



UNIVERSITI PUTRA MALAYSIA

***COMBINATION OF ULTRASOUND AND OZONATION ON EXTRACTION
AND PRESERVATION OF ROSELLE (*Hibiscus sabdariffa* L.) FRUIT
JUICE***

ONG MIOW CEEN

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**PROJECT REPORT SUBMITTED IN PARTIALLY FULFILLMENT
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ABSTRACT

Presently, fruit juices are pasteurized by thermal in food industry to inactivate enzymes and microorganisms. However, application of high temperature affects the nutritional and sensory qualities of juice. Ultrasound extraction and ozone treatment are promising non-thermal technologies which minimize the loss of nutritional qualities of fruit juice. The study focused on optimization of Roselle fruit juice extraction using ultrasound treatment. Response surface methodology (RSM) was used to investigate the effect of ultrasonic time and sonication temperature on juice yield. Sonication of Roselle fruit juice had increased the extraction yield by 11.53% in comparison with the sample without ultrasonic treatment. The study also investigated the effect of ozone processing on physicochemical characteristics (pH, titratable acidity (TA), total color difference (TCD), ascorbic acid (AA), total phenolic content (TPC), and total anthocyanin content (TAC)) of Roselle fruit juice. Roselle fruit juices were ozonated with processing variables of juice sample (Filtered I and Filtered II) and treatment time (0 to 30 min). Ozonated Roselle fruit juice showed similar pH and TA to the untreated juice. Significant degradation ($p < 0.05$) in TCD, AA, TPC and TAC over ozone processing time were observed. However, degradation of AA was less than 50% showed that ozone has the potential to prolong the shelf life of Roselle fruit juice. Filtered I Roselle fruit juice showed higher nutritional value than Filtered II Roselle fruit juice. The results of this study demonstrate the effectiveness of ultrasound extraction and ozonation in preserving the nutritional quality attributes of fruit juice compared to thermal pasteurization.

ABSTRAK

Pada masa ini, pengawetan jus buah-buahan dalam industri makanan dijalankan dengan mengguna haba untuk menghalang tindak balas enzim dan mikroorganisma. Walau bagaimanapun, penggunaan suhu tinggi akan mempengaruhi kualiti makanan dan deria jus. Pengekstrakan melalui teknik ultrasonik dan rawatan ozon merupakan teknologi non-termal yang meminimalkan kehilangan kualiti jus buah-buahan. Kajian ini memberi tumpuan kepada pengoptimuman proses pengekstrakan jus Roselle melalui teknik ultrasonik. Kaedah tindak balas permukaan (RSM) telah digunakan untuk menilai kesan penghasilan Roselle jus terhadap masa ultrasonik dan suhu sonication. Roselle jus yang diekstrak melalui ultrasonik meningkat hasil pengeluaran 11.53% berbanding dengan sampel yang tanpa rawatan ultrasonik. Kajian ini juga menyiasat kesan pemprosesan ozon terhadap ciri fizikokimia (pH, keasidan titratable (TA), jumlah perbezaan warna (TCD), asid askorbik (AA), jumlah kandungan fenolik (TPC), dan jumlah kandungan antosianin (TAC)) jus Roselle. Jus Roselle diozon dengan mengikut jenis jus sampel (Ditapis I dan Ditapis II) dan masa rawatan ozone (0 hingga 30 min). Terdapat jus Roselle yang diozon mempunyai pH dan TA yang sama dengan jus yang tidak dirawat. Terdapat degradasi yang ketara ($p < 0.05$) dalam TCD, AA, TPC and TAC semasa rawatan ozon. Walau bagaimanapun, degradasi AA kurang daripada 50% menunjukkan bahawa ozon mempunyai potensi untuk memanjangkan jangka hayat jus Roselle. Jus Roselle Ditapis I menunjukkan nilai pemakanan yang lebih tinggi daripada jus Roselle Ditapis II. Hasil kajian ini menunjukkan keberkesanan ultrasonik pengekstrakan dan rawatan ozon dalam pengawetan sifat-sifat kualiti pemakanan jus buah-buahan berbanding dengan pasteurisasi.

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LIST OF ABBREVIATIONS

AA	Ascorbic Acid
ANOVA	Analysis of Variance
DNA	Deoxyribonucleic Acid
DOA	Department of Agriculture
FDA	Food and Drug Administration
GAE	Gallic Acid Equivalents
GRAS	Generally Recognized as Safe
HPP	High Pressure Processing
HTST	High Temperature Short Time
PAL	Phenylalanine Ammonium Lyase
PEF	Pulsed Electric Field
PME	Pectin Methylesterase
POD	Peroxidase
PPO	Polyphenolic Oxidase
RNA	Ribonucleic Acid
RSM	Response Surface Methodology
TA	Titratable Acidity
TAC	Total Anthocyanin Contnt
TCD	Total Color Difference
TPC	Total Phenolic Content
UAE	Ultrasound-Assisted Extraction
UV	Ultraviolet

CHAPTER 1

INTRODUCTION

Traditionally, most foods are preserved by thermal food processing such as retort or pasteurization, where they are subjected to a very high temperature for a long time in order to kill microorganism and make food safe to eat. However, undesirable changes were observed during high-energy treatments, such as the change of food flavors and loss of vitamins, essential nutrients and freshness in the product affect the consumer preferences and demands (Xiao et al., 2017; Barbosa Canovas, 2009). Hence, non-thermal food processing was conceptualized to overcome or minimize such disadvantages. Non-thermal processes produce minimally processed food without the use of heat or keep temperature lower (-5°C to 45°C) (Bevilacqua et al., 2018) than thermal pasteurization (60°C to 100°C) (Agcam et al., 2018). Therefore, organoleptic characteristics of fresh produce such as flavors, essential nutrients and vitamins are retained during processing. Moreover, non-thermal processes utilize less energy than thermal processes (Barbosa Canovas, 2009). Examples of non-thermal technology that can be used in food processing are high pressure processing (HPP), pulsed electric field (PEF), ohmic heating, ultrasound, and ozone treatment. Each of these techniques can be used either alone or in combination to optimize product quality, processing time, and

bacterial and enzyme inactivation. In conclusion, non-thermal processes have attracted the attention of many food manufacturers in search of new food processing methods (Brennan & Grandison, 2012).

1.1 Overview of Ultrasound

The use of ultrasound energy in solid and liquid media has been extensive in food-processing applications and preservation (Ali & Maryam, 2016; Picó, 2012). Ultrasound is a clean, cost-effective, green-extraction technology not only able to protect both consumers and environment but also to encourage the adoption of the innovative technology. Moreover, ultrasound-assisted extraction (UAE) can be used as a procedure for the preparation and extraction of analytes (Ali & Maryam, 2016). The power of ultrasound gives a greater solvent penetration into cellular materials and further improves disruption of the cell walls to release the cell contents (Chen et al., 2012). Overall, UAE can be used as an alternative technique over traditional extraction because it enhances extraction yield, reduces processing times, decreases solvent consumption, and increases quality of the extracts.

The principle of ultrasound-assisted extraction (UAE) is mainly related to the formation of acoustic cavitation. The study of Kentish and Ashokkumar (2011) stated that acoustic cavitation refers to the bubble formation, growth and implosion occurring during the propagation of an ultrasound wave in a liquid media. When ultrasound propagates through any medium, it induces a series of compressions and rarefactions (expansion) in the molecules of the medium (Tiwari, 2015). Thus, the high pressure

occurs during implosion of cavitation micro-bubbles will destroy the cell wall of plant matrix. This can improve solvent penetration as there are more contact between content of medium and solvent. Nevertheless, cavitation process can be influenced by different parameters, including medium properties, operating frequency, wavelength, ultrasonic intensity, and ambient conditions such as temperature and pressure (Chemat et al., 2017; Ali & Maryam, 2016; Ashokkumar, 2015; Tiwari, 2015; Picó 2013).

Ultrasound techniques for extraction can be divided into two categories indirect and direct. The similarity is that both systems are based on a transducer as a source of ultrasound power. Ultrasonic baths (indirect) transmit energy and frequency sound waves indirectly through the wall of the sample container. In contrast, ultrasonic probes (direct) are immersed directly into the solution and able to develop a higher ultrasound power. Therefore, it can be concluded that high power ultrasonic probes are generally preferred for extraction applications. The probe system is more powerful due to an ultrasonic intensity delivered through a smaller surface (only the tip of the probe), as compare to the ultrasonic bath (Chemat et al., 2017).

The application of ultrasound-assisted extraction (UAE) improves the extraction yield and reduces processing time. For example, Gonzalez-Centeno et al. (2015) observed a 2.3 – 3.0 fold increase in total phenolic content from grape pomace for 2.5 minutes for UAE compared to conventional mechanical agitation. The reported application shows that UAE is a viable alternative to conventional techniques. Furthermore, the use of ultrasound as a sample preparation method for solid-liquid extraction is widespread in many laboratories and can be regarded as fast and effective (Bendicho & Lavilla, 2000).

1.2 Overview of Ozone

Globally, the demand for innovative processing technologies is currently on the rise in order to meet consumers' needs of fresher, healthier, and safer food products. Attention is now focused on ozone as a powerful sanitizer, safe and environmental friendly that may meet expectations of the industry, approval of the regulatory agencies, and acceptance of the consumer (Khadre et al., 2001). Ozone as a power oxidant has been widely used in food and beverage industries to protect against microorganisms and prolong the shelf life of fruits, vegetables, meat grains and their products. Furthermore, ozone treatment assures the retention of sensory, nutritional and physicochemical characteristic of food (Prabha, et al., 2015).

Ozone is a gaseous compound which is naturally present in the atmosphere and formed as a result of lightning or high energy UV radiation (Jakob & Hansen, 2005). It is a bluish gas with pungent and characteristic odor. However, the gas produced is very unstable and decomposes quickly in the air (Khadre et al., 2001). Therefore, ozone is generated by addition of free oxygen radical to molecular oxygen forming tri-atomic oxygen. Several techniques are used to generate ozone such as corona discharge, UV radiation and electrolysis (Prabha et al., 2015; Nath et al., 2014; Guzel-Seydim et al., 2004; Xu, 1999).

Notably, when ozone is applied to food, it leaves no residues since it decomposes quickly (Guzel-Seydim et al., 2004). Relatively low concentrations of ozone and short contact time are sufficient to inactivate bacteria, molds, yeasts, parasites and viruses (Kim et al., 1999). In addition, Guzel-Seydim et al. (2004) states that food preservation

through ozone is a non-thermal processing technology in which helps in enhancing food safety without compromising quality and desirability of food products.

In 1997, United States Food and Drug Administration (FDA) has declared that ozone as Generally Recognized as Safe (GRAS) for use in food processing. Furthermore, ozone is now recognized and allowed as an antimicrobial food additive by the FDA in 2001 (Nath et al., 2014). Thus, ozone treatment has started to be utilized in liquid food applications. A number of commercial fruit juice processors in the USA have started to employ ozone to meet the recent FDA mandatory 5-log reduction of the most resistant pathogens in their finished products (Cullen et al., 2009). For example, application of gaseous ozone treatment of orange juice resulted in *E.coli* population reduction of 5-log cycles (Patil et al., 2009). Also, spontaneous decomposition without forming hazardous residues in the treatment medium makes ozone safe in food applications (Prabha et al., 2015).

1.3 Problem Statement

Roselle is one of the commercial crops of Malaysia, where it was reported to have been brought in from India (Halimatul et al., 2007). The Department of Agriculture first promoted commercial planting of Roselle in Terengganu in the 1993, and other states soon followed suit (Elsaminejad & Zakaria, 2011). Roselle has been widely used as cold beverage or hot drink as it contains high vitamin C and anthocyanin. These extracts are also used in folk medicine to treat many complaints such as high blood

pressure, liver disease, heart ailment, cancer, and diabetes and nerve disorders (Patel, 2014; Ben Amor & Allaf, 2009).

Traditionally, thermal techniques such as pasteurization are applied in food processing and preservation. These high-energy treatments inactivate enzyme and microorganisms ensuring food safety and improving the shelf life of product, but also result in depletion of food nutrient, especially antioxidant (Saeeduddin et al., 2015). As the global consumers are becoming more conscious about the need of fresh and healthy juice, development of new and powerful technologies arise. To meet the consumer demand of minimally processed nutritious food, the concept of non-thermal innovative technologies are developed, including high pressure processing (HPP), pulsed electric field (PEF), ohmic heating, ultrasonication, ozonation and microwave heating.

Several studies have been done to investigate the effect of ultrasound-assisted extraction (UAE) and ozone treatment toward the quality of fruit juices. For example, sonication of pineapple mash in juice processing increased extraction yield of 10.8% in comparison with the control without ultrasonic treatment (Nguyen & Le, 2012). In addition, according to Patil et al., (2009), fast inactivation rates for total reduction of *E. coli* were achieved in model orange juice (60 s) and in juice with low pulp content (6 min). At the moment of writing, there have been no studies on application of sonication to the production of Roselle fruit juice and ozone disinfection to the Roselle fruit juice. Therefore, the objective of this study is to investigate the effects of different variables of sonication on the extraction yield in Roselle juice processing as well as to determine maximum extraction yield. Moreover, ozone treatment is applied to prolong the shelf-life and to investigate the physicochemical properties of Roselle fruit juice.

1.4 Objectives

The general objective of this study is to evaluate the effects of combination of ultrasound and ozonation on extraction and preservation of Roselle fruit juice. To be specific, the aims of this research are:

- a) To analyze optimization of Roselle fruit juice extraction by using ultrasound.
- b) To determine the effect of gaseous ozone on the physicochemical characteristics (pH, titratable acidity (TA), total color difference (TCD), ascorbic acid (AA), total phenolic content (TPC), and anthocyanin) of Roselle fruit juice.

1.5 Scope of Study

This study is to analyze the application of ultrasound on extraction yield of Roselle fruit juice and the effect of nutritional quality of Roselle fruit juice by ozone treatment. Limitations of this study include ultrasound machine, where the power (200W) and frequency (20 kHz) are fixed. Other than that, limitation of ozone gas generator and ozone gas analyzer, where the maximum ozone processing time is 30 minutes and concentration of ozone (400 mg/h) is fixed.

