UNIVERSITI PUTRA MALAYSIA

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ABSTRACT

Response surface methodology (RSM) was used to determine the effects of ozone treatment on pink pitaya fruit juice physicochemical properties and to determine optimum condition to pasteurize the pink pitaya fruit juice with minimal physicochemical changes using RSM. An optimal experimental design was employed with control variables of ozone concentration (0.84-1.29 mg/min) and type of juice (filtered and unfiltered). All of predicted models were found to be significant ($p < 0.001$) with regression coefficients of 0.96, 0.99, 0.87, 0.99, 0.94 and 0.96 for total colour difference (TCD), pH, total soluble solids (TSS), ascorbic acid, DPPH scavenging activity and total phenolic content (TPC). Ozone concentration was found to be an effective factors influencing TCD, DPPH, ascorbic acid and TPC whereas the type of juice did not influence the result is insignificant (p-value >0.05). These responses illustrated a similar trend of inversely proportional as ozone concentration increases. Meanwhile, pH and TSS recorded no observable change for both type of juices. Synergistic effects of ozone concentration and type of juice were shown significantly ($p < 0.05$) in TCD, ascorbic acid, DPPH scavenging activity and TPC. It also shows that unfiltered ozone treated pink pitaya fruit juice had a better effect in retaining its TPC, DPPH scavenging activity and ascorbic acid content. Results from optimal design validate the previous results with optimum point was recorded at 0.84 mg/min of ozone concentration and unfiltered juice with desirability of minimum colour difference.

ABSTRAK

Permukaan tindak balas (RSM) digunakan untuk menentukan kesan rawatan ozon pada sifat fizikokimia jus buah pitaya merah jambu dan untuk menentukan keadaan optimum untuk memasteurkan jus buah pitaya merah jambu dengan perubahan fizikokimia yang minimum menggunakan RSM. Reka bentuk eksperimen yang optimum digunakan dengan pembolehubah kawalan kepekatan ozon (0.84-1.29 mg /min) dan jenis jus (ditapis dan tidak ditapis). Semua model ramalan telah didapati signifikan (p <0.001) dengan pekali regresi 0.96, 0.99, 0.87, 0.99, 0.94 dan 0.96 untuk jumlah perbezaan warna (TCD), pH, jumlah pepejal larut (TSS), asid askorbik, DPPH aktiviti memerangkap dan jumlah kandungan fenolik (TPC). Kepekatan ozon telah didapati berkesan mempengaruhi TCD, DPPH, asid askorbik dan TPC. Manakala jenis jus tidak mempengaruhi hasilnya adalah tidak penting (nilai-p> 0.05). Tindak balas ini menggambarkan trend yang sama perkadaran songsang peningkatan dengan kepekatan ozon. Sementara itu, pH dan TSS tiada perubahan boleh dilihat daripada maklumat untuk kedua-dua jenis jus. Kesan sinergi kepekatan ozon dan jenis jus telah ditunjukkan dengan ketara (p <0.05) dalam TCD, asid askorbik, aktiviti memerangkap DPPH dan TPC. Juga, ia menunjukkan buah pitaya merah jambu yang tidak ditapis dirawat ozon mempunyai kesan yang lebih baik dalam mengekalkan TSS TPC, DPPH aktiviti memerangkap dan asid askorbik kandungan. Hasil daripada reka bentuk optimum sebelum mengesahkan keputusan dengan titik optimum dicatatkan pada 0.84 mg /min kepekatan ozon dan jus yang tidak ditapis dengan kebaikan minimum perbezaan warna.

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LIST OF ABREVIATIONS

ANOVA	Analysis of Variance
CAM	Crassulacean Acid Metabolic
CDCIS	Communicable Diseases Control Information System
CFU	Colony Forming Unit
DCPIP	dichlorophenol indophenol
DPPH	2,2-Diphenyl-1-Picrylhydrazyl
FDA	Food and Drug Administration
GAP	Good Agriculture Practices
GMP	Good Manufacturing Practices
GRAS	Generally regarded as safe
HACCP	Hazard Analysis and Critical Control Point
HPP	High pressure processing
MOH	Ministry of Health
PEF	Pulsed Electric Fields
RSM	Response Surface Methodology
TCD	Total Colour Difference
TPC	Total Phenolic Content
TSS	Total Soluble Solid
UK	United Kingdom
UPM	Universiti Putra Malaysia
UV	Ultraviolet
USFDA	United State Food and Drug Administration
USA	United State of America
WHO	World Health Organization

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CHAPTER 1

INTRODUCTION

1.1 An Overview on Ozonation and Pink pitaya fruit juice

Ozone systematically named 2λ-trioxidiene and *catena*-trioxygen, or trioxygen, is an inorganic molecule with the chemical formula O. Ozone is a bluish gas with pungent odor and strong oxidizer (Muthukumarappan et al., 2000). Ozone is an allotrope of oxygen that is much less stable than the diatomic allotrope O, breaking down in the lower atmosphere to O or di-oxygen. Ozone is formed from di-oxygen by the action of ultraviolet light and also atmospheric electrical discharges. The physical properties of ozone is colourless or slightly bluish, soluble in water and inert non-polar solvent, which this properties help the ozone gas to be dispersed in liquid product.

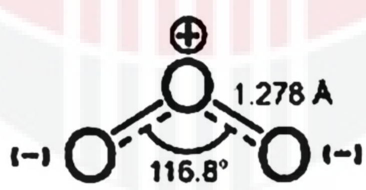


Figure 1.1: Ozone structure

This technology is relatively new to considered as food pasteurizer but there are a lot of interests and researches that have been made to fulfil the demand of pasteurized fruit juice in the market. The demand of pasteurized juice is high because the awareness of community to gain healthy consumable of juice. In USA, it has been required by USFDA for fruit juice producer to reduce the most resistant pathogen by 5 log and FDA have since approved the usage of ozone as a direct additive to food and beverages (Tiwari et al., 2009). The ozone treatment is imperative to the fruit and vegetables producer to preserve them from degradation of nutrient which is crucial for their industry to serve the consumer with freshly harvested produce from plantation. This ozone processing is being considered as one of the options for food industry to serve the customer with high value content of nutrient from fruit and vegetables regardless of their form of finished product in term of liquid or solid to meet the demand of healthier consumption.

Extensive review by Karaca and Velioglu (2007) on ozone has been reported towards fruit and vegetable and various researchers have progressed on designing experimental approach of using ozone as a mean to pasteurize fruit juices including; apple cider (Steenstrup and Floros, 2004); apple cider (Choi and Nielsen, 2005); orange juice (Tiwari et al., 2008a); blackberry juice (Tiwari et al., 2008b); and strawberry juice (Tiwari et al., 2009). The application of ozone also experimented by Palou et al. (2000) for storage of fruits and vegetables including grapes. Others have also recorded extend of ozone application on strawberries (Perez et al., 1999) and blackberries (Barth et al., 1995). Most of the researches are interested to find out the effect of ozone treatment in fruits and vegetables based on their colour changes and antioxidant properties which can be correlated with the quality of finished product.

Pink pitaya is a type of fruit that has a radiant colour of flesh and originates from South America. Pink pitaya also called as '*buah naga*' in Malaysia. Moreover, pink pitaya flesh contains moisture, fat, protein, crude fibre and ascorbic acid. Phytochemical present in pink pitaya (betalins) plays a vital role as the major antioxidant contributor whereas non-betalainic phenolic compounds play a minor role (Esquivel et al. 2007). Betacyanin are unstable and their stability is affected by heat, oxygen, light, pH and moisture (Woo et al. 2011) as these factors make this pigment discoloured are playing as main cause (Liu et al. 2008).

As mentioned above, Food and Drugs Administration (FDA) is amending the food additive regulations to provide for the safe use of ozone in gaseous and aqueous phases as an antimicrobial agent on food, including meat and poultry. FDA has prescribed condition that allows ozone to be used in such that the additive is an unstable, colourless gas with a pungent, characteristic odour, which occurs freely in nature. It can be produced commercially by passing electrical discharges or ionizing radiation through air or oxygen. Specifically, Malaysian has not established ozone as one of food pasteurization process for liquid food. Therefore, assumption was made to fulfil this requirement by following FDA regulation under liquid food. Nevertheless, any food premise should comply with FDA regulation at all stages of food process including Food Act 1983 and Food Regulations 1985.

1.2 **Problem Statement**

The pink pitaya was introduced in Setiawan, Johor and Kuala Pilah, Negeri Sembilan as pioneer in plantation of pink pitaya in 1999. In 2006, the production of dragon fruit in the country has reached 2,534.2 tons (Cheah & Zulkarnain, 2008). Interests toward pink pitaya have grown as many small farmers have tried to meet its high demand in market. Malaysia itself has high potential to plant pitaya fruit due to the distribution and amount of rain which greatly affect the growth and yield of dragon fruit in Malaysia. Pink pitaya also has abundant of nutritional qualities, from its stem to fruit of pink pitaya can be made a product.

Table 1.1: Nutritional values of pink pitaya (Source: Taiwan Food Industry Develop & Research Authorities)

Food Value for 100g serving of Red Pitaya	
Moisture	82.5-83g
Protein	0.159-0.299 g
Fat	0.21-0.61 g
Crude fiber	0.7-0.9 g
Carotene	0.05-0.012 mg
Calcium	6.3-8.8 mg
Phosphorus	30.2-36.1 g
Iron	0.55-0.65 mg
Vitamin B1	0.28-0.043 mg
Vitamin B2	0.043-0.045 mg
Vitamin B3	0.297-0.43 mg
Vitamin C	8-9 mg
Thiamine	0.28-0.30 mg
Riboflavin	0.043-0.044 mg
Niacin	1.297-1.300 mg
Ash	0.28 g
Others	0.54-0.68 g

This study focuses on *Hylocereus polyrhizus* (pink pitaya) because it has a higher phenolic contents rather than white flesh dragon fruit (*Hylocereus undatus*). They are rich in protein, vitamin B1, vitamin B2 and B3 for role in maintaining health, growth and metabolism of the body. Its fruits that contain calcium and phosphorus minerals function for strengthen bone, prevent osteoporosis and iron which can prevent anaemia. Moreover, this fruit is also believed to promote blood circulation, reducing inflammation nerves, helps detoxify, prevent oral infections, prevent bleeding and cure lumps. Studies have shown that dragon fruit can improve vision eye and controlling sugar. Dragon fruit seeds can help control sugar levels diabetic patients, reduced stomach and endocrine problems. Even, its skin can be used as a natural food colour, emulsifier, thickener, texturizer and stabilizer in food. Other than that, the study found that red dragon peel extracts potentially inhibit tumor cell growth (Zainudin Hj. M., 2014). These all shown that pink pitaya has a lot of value in term of health especially. Thus, for maintaining the nutrient inside pink pitaya needs a treatment that can preserved and prolonged the shelf life due the time. In food processing, it is common to used thermal pasteurization as preservation technique. Reduction of betacyanin contents inside the pink pitaya was found to be due to the high temperature exposure for 30 min (85°C) compared to lower temperature (Wong & Siow, 2014). Effect such diminishes vitamins, essential nutrients, and food flavors in the product have been reported to occur due to the high energy treatment on them (Cánovas et. al., 2009). Suggested method of pasteurization derived from non-thermal processing might help in maintaining its nutrients and further ensure the quality of juice produce to be as high as possible. Introduction to non-thermal technologies such as pulsed electric fields (PEF), ultraviolet (UV) light and high pressure processing have been applied in food processing as an alternative to thermal processing (Guerrero-Beltrán & Barbosa-Cánovas, 2004). Non-thermal technology which absence of heat in produces better quality food products such as fruit juices (Mohd Adzahan & Benchamaporn, 2007). Based on past literature review on several fruit, the ozonation methods manipulate the effect on quality attribute of fruit juices. The study was conducted currently on apple juice (Torres et. al., 2010), orange juice (Tiwari et. al., 2008a), grape juice (Tiwari et. al., 2009), blackberry juice (Tiwari et al., 2008b), and strawberry juice (Tiwari et. al., 2008). Currently, no research has been done to study the effects of ozonation on tropical fruit juices particularly pink pitaya juice, therefore this study is imperative to determine the effect of gaseous ozone on the physicochemical antioxidant properties of pink pitaya. Thus, the objectives of this study were to determine the effect of ozone treatment on pink pitaya fruit juice physicochemical properties and to determine optimum condition to pasteurize the pink pitaya fruit juice with minimal physicochemical changes using RSM.

1.3 **Objectives**

The objectives of this study;

a) To determine the effect of ozone treatment on pink pitaya fruit juice physicochemical properties.

b) To determine optimum condition to pasteurize the pink pitaya fruit juice with minimal physicochemical changes using RSM.

1.4 **Scope of Research**

In this study, ozone was chosen as preservation method of pink pitaya. However, the gaseous ozone concentration and treatment time was limited due to the incapability of ozone generator (Model SY- 004, Taiwan) itself to produce ozone gaseous at 30 minutes treatment time and 200 mg/hour was maximum condition to generate ozone.

The parameter of experiment were ozone concentration and type of juice, which an unfiltered and filtered were chosen for this study. The reason why unfiltered and filtered juice were chosen is because to investigate significant effect of ozone on physicochemical and antioxidant properties in both typed of juice.

In addition, pink pitaya was believed to have abundant of antioxidant (betalin) which anti-inflammatory and antidiabetic properties with a suppression effect on cardiovascular disease including cancer. Furthermore, pink pitaya has an attractive physically such as bright in colour and its flesh can be a natural colouring agent for many type of product such as ice-cream and cosmetic products (Lourith & Kanlayavattanakul, 2013).

1.5 Contribution of Thesis

This study will contribute on knowledge and understanding more about ozonation towards tropical fruit juice in region of Southeast Asian, Malaysia. Ozonation is one of technique to preserve the food, as known the thermal technique will give out the side effect toward food, it nutrient, and quality. Therefore, in advanced ozonation provide the food without no toxic residue after treatment which is not dangerous to be consumed and environmental friendly (Tiwari et al., 2008). Ozonation is not widely used in Malaysia due to declaration and assumption that ozone is dangerous to health and body in long term effect.

CHAPTER 2

LITERATURE REVIEW

In this chapter, reviews of past studies are discussed extensively focusing on the related ozone treatment as an alternative pasteurization method. In details, this chapter consisted of five sections which are; 2.1 Pink Pitaya, 2.2 Foodborne Illness Outbreaks, 2.3 Physicochemical Properties of Pink Pitaya and 2.4 Ozone Treatment and 2.5 Effect of Ozone in Fluid Food Quality.

2.1 **Pink Pitaya**

2.1.1 **The Background and Varieties of Pink Pitaya**

Pink Pitaya or scientific name *Hylocereus polyrhizus* belong to family Cactaceae which is one of the fruit from cactus family. In Malaysia, pink pitaya well known as "*buah naga*". They are also called as *queen of the night* (North American) because its flowers open only after night fall, at the same time exhaling a very sweet scent in the air and will close up with the risen of the sun. It is native in South America but most of the commercial plantation of pink pitaya is currently dominated by Asian countries such as Malaysia, Vietnam and Thailand. Pink Pitaya fruit grows on trunk and its adventitus root will come out of the stalk to retrieve water and food. Pink pitaya also has physiological properties of long day plant (LDP) and requires a lot of sunlight. In addition, this plant is from the Crassulacean Acid Metabolic (CAM) group that conducts food synthesis at night.

Figure 2.1: Plantation of Pink Pitaya

The size of fruit is oblong to oval, up to 12 cm long and up to 9 cm thick. Besides, the pink pitaya has horny peel with colour of red enclosed the flesh of fruit. The flesh varies with two different colour; white or red and full of a seed distributed on the flesh of fruit. The moderate sweet and attractive colour of flesh may give a sensation to our taste buds. Some dragon fruits have red or yellow skin (which looks a little like a soft pineapple with spikes) and white or red flesh, but always the beginnings of overlaid leaves, similar to an artichoke, and an abundance of small, black, edible seeds. The flavour is mildly sweet, like a blend of kiwifruit and pear, and it has a crunchy texture (http://foodfacts.mercola.com/dragon-fruit.html).

Figure 2.2 Pink Pitaya

2.1.2 Health Nutrition of Pink Pitaya

Dragon fruit (pink pitaya) is gaining much attention recently because of its micronutrient enrichment regulated by the phenolic that possess antioxidant and anti-proliferative activities (Wu, 2006). Its attractive colour is also one of the factors that has gained attention. The pulp is popularly consumed fresh, and the juice can be an additive to ice-cream colouring application (Wybraniec et al., 2001). It exhibits anti-inflammatory and antidiabetic properties with a suppression effect on cardiovascular disease including cancer prevention potential (Stintzing et al., 2004). Dragon fruit juice is also industrially manufactured as a functional drink (Dartsch et al., 2009) to serve the consumers' requirement of a natural supplement promoting good health. Therefore, accounted as a food product with high economic value (Kim et al., 2011). Pink pitaya well known as the fruit that rich on nutrition and antioxidant that provides in their flesh and other part of fruit could be turn into a beneficial product. The demand for the presence of natural antioxidants and natural food colorants because the red or pink pitaya flesh varieties are rich in betalains (Le Bellec et al., 2006). In 37 type of fruits such as dragon fruit, jackfruit, guava, apple, durian, banana and more fruits but, some of this fruits have high content of beta-carotene, lycopene and vitamin E (Charoensiri et al., 2009).

Table 2.1: Comparison between stem and flesh of dragon fruit

Nutritional Contents	Flesh	Stem
Moisture	82.5-83g	96.0-98.0g
Protein	0.159-0.229g	0.12-0.27g
Fat	0.21-0.61g	0.09-0.23g
Crude fibre	0.7-0.9g	0.02-0.05g
Ascorbic acid (vitamin C)	8-9mg	63.71-132.95mg/L
Ash	-	0.03-0.09g
Water activity	-	0.545-0.865 A_w
Glucose	-	0.263-0.522g/L

(Jaafar et al. 2009)

From table above, proximate analysis of pink pitaya was done by Jaafar et al.(2009) to compare the values such as moisture, protein, fats, crude fibre, ascorbic acid, water activity and glucose content between stem and flesh of pink pitaya. In this research, the value of ascorbic acid will be determined as effect of ozone on pink pitaya juice with respect to process as compare to table above for untreated pink pitaya as reference. There are three types of dragon fruits which are white flesh dragon fruit (*Hylocereus undatus*), red dragon fruit (*Hylocereus polyrhizus*) and yellow dragon fruit (*Selenicereus megalanthus*). It also exported and grown al several Southeast Asian countries, such as Thailand, Malaysia and Vietnam. In Malaysia, pink pitaya well known as *buah naga* and there are several name given to them according country as example *huo long guo/hong long guo* (Taiwan), *pitaya/kaew mangkorn* (Thailand), *than long* (Vietnam), *strawberry pear, pitahaya* (Amerika Latin), *queen of the night* (Amerika Utara), *red dragon fruit* and *fire dragon fruit* (Asia) (Zainudin Hj. M., 2014).

