



**UNIVERSITI PUTRA MALAYSIA**

***CLARIFICATION OF BANANA JUICE BY MEMBRANE-BASED  
PROCESS***

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## **ABSTRACT**

**Banana is well-known as one of the world's most popular fruits due to its delicious taste and nutritional contents. The processing of banana, as fresh juice, can offer benefits to human health by utilizing its functional properties. This can be done using a pressure-driven membrane processes to enhance of the quality of this natural juice by preserving its nutritional and biological properties. Prior to the membrane-based process, it is often begins with enzymatic treatment to facilitate the filtration process. Thus, this study explored the effects of pre-treatment with different concentrations of pectinase enzyme on the attribution of banana juice. Moreover, this study also evaluated the effects of trans-membrane pressure on the permeate flux performance and quality attributes of the filtered banana juice. It was found that the enzymatic pre-treatment of banana juice has profound influence on the permeate flux and membrane fouling. The enzyme hydrolysis of the banana juice resulted in higher flux. The study discovered that the most effective concentration of enzyme was 0.1%. The enzymatic treated banana juice was later undergone the ultrafiltration process in which the physical and chemical properties analyses was done on the pH, total soluble solids, turbidity, clarity, color and total phenolic content. The integrated membrane process in this study had facilitated the production of clarified banana juice with high quality in terms of turbidity, clarity and color. The enhancement on color properties was observed in which the membrane process was successfully in producing a very clear and translucent juice.**

## **ABSTRAK**

Pisang terkenal sebagai salah satu buah-buahan yang paling popular di dunia kerana rasa yang lazat dan kandungan nutrisi. Pemprosesan pisang, seperti jus segar, boleh member manfaat kepada kesihatan manusia dengan menggunakan sifat-sifat fungsinya. Ini boleh dilakukan dengan menggunakan proses membrane tekanan yang didorong untuk meningkatkan kualiti jus semula jadi ini dengan mengekalkan kandungan nutrisi dan sifat biologi. Rawatan enzim selalu dilakukan sebelum proses membrane untuk memudahkan proses penapisan. Oleh itu, kajian ini adalah untuk meneroka kesan-kesan pra-rawatan dengan kepekatan enzim pectinase yang berbeza terhadap sifat pemakanan jus pisang. Selain itu, kajian ini juga menilai kesan tekanan trans-membran pada prestasi penyerapan membran dan sifat-sifat kualiti jus pisang. Berdasarkan kajian, mendapati bahawa pra-rawatan enzim terhadap jus pisang mempunyai pengaruh yang amat mendalam kepada fluks penyerapan dan membran fouling. Hidrolisis enzim jus pisang menyebabkan fluks yang lebih tinggi. Kajian ini mendapati bahawa peratusan kepekatan yang paling berkesan enzim adalah 0.1%. Jus pisang tersebut kemudian telah menjalani proses ultrafiltrasi di mana analisis kimia dan fizikal dilakukan pada pH, jumlah pepejal larut, kekeruhan, kejelasan, warna dan jumlah kandungan fenolik. Proses membrane bersepadu telah membenarkan produk jus-jus ini terutamanya jus pisang adalah berkualiti tinggi dari segi kekeruhan, kejelasan dan warna. Peningkatan keatas sifat-sifat warna diperhatikan dalam proses membrane tekanan telah didorong dengan penghasilan jus yang sangat jelas dan telus.

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## **LIST OF ABBREVIATIONS**

<b>MF</b>	<b>microfiltration</b>
<b>UF</b>	<b>ultrafiltration</b>
<b>NF</b>	<b>nanofiltration</b>
<b>RO</b>	<b>reverse osmosis</b>
<b>PME</b>	<b>pectinmethylesterase</b>
<b>ED</b>	<b>electrodialysis</b>
<b>MWCO</b>	<b>molecular weight cut off</b>
<b>TMP</b>	<b>trans-membrane pressure</b>
<b>TSS</b>	<b>total soluble solid</b>

## **CHAPTER 1**

### **INTRODUCTION**

#### **1.1 Background**

**Banana (*Musa spp*) is a herbaceous plant of the family *Musaceae* which is a type of tropical fruits that are widely planted in Asia and South America. It consists of high sugar content which gives high nutritional value to the fruits. It also contains various essential nutrients such as sodium, potassium, magnesium, Vitamin A and C (Tchewonpi, Jong, Karmakar, & De, 2014). Figure 1 showed the image of the Cavendish banana that was selected to be used in the experiment.**



**Figure 1: Cavendish banana**

Banana can be well-known as the world's leading crop due to its nutrition aspect and economic value. It can be considered as the world's largest production crop that grown in most of the developed country. Production of banana had highly contribute to the economic gain to the country as it been the most consumed fruit in the world due to its nutrients composition. It is also consider as an important worldwide staple food.

Banana is mostly consumed as fresh fruits. It can be processed to become various types of food products which are banana puree, banana chips, banana slices, banana jam, banana figs, banana powder and banana vinegar (Tsen & King, 2002). Besides this, it is also being processed into different products at small and industries scales, such as dried fruit, chip, smoothie, ice cream, wine and ingredients for functional foods (Jackson & Badrie, 2003; Sodchit, Tochampa, Kongbangkerd, & Singanusong, 2014). Banana is also suitable to be processed into fruit juice.