1.6 Significance of Study

The findings of this study can be utilized by any fruit juice processing industry considering that non-thermal technology may provide an ideal alternative in fruit juice

extraction and preservation. Ultrasound technology presents a simple and inexpensive alternative in extraction of fruit juices as compared to traditionally extraction of fruit juices. Moreover, ultrasound technology increases the yield of fruit juice extraction and reduces the processing time. In addition, unlike traditionally pasteurization, ozone treatment is a non-thermal process which was proven to effectively kill microbes while minimally affecting the quality and physicochemical properties of fruit juice. Therefore, utilization of ultrasound and ozone technology for extraction and preservation of fruit juice are strongly recommended. This study will focus on the application of ultrasound extraction for Roselle fruit juice, and the effect of ozone treatment on physicochemical properties of Roselle fruit juice.

CHAPTER 2

LITERATURE REVIEW

2.1 Roselle

Roselle (*Hibiscus sabdariffa* L.) is a tropical wild plant belonging to Malvaceae family, is widely grown in Central and West Africa and South-East Asia. It is an annual or perennial plant with red stems, serrate leaves and red calyces as illustrated in Figure 2.1. Nowadays, it has been widely distributed in tropics and subtropics of hemisphere (Zaman et al., 2017; Morton, 1987) including India, Saudi Arabia, Malaysia, China, Indonesia, Thailand, Philippines, Vietnam, Sudan, Egypt, Nigeria and Mexico (Zaman et al., 2017; Elsaminejad & Zakaria, 2011; Ismail et al., 2008; Halimatul et al., 2007; Rao, 1996; Morton, 1987).

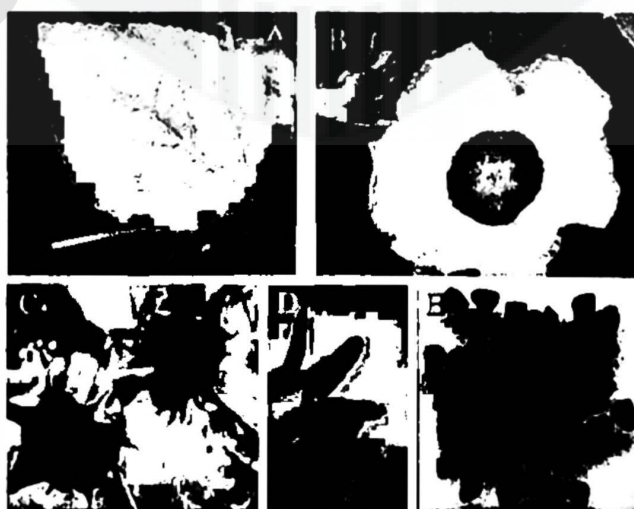


Figure 2.1 Morphology of Roselle plant. (A) Leaves; (B) pink and yellow flower; (C) red fresh calyces; (D) fruit; (E) dark brown seeds (Source: Ansari et al., 2013).

Roselle is locally known by different names in different countries. In Malaysia, Roselle is known as *asam paya* or *asam susur*. Roselle is an ideal crop for developing countries as it is relatively easy to grow, can be grown as part of multi-cropping systems and can be used as food and fibre (Da-Costa-Rocha et al., 2014). In 1993, Roselle was introduced in Malaysia and its commercial planting was first promoted in Terengganu. Due to the global demand, cultivation of Roselle in Malaysia is on the increase. Based on the data of Industrial Crops Statistic from DOA (2015), Pulau Pinang is now the largest Roselle production with 163.8 tonnes followed by Terengganu and Selangor at second and third place respectively (Table 2.1). Most of the Roselle produced in Malaysia is processed to make syrup or juice for healthy drinks. Other usages of Roselle are to make Roselle tea, beverages, jam, health food and natural food coloring.

Table 2.7 Planted area and production of Roselle by state, Malaysia (Source: DOA, 2015).

State	Planted Area (ha)	Harvested Area (ha)	Production (Tons)
Johor	12.3	8.8	41.1
Kedah	1.0	1.0	5.0
Kelantan	-	-	-
Melaka	1.0	1.0	0.5
Negeri Sembilan	-	-	-
Pahang	7.0	6.4	20.7
Perak	3.9	1.2	13.4
Perlis	-	-	-
Pulau Pinang	24.6	18.2	163.8
Selangor	6.5	6.5	58.9
Terengganu	26.9	26.7	107.6
Peninsular Malaysia	83.2	69.8	411.0
Sabah	-	-	-
Sarawak	-	-	-
WP Labuan	-	-	-
Malaysia	83.2	69.8	411.0

2.1.1 Nutritional Values and Usages of Roselle

The most exploited part of a Roselle plant is its calyces (Ismail et al., 2008). The calyces are separated from seed capsule. The brilliant red color and unique flavor make it a valuable food product (Tsai et al., 2002). For instance, fleshy calyces are used fresh for making wine, jelly, syrup, gelatin, and cakes; dried calyces are used for tea, jelly, marmalade, ice-cream, sauces, and other desserts (Ismail et al., 2008; Janick & Paull, 2008; Morton, 1987). Furthermore, calyces from the Roselle has high content of Vitamin C are processed to produce juices. Ismail et al., (2008) reported that Roselle contains three times more vitamin C than blackcurrant (*Ribes nigrum, L*) and nine times more than citrus (*Citrus sinensis L.*) fruit. For non-food application, other studies have reported that it is good for treatment of hypertension and hyperlipidemia (Hopkins et al., 2013; Wahabi et al., 2010). The presence of anthocyanin and proanthocyanidin compounds in Roselle calyces makes it a good colorant and good source of antioxidant component. In addition, the seeds of Roselle have been exploited as a substitute for coffee for human consumption and considered as excellent feed for chicken (Morton, 1987). In Malaysia, the seeds of Roselle are used to produce scrubs and soaps while the leaves are eaten as vegetables after cooking (Ismail et al., 2008). Table 2.2 shows the nutrient compositions of different parts of Roselle.

Table 2.8 Proximate and nutritional information from different parts of Roselle (Source: Ansari et al., 2013).

Nutritional value (unit/100g)	Flower	Red Calyces	Green Calyces	Seeds
Ash Content (g)	9.75±0.59	12.24	6.83	6.89
Fat Content (g)	0.59±0.06	2.01	2.17	21.60
Crude Fibre (g)	33.9±3.59	4.69	6.75	4.12
Protein Content (g)	9.87±0.28	4.71	6.45	31.02
Moisture content (g)	4.38±0.05	7.60	6.24	9.25
Carbohydrate (g)	4.38 ±0.05	68.75	71.56	36.37
Sodium (mg)	ND	96.66	48.1	ND
Potassium (mg)	ND	49.35	49.59	ND
Calcium (mg)	ND	12.65	21.58	6.6
Magnesium (mg)	ND	38.65	47.54	ND
Iron (mg)	ND	3.22	3.37	ND
Zinc (mg)	ND	12.22	16.28	ND
Manganese (mg)	ND	2.39	5.61	ND
Nickel (mg)	ND	1.78	3.57	ND
Phosphorus (mg)	ND	36.30	15.05	6.8
Ascorbic acid (mg)	ND	16.67	12.50	ND

*ND: Non Determine

2.1.2 Physicochemical and Antioxidant Properties of Roselle

Roselle calyces are used to prepare drinks, jam, jelly, and chutney. Today, several studies have shown that compounds found in Roselle calyces may have ascorbic acid, total phenol, total flavonoid, total anthocyanin and antioxidant properties (Zaman et al., 2017; Cid-Ortega & Guerrero-Beltrán, 2014). Table 2.3 shows the physicochemical properties of Roselle calyces. From the investigation, it can be concluded that Roselle is a good source of antioxidant agents as anthocyanins and ascorbic acids.

Table 2.9 Physicochemical properties of fresh and dried Roselle calyces (Source: Zaman et al., 2017).

Parameters	Fresh Calyces	Oven-Dried Calyces	Sun-Dried Calyces
pH	2.07 ^a ± 0.01	2.71 ^b ± 0.02	2.67 ^b ± 0.01
Titrateable acidity (% Citric acid)	20.22 ^a ± 0.03	18.82 ^b ± 0.02	19.12 ^b ± 0.03
Ascorbic acid (mg/100g)	66.52 ^a ± 0.04	58.48 ^c ± 0.01	52.42 ^c ± 0.02
Total phenol (mg/100g)	1018 ^a ± 0.32	2019 ^b ± 0.34	2010 ^b ± 0.32
Total flavonoids (mg/100g)	388 ^a ± 0.11	322 ^b ± 0.16	310 ^b ± 0.12
Total anthocyanins (mg/100g)	326 ^a ± 0.12	298 ^c ± 0.13	282 ^c ± 0.14
DPPH inhibition (%)	92.12 ^a ± 0.10	86.14 ^b ± 0.14	79.28 ^b ± 0.13
Color			
L*	28.76 ^a ± 0.01	23.74 ^b ± 0.01	21.76 ^c ± 0.02
a*	18.82 ^a ± 0.04	15.82 ^b ± 0.03	14.78 ^c ± 0.01
b*	3.22 ^a ± 0.02	5.38 ^b ± 0.02	6.49 ^c ± 0.01
H*	9.73 ^a ± 0.04	18.79 ^b ± 0.03	23.76 ^c ± 0.04
C	19.09 ^a ± 0.03	16.70 ^b ± 0.02	16.14 ^c ± 0.02

2.2 Ultrasound-Assisted Extraction (UAE)

Application of ultrasound-assisted extraction (UAE) has found increasing attention as it enhances extraction yield and reduces extraction time. There are a lot of studies on UAE, however the authors were only interested in certain valuable components in the plant extract such as saponin, anthocyanins, isoflavones, lycopene, oil, and xyloglucan (Nguyen & Le, 2012). In juice processing, water-soluble components in the fruit flesh must be completely recovered for improvement in extraction yield. Nguyen and Le (2012) proved that sonication of pineapple mash in juice processing increased the extraction yield by 10.8% in comparison with the control sample - without ultrasonic treatment.

Furthermore, ultrasound-assisted extraction (UAE) is known as cold extraction techniques as temperature during the extraction process is comparatively low and does

not affect the stability of extracted compounds. Due to the effect of implosion of cavitation, ultrasound provides a greater solvent penetration into cellular materials thus improving the mass transfer, while the disruption of biological cell walls facilitates the release of its contents (Chen et al., 2012). Overall, UAE has two main advantages compared with conventional extraction; reduced processing time and solvent volume usage, as shorter operating times are obtained compared with conventional methods (Bonfigli et al., 2017). However, there have been no studies on application of sonication to the production of Roselle fruit juice. Table 2.4 shows the optimization of ultrasound-assisted extraction of various juices.

Table 2.4 Optimum conditions for ultrasound-assisted extraction of juice.

Fruit Juices	Ultrasound Parameters	Optimum Condition	Reference
Apple juice	Power: 0 – 25 W/g Time: 0 – 8 min Temperature: 30°C	Power: 20 W/g Time: 7.3 min Extraction yield: 67.5% higher than control (without ultrasonic treatment)	Quang et al., 2014.
Rose myrtle juice	Power: 0 – 35W/g Time: 2 min Temperature: 30°C	Power: 25 W/g Time: 6.5 min Extraction yield: 86% higher than control sample (without ultrasonic treatment)	Vo & Le, 2014.
Pineapple juice	Power : 0 – 300 W at ambient temperature for 1 min Time: 0 – 5 min at ambient temperature and 225W Temperature: 30 – 80°C at 225W for 2 min	Power: 247.4 W Time: 2.7 min Extraction yield: 85.7%.	Nguyen & Le, 2012.

2.3 Thermal Pasteurization

Thermal pasteurization is one of the unit operation used throughout the food industry. Generally, thermal pasteurization of fruit juices is related to heat treatments which are between 60°C and 100°C to destroy the heat sensitive microorganism such as non-spore forming bacteria, yeast and molds as well as retards the enzymatic reaction in order to prolong the shelf life of food (Agcam, et al., 2018).

Thermal processes can be classified according to the intensity of the heat treatment (Miller & Silva, 2012). High temperature long time (HTLT) heat treatment is the most commonly used method in the processing of juices and beverages, operated at 80 to 100°C and holding times is more than 30 seconds (Miller & Silva, 2012). In order to ensure the product safety and maintain the desired bioactive compounds, high temperature short time (HTST) thermal pasteurization has been proposed, because temperature dependency is more significant for microorganism destruction than for nutrient degradation (Achir et al., 2016). Thermal processes used for juice pasteurization is generally based on a 5 log reduction of the most resistant microorganisms (Petruzzi et al., 2017; Rupasinghe & Yu, 2012).

Nevertheless, thermal treatments may induce several chemical and physical changes that impair the organoleptic properties and may reduce the content or bioavailability of some nutrients (Petruzzi et al., 2017). Conventional thermal processing such as pasteurization was reported to degrade the level of phytochemicals in processed food products (Tiwari & Cummins, 2013). Moreover, Juárez et al. (2016) reported that polyphenolic compounds and antioxidant capacity can also be affected by thermal

processes. The reduction of the antioxidant capacity was generally due to a loss in total anthocyanins and vitamin C (Miller & Silva, 2012). For instance, content of ascorbic acids in grapefruit juice was found to decrease after HTST heat treatment (Igual et al., 2010). Besides, total anthocyanins of fresh blueberries after pasteurisation was found to reduce about 43% compared to the original levels (Tiwari & Cummins, 2013). The benefits and the drawbacks of heat treatments in juices were extensively reported in many papers. Table 2.5 shows the compounds of juice reduced by thermal processing.

Table 2.5 Compounds of juice reduced by thermal processing. (Source: Petruzzi et al., 2017).

Compounds / nutritional attributes	Product	Thermal treatment	Reference
Anthocyanins	Jamun juice	HTLT	Shaheer et al., 2014.
	Pomegranate juice	HTLT	Pala & Toklucu, 2011.
Antioxidant capacity		HTST	Woodward et al., 2011.
	Amla juice	HTLT	Bansal et al., 2015.
Ascorbic acid	Amla juice	HTLT	Bansal et al., 2015.
	Pomegranate juice	HTST	Mena et al., 2013a.
Color	Lychee juice	HTLT	Guo et al., 2011.
Carotenoid	Lemon juice	HTST	Ucan et al., 2016.
	Peach juice	HTLT	Oliveira et al., 2012.
Phenolic content	Pear juice	HTST	Jimenez-Aguilar et al., 2015
Overall quality	Mixed fruit juice	HTLT	Jayachandran et al., 2015.
	Mango juice	HTLT	Santhirasegaram et al., 2015.
	Beetroot juice	HTLT	Kathiravan et al., 2014.

