



**UNIVERSITI PUTRA MALAYSIA**

***GASEOUS OZONE PASTEURIZATION OF BROWN RICE MILK***

**PRESLEY JALI ANAK SATAP**

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by



**PRESLEY JALI ANAK SATAP**

**182083**

**BACHELOR OF ENGINEERING (PROCESS AND FOOD)**

**FACULTY OF ENGINEERING**

**UNIVERSITI PUTRA MALAYSIA**

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## ABSTRACT

The application of thermal processing technology to kill or inactivate microorganism are commonly used in liquid food industries. However, the conventional technology will negatively affect the nutritional and qualitative characteristics of food products. Thus, to overcome this problem, ozone treatment has been considered as it has shown potentials to reduce the loss of nutritional characteristics while minimizing pertinent microorganisms within liquid food products. The objectives of this study are to determine the inactivation kinetics of aerobic plate count (APC) and yeast and mold count (YMC) on ozone-treated brown rice milk beverage and determine the effect of gaseous ozone on the physicochemical characteristics (pH, total soluble solid (TSS) and total colour difference (TCD)) of brown rice milk. Brown rice milk were exposed to ozone treatment time of 0 (control), 10 to 30 minutes with 10 minutes interval. These results showed that both APC and YMC can be inactivated through the process of ozonation significantly ( $p < 0.05$ ). However, the synergistic combination of ozone concentration and treatment time also shows significant effect ( $p < 0.05$ ) on total colour difference but no significant ( $p > 0.05$ ) effect has been observed on pH and TSS. These results showed the possibility of ozone as one of the option to pasteurize while preserving the quality of brown rice milk.

## ABSTRAK

Pengaplikasian teknologi pemprosesan berasaskan haba untuk membunuh atau menyahaktifkan mikroorganisma adalah perkara biasa. Namun, teknologi ada kekurangannya kerana ia memberi kesan negative kepada ciri-ciri nutrisi dan kualitatif produk makanan.. Jadi, untuk mengatasi masalah ini, rawatan ozon telah menjadi pilihan kerana teknik ini menunjukkan potensi untuk mengurangkan kehilangan kandungan berkhasiat dlama makanan berasaskan cecair di samping turut mengekang pembiakan mikroorganisma dalam produk makanan. Objektif kajian ini ialah untuk menentukan kinetic penyahaktifan menerusi kaedah “aerobic plate count”(APC) dan “yeast and mould count”(YMC) ke atas susu beras perang yg telah diozonkan dan menentukan kesan gas ozone ke atas ciri-ciri psikokimia(pH, jumlah pepejal terlarut(TSS) dan jumlah perbezaan warna(TSD)) ke atas susu beras perang. Beras perang akan dideahkan dengan rawatan ozon dengan masa 0(sampel kawalan),10,20 dan 30 dengan selang masa 10 minit. Keputusan ujian menunjukkan APC dengan YMC boleh dinyahaktifkan menerusi proses pengozonan secara signifikan( $p>0.05$ ). Selain itu, rawatan ozon juga menunjukkan kesan secara signifikan kepada ( $p>0.05$ ) kepada jumlah perbezaan warna tetapi tiada kesan signifikan( $p<0.05$ ) ke atas pH dan TSS. Ini menunjukkan kebarangkalian ozon mampu dijadikan sebagai satu pilihan untuk mempasteur produk makanan disamping mengekalkan kualiti susu beras perang.

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## CHAPTER 1

### INTRODUCTION

Preservation of food by thermal food processing such as pasteurization is a common practice by the industries where food products are exposed to high temperature for a short time (HTST) with the aim to kill the pertinent microorganism and to ensure that the food is safe for a long period of time. However, there are some negative impacts of this preservation technique has been proven to alter the flavours and causes loss of vitamins, essential nutrients and the freshness of the products that subsequently consumer preferences and demands (Xiao et al,2017; Barbosa Canovas.,2009). Hence, the concept of non-thermal processes was developed to overcome these obstacles. Essentially, non- thermal processes of food products does not involve heat was minimally use with low temperature ranging from  $-5\text{ }^{\circ}\text{C}$  to  $45\text{ }^{\circ}\text{C}$  (Bevilacqua et al.,2018) which is lower than the range require for thermal pasteurization which required around  $60\text{ }^{\circ}\text{C}$  to  $100\text{ }^{\circ}\text{C}$ (Agcam et al.,2018). Therefore, important aspects for fresh products such as flavours, essential nutrients and vitamins are able to be retain during the processing.

In addition, non-thermal processes requires less energy than thermal processes (Barbosa Carnovas, 2009). Other technologies food processing are high pressure

processing (HPP), pulsed electric field (PEF), ohmic heating, ultrasound and cold plasma. With each of these technologies, treatment time for techniques can be combined or use alone with the objective to optimize the product quality, processing time and to inactivate bacteria and enzyme. In a nutshell, non-thermal has captured the attention of food manufacturers that needs new food processing methods with less carbon footprints and its beneficial effects towards food products (Brenann & Grandison, 2012).



## **1.1 Overview of ozone**

The demand for new, innovative processing technologies is rapidly increasing as the demand from the consumers for fresher, healthier and safe food products continue to rise. Ozone has been identified as a powerful sanitizer, safe and environmental friendly which may meet the presupposition of the industry, approval of the regulatory agencies and acceptance of the consumers (Khadre et al., 2001). Ozone is often used in food and beverages industries to protect against microorganism and to lengthen the shelf life of fruits, vegetables, meat grains and their products. Furthermore, ozone treatment has proven to give out satisfied results in terms of maintaining the sensory, nutritional and physicochemical characteristics of food (Prabha et al,2015).

Ozone is a gaseous compound which can be found naturally in our atmosphere and it is formed due to lightning or high energy UV radiation (Jakob & Hansen,2005). It is a bluish gas with pungent odour. However, ozone is an unstable gas and decomposes very quickly in the water (Khadre et al.,2001). Thus, generation of ozone can be done through addition of free oxygen radicals with molecular oxygen that will form tri-atomic oxygen. Techniques such as corona discharge, UV radiation and electrolysis can be used to generate ozone (Prabha., et al., 2015; Nath et al., 2014; Guzel –Seydim et al., 2004; Xu,1999).

According to Guzel –Seydim et al. (2004), when ozone is exposed to food, no residues are found since it decomposes quickly. Low concentration of ozone and short time of exposure are enough to inactivate bacteria, molds, yeast, parasites and viruses (Kim et al., 1999). In addition, Guzel –Seydim et al. (2004) stated that ozone treatment is one of

non-thermal technologies that will help to increase food safety without compromising in terms of quality and the desirability of food products.

## **1.2 Problem statement**

Rice milk is chosen for this study because rice, the raw material for rice milk are available in abundance amount in the market. According to FAO (2016), global production area for rice is about 160.6 million ha with annual production of 490 million tonnes. Besides that, rice also has chosen due to its nutritional content which largely consists of carbohydrates, proteins, fats, fibers, minerals, vitamins (Juliano, 1993) and this causes rice to be regarded as major sources of nutrients. Next, the ozone treatment method is chosen due to high degree of safety as ozone reaction will produce oxygen when it decomposes, and as a consequence, food products that are treated with ozone are free of disinfectant residue (Prabha et al., 2015; Guzel-Seydim et al., 2004). Besides that, ozone treatment has high decomposition rate in water phase which is needed to inactivate bacteria, molds, yeast, parasites and viruses (Kim et al., 1999). Ozone treatment also chosen due cost-effective and high availability of ozone. Currently, there is no study regarding the effects of ozone treatment on non-dairy products has been carried out although ozone has big potential to be exploited as method to preserve the quality of non-dairy products.

### **1.3 Objectives**

The objectives of this study are:

- a) To determine the inactivation kinetics of aerobic plate count (APC) and yeast and mold count (YMC) ozone-treated brown rice milk beverage;
- b) To determine the effect of gaseous ozone on the physicochemical characteristics (pH, total soluble solid (TSS) and total colour difference (TCD)) of ozone-treated brown rice milk.

### **1.4 Scope of study**

This study is to analyse the application of ozone on inactivation kinetics of APC and YMC to study the effect of ozone on the nutritional content of rice milk. There are some parameters has been set for this study. The parameters involved were treatment time (0,10,20 and30 minutes). The ozone generator that was used in this study are Model GL-3189(China). The limitation of this study is the ozone generator could only produce ozone around 400mg/hour with treatment time limited to 30 minutes.

### **1.5 Significance of study**

The findings of this study can be utilized for non-dairy food industry if ozone can be proven to be alternatives to inactivate microorganism in non-dairy product. Ozone treatment technology is a simple and cost-effective option in comparison to conventional thermal technology method which uses high energy in heating and cooling treated food products. In addition, ozone is also expected to minimally affecting the quality and nutritional properties of non-dairy products. This study can be introduced and implemented in Small Medium Industries in Malaysia in Malaysia while increasing the income of SMEs providing liquid food products that are safe, healthy and convenient to be consumers at large.

### **1.6 Thesis structure**

This study are mainly consists of 5 chapters which comprises introductions for chapter 1, literature review for chapter 2, methodology for chapter 3, result and discussion for chapter 4 and conclusion and recommendation for chapter 5. Chapter 1 consist of overview of ozone, problems statements, objectives, scopes and significant of study, and thesis structure. Next, chapter 2 consist of literature review of rice milk and introduction of ozone. In addition, chapter 3 comprises the methodology of research design, preparation of brown rice milk, ozone treatment, microbial analysis physicochemical analysis of ozone-treated rice milk and statistical analysis .Next, chapter 4 consists of result and discussion of enumeration of aerobic plate count(APC) and yeast and mold count (YMC), statistical analysis and effect of ozone treatment on physicochemical analysis characteristics of ozone treated brown rice milk. Finally, for chapter 5 is the conclusion and recommendation that can be drawn from this study.