Banana is a delicious food and loved by most of the people. People are likes to drink juice because it is easy to prepare. Drinking banana juice able to increase the digestion of food particles in our body and prevent constipation as it contains high

amount of dietary fiber. Furthermore, banana contains high sugar content and it able to boost energy level in our body. Banana juice contains high content of vitamin and mineral. High level of potassium in the banana able to maintain the blood pressure and protect against hypertensions, as well as stroke and heart attack.

Normally, fruit juices can be clarified with the traditional method which is enzymatic treatment and coagulation method. For the enzymatic treatment, juices are being extracted using enzymes such as pectinase, cellulase and amylase. Pectinase breaks down the pectin, reduce the viscosity and cluster formation of the juice and thus facilitate the filtration or centrifugation process. At the low temperature, clarity of banana juice tends to increase rapidly at the beginning stage but become slower to the end. On the contrary, there is problem encounter during the cold storage of the juices where it will become cloudy and unstable.

Membrane-based process is a method to separate particles in liquid solutions or gas mixtures. It can operate without the usage of heat. It had been widely used to substitute the traditional juice clarification method as it requires less man power and efficient process. Membrane processes can lower thermal damage to the product and thus enhance the juice aroma retention. The membrane processes are pressure-driven process which it includes microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO). The greatest advantages of the process as it lower energy usage which in turn able to cut down the operating cost. Besides this, it allows significant improvement on process efficiency and quality control.

Membrane-based process has a potential in the banana juice clarification. This method requires fewer chemical additives to remove impurities and it able to performed isothermally at low temperature and thus reducing the energy

**consumption comparing to the traditional methods. This process is able to retain the original nutritional value and quality of the fruit juice. This is because the retention of natural color and pleasant aroma of the juice is significantly lost during thermal evaporation. Moreover, membrane-based operation does not require any phase changes. These features had contributes an essential factors to produce natural fresh taste banana juice. Thus, it leads to objective of my study.**



## **1.2 Problem Statement**

**Banana is considered as very important crop in many countries, but a large percentage of the crop is typically unsuitable for the fresh market since it is too mature for shipment. Since bananas are high in sugar content and have a recognizable and desirable flavor that are pleasant for most consumers. Thus, this high value clarified juice from these excess bananas could become valuable products from otherwise rejected bananas. There is some problems are associated with the banana juice processing. A high viscosity, problems with juice extraction and browning problems seem to be the most severe. Studies conducted is to address these problems associated with banana juice are summarized in this paper.**

### **1.3 Objective**

**The objectives of this project are:**

- 1. To determine the effect of pre-treatment using different concentrations of pectinase enzyme on the attribution of banana juice.**
- 2. To evaluate the effects of different trans-membrane pressure during the membrane-based process on the permeate flux performance and the quality attributes of banana juice.**



## **1.4 Scope of Study**

The main focus on this study is on the Cavendish banana which covers the clarification of the juice by membrane-based process. We study on the effect of trans-membrane pressure (TMP) on the flux performance and quality attributes on the banana juice. The study is to examine how membrane pore size and trans-membrane pressure to affect the flux performance and quality attributes on the banana juice.



## **CHAPTER 2**

### **LITERATURE REVIEW**

#### **2.1 Banana**

**Banana (*Musa spp*), is a type of giant monocotyledon herbs (Li & Ge, 2017). It is categorized as the family of Musaceae which comprised from a number of hybrids in the genus *Musa* (Pereira & Maraschin, 2015). Genus *Musa* is divided into four, which is *Eumusa*, *Rhodochlamys*, *Australimusa* and *Callimusa* (Vu, Scarlett, & Vuong, 2018). Banana is the world's second largest production among the fruits after citrus (Mohapatra, Mishra, & Sutar, 2010). World banana production had significantly increased every year and almost reached 100 million tones on 2014 (Baini & Langrish, 2007). Moreover, it had become the major crop for the fruit production in Brazil (Saraiva, Castelan, Gomes, Purgatto, & Cordenunsi-Lysenko, 2017).**

**Banana is being widely consumed by all ranges of people (Ghag & Ganapathi, 2017). The banana plantation is mostly grown by small-scale farmers and it had been played an significant socioeconomic role in most developed countries (Panis, 1995).**

**As studied by researchers, over 300 types of banana are cultivated around the world (Singh, Singh, Kaur, & Singh, 2016a).**



## **2.2 Nutritional Values**

Banana contains of high nutritional value and it is popular among the consumers due to its unique aroma and texture (Mohapatra et al., 2010). Banana consists of high amount of carbohydrates and important vitamins such as vitamin B and C and minerals such as phosphorus and potassium (Ghag & Ganapathi, 2017). According to Lim, Lim, & Tee(2007), banana also is a sources of phytonutrients which including vitamins and phenolic compounds. Based on several researchers, they proved that banana was a source of phytochemicals for health enhancer (Davey et al., 2009; Someya, Yoshiki, & Okubo, 2002).

Singh et al. (2016) indicated that banana consists of bioactive components, including carotenoids, biogenic amines and phytosterols, which are a good source for diet that able to enhance human health. It is claimed that banana have a higher antioxidant levels compare to some berries, herbs and vegetables. Banana consists of antioxidant compounds and people from the past are used to cure various diseases. This including minimize the risk of chronic degenerative disorders (Singh et al., 2016a).