2.4 Ozone Generation

Ozone is formed by a high energy input that splits the diatomic oxygen molecule into free radical oxygen. Single oxygen molecule is thereby free to react with another

diatomic oxygen to form the triatomic ozone molecule. Ultraviolet radiation (188nm wavelength) and corona discharge methods can be used to initiate free radical oxygen formation and, thereby generate ozone. In order to generate commercial levels of ozone, the corona discharge method is usually used as illustrated in Figure 2.3 (Nath et al., 2014; Guzel-Seydim et al., 2004).

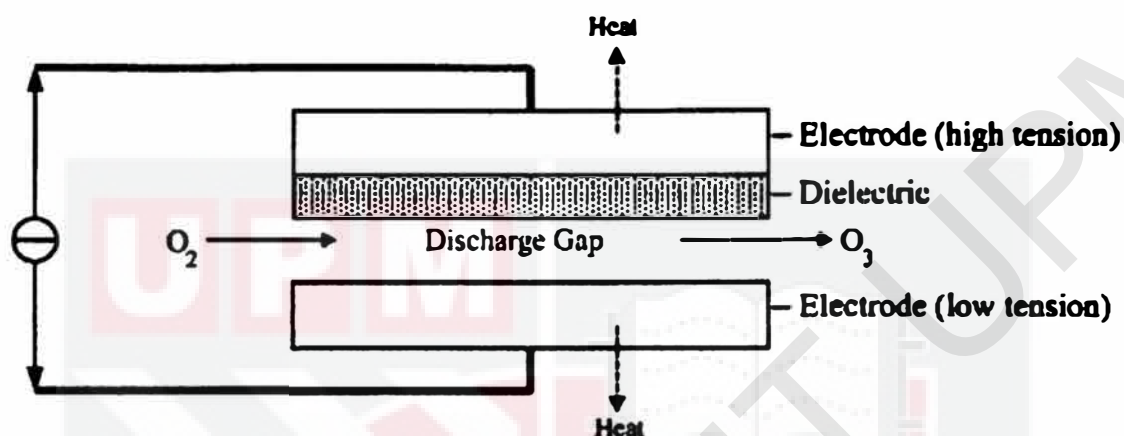


Figure 2.2 Schematic diagram of ozone generation by corona discharge method (Guzel-Seydim et al., 2004).

There are two electrodes in corona discharge, the high tension and low tension (ground) electrode, separated by a ceramic dielectric medium in a narrow discharge gap. When dry air or oxygen gas is fed through the gap between two electrodes, a high voltage alternating current is applied causing the dissociation of molecular oxygen to atomic form. Thus, atomic oxygen collides with molecular oxygen to form ozone (Chawl et al., 2012). The concentration of ozone produced by corona discharge depend on voltage, current frequency, dielectric material, discharge gap and absolute pressure within the discharge gap (Prabha et al., 2015; Nath et al., 2014; Chawla et al., 2012; Khadre et al., 2001; Kim et al., 1999).

Ozone is a very pungent bluish gas with strong oxidizing properties. Ozone readily degrades but has a longer half-life in the gaseous state than in aqueous solution

(Pranba et al., 2015). However, ozone is unstable and decomposes in a very short time, thus, it is generated continuously either by ultraviolet radiation or corona discharge method. The only result of ozone, when it decomposes, is oxygen; so, food products treated with ozone are free of disinfectant residue (Prabha et al., 2015; Guzel-Seydim et al., 2004). Moreover, decomposition of ozone is so rapid in the water phase of foods that is sufficient to inactivate bacteria, molds, yeasts, parasites and viruses (Kim et al., 1999).

2.4.1 Antimicrobial Effect of Ozone

The use of ozone as an antimicrobial agent in food processing was reviewed by various researchers (Khadre et al., 2001; Kim et al., 1999; Xu, 1999). Its efficacy against Gram positive and Gram negative bacteria, fungi, viruses, protozoa, and bacterial spores has been reported (Cullen et al., 2009; Khadre et al., 2001; Restaino et al., 1995). Microbial inactivation by ozone is mainly due to the irreversible damage to fatty acid in cell membrane and to cellular macromolecules, such as proteins, and DNA (Prabha et al., 2015). Scott and Leshner (1963) demonstrated that ozone reacts with unsaturated lipids of *E. coli* cell membrane, ultimately resulting in leakage of cell contents and death. Other researcher revealed that treatment of viruses with ozone will cause damage due to the destruction of capsid protein, compromised integrity of the lipid envelopes as well as damage to RNA or DNA (Kim et al., 1980).

With the FDA approval of ozone as a direct additive to food, some researchers suggested using ozone as an alternative to thermal pasteurization of fruit juices. Also, a number of commercial fruit juice processors in the USA have started to employ ozone to

meet the recent FDA mandatory 5-log reduction of the most resistant pathogens in their finished products (Cullen et al., 2009). Steenstrup and Floros (2004) observed a rapid inactivation of *E. coli* O157:H7 in apple cider when treated with 860 ppm (v/v). Another author observed 6-log reduction of *E. coli* O157:H7 in apple cider and orange juice when treated with 0.9 g/h ozone under mild heating of 50°C (William et al., 2004). However, the effectiveness of ozone against microorganisms depends not only on the ozone concentration, but also on the residual ozone in the medium, various environmental factors such as medium pH, temperature, humidity, additives, and the amount of organic matter surrounding the cells. While ozonation of liquid foods is still in its infancy, it has been reported for processing of various fruit juices, including apple cider and orange juice (Cullen et al., 2009). Table 2.6 shows microbial inactivation in fruit juice by ozone whereas Table 2.7 shows microbial inactivation in fruit juice with ozone parameters.

Table 2.10 Microbial inactivation in fruit juice by ozone (Source: Cullen et al., 2009).

Fruit Juices	Phase / Form	Target Microbial Population	Reference
Apple cider	Ozone gas (pumped into juice)	<i>Escherichia coli</i> 157:H7 (0.9 LR); Salmonella (1.0LR)	Williams et al., 2005.
Orange juice	Ozone gas (pumped into juice)	<i>Escherichia coli</i> 157:H7 (0.4 LR); Salmonella (1.8 LR)	Williams et al., 2005.
Apple cider	Ozone gas (pumped into juice)	Moulds (↓); yeast (↓)	Choi & Nielsen, 2005.
Apple cider	Ozone gas (pumped into juice)	<i>Escherichia coli</i> O157:H7 (5.0 LR)	Steenstrup & Floros, 2004.

(↑), increases; (↓), decreases; LR, log reduction.

Table 2.11 Microbial inactivation in fruit juice with ozone parameters.

Fruit Juices	Ozone Parameters	Target Microbial Population	Reference
Apple juice	Concentration: 2.8 mg/L Temperature: 4°C Time: 40 min	<i>A. acidoterrestris</i> (2.2 LR)	Torlak, 2014.
Apple juice	Concentration: 5.3 mg/L Temperature: 4°C Time: 40 min	<i>A. acidoterrestris</i> (2.8 LR)	Torlak, 2014.
Apple juice	Concentration: 2.8 mg/L Temperature: 22°C Time: 40 min	<i>A. acidoterrestris</i> (1.8 LR)	Torlak, 2014.
Apple juice	Concentration: 5.3 mg/L Temperature: 22°C Time: 40 min	<i>A. acidoterrestris</i> (2.4 LR)	Torlak, 2014.
Apple juice	Concentration: 0.048 mg/min/mL pH: 3 Time: 4 min	<i>E. coli</i> ATCC 25922 (5.66 LR)	Patil et al., 2010.
Apple juice	Concentration: 0.048 mg/min/mL pH: 5 Time: 18 min	<i>E. coli</i> ATCC 25922 (5.24 LR)	Patil et al., 2010.
Orange juice (Unfiltered)	Concentration: 75–78 µg/mL Time: 11.86 min	<i>E. coli</i> ATCC 25922 (5.0 LR)	Patil et al., 2009.
Orange juice (Filtered)	Concentration: 75–78 µg/mL Time: 4.76 min	<i>E. coli</i> ATCC 25922 (5.0 LR)	Patil et al., 2009.
Apple cider	Concentration: 0.9 g/h Temperature: 50°C Time: 45 min	<i>E. coli</i> O157:H7 (6.0 LR)	Williams et al., 2004.
Orange juice	Concentration: 0.9 g/h Temperature: 50°C Time: 75 min	<i>E. coli</i> O157:H7 (6.0 LR)	Williams et al., 2004.
Apple cider	Concentration: 0.9 g/h Temperature: 4°C Time: 45 min	<i>E. coli</i> O157:H7 (4.8 LR)	Williams et al., 2004.
Orange juice	Concentration: 0.9 g/h Temperature: 4°C Time: 75 min	<i>E. coli</i> O157:H7 (5.4 LR)	Williams et al., 2004.

LR, log reduction.

2.4.2 Effect of Product Quality and Nutrition by Ozone Treatment

Applying ozone as doses that are enough for effective decontamination, however if improperly used, can cause deleterious effect on the quality of food such as sensory quality. Surface oxidation, discoloration and undesirable odors may result from excessive use of ozone (Kim et al., 1999). The authors also stressed that ozone is not universally beneficial and, in some cases, may promote oxidative spoilage in foods, if used excessively (Prabha et al., 2015; Chawla et al., 2012; Kim et al., 1999).

Ozonation is expected to lead to the loss of ascorbic acid content due to its strong oxidizing activity. Tiwari et al. (2008) reported that treatment with gaseous ozone degrade ascorbic acid content of orange juice. There is also a significant reduction in anthocyanin content and ascorbic acid of ozonated strawberry juice that were observed (Tiwari et al., 2009a). Moreover, ozone-treated apple cider had increased sedimentation, decreased soluble solids by day 21 (Choi and Nielsen, 2005). However, Dock (1999) observed no detrimental changes in quality of ozone-treated apple cider with ozone concentration of 860 ppm.

Furthermore, color change in food also serves as an indicator of quality loss. Oxidation of carotenoids or anthocyanin during ozone treatment produced color changes in orange juice (Tiwari et al., 2008), blackberry juice (Tiwari et al., 2009b). Other researcher observed a significant reduction on color, rheological properties and phenolic content of ozonated apple juice (Torres et al., 2010). Therefore, for effective and safe use in food processing, optimum ozone concentration, contact time and other treatment

conditions should be defined for all products. Table 2.8 shows the effect of ozone on quality and nutritional parameter of fruit juices.

Table 2.12 Effect of ozone on quality and nutritional parameter of fruit juices (Source: Cullen et al., 2009).

Fruit Juices	Phase / Form	Quality and nutritional attributes	Reference
Strawberry juice	Ozone gas (bubble column reactor)	Colour (↓), pH (~), TA (~), AA (↓), anthocyanins (↓)	Tiwari et al., 2009a.
Blackberry juice	Ozone gas (bubble column reactor)	Colour (↓), pH (~), TA (~), AA (↓), anthocyanins (↓)	Tiwari et al., 2009b.
Orange juice	Ozone gas (bubble column reactor)	Colour (↓), NEB (~), cloud value (~), pH (~), TA (~), AA (↓)	Tiwari et al., 2008b.
Apple cider	Ozone gas (pumped into juice)	Sediment (↑), colour (x)	Choi & Nielsen, 2005.
Orange juice	Ozone gas (pumped into juice)	AA (↓), colour (x)	Angelino et al., 2003.

AA, ascorbic acid; TA, titratable acidity; (x), significant difference; (↑), increases; (↓), decreases; (~), no change.

CHAPTER 3

METHODOLOGY

3.1 Research Design

Research design of ultrasound and ozone treatment for extraction and preservation of Roselle fruit juice was summarized in Figure 3.1. There were three parts in conducting the experiment. The first part was optimization of Roselle fruit juice extraction using ultrasound at different temperature and processing time. The second part was ozone treatment to Roselle fruit juice (Filtered I and Filtered II) at different processing time. Then the ozonated juice undergone physicochemical analysis.

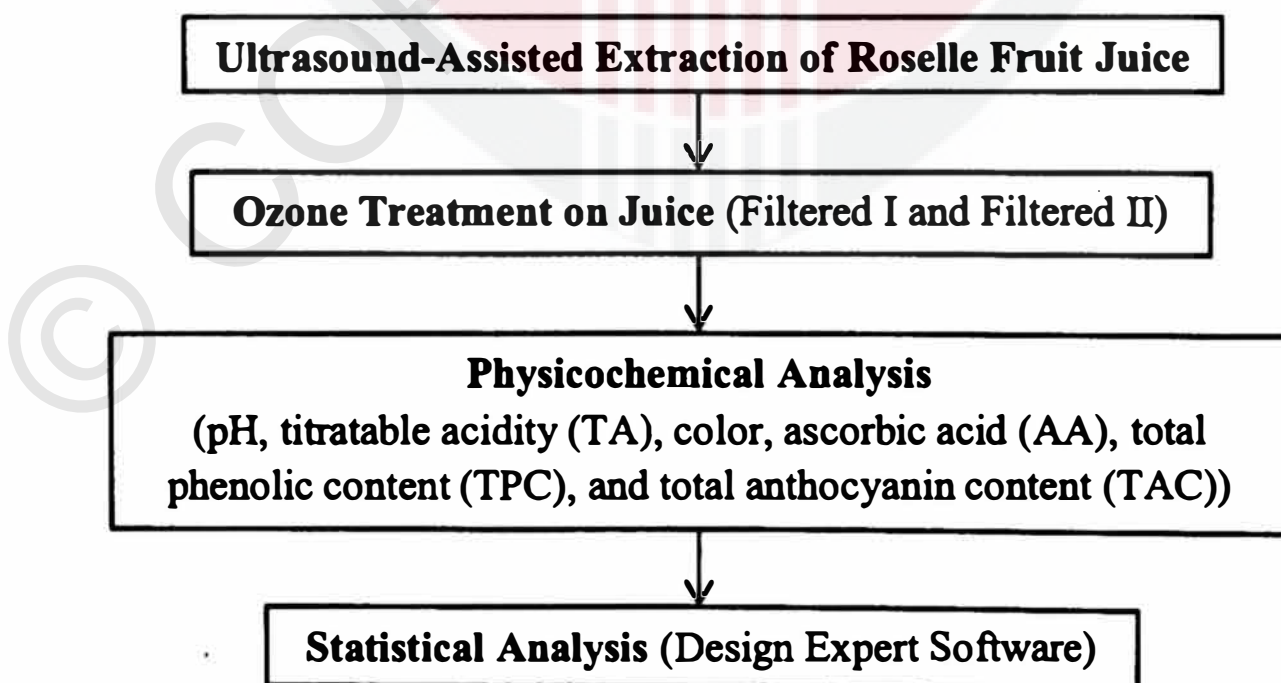


Figure 3.1 Research design.