## **CHAPTER 2**

### **LITERATURE REVIEW**

#### **2.1 Rice milk**

Rice milk can be made from boiled rice, brown rice syrup and brown rice starch. Generally, rice milk will taste sweeter to cow's milk (Anonymous, 2018). Furthermore, majority of the rice milk varieties are sweet due to natural fermentation process when the carbohydrate compound in rice are broken down into sugars which is quite similar to amazake, traditionally sweet, low-alcohol Japanese drink that has has sweet taste (Robertson & Ross, 2000). Rice milk are quite foamy and its appearance are indistinguishable when compared to other type of milks that exist in the market (Sarika Raka,2017).

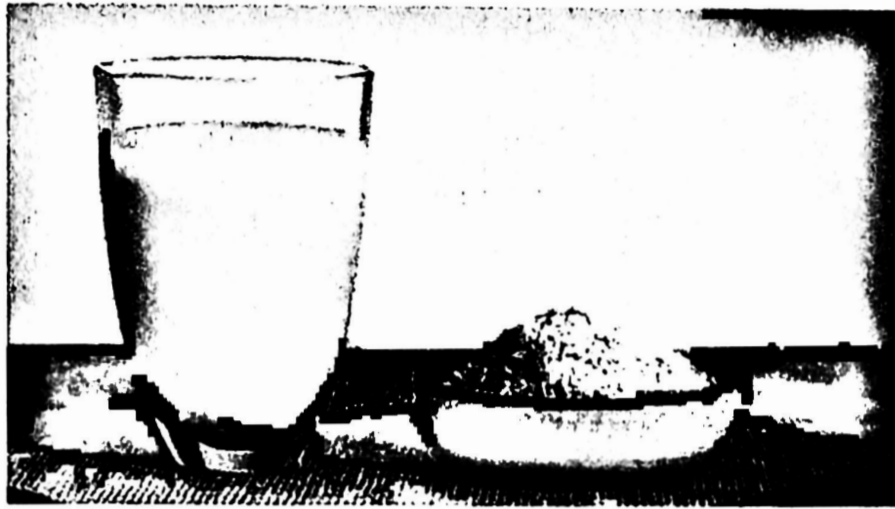


Figure 2.1 Rice milk (Source: Phoebe Jenkins,2015)

Rice milk is well known as lactose free milk which make it suitable for people with lactose intolerance (Lomer et al. 2008). Next, rice milk also suitable as an option for peoples that are allergic to other non-dairy milk sources such as soybean and almonds. (Gizzarelli et al. 2006; Roux et al. 2003). In addition, according a research firm, Mintel, plant-based dairy product has been experienced consistency in terms of growth in America for the past five years for about 61% and estimated to reach about \$2.11 billion in 2017 and up to \$38 billion by 2024. Almond milk (64% percent market), soymilk (13% market share) and coconut milk (12% market share) are the most popular choice by the American to replace cow's milk. Likewise, non-dairy milk product also experienced significant growth about 50% which is equivalent to 15% of dairy product in United States. In addition, rice milk is reported to experience compound annual growth rate (CAGR) for above 15% for the period of 2018 to 2023 which focus targeted to vegan and lactose-free population, with North America will remain as biggest market for rice milk, according to a report. In fact, with the price of \$4.35, one can get 1kg of rice which is the raw material for rice milk.

Next, rice milk was very easy to be prepared. Firstly, 2 cups of rice were soaked for 2 hours. Then, the rice will be blended by using blender. Finally, the rice milk will put in a bottle and refrigerated. Rice milk is suitable to be consume by everyone compare to soymilk or almond milk which has allergy to nuts (Anonymous,2018).



### 2.1.1 Nutritional Value of Rice Milk

Rice milk is found to contain the least amount of fat which is in contrast to other non-dairy milk. In addition, rice milk also supplies 30 percent of the daily value for bone- building calcium per 8-ounce serving (Anonymous, 2018c) Furthermore, it is found that rice milk contain only 1 gram of fat per cup and all of its fat are unsaturated. For those who are restricted to consume high fat and cholesterol food, rice milk is the best alternative (Anonymous, 2018d). Based on the data of nutritional profile of rice milk (Table 1), it is found that it is very rich in carbohydrates ranging between 23 and 27 g.

**Table 1 Nutritional profile of rice milk (Source: Vanga, 2017)**

Component	EAR	Mean	SD	Median	Min	Max
Carbohydrates(g)	130	25.28	1.7	26	23	27
Sugars		13.12	2.48	14	10	15.6
Fibers	35	0	0	0	0	0
Fats(g)	35	2.33	0.31	2.5	2	2.64
Saturated		0.16	0.22	0	0	0.48
MUFA		1.16	0.59	1.5	0.48	1.5
PUFA		0.83	0.75	0.5	0.3	1.68
Cholesterol		0	0	0	0	0
Proteins(g)	55	0.85	0.75	1	0	2
Minerals(mg)						
Calcium	1100	245.5	149.67	315	22	330
Iron	6.5	0.13	0.18	0.065	0	0.39
Phosphorus	600	63	38.19	63	36	90
Potassium	4700	50	-	50	50	50
Sodium	1500	72	22.53	65	45	100
Zinc	9.4	0.75	0.27	0.75	0.56	0.94
Vitamins						
Vitamin C(mg)	75	0	0	0	0	0

Thiamine(mg)	1	-	-	-	-	-
Riboflavin(mg)	1.1	0.3025	0.039	0.30	0.28	0.33
Niacin(mg)	11	-	-	-	-	-
Vitamin B6(mg)	1.2	-	-	-	-	-
Folate,DFE( $\mu$ g)	320	-	-	-	-	-
Vitamin B-12( $\mu$ g)	120	-	-	-	-	-
Vitamin A( $\mu$ g)	2	1	0	1	1	1
Vitamin E(mg)	12	3	-	3	3	3
Vitamin D( $\mu$ g)	10	2.09	1.48	2.5	0.45	3.33
Energy(kcal)		133	13.04	130	100	140



## **2.2 Introduction to ozone**

### **2.2.1 Physical and Chemical properties of Ozone**

In 1840, According to Manley and Niegowski (1967), Schoebein discovered and named a molecule called ozone. It consists of tri-atomic oxygen which was formed from addition of a free radical of oxygen with molecular oxygen. The three atoms of oxygen in the ozone molecule were arranged at obtuse angle. The ozone has boiling point of  $111.9 \pm 0.3$  °C, melting point was  $192.5 \pm 0.4$  °C, critical temperature was  $12.1$ °C and the critical pressure is 54.6 atm. Besides that, in room temperature, ozone existed as gaseous state and it was partially soluble in water. Next, ozone has pungent odour which similar to “fresh air after a thunderstorm”. The ozone exists as blue colour gas at ordinary temperature when generated from dried air, but will be colourless when generated from high- purity oxygen (Prabha et al,2015)

### **2.2.2 Generation of ozone**

Ozone is formed by high energy input that causes diatomic oxygen molecule to split into free radical oxygen. Then single oxygen molecule will react with another diatomic that will produce triatomic molecule. There are two ways to initiate free radical oxygen formation that is through ultraviolet radiation with wavelength of 188nm and corona discharge which generates ozone. Usually for commercial scale, corona discharge is used to generate commercial levels of ozone as illustrated in Figure 2.2 (Nath et al., 2014; Guzel—Seydim et al; 2004).

There are two electrodes that are used in corona discharge, high tension and low tension(ground) electrode, which is separated by a ceramic dielectric medium in a narrow discharge gap. When dry air or oxygen gas is fed through the gap between those electrodes, it will create a high voltage of alternating which will causes dissociation of molecular oxygen to atomic form. The collision between atomic oxygen with molecular oxygen will form ozone (Chawla et al.,2012). The concentration of ozone produced by corona discharge depend on several parameter such as voltage, current frequency, dielectric material, discharge gap and absolute pressure (Prabha et al., 2015; Nath et al., 2014; Chawla et al.,2012; Khadre et al., 2001&; Kim et al., 1999).

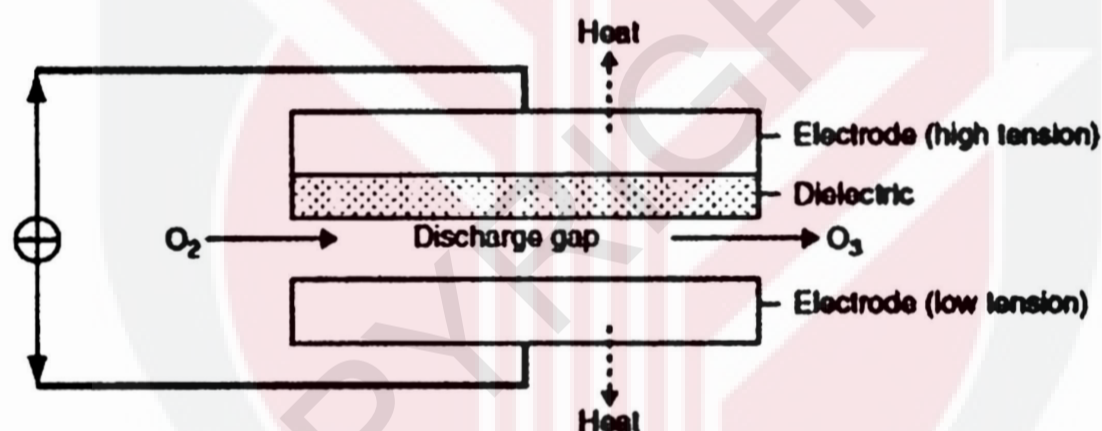


Figure 2.2 Schematics diagrams of ozone generation by corona discharge method (Guzel-Seydim et al., 2004)

Ozone is well known for its pungent bluish gas with strong oxidizing properties. Ozone readily degrades but has longer half-life when it exists in the gaseous states compared to aqueous solution (Prabha et al.,2015). However, ozone is unstable and will decomposes in very short period of time and due to this circumstance, to ensure continuous generation of ozone, ultraviolet radiation or corona discharge method are used. As a result of ozone, when it decomposes, it will produce oxygen, and as a consequence, food products that are treated with ozone are free of disinfectant residue (Prabha et al.,2015; Guzel-Seydim et al.,2004). However, decomposition of ozone is so fast in water phase it is still sufficient

to inactivate bacteria, molds, yeast, parasites and viruses (Kim et al., 1999)



### **2.2.3 Antimicrobial Effect Mechanisms and Effect of Ozone**

The use of ozone as an antimicrobial agent in food processing was reviewed by various researchers (Khadre et al.,2001; Kim et al., 2009; Xu,1999). Its efficiency against both Gram positive and Gram negative bacterias, viruses, protozoas and bacterial spores has been reported throughout the years (Cullen et al.,2009; Khadre et al.,2001; Restaino et al., 1995). The microbial inactivation mechanism using ozone is mainly due to irreversible damage to fatty acids in cell membrane and to cellular macromolecules like proteins and DNA (Prabha et al.,2015). Scott and Leshner (1963) shows that ozone reacts with unsaturated lipids of *E.coli* cell membrane, which causes leakage to the cell content and it will die. Other researcher revealed that treatment of viruses with ozone will cause damage to the capsid protein will be destroy, the integrity of the lipid enveloped will be compromised subsequently causes damages and damage to RNA and DNA (Kim et al., 1980).