Banana also contains high amounts of total phenolic compounds and flavonols. These compounds are important for the banana fruits as it imparts astringent taste to the unripe banana (Singh, Singh, Kaur, & Singh, 2016b). There is many researchers had found out that flavonoids present in banana are advantageous for human health. Flavonoids act as protective scavengers against oxygen-derived free radicals and reactive oxygen species (ROS) in which responsible for aging and various disease (Singh et al., 2016a).

**Additionally, banana had been classified as the most affordable fruits for human consumption due to its immense nutritional and medicinal value (Borges et al., 2014). Generally, banana is often being recommender for anemic patients due to its high iron content. Besides, it is also proven by researchers that being beneficial in control blood pressure (Singh et al., 2016b).**



### **2.3 Uses and Potentials**

Despite being consumed freshly, banana also can be processed (W.C. Lee, S. Yusof, 2006). Banana is normally processed into puree, jam, juices, chips, powder or vinegar. Besides, banana can also be used to produce banana fiber and paper. Apart from that, banana can also used to process beer, edible dried chips and flour (Ghag & Ganapathi, 2017). It also being utilized as constituents for various food products (Singh et al., 2016a).

Unripe banana fruits are commonly used to make banana chips by deep-frying the banana's slices. Besides, banana puree can also be used in the drinks, dairy desserts, sausages and other processed foods (Singh et al., 2016a).

Despite all the benefits offered, there are many drawbacks in the banana industry that require further improvement. Commonly, the uneven size and shape and external injury of fresh fruits are often getting rejected in the supermarket. Moreover, a fully ripened banana deteriorate very quickly and it is not suitable for cold storage (Bora, Handique, & Sit, 2017). Furthermore, it is hard to extract and clarify the banana juice due to its pectic content of the fruits (Tchewonpi et al., 2014). Thus, banana has potential to become the source of raw materials to be processed into juice (W.C. Lee, S. Yusof, 2006).

## **2.4 Traditional Juice Extraction**

Methods such as normal hydraulic pressing, centrifugation or hot water extraction is the traditional way to extract fruit juice (W.C. Lee, S. Yusof, 2006). Some traditional methods involved of fruits mashing in mechanical way and then squeeze out the juice which is cloudy (Bora et al., 2017). However, this method is complicated and require great amount of energy that only result a low yield of juice (Egwim, Ogudoro, & Folashade, 2013). Furthermore, the juices obtained are turbid, viscous and cloudy in color (Bora et al., 2017). Hence, clarification process is mandatory in order to improve its appearance and marketability in the fruit juice processing (Cerreti et al., 2017).

Ripe peeled or unpeeled bananas is being extracted by a traditional methods which is hand mashing in plastic basins using *Imperata cylindria* grass to produce banana juice (Gensi, Kyamuhangire, & Carasco, n.d.). Researchers had evolved a mechanical juice extractor by mixing peeled banana with spear grass in order to minimize the extraction time. However, the yield is still low as compared to the traditional method (Kasozi & Kasisira, 2005).

Some common clarification process involves several steps. These included centrifugation in order to eliminate suspended solids, hydrolysis of pectin with specific enzyme (depectinization), flocculation of turbidity with clarifying agents and filtration by diatomaceous earth to remove fining agents (Rai & De, 2009).

## **2.5 Enzyme Pre-treatment**

The most critical issues in fruit juice processing is the unstable color after bottling (Lee, Yusof, Hamid, & Baharin, 2007). Extracted fruit juices are normally turbid due to the presence of plant residues and colloid macromolecules (pectin and protein) that are not water soluble (fiber, cellulose, starch and hemicelluloses) (Urošević, Povrenović, Vukosavljević, Urošević, & Stevanović, 2017). Additionally, the enzymatic and non-enzymatic browning will give rise to the darkening of the banana juice during processing and storage (Beveridge & Wrolstad, 1997).

For the sake of avoid the browning and haze formation and optimize the taste characteristics in the banana juice (T. & E., 2007). Thus, it is require to clarify the commercialized fruit juice to remove the macromolecules in order to prolong the storage period of the products (Bhattacharjee, Saxena, & Dutta, 2017). Enzyme pre-treatment is normally applied in the raw juices to reduce the pectic substances and starch content (Bhattacharjee et al., 2017). This method reduced the viscosity and cloudiness of the juice and thus aids the clarification process (Bhattacharjee et al., 2017). Clear and attractive juices can be obtained when extracted the raw fruits with enzymes (Sharma, Patel, & Sugandha, 2016). There is many researches had been used pectinase enzyme to improve the clarification of banana juice (Egwim et al., 2013; Tchewonpi et al., 2014).

Researchers had found out that enzymatic pretreatment will assist in obtaining high flux performance during fruit juice clarification by membrane process with the aids in lowering the viscosity and pectic materials reduction (Maktouf et al., 2014; Nandi, Das, Uppaluri, & Purkait, 2009; Youn, Hong, Bae, Kim, & Kim, 2004). Domingues, Ramos, Cardoso, & Reis (2014) indicated that enzymatic pretreatment

**is able to improve the clarity of juice without modifying the total soluble solids content in the juices. Clarification contributes to a low viscosity product that is clear and bright. In a fluid system, viscosity is one of the important physical properties (Giap, 2010).**



## **2.6 Pectinase**

According to previous studies, pectinase is another effective option in the depectinization of fruit juices to reduce the turbidity (Kashyap, Vohra, Chopra, & Tewari, 2001). Pectinase helps to break down pectin, thus, it reduce the viscosity and formation of cluster in the juice which ease for the separation and clarification process (Sharma et al., 2016). Consequently, resulting a juices with high degree of clarity with more concentrated flavor and color (Kaur, Kumar, & Satyanarayana, 2004; Liew Abdullah, Sulaiman, Aroua, & Megat Mohd Noor, 2007).

