiency in different juice characteristics. Additionally, previous studies have proven that ozone is safe and suitable for usage in food industry as it has ability to pasterurize the drinks from microorganism.

CHAPTER 2

LITERATURE REVIEW

This chapter discusses more detailed about this study background as described in Chapter 1 based on variety literatures. Therefore, this section includes section 2.1, that explaining the background of ozone which explains its ozone generation and its application in food industry. Besides, the previous studies involving ozone and fruit juice, ozone as preservation or disinfectant material, and ozone treatment limitations in food industry are been discussed in section 2.2, 2.3 and 2.4 respectively. In section 2.5 and 2.6, are discussing about the toxicity of ozone and half-life of ozone. Then, physicochemical properties that affect ozone half-life has been elaborated in section 2.7. For the last section, 2.8, is explaining the background of citrus fruit juices (orange, lime and lemon) and their nutritional contents that have been used for this study.

2.1.1 Ozone Generation

Ozone is produced by the response of free oxygen radicals with diatomic oxygen to shape triatomic oxygen molecules. The solid O-O bonds are broken with the assistance of vitality contribution to create free oxygen radicals. Ultraviolet ozone generation and corona discharge techniques can be utilized to start free radical oxygen development and in this mannered produce ozone (Patil et al., 2010). Briefly, the distinction between this two techniques are ultraviolet ozone generation is shaped when oxygen atom is presented to UV light of 140-190 nm wavelength (Muthukumarappan et al., 2000). However, UV lights solarize after some time and required intermittent substitution making it not a temperate or productive approach to create ozone. In this way, in this research think about, corona discharge technique has been utilized as a part of supplanted the UV ozone generation which give more productive approach to create ozone.

Corona release method in ozone generation is made when the oxygen particle is passed between two high-voltage electrodes which isolated by a dielectric material, permitting the development of triatomic oxygen atoms (ozone) (Patil et al., 2010). Figure 2.1 beneath demonstrates the ozone generation mechanism of corona discharge.

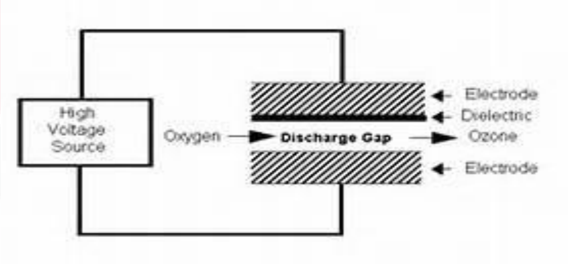

Figure 2.1: The ozone generation utilizing corona discharge

The power scattering in the corona discharge causes the generation of ozone. This is because of the electrons that quickened crosswise over give an air crevice in order to give adequate energy to part the oxygen double bond, along these lines delivering nuclear oxygen. At that point, these oxygen atoms are responds with other diatomic oxygen particles to frame ozone.

2.1.2 Application of Ozone in Food Industry

As an environmentally friendly disinfectant, ozone now is being studied as an alternative to chlorine sanitizers in many segments of the food industry. Ozone-enriched water kills microbes as effectively as chlorine, and since it is generated on-site its use eliminates the need for personnel to handle, mix and dispose of harsh chemicals for sanitation. Further, since ozone readily reverts to oxygen, an end-product that leaves no residue on contact surfaces.

The potential utility of ozone to the food industry lies in the fact that ozone is 52% stronger than chlorine and has been shown to be effective over a much wider spectrum of microorganisms than chlorine and other disinfectants. Complementing the effectiveness, is the fact that ozone, unlike other disinfectants, leaves no chemical residual and degrades to molecular oxygen upon reaction or natural degradation. The fact that ozone has a relatively short half-life is both an asset and a liability to practitioners. This is particularly true in treatment of drinking water where ozonation is employed to enhance filtration and provide primary disinfection but requires the addition of chlorine as the terminal disinfectant to maintain a residual in the distribution system.

Ozone is effective killing microorganisms through oxidation of their cell membranes and most of the pathogenic, foodborne microbes are susceptible to this oxidizing effect. During food processing operations, surface disinfection of the product (raw or partially processed) is very important. In response to a Food Additive Petition submitted during August 2000, the U.S. Food and Drug Administration (USFDA) formally endorsed the utilization of ozone as an Antimicrobial Agent for the Treatment, Storage and Processing of Foods in Gas and Aqueous Phases. On December. 21, 2001, the U.S. Department of Agriculture's Food Safety and Inspection Service (USDA/FSIS) affirmed the use of ozone in contact with meats and poultry, from raw product up to fresh cooked and products just prior to packaging (USDA/FSIS, 2001). In addition to immediate contact disinfection of foods, ozone also can be applied to food processing equipment and non-food contact surfaces as major aspect of a nourishment organization's sanitation endeavors.

Ozonation has been used to disinfect water for drinking purpose in Europe. (Zeyneb et al., 2004). In the US ozone application in the food industry has not been widely used; however, the United States Food and Drug Administration granted generally recognized as safe (GRAS) status for use of ozone in filtered water in 1982. Ozone use was approved by the US Department of Agriculture for reconditioning reused poultry chilling water in 1997. After a year of evaluating the worldwide database on ozone, an expert panel in 1997 announced that ozone was a GRAS substance for use as a disinfectant or sanitizer for foods when used in accordance with good manufacturing practices. Since the US Food and Drug Administration did not protest to the expert panel's findings, ozone has now been approved for use as a disinfectant or sanitizer in foods and food processing in the United States (USDA, 1997).

2.2 Previous Studies on Ozone Treated Fruit Juice

Fruits and vegetables are an important group of foods that represent a substantial segment of the food market. Their high consumption is related with the recognition that fresh produce is essential for a well-balanced diet, due to their nutritional value, as well as presenting color, shape, taste, aroma, and texture attractive characteristics. However, the perishable nature of these products, which determines its fast consumption and also the number of associated outbreaks, identifies the importance of applying efficient decontamination treatments. In order to extend the shelf life of these products, conventional processes, which include the application of moderate thermal treatments, are usually applied. However, heat processing can induce several biological, physical, chemical, and microbiological changes, leading to sensorial, nutritional, and textural modifications that can negatively affect the product quality. Consequently, thermal-based treatments are not suitable for all kinds of products. Due to these drawbacks and the rising demand for fresh produce, other preservation techniques are being used. The application of several sanitizers that act as antimicrobial solutions is standard. Among them, chlorine and associated compounds are the most routinely used by the food industry (Artes et al., 2009). Nevertheless, due to the formation of carcinogenic chlorinated by-products, some European countries have forbidden its use (Betts and Everis, 2005). Additionally, it has been attested the inability of chlorine to effectively act on foodborne pathogens (Ijabadeniyi et al., 2011)

In light of all this knowledge, considerable research efforts have been made in the recent years to find feasible processing alternatives with the potential to produce fresh products with high quality and safety standards. The food industry is searching for a technology that ensures high levels of quality retention of the products, while extending their shelf life by the reduction of enzymatic activity, the destruction of pathogens and spoilage microorganisms and their toxins and by the elimination of insects, parasites, and pesticides. From the available technologies, ozone application is a promising one, which is gaining interest in the fruit and vegetable industry. The efficacy and usefulness of ozone has been proved over the years with its widespread application in the treatment of water and food (Guzel-Seydim et al., 2004). In 1997, an Expert Panel of Food Scientists convened by the Electric Power Research Institute (EPRI) supported a generally recognized as safe (GRAS) classification of ozone as a disinfectant for foods when used at levels and by methods of application in accordance with good manufacturing practices. Although the Food and Drug Administration (FDA) did not objected this GRAS status, in 2000, a Food Additive Petition filled by the EPRI requested FDA approval of ozone for direct contact with foods. In 2001, FDA finally approved the application of ozone as a direct food additive for the treatment, storage, and processing of foods in gas and aqueous phases (FDA, 2001). Since then, several studies had been conducted to evaluate the effectiveness of ozone in various fruits and vegetables. The ozone treatments are mainly applied in two forms: (1) the gaseous ozone can be added continuously or intermittently to an atmosphere where the produce is stored or (2) the product can be washed or dipped in water containing ozone. Nevertheless, this work intends to collect and summarize all the studies and results obtained for fruits and vegetables treated with ozone that are not covered in previous works. It is also approached the effect of ozone on quality and safety features of the products. This review intends to compile information that will contribute to identify main achievements and what still needs to be exploited. The industrial application of ozone can be standardized to a certain product, if significant improvements in quality and safety features are attained as well as economic and competitive advantages of the technology.

The effectiveness of ozone treatment on microbial load depends on several factors, which may explain the diversity of published results (Table 2.1). The main influential factors are as follows: (1) type of product; (2) target microorganism; (3) initial microbial load level; (4) physiological state of the bacterial cells; and (5) ozone physical state. The sensitivity of microorganisms to ozone is also extremely affected by the organic nature of the medium, with protection caused by physical factors, as in the case of agar, and by reduced ozone levels due to ozone demand of organic nutrients in the medium (Restaino et al., 1995). Nevertheless, Restaino et al. concluded that more important than the amount of organic material is the type of organic material present during ozonation.