3.2 Ultrasound Extraction of Roselle Fruit Juice

3.2.1 Sonication Variables on Extraction Yield in Roselle Fruit Juice

Roselle was first washed with potable water and cut into small pieces. Samples of 15 gram Roselle mash were taken and placed into 100 ml beaker. Roselle was suspended with distilled water; the weight ratio of Roselle to water was 1:2 (Chumsri et al., 2008). The ultrasonic probe was put into the beaker and ultrasonic treatment started at frequency of 20 kHz with power of 200 W for different processing time (0, 2, 4, 6, and 8 minutes). The sonication was carried out at ambient temperature and controlled using a water-bath filled with ice cubes. At the end of ultrasound treatment, the sample was filtered through a wire mesh sieve. Next, the procedure was repeated by changing sonication temperature to 10, 20, 30, and 40°C. The ultrasound power and treatment time were 20 kHz and 2 minute, respectively. The extraction yield can be calculated by the following equation;

$$\text{Extraction yield (\%)} = \frac{\text{Amount of juice recovered (g)}}{\text{Amount of initial mixture (g)}} \times 100$$



Figure 3.2 Experiment set-up for ultrasound extraction of Roselle fruit juice.

3.2.2 Optimization of Sonication Conditions for Maximizing the Extraction Yield of Roselle Fruit juice by Response Surface Methodology (RSM)

The quadratic central composite circumscribed response surface design was used to determine the optimal sonication temperature and ultrasonic time for maximizing the extraction yield of Roselle fruit juice. Five level of independent variables and software Design Expert Version (11 Stat – Ease Inc, MN, USA) were used to generate the experimental planning and to analyze data. The complete design consisted of 13 experimental points including 4 factorial points, 4 axial points and 5 centre points. The experiment steps were similar to those of the previous experimental section. A control was also carried out for comparing the extraction yield in Roselle fruit juice with and without ultrasonic extraction.

3.3 Preparation of Roselle Fruit Juice

The extracted Roselle fruit juice was thawed in refrigerator for 24 hours before conducting ozone treatment. Roselle fruit juice was divided into two categories which is Filtered I and Filtered II. Filtered I indicates that the Roselle fruit juice was filtered through a wire mesh sieve after ultrasound treatment. Filter II indicates that the filtered Roselle fruit juice after ultrasound treatment was centrifuged at 6500 rpm for 10 minute by centrifuge (Universal 320) and then filtered again through a cloth mesh to remove the pulp.

3.4 Ozone Treatment

Roselle fruit juice was treated with gaseous ozone in a 500 ml conical flask by ozone generator (Model GL-3189). Ozone output used in this experiment was 400 mg/h. The delivery tube was then connected to ozone generator. Ozone gas was pumped into juices (Filtered I and Filtered II) for 0, 10, 20, and 30 minutes and the juices were stirred. The experiment was conducted in the fume board to prevent excessive exposure of ozone gas. Ozone generator was left to rest for 3 to 5 minutes after every test.

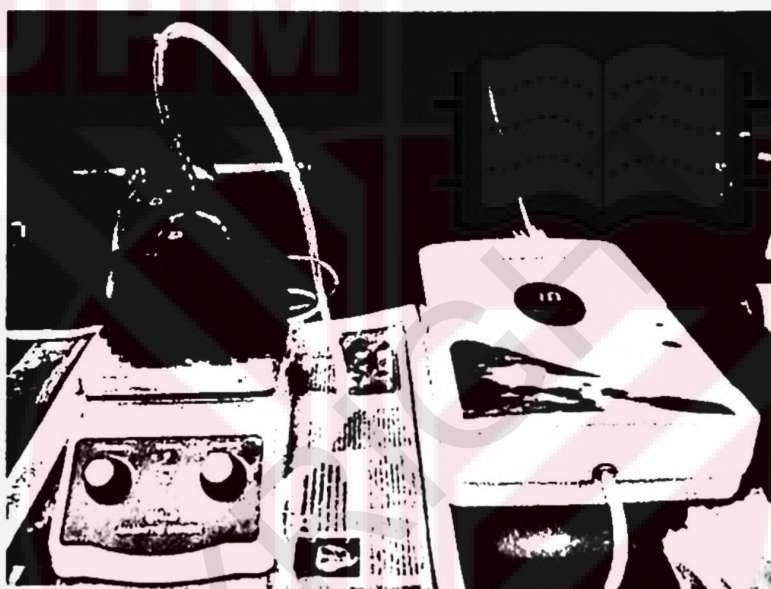


Figure 3.3 Experimental set-up for ozone treatment.

3.4 Physicochemical Analysis

3.4.1 pH

The pH of Roselle fruit juice was determined by using pH meter (SevenMulti pH Conductivity Meter, Mettler Toledo, Switzerland) and it was calibrated with standard buffer solution of pH 4 and 7. Roselle fruit juice with 10 ml was prepared in a beaker

and an electrode was submerged directly in the sample solution until a steady reading is reached.

3.4.2 Titratable Acidity (TA)

Titrateable acidity of Roselle fruit juice was determined by titration using a pH meter (Youssef et al., 2015). pH meter was calibrated with standard buffer solution of pH 4 and 7. Distilled water with 100 ml was placed into a 250 ml beaker. The pH of water was adjusted to 8.1 by adding 0.1 N sodium hydroxide, NaOH. Then, Roselle fruit juice with 5 ml was added into beaker and stirred. The mixture is titrated to a pH of 8.2 by slowly adding 0.1 N NaOH. The volume of 0.1 N NaOH was recorded. Percentage of citric acid content was calculated by using the following formula;

$$\% \text{ Titratable Acid (w/w)} = \frac{(\text{Net ml Titrant})(N \text{ Titrant})}{(\text{g Sample})} \times 6.4$$

3.4.3 Total Color Difference (TCD)

CIE (Commision Internationale de l'éclairage) Lab scale: L^* (lightness, 0 - 100), a^* (green to red) and b^* (blue to yellow) of Roselle fruit juice were determined in a HunterLab Ultra Scan Pro (D65, HunterLab Assoc Lab Inc, Reston, VA, USA). For color determination, the colorimeter was previously calibrated using black and white mosaics. Triplicate measurements were taken for each set of samples and values of L^* ,

a^* , and b^* were recorded. Total color difference (TCD) which indicates the magnitude of color change after treatment was 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.

3.4.4 Ascorbic Acid (AA)

Vitamin C of Roselle fruit juice was determined by reducing 2,6-dichlorophenol-indophenols solution from a blue color to a colorless or very faint endpoint with addition of ascorbic acid. Dye solution with 0.5% was prepared by dissolving 0.042 g of sodium hydrogen carbonate, NaHCO_3 in distilled water and adding 0.050 g of sodium 2,6-dichloroindolphenol. When dye was dissolved, make up to 200 ml. To prepare ascorbic acid standard solution (1 mg/ml), 0.100 g of ascorbic acid was placed into a 100 ml volumetric flask and dilute to 100 ml. Then, 10 ml of dye solution was transferred to a beaker. Ascorbic acid solution was added drop by drop into dye solution using a Beral-type pipet and stirred. The number of drop added into the dye solution was counted when color changes from blue to very light amber endpoint. Next, the procedure was repeated using Roselle fruit juice. All the experiments were carried out in triplicate. The concentration of ascorbic acid content was calculated by using the following formula;

Ascorbic Acid (mg/100ml)

$$= \frac{(\text{Drops of standard})(\text{Concentration of standard})}{(\text{Drops of fruit juice})} \times 100$$

3.4.5 Total Phenolic Content (TPC)

Total phenolic content of Roselle fruit juice was determined according to the Follin-Ciocalteu procedure that was adopted from Youssef et al., (2015). Folin-Ciocalteu reagent (10%) with 1.5 ml was mixed with 1.2 ml of 7.5% sodium carbonate, Na_2CO_3 solution, then 0.3 ml of Roselle juice sample was added. The test tubes were allowed to stand for one hour at ambient temperature, and the absorption was measured 765 nm using spectrophotometer (Ultraspec 3100 Pro, Amersham Pharmacia Biotech, UK) against a blank. All the experiments were carried out in triplicate. TPC were obtained from the calibration curve prepared with gallic acid at concentrations of 0, 50, 100, 150, 250 and 500 mg/L, as shown in Figure 3.3. The calibration equation for gallic acid was $y = 0.0012x + 0.0023$ ($R^2 = 0.9897$), where y is absorbance and x is concentration of gallic acid in mg/L.

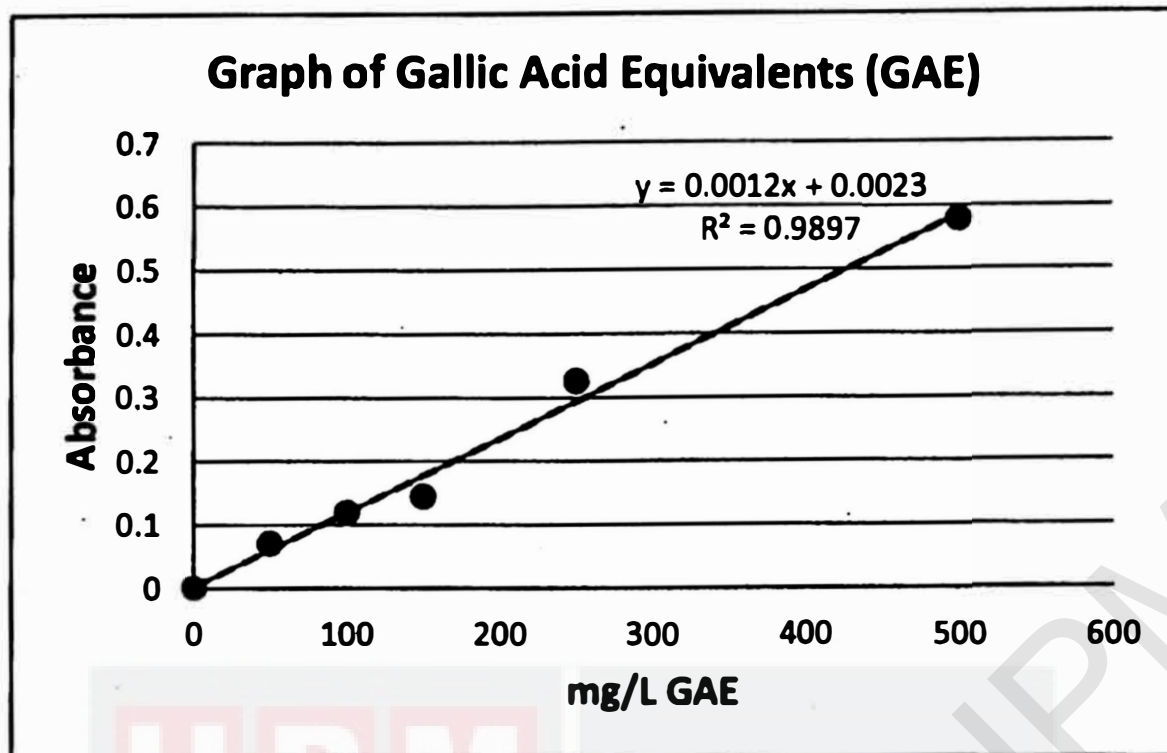


Figure 3.4 Folin Ciocalteu gallic acid standard curve.

3.4.6 Total Anthocyanin Content (TAC)

The total anthocyanin content of Roselle fruit juice was measured using a spectrophotometric pH differential protocol (Wu et al., 2006). The Roselle fruit juice was thoroughly mixed with 0.025M potassium chloride (pH 1.0) in a known dilution. The absorbance of the mixture was measured at 515 and 700 nm using distilled water to zero the spectrophotometer. The Roselle fruit juice was then combined with 0.4M sodium acetate buffer (pH 4.5), and the absorbances were measured at the same wavelengths. The absorbance of the diluted sample (A) was as follows;

$$A = (A_{515} - A_{700})_{pH\ 1.0} - (A_{515} - A_{700})_{pH\ 4.5}$$

The anthocyanin content was calculated as follow;

$$\text{Total anthocynin content (mg/l)} = \frac{A \times MW \times DF \times 1000}{\epsilon \times 1}$$

Where;

A = absorbance of the diluted sample

DF = dilution factor

MW= 449.2

e = 26,900

Since the sample composition was unknown, pigment content was calculated as cyanidin- 3-glucoside.

3.5 Statistical Analysis

In this study, experiment design from Design Expert Software Version 11 (Stat – Ease Inc, MN, USA) was used to perform statistical analysis. All experiments in all combinations of factors are performed in triplicate. Circumscribed central composite design was used to study the effects of sonication temperature and ultrasonic time on extraction yield of Roselle fruit juice. Moreover, general factorial design of ozone treatment was used where two independent variables: juice sample (Filtered I and Filtered II) and processing time were investigated for the influence on physicochemical properties of Roselle fruit juice as shown in Table 3.1. The analysis of the results was performed by using ANOVA analysis.

Table 3.1 General factorial design of ozone treatment.

Block	Run	Factor 1	Factor 2
		A:Juice Sample	B:Processing Time (Min)
1	1	Filtered I	30
1	2	Filtered I	0
1	3	Filtered II	20
1	4	Filtered I	20
1	5	Filtered II	0
1	6	Filtered I	10
1	7	Filtered II	10
1	8	Filtered II	30
2	9	Filtered I	20
2	10	Filtered II	20
2	11	Filtered I	30
2	12	Filtered II	30
2	13	Filtered I	0
2	14	Filtered II	0
2	15	Filtered II	10
2	16	Filtered I	10
3	17	Filtered I	30
3	18	Filtered II	0
3	19	Filtered I	20
3	20	Filtered II	30
3	21	Filtered II	10
3	22	Filtered I	10
3	23	Filtered I	0
3	24	Filtered II	20

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Impact of Sonication Variables on Extraction Yield in Roselle Fruit Juice

The impact of ultrasonic time on the extraction yield of Roselle fruit juice is shown in Figure 4.1. The graph shows that the extraction yield in Roselle fruit juice after 4 minutes had increased 11.41% in comparison with the control samples. However, extraction yield in Roselle fruit juice decreased slightly when sonication treatment was prolonged more than 4 minutes. Longer treatments time did not make significant differences in extraction yield. Similar observation was reported by Lieu and Le (2010) who applied ultrasound to grape mash extraction. Therefore, the appropriate ultrasonic time to achieve maximum extraction yield in this study was 4 minutes. There are various ultrasound-assisted extraction time was found for different fruit juices. For example, the sonication time for the extraction of pineapple juice was only 2 minutes (Nguyen & Le, 2012). The ultrasonic treatment for maximizing total phenolic and ascorbic acid from rose myrtle juice was 6 minutes (Vo & Le, 2014). The ultrasonic time of Roselle calyxes in this research was slightly different with the findings of various authors. This is because the optimal sonication time will depend on temperature, ultrasonic power, and

weight of plant material used in the experiment (Lieu & Le, 2010; Wang *et al.*, 2008).