Pectinase had been used for guava juice clarification and based on the studies, it clearly showed that viscosity and turbidity of guava juice is decreased through enzymatic depectinization, degrade pectin and polysaccharides (Ninga et al., 2018). Cerreti et al(2017) had done studies on the pectinase and protease clarification treatment of pomegranate juice. The study discovered the effects of incubation time (30 – 120 minutes), temperature (25 – 50°C) and complex enzyme amount (0.1 – 0.4g/100g of juice) on physical characteristics of pomegranate juice using response surface method.

In order to extract banana juice, enzyme pectinase and amylase was used (Zaker, Syed, & Harkal, 2014). The enzyme hydrolysis of pectic substances are rely on several operating conditions, this including enzyme concentration, incubation time and temperature (W.C. Lee, S. Yusof, 2006). The advantages of degradation of pectin by enzyme action are includes enhance the efficiency of the process, minimize energy costs and waste, improve the yield of juice extraction and promote an attractive appearance of the final products (Danalache, Mata, Alves, & Moldão-Martins, 2018).

Pectic substances are naturally degraded by pectic enzymes (Alkorta, Garbisu, Llama, & Serra, 1998). Generally, pectic enzymes can affect the viscosity, cloud stability and other food properties of a fruit juices. The increasing of viscosity of fruit juice is due to the presence of pectic substances (Alkorta et al., 1998).

This pectic enzyme, particularly pectinmethylesterase (PME), which is a ubiquitous enzyme present in plants. It deesterifies the methoxylated pectin in the plant cell wall and forming pectic acid (Tiwari, Muthukumarappan, O'Donnell, & Cullen, 2009). The PME is then released into the juice during extraction. PME hydrolyses ester bonds of pectin in the fruits, resulting in the cloud stability reduction (Tiwari et al., 2009).

Majority of commercial pectic enzymes are acquired from fungal sources (Alkorta, Llama, & Serra, 1994). Apart from this, pectic enzymes can also apply in the maceration and solubilization of fruit tissues. This is to maintain the integrity of the plant cell wall in which the process is called the liquefaction of tissues (Alkorta et al., 1998).

## 2.7 Membrane-Based Process

Membrane-based process technology had developed swiftly for the past few decades (Wee, Tye, & Bhatia, 2008). It is believed that it is a sustainable process and had been developed as green engineering (Koros, 2004). Most of the researchers had found out that the capability of this technology is better than conventional separation method. This process is the separation of a mixture of two or more components of a feed with the semi-permeable barrier (membrane) (Wee et al., 2008). These membrane separation is includes microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), reverse osmosis (RO) and electrodialysis (ED) (R. W. Baker, 2000). Table 1 showed the summary of established membrane separation technology.

**Table 1: Summary of established membrane separation technology(R. W. Baker, 2000)**

Process	Principle	Type of Membrane	Feed Phase	Driving Force
Microfiltration	Separation of organic and polymeric compounds with micropore ranges of 0.1–10 $\mu\text{m}$	Finely microporous 0.1–10 $\mu\text{m}$	Liquid or gas	Pressure difference 35–350 kPa
Ultrafiltration	Separation of water and microsolute from macromolecules and colloids	Finely microporous 1–100 nm	Liquid	Pressure difference 140–700 kPa

<b>Reverse osmosis</b>	<b>Passage of solvents through a dense membrane that is permeable to solvents but not solutes</b>	<b>Dense solution-diffusion</b>	<b>Liquid</b>	<b>Pressure difference 700-7000 kPa</b>
<b>Electrodialysis</b>	<b>Ions are transported through a membrane from one solution to another under the influence of an electrical potential</b>	<b>Electrically charged films</b>	<b>Liquid</b>	<b>Voltage difference 1-2 V</b>

**Pressure-driven process including microfiltration, ultrafiltration and nanofiltration is normally applied in clarification and extraction for fruits and vegetables juices. While reverse osmosis is proposed to concentrate juice extracts (Yilmaz & Bagci, 2018).**

**The greatest advantages for membrane process is that it is a continuous (simple process) and environmental friendly operation (no chemical reactions involved) and low footprint (intensified process) (Castel & Favre, 2018). In fact, high energy efficiency of the membrane process had been always mentioned. This is due to the process require no phase change (Koros & Lively, 2012).**

**Membrane-based process had become a substitute for juice clarification method and it had been widely applied in the dairy and beverage industries (Bhattacharjee et al., 2017). Membrane separation become advantageous due to its**

high efficiency as it require less man-power and less processing time compare to the conventional filtration (Bhattacharjee et al., 2017). Moreover, the operating and maintenance cost for membrane separation is extremely lower than the conventional processes (R. Baker, 2001). It is also an energy efficient method as there is no phase change occurs during membrane processes (Urošević et al., 2017).

Membrane-based process is normally works together with pressure-driven operation in food and beverage industry. Membrane process application had become the main concerns in vegetable and fruit juice clarification (Daufin et al., 2001).