Table 2.1: Previous Studies on Ozone Treated Fruit Juices

Fruit Juice	Treatment Conditions	Quality/Safety Characteristics	Results	References
Apple	Exposure to 33–40 ppm ozone for 8 min at 15–18 C and storage at 4, 8, 12, and 16 C during 30 days A dynamic storage temperature study was also carried out.	*Saccharomyces cerevisiae* ATCC 9763	A lag phase was observed for all ozone-processed samples, probably due to the oxidizing action of the applied ozone treatment, which may exert additional stress prior to allowing growth. Authors reported the effectiveness of ozone for the extension of shelf life of apple juice	(Patil et al. 2011)
Blackberry	Ozone exposure (0–7.8 %) at 20°C up to 10 min	Anthocyanins Color	Ozone concentration and treatment time were found to be critical factors influencing both anthocyanins and color degradation.	(Tiwari et al. 2009)
Grape	Ozone exposure (0–7.8 %) at 20°C up to 10 min	Anthocyanins Color pH	No significant changes in pH, Brix and titratable acidity of ozonated samples were observed.	(Tiwari et al. 2009)

		Brix Titratable acidity	However, significant changes in the juice color and anthocyanins content were observed during ozone treatment.	
Orange	Exposure to 75–78 ppm ozone for different time periods (0–18 min)	*E. coli* ATCC 25922 and NCTC 12900	Generally, ozone treatment of orange juice resulted in a population reduction of 5 log-cycles	(Patil et al. 2009)
Strawberry	Ozone exposure (1.6–7.8 %) at 20°C up to 10 min	Anthocyanins Color Ascorbic Acid	Significant reductions in anthocyanins (98.2 %) and ascorbic acid (85.8 %) were observed at an ozone concentration of 7.8 % w/w and a treatment time of 10 min	(Tiwari et al. 2009)

2

2.3 Ozone as Preservation or Disinfectant Material

Ozone as an antimicrobial agent has numerous potential applications in the food industry because of its significant advantages over traditional antimicrobial agents such as chlorine, potassium sorbates, and many more. It is mainly by gaseous ozone or ozonated water that food products are treated. The choice of the type of the applied treatment appears to be product dependent, i.e., liquid or solid food, and process dependent, i.e., washing/cleaning or direct processing. Previous studies have shown that aqueous ozone was more effective than gaseous ozone on decontamination of intact products (Bialka and Demirci, 2007) while the advantage of gaseous ozone is its use as a direct antimicrobial additive on liquid foods after its diffusion in the studied system (Patil et al., 2009a).

Ozonation of fruit juices is still in its infancy. Ozonation of liquid phases is most frequently accomplished by injecting ozone gas (mixtures of air/ozone or oxygen/ozone) through a sparger into a liquid. Usually the studies on ozone absorption in the aqueous systems are carried out in stirred-tank reactors or bubble columns (Cullen et al., 2009). Subsequent approval of ozone as a direct additive led to the application of ozone for processing of various fruit juices including; apple cider (Steenstrup and Floros, 2004; Choi and Nielsen, 2005) orange juice (Angelino et al., 2003; Tiwari et al., 2008a; Sonal et al., 2009), blackberry juice (Tiwari et al., 2009b), strawberry juice (Tiwari et al., 2009c) etc. The specific ozonation of fruit juices is reported to meet the USFDA's requirement of a mandatory 5-log reduction of the most resistant pathogens (*E. coli, Salmonella, Listeria monocytogens*) in juices. They reported that a 5-log reduction of *E.coli* O157:H7 could be achieved using ozone in combination with dimethyl dicarbonate. The uses and efficacy of ozone for the preservation and quality retention of several fruit juices are shown in Table 2.2. As presented in Table 2.2, it appears that most studies have been conducted for Gram-negative bacteria (*E. coli*), which appear to be more resistant than Gram-positive bacteria. Bacteria are also more sensitive than yeasts and fungi. The microbial resistance is also dependent on the acidic environment of the fruit juices which has an impact on the ozone efficiency. Quality effects of ozonation of several fruit juices is reported explicitly here. Efficacy of ozone depends upon its composition; it is difficult to produce pure ozone due to technical limitation maximum ozone concentration that can be achieved is about 10–14 % w/w of oxygen. In addition, ozone is sparingly soluble in water (i.e., 10 times less than chlorine and 130 times less than chlorine dioxide (Tizaoui et al., 2008)). The oxygen in the ozone treatment gas mixture will naturally not play a role in microorganism inactivation; however, it will significantly influence the degradation of organic compounds present in juice sample.

Table 2.2: Ozone Preservation in Fruit Juices

Fruit juice	Ozone application	Target-microorganism	Reference
Apple cider	Ozone gas (pumped into juices) (Ozone concentration: 9 g/h)	*Escherichia coli* O157:H7 (0.9 LR) *Salmonella* (1.0LR)	(Williams et al., 2005)
Orange juice	Ozone gas (pumped into juices)	*Escherichia coli* O157:H7 (0.4 LR) *Salmonella* (1.8LR)	(Williams et al., 2005)
Orange juice	Ozone gas (pumped into juices)	*Yeast (S. cerevisiae)* (↓)	(Angelino et al., 2003)
Apple cider	Ozone gas (pumped into juices)	*Moulds* (↓) *Yeast* (↓)	(Choi and Nielsen, 2005)
Apple cider	Ozone gas (pumped into juices)	*Escherichia coli* O157:H7 (5.0 LR)	(Steenstrup and Floros, 2004)
Apple cider	Ozone (Bubble column reactor)	*Escherichia coli* ATCC 25922 and NCTC 12900 (5.0 LR)	(Patil et al., [upublished])
Orange juice	Ozone (Bubble column reactor)	*Escherichia coli* ATCC 25922 and	(Patil et al., 2009a)

		NCTC 12900 (5.0 LR)	
Orange juice	Ozone (Bubble column reactor)	*Listeria monocytogenes Listeria innocua* (5.0 LR)	(Patil et al., [unpublished])
Apple cider	Ozone gas (bubbled into juice)	*Patulin (mycotoxin)* (↓)	(Cataldo, 2008)
Apple juice (model)	Ozone gas (sparged into juice system	*Patulin (mycotoxin)* (↓)	(Ashirifie-Gogofio et al., 2009)

APC: Aerobic plate count; Significant difference; (√) Insignificant; (↑): Increases; (↓): Decreases & (~): No change; LR: Log reduction

2.4 Ozone Treatment Limitations in Food Industry

Ozone is a highly unstable triatomic oxygen molecule (O_3), arranged to form an obtuse angle. It is formed by a high energy input that splits the molecular oxygen (O_2) into two O. These single O molecules rapidly combine with available O_2 to form O_3. The source of this high energy is usually electrochemical, ultraviolet radiation or electrical (corona) discharge. The first two methods have limited use due to the very high associated costs and to the poor ozone yields achieved, respectively. The electrochemical method usually applies an electrical current, between an anode and cathode, in an electrolytic solution containing water and a solution of highly electronegative anions. This results in the production of a mixture of oxygen and ozone at the anode (Mahaptra et al., 2005). The ultraviolet (UV) method is based on ozone formation throughout O_2 exposure to UV radiation at a wavelength of 140–190 nm. Corona discharge is the most commonly used method as, although it consumes large amount of electricity, it can generate the required commercial ozone levels. In this method, the feed gas (such as dry air, oxygen or a gaseous mixture) is passed between two electrodes separated by a dielectric material. For achieving higher ozone yields, oxygen must be used as the feed gas instead of air. Once produced, ozone can be inlet directly in the gaseous form into an atmosphere or pulverized into water to produce aqueous ozone for rising and washing applications (Perry and Yousef, 2011). Although ozone is relatively stable in the gaseous state, it is highly unstable in aqueous solution. In water, ozone quickly degrades to oxygen. Although ozone is extremely soluble in water (at 27 C, ozone solubility is 580 mg/L), its solubility rate is dependent on several factors, such as pressure, temperature, pH, ozone bubble sizes, the flow rate of ozone and contact time, the purity of the water and also the technology of interchange gas/liquid (Khadre et al., 2001). The higher the temperature and pH of the solution, the lower the stability of ozone (i.e., shorter half-life). Ozone solubility increases when the purity of water increases, because the presence of minerals and organic matter in the water catalyzes ozone decomposition. Ozone solubility also increases when smaller bubble sizes are formed due to the resulting larger contact surface area. At room and cold temperatures, ozone is a gas with a pungent characteristic odor, which is detectable by humans at concentrations as low as 0.02 ppm (Horvath et al., 1985). Although in low concentrations ozone is not an extremely toxic gas, at high concentrations ozone can cause severe detrimental health effects and can even be fatal. Several federal agencies have established health standards or recommendations to limit human exposure to ozone. These exposure limits are summarized in Table 2.3.

Table 2.3: Ozone Treatment Limitations (Adapted from Golcaves and OzoneSolutions)

Institution	Health Standards
Food and Drug Administration (FDA)	Requires a concentration limit exposure of 0.05 ppm during 8 hours
Occupational Safety and Health Administration (OSHA)	Requires a concentration limit exposure of 0.10 ppm during 8 hours
National Institute of Occupational Safety and Health (NIOSH)	Recommends an upper limit of 0.10 ppm, not to be exceeded at any time
Environmental Protection Agency (EPA)	Requires a concentration limit exposure of 0.08 ppm during 8 hours

2.5 Toxicity of Ozone

Ozone, along with reactive forms of oxygen such as superoxide, singlet oxygen, hydrogen peroxide, and hypochlorite ions, reacts directly with organic double bonds. Also, when ozone breaks down to dioxygen, it gives rise to oxygen free radicals, which are highly reactive and capable of damaging many organic molecules. Moreover, it is believed that the powerful oxidizing properties of ozone may be a contributing factor of inflammation. The cause-and-effect relationship of how the ozone is created in the body and what it does is still under consideration and still subject to various interpretations, since other body chemical processes can trigger some of the same reactions

When inhaled, ozone reacts with compounds lining the lungs to form specific, cholesterol-derived metabolites that are thought to facilitate the build-up and pathogenesis of atherosclerotic plaques; a form of heart disease. These metabolites have been confirmed as naturally occurring in human atherosclerotic arteries and are categorized into a class of secosterols termed atheronals, generated by ozonolysis of cholesterol's double bond to form a 5,6 secosterol (Smith, 2004) as well as a secondary condensation product via aldolization (Paul et al, 2003).