United States Food and Drug Administration (USFDA) (2001) has approved the usage of ozone as directive additive to food, and for that reason many researchers suggested to use ozone as an alternative to thermal pasteurization of fruit juices. Fruit juices processors in USA are required to meet the Food and Drug Administration mandatory 5-log reduction of the most resistant pathogen in their finished products (Cullen et al., 2009). Steenstrup and Floros (2004) in their study revealed that a rapid inactivation of *E.coli* O157:H7 in apple cider and orange juice when treated with 0.9 g/h ozone under mild heating of 50 °C (William et al.,) Besides that, several studies have been carried out to investigate the inactivation

kinetics of microorganisms using ozone. Menzel (1971) and Kim (2003) stated that ozonated water has higher oxidizing potential than oxidizing agent that normally used to kill spores. A study by Farooq et al. (1977), found that the initial population of organism has great influence on degree of inactivation. Farooq et al. (1977) use *Candida parapsilosis* as its organism to be test its inactivation by using ozone. The results showed that when the initial density of *Candida parapsilosis* was  $1.4 \times 10^5$  colony forming unit (CFU)/ml, a 4-log reduction was noticed. But when the initial density was  $1.6 \times 10^7$ , no inactivation was detected. Zorlugenc et al. (2008) in a study shows that yeast was destroyed when the treatment time were 15 minutes while gaseous ozone with concentration of 13.8 mg/L was found not enough at 15 minutes.

Figure 2.2.3: Microbial inactivation in food products by ozone (Prabha et al.,2015)

Product	Treatment	Findings	Reference
Orange and Lemon	Gas ozone (0.3ppm) for 4 weeks	Spore was reduced of <i>P. Italicum</i>	Palou <i>et al.</i> , 2001
Peach	Ozone atmosphere storage (0.3ppm, 5°C) for 4 weeks	Aerial mycelial growth and sporulation were inhibited	Palou <i>et al.</i> , 2002
Strawberry	Storage for 3 days at 2°C with 1.5ppm ozone	Mycelial growth developed more slowly	Nadas <i>et al.</i> , 2003
Apple	Ozone bubbling and dipping in pre-ozonated water, 3min	Decrease in counts of <i>E coli</i> (<1 log CFU)	Achen <i>et al.</i> , 2001
Citrus	Basket immersed in ozonated water at 1.5-10ppm, stored for 7-21 days at 10° C and 20° C	5 ppm for 5 min reduced aerobic bacteria population	Smilanick <i>et al.</i> , 2002
Celery	Dipping to (0.03,0.08,0.18 ppm) ozonated water for 5 min and storing at 4°C for 9 days	Populations of total bacteria reached to 5.72, 5.64 and 5.63 from 5.08 log CFU/g	Zhang <i>et al.</i> , 2005
Lettuce	Soaking in ozonated water with 5 ppm ozone	Reduced aerobic bacteria and yeast within 10 min	Koseki <i>et al.</i> , 2001
Orange juice	Ozone gas pumped into juice	<i>E. coli</i> was reduced to 5 log cycle, Ascorbic acid decreased	Angelino <i>et al.</i> , 2003, Williams <i>et al.</i> , 2004
Cheese	Gaseous ozone at 3 different levels	Reduction of fungal counts on ripening room and on cheese surface	Serra <i>et al.</i> , 2003; Pinto <i>et al.</i> , 2007
Orange and lemon	Ozone gas (0.1-2g/m <sup>3</sup> ) at 4.5-10° C	Decrease of <i>P. digitatum</i> and its sporulation	Palou <i>et al.</i> , 2001
Barley	Gaseous ozone	Reduction mycelia grow and spores germinates	Allen <i>et al.</i> , 2003

#### **2.2.4 Factors Affecting the Efficiency of Ozone**

But there are several factors that affects the effectiveness of ozone to against microorganisms like flow rate of ozone, medium pH, temperature humidity, ozone concentration and the amount of organic matter surrounding the cells.

The first factor was flow rate of ozone. The flow rate of ozone will affect the size of bubbles produced which will have direct effect on ozone solubilisation rate and disinfection efficiency. This can be proved from a study by Ahmad and Farooq (1985) that showed that the ozone mass transfer and disinfection efficiency increased when the bubble size decreased while the other factors were constant. Ogden (1970) stated that this might be caused by high interfacial area available for mass transfer at smaller bubble size. Next, the ozone concentration present or available was another factor that can be used to determine the efficiency of ozonation. The higher the ozone concentration will caused saturation to happened and further ozonation will caused ineffectiveness to achieve same log-reduction values.

The next factor was the pH of the medium. This factor correlates to ozone inactivation as ozone has decomposition rate changes significantly with changes in pH (Farooq et al., 1977b; Roy et al., 1980). This can be proved from a study by Patil et al. (2010a) that showed that *E.coli* were able to achieve 5- log reduction number at lower pH. In addition, amount of organic matter surrounding the cells also has significant effect on ozone demand. The dissolved organic matters will caused reduction in disinfection activity which will reduced the concentration of available active species needed to react with the microorganisms. This can be observed from experiments conducted by Williams et al. (2005) on inactivation of

*E.coli* in orange juices and he observed that when ascorbic acid and others organic matters were present, the efficiency will reduced as the bacteria will react with the organic matter .Besides that, the temperature also has its own role in ozonation process. Rice (1986) reported that the ozone will be more soluble in in colder water and 13 times more soluble in water when compared oxygen at temperature 0-30°C. Besides than solubility, the temperatures of the medium also influenced the ability of the ozone to inactivate microorganism. This can be proved from a study from Farooq et al. (1977) that reported inactivation ability of ozone were under control with decreased in temperature.

## **Chapter 3**

### **METHODOLOGY**

#### **3.1 Research Design**

Research design for to determine the inactivation kinetics of aerobic plate count (APC) and yeast and mold count (YMC) on ozone-treated rice milk beverage was summarized in Figure 3.1 below. There were four parts in carrying out the experiments. The first part was the physicochemical analysis of brown rice milk before and after treatment using ozone. Next part was microbial analysis which include before and after the rice milk was treated with ozone.

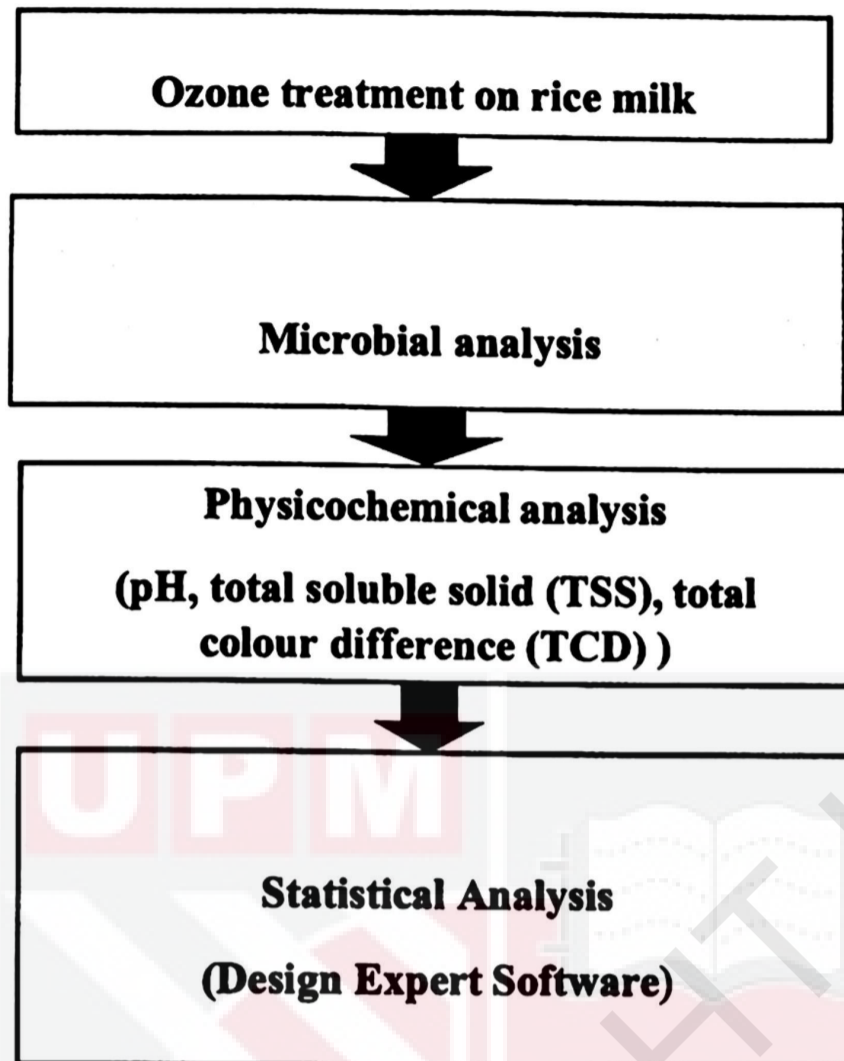


Figure 3.1 Research Design

### 3.2 Preparation of Brown Rice Milk

Firstly, the brown rice with ratio of 1 kilogram to 1 litre of water was soaked in the water for 2 hours. Then, the brown rice was blended until the rice grains were broken completely. After that, the blended brown rice was sieved by using cheese cloth. The next step was keeping the rice milk in a deep freezer at  $-20^{\circ}\text{C}$ . Prior to the experiment, the rice milk was thawed in a chiller for 24 hours at  $4^{\circ}\text{C}$  before being use.