2.1.3 **Fruit Market, Potential, and Malaysia Production for Pink Pitaya**

Estimated production size of pink pitaya plantation is around RM28,200 per hectare (0.4 ha) according to MARDI (Malaysian Agricultural Research and Development Institute, 2014). Average yield per hectare (0.4 ha) in the first year is 500 - 800 seeds. Profits depending on the market price of RM1 - RM2 per kg for white varieties and red varieties are RM5 - RM7 per kg. Financial analysis shows that the return on investment is estimated in the third year at a price of RM5 per kg in the estate (Zainuddin Hj M., 2014).

Market price of pink pitaya has a high price until now, however, the price sometimes fluctuate from RM7- RM8 per kg of pink pitaya because of demand of this fruit is high even if it is not during harvesting season. In 2012, the pink pitaya became one of the popular plantation due to the character of pink pitaya that can withstands high temperature at a range of 25 to 30°C (Cheah & Zulkarnain 2008). Pink pitaya plantation is also known as organic plantation and it does not require high capital cost compared to other fruits plantation (Taman Pertanian Universiti (UPM), 2011).

2.2 **Foodborne Illness Outbreaks**

2.2.1 **Diseases**

Fruit juices sold by the street vendors (as thirst quenching aid) are consumed regularly by the local population in most of the tropical countries (Suguna et al., 2011). Often, consumer preference is for fresh-cut fruits and juices rather than their processed counterparts which also referred to as minimally processed fruits in Malaysia in the last two decades (Osman, 2011). Generally, the consumer believe that fresh fruit juice retains the original nutritional and sensory attributes. However, available reports on the outbreak of foodborne diseases (attributed to cross-contamination) have raised serious concerns regarding the safety of consuming unpasteurized or unprocessed fruit juices. There are many of reports stated on the presence of pathogenic or toxin producing microorganisms such as *E. coli* 0157:H7, *Salmonella* sp., *Penicillium expansum*, *Aspergillus* sp., *Byssochlamys* sp., and *Fusarium* sp., in the unprocessed apple, orange and grapes fruit juices (Tournasa et al., 2006; Bae et al., 2009; Tribst et al., 2009; Sant'Anab et al., 2010).

Recently, the typhoid fever has been reported in Belaga, Kapit division, Sarawak. According to a Malaysian Digest report, nine cases on November, 2017 were revealed but no death has been reported. All patients were hospitalized at Bintulu Hospital for further treatment. Typhoid is a communicable disease and this fever disease can spread out through consuming food or beverages that have been handled by a person who is shedding *Salmonella Typhi* or if sewage contaminated with *Salmonella Typhi* bacteria leaches into the water that use for drinking or washing food.

Figure 2.3: Food and Vector Borne Diseases in Malaysia (Source: Ministry of Health Malaysia, 2016)

In many countries, the food borne outbreaks become one's of high in number in cases reported year by year even in Malaysia, in 2015 there are 32 typhoid cases over a month of August reported. The above figure (Figure 2.3) shown that typhoid illness comes as the second highest cases of food and vector borne disease with 434 cases on 2016 as reported by Ministry of Health Malaysia. Approximately, 1210 food borne cases per 100,000 inhabitants in France, 2600 cases per 100,000 in the United Kingdom and more than 25,000 cases per 100,000 inhabitants in Australia and United States are reported (Teisl et al., 2010). Based on Food and Drug Administration (2012), there is also multistate of outbreak *Salmonella spp.* infections associated with mangoes from Agricola Daniella which causes the United States of Food and Drug Administration to ban the export and import of these mangoes.

In Malaysia, the food borne outbreaks does not indicate a significant increment of cases reported ahead of time and the incidence that notify case of food borne diseases, namely cholera, typhoid, food poisoning, hepatitis A and dysentery are food borne diseases that has less than 5/100,000 population statically, sporadic in nature and outbreaks are confined to certain areas only. According to Soon et al. (2011) the food borne cases in Malaysia is low due to unreported cases and some of the cases need to be addressed before released to the public. The main contributing factor to foodborne diseases was identified as unsanitary food handling procedures which accounted for more than 50% of the poisoning episodes especially in Malaysia. The prevention of food poisoning during food preparation should be addressed to food handlers as they play a major role; hence, food handler training is seen as one of the main strategies to increase food safety practices. This factor can also be eliminated and controlled by a proper process to kills the microbes in stage of production usually by pasteurization (heat treatment process).

2.2.2 Regulation of Fruit Juice

The awareness of foodborne illnesses have become severe complication as to be seen high in number reported cases year by year even can lead to fatality. A significant approach was determined to control this issue. Thus, food regulations were gazetted and studies were made to comply with the current issue. Since fruit juice is one of the favourite drinks with regards to its nutrient and benefit on health, FDA has published a regulation which requires processors of juice to develop and implement Hazard Analysis and Critical Control Point (HACCP) system for their processing operation in 2001. Labelling in juices product play an important role that help consumer to choose right product to be consume. FDA has stated that unpasteurized or untreated juice - normally found in the refrigerated sections of grocery stores, health-food stores, cider mills, or farm markets, must have the warning on the label (FDA, 2017).

There are many available reports on the overall quality of pink pitaya, but there are no scientific reports available on the microbial safety of this fruit or their juices (Suguna et al., 2011). The demand for safer product increase gradually as many of consumer voice their concerns. Implementation of HACCP (Hazard Analysis and Critical Control Point), GAP and GMP (Good Agricultural and Manufacturing Practices) in juice manufacturing, making it a step closer to produce a safer product. In Malaysia, there are two laws that were gazetted under food and safety of surveillance which are Food Act 1983 and Food Regulation of 1985. These laws regulation was made up to comply with HAACP, GMP and GAP programs in order to synchronize them with the laws stated in USFDA. The Fruit Juices and Fruit Nectars (England) Regulations in 2013 has highlight the regulation implement to fruit juice such labelling, use of the name fruit juice, use of the name fruit juice from concentrate, use of the name concentrated fruit juice, use of the name water extracted fruit juice, indication of kinds of fruits seed, indication of added extra pulp or cells, enforcement and many more. This regulation provide a numerous schedule from 1 to 15 for explaining the specification for variety type of juice. Fruit juice can be defined as fermentable but unfermented product obtained from the edible part of fruit which is sound, ripe and fresh or preserved by chilling or freezing of one or more kinds mixed together having the characteristic colour, flavour and taste typical of the juice of the fruit from which it comes (Fruit Juices and Fruit Nectars (England) Regulations, 2013). Thus, pink pitaya should obey this regulation in order to produce the juices.

2.3 Physicochemical Properties of Pink Pitaya

Physicochemical properties is physical and chemical properties of food products have central roles in biotechnology and the pharmaceutical and food industries. The properties include, colour, pH, the antioxidant content, and chemical analysis of vitamin content of pink pitaya fruit juice. Besides, these properties of fruit juices help to maintain their quality and shelf life

2.3.1 Colour

Color or colour is the characteristic of human visual perception described through colour categories, with names such as red, blue, yellow, green, orange, or purple. This perception of colour derives from the stimulation of cone cells in the human eye by electromagnetic radiation in the spectrum of light. It is a beam of light composed of irregularly distributed energy emitted at different wavelengths (Yap, 2012). Each of objects does reflect their colour through wavelength. This reflection is governed by the object's physical properties such as light absorption, emission spectra, and others medium.

Quality is a term which denotes a degree of excellence, a high standard or value. Kramer (1965) stated that quality of foods may be defined as the composite of those characteristics that differentiate individual units of a product, and have significance in determining the degree of acceptability of that unit to the user. For vegetable and fruits, the characteristics that express their quality from different attributes such as colour and appearance, flavour (taste and aroma), texture and its nutritional value (Barrett et al., 2010). Colour can also define the food quality attribute from outer appearance where is the colour of fruit or vegetable deteriorate causing by browning reaction. Others, the changes of colour of fruit become ones of factors to determine the maturity and ripening process of fruit which derived from natural pigments of plant. The primary pigments imparting colour quality are the fat soluble chlorophylls (green) and carotenoids (yellow, orange, and red) and the water soluble anthocyanins (red, blue), flavonoids (yellow), and betalains (red).

The determination of colour may use non-destructive methods founded on visual or physical measurements. These methods are based on evaluation of either the light reflected from the surface of a product or transmitted through it (Barrett et al., 2010). There are three components necessary to the perception of colour a source of light, an object that modifies light by reflection or transmission and the eye/brain combination of an observer (Leggett, 2004). Analytical sensor methods can also be used to determine the colour which no specialized instrument used and this method is fast and easier way. Unfortunately, it may vary depending human perception and errors. Tristimulus colorimeters simulate the human eye by replacing the receptors with filters—one for each primary hue (Barrett et al., 2010). This method comprises further characterized by determining lightness or degree to which an object reflects light, and chroma or saturation, which is the intensity of colour or difference from grey of the same lightness.

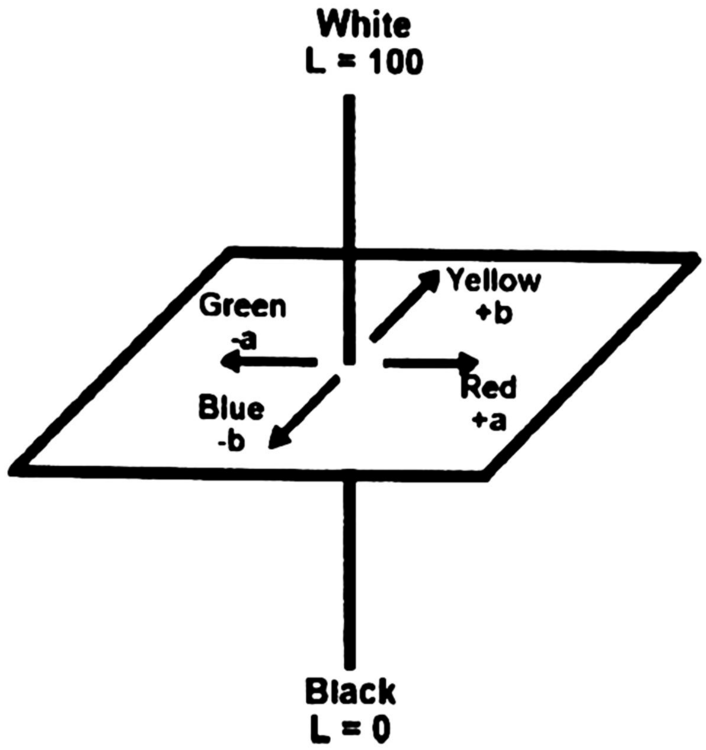


Figure 2.4: Diagram depicting three dimensional L, a and b colour space.

2.3.2 pH

pH is a function of expressing the acidity or alkalinity of a solution on a logarithmic scale on which 7 is neutral, lower values are more acid and higher values more alkaline. The pH is equal to $-\log_{10} c$, where c is the hydrogen ion concentration in moles per litre. The pH can be as indicator to degradation ascorbic acid in fruit juice, according to Patkai et al. (2002) recommended that ascorbic acid degradation to 2,3-diketogulonic acid under aerobic conditions at pH lower than 5 is a 1-step reaction, but at pH 8, oxidation proceeds as a 2-step reaction. Nevertheless, the change in pH value will also affect the microbial stability and susceptibility to mould and bacterial spoilage. Besides, pH also determine to produce products with consistent well defined properties.

2.3.3 Total Soluble Solids

Sugars are the major soluble solids in fruit juice. Other soluble materials include organic and amino acid, soluble pectins, etc. Soluble solids concentration (SSC%, ^{o}Brix) can be determined in a small sample of fruit juice using a hand held refractometer. This instrument measures the refractive index, which indicates how much a light beam is "bent" when it passes through the fruit juice. Refractometer equipped with a scale, based on the relationship between refractive indices at 20°Bx (pronounced as 20 degrees brix) and the percentage by mass of total soluble solids of a pure aqueous sucrose solution.

2.3.4 **Ascorbic Acid**

Ascorbic acid (vitamin C) is a water-soluble vitamin which can be discovered in many biological systems and food stuffs (fresh vegetables and fruits, namely, citrus). It functions as collagen biosynthesis, iron absorption, and immune response activation and is involved in wound healing and osteogenesis. Furthermore, it also acts as a powerful antioxidant which fights against free-radical induced diseases (Fox and Cameron, 1989). Ascorbic acid is a subtle substance, as it is easily degraded by enzymes and atmospheric oxygen. An excessive heat, light, and heavy metal cations accelerated the oxidation of ascorbic acid (Bhagavan, 2001). Therefore, ascorbic acid content of foodstuffs and beverages represents a relevant indicator of quality which has to be carefully monitored, regarding its variation during manufacturing and storage.

Classically, ascorbic acid can be determine by volumetric methods titration with an oxidant solution such as dichlorophenol indophenol (DCPIP) (Şerban et al., 1993 and Papuc et al., 2001), potassium iodate (Balan et al., 2005), or bromate (Matei et al., 2004). This method are more convenience but lack of specificity.

2.3.5 DPPH (2,2-Diphenyl-1-Picrylhydrazyl)

DPPH is a common abbreviation for the organic chemical compound 2,2-diphenyl-1-picrylhydrazyl. It is a dark-coloured crystalline powder composed of stable free-radical molecules. DPPH is a well-known radical and a trap ("scavenger") for other radicals. Antiradical scavenging activity was tested by the DPPH model inside fruits juice. DPPH radical will undergoes reduction of electron transfer or radical quenching via hydrogen atom transfer which in this case acid medium could help the mechanism (Prior et al., 2005). The test will be conducted by dissolving the DPPH into methanol solution and react with juices. The absorbance result from this test was measured at 517 nm using UV spectrophotometer.

2.3.6 **Total Phenolic Content**

Total phenolic content are a class of chemical compounds consisting of a hydroxyl group (—OH) bonded directly to an aromatic hydrocarbon group. The simplest of the class is phenol, which is also called carbolic acid C_6H_5OH. Phenolic compounds are classified as simple phenols or polyphenols based on the number of phenol units in the molecule. ingestion of natural antioxidants has been inversely associated with morbidity and mortality from degenerative disorders could be avoid by the presence of antioxidants such as phenolic, flavonoids, tannins and pro-anthocyanidins in plants may provide protection against a number of diseases (Gulcin, 2012). TPC test was conducted by Folin–Ciocalteu reagent and external calibration with gallic acid.

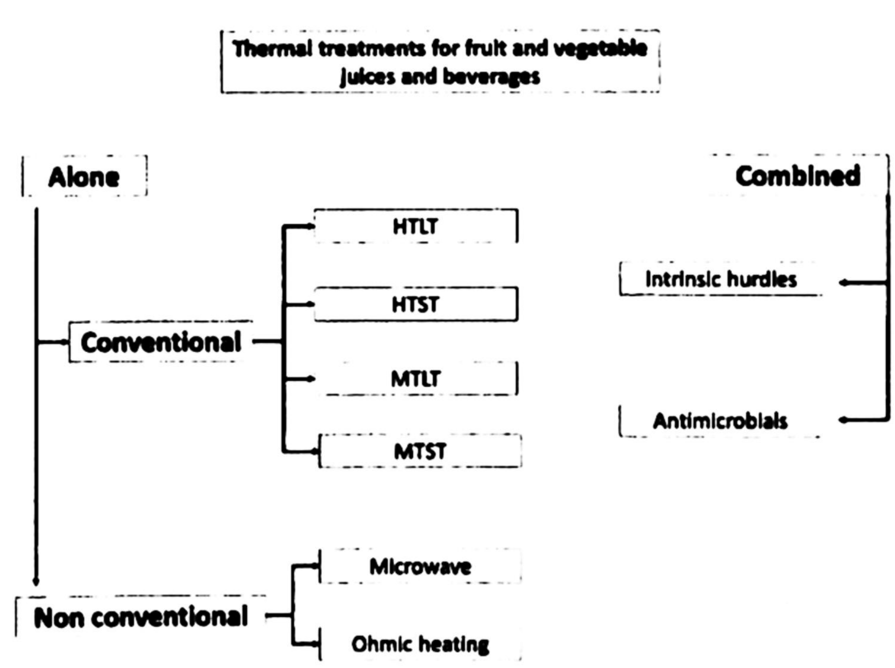
2.4 **Thermal VS Non-Thermal Process**

Fruit and vegetable juices and beverages are generally preserved by thermal processing, currently being the most cost-effective means ensuring microbial safety and enzyme deactivation. However, thermal treatments may induce several chemical and physical changes that impair the organoleptic properties and may reduce the content or bioavailability of some nutrients; in most cases, these effects are strongly dependent on the food matrix. As recommended, 2010 Dietary Guidelines for

Americans suggest in a 2000-kcal diet 9 servings of fruits and vegetables per day, 4 servings of fruits and 5 servings of vegetables (Liu, 2013). The European Union supports the WHO recommendation for at least 400 g/d (Tennant et al., 2014). This mean intake of fruit and vegetables are increase to avoid chronic diseases. Furthermore, this intake could be in liquid (juice) form to facilitate us to consume more nutrient from fruits and vegetables.

Thermal processing is the most common and traditional technique used for food processing. An adverse effect on organoleptic properties of foods caused by thermal processing has been reported. Temperature is well known to be one of the major preservation mechanisms that can be applied by the food processor to render food products commercially sterile – that is, free from pathogenic and spoilage microorganisms likely to grow during the normal distribution and shelf-life of the product. Others than that, thermal processing also permitting the application of overpressure around containers such retort. There are some thermal process used to achieve certain condition regard to safety in consuming fruits juices and beverages such 'HTLT'(high temperature long time), 'HTST' (high temperature short time), 'MTLT' (mild temperature long time), 'MTST' (mild temperature short time), microwave heating and ohmic heating process.

Figure 2.5: Thermal treatments for fruits and vegetable juices and beverages.