According to Wagner (2001), MF is a process that only proteins are able to pass through the membrane while others suspended solids are remained on the surface of the membrane. On the other hand, UF is a process where only low molecular weight components are free to pass through the membrane. High molecular weight component such as proteins and suspended solids are rejected (Wagner, 2001). In order to select a membrane filtration, permeate flux and product quality is the main features to be considered (Urošević et al., 2017). UF able to separate juices into a clarified fraction free of spoilage microorganisms and a fibrous concentrated pulp. This clarified juice with the improved sensorial properties could bring a strong impact to the new market areas (de Barros, Andrade, Mendes, & Peres, 2003).

On the other hand, MF and UF are played a significant role in the juice and beverage industry. The process enhances the microbiological quality of the permeate by preserved the natural fruit constituents and the volatile aroma profile of the fruit juices. Thus, the presentation of the final products were appealing to the consumers (Cassano, Conidi, Timpone, D'Avella, & Drioli, 2007). The preserved nutrient

content in the permeate juice is depends on the membrane pore diameter, cut-off material, configuration and trans-membrane pressure applied (de Carvalho, de Castro, & da Silva, 2008).

Conidi, Drioli, & Cassano, (2017) investigated that MF is the separation of particles from a solvent or other lower molecular weight compounds with the aids of 0.05-10 $\mu$ m diameter of symmetry membrane. In a contrary, UF is applied 2-10nm pore size asymmetry membrane that consists of high retention of macromolecules and colloids from a solution (Conidi et al., 2017). Generally, the membrane of UF is denoted by the molecular weight cut-off (MWCO) (van Reis & Zydney, 2007).

MF and UF had been widely used in the fruit juice clarification for recent years (Bhattacharjee et al., 2017). During membrane filtration, all undesirable microorganisms are remained on the membrane surface (Urošević et al., 2017). Thus, the juice can be considered as microbiologically stable and high quality of juice can be obtained (Urošević et al., 2017).

Cassano et al. (2003) stated that UF is an effective method which is able to get rid of natural polymers (polysaccharides and proteins) that found in fruits and vegetables juices. UF process able to remove the total suspended solids and turbidity of the fresh juice (Cassano, Donato, & Drioli, 2007). Maktouf et al. (2014) analyzed that UF in lemon juice able to achieve high level of clarity with a great reduction in terms of 99% turbidity and 98% viscosity.

## **2.8 Effect of Operating Parameters on Permeate Flux**

Many studies had shown that there is a close relationship between operating parameters during MF and UF. These parameters, for example, trans-membrane pressure, cross-flow velocity and temperature on MF and UF could affect the flux on various fruit juice clarification (Vladislavljević, Vukosavljević, & Bukvić, 2003). Among the parameters, only trans-membrane pressure and temperature is able to be controlled. Researchers had found that the increase in temperature could enhance the flux performance on juice clarification (Vladislavljević et al., 2003). High temperature over 55°C during juice clarification could degrades many compounds in the juices (R. W. Baker, 2004; Girard, Fukumoto, & Sefa Koseoglu, 2000).

Based on the research, it stated that operating UF at high pressure does not affect the permeate flux but it does increase the thickness and density of the retained substances at the membrane surface layer. The trans-membrane pressure is independently affects on the permeate flux at steady state (Porter, 1972).

Table 2 - Operating parameters reported during ultrafiltration of various fruit juices.							
Juice	Membrane and pore size	Operating parameters					Ref.
		TMP (kPa)	CFV (m/s)	T (°C)	VCR	Flux (L/m <sup>2</sup> h)	
Chokeberry, cherry, redcurrant	Flat sheet PES	400-1600	1-1.2	25-35	-	36-38	Karcknai et al. (2008)
Kiwifruit	MWCO 45 kDa Flat sheet CA	60-65	-	25	2.76	15-40	Casano et al. (2008)
Momambi	MWCO 30 kDa Flat sheet PES	270-635	0.09-0.18	30±2	-	45-10	Sarkar et al. (2008b)
Strawberry fruit juice	MWCO 50 kDa Organic PPES	350-550	0.11-0.275	-	-	9.25-48.7	Pal et al. (2008)
Pineapple	MWCO 30 kDa Tubular PVDF	150-750	-	25±2	-	35.7	Carvalho et al. (2008)
	MWCO 30-80 kDa Flat sheet PS					13.4-19.8	
Kiwifruit	MWCO 50 and 100 kDa Hollow fiber PEEK Fiber d <sub>i</sub> /d <sub>o</sub> = 1.41/1.96 mm	0-125	0.59-2.96	15-35	2.05	90-16	Mandal et al. (2011)
Pomegranate	Hollow fiber PEEK Fiber d <sub>i</sub> /d <sub>o</sub> = 1.64/2.11 mm	96	-	25	2.43	29-20	Casano et al. (2011)
Cactus pear	Flat sheet PVDF	220	0.123	25±2	-	41.8	Casano et al. (2010)
Apple	MWCO 200 kDa Tubular PVDF	250	1.21	50	25-32	129.6-72	Yazdanshenas et al. (2010)
Pineapple	MWCO 18 kDa Hollow fiber PS	5-100	1.5-3.4	20±2	-	35-9	Laorto et al. (2010)
	MWCO 30 kDa and 100 kDa						
Sugar cane	Tubular ZrO <sub>2</sub> 0.02 and 0.05 μm ceramic	100	3	60	-	66-54	Jegatheesan et al. (2009)
Clementine mandarin	Hollow fiber PEEK and PS diameter of fiber 1.64 mm and 1.43 mm	30	3-4	25±2	2	33-38	Casano et al. (2009)
Synthetic fruit juice	Spiral wound polyamide	414-690	-	28±2	2.09-2.14	30.6-53.5 (with permeate recycle)	Singh et al. (2013)
	MWCO 30 kDa				2.38-2.92	33.5-49.6 (without permeate recycle)	