As a result, ultrasonic time can be varied in range.

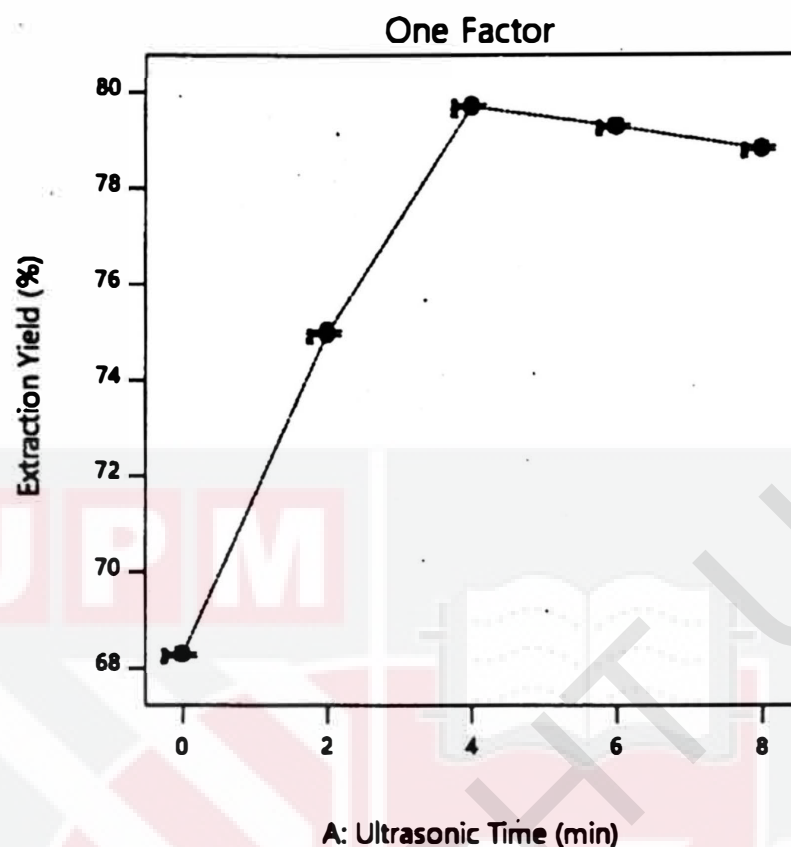


Figure 4.1 Effect of ultrasonic time on extraction yield in Roselle fruit juice.

Figure 4.2 represents the effect of sonication temperature on the extraction yield in Roselle fruit juice. When the treatment temperature increased from 10°C to 20°C, the extraction yield just increased slightly from 73.99 to 74.84%. In the temperature range of 20°C to 40°C, the extraction efficiency reduced slightly. As sonication temperature increased, the collapse of cavitation bubbles reduced due to an increase of vapor pressure. In contrast, increased temperature decreases the viscosity allowing for a more violent collapse of bubbles. Thus, there is an optimum temperature at which the viscosity and temperature is low enough to form violent cavitation bubbles and prevent the dampening effect on bubble collapse (Chemat *et al.*, 2017; Tiwari, 2015; Patist & Bates, 2008). In this study, the appropriate temperature of ultrasonic treatment is at 20°C where the extraction yield reached its maximum point. Nevertheless, the sonication

temperature of Roselle fruit juice extraction was much lower in comparison with some studies. For example, Lieu and Le (2010) stated that the optimal sonication temperature of grape juice extraction was 74°C. Chemat et al. (2017) reported that the degree of cavitation effects depend on the structure of plant tissue, plasticity and its chemical composition. Hence, the optimal sonication temperature can be varied in a large range depending on type of product in order to obtain the highest yield of target compound. It can be concluded that the low sonication temperature during Roselle extraction gives low energy input and low degradation of nutrient compound in Roselle fruit juice.

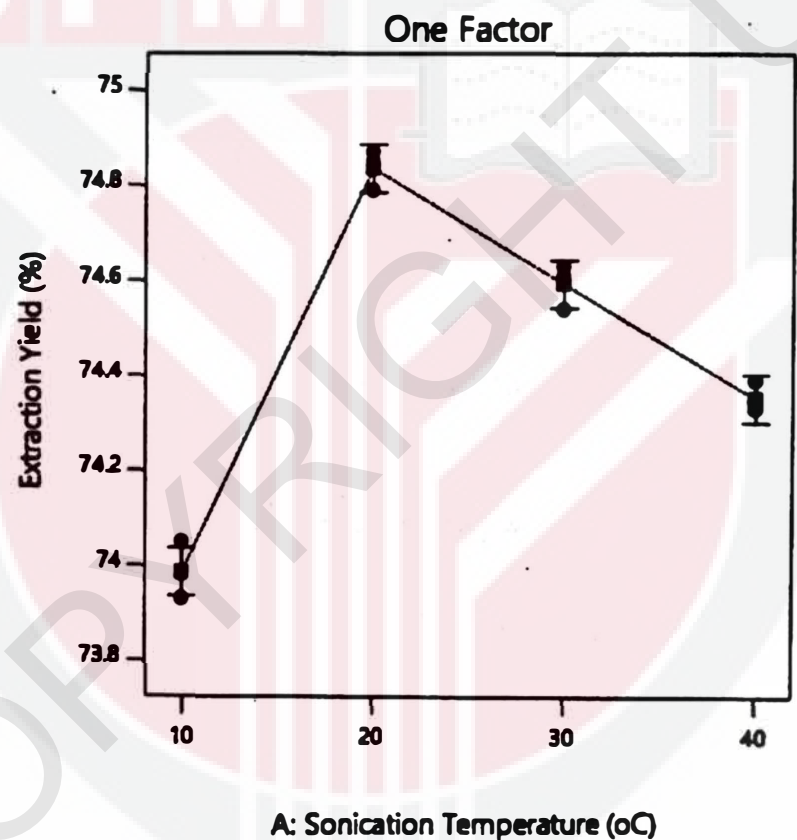


Figure 4.2 Effect of sonication temperature on extraction yield in Roselle fruit juice.

4.2 Optimization of Sonication Conditions for Maximizing the Extraction Yield of Roselle Fruit juice by Response Surface Methodology (RSM)

Based on the results of the previous sections, ultrasonic time of 4 minutes and sonication temperature of 20°C were selected as the central conditions of the optimization experiment. Table 4.1 shows the experimental design and results of extraction yield of Roselle fruit juice. Multiple regression analysis was performed on the experimental data and the coefficients of the model were evaluated for their significance. Table 4.2 shows that the linear coefficients (A, B) and the pure quadratic coefficients (A^2 , B^2) were significant ($P < 0.05$). The cross product coefficient (AB) was eliminated in the refined equation as its effect was not significant ($P > 0.05$). The final predictive equation (1) obtained was as follow;

$$\text{Extraction Yield} = 79.69 + 0.8966A + 0.9073B - 3.35A^2 - 1.72B^2 \quad (1)$$

Where A, B were sonication temperature and ultrasonic time, respectively. The analysis of variance of the fitted model was performed. The coefficient of determination (R^2) of the model is 0.9735 which indicated that a reasonable agreement between the experimental and the predicted values.

Table 4.1 Experiment design and results of extraction yield of Roselle fruit juice from ultrasound-assisted extraction.

Std	Run	Factor 1	Factor 2	Response 1
		A:Sonication Temperature (°C)	B:Ultrasonic Time (min)	Extraction Yield (%)
1	12	10	2	73.95
2	9	30	2	74.59
3	10	10	6	74.23
4	5	30	6	75.94
5	4	5.85786	4	71.23
6	3	34.1421	4	74.64
7	2	20	1.17157	74.21
8	13	20	6.82843	78.19
9	6	20	4	79.67
10	1	20	4	79.77
11	7	20	4	79.72
12	11	20	4	79.65
13	8	20	4	79.62

Table 4.2 Analysis of variance for the model representing the extraction yield of Roselle fruit juice.

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	102.85	5	20.57	51.41	< 0.0001
A-Sonication Temperature	6.43	1	6.43	16.07	0.0051
B-Ultrasonic Time	6.59	1	6.59	16.46	0.0048
AB	0.2862	1	0.2862	0.7153	0.4257
A ²	77.98	1	77.98	194.87	< 0.0001
B ²	20.47	1	20.47	51.16	0.0002
Residual	2.80	7	0.4001		
Lack of Fit	2.79	3	0.9290	263.16	< 0.0001
Pure Error	0.0141	4	0.0035		
Cor Total	105.65	12			
R ² = 0.9735					
Adjusted R ² = 0.9546					

Surface response graph of the equation (1) is illustrated in Figure 4.3. Based on the results, the change in sonication temperature or ultrasonic time had significant positive effect on the extraction yield of Roselle fruit juice. According to the model, the extraction yield of Roselle fruit juice achieved maximum of 79.86% at optimal sonication temperature of 22°C and ultrasonic time of 4.4 minute. Three independent replicates were carried out for measuring extraction yield of Roselle fruit juice under the optimal conditions in order to verify the accuracy of the model. The experiment showed that the average extraction yield of Roselle fruit juice was of 79.81%. The predicted and experimental values were nearly similar. Moreover, a control sample without ultrasonic treatment was also performed with an extraction yield of 68.28%. Thus, the application of sonication to Roselle fruit juice increased the extraction yield 11.53% in comparison with the control sample.

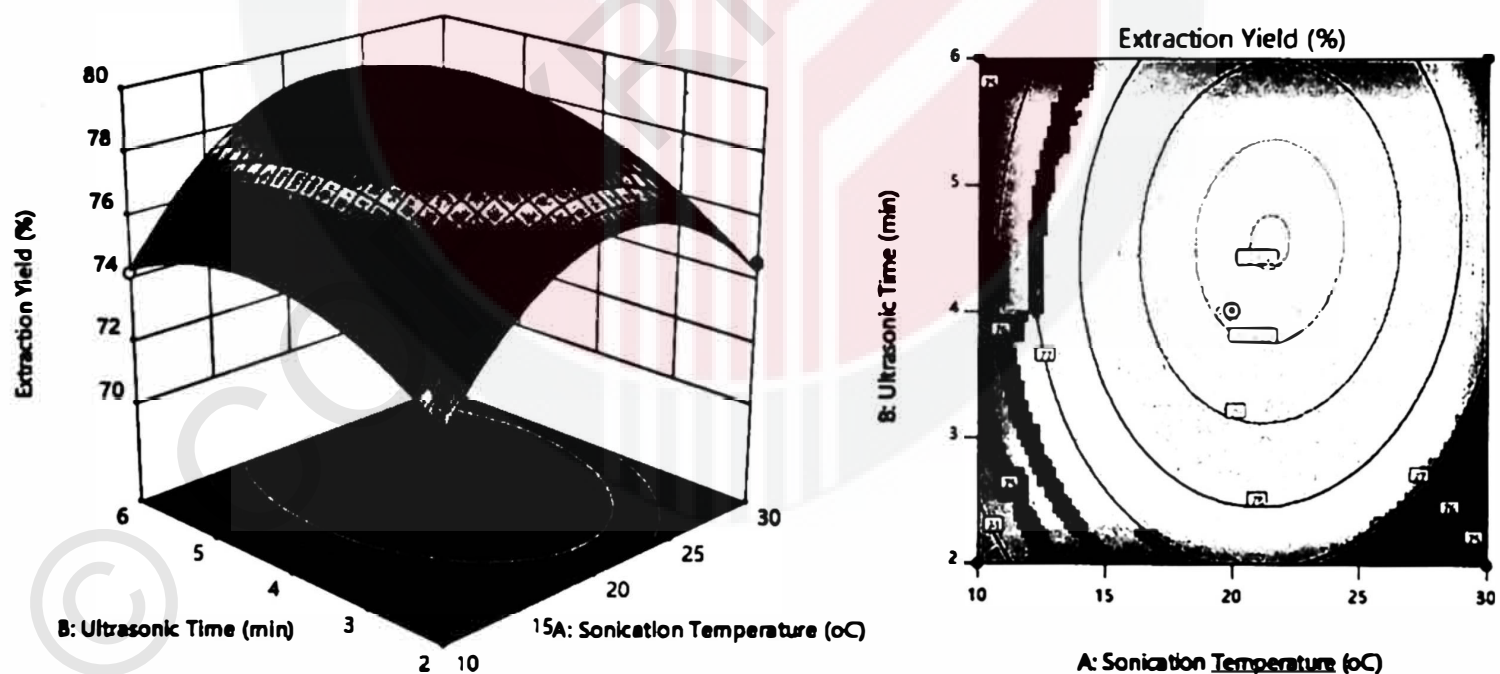


Figure 4.3 Effect of ultrasonic time and temperature on the extraction yield of Roselle fruit juice.

4.3 Effect of Ozone Treatment on Physicochemical Characteristics of Roselle Fruit Juice.

4.3.1 Statistical Analysis

General Factorial experimental design was used to study on the effect of ozonation on physicochemical properties of Roselle fruit juice. Table 4.3 showed General Factorial experimental design on this study with all its dependent variables (pH, titratable acidity (TA), total color difference (TCD), ascorbic acid (AA) total phenolic content (TPC), and total anthocyanin content (TAC)).

Based on the analysis of regression using ANOVA in Table 4.4, the value of R^2 for all respective responses were 0.5628, 0.4643, 0.8245, 0.9481, 0.9144, and 0.9545, accordingly. It showed that the pH, TCD, AA, TPC, and TAC satisfied well with the fitted polynomial models and were significant ($p < 0.05$) except for TA ($p > 0.05$). Furthermore, the lack of fit for all models were considered insignificant as F-values were greater than 0.1. Based on Table 4.4, AA, TPC and TAC showed significant variation for both factors (juice sample and processing time) while pH and TA showed no significant effect for both factors. TCD only affected by processing time.

Table 4.3 General factorial design for ozone treatment of Roselle fruit juice.