HTLT is the one of thermal treatment that operate at temperature ≥80 °C and holding times >30 s) is commonly used for processing juices and beverages (Petruzzi et al., 2017). The product of juice pasteurization is based on a 5 log reduction of the most resistant microorganisms cab be eliminated. This method relies on heat generated outside and then transferred into the food through conduction and convection mechanisms (Chen et al., 2013). Some research were done on this methods such apple, grape, or orange juices enriched with hydrolyzed collagen (Bilek and Bayram 2015), mango (Santhirasegaram et al., 2015), pear (Saeeduddin et al., 2015) and tomato juices (Stratakos et al., 2016). Due to high exposure of temperature lipid phase transitions and protein conformation changes, eventually causing cell death. In order to ensure product safety and desired bioactive compounds, HTST was introduced because temperature dependency is more significant for microorganism destruction than for nutrient degradation (Achir et al., 2016). This methods contribute to microbiological quality of products which it can control the growth of *Lactobacillus plantarum* CECT 220 in orange juice added with milk (Zulueta et al.,2013), or the native microorganisms in orange/sweet pepper juice blend (Xu et al., 2015). For MTLT and MTST studies was made over the years in order to increase the shelf life of minimal processed products by increase the stability of colour attributes and control the microbial growth. Heat treatment, MTST reported to achieve larger than 6 to 7 log reduction of microbial *Listeria innocua* population in apple/mango/orange/pineapple smoothie (Palgan et al., 2012). However, this method can alter physicochemical, sensory, and functional properties of beverages. Then, new technologies introduced to reduce the effect of thermal on food product called microwave heating to inactivate the microorganism (Arjmandi et al., 2016). According to Arjmandi et al. (2016), microwave heating can destroyed vegetative bacteria (*L. monocytogenes)* in smoothies without alteration of quality throughout their shelf life. However, microwave heating also has disadvantages such formation of coloured decomposition products in beetroot juice (Goncalves et al., 2013) and decreasing pH and colour of pomegranate juice (Dhumal et al., 2015). Another approaches of thermal process is ohmic heating, this OH is based on the passage of electrical current through a food product that provides electrical resistance (Baysal and Icier, 2010). Most of foods has electrical conductivity that increase with temperature in which contain of juice itself is water and ionic compounds that can conduct electricity. OH has a lot of advantages such as better energy efficiency, uniform and rapid heating, lower capital cost and shorter time treatment. However, OH is not an excellent method to kill the foodborne pathogen and bacteria, as reported in Petruzzi et al. (2017) under condition at 30 s and 58 °C resulted log reduction for *E. coli* O157:H7, *S. Typhimurium*, and *L. monocytogenes* organisms were recorded at 4.00-, 4.63-, and 1.11-log of reduction respectively.

As mentioned above, thermal processing has benefit on eliminating microorganism growth in juice, however the side effect of this treatment could be vary depending of methods being used. The most significant effect from thermal processing is the degradation of nutrients and colour attributes changes as the temperature increase. Even worst, it can destroy the cell inside juices after treatment which this offer an unhealthy fruit juices. Therefore, an alternative method was introduced to reduce the effect of thermal process on physicochemical properties, browning reaction, attributes of juices and quality. Non-thermal technologies are food processing that offers reduction of microbial growth with extending the shelf life of food products and also retains physical, nutrition and sensory qualities. These technologies including pulsed or radio frequency electric fields (PEF/RFEF), ultraviolet light (UV), ultrasound (US), pulsed light (PL), high pressure processing (HP), ionizing radiation, dense phase carbon dioxide (DPCO2) and ozone. Since Southeast Asian countries has foods that stronger taste and aroma with flavourful taste such as fruit juices therefore non-thermal technology treatment can be applied as to maintain the flavour and kills microorganism form deteriorate the juice. Commonly, food passes through many hands from growing, plucking, packaging, shipping, to final processing prior to reaching the consumer. Because of the incidence of food contamination along the entire chain of production, and the recognition that many pathogens some have recently emerged are found in even healthy animals, the industry has realized that some form of disinfection, perhaps at multiple points, is necessary (Muredzi, 2012). In this study, the non-thermal methods is focused on ozone.

2.5 **Ozone Treatment**

Ozone can be defined as a triatomic form of oxygen and is characterized by a high oxidation potential that conveys bactericidal and virucidal properties (Burleson, 1975; Horvath et. al., 1985; Kim et. al., 2001, Patil and Bourke, 2012). Xu (1999) and Patil and Bourke (2012) reported that ozone is 1.5 times stronger than chlorine and is effective over a much wider spectrum of microorganisms than chlorine and other disinfectants.

There are a lot of research done to study the effectiveness of ozone in varies type of juice and optimize the application so that a new emergent product of fruit juices with a longer shelf and storage life. Naturally, the factor are used to determine the reduction of number in microorganism in fruit juice are ozone variable defined by concentration and ozone dissolved also another factor that become the control of experiment is time processing dependent of type of fruit juices used. Interest in ozone technology has become one of approach that result in of logarithm reduction (CFU/mL) among researcher.

Table 2.2: The inactivation of various microorganisms via ozone treatment

Sample Fruit Juices	Ozone Variables	Microorganisms	Log Reduction (CFU/mL)	References
Peach juice	Concentration: 18 ppm Time: 12 min	*Listeria innocua ATCC 33090*	4.9	Alzamora *et al.*(2015)
Peach juice	Concentration: 10 ppm Time: 12 min	*Listeria innocua ATCC 33090*	3.9	Alzamora *et al.*(2015)
Apple juice	Concentration:5 .3mg/L Time: 40 min	*Alicyclobacillusacidote rrestris*	2.4	Torlak.(2013)
Apple juice	Concentration: 2.4 mg/L Time: 40 min	*Alicyclobacillusacidote rrestris*	1.8	Torlak.(2013)
Orange juice (Model)	Concentration: 0.9g/h Time: 0.5 min	*E.coli*	5.0	Patil *et al.*(2009)
Orange juice without pulp (Filtered)	Concentration: 0.9g/h Time: 4.9 min	*E.coli NCTC 12900*	5.0	Patil *et al.*(2009)
Orange juice	Concentration: 0.9g/h Time: 15 min	*Salmonella (five-serovar mixture)*	5.0-6.0	Williams *et al.*(2004)
Apple cider	Concentration: 0.9g/h Time: 15 min	*Salmonella (five-serovar mixture)*	4.8	Williams *et al.*(2004)
Orange juice	Concentration: 0.9g/h Time: 75 min	*E.coli O157:H7 (five strain mixture)*	6.0	Williams *et al.*(2004)
Apple cider	Concentration: 0.9g/h Time: 45 min	*E.coli O157:H7 (five strain mixture)*	6.0	Williams *et al.*(2004)
Orange juice	Concentration: 0-0.25 L/min Time: 2-10 min	Yeast (*S.cerevisiae*)	5.7-6.0	Angelino *et al.* (2003)

Although ozone is significantly more effective than chlorine at inactivating and or killing viruses, bacteria and cysts (e.g., Cryptosporidium and Giardia) and has been widely used in Europe for many years to treat municipal drinking water, it has not had similar acceptance in the US. Reasons include its higher cost and the fact it does not remain present long in water.

There are some factor need into consideration of controlling the ozone concentration which is depending on the atmospheric pressure, temperature and relative humidity of the surrounding environment (Ozone Solution, 2011). Ozone is created by charging the air (O_2) with a burst of high negative voltage. Then, O_2 will split into oxygen atom (O) and the individual oxygen atom is combined with remaining molecules to form ozone (O_3) (Muthukumarappan et al., 2008). The residual ozone will spontaneously decomposes into oxygen molecule which is not harmful to the human beings (Kim et al., 1999).

According to Muthukumarappan et al. (2000), ozone rapidly decomposes into oxygen leaving no toxic residues making it environment friendly. According to Kim et al. (1999) ozone can be used in food industry purpose as antimicrobial agent as it can inactivates microorganisms through oxidization, and residual ozone spontaneously decomposes to non-toxic products (i.e. oxygen), making it an environmentally friendly. Since the free hydroxyl is so highly reactive, the contact time necessary is minimal, as compared to other disinfectants.

Ozone is 1.5-times stronger than chlorine and is effective over a much wider spectrum of microorganisms than chlorine and other disinfectants (Xu, 1999). This technology was recognized well as GRAS (generally recognized as a safe substance) by FDA for treatment of bottled water for drinking when used in accordance with good manufacturing practices (FDA, 1995). The affirmation as GRAS triggered broad usage of ozone gas in the food industry. The interest in ozone as an alternative to chlorine and other chemical disinfectants in cleaning and disinfection operations is based on its high biocidal efficacy, wide antimicrobial spectrum, absence of by-products that are detrimental to health and the ability to generate it on demand, in situ, without needing to store it for later use. The application of ozone in development, and industry guidelines for apple juice and cider were published by USFDA, 2004 and an approval of direct additive to food trigger the interest in ozone itself (US FDA, 2004).

Further explanation on ozone generation requiring that it be generated on-site, since ozone is a highly unstable molecule with a relatively short half-life preventing it from being stored or transported. It can be generated from any source of gas which contains oxygen molecules. The most common sources for ozone generation are commercially prepared liquefied compressed oxygen or air in the atmosphere. Use of pure oxygen gas results in a higher efficiency of ozone generation but it increases the production cost. Using air as an oxygen source requires that the air be compressed and cleaned and dried (i.e., dehumidified). Compression of the air serves to increase the concentration of oxygen. Use of filters to remove foreign particulate such as dirt and dust is accomplished. Dehumidification is accomplished by lowering the dew point by refrigeration. Most ozone generators require clean, dry gas for optimal production. Clean dry gas is passed through a chamber where the continuous discharge (arcing) from a high voltage electric current disperses electrons into the air. The electrons convert oxygen molecules to ozone molecules and oxygen atoms. The highly unstable oxygen atoms bond with hydrogen atoms in the air to form hydroxyl radicals. It is these hydroxyl radicals that give ozone its oxidation characteristic.

Ozone has effect on physical quality, nutritional quality and enzyme inactivation of food products. This method showed a significant colour degradation due from loss of pigment inside juices during ozonation. These including apple cider (Choi and Nielsen, 2005; Williams et al., 2005), orange juice (Angelino et al., 2003; Tiwari et al., 2008a), strawberry juice (Tiwari et al., 2009a), and blackberry juice (Tiwari et al., 2009b), Most of organic compound were degrade as high oxidation potential was applied. Nebel (1975) found that loss of colour in dyes was due to the oxidative cleavage of chromosphere as it attack on conjugated double bonds. Ozone and hydroxyl radicals (OH_2) generated in the aqueous solution may open these aromatic rings and lead to partial oxidation of products such as organic acids, aldehydes, and ketones. Besides, ozone show a minor effect on anthocyanin content of strawberries (Perez et al., 1999) and blackberries (Barth et al., 1995). Thus, observation alter the content of nutrient as some of components might be degrade after treatment. For ascorbic acid content will be degrade as result from direct reaction with ozone or indirect reactions of secondary oxidators. A direct reaction is explained by the Criegee mechanism (Criegee, 1975), formation of ozonides (1,2,4-trioxolanes) from alkenes and ozone with aldehyde or ketone oxides as decisive intermediates where ozone molecules undergo 1-3 dipolar cyclo addition with double bonds present, then which it have finite lifetimes. This leads to the oxidative disintegration of ozonise and the formation of carbonyl compounds, while oxidative work-up leads to carboxylic acid or ketones. In previous study reported that ozone does not effecting non-enzymatic reaction. Enzymes such as poly-phenoloxidase, peroxidise, and β-glucosidase have been implicated in the degradation of anthocyanins. The colour of juice was affected by the loss of the glycosidic moiety leading to the formation of aglycon (anthocyanidin) was found by Barbagallo et al. (2007).

CHAPTER 3

METHODOLOGY

This chapter discusses in details the materials, equipment/machines and methodology used for this study. Experimental design is explained in detail in which all aspects are taken into consideration. Section 3.1 concentrated on the material used in this experimental design which is the preparation of pink pitaya fruit juice. Section 3.2 describes the summary of overall experimental design throughout the research study whereas, Section 3.3 explained the steps and processes that involves ozone concentration as disinfectant to the microorganisms in pink pitaya fruit juice. Section 3.4 defined the physicochemical properties analysis which explain the methods to determine colour and pH of pink pitaya used in this research study. Section 3.5, explains the methods and steps define the antioxidant properties exist in the flesh of pink pitaya. Lastly, Section 3.6 describes the statistical analysis used for this research study which is Optimal Design analysis by using Design Expert 11.0 Software.

3.1 **Preparation of Pink Pitaya Juice**

Pink pitaya fruits (*Hylocereus polyrhizus*) were purchased from Kompleks Pasar Borong, Selangor under management of Selangor Agricultural Development Corporation and maintained at the temperature 4 ± 1°C until they are used for the experimental work. Pink pitaya fruits must be rinsed and washed with distilled water to eliminate any microbial contaminations which might affect the experimental research before conducting the experiment. Then, pink pitaya fruits were manually peeled and cut using a knife. The white membrane were removed completely as the peel of pink pitaya fruit was removed by using hand cover with the rubber glove as precaution step to avoid of any contaminant during peeling. Russelle Taylors Slow-Juicer SJ-33 (cold pressed juicer) was used to extract the pink pitaya juice and the extractions were repeated three times (Shah et al., 2015) to get fully extracted pulp and juice from the fruit itself. Juices extracted were then kept in the polyethylene bottle to be stored in a freezer with the temperature of -20°C until further experimental research.

3.2 Research Design

The overall research design of ozone treatment towards pink pitaya fruit juice is summarized into a flow chart below (Figure 3.1 & Figure 3.2). The research designs are divided into two parts; Part 1 describes the preparation of juice and preliminary analysis on physicochemical and antioxidant properties done on pink pitaya juice while Part 2 included the preparation of juice, treatment of ozone, physicochemical and antioxidant properties and statistical analysis.

Juice Preparation

- Storage at -20 ± 1°C and thaw at 4 ± 1°C
- Sample A: Unfiltered juice
- Sample B: Filtered juice (using Whatman No.1 filter paper)

↓

Physicochemical properties

- Colour determination using spectrophotometer Hunter lab.
- Measuring pH
- TSS (total soluble solids)

Antioxidant properties analysis

- Ascorbic acid test (vitamin C)
- TPC (total phenolic content)
- DPPH (2,2-diphenyl-1-picrylhydrazyl)

Figure 3.1: Part 1: Preparation of juice, physicochemical and antioxidant properties analysis

Juice Preparation

- Storage at -20 ± 1℃ and thaw at 4 ± 1℃
- Sample A: Unfiltered juice
- Sample B: Filtered juice (using Whatman No.1 filter paper)

↓

Ozone Treatment

- Ozone parameter: Processing time
- Design of Experiment: General Full Factorial Design
- Apply ozone on the juice to reduce microbial growth

↓

Physicochemical properties

- Colour determination using spectrophotometer Hunter lab.
- Measuring pH
- TSS (total soluble solids)

↓

Antioxidant properties analysis

- Ascorbic acid test (vitamin C)
- TPC (total phenolic content)
- DPPH (2,2-diphenyl-1-picrylhydrazyl)

Figure 3.2: Part 2: Preparation of juice physicochemical and antioxidant properties analysis after ozone treatment.

3.3 **Ozone Concentration Determination**

The method followed American Public Health Association (1999) to determine ozone concentration that generated by ozone generator with slight modification. Determination of output of ozone generator by passing the zone gas through potassium iodide, KI solution traps for about 5, 15 and 25 minutes. Each conical flask traps is a gas washing bottle containing a known volume by 100 mL of 2% potassium iodine solution KI with magnetic stirrer to ensure ozone fully dissolved in solution. Quantitatively transfer contents of each trap into a beaker, add 2.805 mL of 2N H_2SO_4 and then titrate with standardised 0.05 N $Na_2S_2O_3$ until the yellow iodine colour almost disappears. Add 1 to 2 mL starch indicator solution and continue titrating to the disappearance of blue colour. The experiment is triplicate for each time treatment. Ozone concentration are calculated as formula below;

$$\text{Ozone concentration mg/min} = \frac{(A)\ x\ N\ x\ 24}{T}$$

A = mL titrant for trap A

N= normality of $Na_2S_2O_3$

T= ozonation time, minute.

3.4 **Ozone Treatment**

The main purpose of this is to determine the effect of pink pitaya fruit juice that has been extracted then dissolve into an ozone gas according to ozonation treatment time in which gaseous ozone is generated using ozone gas generator (Model SY- 004, Taiwan) in a 500 mL beaker. Prior to the experiment, pink pitaya fruit juice sample were thawed about 3 to 4 hours to completely defrost the juices. In this experiment, ozone as disinfectant agent is tested for unfiltered and filtered pink pitaya fruit juices. The ozone treatment is conducted in a clean place to avoid any cross-contamination and the sample is then filled into a 250 mL beaker to be ozonated according to the treatment time specified in design of experiment (DOE). The gaseous ozone was pumped directly into the juice through a transparent delivery tube and stirred using a magnetic stirrer to make sure the ozone molecules are completely mixed with fruit juice. Samples are treated for 0 (control), 5, 15 and 25 minutes processing times and the concentration of ozone varies from 0, 0.84, 1.067 to 1.29 mg/min. The ozone generator used in this experiment generated 400 mg/ hour of gaseous ozone.

3.4 Physicochemical Properties of Pink Pitaya

3.4.1 Colour Determination

Colour of pink pitaya juice is measured based on three colour coordinates which are L, a, and b using colorimeter. Each coordinates expresses colour values where L refers to whiteness or brightness / darkness, a refers to redness / greenness, and b expresses yellowness/blueness. White reference tiles (L = 97.55; a = -0.11; b = -0.04) and light trap were used for calibration of colour spectrophotometer of Hunter Lab Ultra Scan Pro (D65, 28 Hunter Lab Assoc Lab Inc, Reston, VA, USA). Measurements of colour is triplicate and the result are taken for each set of samples and values of L, a, and b were recorded. Indication total colour difference (TCD) of the magnitude of colour change after treatment is calculated using equation below:

$$TCD = \sqrt{(L - L_O)^2 + (a - a_o)^2 + (b - b_o)^2}$$

Where

L_O, a_o, b_o initial values obtained from untreated juice

3.4.2 pH

pH meter (Seven Multi pH Conductivity Meter, Mettler Toledo, Switzerland) is applied to measure pH of pink pitaya juice sample. At first, pH meter was calibrated with commercial buffer solution at pH 7.0 and pH 4.0. Then, samples of 10 mL were placed in a beaker with a magnetic stirrer and measured at 27 ± 0.5°C.