Figure 2: Operating parameters of UF of various juices (Urošević et al., 2017)

In Figure 2 we can observed that the permeate flux are best operated with tubular membranes while the worst one is with flat sheet membranes. A researcher had done experiment with flat sheet membrane. He claimed that high pressure must be applied on the flat sheet membrane as fouling occurs more rapidly compare to the membrane with other configurations and module geometries (tubular, hollow fiber) (de Carvalho et al., 2008).

de Oliveira et al. (2012) deduced that vitamin C able to pass through the tubular membrane with the aids of TMP. While for the hollow fiber membrane, TMP (0.5 and 1.0 bar) did not affect the permeation. According to the studies, the experiment showed that tubular polysulfone membrane contributes to the highest

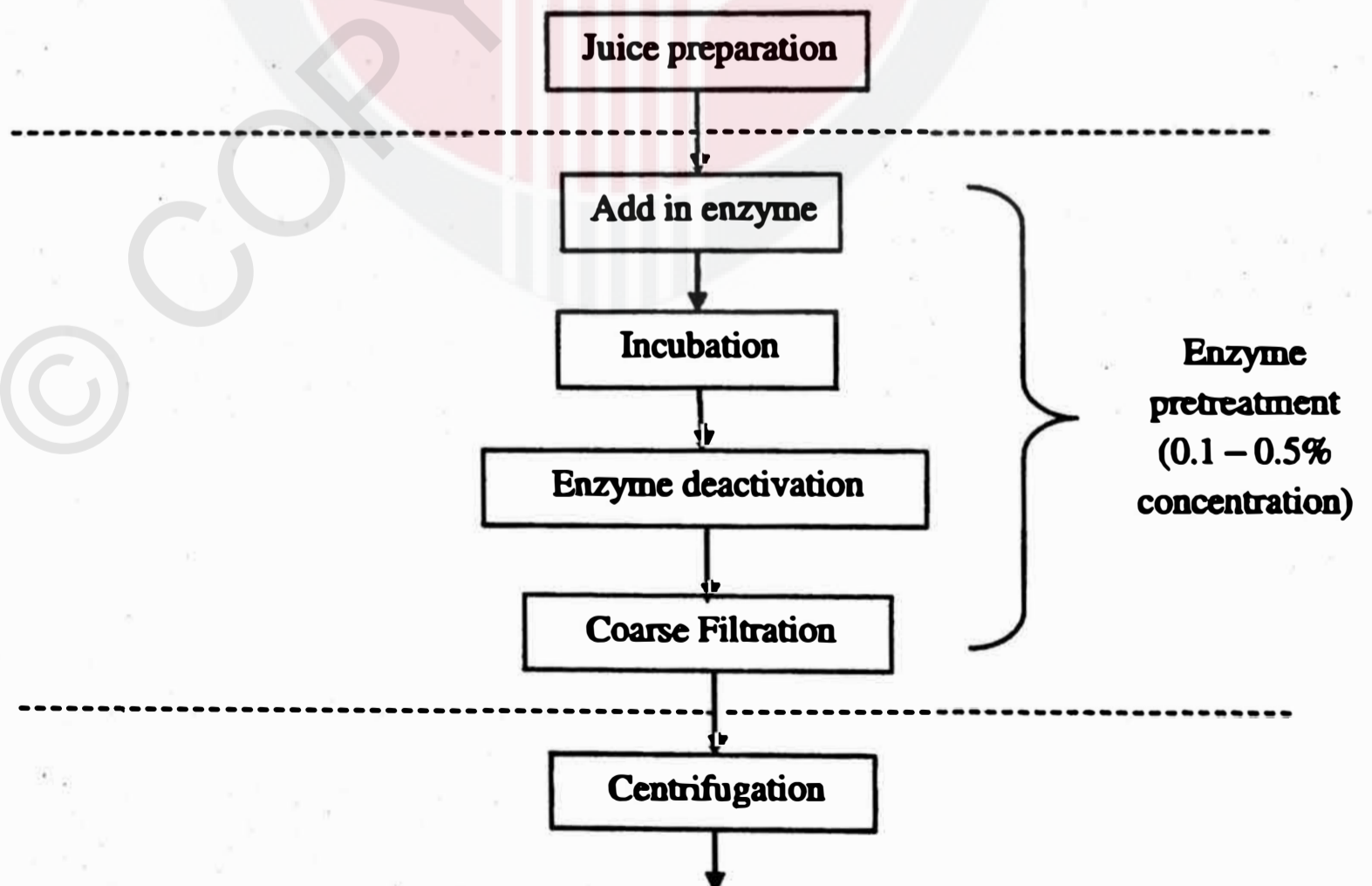
**sugar recovery. It stated that UF with hollow fiber membrane allowed for good preservation of antioxidant compounds (de Carvalho et al., 2008).**