Ozone is very unstable, but has strong oxidation capability and high reactivity substance. In case the concentration of ozone is high, it is highly toxic gas. In Japan, the acceptable concentration of ozone in workspace is not more than 0.1ppm (Nikki, 2015). The example of influence on the body at ozone concentration is the following in the Table 2.4.

Table 2.4: Ozone Concentrations and the Effects on the Body

Ozone concentration	Influence/action/effects on the body
0.01~0.02 ppm	Feel smell
0.1 ppm	Strong smell. Irritation at nose and throat
0.2~0.5 ppm	Visual deterioration in 3-6 hours
0.5 ppm	Upper respiratory system
1~2 ppm	Headache and chest pain in 2 hours
5~10 ppm	Increase in pulse rate
15~20 ppm	Small animals will die within 2 hours
50 ppm	Threat to life within 1 hour

Ozone chemical reactions are rapid, compared to the time required for breathing and demonstration of the pathophysiological effects of ozone. In both animals and humans, shallow rapid breathing occurs on ozone exposure and, in short-term exposures, disappears within 30 minutes on cessation of exposure. In both animals and humans, a delay of 12-24 hours occurs between a single short-term exposure and maximum expression of toxicity, as measured by more complex biochemical or physiological indices. In short-term continuous exposures, maximum anatomical lesions in animals occur after 3 days of exposure. The time sequence beyond 7 days of continuous exposure is not well documented, but after intervals of 14-30 days, and up to 180 days, of exposure, the anatomical lesions persist and become more pronounced. Inflammation persists, although at a reduced intensity, after about 7 days and throughout continued ozone exposure. Periods of exposure of as short as 2 hours to 0.2 ppm produce observable changes (Menzel, 2009). Intermittent and continuous exposures produce anatomical alterations of comparable intensity, but only limited concentrations and times of exposure have been investigated. If ozone was not safe, humans would not be able to go outside and breathe the air, especially during sunshine, a thunderstorm, lightning, or after the rain. The fact is, when used responsibly, ozone is very safe just like oxygen and very beneficial to the planet and all of the living things.

2.6 Half-Life of Ozone

Ozone is a highly reactive gas that makes it useful in sterilizing surfaces, as it will react with many compounds. Due to the highly reactive nature of ozone, it is necessary to better understand key parameters about the gas before further investigation into its sterilizing abilities is undertaken. The half-life time (HLT) of ozone is the amount of time it takes to reduce the initial concentration by half. A lower HLT of ozone could result in less effective treatments during exposure.

Ozone is a strong oxidizing agent, which is used in a growing number of industrial applications to control harmful microbes and volatiles. Thus, a better understanding of the properties of ozone is needed, especially with respect to the half-life. Half-Life Time (HLT) averaged ~ 1500 minutes in still air at room temperature (24°C) and zero humidity, which was substantially longer than previously published data (i.e., 30-40 minutes) (Engineering and Lafayette, 2010). As air speed, temperature and humidity increased, HLT decreased to ~40, 800 and 450 minutes, respectively. The results suggest that ozonation will be more effective in still air at low temperature and humidity.

Ozone decomposition at pH and temperatures ranging between 2.5 and 9 and 10 and 40 °C, respectively. It was shown that at pH lower than 7, whatever the temperature was, direct ozone decomposition and the initiation step involving the hydroxyl radicals are the main cause of ozone decomposition. At higher pH, the importance of the peroxy radicals and of the hydroxide ion initiation step increases. Thus, at pH around 9, the ozone decomposition rate depends on two major contributions: direct ozone decomposition, which leads to the formation of hydroxyl radicals, $OH^{\bullet}$, and hydroxide ion action, which produces not only peroxy radicals, $HO_2^{\bullet}$, but also hydroxyl radicals. Finally, the nature of the ionic species (carbonate, sulfate, phosphate, etc.) present in the water greatly influences ozone decomposition, inhibiting some of the reactions in the mechanism proposed. (Sotelo, Beltrln, Benitez, & Beltrkn-heredia, 1987)

2.7 Physicochemical Properties that Affect Ozone Half-Life

Ozone is used in many applications in the industry as an oxidising agent for example for non-thermal pasteurization in food industry. The decomposition of ozone in aqueous solutions is complex, and is affected by many properties such as, pH, temperature and substances present in the water. Additives can either accelerate the decomposition rate of ozone or have a stabilising effect of the ozone decay. By controlling the decomposition of ozone, it is possible to increase the oxidative capacity of ozone.

Ozone is an allotrope of oxygen, which at room temperature is a blue, explosive gas that absorbs UV-radiation in the range of 220-290 nm. It is a bent molecule with a bond angle of 116.8° and an interatomic distance of 1.278 Å. The melting point is -193°C and boiling point -112°C. The solubility of ozone in aqueous solutions is 14 mmol L-1 at 20°C, but it is more soluble in organic solution. Thus, it can be regarded as a hydrophobic molecule. (Horvath et al., 1984)

In a pure aqueous solution ozone slowly decomposes in multiple steps involving radical formation. The depletion is a chain process and has been described by two different mechanisms, by Hoigné-Staehelin-Bader (HSB) and Gordon-Tomiyasu-Fukutomi (GTF). (Langlais et al., 1991)

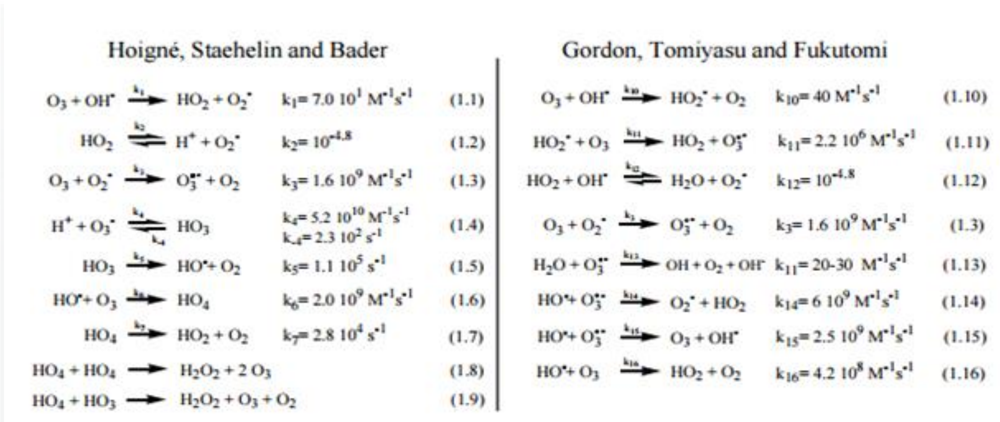

Figure 2.2: The decomposition of ozone in aqueous solution

HSB state that the initial step is an oxygen-atom transfer from ozone to a hydroxide ion, followed by a reverse one-electron transfer. In contrast, GTF only state an oxygen-atom transfer. However, the fundamental reaction in both mechanisms is the initial step, where ozone reacts with OH^-. Furthermore, removal of the superoxide anion radical, O_2^-, and the HO radical in the chain reaction reduces the speed of ozone decomposition. Since reaction with OH^- is the initial decomposition step, the stability of an ozone solution is thus highly dependent on pH and decreases as alkalinity rises (Roth et al, 1983). At pH above 8 the initiation rate has, in the presence of radical scavengers, been shown to be proportional to the concentrations of ozone and OH^- (Hoigne et al., 1985). However, in acidic solutions the reaction with OH^- cannot be the only initiation step. Predicted reaction rates below pH

4 including a mechanism based only on reaction with OH^- are much lower than those determined experimentally. Contradictory to the behavior in weakly alkaline solution, the depletion rate of ozone is reduced in strongly alkaline solutions. The half-life of ozone at room temperature is about 2 minutes in 1 M NaOH solutions, compared to 40 minutes in 5 M and 83 hours in 20 M solutions (Heidt and Landi, 1964) One reason for the observed decrease in overall decomposition rate can be the formation of ozonide, O^{3-}, which reacts with H_2O_2 or OH^- radicals that are produced in the ozone decomposition, reforming ozone (Greenwood and Earnshaw, 2001) Dissolved ozone can react with a variety of matter, such as organic compounds, viruses, bacteria, and so on. As a result, ozone decomposes to other matter; see Figure 2.3. This figure illustrates that the half-life of ozone in distilled water is much shorter, compared to tap-water.