### **3.3 Ozone treatment**

Rice milk was treated with gaseous ozone in a 200 ml beaker by ozone generator (Model GL-3189, China). Ozone output in this experiment was 400 mg/h. The delivery tube was then connected to ozone generator. Ozone gas was pumped into the rice milk for 0, 10, 20 and 30 minutes. The experiment was conducted in the fume board to prevent excessive exposure of ozone gas. After every test, the ozone generator was left to rest for 3 to 5 minutes.



Figure 3.3 Experimental set up for ozone treatment

### **3.4 Microbial analysis**

#### **3.4.1 Medium Preparation**

Platecount agar was prepared by adding 17.5g with 1L of distilled water. Then, it will boil with frequent stirring and sterilised in an autoclave at 121°C for 15 minutes. Meanwhile, to prepare malt extract agar, 33.5 g of the powder were suspended in 1 litre of distilled water and was boiled to dissolve it. Finally, the suspension will autoclaved at 115 °C for 15 minutes

### **3.4.2 Microbial Enumeration**

Two hundred millilitres of rice milk which was treated for treatment time 0 min was homogenized for 30 seconds in 90mL of 0.1% peptone Water. Then, further dilutions of the homogenate were made in 0.1% peptone water. After that, 0.1ml amounts of the  $10^{-1}$  and higher dilutions was inoculated on to the surface of the medium plate count agar and malt extract agar.. After that, the petri dish was incubated at 37°C for 24 hours. Then, the process was repeated by using ozone treatment time 0,10,,20, and 30 minutes and the log count were done by calculating the white spots appears at the surfaces of the agar and the samples were triplicated.

### 3.5 Physicochemical Analysis of Ozone-Treated Rice Milk

#### 3.5.1 pH

The pH of rice milk was determined by using pH meter (SevenMulti pH Conductivity Meter, Mettler Toledo, Switzerland) and it was calibrated with standard buffer solution of pH 4 and 7. Rice milk then was prepared in a beaker an electrode was submerged directly in sample solution until a steady reading is reached.(Anonmyous,2018)



Figure 3.5.1 Measurement of pH using digital pH mete

#### 3.5.2 Measurement of Color

International Commission on Illumination color parameters  $L^*$  (0-100, Black-Lightness),  $a^*$  (positive values – red, negative values- green and 0 is neutral) and  $b^*$  (positive values – yellow, negative values- blue and 0 is neutral) was used to measured the color of rice milk. Hunterlab Ultra Scan Pro(D65,Hunterlab Assoc Lab Inc, Reston,VA,USA) was used for color determination. Results were expressed as the mean of three measurements. The overall color difference ( $\Delta E^*$ ) can be calculated using the following equation:

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

### 3.5.3 Total soluble solid

A drop of ozonated rice milk with treatment time of 0,10,20 and 30 minutes are dropped into digital handheld refractometer. Each of treatment time reading are triplicated. The readings are expressed as degree Brix.



Figure 3.5.3 Experimental set up for TSS test.

### 3.6 Statistical Analysis

In this study, experiment design from Design Expert Software Version 11 (Stat-Ease Inc, MN,USA) was used to perform statistical analysis. General Full Factorial design was used and all experiments in combinations of factors are triplicate. One factor design was used to study the effect treatment time of ozone was used to investigate the influence on the physicochemical properties of ozone treated rice milk. The analysis of the results was performed using ANOVA analysis.

## CHAPTER 4

### RESULTS AND DISCUSSION

#### 4.1 Enumeration of Aerobic Plate Count (APC)

To study the effect of treatment times of ozone and the concentration of bacteria during treatment time, graph in Figure 12 between the logarithm aerobic plate count and ozone treatment time was plotted. A linear correlation between the logarithm of aerobic plate count concentration (N) and ozone treatment time. Under the experimental condition of this study, inactivation followed a first order kinetic law with respect as follow:

$$\frac{dN}{dt} = -k_1t$$

Or integrating

$$\text{Log } N_0 = \text{Log } N - k_1t$$

Where;

$K_1$ =First order inactivation  
constant  $N_0$ =Initial number  
of bacteria

From the graph, it can be observed that when the ozone treatment time is 10 minutes, the bacteria experienced 0.3- log reduction number. As the ozone treatment time increase to 20 minutes, the bacteria experienced 5- log reduction number and when the ozone treatment increase to 30 minutes, the bacteria experienced 5.3 log-reduction number. The value of  $k$  for this experiment is 0.1905 which is the gradient of the slope and the value of  $R^2 = 0.8285$ . This indicated a reasonable agreement between the experimental and predicted values.



**Table 2 Average aerobic plate count and ozone treatment times**

Ozone treatment times	Total count (log cfu/ml)			Average	Standard deviation
	10 <sup>-1</sup>	10 <sup>-2</sup>	10 <sup>-3</sup>		
0	1600000	15500000	15300000	15600000	360555.13
10	8700000	8200000	8000000	8300000	360555.13
20	1000	1000	1000	1000	0.00
30	1000	900	800	900	100.00

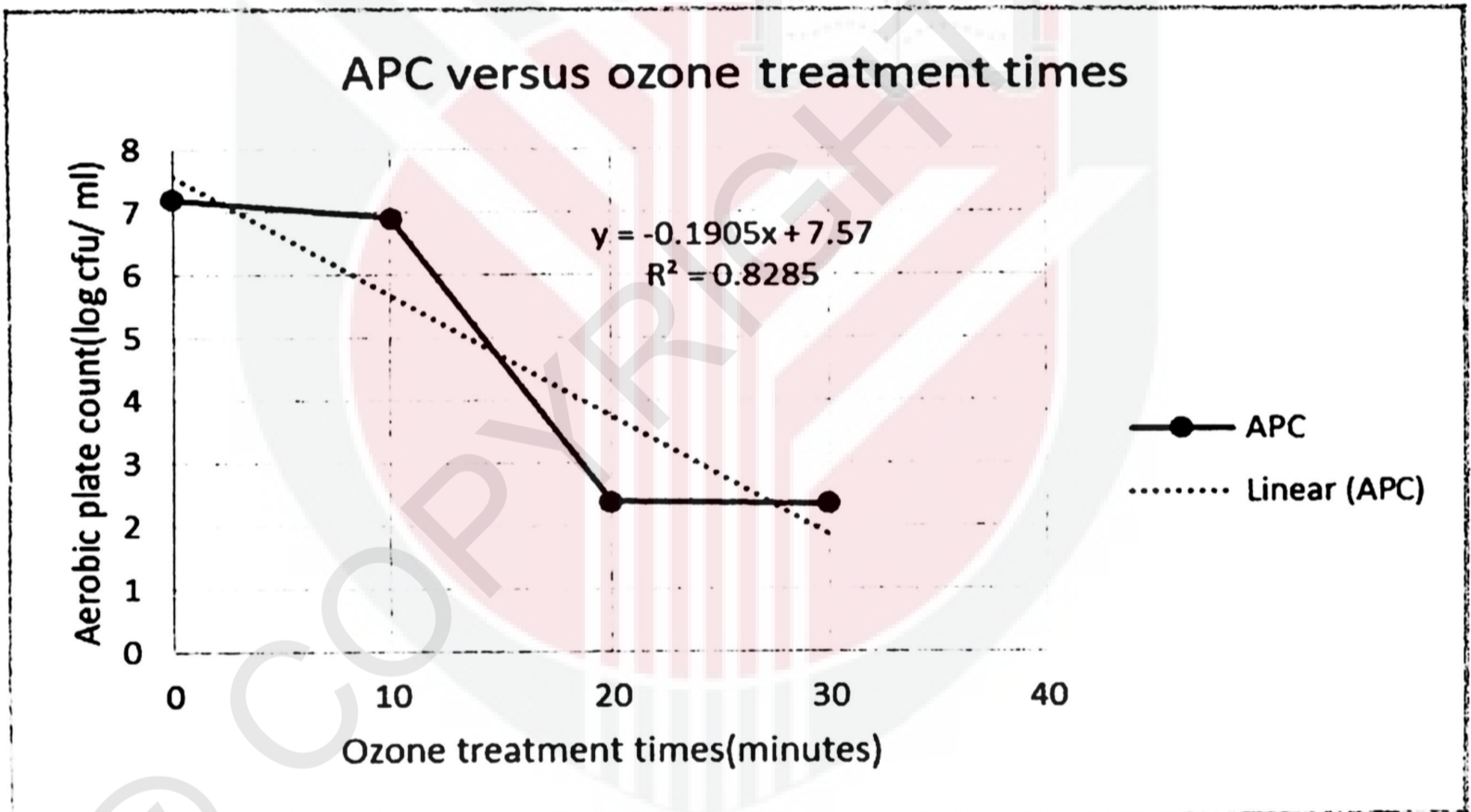
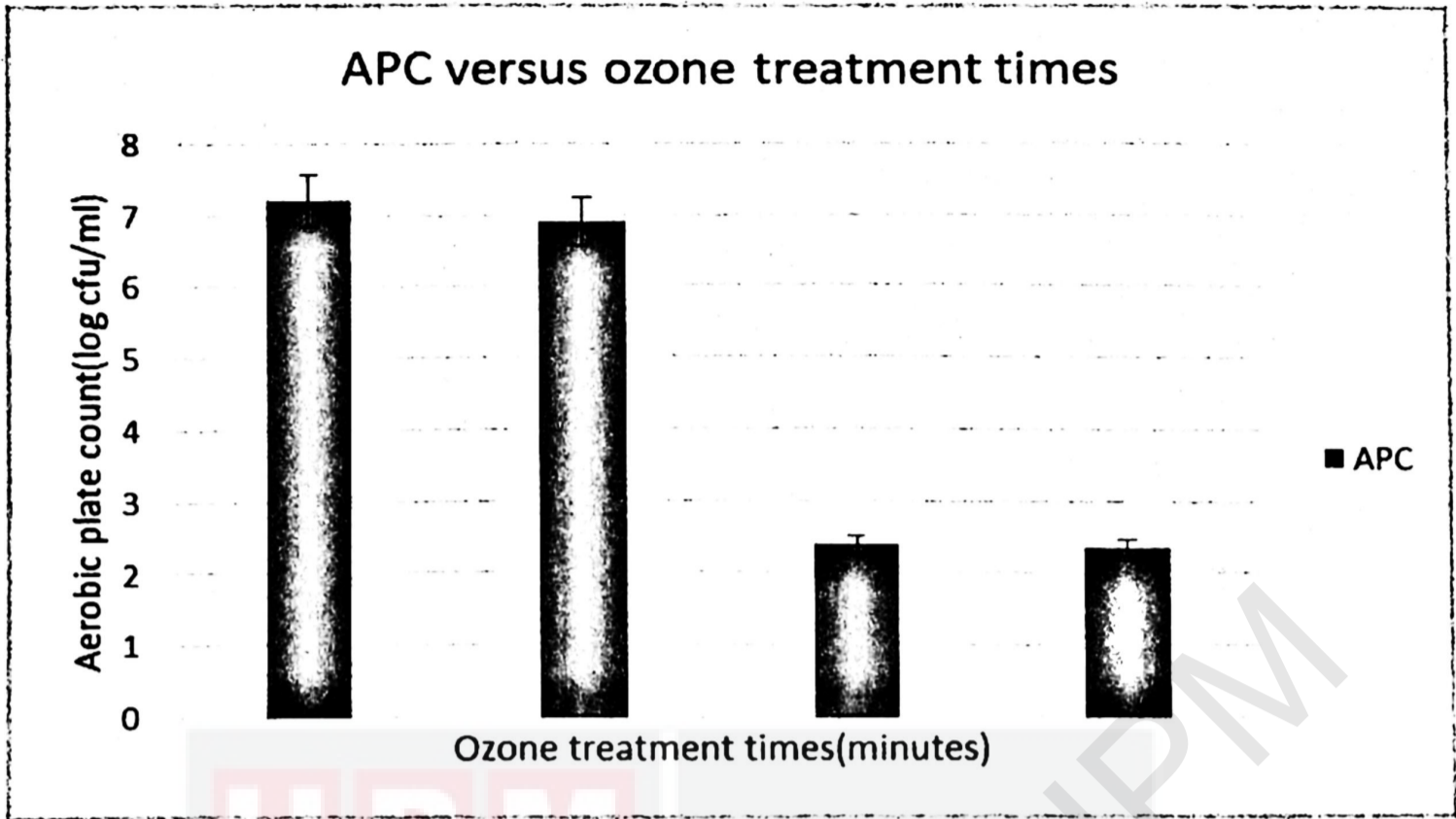