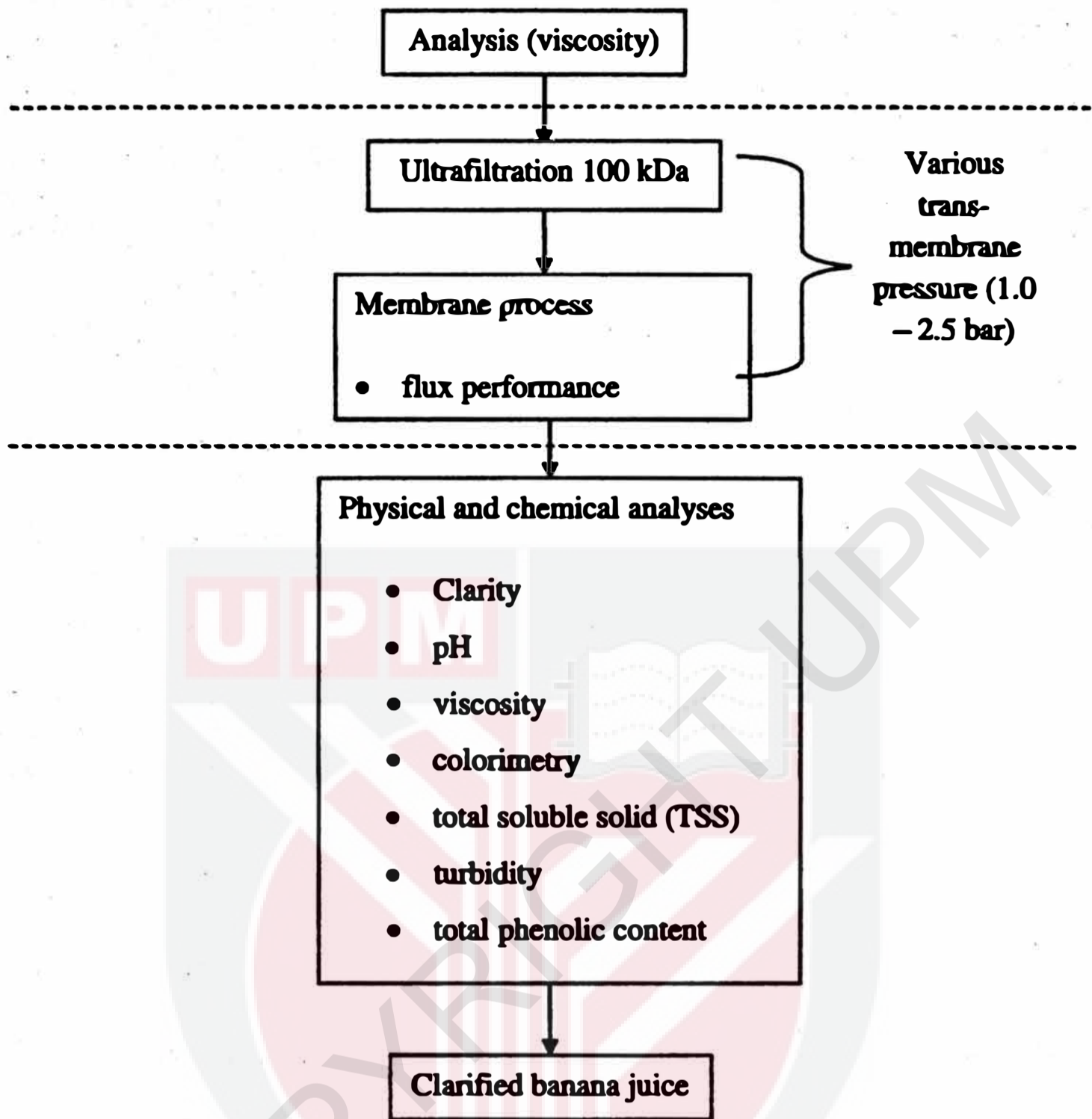


## CHAPTER 3

### EXPERIMENTAL DESIGN & METHODOLOGY

The overall flow of this study is shown in Figure 3, in which it was divided into 2 phases namely, 1) enzymatic pretreatment of the raw banana juice using pectinase; and 2) clarification of banana juice using ultrafiltration.





**Figure 3: Overall methodology of banana juice clarification**

### **3.1 Materials**

Fresh and fully ripened banana fruits (Cavendish) at stage 7 (completely yellow in color) (W.C. Lee, S. Yusof, 2006) are purchased from local store in Serdang, Malaysia.

### **3.2 Methodologies**

#### **3.2.1 Sample Preparation**

The banana was manually peeled with knife and cut into pieces and processed with juice extractor for 1 minute. Distilled water was added in to the juice extractor with a ration of 1:1 (weight/volume) with the banana to form a raw banana juice.

#### **3.2.2 Pectinase**

Pectinase (Pectinex Ultra SPL, Novozymes Denmark) used for the enzymatic treatment of the banana juice. This enzyme was produced from a selected strain of fungus, *Aspergillus niger* and with an activity of 3800 units/ml.

### 3.2.3 Enzyme Pre-treatment

500ml sample of juice was prepared for each set of experiment and was subjected to pectinase of different concentration (0.1, 0.2, 0.3, 0.4 & 0.5%). Three control samples were prepared (non-enzyme treated, centrifuged at 6500rpm and 9000rpm). The sample preparation is shown in Table 2. The quantity of enzyme at different enzyme concentration is shown in Table 3.