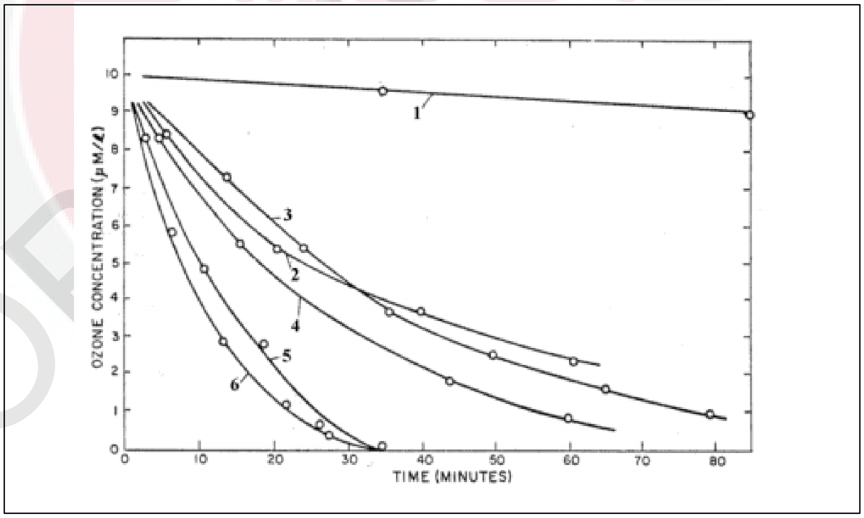

Figure 2.3: Ozone decomposition in different types of water at 20 °C. 1 = double-distilled water; 2 = distilled water; 3 = tap water; 4 = groundwater of low hardness; 5 = filtered water from Lake Zurich; 6 = filtered water from Bodensee

Ozone decomposes in water in OH-radicals. Dependent on the nature of the dissolved matter, these can accelerate (chain-reaction) or slow down the decay of ozone. Substances that accelerate this reaction are called promoters. Inhibitors are substances that slow down the reaction. When water is ozonized, one often uses the term 'scavenging capacity'. Scavengers are entities that react with OH-radicals and slow down the chain-reaction (Lenntech B.V, 1998).

Temperature has an important influence on the half-life of ozone. Table 2.5 shows the half-life of ozone in air and water. In water, the half-life of ozone is much shorter than in air, in other words ozone decomposes faster in water. The solubility of ozone decreases at higher temperatures and is less stable. On the other hand, the reaction speed increases with a factor 2 or 3 per 10 °C. Principally, ozone dissolved in water cannot be applied when temperatures are above 40 °C, because at this temperature the half-life of ozone is very short.

Table 2.5: Half-life of ozone in gas and water at different temperatures

Air		Dissolved in water	
Temp (°C)	Half-live	Temp (°C)	Half-live
-50	3 months	15	30 minutes
-35	18 days	20	20 minutes
-25	8 days	25	15 minutes
20	3 days	30	12 minutes
120	1.5 hours	35	8 minutes
250	1.5 seconds		

2.8 Background of Orange, Lemon and Lime Juice

2.8.1 Orange Juice

Orange juice is the liquid concentrate of the product of the orange tree, produced by pressing oranges. It comes in a few different varieties, including blood orange, navel orange, Valencia orange, clementine, and tangerine. As well as varieties in oranges utilized, some varieties incorporate varying measures of juices vesicles, known as “pulp” in American English, and “juicy bits” in British English. These vesicles contain the juice of the orange and can be left in or evacuated during the manufacturing process. How juicy these vesicles are relied on many variables, for example, species, assortment, and season.

Commercial orange juice with a longer time span of usability is made by drying and later rehydrating the juice, or by concentrating the juice and later adding water to the concentrate. Before drying, the juice may likewise be sanitized and oxygen expelled from it, requiring the later expansion of a flavor pack, for the most part produced using orange items.

The health value of orange juice is debatable. It has a high convergence of vitamin C, additionally a high grouping of simple sugars, practically identical to delicate drinks. Subsequently, some legislature wholesome guidance has been balanced thus, to encourage substitution of orange juice with raw fruit, which is processed all the more gradually, and limit daily consumption.

A cup serving of raw, fresh orange juice, adding up to 248 grams or 8 ounces, has 124 mg of vitamin C. It has 20.8 g of sugars and has 112 calories. It likewise supplies potassium, thiamin, and folate. Citrus juices contain flavonoids (particularly in the pulp) that may have medical advantages. Orange juice is additionally a source of the antioxidant hesperidin. In light of its citrus extract content, orange juice is acidic, with common pH of around 3.5.

2.8.1.1 Health Nutrition of Orange Juice

The antioxidants in orange juice are accepted to be behind a significant number of its medical advantages, including counteractive action of tumor and stroke, A recent report in the journal Nutrition and Cancer: An International Journal reviewed all the exploration on fresh orange and tumor anticipation, concluding that antioxidants in orange juice helped prevent cancer of the blood, breast, color and liver. Table 2.6 below shows the amount of nutritional fact of orange juice from the USDA.

Table 2.6: Nutritional fact of orange juice (Source: USDA)

Nutrients	**Unit**	**Serving Size: 100 ml**	**Serving Size: 1 cup**
Total fat	g	0	0
Saturated fat	g	0	0
Polyunsaturated fat	g	0.011	0.025
Monounsaturated fat	g	0.011	0.025
Cholesterol	mg	0	0
Sodium	mg	2	5
Potassium	mg	44	105

Total carbohydrate	g	14.1	33.39
Dietary fiber	g	0.2	0.5
Sugar	g	9.84	23.31
Proteins	g	0.21	0.5
Vitamin A	%	1	2
Vitamin C	%	26	62
Calcium	%	0	0
Iron	%	1	2

2.8.2 Lime Juice

A lime (from French *lime*, from Arabic *līma*, from Persian *līmū*) is a hybrid citrus fruit, which is typically round, lime green, 3-6 centimetres in diameter, and containing acidic juice vesicles. Lime juice is a sour, tart liquid that is obtained from the fruit of the Citrus aurantifolia, a shrubby citrus tree that is native to Asia. A small, green fruit related to the lemon, lime fruit is pale green with a juicy pulp. Widely used for both culinary and non-culinary purposes, it is among the most popular citrus juice available in the market today.

Drinking lime juice helps to meet the body's daily requirement for vitamin C. The USDA reports that a cup of freshly squeezed lime juice contains 123 milligrams of Vitamin C. Vitamin C is an essential nutrient that is needed for the growth and repair of tissues, as well as the repair and maintenances for the bones and teeth (University of Maryland Medical Centre, n.d.). It also helps the body to produce collagen, a protein that plays a vital role in the production of blood vessels, skin, tendons, ligaments, and cartilage.

Besides, lime contain unique active compounds called flavanol glycosides, which not only offer anticancer and antioxidant benefits, but also have antibiotic properties. The antibiotic properties of these compounds have been shown to protect against the contraction of cholera, a disease caused bacteria called Vibrio cholera. Research conducted in West Africa, published in "Tropical Medicine and International Health", shows that drinking lime juice with the main meal protects participant against this disease.

2.8.2.1 Health Nutrition of Lime Juice

Lime juice has potent anti-carcinogenic properties. Lime and other citrus fruit contain a variety of cancer-fighting compounds called flavonoids (Hatherill, n.d.) Flavonoids are a family of naturally-occurring compounds found in many fruit and vegetables. The biological activity of citrus juice flavonoids has anti-cancer effects that prevent the invasion of cancer cells. These compounds also powerfully inhibit the growth of tumor cells. Table 2.7 below shows the amount of nutritional fact of lime juice from the USDA.

Table 2.7: Nutritional fact of lime juice (Source: USDA)

Nutrients	Unit	Serving Size: 100 ml	Serving Size: 1 cup
Total fat	g	0.07	0.17
Saturated fat	g	0.008	0.02
Polyunsaturated fat	g	0.024	0.057
Monounsaturated fat	g	0.008	0.02
Cholesterol	mg	0	0

Sodium	mg	2	5
Potassium	mg	122	288
Total carbohydrate	g	8.77	20.71
Dietary fiber	g	0.4	1
Sugar	g	1.76	4.16
Proteins	g	0.44	1.03
Vitamin A	%	1	2
Vitamin C	%	52	123
Calcium	%	2	3
Iron	%	0	1

2.8.3 Lemon Juice

The lemon, *Citrus limon* (L.) Osbeck, is a species of small evergreen tree in the flowering plant family Rutacea, native to Asia. The tree's ellipsoidal yellow fruit is utilized for culinary and non-culinary purposes throughout the world, mainly for its juice, which has both culinary and cleaning uses. The pulp and rind (zest) are also used in cooking and baking. The juice of the lemon is about 5% to 6% citric acid, which gives a sour taste. The distinctive sour taste of lemon juices makes it a key ingredient in drinks and food.

Lemon juice offers powerfully healthy physical properties along with a pleasingly tart taste, which is acidic, yet soothing to an upset stomach. It is valued for its vitamin C and potassium content as well as its versatility in cooking and baking. Lemon water and lemonade help settle morning sickness during pregnancy and other sources of nausea. Researchers are also exploring the ability of lemonade to prevent kidney stones.

Lemon juice is rich in vitamin C. The body needs vitamin C to resist colds, fight disease and aids absorption of iron from vegetables and grains. It wards off a broad range of health problems including the condition called scurvy that once plagued seafarers who lacked access to fresh fruits and vegetables. The juice in lemon can weight-up to 1.5 ounces, which is equal to about 43 grams. It takes roughly three lemons to gather 100 g of juice, which contains about 50 mg of vitamin C.