Figure 4.1 Aerobic plate count (APC) versus ozone treatment count

#### 4.2 Enumeration of Yeast and Mold Count(YMC)

To study the effect of treatment times of ozone and the concentration of bacteria during treatment time, graph in Figure 12 between the logarithm aerobic plate count and ozone treatment time was plotted. A lineal correlation between the logarithm of aerobic plate count concentration (N) and ozone treatment time. Under the experimental condition of this study, inactivation followed a first order kinetic law with respect as follows:

$$\frac{dN}{dt} = -k_1t$$

Or integrating

$$\text{Log } N_0 = \text{Log } N - k_1t$$

Where;

$K_1$ =First order inactivation constant

$N_0$ =Initial microbial concentration

From the graph, it can be observed that when the ozone treatment time is 10 minutes, the bacteria experienced 0.3-log reduction number. As the ozone treatment time increase to 20 minutes, the bacteria experienced 1.64-log reduction number and when the ozone treatment increase to 30 minutes, the bacteria experienced 5 log-reduction number. The value of k for this experiment is 0.1634 which is the gradient of the slope and the value of  $R^2=0.8482$ . These results indicated a reasonable agreement between the experimental and predicted values.

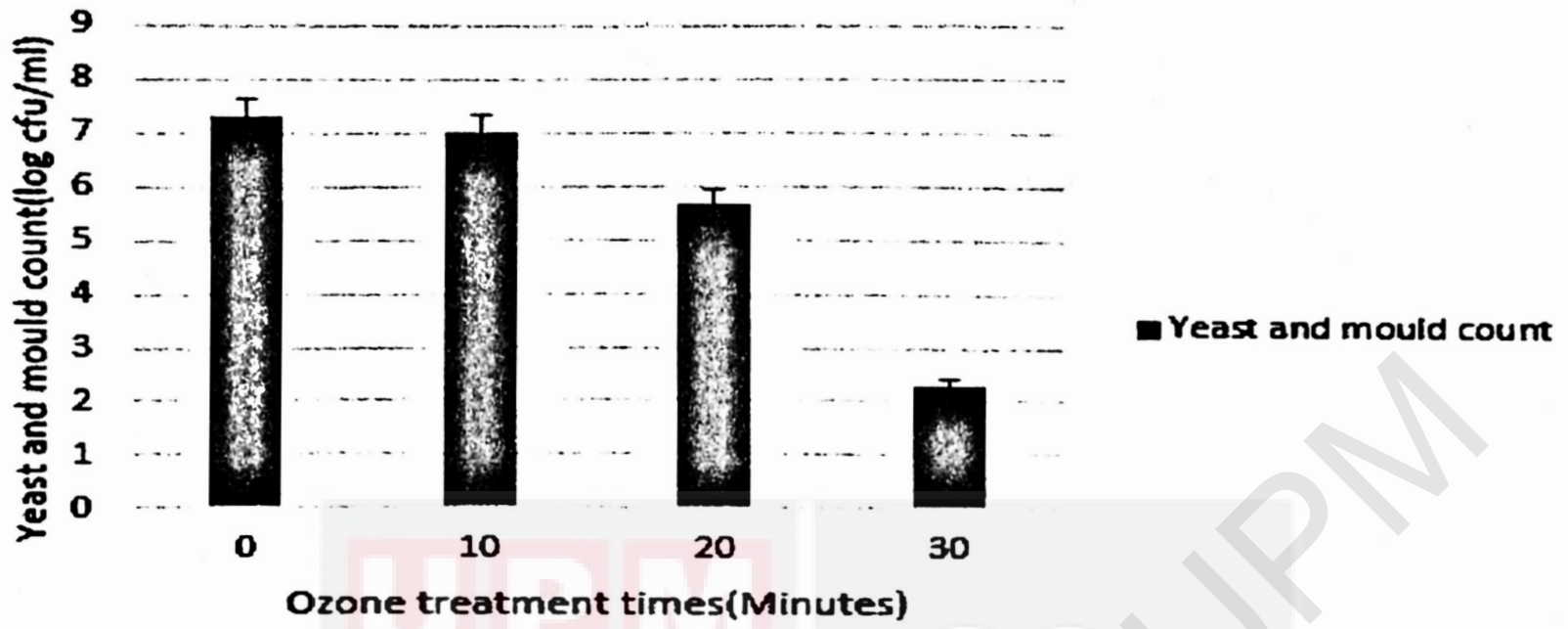
Based on both tests, it is found that ozone managed to inactivate bacteria faster

compared to yeast and mold. This, due to the fact, that yeast and mold were more resistant compare to vegetative bacteria (USEPA., 1999; Sobsey., 1989; Zoutman et al.,2011). Furthermore, yeast and mold has thicker coat that acts as barrier to ozone (Foegeding,1985), so, yeast and mold were more resistant than bacteria.

**Table 3 Yeast and mold plate count versus ozone treatment times**

Ozone treatment times	Total count (log cfu/ml)			Average	Standard deviation
	10 <sup>-1</sup>	10 <sup>-2</sup>	10 <sup>-3</sup>		
0	20000000	18000000	17800000	15600000	1216552.51
10	10000000	9800000	9000000	9600000	529150.26
20	4000	4300	4000	4100	173.20
30	2500	2400	2200	2367	152.80