3.4.3 **Total Soluble Solids (TSS)**

Total soluble solids content of a solution is determined by the index of refraction. This test measured using a refractometer and is referred to as the degrees Brix which is the term used when a refractometer equipped with a scale, based on the relationship between refractive indices at 20°C ± 0.5°C and the percentage by mass of total soluble solids of a pure aqueous sucrose solution. This test give the amount of solids in the food which is consist of sugars usually exist in food such sucrose, fructose, and glucose. In case for fruit juice the amount of acids and minerals are also included. The refractometer prism was cleaned with distilled water after each analysis to ensure the measurement was taken accurately. As the fruit juice drop on the automatic refractometer (Digital Hand-held "Pocket" Refractometers PAL-79S, Atago), the reading appear directly in 3 seconds and the reading was been recorded for each sample. This is done by triplicate the measurement in order to verify the precise reading.

3.5 **Antioxidant Properties**

3.5.1 **Ascorbic Acid**

2,6-dichlorophenol-indophenol titration method adapted from Amador (2011) was used in determining ascorbic acid content in samples of pink pitaya fruit juice. The titration begin with 0.5% sodium 2,6-dichlorophenol-indophenol dye is prepared using mixture of solution from 0.42 g sodium hydrogen carbonate, $NaHCO_3$ with 200 mL of distilled water prior to the test. At the same time, to prepare 3% acid stabilization solution is made up by dissolving 15 g of meta-phosphoric acid, HPO_3 in a mixture of 40 mL of glacial acetic acid and 200 mL of distilled water. The solution is diluted, made up to 500 mL with distilled water. The test is run by adding 2 mL of juice sample, blank (distilled water), or standard of 1 mg/mL of ascorbic acid solution into 5 mL of acid stabilization solution. At last, the mixture is titrated rapidly with the dye solution until a light but distinct rose pink colour persists for at least 5 seconds. Then, vitamin C or ascorbic acid content in pink pitaya juice is obtained from following equation and expressed as mg/100mL:

Ascorbic Acid

$$= \frac{(ml\ of\ titrant\ for\ sample) - (ml\ titrant\ for\ blank)}{(ml\ of\ titrant\ for\ standard) - (ml\ of\ titrant\ for\ blank)} \times 100$$

3.5.3 2,2-Diphenyl-1-Picrylhydrazyl radical scavenging (DPPH)

For scavenging activity of the pink pitaya juice extracts is determined according to the method described by Tang et al. (2002) and some adaptation method from Zabidah et al. (2011) with slight modifications. At first, three millilitre 3 mL of juice extract is added to 3 mL DPPH (0.1 mM) in methanol solution. Then, the mixture is then shaken vigorously and keep to stand in the dark room for 30 minutes at room temperature. The 517 nm of absorbance is read with solvent as blank by using UV/ Visible Spectrophotometer (Ultra spec 3100 Pro, Amersham Pharmacia Biotech, UK). The reading is compared with the control that contained 20 mL of distilled water, 80% methanol and 3 mL DPPH in methanol solution. The scavenging activity (%) is calculated according to the equation as follows:

$$Scaveging\ activity\ (\%) = \left(1 - \frac{A}{B}\right) \times 100$$

Where, A = absorbance of sample at 517 nm,

B = absorbance of control at 517 nm.

3.5.4 **Total Phenolic Content**

Procedure of Folin-Ciocalteu that was adopted from Shah (2015) was applied in determining total phenolic contents. Samples were centrifuged at 5000 rpm for 5 minutes at 4°C. A mixture of one mL of juice sample with one mL Folin-Ciocalteau reagent and 10 mL of 20% sodium carbonate was prepared before being diluted to 100 mL with distilled water. The mixture was mixed thoroughly and incubated for one hour at room temperature in the dark prior to the measurement. UV/ Visible Spectrophotometer (Ultraspec 3100 Pro, Amersham Pharmacia Biotech, UK) was then used to measure the absorbance of mixture at 765nm. Data for total phenolic content were obtained from the calibration curve prepared with gallic acid at concentration of 0 to 500 mg/L and are expressed as gallic acid equivalents (GAE).

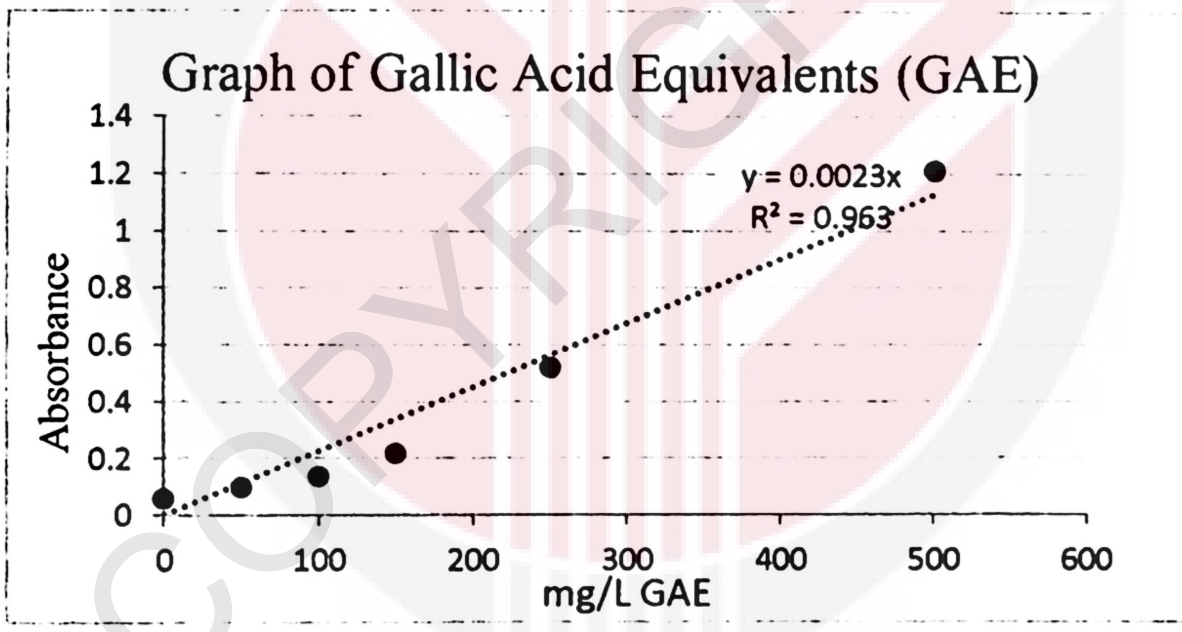


Figure 3.3: Gallic Acid Equivalent Standard Curve (0-500mg/L)

3.6 **Statistical Analysis**

The data collected were used for execution in Design Expert 11.0 Software for further statistical analysis of Response Surface Methodology and colour degradation optimization. Statistical analysis was performed by means of analysis of variance (ANOVA).

An optimal design was selected for RSM option, while numeric factor inserted was 3 levels of -1, 0 and 1. Replicate points and lack-of-fits points inserted was 5 for each and resulted to 15 runs. The experimental data A and B then inserted according to number of run. The design is shown in APPENDIX D.

Subsequently, from data execution in Design Expert 11.0 Software result to ANOVA for response quadratic model. Therefore, the expected empirical model was quadratic model. Refer in each of responses, ANOVA shows the values of model term, of A, B, AB, A^2, A^2B, lack-of-fit, regression coefficient (R^2), Adj. R^2 for p-value and ratios. From analysis of various (ANOVA), the adequacy of the model was determined by evaluating the lack of fit, R^2 and Fisher test value (F-value). Model variables and statistical significance of model were determine at 5% probability level ($p<0.05$). Each model terms explained the degree of how significant they are to the model by showed significant value.

3.7 Experimental Design

In this research study, the statistical data were performed using the response surface methodology (RSM) of Design Expert Software Version 11.0 (Stat – Ease Inc., MN, and USA). This software determined the number of samples based on RSM analysis. RSM methodology in this study was based on a two-factor optimal design with independent variables of ozone concentration (0.84, 0.96, 1.29 mg/min) and type of juice (filtered and unfiltered). Figure 3.2 shows the 15 run of experiment steps with ozone concentration and type of samples as parameters. The treated pink pitaya juice undergoes physicochemical and antioxidant properties analysis by using six responses which are total colour difference (TCD), pH, total soluble solids (TSS), ascorbic acid content, 2,2-diphenyl-1-Picrylhydrazyl radical scavenging (DPPH) and total phenolic content analysis. Experimental data from the optimal design was analysed using response surface regression and repeated three times for each experiment conducted.

Table 3.1: The optimal design of ozone treatment

Run Order	Ozone conc. (mg/min)	Type of juice
1	0.84	Unfiltered
2	0.84	Filtered
3	1.07	Unfiltered
4	0	Filtered
5	0.84	Filtered
6	1.29	Filtered
7	0	Unfiltered
8	0.84	Filtered
9	1.07	Unfiltered
10	0	Filtered
11	0.84	Unfiltered
12	0	Unfiltered
13	1.29	Unfiltered
14	1.07	Filtered
15	1.29	Filtered

CHAPTER 4

RESULT AND DISCUSSION

In this chapter, the results obtained from experiments will be analysed and discussed on the physicochemical properties and antioxidant activity on ozone-treated pink pitaya fruit juice. This chapter consisted of discussion on Statistical Analysis on the Effects of Ozone Treatment on Physicochemical and Antioxidant Properties of Pink Pitaya Juice (Section 4.1) and Optimization of Response Surface Methodology (RSM) (Section 4.2) that elaborated on the optimization of ozone treatment in which all parameters are included to choose the best condition of pink pitaya to be treated.

4.1 Effects of Ozone Treatment on Physicochemical and Antioxidant Properties of Pink Pitaya

In this study, effects of gaseous ozone on the physicochemical properties pink pitaya juices such as colour, pH and total soluble solids to be discussed in details. Furthermore, the antioxidant tests were conducted to determine the ascorbic acid content, DPPH scavenging activity and total phenolic content. Detailed results were recorded in the table below (Table 4.1) for all responses with respects to ozone concentration and type of juice.

Table 4.1: Ozone Treatment Utilizing Optimal design of Response Surface Methodology (RSM).

Run Order	Ozone concentration (mg/min)	Type of Juice	Response 1: TCD*	Response 2: TSS** (%)	Response 3:TPC*** (mg/L GAE)	Response 4: DPPH**** (%)	Response 5: Ascorbic Acid	Response 6: pH
1	0.84	Unfiltered	1.80	11.7	57.39	62.6	9.4	4.86
2	0.84	Filtered	2.46	11	37.39	61.2	7.5	4.36
3	1.07	Unfiltered	1.50	11.5	42.61	42.4	8.7	4.96
4	0.00	Filtered	0.00	11.1	33.04	72.8	11.1	4.36
5	0.84	Filtered	2.40	11	29.13	70.5	7.6	4.38
6	1.29	Filtered	11.67	10.9	19.57	68.4	6.5	4.36
7	0.00	Unfiltered	0.00	11.7	86.09	83.2	11.2	4.94
8	0.84	Filtered	2.70	10.8	23.48	68.4	7.7	4.37
9	1.07	Unfiltered	2.60	11.6	41.74	43.1	8.2	4.92
10	0.00	Filtered	0.00	11.1	35.65	72.5	11	4.37
11	0.84	Unfiltered	1.50	11.2	47.39	70.9	8	4.84
12	0.00	Unfiltered	0.00	11.8	80.87	80.9	11	4.90
13	1.29	Unfiltered	2.00	11.8	36.52	24.5	7.4	4.97
14	1.07	Filtered	10.96	11.1	16.09	55.4	7.1	4.36
15	1.29	Filtered	11.70	11.2	23.04	52.3	6.9	4.37
p-value			< 0.0001	0.0009	< 0.0001	0.0002	< 0.0001	< 0.0001
F-value			35.00	11.89	39.35	20.33	207.09	0.0838
R^2			0.9633	0.8686	0.9563	0.9384	0.9936	0.9971
SD			1.08	0.1566	5.36	5.23	0.1745	0.0204

*Total Colour Difference ** Total Soluble Solids *** Total Phenolic Content **** Scavenging activity

p-value <0.05 is significant F-value <0.05 significant

4.1.2 **Total Colour Difference (TCD)**

Table 4.2: Initial value for untreated sample of filtered and unfiltered pink pitaya juice.

Type of juice	$L_o(\pm 0.1)$	$a_o(\pm 0.1)$	$b_o(\pm 0.1)$
Unfiltered	19.2	7.0	0.1
Filtered	23.39	1.01	-0.82

Total colour difference can be classified into three distinct group which are very distinct (TCD>3), distinct (1.5< TCD<3), and small differences (TCD<1.5) based on Tiwari et al. (2008). From table above shows result on unfiltered pink pitaya analysis for colour degradation between L, a* and b* relation show the total colour difference lies in between 0 to 2.60 where classified under distinct group for unfiltered juice. Hence, ozone-treated sample give an outcome a distinct difference colour of pink pitaya juice. These changes are a function of type of juice and ozone concentration (mg/min). Colour values for unfiltered pink pitaya (untreated) were 19.2 ± 0.1, 7.0 ± 0.1, and 20.4 ± 0.1 shows values denoted for L_o, a_o and b_o respectively. In contrast, for filtered juice the TCD lies in between 2.46 to 11.70. Therefore, this group under very distinct class thus ozone treatment does change the colour of filtered juice very distinctly. L_o, a_o and b_o for filtered juice is much lighter than unfiltered juice which give a result of 23.39 ± 0.1, 1.01 ± 0.1 and -0.82 ± 0.1 respectively. After ozonation, the colour degrade directly proportional with time treatment of ozone. The result were observed to be lighter in colour of filtered fruit juices as give the value of L, b* (yellowness) and a* value increases as ozone concentration increases based on Table 4.1.

Table 4.3: L, a*, b*, hue and chroma values of pink pitaya.

Ozone concentration (mg/min)	Type of juice	L (± 0.1)	a*(± 0.1)	b*(± 0.1)	*TCD
0.84	Unfiltered	19.8	5.5	21.1	1.80

0.84	Filtered	23.95	3.10	0.29	2.46
1.07	Unfiltered	20.1	5.8	20.4	1.50
0	Filtered	23.29	1.01	-0.82	0.00
0.84	Filtered	23.87	3.02	0.35	2.40
1.29	Filtered	31.42	-0.61	7.40	11.67
0	Unfiltered	19.3	6.6	20.4	0.00
0.84	Filtered	23.89	3.52	-0.02	2.70
1.07	Unfiltered	19.8	4.5	20.8	2.60
0	Filtered	23.92	0.99	-0.88	0.00
0.84	Unfiltered	19.8	5.5	21.1	1.50
0	Unfiltered	19.2	7.3	20.4	0.00
1.29	Unfiltered	20.4	5.8	21.4	2.00
1.07	Filtered	28.80	4.15	8.12	10.96
1.29	Filtered	31.32	-0.59	7.52	11.70

Meanwhile, unfiltered juice also show a lighter in colour of juice but L and b* (yellowness) increase while for a* value because it seem to be decrease over ozonation. The correlation of colour degradation might have been the direct effect of total phenolic content and ascorbic acid after being treated with gaseous ozone. Tiwari et al. (2008a), Tiwari et al. (2009), and Torres et al. (2011) have also reported colour degradation on orange, strawberry, and apple juices due to ozone processing.

Figure 4.1 also shows that increment of TCD values were affected by increasing ozone concentration over treatment time. Thus, this finding is coherent to the previous studies as TCD was shown to increase as treatment time and ozone concentration increase due to oxidative cleavage of the chromophores (Nebel, 1975).

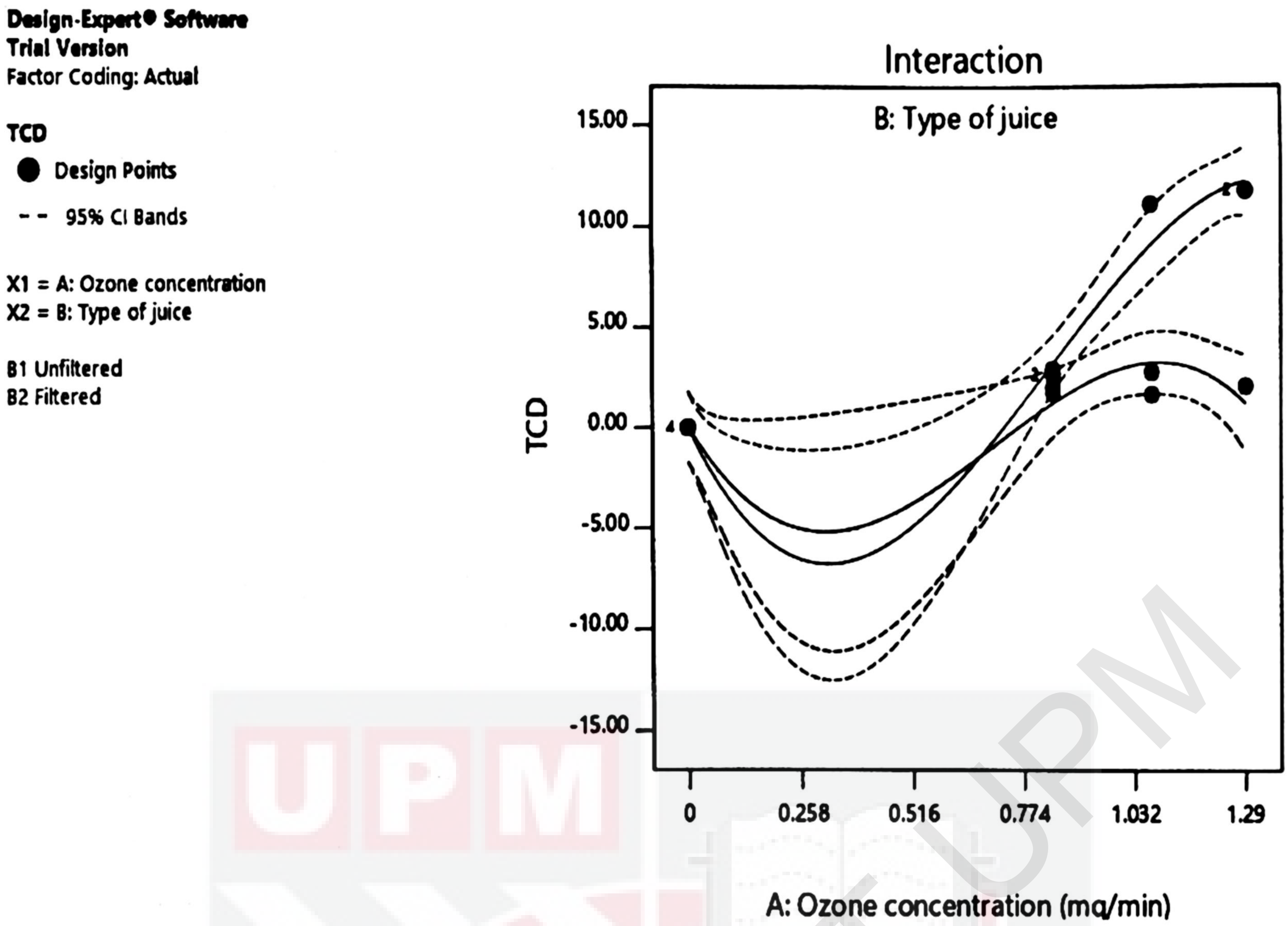


Figure 4.1: Interaction graph of types of juice and ozone concentration (mg/min) on TCD.