Block	Run	Factor 1 A:Juice Sample	Factor 2 B:Processing Time (Min)	Response 1 pH	Response 2 TA* (% citric acid)	Response 3 TCD**	Response 4 AA*** (mg/100ml)	Response 5 TPC**** (mg/L GAE)	Response 6 TAC***** (mg/L)
1	1	Filtered I	30	2.37	0.7424	4.50999	58.33	244.75	55.19
1	2	Filtered I	0	2.36	0.7424	0	72.92	408.92	286.94
1	3	Filtered II	20	2.40	0.7552	2.9749	58.33	287.25	49.68
1	4	Filtered I	20	2.40	0.7680	3.49571	67.31	354.75	105.93
1	5	Filtered II	0	2.41	0.7424	0	70.00	318.92	210.65
1	6	Filtered I	10	2.35	0.7552	1.23693	64.81	444.75	202.41
1	7	Filtered II	10	2.39	0.768	4.52217	62.50	263.08	135.24
1	8	Filtered II	30	2.44	0.7936	4.70532	52.24	154.75	109.63
2	9	Filtered I	20	2.38	0.7424	4.9689	69.09	329.75	88.67
2	10	Filtered II	20	2.39	0.7424	4.28602	61.29	254.75	62.82
2	11	Filtered I	30	2.35	0.7680	5.70263	59.38	268.08	80.42
2	12	Filtered II	30	2.41	0.8064	5.59285	54.29	160.58	120.80
2	13	Filtered I	0	2.37	0.7424	0	74.51	393.92	257.23
2	14	Filtered II	0	2.41	0.7424	0	70.37	300.58	245.84
2	15	Filtered II	10	2.42	0.7680	3.94081	66.67	242.25	117.59
2	16	Filtered I	10	2.38	0.7680	1.23288	70.37	458.08	174.85
3	17	Filtered I	30	2.38	0.7424	4.08901	55.38	260.58	78.53
3	18	Filtered II	0	2.39	0.7552	0	70.59	282.25	221.57
3	19	Filtered I	20	2.38	0.7296	2.57876	64.29	316.42	81.04
3	20	Filtered II	30	2.40	0.7936	7.62234	52.17	144.75	123.24
3	21	Filtered II	10	2.42	0.7936	3.49428	61.02	231.42	133.06
3	22	Filtered I	10	2.36	0.7680	3.45832	67.92	428.92	186.74
3	23	Filtered I	0	2.39	0.7168	0	72.00	403.92	261.54

3	24	Filtered II	20	2.38	0.7424	3.71214	58.06	276.42	57.12
		<i>p-value</i>		0.0135	0.0546	< 0.0001	< 0.0001	< 0.0001	< 0.0001
		<i>F-value</i>		4.12	2.77	15.03	58.45	34.19	67.70
		<i>R²</i>		0.5628	0.4643	0.8245	0.9481	0.9144	0.9549
		<i>SD</i>		0.0181	0.0195	1.10	1.82	31.05	18.77

* Titratable Acidity

p-value < 0.05 is significant

** Total Color Difference

lack of fit insignificant at F-value > 0.10

*** Ascorbic Acid

**** Total Phenolic Content

***** Total Anthocyanin Content

Table 4.4 Regression coefficient, R^2 and p-values for six dependent variables.

Dependent Variables	Regression Coefficient					p-value				
	<i>A</i>	<i>B</i>	<i>AB</i>	<i>B</i> ²	<i>AB</i> ²	<i>A</i>	<i>B</i>	<i>AB</i>	<i>B</i> ²	<i>AB</i> ²
pH	0.0116	0.0017	0.0013	0.0028	0.0084	0.0688	0.7292	0.8045	0.7400	0.3262
TA* (% citric acid)	0.0017	0.0118	0.0067	-0.0012	0.0132	0.0739	4.91	1.58	0.0179	2.17
TCD**	0.5209	2.52	0.1189	-0.7200	-0.2194	0.1675	<0.0001	0.6989	0.1743	0.6707
AA*** (mg/100ml)	-3.13	-7.77	-0.6965	-0.8887	1.22	<0.0001	<0.0001	0.1809	0.3029	0.1619
TPC**** (mg/l GAE)	-66.45	-71.86	9.69	-51.17	14.92	<0.0001	<0.0001	0.2713	0.0025	0.3109
TAC***** (mg/L)	-26.76	-81.31	21.84	61.54	27.75	0.0005	<0.0001	0.0006	<0.0001	0.0054

* Titratable Acidity

p-value < 0.05 is significant

** Total Color Difference

A: Juice Sample

*** Ascorbic Acid

B: Processing Time

**** Total Phenolic Content

***** Total Anthocyanin Content

4.3.2 pH

pH is a quantitative measure used to specify the acidity or basicity of an aqueous solution. pH value depends on the concentration of free hydrogen ions, H^+ which is dissociated from carboxyl group, COOH. A solution with a pH less than 7 is considered acidic and a solution with a pH greater than 7 is considered basic, or alkaline. In this study, the pH value of ozonated Roselle fruit juice was ranged from 2.35 to 2.40 for Filtered I whereas 2.38 to 2.44 for Filtered II within the range recommended by the Mexican Standard (a maximum pH 3)

An ANOVA for pH determination showed both independent variables (juice sample and processing time) were no significant ($p > 0.05$), as shown in Table 4.4. Hence, Figure 4.4 showed the pH value of Roselle fruit juice for Filtered I and Filtered II exhibited insignificant difference over processing time. Similar results were also observed in ozonated strawberry juice (Tiwari et al., 2009a), blackberry juice (Tiwari et al., 2009b), grape juice (Tiwari et al., 2009c) and orange juice (Tiwari et al., 2008) which showed no significant change on pH value over the ozone treatment time. In contrast, previous findings found that there were no significant changes in pH value for Roselle fruit juice which pasteurized at 75°C for 15 seconds and 70°C for 25 minutes, respectively (Ramírez-Rodrigues et al., 2012; Egbere et al., 2007). Therefore, ozone treatment and pasteurization can preserve fruit juice by maintaining the pH at low value in order to prevent the microbial growth in fruit juice.

Nevertheless, Filtered I Roselle fruit juice showed lower pH value in comparison with Filtered II Roselle fruit juice. This can be explained by the hydrolysis of

methylesters by pectin methylesterase (PME) in fruit juice results in the formation of acidity. Furthermore, Filtered I Roselle fruit juice may contain more pectin than Filtered II Roselle fruit juice. Therefore, it was believed that the PME activity in Filtered I Roselle fruit juice is higher giving a lower pH value than Filtered II Roselle fruit juice. This finding agreed with an investigation by Anthon and Barrett (2012) who observed that de-esterification of pectin by PME in tomato juice causing the drop of pH value with the increase of methanol concentration.

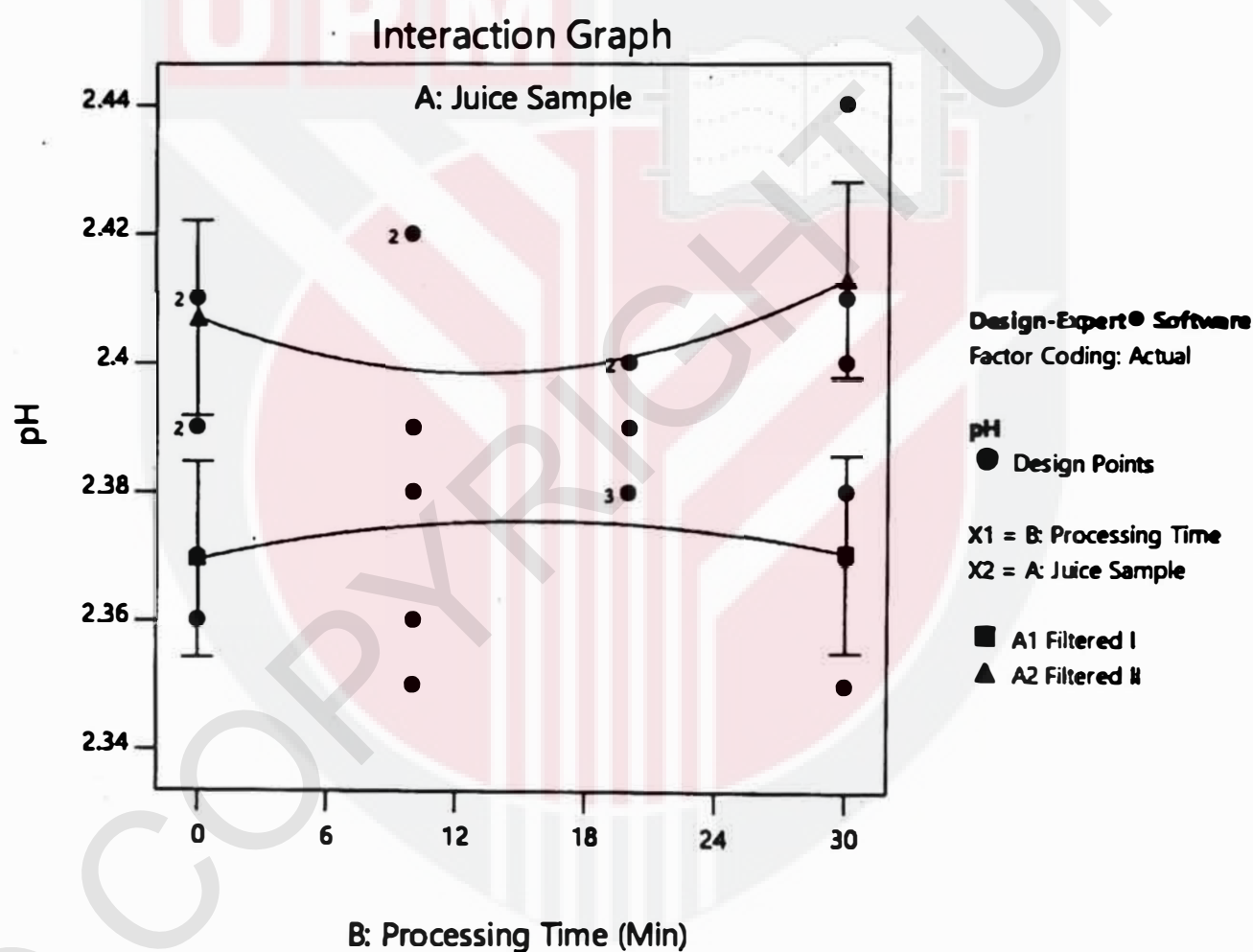


Figure 4.4 Interaction graph of juice sample and processing time on pH.

4.3.3 Titratable Acidity (TA)

Titrateable acidity is a simple measure of the amount of acid in a juice. The high acidity in the roselle calyxes extracts is given by organic acids such as citric, malic, tartaric, hibiscus, succinic, oxalic and ascorbic acid (Youssef et al., 2015). In this study, titrateable acidity of Roselle fruit juice was expressed as percentage of citric acid. Table 4.3 showed that the titrateable acidity of Roselle fruit juice for Filtered I was within the range of 0.7168 – 0.768% whereas for the Filtered II which lied between 0.7424 – 0.8064%.

Based on the ANOVA analysis, ozone treatment had no significant effect ($p > 0.05$) on titrateable acidity in Roselle fruit juice. It can be concluded that the titrateable acidity for both types of Roselle fruit juice exhibited no change over processing time. This finding was in agreement with ozonated strawberry juice (Tiwari et al., 2009a), blackberry juice (Tiwari et al., 2009b), grape juice (Tiwari et al., 2009c) and orange juice (Tiwari et al., 2008) which showed no change on titrateable acidity over the ozone treatment time. In comparison with thermal pasteurization, Mashkour et al. (2013) reported that Roselle fruit juice showed no significant difference on titrateable acidity after pasteurized at 85°C for 10 minutes. Similar result was also observed by Ramírez-Rodrigues et al. (2012) where the Roselle fruit juice was pasteurized at 75°C for 15 seconds. Hence, it showed that ozone treatment and pasteurization have positive effect in preserving the titrateable acidity of fruit juices.

Furthermore, titrateable acidity of Filtered I Roselle fruit juice was slightly lower than Filtered II Roselle fruit juice, as illustrated in Figure 4.5. Plotto et al. (2008)

reported the similar trend that citric and malic acid were found lower in juice with pulp as compared with filtered juice, thus explaining the perception of tasting sweeter. Titratable acidity is also responsible for tasting sensation, for instance, too much acidity may render the juice or cider too sharp and unpleasant to drink (Jolicoeur, 2015). This study indicated that the taste of Roselle juice with low pH value is not acidic and it is organic in nature therefore less corrosive to gastro intestinal tract.

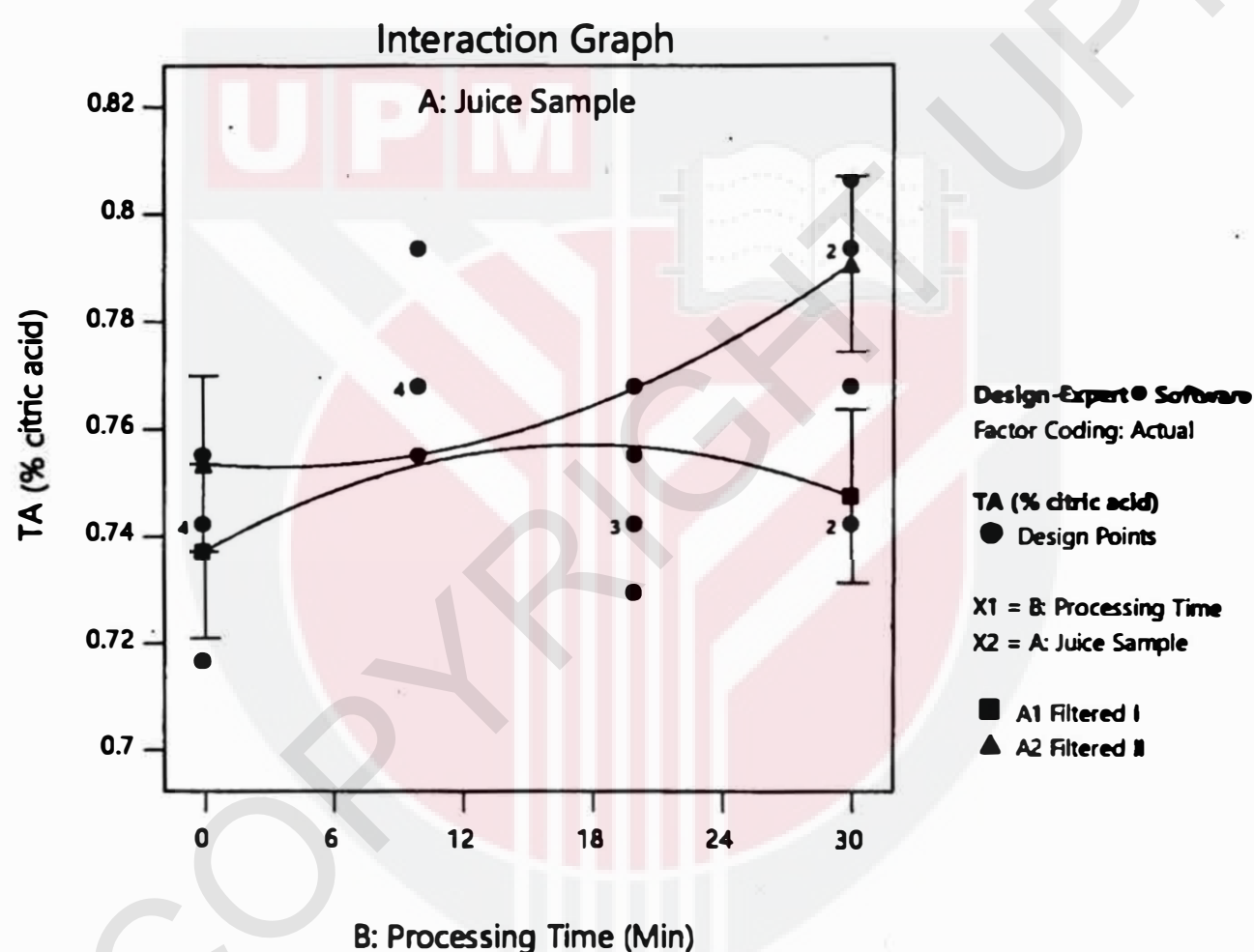


Figure 4.5 Interaction graph of juice sample and processing time on TA.