### Yeast and mould versus ozone treatment time



### Yeast and mould count versus ozone treatment times

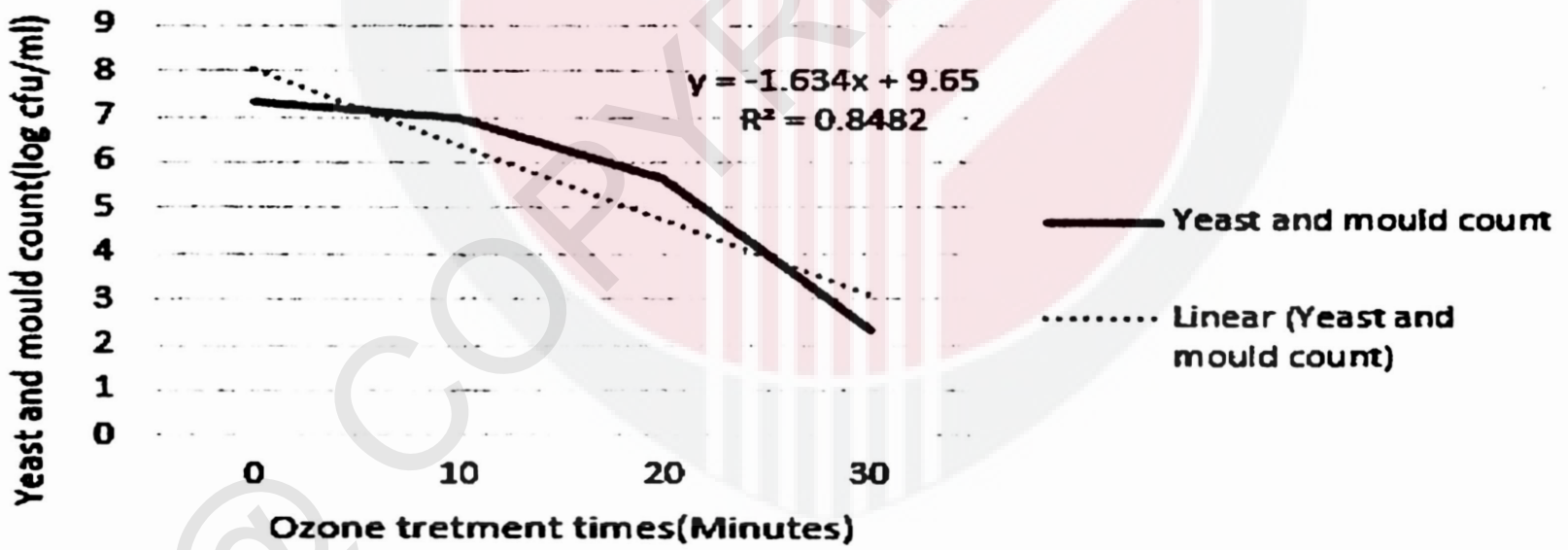


Figure 4.2 Yeast and mould count versus ozone treatment times

### 4.3 Effect of Ozone Treatment on Physicochemical Characteristics of Rice Milk

#### 4.3.1 Statistical analysis

General factorial experimental design was used to study the effect of ozonation on physicochemical properties of rice milk. Table 3 show General Factorial experimental design for this study with all dependent variable like pH, total colour differences and total soluble solid.

Based on the analysis by using ANOVA in Figure 12, the value of  $R^2$  for each response for pH, TSS and TCD are 0.8909, 0.6 and 0.9981. It showed that only total soluble solid was insignificant while pH and total colour difference were significant.

Next, the lack of fit was insignificant since F-values were greater than 0.1.

**Table 4 General factorial design for ozone treatment of rice milk**

Block	Run	Factor: Treatment time	Response 1: pH	Response 2:TSS	Response 3:TCD
1	1	0	4.1	1.0	
1	2	10	4.12	1.0	30.41
1	3	20	4.13	1.0	45.51
1	4	30	4.13	1.0	58.5
2	5	0	4.1	1.0	
2	6	10	4.12	1.1	30.41
2	7	20	4.13	1.1	45.51
2	8	30	4.13	1.1	58.5
3	9	0	4.10	1.0	
3	10	10	4.12	1.2	30.41
3	11	20	4.13	1.1	45.51
3	12	30	4.13	0.9	58.5
p- value			0.8909	0.6	0.0276

F-value			6.36x10 <sup>7</sup>	18.00	531.69
R <sup>2</sup>			0.8909	0.6	0.9981
SD			0.00	0.032	0.86

p-value<0.05 is significant

Lack of fit insignificant at F-value>0.10

Full factorial experimental design was used to study the effect of ozonation towards TPC and YMC. Table showed General Factorial experimental design for this study. Based on the analysis by using ANOVA in Figure 12, the value of R<sup>2</sup> for each response for TPC and YMC are 0.8282 and 0.8282. Next, the lack of fit was insignificant since F-values were greater than 0.1.

**Table 5 Full factorial result for APC and YMC**

Block	Run	Factor: Treatment time	Response: APC(cfu/ml)	Response: YMC(cfu/ml)
1	1	0	2000000	20000000
1	2	10	870000	10000000
1	3	20	1000	4000
1	4	30	1000	2500
2	5	0	1700000	18000000
2	6	10	820000	9800000
2	7	20	1000	4300
2	8	30	900	2400
3	9	0	1650000	17800000
3	10	10	800000	9000000
3	11	20	1000	4000
3	12	30	2400	2200
p-value			<0.001	<0.001
F-value			1657.54	1757.10
R <sup>2</sup>			0.8282	0.8484

SD			17620	18739
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**p-value<0.05 is significant    Lack of fit insignificant at F-value>0.10**



#### 4.3.2 pH

pH is a quantitative measure used to specify the acidity or basicity of an aqueous solution. pH value depends on the concentration of free hydrogen ions,  $H^+$  which is dissociated from carboxyl group, COOH. A solution with pH less than 7 is considered acidic and a solution with a pH greater than 7 is considered basic, or alkaline. In this study, the pH value control sample is 4.11 and the pH value of ozonated rice milk is ranged from 4.12 to 4.13.

The coefficient of determination ( $R^2$ ) of the model is 0.8909. This indicated a reasonable agreement between the experimental and predicted values. ANOVA for pH determination showed that the independent variable (treatment time) were not significant ( $p > 0.05$ ) in determination of pH of rice milk. Figure 15 showed that insignificant differences of pH were detected during difference treatment time. Similar results also been recorded by Tiwari et al. (2009a) during ozonation of strawberry juice, blackberry juice (Tiwari et al., 2009b), grape juice (Tiwari et al., 2009c) and orange juice (Tiwari et al., 2009) where no significant change of pH over ozone treatment time were observed. But, in contrast, skimmed milk that were exposed to sterilization, a type of heat treatment processing, shows significant effect ( $p < 0.05$ ) in determination of pH (Israr ul Haq et al., 2013). Therefore ozone is better than pasteurization to maintain the pH low which is very important to prevent the microbial growth in liquid product.

**Table 6 Readings of pH versus ozone treatment times**

No. of trials	Ozone treatment times(Minutes)			
	10	20	30	40
1 <sup>st</sup> trial	4.11	4.12	4.13	4.14
2 <sup>nd</sup> trial	4.13	4.12	4.14	4.13
3 <sup>rd</sup> trial	4.12	4.13	4.13	4.12
Mean	4.11	4.12	4.13	4.13
Standard deviation	0.01	0.005	0.005	0.01

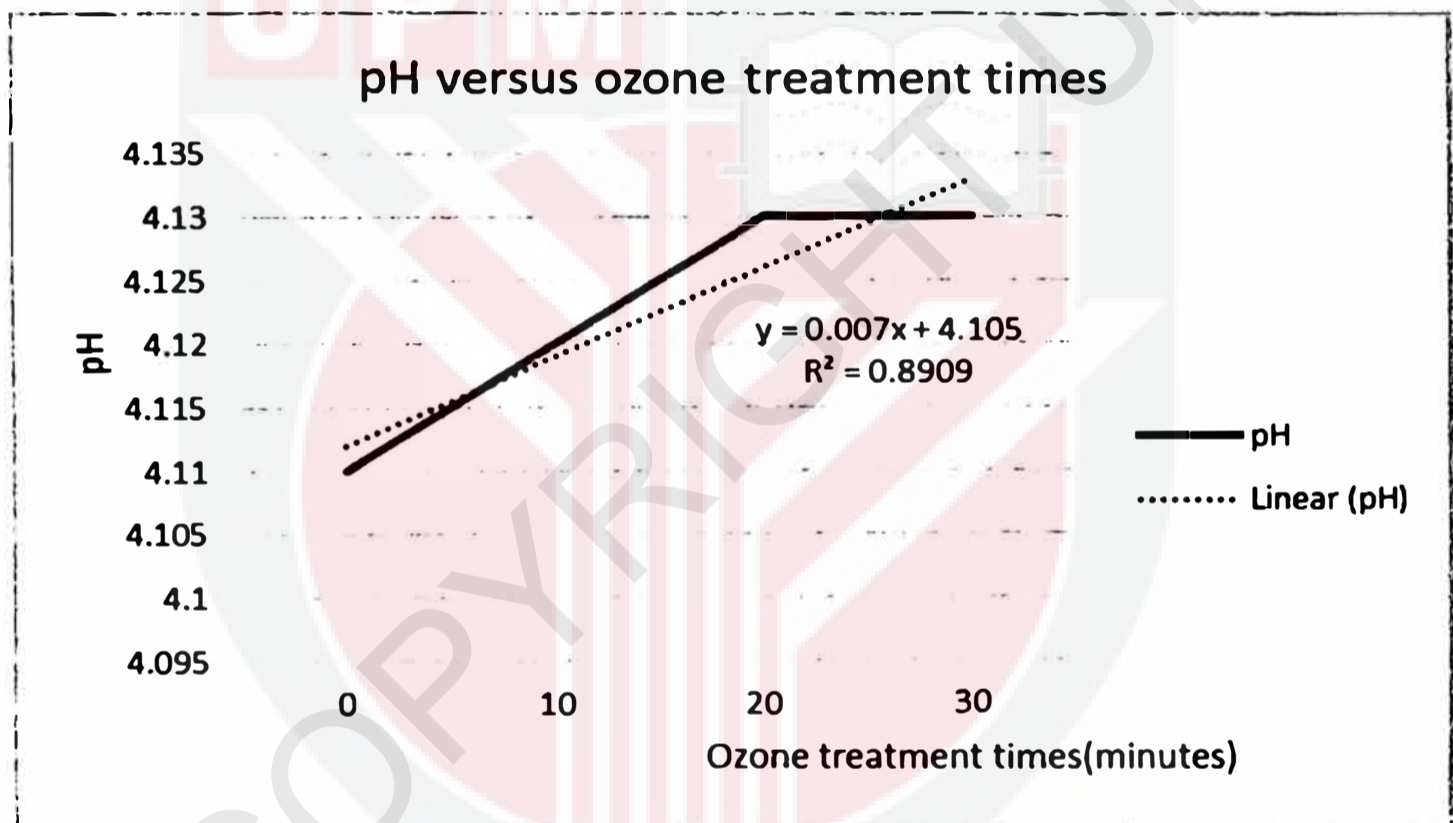
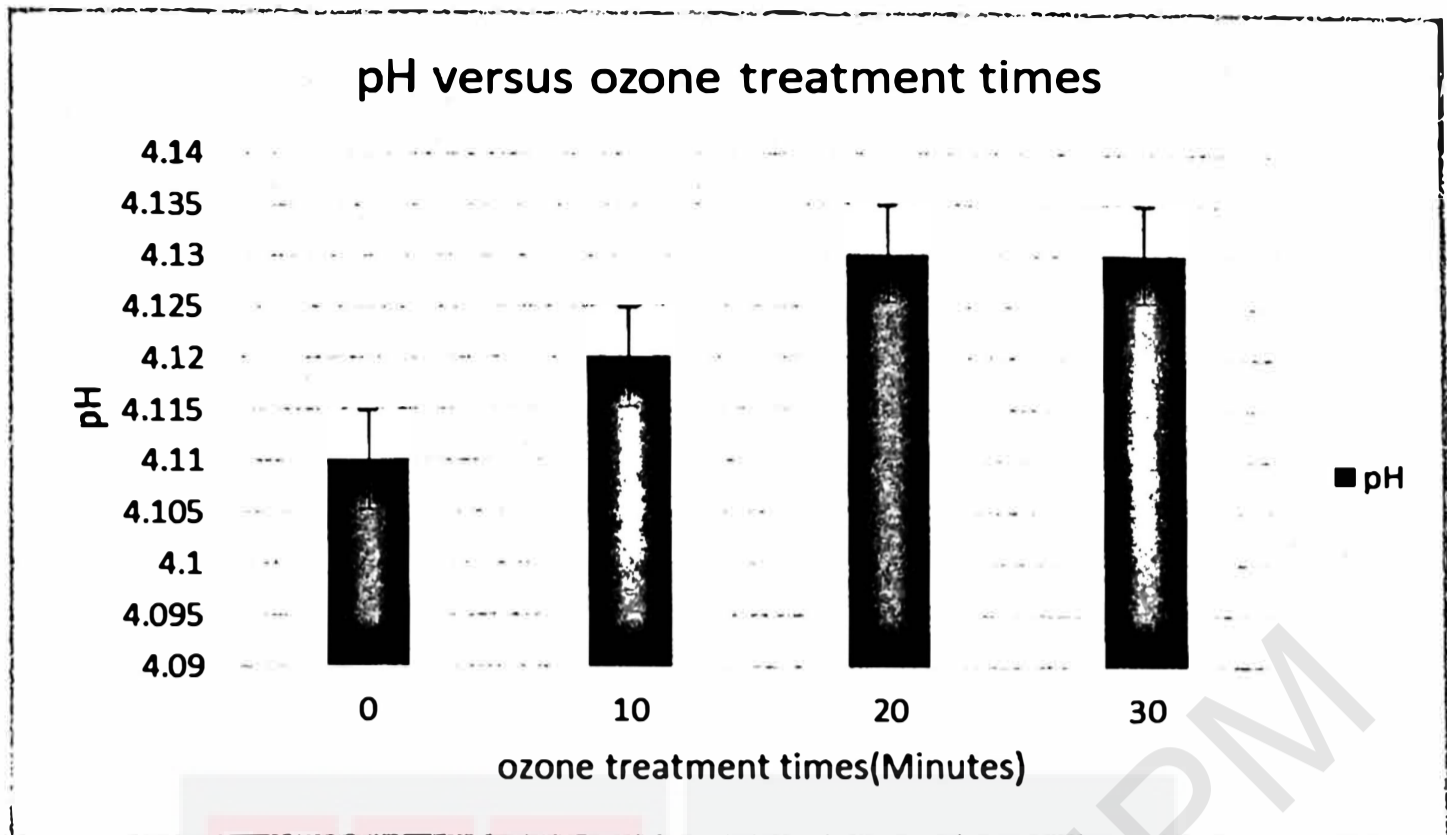