A glass of lemonade also provides lots of potassium. There are about 145 mg of potassium in 100 g of lemon juice. Potassium works to balance acid and alkali blood chemistry known as body pH. It helps nourish the body by aiding in synthesis of amino acids from protein and use of carbohydrate.

2.8.3.1 Health Nutrition of Lemon Juice

Drinking lots of lemon juice can help prevent kidney stones, according to Roger L. Sur, M.D., of the Comprehensive Kidney Stone at Center at the University of California at San Diego. Lemon juice inhibits the growth of kidney stones due its citrate, a substance that also occurs in other citrus fruit but is highest in lemons. Drinking about 2 quarts of lemonade daily made with 4 ounces of lemon juice and artificial sweeteners can decrease kidney stone formation. Other citrus juices are helpful as well, but not if they are supplemented with calcium or if they are supplemented with calcium or if they contain oxalate, a natural substance that promotes the formation of kidney stones, Sur says, as do salt and protein. Table 2.8 below shows the amount of nutritional fact of orange juice for 1 cup serving.

Table 2.8: Nutritional fact of lemon juice (Source: USDA)

Nutrients	Unit	Serving Size: 100 ml	Serving Size: 1 cup
Total fat	g	0	0
Saturated fat	g	0	0
Polyunsaturated fat	g	0	0
Monounsaturated fat	g	0	0
Cholesterol	mg	0	0
Sodium	mg	1	2
Potassium	mg	128	303
Total carbohydrate	g	8.9	21.06
Dietary fiber	g	0.4	1
Sugar	g	2.48	5.86
Proteins	g	0.39	0.93
Vitamin A	%	0	1
Vitamin C	%	79	187
Calcium	%	1	2
Iron	%	0	0

CHAPTER 3

METHODOLOGY

This chapter explains the materials and methods used throughout in this experimental work. It consists of Section 3.1 Research Design, 3.2 Design Experiment, 3.3 Ozone Treatment, 3.4 Gas Analyzer Method, 3.5 Iodine Method and 3.6 Physicochemical Analysis for p H, TSS, Vitamin C, Turbidity and TPC.

3.1 Research Design

The overall research design of the validation of gaseous ozone (O_3) concentration and its half-life in various fruit juices are summarized into a flow chart below (Figure 3.1). It consists of three parts in conducting this study. The first part is the test on preliminary studies to determine the pH, TSS, vitamin C, turbidity and TPC on the selected citrus fruit juices. The second part is validation of ozone using gas analyzer and iodine method. This is to test the production of ozone from the ozone generator. For this part, gas analyzer is used to measure the gaseous ozone (O_3) concentration in the air since the reading could not be obtained when the tube is submerged into water whereas for iodine method is used to measure the gaseous ozone (O_3) concentration in the distilled water. Based on the second part, iodine method is used to measure the gaseous ozone (O_3) concentration in citrus fruit juice after being treated with ozone at different exposure time and then determine the half-life of ozone too.

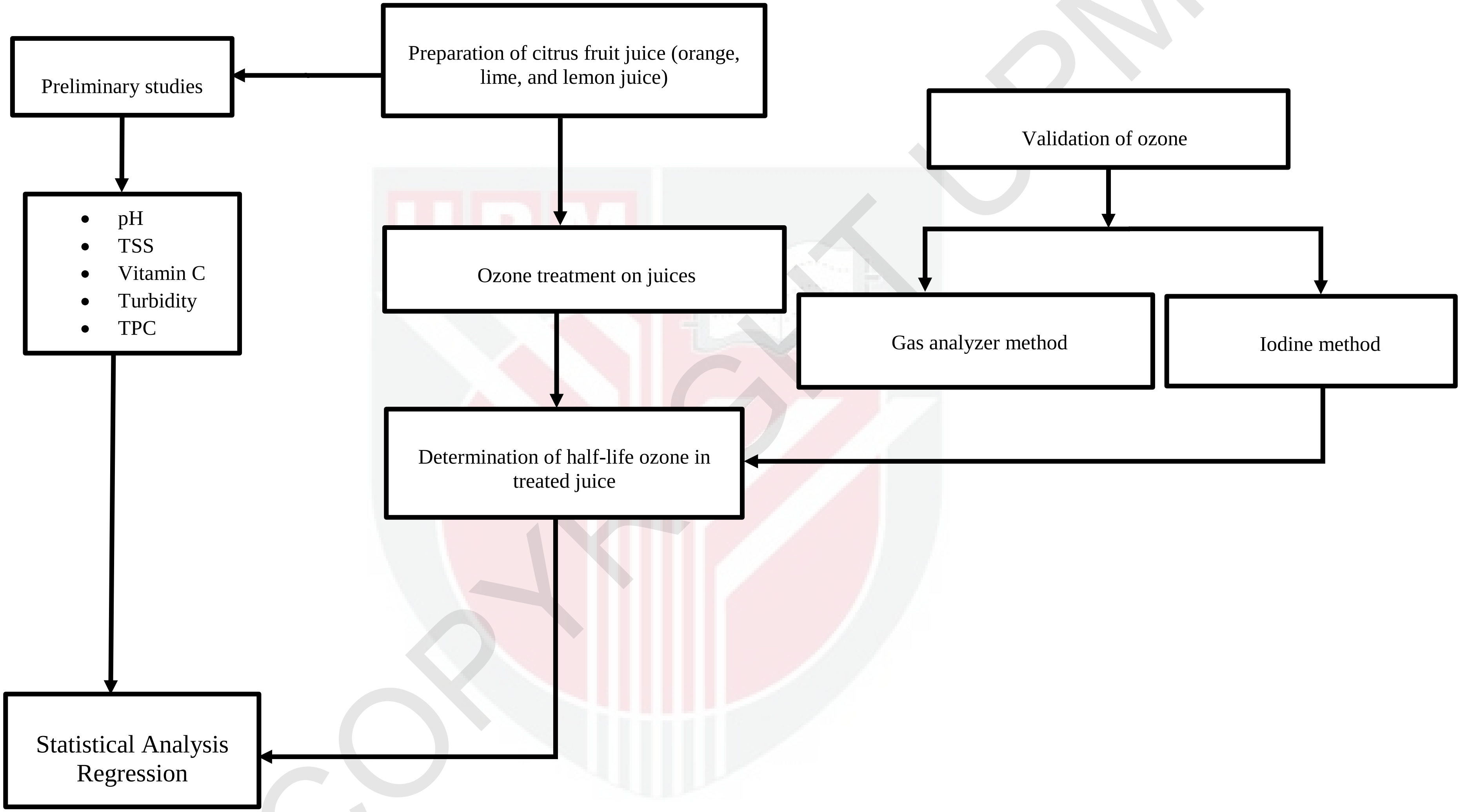

Figure 3.1: Summary of experiment

3.2 Design of Experiment

The correlation between the two factors (i.e types of fruit juices and time) on validation of gaseous O_3 concentration and its half-life in fruit juices will be assessed using Full Factorial Design of Experiment (Table 3.1). A full factorial design will provide the most response information about factors main effect and factors interaction. It also will provide the process model's coefficient for all factors and all interaction. Furthermore, when validated, it allows the process to be optimized. The statistical analysis of the result will be done using regression analysis, which will provide the coefficients for the prediction equations; means and standard deviations.

Table 3.1 General Full Factorial Experimental Design (RSM)

Std	Run	Block	Type of sample	Time (minutes)
46	1	Block 1	Orange juice	25
55	2	Block 1	Orange juice	30
49	3	Block 1	Lime juice	25
31	4	Block 1	Lime juice	15
1	5	Block 1	Orange juice	0
25	6	Block 1	Lemon juice	10
4	7	Block 1	Lime juice	0
52	8	Block 1	Lemon juice	25
28	9	Block 1	Orange juice	15
34	10	Block 1	Lemon juice	15
22	11	Block 1	Lime juice	10
7	12	Block 1	Lemon juice	0

61	13	Block 1	Lemon juice	30
19	14	Block 1	Orange juice	10
58	15	Block 1	Lime juice	30
13	16	Block 1	Lime juice	5
43	17	Block 1	Lemon juice	20
16	18	Block 1	Lemon juice	5
37	19	Block 1	Orange juice	20
40	20	Block 1	Lime juice	20
10	21	Block 1	Orange juice	5

[1]

3.3 Ozone Treatment

In this experiment, ozone gas is generated using water ozonizer (Model SY- 004, Taiwan), in a Figure 3.2, in a 200 mL beaker. The sample of orange juice is filled in the beaker and ozone treatment is conducted in the experiment. The ozone is pumped specifically into the juice through conveyance tube into a beaker and stirred physically to ensure the ozone molecule are totally blended with orange juice. This can be shown like the Figure 3.2 below.

Ozone is makes by accusing the air (O_2) of a burst of high negative voltage. At that point, O_2 will part into oxygen particle O_2 and the oxygen atoms (O) is joined with remaining atoms to formed ozone (O_3) (Muthukumarappan et al., 2008). The residual

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[1] Three blocks of ozonation treatment was done throughout the experiment.

ozone will spontaneously disintegrate into oxygen atom which is safe to the people (Kim et al., 1999).

The parameters for this experiment are the type of fruit juices (orange, lime and lemon) and exposure time of ozone to supply into the juice. The range is starting from 0 to 30 minutes. Then, every 5 minutes, the ozone-treated orange juice is being tested its ozone efficiency by using iodine method. The experiment is proceeded with another sample, lime juice and lemon juice, which have different physicochemical and quality characteristics.