4.3.4 Total Color Difference (TCD)

Total color difference is the magnitude of color change after treatment. Differences in perceivable color can be analytically classified as very distinct (TCD > 3),

distinct ($1.5 < \text{TCD} < 3$), and small differences ($\text{TCD} < 1.5$) (Tiwari et al., 2009b). Table 4.3 showed that the range of TCD value is from 0 to 5.70 for Filtered I Roselle fruit juice and 0 to 7.62 for Filtered II Roselle fruit juice over the ozone treatment time. Thus, overall color changes for both types of Roselle fruit juice during ozonation were very distinct, indicating major changes in the appearance of Roselle juice color.

In this study, TCD values were significantly influenced by processing time ($p < 0.0001$) as shown in Table 4.4. A trend towards increased TCD values for both types of Roselle fruit juice at increasing ozone processing time was observed, as illustrated in Figure 4.6. Similar results have been observed in ozonated apple juice (Torres et al., 2011), strawberry juice (Tiwari et al., 2009a), blackberry juice (Tiwari et al., 2009b), grape juice (Tiwari et al., 2009c) and orange juice (Tiwari et al., 2008) which showed a significant color degradation over the ozone treatment time. On the other hand, Zaman et al. (2017) reported that thermal treatment (90°C for 2 minutes) of extracted Roselle fruit juice showed a massive decrement with L^* (lightness) due to heating cause the accumulation of dark color compound in juice. Besides, Roselle fruit juice which pasteurized at 82.5°C for 20 minutes obtained L^* value of 14.3 (Mgaya et al., 2014), which was lower as compared to this study with L^* value ranging from 19.4 to 22.8. Both ozone treatment and thermal pasteurization result in a significant degradation of fruit color. Thus, an improved understanding of ozone effect on retaining the color of Roselle fruit juice is important in order to facilitate the adoption of this preservation technique.

Nevertheless, TCD value for Filtered II Roselle fruit juice was higher than Filtered I Roselle fruit juice. According to Tiwari et al. (2018), ozone can react and

attack the conjugated double bond in chromophores of some organic dyes, carotenoid or anthocyanin which results in loss of color. Similarly the chromophore of conjugated double bond for anthocyanin which is responsible for Roselle juice color maybe degraded as above. Furthermore, Stein-chisholm (2015) stated that filtered juices retained less anthocyanidins from raw due to the removal of copigmentation of anthocyanins by filtration. It can be related to the decreasing trend of anthocyanin (TAC) which gave a lower TAC value in Filtered II Roselle fruit juice, as shown in Figure 4.9. Therefore, Filtered II Roselle fruit juice had higher TCD value than Filtered I Roselle fruit juice after ozone treatment.

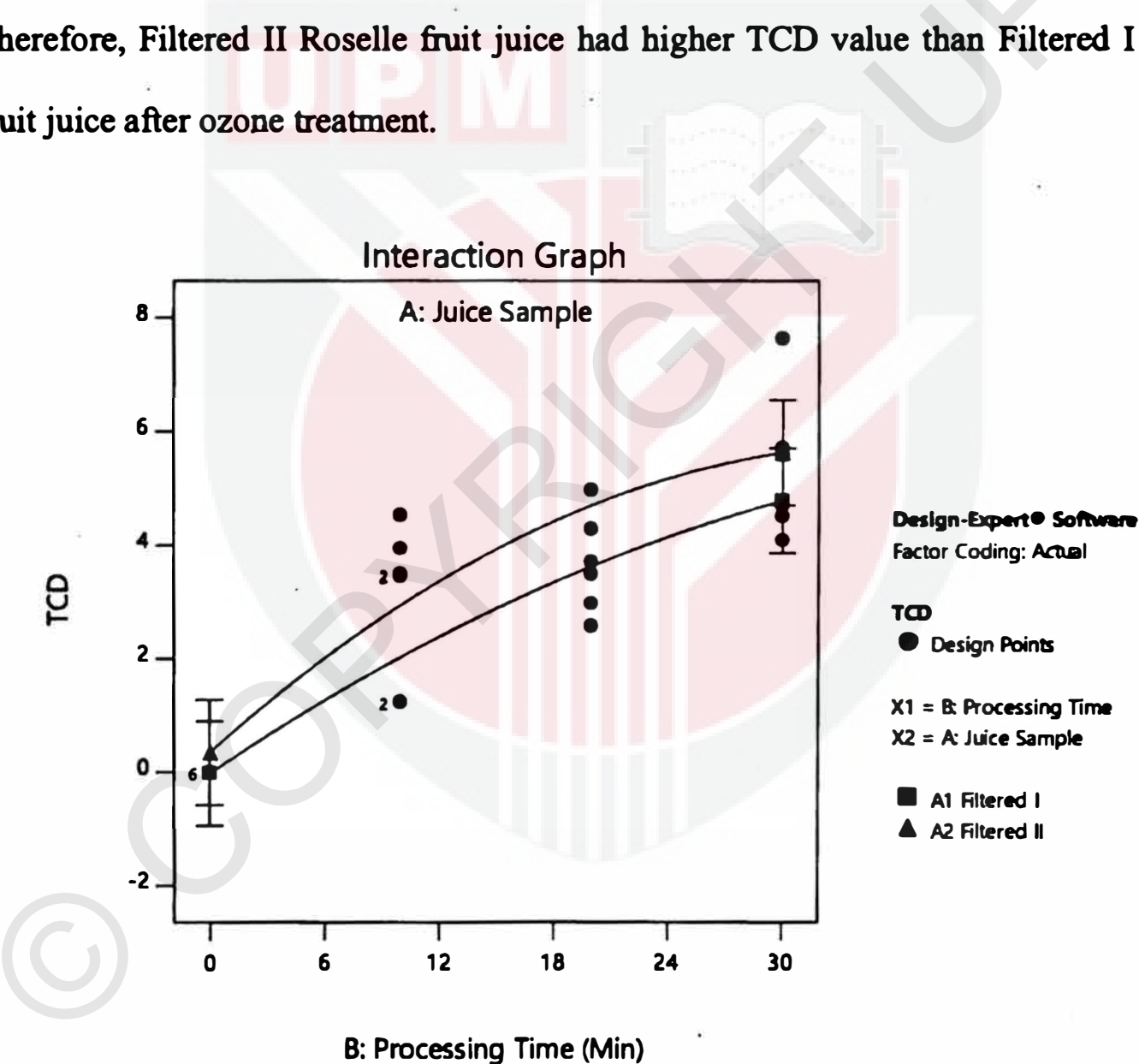


Figure 4.6 Interaction graph of juice sample and processing time on TCD.

4.3.5 Ascorbic Acid (AA)

Ascorbic acid is important antioxidants which provide a beneficial biological defence mechanism against free radical damage of biological compound, such as DNA (Range, 2015). Ascorbic acid as a quality indicator in fruits and vegetables as it is sensitive bioactive compound providing an indication of the loss of vitamins and therefore acting as a valid criterion for other organoleptic or nutritional components (Zulueta et al., 2010). In this study, the initial ascorbic acid of 74.51 mg/100ml reduced about 25.67% (55.38 mg/100ml) for Filtered I Roselle fruit juice while initial ascorbic acid of 73.08 mg/100 ml reduced about 28.61% (52.17 mg/100ml) for Filtered II Roselle juice as shown in Table 4.3.

ANOVA for ascorbic acid determination of Roselle fruit juice showed statistical significant effect ($p < 0.05$) by both independent variables; juice sample and processing time as shown in Table 4.4. Figure 4.7 showed that the ascorbic acid content for both types of Roselle fruit juice decreased significantly throughout the ozone processing time. A similar study taken by Tiwari et al. 2009a, found that ozonation of strawberry juice with sufficiently large dose to meet FDA regulations giving a significant degradation of ascorbic acid. In contrast, thermal treated (90°C for 2 minutes) Roselle fruit juice showed a reduction in ascorbic acid content about 42.0% reported by Zaman et al. (2017), which was greater than both ozonated Filtered I and Filtered II Roselle fruit juice. In addition, a previous study found that Roselle fruit juice resulted in a significant reduction in ascorbic acid of about 53.41% after pasteurized at 70°C for 25 minutes (Egbere et al., 2007). Therefore, it proved that ozone treatment preserved better quality of Roselle fruit juice in respect to ascorbic acid content than thermal pasteurization.

Moreover, degradation of ascorbic acid in Roselle fruit juice was lower than 50% which means it is able to prolong the shelf life of juice.

Nevertheless, ascorbic acid of Filtered I Roselle juice was higher than Filtered II Roselle fruit juice due to the removal of antioxidant compound by filtration. Degradation of ascorbic acid is caused by the formation of free hydroxyl radicals by photochemical reaction, related to oxidative processes (Santhirasegaram et al., 2015; Chia et al., 2012). In addition, the presence of oxygen, light, and enzyme activities such as ascorbate oxidase and peroxidase contribute to detrimental effects on ascorbic acid (Santhirasegaram et al., 2015).

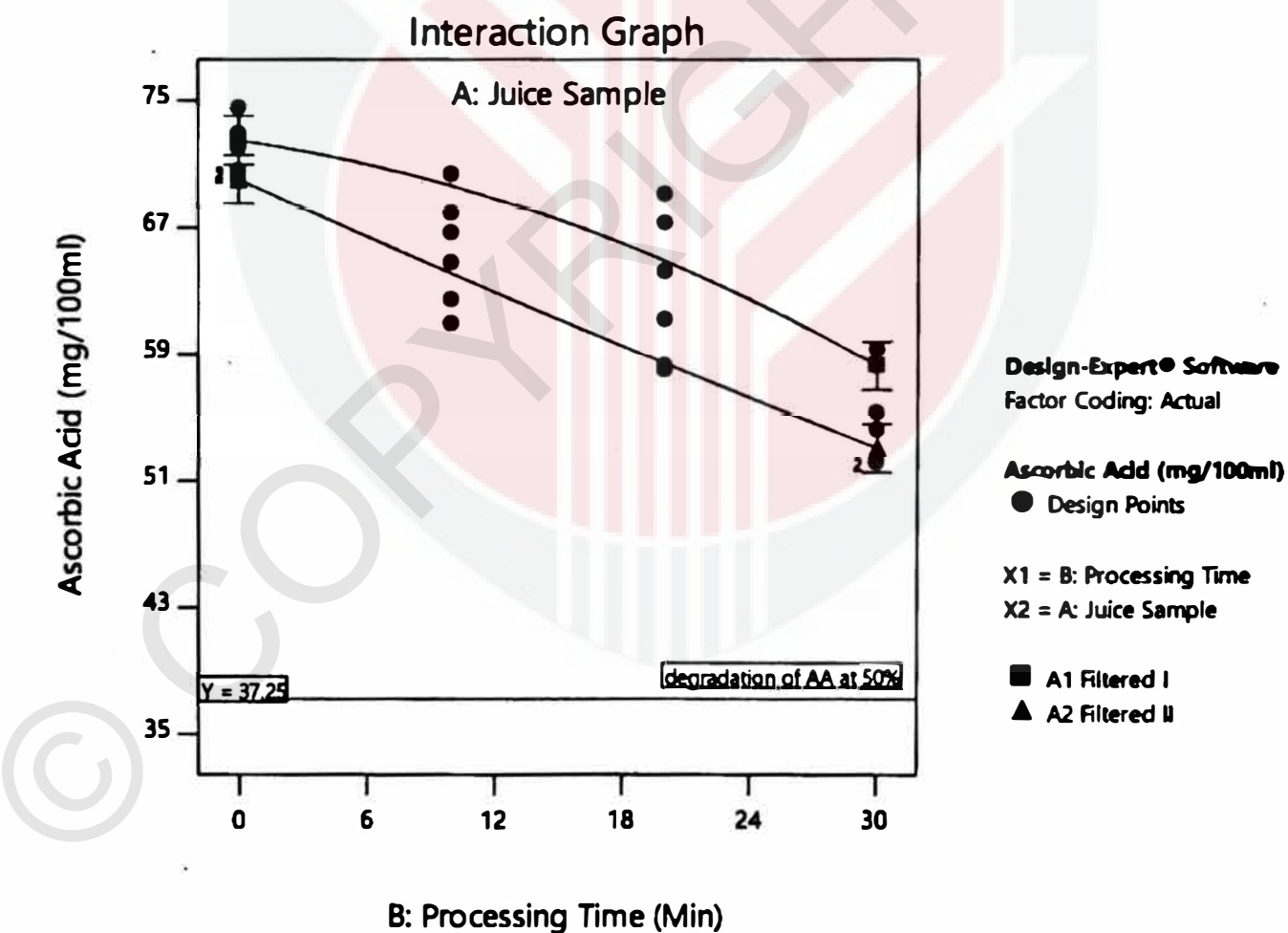


Figure 4.7 Interaction graph of juice sample and processing time on AA.