Analysis of variance (ANOVA) for response of TCD varied by ozone concentration and type of juice was used to investigate the effect of each independent variables towards forming an empirical model that yield to minimize the colour degradation of pink pitaya and optimization of each independent variables is shown in Table 4.4.

Table 4.4: Analysis of variance (ANOVA) and estimated regression coefficient of TCD for RSM.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	246.05	6	41.01	35.00	< 0.0001*	significant
A-Ozone concentration	14.34	1	14.34	12.24	0.0081*	
B-Type of juice	0.0726	1	0.0726	0.0619	0.8097*	
AB	53.84	1	53.84	45.96	0.0001*	
A^2	17.57	1	17.57	15.00	0.0047*	
A^2B	17.00	1	17.00	14.51	0.0052*	
Residual	9.37	8	1.17			
Lack of Fit	8.67	1	8.67	86.61	< 0.0001***	significant

Pure Error	0.7009	7	0.1001			
Cor Total	255.42	14				
R^2	0.9633					
Adj. R^2	0.9358					

*p-value<0.05 significant

From the ANOVA diagnostic, the model suggested is quadratic with p-value less than 0.0001 which shows that the model is significant, thus the empirical model build is considered as a fit model. Based on Table 4.4, each model terms of A, AB, A^2 and A^2B are significant as presented with p-value are less than 0.1 and can be important factors affecting mathematical model TCD. However, model term of type of juice (B) shown in Table 4.4 is not significant. From all the model terms, this indicated that the effect of ozone concentration, interaction between type of juice and ozone concentration, quadratic equation of ozone concentration and quadratic equation of ozone concentration and type of juice does influenced TCD result. The following equation of model representing the TCD as function of independent variables is expressed in coded factors as follows:

$$Y= -2.19+13A-0.1351B+2.77AB+5.50A^2++2.86A^2B \quad 4.1$$

Where;

A is ozone concentration (mg/min), B is type of juice, and Y is the TCD.

In this case, R^2 obtained is 0.9633; which in range if 0.6 to 1.0. Thus 9.633 indicated the model of TCD formed is fit.

4.1.2 **pH**

An unfiltered pink pitaya juice already has an acidic pH at 4.82 as a control measure before experiment was conducted and filtered pink pitaya juice at 4.37 which was lower than unfiltered juice due to absence of pulp due to filtration process. The changes were recorded in Table 4.1 that showed a slight changes in values of pH for both type of juice which also indicated that the pH was not affected by the ozone treatment ($p > 0.05$). Previous related study have shown that no effect on pH value was found after the ozonation process (Tiwari et al., 2008a; Tiwari et al., 2008b; Tiwari et al., 2009). pH values was shown to range between 4.84 to 4.97 as the highest value of pH from the experiment for unfiltered juice. Whereas, the pH for filtered juice lies in between 4.36 to 4.38. The value at 0 mg/min (control) of ozone concentration was untreated pink pitaya fruit juice.

However, the increased of pH value does occurs after ozonation due to the ascorbic acid degradation and accumulation in the juice. Other research study has also discovered this when gaseous ozone was applied on beetroot juice (Karthivaran et. al., 2014).

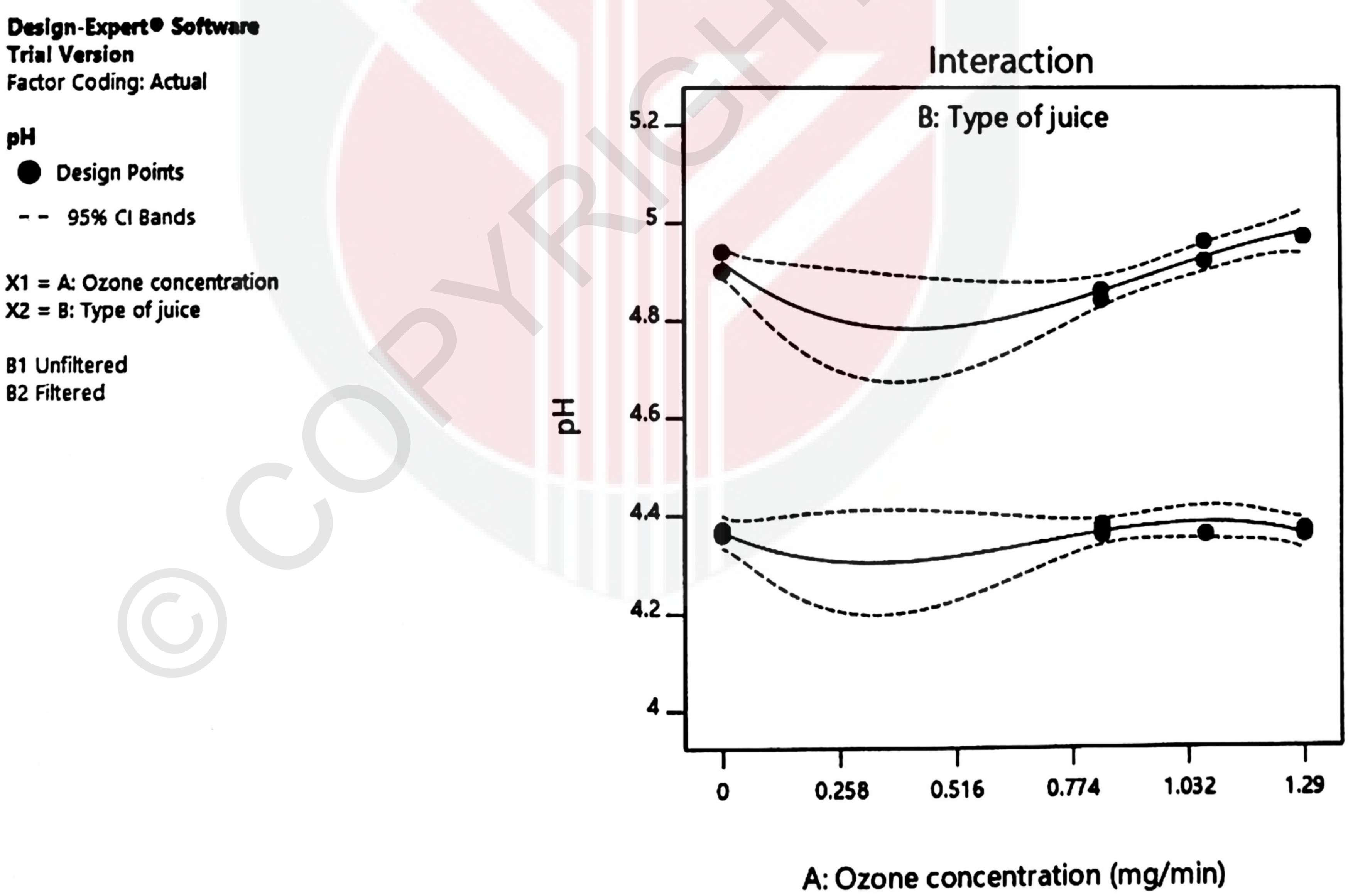


Figure 4.2: Interaction graph of types of juice and ozone concentration (mg/min) on pH.

Analysis of variance (ANOVA) for response pH varied by ozone concentration and type of juice was used to investigate the effect of each independent variables from pink pitaya fruit juice is shown in Table 4.5.

Table 4.5: Analysis of variance (ANOVA) and estimated regression coefficient of pH for RSM.

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	1.13	6	0.1878	452.94	< 0.0001*	significant
A-Ozone concentration	0.0012	1	0.0012	2.92	0.1261*	
B-Type of juice	0.2214	1	0.2214	533.91	< 0.0001*	
AB	0.0019	1	0.0019	4.68	0.0626*	
A^2	0.0043	1	0.0043	10.38	0.0122*	
A^2B	0.0069	1	0.0069	16.67	0.0035*	
Residual	0.0033	8	0.0004			
Lack of Fit	0.0012	1	0.0012	4.06	0.0838***	not significant
Pure Error	0.0021	7	0.0003			
Cor Total	1.13	14				
R^2	0.9971					
Adj. R^2	0.9949					

*p-value<0.05 significant

From the ANOVA diagnostic, the model suggested is quadratic model with p-value less than 0.0001which shows that the model is significant, thus the empirical model build is considered as a fit model. Based on Table 4.5, each model terms of B, AB, $A^2$and A^2B are considered significant as presented p-value are less than 0.1 and can be important factors affecting mathematical model pH. However, model term of ozone concentration (A) shown in Table 4.5 is not significant since the p-value is higher than 0.1. From all the model terms, this indicate the effect of type of juice, interaction between type of juice and ozone concentration, quadratic equation of ozone concentration and quadratic equation of ozone concentration and type of juice does influence the pH value. The following equation of model representing the pH as function of independent variables is expressed in coded factors as follows:

$$Y= +4.57+0.1194A-0.2360B-0.0166AB+0.0861A^2-0.0576A^2B \quad 4.2$$

Where;

A is ozone concentration (mg/min), B is type of juice, and Y is the pH.

In this case, R^2 obtained is 0.9971; which in range if 0.6 to 1.0. Thus 9.971 indicates the model of pH formed is fit.

4.1.3 **Total Soluble Solid (TSS)**

The presence of solids such as sucrose, fructose, acids, and mineral. Based on Table 4.1, the results have shown that TSS was highly influenced by the ozone concentration and type of juice ($p < 0.05$). In addition to that, TSS might also been influenced by the concentration of unfiltered juice as juice that contains coagulated pulp tends to have a higher percentage of Brix (Qiao et al., 2010).

The result also showed that Brix values of unfiltered pink pitaya samples lies in range of 11.5 to 11.8 which the highest TSS recorded (Figure 4.3). For filtered juice has a slightly lower value of TSS which in range of 10.8 to 11.2. The Brix percentage in each of sample had no significant changes after the juice undergoes ozonation process. This is related to the breakdown of polysaccharides into smaller molecule such monosaccharide or disaccharide by depolymerisation process during ozone. According to Patil and Bourke (2012), the load of organic matter with added D-glucose in raw water purification reduced the effectiveness of ozone. Thus, at 15 minutes ozone treated pink pitaya juice show a highest increment in degree of Brix (13.8) as result from rich in pulp content for the first sample treated which leads to fluctuate from predicted model of straight line.

Based on Table 4.6 both factors of ozone concentration and time processing do not affect TSS of pink pitaya juice. Tiwari et al. (2008b), Tiwari et al. (2009) and Choi and Nielsen (2005) found no changes in pH, Brix and titratable acidity in fruits juice after ozone for orange juice, grape juice and apple cider respectively.

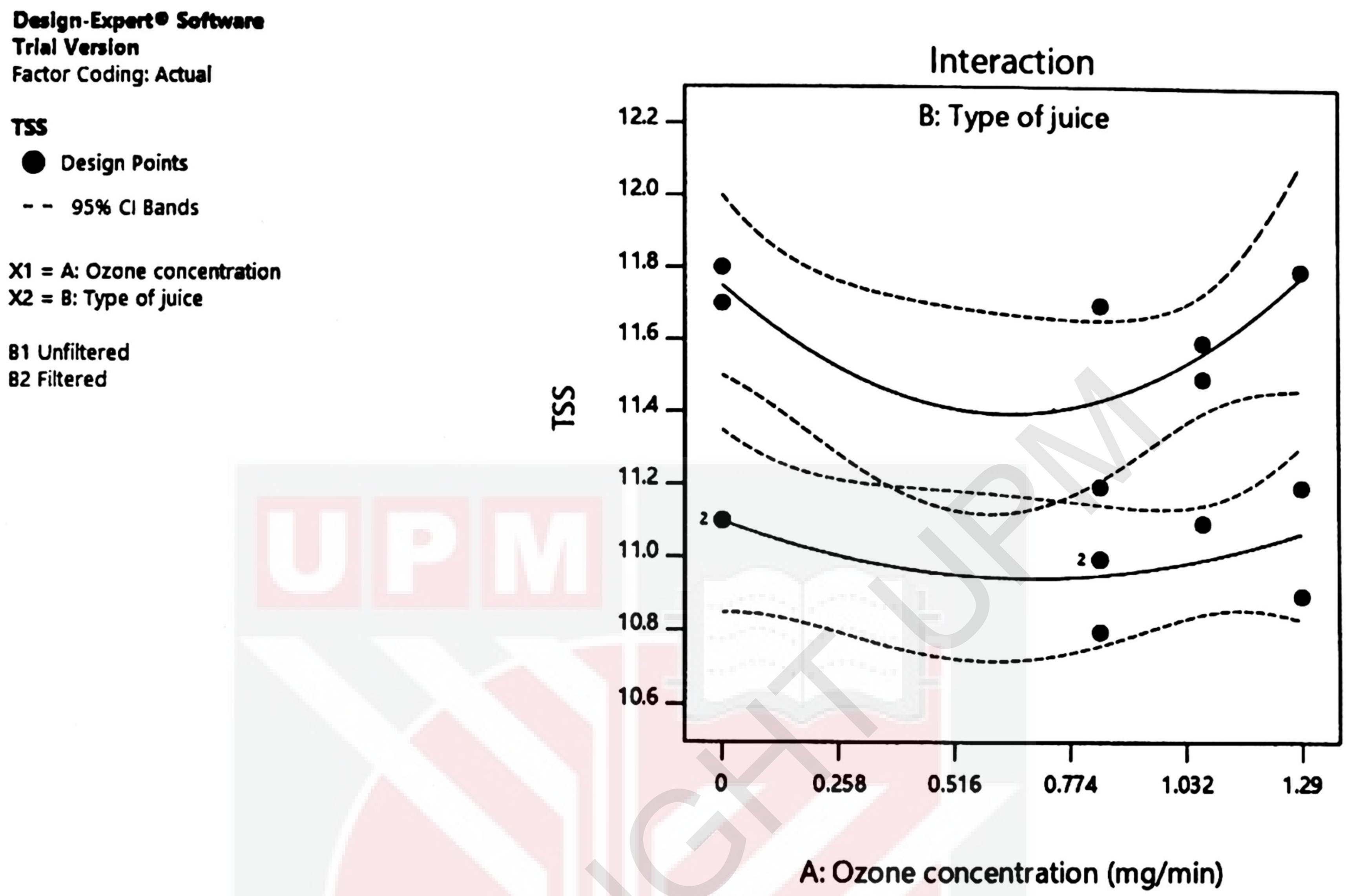


Figure 4.3: Interaction graph of types of juice and ozone concentration (mg/min) on TSS.

Both factors were plotted against TSS (Brix %) of unfiltered and filtered juice. The interaction graphs illustrate that, as the ozone concentration increase, TSS value slightly increases for unfiltered juice. However, for filtered juice show no significant changes over ozone concentration increase. The changes is not really noticeable as the ozone treatment used to be found that has no effect on TSS as mention above.

Table 4.6: Analysis of variance (ANOVA) and estimated regression coefficient of TSS for RSM.

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	1.46	5	0.2918	11.89	0.0009*	significant
A-Ozone concentration	0.0000	1	0.0000	0.0008	0.9783*	
B-Type of juice	0.2063	1	0.2063	8.41	0.0176*	

AB	0.0015	1	0.0015	0.0623	0.8085*	
A²	0.1338	1	0.1338	5.45	0.0443*	
A²B	0.0273	1	0.0273	1.11	0.3186*	
Residual	0.2208	9	0.0245			
Lack of Fit	0.0142	2	0.0071	0.2397	0.7931***	not significant
Pure Error	0.2067	7	0.0295			
Cor Total	1.68	14				
R^2	0.8686					
Adj. R^2	0.7955					

*p-value<0.05 significant

From ANOVA diagnostic, the model suggested is quadratic model with p-value less than 0.0001. Based on Table 4.6, model term of ozone concentration (A) and AB shown are not significant since the p-value is higher than 0.1. Thus, validating the results shown in Figure 4.3, that only the type of juice had the significant effect on the TSS of pink pitaya. The following equation of model representing the TSS as function of independent variables is expressed in coded factors as follows:

$$Y= +11.17+0.0016A-0.2267B-0.0146AB+0.2534A^2-0.1145A^2B \quad 4.3$$

Where;

A is ozone concentration (mg/min), B is type of juice, and Y is the TSS.

The fitness of the model is assured by ratios of R^2, Adj. R^2 are used for verification. In this case, R^2 obtained is 0.8686; which in range if 0.6 to 1.0. Thus 8.686 indicates the model of TSS formed is fit.

4.1.4 **Ascorbic Acid**

Figure 4.4 show the effect of ascorbic acid after ozone in progress with different type of juice treated. From Table 4.1, the ascorbic acid content in unfiltered pink pitaya juices lies in range of 7.9 to 11.2 mg/100 mL which was the highest value of ascorbic acid content for the control sample. The ascorbic acid content for filtered juice was found to be lower, between 6.5 to 11.1 mg/100 mL. This might be caused by the unfiltered juice has rich in pulp as it is related to higher ascorbic acid content. According to Nerd et al. (1999) and Islam et al. (2012) state that red-fleshed dragon fruit has ascorbic ranging from 7.00 to 11.4 mg/100 mL. Thus, this illustrated significant degradation of ascorbic acid in pink pitaya juice over ozone concentration. The content of ascorbic acid in pink pitaya juice sample has been studied by several researches where this fruits kind of relatively new to Bangladesh country (Islam et al., 2012). The studies had showed that red dragon fruit weighing up to 1 kg, is a rich source of nutrients and minerals such as vitamin B1, vitamin B2, vitamin B3 and vitamin C, protein, fat, carbohydrate, crude fiber, flavonoid, thiamin, niacin, pyridoxine, kobalamin, glucose, phenolic, betacyanins, polyphenol, carotene, phosphorus, iron and phytoalbumin (Bellec et al., 2006 and Wu et al., 2006).