Figure 4.3.2 pH of rice milk versus ozone treatment time (Minutes)

#### **4.3.3 Total Soluble Solid**

Total soluble solid (TSS) is used to determine the solid concentration of a sucrose containing solution. The unit for this quantitative measurement is °Brix. It usually being measured by a device called digital handheld refractometer.

The coefficient of determination ( $R^2$ ) of the model is 0.6. This indicated a worthwhile agreement between the experimental and predicted values. An ANOVA for TSS determination shows that independent variables (treatment time) has no significant ( $p > 0.05$ ) effect on total soluble solid content in rice milk sample. This observation can be supported by a result from an experiment conducted by Zhang et al. (2005) that found that no significant decrease in total soluble solid were found when celery was treated with water that contain ozone. As a comparison with thermal pasteurization, Shikha et al. (2014) reported that low calorie milks that were exposed to pasteurization with temperature 75°C for 15 seconds and 116°C for 15 minutes showed significant differences of total soluble which assume due to hydrolysis of the component of the milk. Hence, ozone has shown to have a positive impact toward retaining the sugar concentration inside food product compared to pasteurization as it does not have any significant effect towards total soluble solid.

**Table 7 Total soluble solid versus ozone treatment times**

No. trials(TSS) (°Brix)	Ozone treatment times(Minutes)			
	0	10	20	30
1 <sup>st</sup> trial	1.2	1.0	1.1	0.9
2 <sup>nd</sup> trial	1.1	1.0	0.9	1.1
3 <sup>rd</sup> trial	1.1	1.0	1.0	1.1
Mean	1.1	1.0	1.0	1.0
Standard deviation	0.05	0.05	0.1	0.05

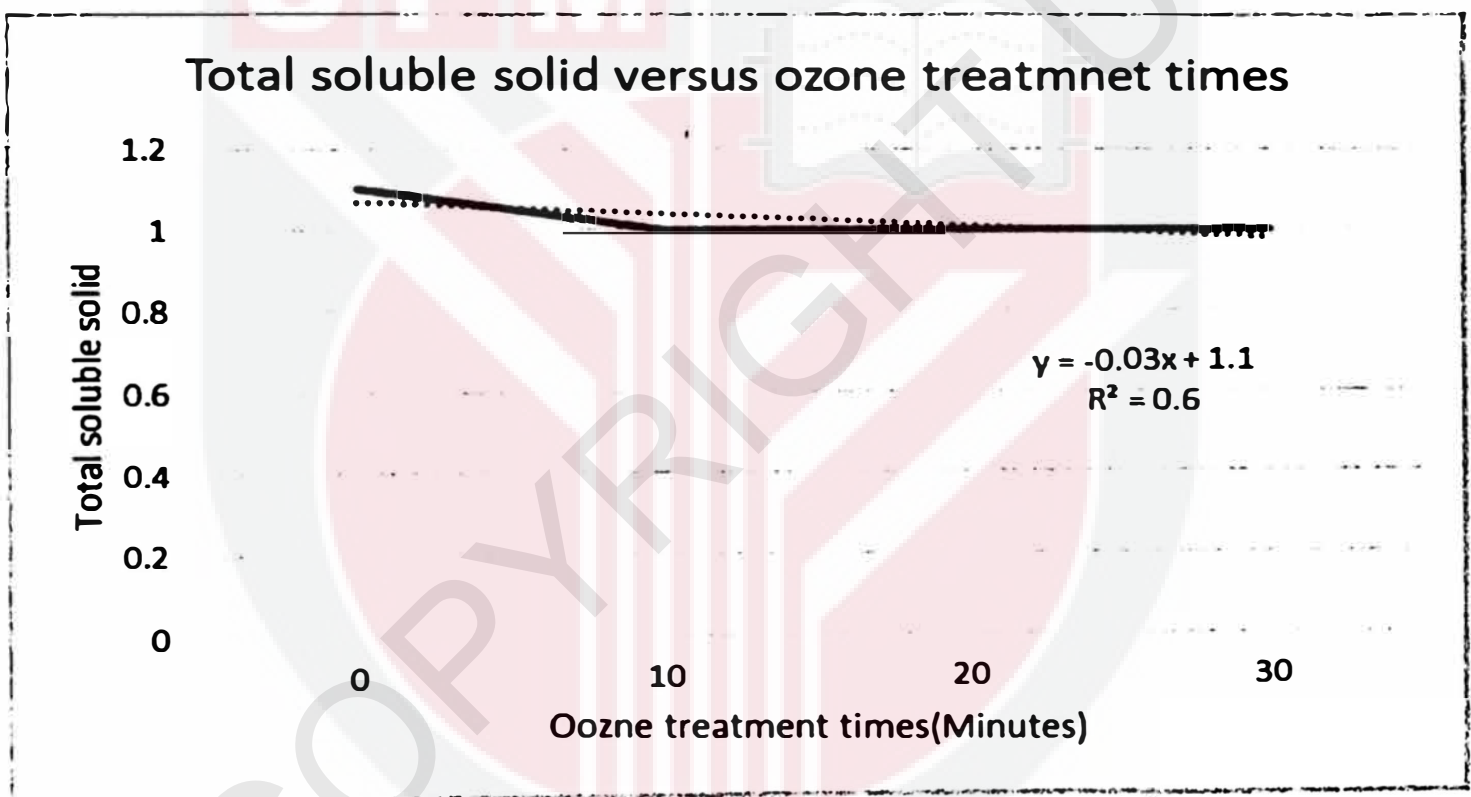
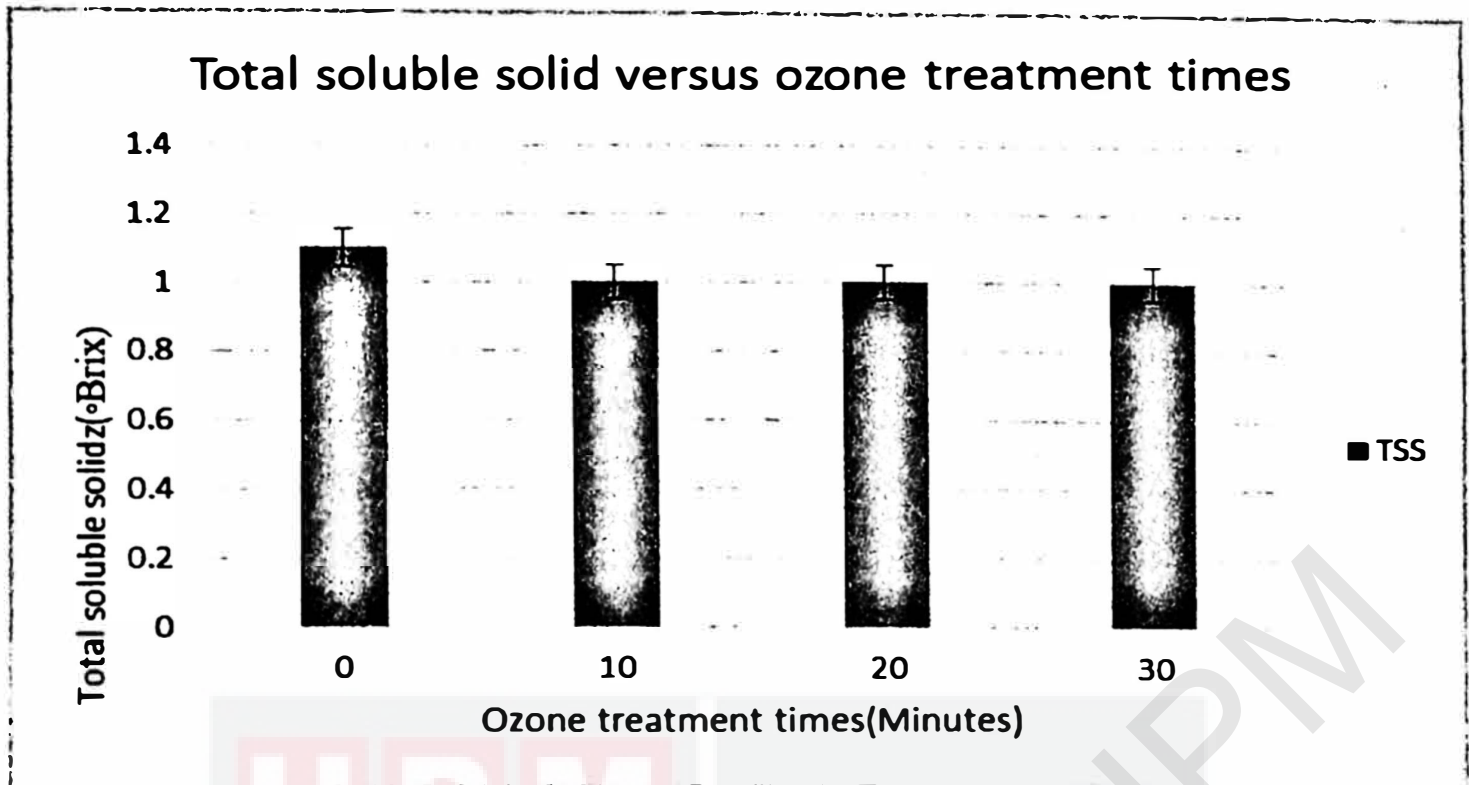