4.3.6 Total Phenolic Content (TPC)

Phenols are aromatic compounds containing one or several hydroxyl groups directly attached to the benzene ring (Keskin et al., 2012). Natural phenolic compounds are capable of performing the repair and adaptation functions in human organism due to the antioxidant activity (Owczarek et al., 2004). Roselle is a rich source of phenol compounds, including phenol acids, flavonoids, and anthocyanidin (Borrás-Linares et al., 2015; Youssef et al., 2015).

From statistical analysis of data, it showed that sample juice and processing time affected significantly ($p < 0.05$) the TPC value of Roselle fruit juice. Figure 4.8 showed that the TPC value of Filtered I Roselle fruit juice decreased significantly from 402.25 to 257.80 mg/L GAE while for Filtered II Roselle fruit juice decreased from 300.58 to 153.36 mg/L GAE over 30 min of ozone treatment. Similarly, treatment of ozonated apple juice for 10 min resulted in a significant reduction in TPC value (Patil et al., 2010). The degradation of polyphenol is due to the strong oxidative potential of ozone, resulting in direct reaction with target compounds or its intermediates and radical reactions between hydroxyl radicals through ozone decomposition catalyzed mainly by the hydroxide ion (Torres et al., 2011; Patil et al., 2010; Cullen et al., 2009). According to Ramírez-Rodrigues et al. (2012), TPC value of Roselle fruit juice after HTST (75°C for 15 seconds) was 50.79 mg/L, which is much lower than this study. Therefore, it is important to understand the ozone effect in order to retain the total phenolic content of juice.

However, Figure 4.8 also showed that the TPC value in Filtered I and Filtered II Roselle fruit juice increased slightly after 10 min and 20 min of ozone treatment, respectively. The increase of TPC value due to ozone treatment was also confirmed in the study of Onopiuk et al. (2017), where the polyphenolic content in pineapples and bananas increased significantly after 20 min exposure to ozone. This growth tendency may be caused by the activation of phenylalanine ammonium lyase (PAL), as enzyme can be stimulated by different abiotic stresses besides wounding (Onopiuk et al., 2017; Yeoh et al., 2014; An et al., 2007). PAL is an enzyme participating in synthesis of phenolic compounds in plant tissues. Furthermore, several studies have reported that the presence of ozone may contribute to the inhibition of enzymes such as polyphenolic oxidase (PPO) and peroxidase (POD), which often cause oxidation of phenolic compounds (Onopiuk et al., 2017; Yeoh et al., 2014).

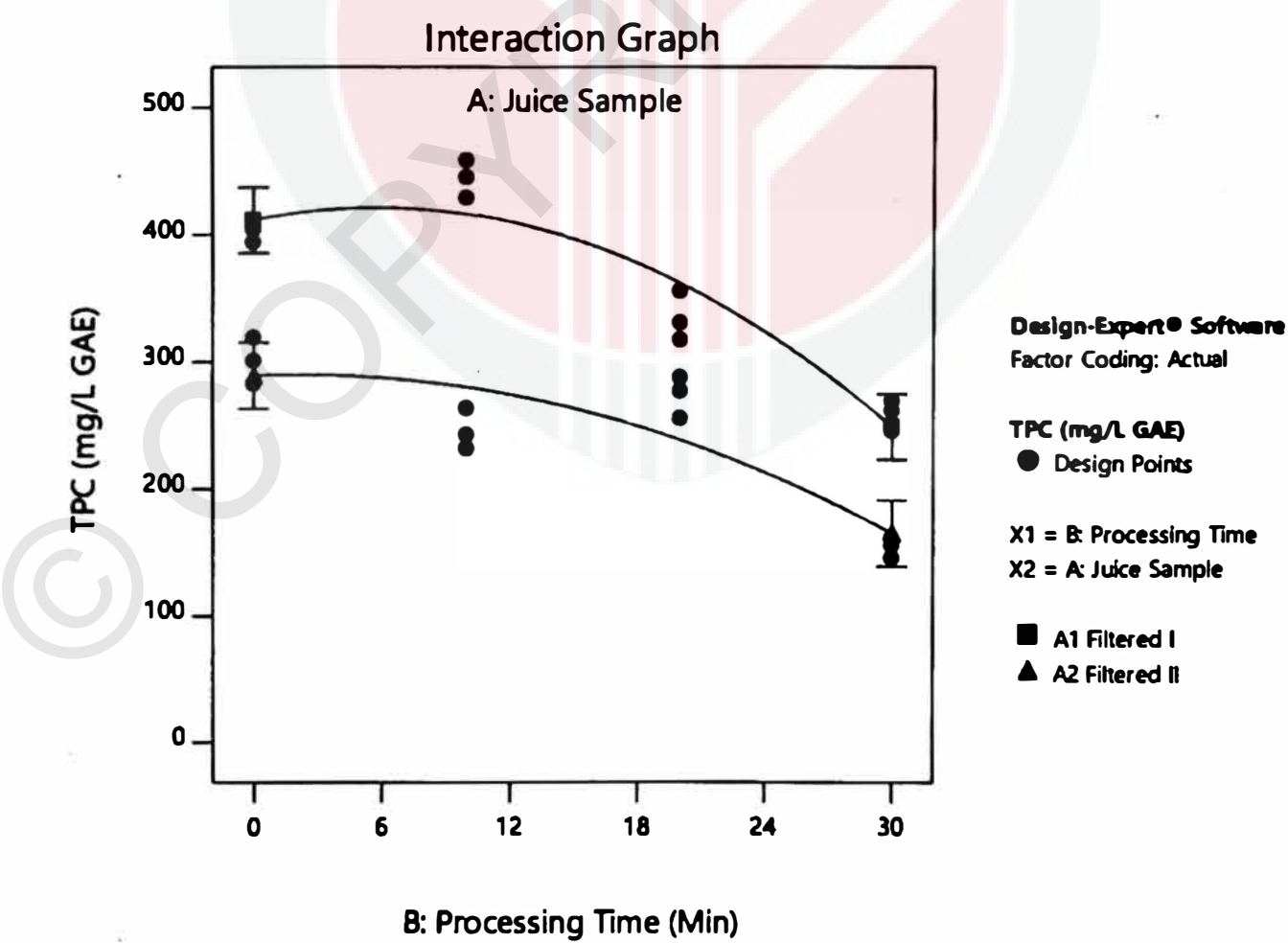


Figure 4.8 Interaction graph of juice sample and processing time on TPC.

4.3.7 Total Anthocyanin Content

Anthocyanin is the major compounds attributed to the protective health benefits associated with fruits and vegetables (Khanal et al., 2010). A significant property of anthocyanin is their antioxidant activity, which plays an important role in the prevention of cardiovascular disease, cancer, diabetes, obesity and anti-inflammatory (Steinchholm, 2015; Patras et al., 2010). It is this factor that contributes to the importance of juice processing to preserve these compounds.

ANOVA for anthocyanin (TAC) determination of Roselle fruit juice showed statistical significant effect ($p < 0.05$) by both independent variables; juice sample and processing time, as shown in Table 4.4. Figure 4.9 showed that the TAC value of Filtered I Roselle fruit juice decreased significantly from 268.57 to 71.38 mg/L (73.42%) over 30 min of ozone treatment. Filtered II Roselle fruit juice decreased from 226.02 to 56.54 mg/L (74.98%) over 20 min of ozone treatment, but increased again to 117.89 mg/L after 30 min of ozone treatment. This finding was in agreement with the reduction of 98.2% in anthocyanin of strawberry juice (Tiwari et al., 2009a) and 78.0% in anthocyanin of grape juice (Tiwari et al., 2009c) with increasing treatment time and ozone concentration. Nevertheless, the increase of anthocyanin of Filtered II Roselle fruit juice after 30 min of ozone treatment may cause by storage period. Onopiuk et al. (2017) reported that the content of raspberries anthocyanin increased during storage. For thermal pasteurization, the magnitude and duration of heating has a strong influence on anthocyanin stability (Zaman et al., 2017; Patras et al., 2010). The half-life of Roselle anthocyanin decreased with increasing heating temperature from 50°C to 98°C, which proved that Roselle anthocyanin exhibited a significant degradation (Lee et al., 2015;

Idham et al., 2012). Therefore, it is important to understand the ozone effect in order to retain the total anthocyanin content of juice.

Moreover, Filtered I Roselle fruit juice retained more anthocyanidins from raw than Filtered II Roselle fruit juice due to the removal of copigmentation of anthocyanins by filtration (Stein-chisholm, 2015). Degradation of anthocyanin undergo oxidative cleavage either to direct reaction with ozone or indirect reaction due to the formation of intermediate radicals ($\bullet\text{OH}$, $\text{HO}^2\bullet$, $\bullet\text{O}_2^-$, and $\bullet\text{O}_3^-$). This may lead to electrophilic and nucleophilic reactions occurring with aromatic compounds that are substituted with an electron donor (OH^-) having high electron density on the carbon compounds in ortho and para positions (Cullen et al., 2009). Thus, the formation of ozonide broken down the aromatic ring and degradation efficiency rises quickly (Tiwari et al., 2009a; Tiwari et al., 2009c).

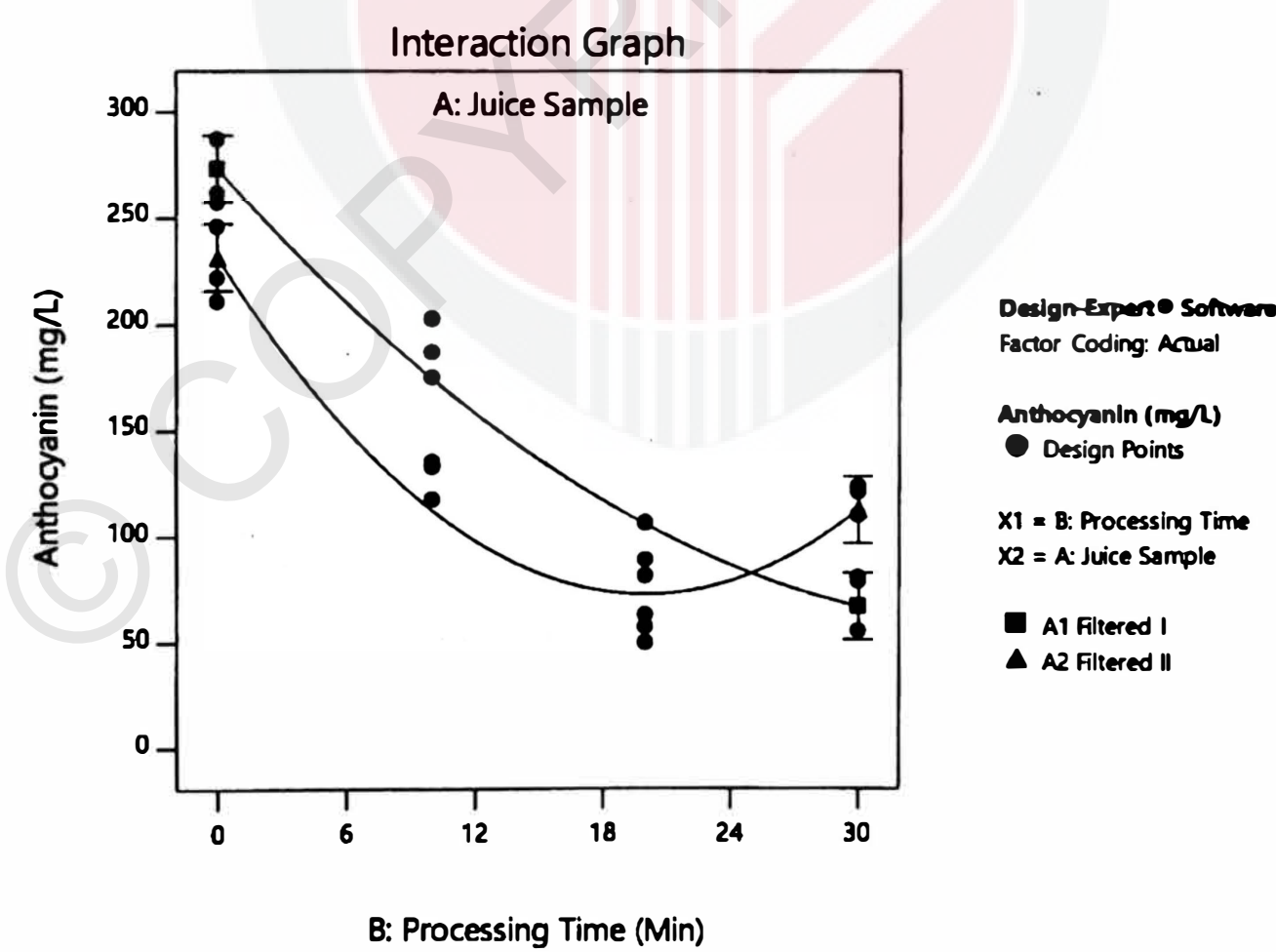


Figure 4.9 Interaction graph of juice sample and processing time on TAC.

CHAPTER 5

CONCLUSION

5.1 Conclusion

In comparison with traditionally extraction techniques, application of ultrasound in Roselle fruit treatment enhanced extraction yield and shortened treatment time. The extraction yield of Roselle fruit juice increased 11.53% in comparison with the control at optimal sonication temperature of 22°C and ultrasonic time of 4.4 min. Low sonication temperature during Roselle extraction gives low energy input and low degradation of nutrient compound in Roselle fruit juice. The effects of juice sample and ozone processing time on physicochemical properties of Roselle fruit juice were evaluated. No significant differences ($p > 0.05$) in pH and TA of Roselle fruit juice were observed. However, ozonation was found to have a significant effect ($p < 0.05$) on TCD, AA, TPC and TAC. Nevertheless, ozone treatment showed lower degradation of ascorbic acid as compared to thermal pasteurization. From this study, ozonated Filtered I Roselle fruit juice was found to retain higher nutrient than Filtered II Roselle fruit juice. Overall, the results demonstrate the effects of ultrasound extraction and ozonation in preservation of fruit juice compared to thermal pasteurization.

5.2 Recommendation for Future Work

The use of ultrasound in extraction of Roselle fruit juice can be regarded as fast and effective. Further studies are warranted to clarify the effects of sonication variables (ultrasonic power, medium properties, and operating frequency) on extraction yield of juice. Direct ozone treatment was found to be a non-thermal processing method to preserve Roselle fruit juice. However, the effect of ozonation on inactivation of microorganisms is needed to be determined before it can be considered by processors as a preservation technology in liquid food applications. Moreover, other extrinsic and intrinsic parameters such as ozone concentration, temperature, ozone flow rate, and pH on ozone treatment warrant further study in order to determine the optimal condition for preservation of fruit juice.

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