The ascorbic acid content shown in Figure 4.4, illustrated a decreasing trend of ascorbic acid as the ozone concentration increased. From the graph shown, an ascorbic acid at 0 mg/min (ozone concentration) which was a control of untreated pink pitaya juice. At 0.84 mg/min ozone treated show a degradation of ascorbic acid of unfiltered juice from 11.2 mg/100 mL to 9 mg/100 mL followed by decrease in amount of ascorbic acid further to 7.9 mg/100 mL.

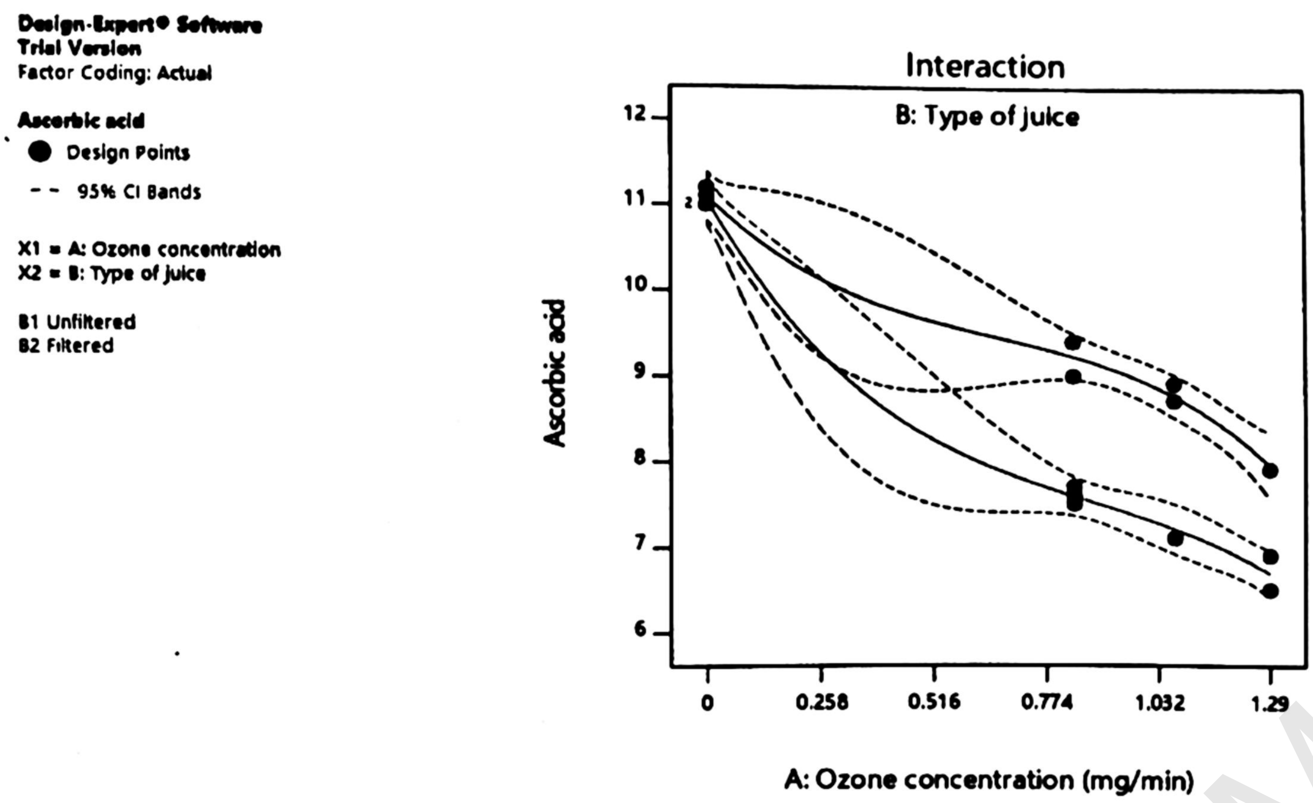


Figure 4.4: Interaction graph of types of juice and ozone concentration (mg/min) on ascorbic acid.

Furthermore, at 0.84 mg/min treatment of ozone filtered pink pitaya juice give a result of further degradation of ascorbic acid from 11.1 mg/100 mL (untreated filtered juice) to 7.5 mg/100 mL.

Degradation of anthocyanins or ascorbic acid might be caused by oxidation either by direct interaction with ozone or formation of various intermediate radicals which causes to electrophilic or nucleophilic reactions occurring with the aromatic compounds, thus resulting in chemical reactions involving breakage of old bonds and formation of new bonds (Cullen et al., 2009). Studies have also reported that degradation of ascorbic acid was due to the presence of oxygen (Kennedy et al., 1992; Samaniego-Esguerra et al., 1991; Zimeri & Tong, 1999). These findings convey that ascorbic reduced as being exposed to the presence of oxygen during ozonation, since an ozone gas react to form high relative species such as OH ion, HO^{2-} ion, $O2^{1}$ ion, and $O3^{-}$ion which helps in further degradation of ascorbic acid content in ozone-treated pink pitaya.

Analysis of variance (ANOVA) for response ascorbic acid varied by ozone concentration and type of juice is shown in Table 4.7 below.

Table 4.7: Analysis of variance (ANOVA) and estimated regression coefficient of Ascorbic acid for RSM.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	38.28	5	7.66	48.29	< 0.0001	Significant
A-Ozone concentration	27.55	1	27.55	173.78	< 0.0001	
B-Type of juice	1.39	1	1.39	8.75	0.0160	
AB	0.3269	1	0.3269	2.06	0.1848	
A^2	0.2160	1	0.2160	1.36	0.2731	
A^2B	0.2623	1	0.2623	1.65	0.2304	
Residual	1.43	9	0.1585			
Lack of Fit	0.1967	2	0.0983	0.5596	0.5950	not significant
Pure Error	1.23	7	0.1757			
Cor Total	39.70	14				
R^2			0.9641			
Adj. R^2			0.9441			

*p-value<0.05 significant

From ANOVA diagnostic, the model suggested is quadratic model with p-value less than 0.0001 which shows that the model is significant. Based on Table 4.7, only model terms of ozone concentration (A), type of juice (B) are considered significant as presented p-value are less than 0.1 and can be important factors affecting mathematical model ascorbic acid. However, the model of AB, $A^2$and A^2B are not significant which p-value larger than 0.05.

Final equation in terms of coded factors:

$$Y=+8.72-1.10A-0.7735B-0.0336AB+0.4765A^2+0.4472A^2B \quad 4.4$$

Where; A is ozone concentration (mg/min), B is type of juice, and Y is the Ascorbic acid content.

The fitness of the model is assured by ratios of R^2, Adj. R^2 are used for verification. In this case, R^2 obtained is 0.9641; which in range if 0.6 to 1.0. Thus 9.641 indicates the model of ascorbic acid formed is fit.

4.1.5 2,2-Diphenyl-1-Picrylhydrazyl radical scavenging (DPPH)

DPPH of pink pitaya sample juices shows significant changes after ozone treatment on filtered and unfiltered pink pitaya juice. The results lies in between 24.5 to 83.2 % of DPPH recorded for unfiltered juice and filtered juice show a scavenging activity from 53.2 to 72.5%. Based on graphs plotted (Figure 4.5), DPPH (%) against ozone concentration and type of juice were found to decrease from 83.2% to 62.6% at 0.84 mg/min treatment of ozone. The degradation of DPPH continued at higher ozone concentration 1.07 mg/min which show a huge in percentage difference between 0.84 mg/min ozone treated and 1.07 mg/min, dropping from 62.6% to 43.1%. This might be caused from human error during handling the experiment as time is a crucial measure to preserve reaction of free radicals of DPPH.

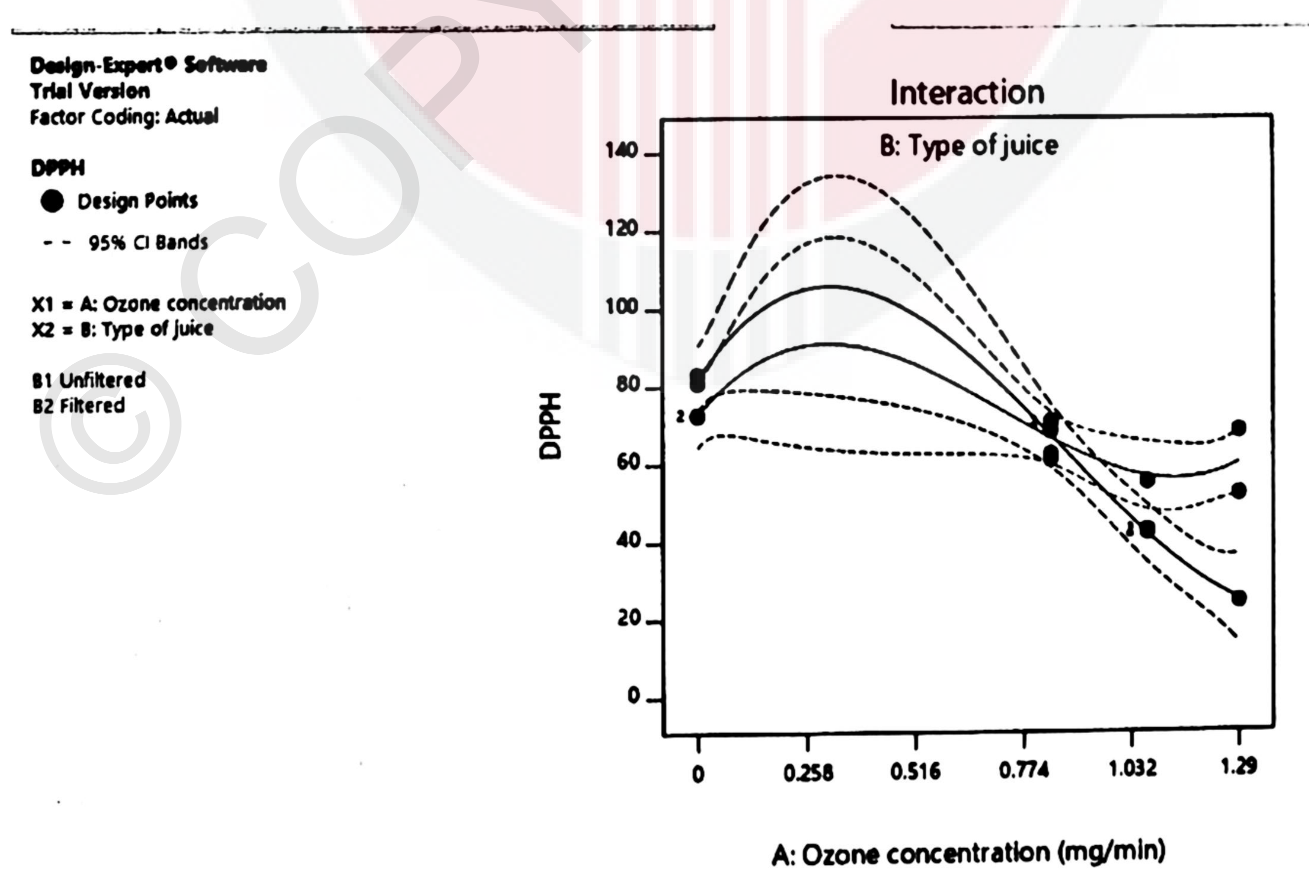


Figure 4.5: Interaction graph of types of juice and ozone concentration (mg/min) on scavenging activity.

As the ozone concentration increased at 1.29 mg/min, DPPH resulted in a reduction up to 24.5 %. The total antioxidant activity from DPPH assay shows a high correlation with total phenolic content. These results suggested that total phenolic content may have an influence on DPPH assay results. Many of studies reported a relationship between total phenolic content and antioxidant activity. Furthermore, some of them recorded a high correlation between total phenolic content with antioxidant activity (Kuskoski et al., 2005 (blackberry, grape, strawberry and guava); Mahattanatawee et al., 2006 (14 fresh fruits such as apple, banana, mango, pineapple, grape, and guava); Reddy et al., 2010; Silva et al., 2007 (*Eugenia patrisii* Vahl. Fruit and *Inga edulis* Mart); Thaipong et al., 2006 (guava fruit)).

Analysis of variance (ANOVA) for response of DPPH varied by ozone concentration and type of juice was used to investigate the effect of each independent variables is shown in Table 4.8 below.

Table 4.8: Analysis of variance (ANOVA) and estimated regression coefficient of DPPH for RSM.

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	3334.40	6	555.73	20.33	0.0002*	significant
A-Ozone concentration	213.39	1	213.39	7.80	0.0234*	
B-Type of juice	90.05	1	90.05	3.29	0.1071*	
AB	861.14	1	861.14	31.50	0.0005*	
A^2	313.08	1	313.08	11.45	0.0096*	
A^2B	259.80	1	259.80	9.50	0.0151*	
Residual	218.74	8	27.34			
Lack of Fit	4.17	1	4.17	0.1361	0.7231***	not significant
Pure Error	214.57	7	30.65			
Cor Total	3553.14	14				
R^2	0.9384					
Adj. R^2	0.8923					

*p-value<0.05 significant

From ANOVA diagnostic, the model suggested is quadratic model with p-value less than 0.0001. Thus, showing the model is significant, and the empirical model build is considered as a fit model. Based on table 4.8, each model terms of A, AB, A^2 and A^2B are considered significant as presented p-value are less than 0.1 and can be important factors affecting mathematical model DPPH. However, model term of type of juice (B) shown is not significant since the p-value is higher than 0.1.

Final equation in terms of coded factors:

$$Y=+83.17-50.14A-4.76B+11.07AB-23.21A^2+11.16A^2B \quad 4.5$$

Where A is ozone concentration (mg/min), B is type of juice, and Y is the DPPH.

The fitness of the model is assured by ratios of R^2, Adj. R^2 are used for verification. In this case, R^2 obtained is 0.9384; which in range if 0.6 to 1.0. Thus 9.384 indicates the model of DPPH formed is fit.

4.1.6 Total Phenolic Content (TPC)

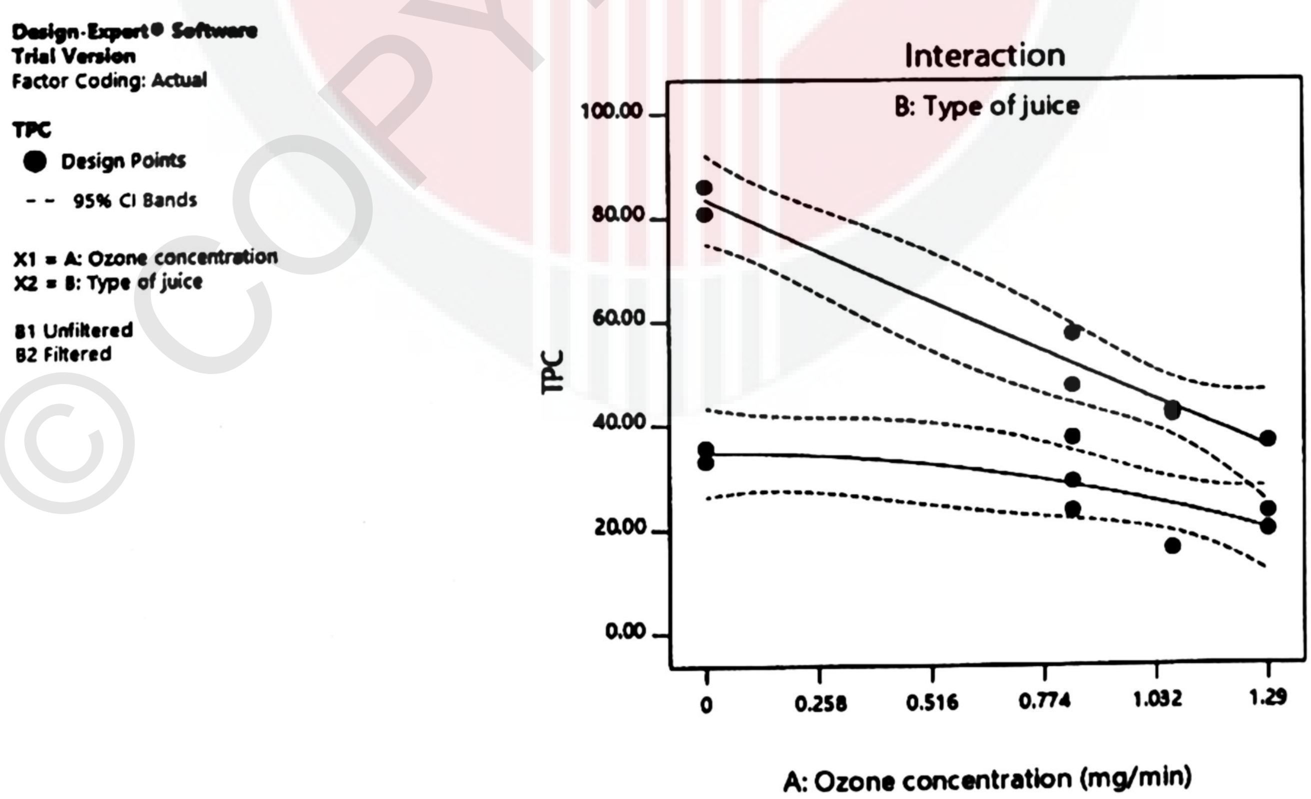


Figure 4.6: Interaction graph of types of juice and ozone concentration (mg/min) on TPC mg/L GAE.