Figure 3.2: Ozone generator and ozone generator setup

3.4 Gas Analyzer Method

The concentration of gaseous ozone (O_3) that has been produced by ozone generator is measured gas analyzer equipment, in a Figure 3.3. Every five minutes, the tube that is connected with the gas analyzer, is inserted into the tube that attached at the ozone generator. The reading is taken until thirty-minutes. The reading that is been displayed is in units of ppm.

Figure 3.3: Gas Analyzer

3.5 Iodine Method

The iodine method or otherwise known as KI method appears to have a standard deviation percentage of 2% when performed in a careful manner using a prescribed procedure. The potassium iodide (KI) wet-chemistry method is based on the principle that iodine ion is oxidized by ozone to form iodine as the ozone gas is bubbled through a solution of KI. The basis of the iodometric titration is the reaction of ozone and alkali iodide solution:

$$2\,KI + O_3 + H_2O \longrightarrow I_2 + 2\,KOH + O_2$$

where iodine is formed from iodine. Iodine makes solution colour yellow or even brown. Amount of iodine is consequently determined by the titration with sodium thiosulphate at the acidid conditions:

$$I_2 + 2\,Na_2S_2O_3 \longleftrightarrow 2\,I^- + Na_2S_4O_6$$

where reduction to iodine to iodide is remarkable by the decoloration of yellow-brownish solution. To make this reaction more sensitive, the starch solution is added before the end of the titration. This solution changes colour of the titrated solution to the blue. The decoloration of blue colour is more visible for the eye than the decoloration of yellow solution.

Flowing gas is led from the discharge region (ozonizer) into the bubling vessel containing 100 mL of 0.2 M KI solution. Generated ozone reacts with iodide to form iodine. Reaction time convenient for the sufficient amount of generated ozone is minutes. Then, the solution with formed iodine is poured into the titration vessel, it is acidified by 10 mL of 2 M HCl and it is titrated by 0.05 M $Na_2S_2O_3$ solution until the whole decolouration. To make this reaction more sensitive, the starch solution is added before the end of the titration. The amount of ozone is determined from the consumed solution of thiosulphate (1 mL of 0.05 M $Na_2S_2O_3$ solution correspond to 1.2 mg of ozone).

3.6 Physicochemical Analysis

3.6.1 pH

pH meter (SevenMulti pH Conductivity Meter, Mettler Toledo, Switzerland) was used to measure pH of citrus fruit juice (orange, lime, and lemon). The meter was first calibrated with commercial buffer solution at pH and pH 4. 40 mL of samples were placed in a beaker and measured at 27±0.5 ºC.

3.6.2 TSS

Total soluble solid of citrus fruit juice were using a digital refractometer (D Series, Graigar Technology, China). The instruments were first calibrated using distilled water prior to test. Then, a substantial amount of extracted juice was dropped onto the refractometer. The resultant reading refers to the reading of total soluble solid of the juice in the terms of degree brix.

3.6.3 Vitamin C

2,6-dichlorophenol-indophenol titration method was utilized in determining vitamin C content in samples of orange, lime and lemon juice (AOAC 1996, Shah 2015). 3% meta-phosphoric acid (w/v) was used to extract the vitamin C from the citrus fruit juice prior to the test. 10 mL of the mixture were titrated against standard 2,6-dichlorophenol-indophenol dye which was already standardized against L-ascorbic acid solution. The ascorbic acid concentration was calculated by comparison with the standard and expressed as mg/L.

3.6.4 Turbidity

The turbidity of the citrus fruit juice was determined using a portable Turbidimeter (Model 2100P, Hach Company, Loveland, Colo, USA) and results were reported in nephelometric turbidity units (NTU).

3.6.5 TPC

Procedure of Follin-Ciocalteu was adopted from Shah (2015) was applied in determining the phenolic contents. 10 mL samples were centrifuged with 10 mL of pure methanol at 5000 rpm at 4 ºC for 5 minutes. A mixture of 0.2 mL of citrus fruit juice sample were made up to 3 mL with distilled water, 0.5 mL Follin-Ciocalteau reagent and 2 ml of 20% sodium carbonate were added. The mixture was mixed thoroughly and incubated for one hour at room temperature in the dark prior to the measurement. UV spectrophotometer was then used to measure the absorbance of mixture at 765 nm. Data for total phenolic content were obtained from the calibration curve prepared with gallic acid at concentration of 0 to 100 mg/L and are expressed as gallic acid equivalents (GAE) is shown in the Figure 3.4 below.

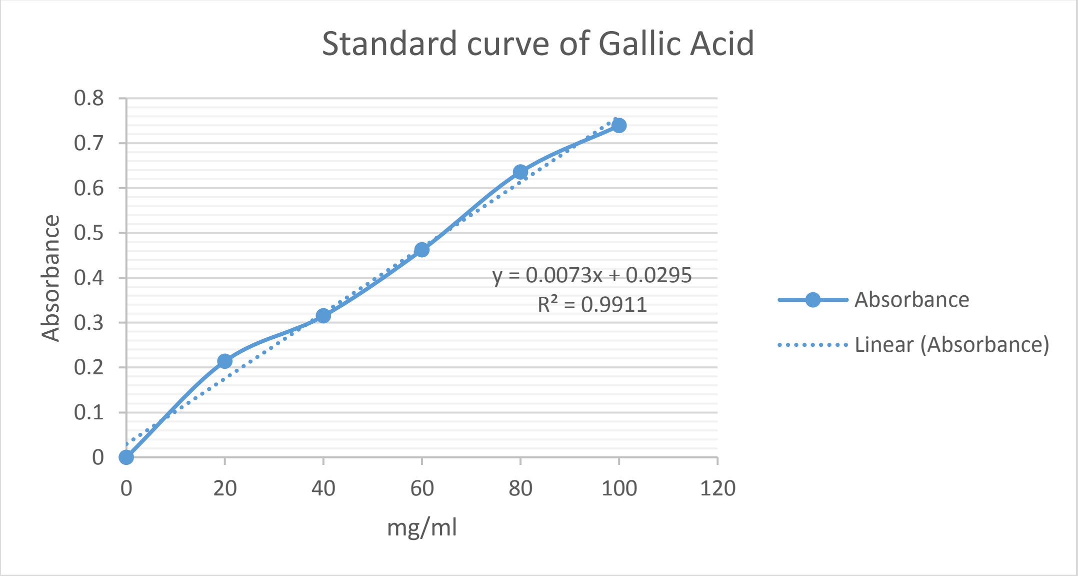

Figure 3.4: Standard curve of Gallic Acid

CHAPTER 4

RESULTS AND DISCUSSION

Chapter 4 discusses on the result obtained from the experiments based on the objectives listed in Chapter 1. Firstly, validation of gaseous ozone (O_3) concentration in various citrus fruit juice which are orange, lime and lemon juice are done by using iodine method and comparison are made with an automated gas analyzer. This chapter also discusses on the correlations of ozone half-life in various citrus fruit juice with different physicochemical and quality characteristic; pH, total soluble solid (TSS), vitamin C, turbidity and total phenolic content (TPC) value.

4.1 Validation Gaseous Ozone (O_3) Concentration using Gas Analyzer and Iodine Method

Validation of gaseous ozone (O_3) concentration using gas analyzer and iodine method were conducted with air and distilled water. This reading was done to validate generator’s efficiency in generating ozone. This was done in the range of time between 0 to 30 minutes. Figure 4.1 shows the reading of ozone concentration via automated gas analyzer and iodine method.

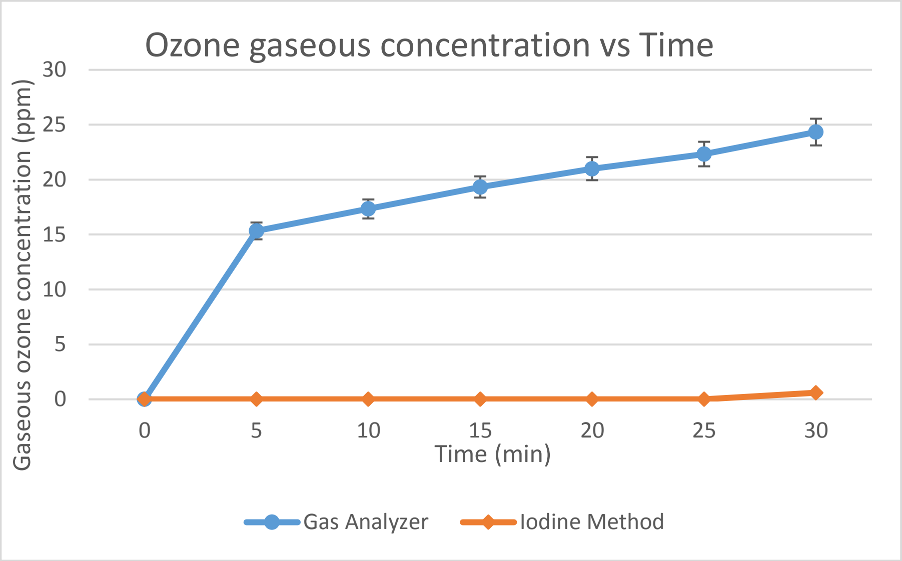

Figure 4.1: Validation of gaseous ozone (O_3) concentration vs time

From the Figure 4.1 above, it shows that the reading of gaseous ozone (O_3) is higher when using gas analyzer method than using iodine method. The reading of gaseous ozone (O_3) in the air could be read by automated gas analyzer after 5 minutes of exposure time. Whereas reading of gaseous ozone (O_3) in the distilled water was proved by titration of iodine method after 30 minutes of exposure time. Overall, in the range time of 0 to 30 minutes, automated gas analyzer could give a reading from 0 to 24 ppm while titration iodine method could give a reading from 0 to 0.6 ppm. This shows that for iodine method, 0 to 25 minutes of exposure time is not enough of ozone (O_3) to stay in the distilled water, hence no result was shown. This could be concluded that the ozone decomposes rapidly in the aqueous solution compare in the air.