Figure 4.4.2 Total soluble solid versus treatment times

#### **4.3.4 Total Colour Difference (TCD)**

Total colour difference can be defined as the magnitude of change of colour after treatment. According to Tiwari et al. (2009b), the differences in colour perceivable can be categorized as very distinct ( $TCD > 3$ ), distinct ( $1.5 < TCD < 3$ ), and small differences ( $TCD < 1.5$ ). Table 4.2 showed that the range of TCD values from 31.4 to 58.5 over ozone treatment time. Hence, it can be concluded that very distinct colour rice milk can be observed during ozonation which gave us hint that major changes in appearances of rice milk has occurred

The coefficient of determination ( $R^2$ ) of the model is 0.9981. This indicated strong agreement between the experimental and predicted values. In this study, TCD were greatly influenced by the treatment time ( $p < 0.005$ ) as shown in Table 4.2. We can observe that significant increase in TCD values for every increase in treatment time which is shown by Figure 4.4.3. Similar result also obtained by Tiwari et al. (2009b) for ozonation apple juice, strawberry juice (Tiwari et al., 2009a) and orange juice (Tiwari et al., 2008) which gave indication that degradation of colour had occurred over periods of ozonation time. However, this finding was differed from study conducted by Bath et al. (1995) that showed total colour difference was insignificant to ozonation of blackberries which were ozonated and stored for 12 days. The significant effect of ozonation towards colour due to its high oxidation potential (2.07V) which caused disintegration of organics compound when organics dye experienced loss of colour because of oxidative cleavage of chromophore (Nebel, 1975) where ozonation attacked on conjugated double

bonds which were responsible for peach juice colour (Melendez-Martinez et al., 2007).



**Table 8 Total colour differences versus ozone treatment times**

TCD	Ozone treatment times (minutes)			
	0	10	20	30
1st trial	-	30.42	45.49	58.6
2nd trial	-	30.41	45.51	58.4
3rd trial	-	30.43	45.50	58.5
Mean	-	30.42	45.50	58.5
Standard deviation	-	0.1	0.1	0.1

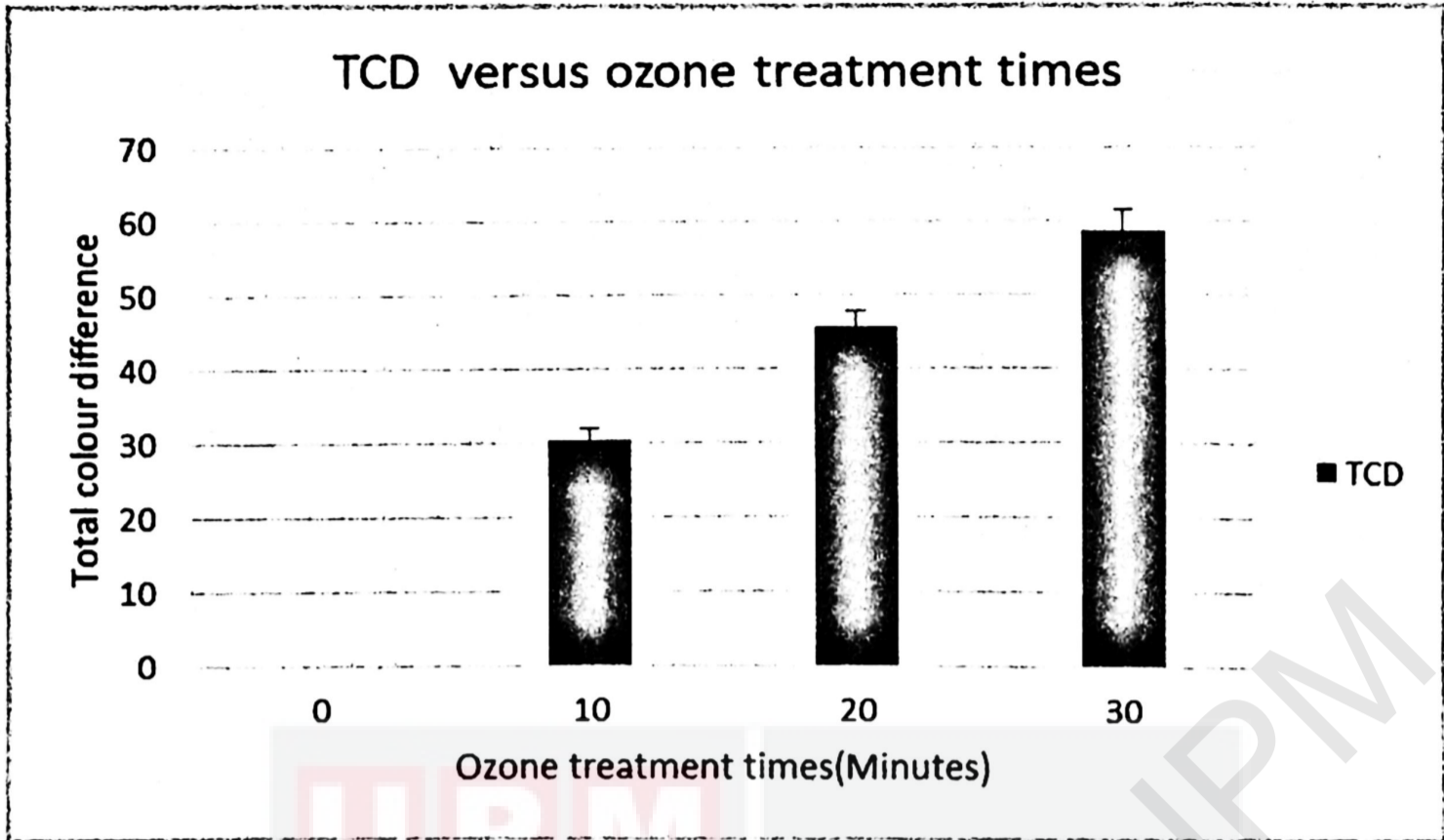


Figure 4.4.3 Total colour differences versus ozone treatment times

## **CHAPTER 5**

### **CONCLUSION AND RECOMMENDATION**

#### **Conclusion**

As a conclusion, gaseous ozone has been proven to be able to inactivate both APC and YMC in brown rice milk to a minimum of 5-log reduction. It was also shown that ozone treatment will reduce APC to 5-log reduction faster when compared to YMC. Furthermore, it was shown that ozone treatment can significantly affect ( $p < 0.05$ ) total colour difference but no significant ( $p > 0.05$ ) effect has been detected on pH and TSS. Overall, the results showed that ozone treatment has big potentials to be exploited to preserve liquid food products.

#### **5.1 Recommendation for Future Work**

Direct exposure of ozone treatment is suitable to preserve the quality of rice milk as it is one of the non-thermal processing technologies that has the potentials as an alternative to pasteurization. Although ozone technology is still expensive to be implemented in liquid food industries, maybe in the near future, with the help of food engineers, non-thermal experts and fabricators, food industry will move forward

**and try to consider gaseous ozone as an alternative to pasteurization. Furthermore, ozone concentration, ozone flow rate and temperature are some of the parameters that might have beneficial effects to preserve rice milk and it is imperative to look into its possibilities.**



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