In studies, implication of ozone treatment does have an effect on TPC of apple juice in overall structure over ozone concentration (Torres et al., 2011). Figure 4.6 shows graph plots for unfiltered and filtered pink pitaya juice and total phenolic content with respect to ozone concentration. Clearly, the TPC of ozone-treated pink pitaya juice appeared to be decreased as the ozone concentration increased. It was found that similar results of reduction in TPC over ozone treatment from other researches on strawberry and blackberry fruits such as Tiwari et al. (2009a) and Tiwari et al. (2009b), respectively. At 0.84 mg/min treatment, TPC start to reduce from 86.09 mg/L GAE to 57.39 mg/L GAE for different sample undergoes ozone of unfiltered juice. TPC at 1.07 mg/min ozone treatment decreased to 42.61mg/L GAE from 57.39 mg/L GAE. Furthermore, the ozone concentration at 1.29 mg/min treatment on unfiltered pink pitaya juices illustrated reduction to 36.52 mg/L GAE. The decreased of TPC of ozone-treated pink pitaya juice might be due to long storage time before performing this analysis. For filtered juice, the same trend of decreasing in TPC content over ozone concentration has been recorded that starts at 0 mg/min (control) which have TPC content at 35.65 mg/L GAE to 23.48 mg/L GAE at 0.84 mg/min. At 1.29 mg/min of ozone concentration, filtered juice again reduced to 23.04 mg/L GAE.

Therefore, a significant reduction of TPC observed as the ozone concentration increases over time. A strong potential of ozone most likely effect the degradation of TPC content in pink pitaya juice. Total phenolic content of pink pitaya evaluated shows a higher value than other finding with 24.22 ± 0.95 mg/100g GAE for fresh pulp of *H. polyrhizus* reported by Wee et al. (2011). The different in result might be caused by different methods used for determining TPC values and different sample preparation of fruit juice extracted by ethanol.

Table 4.9 below shows analysis of variance (ANOVA) for response TPC varied by ozone concentration and type of juice used to investigate the effect of each independent variables towards forming an empirical model that yield to a range of TPC for pink pitaya juice and optimization of each independent variables.

Table 4.9: Analysis of variance (ANOVA) and estimated regression coefficient of TPC for RSM.

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	5650.39	5	1130.08	39.35	< 0.0001*	Significant
A-Ozone concentration	1781.70	1	1781.70	62.04	< 0.0001*	
B-Type of juice	783.92	1	783.92	27.30	0.0005*	
AB	489.90	1	489.90	17.06	0.0026*	
A^2	5.29	1	5.29	0.1843	0.6778*	
A^2B	10.71	1	10.71	0.3730	0.5565*	
Residual	258.45	9	28.72			
Lack of Fit	87.09	2	43.54	1.78	0.2374***	not significant
Pure Error	171.36	7	24.48			
Cor Total	5908.83	14				
R^2	0.9563					
Adj. R^2	0.9320					

*p-value<0.05 significant

Based on Table 4.9, each model terms of A, B, AB, $A^2$and A^2B are considered significant as presented p-value are less than 0.1 and can be important factors affecting mathematical model TPC. From all the model terms, this indicate the effect of ozone concentration, effect of type of juice, interaction between type of juice and ozone concentration does influence to TPC result.

The following equation of model representing the DPPH as function of independent variables is expressed in coded factors as follows:

Final equation in terms of coded factors:

$$Y=+44.89-15.74A-13.97B+8.26AB+-1.59A^2-2.27A^2B \quad (4.6)$$

Where; A is ozone concentration (mg/min), B is type of juice, and Y is the TPC.

The fitness of the model is assured by ratios of R^2, Adj. R^2 are used for verification. In this case, R^2 obtained is 0.9563; which in range if 0.6 to 1.0. Thus 9.563 indicated the model of TPC formed is fit.

4.2 **Optimization of Response Surface Methodology (RSM)**

A decline in lightness (L value) has been reported by Labuza and Baisier (1992) that associated with browning in fruit effect. In addition, minimising colour degradation and browning in juice, the optimisation of non-thermal processing parameters is required. This optimisation processing parameter of pink pitaya has been shown by Figure 4.7 that was analysed by Design Expert 11.0 Software. According to Optimal Design model, the optimal ozone condition to obtain maximum colour of pink pitaya were determined by Response Surface Methodology (RSM) as follows ozone concentration at 0.84 mg/min and used of unfiltered juice. Under this condition, pink pitaya juice colour can be determined at 1.02 of TCD which the lowest value obtained with desirability is equals to 0.913. The suitability of the optimized conditions for predicting the optimum response values was tested using the selected optimal conditions. Additional response was also optimised regarding the colour as important parameter to be minimised. The response such TSS was obtained is 11.4 and it also shows a high pH values of pink pitaya juice determination at 4.86. However, other parameters of antioxidant predicted to be in middle of range, as DPPH (scavenging activity) percentage at 67.19% which is consider as good power of antioxidant to avoid from free radical reaction. In addition, ascorbic acid also lied at high of range which is 9.22 mg/100 mL. For TPC, the result of optimisation shows the highest values at 51.67 mg/L GAE of pink pitaya juice.

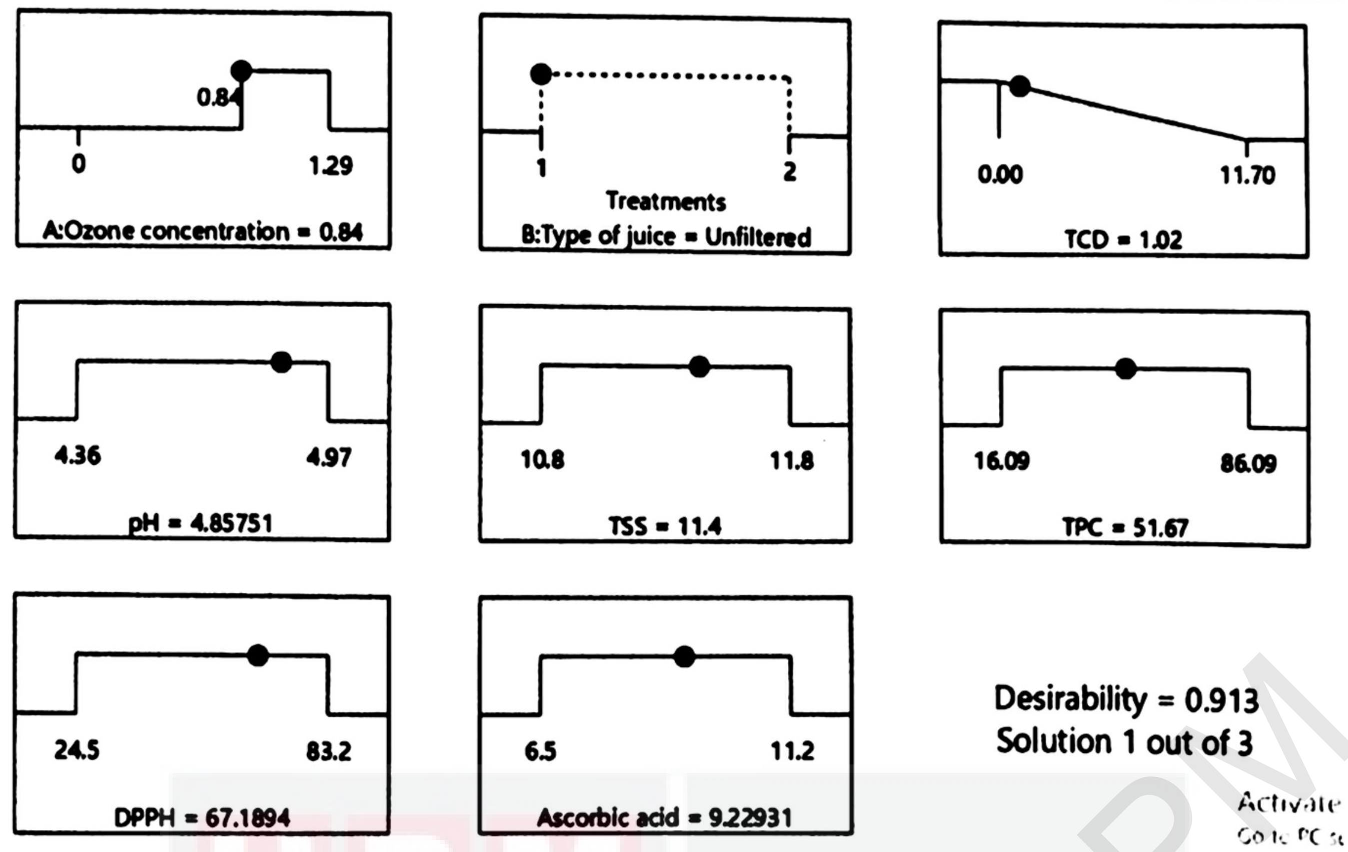


Figure 4.7: Optimisation of pink pitaya parameters after ozonation using Design Expert 11.0 Software

CHAPTER 5

CONCLUSION AND FUTURE RECOMMENDATION

5.1 Conclusion

In conclusion, total colour difference (TCD) values were found to be insignificantly affected for unfiltered juice meanwhile, the degradation of colour in filtered juice was found to be significant over increasing ozone concentration which has resulted in the juice to become lighter. However, pH and TSS values indicated that the gaseous ozone did not significantly affect both type of juices. Whereas, ascorbic acid illustrated a significant decreased with type of juice and ozone concentration as factors of degradation. Total phenolic content (TPC), meanwhile, has shown that both factors (ozone concentration and type of juice) indicated effects towards pink pitaya juice and the results correlated with DPPH scavenging activity significantly.

5.2 Recommendation of future work

Further approach of new parameters can be investigate to optimize the suitable condition that can be applied on pink pitaya juice with different ozone concentrations, time processing, and other antioxidant analysis. In addition, non-thermal hurdle technology with combination of ozone treatment can also be explored to determine the effects of physicochemical and antioxidant properties of tropical fruit juices in future work.

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APPENDICES

APPENDIX A: Preparation of filtered and unfiltered Pink Pitaya juice.

Figure I: Pink Pitaya peel's was removed.

Figure II: Pink Pitaya fruits were cut.

Figure III: Pink pitaya fruits was undergoes cold pressed extraction to produce juice.

Figure IV: Filtered juice for untreated ozone.

APPENDIX B: Filtered and unfiltered juice undergoes ozone treatment.

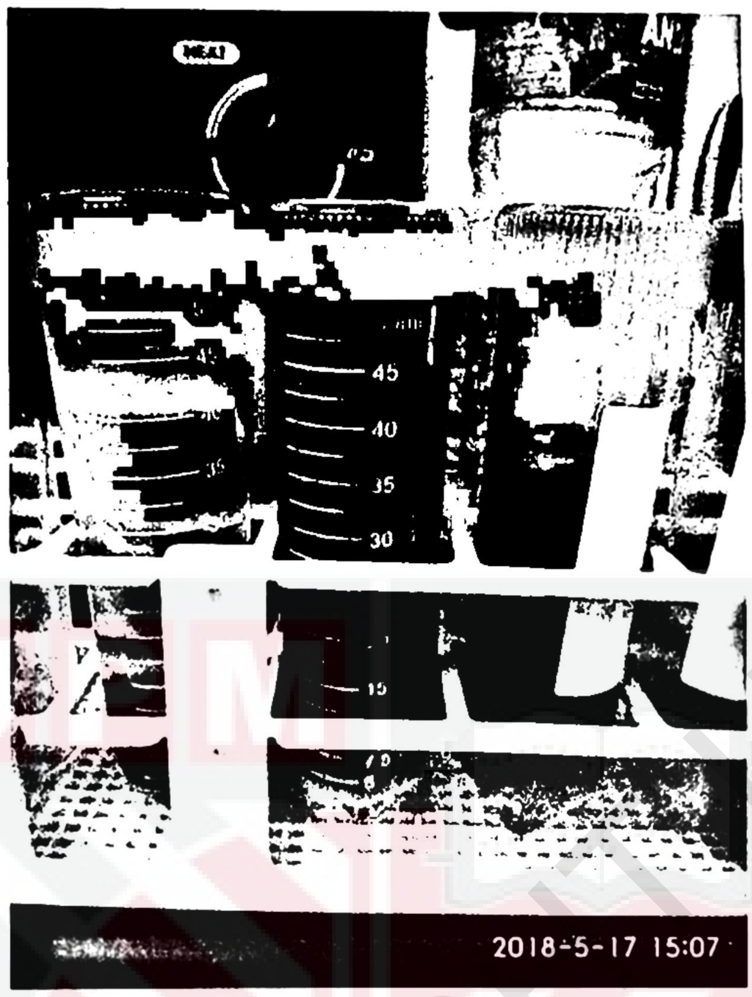


Figure I: Filtered juice after ozone treatment at different ozone concentration.

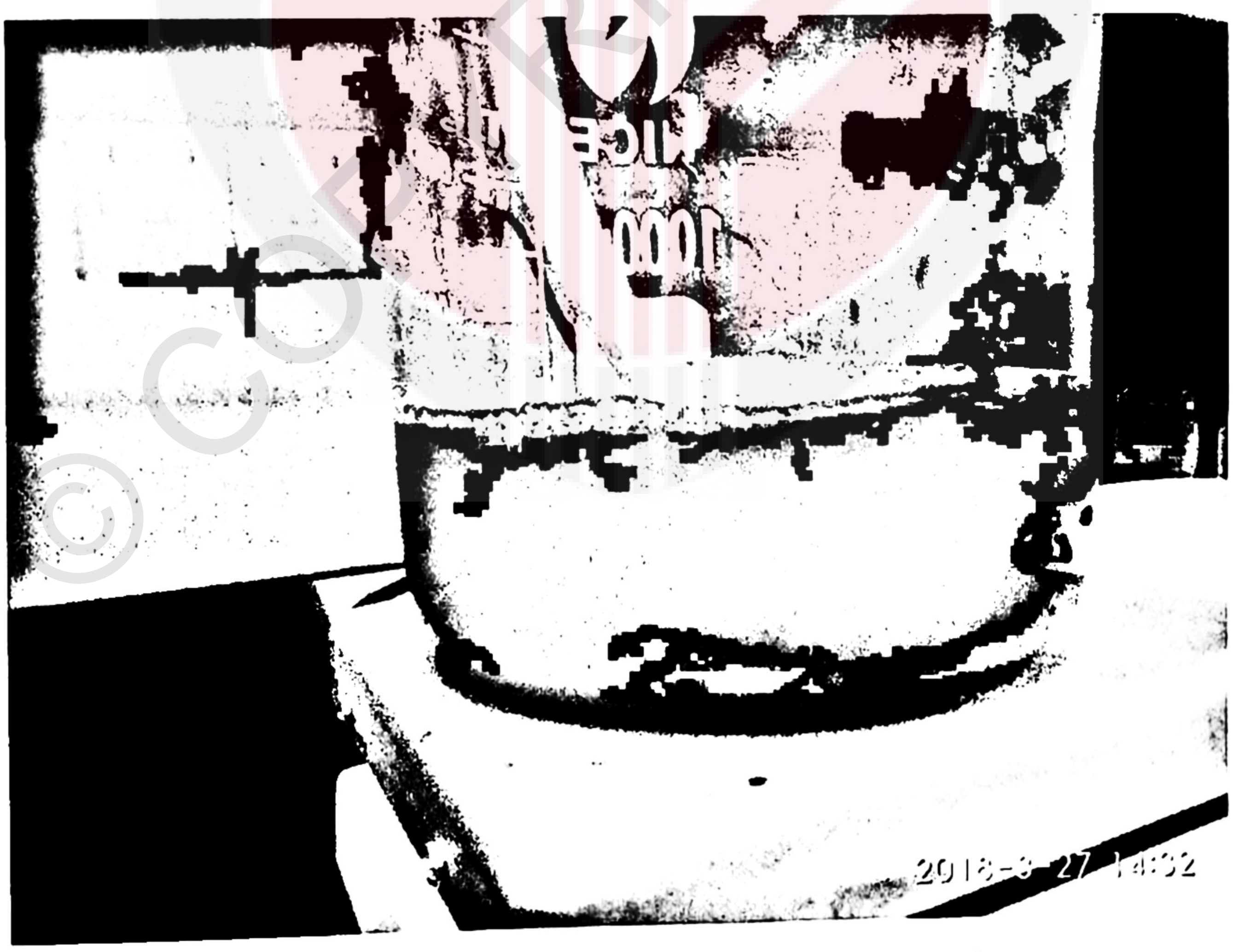


Figure II: Ozone treatment on unfiltered pink pitaya.

Figure III: Colour changes of untreated pink pitaya juice after ozone at 1.29 mg/min treatment.

Figure IV: Creation of bubble in pink pitaya juice after ozone.

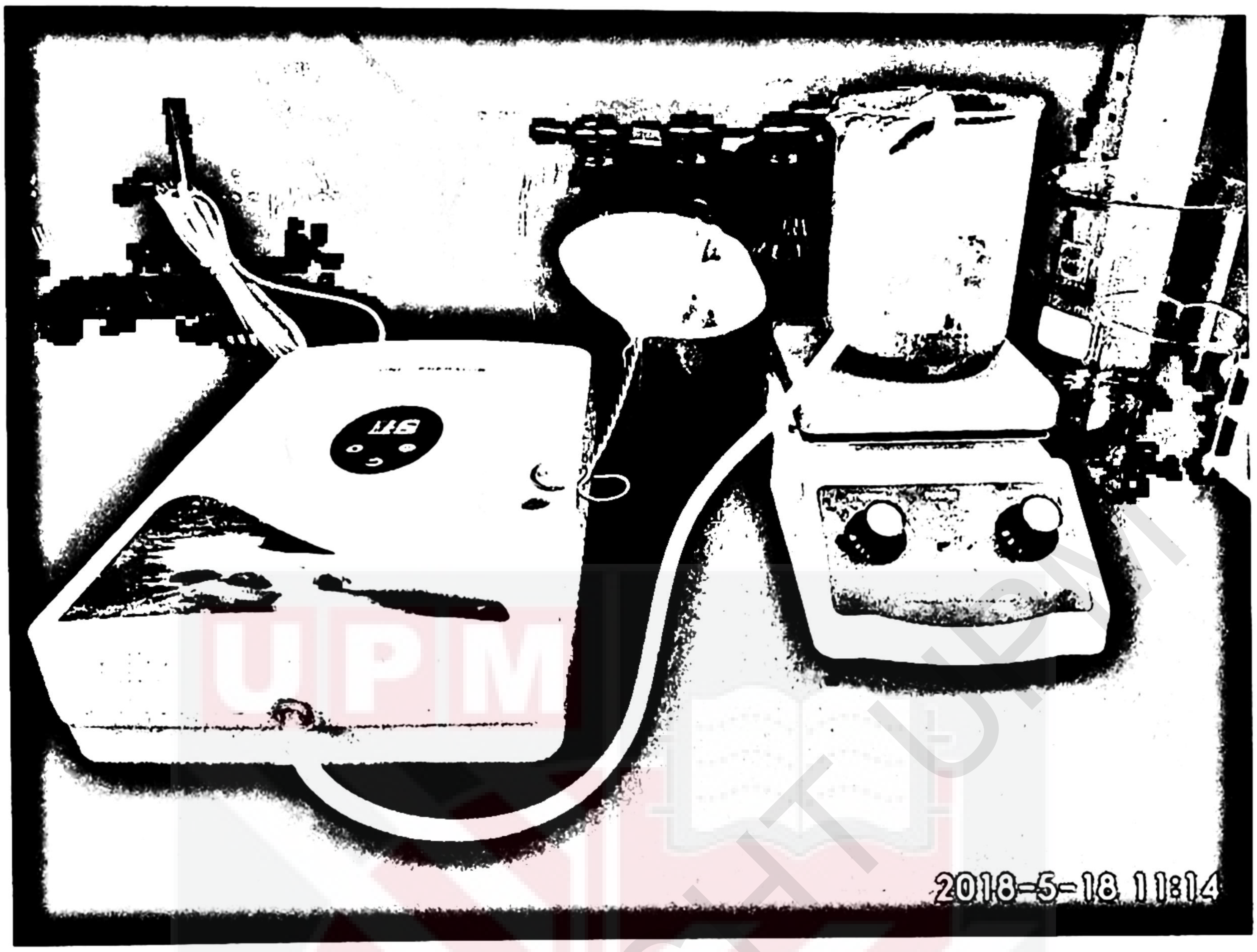


Figure V: Ozone treatment on filtered juice.