4.2 Half-Life of Gaseous Ozone in Various Citrus Fruit Juice which are Orange, Lime and Lemon Using Iodine Method

Orange, lime, and lemon juices were treated with different ozone (O_3) concentration and different time of treatment (0-30 minutes). At an interval of 5 minutes each sample undergone the ozone treatment, the reading of gaseous ozone (O_3) concentration will be done using iodine method. The amount of ozone is determined from the consumed solution of thiosulphate which is 1 ml of 0.05 M $Na_2S_2O_3$ solution corresponds to 1.2 mg of ozone. The reading was taken until the sample of fruit juices undergone 30 minutes of ozone treatment. The reading of every 1 ml of consumed solution of thiousulphate is then converted to the concentration of ozone by multiplying with 1.2 mg ozone. Then, the conversion unit from mg to ppm had been done. Below in Table 4.1 is the reading of concentration of gaseous ozone (O_3) in ppm for 21 runs of samples.

Table 4.1: Response of Ozone Concentration

Run	Block	Type of sample	Time (minutes)	Response 1: Average Concentration of Gaseous Ozone (O_3) (ppm)	Mean ± SD
1	Block 1	Orange juice	25	0.96	0.96 ± 0
2	Block 1	Orange juice	30	1.44	1.44 ± 0.20
3	Block 1	Lime juice	25	2.65	2.65 ± 0.09
4	Block 1	Lime juice	15	0	0 ± 0
5	Block 1	Orange juice	0	0	0 ± 0
6	Block 1	Lemon juice	10	1.64	1.64 ± 0.15
7	Block 1	Lime juice	0	0	0 ± 0
8	Block 1	Lemon juice	25	4.85	4.85 ± 0.07
9	Block 1	Orange juice	15	0	0 ± 0
10	Block 1	Lemon juice	15	2.68	2.68 ± 0.20
11	Block 1	Lime juice	10	0	0 ± 0
12	Block 1	Lemon juice	0	0	0 ± 0
13	Block 1	Lemon juice	30	8.76	8.76 ± 0.10

14	Block 1	Orange juice	10	0	0 ± 0
15	Block 1	Lime juice	30	4.08	4.08 ± 0.20
16	Block 1	Lime juice	5	0	0 ± 0
17	Block 1	Lemon juice	20	4.56	4.56 ± 0
18	Block 1	Lemon juice	5	0	0 ± 0
19	Block 1	Orange juice	20	0	0 ± 0
20	Block 1	Lime juice	20	1.24	1.24 ± 0.06
21	Block 1	Orange juice	5	0	0 ± 0

The data above is then plotted in the Figure 4.2 below. The figure shows that the ozone (O_3) concentrations were significantly different in different sample of citrus juice. The lemon juice is the fastest one that can detect its concentration as early as 5 minutes compared to the rest. For the lime juice, the gaseous ozone (O_3) concentration can be detected after 15 minutes while the orange juice after 20 minutes of exposure time. For the 0 reading in orange and lime juice after 0-20 minutes, exposure time, this is no color changes during the titration. This shows that there is no gaseous ozone (O_3) concentration during that time in the juices. A number of factors could be attributed to the results shown, such as the juice's pH, TSS and vitamin C value. For pH, when the pH value increases, the formation of OH-radicals increased (Lenntech B.V, 1998). In a solution with a high pH value, there are more hydroxide ions present. These hydroxide ions act as an initiator for the decay of ozone. Meanwhile for TSS, it is dissolved solid concentrations within the juice and dissolved ozone can react with variety of matters, such as organic compounds, viruses, and bacteria. As a result, ozone decomposes to other matter hence the ozone decomposed rapidly in high TSS value. And lastly for vitamin C, differences of vitamin C levels in these fruit juices appeared to reflect changes in the rate of gaseous ozone (O_3) degradation in response to the Vitamin C value.

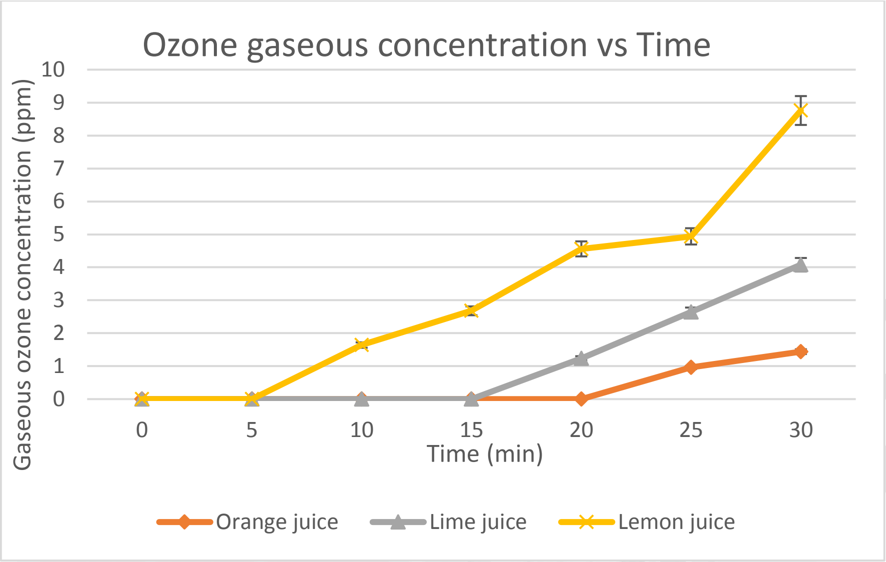

Figure 4.2: Half-life of gaseous ozone (O_3) concentration vs time

4.3 Correlations of pH, TSS, Vitamin C, Turbidity, and Total Phenolic Content (TPC) with Half-Lives of Gaseous Ozone (O_3) Concentration

For the preliminary study, the initial value of physicochemical and quality characteristic; pH, total soluble solid (TSS), vitamin C, turbidity and total phenolic content (TPC) value of orange, lime and lemon juice have been measured. The reading is shown in the Table 4.2 below.

Table 4.2: Initial value of physicochemical and quality characteristic of citrus fruit juices

Type of fruit juices	Physicochemical and Quality Characteristic				
	pH	TSS	Vitamin C (mg/100 mL)	Turbidity (NTU)	TPC (GAEmg/mL)
Orange juice	2.4	11.0	27.6	982.0	144.5
Lime juice	1.4	10.9	22.4	49.9	110.34
Lemon juice	1.1	7.0	12.6	323.0	180.89

From the information in the Table 4.2 above, the correlations of ozone half-life in various citrus fruit juice with different physicochemical and quality characteristic; pH, total soluble solid (TSS), vitamin C, turbidity and total phenolic content (TPC) value has been studied. The relationship between the half-life of gaseous ozone (O_3) concentration and type of fruit juice at different exposure time has been shown in the Figure 4.3 below. Further explanation of this relationship has been discussed in the section 4.3.1 and so on.

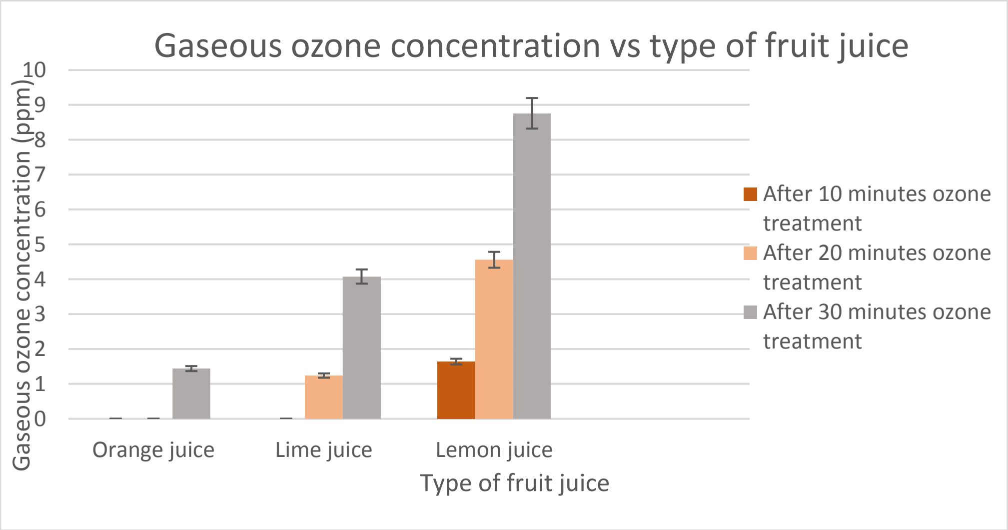

Figure 4.3: Half-life of gaseous ozone (O_3) concentration vs type of fruit juice

4.3.1 pH

The initial pH values of orange, lime and lemon juice are 2.4, 1.4 and 1.1 respectively. Figure 4.3 shows the half-life of gaseous ozone (O_3) concentration against type of fruit juice. It can be seen from the figure that ozone (O_3) concentration in different samples decreased when the pH values are high. This shows that the gaseous ozone decay rapidly in the higher pH value compared in the lower pH value. This is because ozone decomposes partly in OH-radicals. When the pH value increases, the formation of OH-radicals increased (Lenntech, 1998). In a solution with a high pH value, there are more hydroxide ions present. These hydroxide ions act as an initiator for the decay of ozone. The formulas can be seen below;

$$O_3 + OH^- \longrightarrow HO_2^- + O_2 \qquad \text{[Equation 1]}$$

$$O_3 + HO_2^- \longrightarrow {}^{\bullet}OH + O_2^{\bullet -} + O_2 \qquad \text{[Equation 2]}$$

The radicals that are produced during the second reaction can introduce other reactions with ozone, causing more OH- radicals to be formed. The trends can be seen to be consistent as the treatment time progresses.

4.3.2 Total Soluble Solid (TSS)

The relationship between total soluble solid (TSS) and gaseous ozone concentration can be seen in Figure 4.3 above. The initial value of TSS for orange, lime, and lemon are 11.0, 10.90 and 7.0. It was observed from the experiment that gaseous ozone (O_3) concentration decreased when the value of the TSS is high. Sample of orange juice shows the lowest gaseous ozone (O_3) concentration 0-1.44 ppm as it has the highest values of TSS content 11.0. Sample of lemon juice has the highest gaseous ozone (O_3) concentration 1.64-8.76 ppm as it has the lowest value of TSS 7.0. This is because total soluble solid is dissolved solid concentrations within the juice and dissolved ozone can react with variety of matters, such as organic compounds, viruses, and bacteria (Lenntech, 1998). As a result, ozone decomposes to other matter and can be seen in the Figure 4.3 below. The graph illustrates that the half-life of ozone in orange juice is much shorter.

Dependent on the nature of the dissolved matter, these matters can accelerate (chain-reaction) or slow down the decay of ozone. Substances that accelerate this reaction are called promoters. Inhibitors are substances that slow down the reaction. When water is ozonized, one often uses the term ‘scavenging capacity’. Scavengers are entities that react with OH-radicals and slow down the chain-reaction (Lenntech, 1998).

4.3.3 Vitamin C

The initial value of vitamin C for orange, lime and lemon juices are 27.6 mg, 22.4 mg, and lemon 12.6 mg respectively for every 100 mL of fruit juices. From the experiment illustrated in Figure 4.3 above, sample of orange juice has the lowest gaseous ozone (O_3) concentration 1.44 ppm as it has the highest values of vitamin C content 27.6 mg. Whereas, sample of lemon juice has the highest gaseous ozone (O_3) concentration 8.76 ppm as it has the lowest value of vitamin C 12.6 mg. This result indicated that the differences of vitamin C levels in these fruit juices appeared to reflect changes in the rate of gaseous ozone (O_3) degradation in response to the Vitamin C value.

4.3.4 Turbidity

The initial value of turbidity for orange, lime, and lemon juice are 982.0 NTU, 49.9 NTU and 323.0 NTU. As seen as the Figure 4.3 above, orange juice which has the highest value for the turbidity 982.0 NTU shows the lowest value for gaseous ozone (O_3) concentration 0-1.44 ppm. Meanwhile, lemon juice with the initial value of turbidity of 323.0 NTU shows the value of gaseous ozone (O_3) concentration 1.64-8.76 ppm. From the result, it shows that when the turbidity decreased, the gaseous ozone (O_3) concentration increased. Whereas, for lime juice, with the lowest initial value of turbidity, 49.9 NTU, shows slightly increased in gaseous ozone (O_3) concentration. This can be concluded that there is no clear trend was observed for gaseous ozone (O_3) concentration regarding the effects of the differences turbidity value.

4.3.5 Total Phenolic Content (TPC)

The total phenolic content (TPC) values for orange, lime and lemon are 144.5 GAEmg/mL, 110.34 GAEmg/mL, and 180.89 GAEmg/mL. Similar with turbidity, in Figure 4.3, also does not show the clear trend was observed for gaseous ozone (O_3) concentration regarding the effects of the differences total phenolic content (TPC). However, it is directly opposite from the pattern turbidity of the juices sample showed. Lemon juice, which has the highest value for the TPC, 180.89 GAEmg/mL shows the highest value for gaseous ozone (O_3) concentration 1.64-8.76 ppm as seen in Figure 4.3. Meanwhile, lime juice with the initial value of TPC of 110.34 GAEmg/mL shows the value of gaseous ozone (O_3) concentration 0-4.08 ppm. From the result, it shows that when the TPC decreased, the gaseous ozone (O_3) concentration decreased. However, for orange juice, with the initial value of TPC, 144.5 GAEmg/mL, shows decreased in gaseous ozone (O_3) concentration 0-1.44 ppm and it was even lower than in the lime juice. This could be concluded that the TPC does not show any significant effect on the half-life of ozone.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATION

5.1 Conclusions

Ozone treatment is attractive because it does not leave any undesirable residue in fruit juices. Thus, from this study it could prove that ozone has a very short half-life in aqueous and could be used for juice treatment. The result also showed that the gaseous of ozone (O_3) concentration in lemon juice could be detected after 5 minutes exposure time, in lime juice could be detected after 15 minutes exposure time and in orange juice could be detected after 20 minutes exposure time. This could be concluded that half-life of ozone decomposes rapidly in the orange juice, then lime juice and the last one is lemon juice. Furthermore, the decay of ozone is faster in juice with high pH, TSS, and vitamin C value whereas the turbidity and TPC does not show any significant effect on the half-life of ozone. Overall, ozone treatment has the potential to be used as a non-thermal pasteurization to produce a safe and high quality of juice.

5.2 Recommendation for Future Work

Based on this study, it was found that the iodine method can be used to determine the presence of dissolved gaseous ozone in treated fruit juices. Besides, the study also showed that the half-life of gaseous ozone is shorter in a high pH fruit juices compared to in low pH fruit juices. Therefore, further study should be done to investigate other extrinsic and intrinsic parameters such as temperature, ozone concentration, and ozone flow rate on the ozone treatment in order to study the factors that influence the half-life of gaseous ozone. More investigations related with the half-life of gaseous ozone should be done on various types of fruit juice since there are inadequate studies about this knowledge gap. Apart from that, optimization on suitable ozone concentration and processing time without jeopardizing the nutritional content of the juice should be considered.

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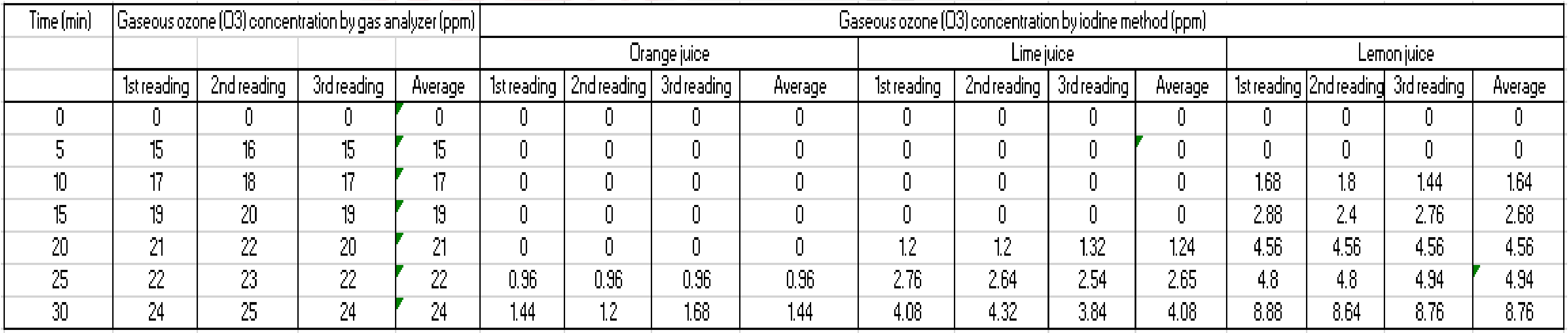
APPENDICES

Table A1: Data for gaseous ozone (O_3) concentration using gas analyzer and iodine method with increasing processing time.



Time (min)	Gaseous ozone (O3) concentration by gas analyzer (ppm)				Gaseous ozone (O3) concentration by iodine method (ppm)											
					Orange juice				Lime juice				Lemon juice			
	1st reading	2nd reading	3rd reading	Average	1st reading	2nd reading	3rd reading	Average	1st reading	2nd reading	3rd reading	Average	1st reading	2nd reading	3rd reading	Average
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	15	16	15	15	0	0	0	0	0	0	0	0	0	0	0	0
10	17	18	17	17	0	0	0	0	0	0	0	0	1.68	1.8	1.44	1.64
15	19	20	19	19	0	0	0	0	0	0	0	0	2.88	2.4	2.76	2.68
20	21	22	20	21	0	0	0	0	1.2	1.2	1.32	1.24	4.56	4.56	4.56	4.56
25	22	23	22	22	0.96	0.96	0.96	0.96	2.76	2.64	2.54	2.65	4.8	4.8	4.94	4.94
30	24	25	24	24	1.44	1.2	1.68	1.44	4.08	4.32	3.84	4.08	8.88	8.64	8.76	8.76