APPENDIX C: Physicochemical and antioxidant test.

Figure I: Ozone concentration test was conducted.

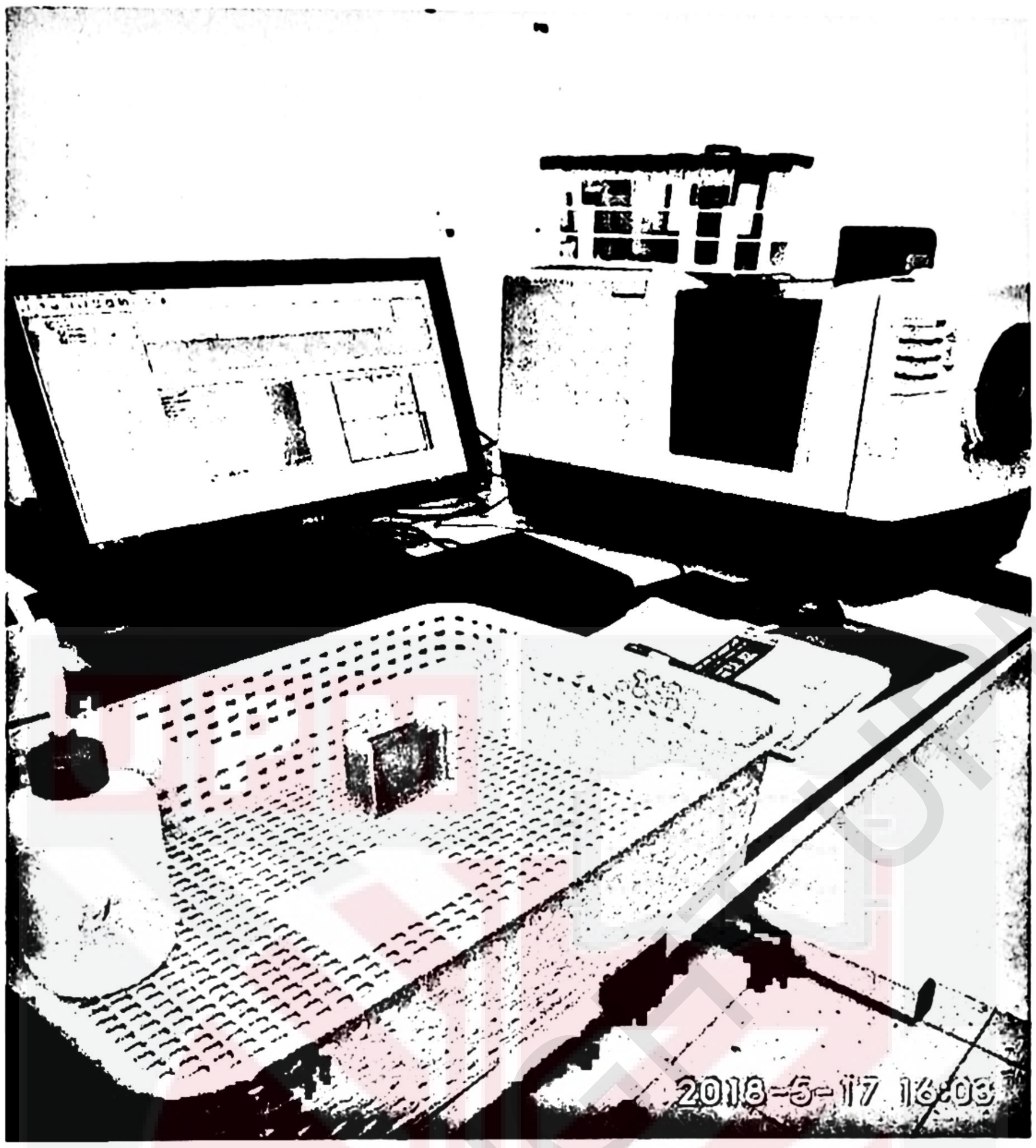


Figure II: Colour test by using colour spectrophotometer of Hunter Lab Ultra Scan Pro (D65, 28 Hunter Lab Assoc Lab Inc, Reston, VA, USA for filtered juice.

Figure III: Pocket refractometer used to determine Brix.

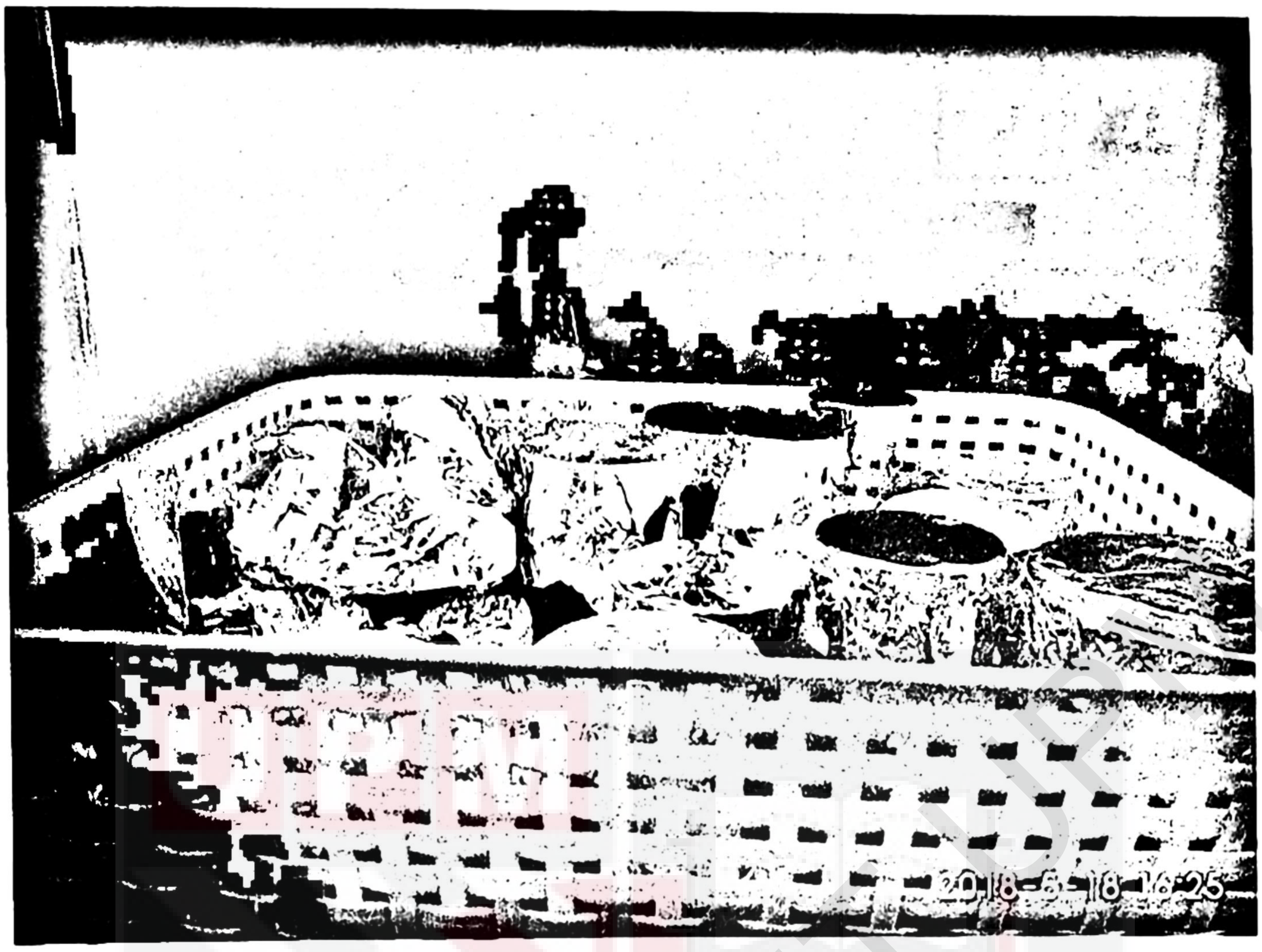


Figure IV: Total phenolic content experiment was incubated for 1 hour.

Figure V: Inserting solution into cuvette of total phenolic content after incubation period.

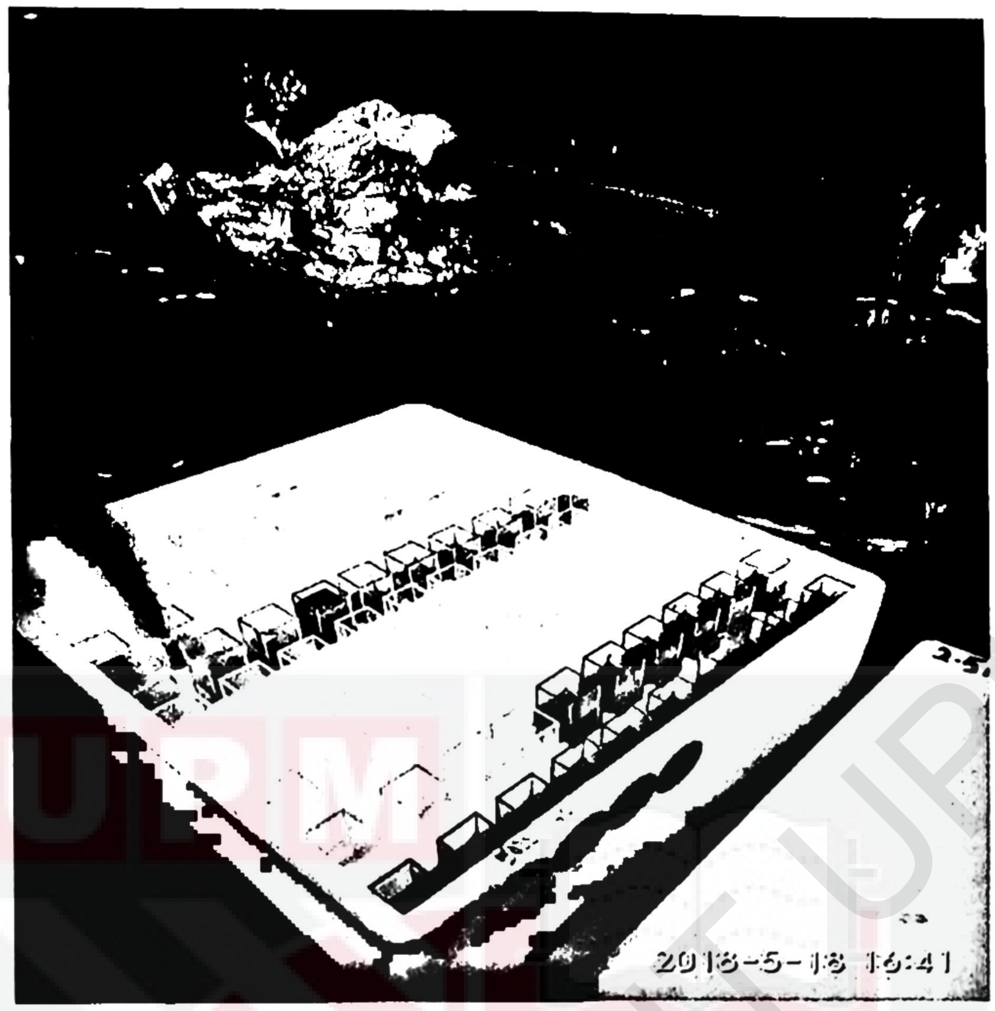
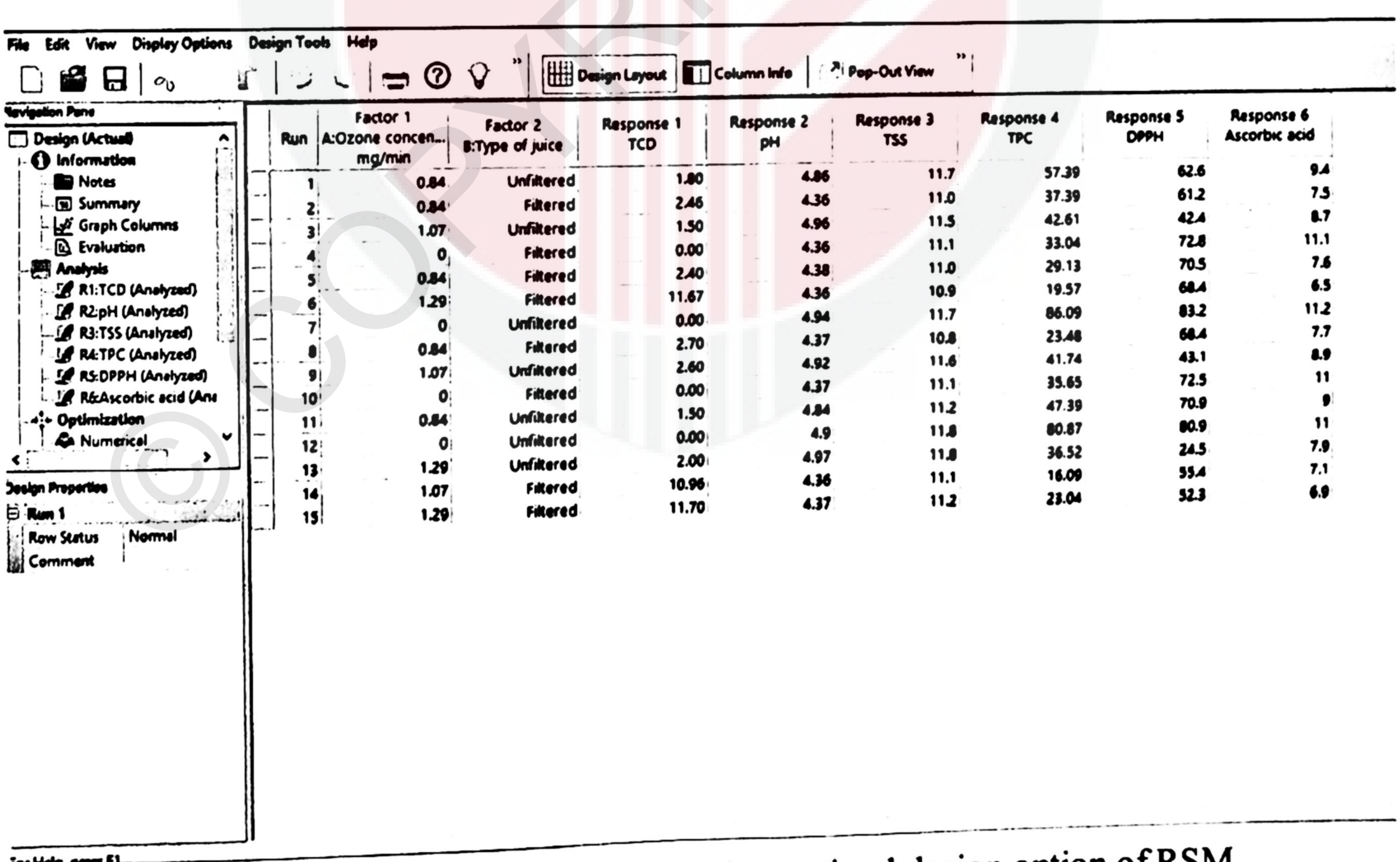


Figure VI: DPPH and total phenolic content solutions were prepared to measure their absorbance.

APPENDIX D: Optimal Design, Response Surface Methodology.



Run	Factor 1 A:Ozone concen... mg/min	Factor 2 B:Type of juice	Response 1 TCD	Response 2 pH	Response 3 TSS	Response 4 TPC	Response 5 DPPH	Response 6 Ascorbic acid
1	0.84	Unfiltered	1.80	4.86	11.7	57.39	62.6	9.4
2	0.84	Filtered	2.46	4.36	11.0	37.39	61.2	7.5
3	1.07	Unfiltered	1.50	4.96	11.5	42.61	42.4	8.7
4	0	Filtered	0.00	4.36	11.1	33.04	72.8	11.1
5	0.84	Filtered	2.40	4.38	11.0	29.13	70.5	7.6
6	1.29	Filtered	11.67	4.36	10.9	19.57	68.4	6.5
7	0	Unfiltered	0.00	4.94	11.7	86.09	83.2	11.2
8	0.84	Filtered	2.70	4.37	10.8	23.48	68.4	7.7
9	1.07	Unfiltered	2.60	4.92	11.6	41.74	43.1	8.9
10	0	Filtered	0.00	4.37	11.1	35.65	72.5	11
11	0.84	Unfiltered	1.50	4.84	11.2	47.39	70.9	9
12	0	Unfiltered	0.00	4.9	11.8	80.87	80.9	11
13	1.29	Unfiltered	2.00	4.97	11.8	36.52	24.5	7.9
14	1.07	Filtered	10.96	4.36	11.1	16.09	55.4	7.1
15	1.29	Filtered	11.70	4.37	11.2	23.04	52.3	6.9

Figure I: Experimental design data using optimal design option of RSM.