**Table 2: Description of sample preparation**

<b>Sample</b>	<b>Percentage of Enzyme (%)</b>	<b>Centrifugation Speed (rpm)</b>
1	-	-
2	-	6500
3	-	9000
4	0.1	9000
5	0.2	9000
6	0.3	9000
7	0.4	9000
8	0.5	9000

**Table 3: Quantity of enzyme at different enzyme concentration**

<b>Enzyme Concentration (%)</b>	<b>Quantity of Enzyme (unit/ml)</b>
0.1	3.8
0.2	7.6
0.3	11.4
0.4	15.2
0.5	19.0

All enzymatic treated samples were incubated in a water bath (Protech Electronic, Model 903, Malaysia) at 33°C for 108 minutes (Tchewonpi et al., 2014). After the enzymatic treatment, the enzyme in the samples was deactivated by heating the samples at 95°C for 5 minutes (Tchewonpi et al., 2014).

All enzymatic treated samples were then centrifuged (Centrifuge Eppendorf 5804R, Malaysia) at 9000rpm for 10 minutes to separate the suspended solids. The obtained supernatants were tested for viscosity.

#### **3.2.4 Membrane-based Process**

The supernatant collected after centrifugation was used for processing for ultrafiltration (UF). The process is carried out in a laboratory scale of ultrafiltration unit. The system is equipped with a polyethersulfone dead end disc membrane with molecular weight cut-off (MWCO) of 100kDa, which had been tested for clarification of natural juices and agro-food by-products.

Each UF experiment performed is run with 100ml of juice. The process is performed at different transmembrane pressures (TMP) (1.0bar, 1.5 bar, 2.0 bar and 2.5 bar) so that we can find the limiting TMP that able to give the maximum permeate flux for the performance of juice clarification. The specification of the membrane used for banana juice clarification is showed in Table 4.

**Table 4: Specifications of the membrane used for clarification of banana juice**

<b>Membrane</b>	<b>Ultrafiltration (UF)</b>
<b>Manufacturer</b>	<b>EMD Millipore Corporation</b>
<b>Membrane Type</b>	<b>PBHK06210</b>
<b>MWCO (Da)</b>	<b>100,000</b>
<b>Filter Diameter (mm)</b>	<b>63.5</b>
<b>Membrane Material</b>	<b>Polyethersulfone (PES)</b>
<b>Configuration</b>	<b>Dead end</b>
<b>Trans-membrane Pressure Operating Range (bar)</b>	<b>1.0 – 2.5</b>

### **3.2.5 Physical and chemical analyses**

#### **3.2.5.1 Viscosity**

The viscosity of the sample is determined with Brookfield viscometer (Model LVDV-11+, Brookfield Engineering Laboratory, Inc., Middleboro, USA). Viscosity is measured at the room temperature with a unit measurement of cps.

### **3.2.5.2 Clarity**

Clarity of the sample is determined by measuring the absorbance at 660nm with a UV-Vis spectrophotometer (Model UV-1201, Shimadzu Corporation, Japan).

Distilled water was used as a reference.

### **3.2.5.3 pH**

pH is determined by using pH meter with buffer solution of pH 4 and 7.

### **3.2.5.4 Turbidity**

Turbidity of the samples was determined using model Microprocessor Turbidity-meter (Eutech Instrument TN-100, Singapore) and was reported as Nephelometric Turbidity Units (NTU). It was calibrated with patron solutions 0-1000 NTU.

### **3.2.5.5 Total Soluble Solid (TSS)**

Total Soluble Solids (TSS) was determined using Atago digital refractometer (Model RP-101, Tokyo, Japan) in terms of °Brix. The scale is ranging from 0 - 45 °Brix.

### **3.2.5.6 Colorimetry**

The color measurement is determined with a Minolta Colorimeter (CS-2000 Konica Minolta Sensing Americas, Inc). 30 ml sample is first placed in the Petri dish and determined the color parameter of the CIELAB system. Data were expressed in

terms of  $L^*$ ,  $a^*$  and  $b^*$  parameters.  $L^*$  represent the lightness index;  $a^*$  and  $-a^*$  redness and greenness, respectively;  $b^*$  and  $-b^*$  yellowness and blueness, respectively.

Chroma is used to determine the degree of difference of a hue in comparison with a gray color with the same lightness, and is considered the quantitative attribute of colorfulness. The chroma (C) and hue angle ( $h^\circ$ ) were calculated using the following equations:

$$h^\circ = \tan^{-1}\left(\frac{b^*}{a^*}\right)$$

$$C = (a^{*2} + b^{*2})^{1/2}$$

### **3.2.5.7 Total Phenolic Content**

Total phenolic content was determined colorimetrically with Folin-Ciocalteu assay which depends on the reduction of Folin-Ciocalteu reagent by phenolic compounds under alkaline condition. 0.3 ml of banana juice sample was added into 1.5 ml of 10% Folin reagent and 1.2 ml of 7.5% sodium carbonate,  $\text{Na}_2\text{CO}_3$ . The absorbance of the solution was measured at 765nm with Shimadzu UV-VIS spectrophotometer (Ultraspec 3100 Pro, Amersham Pharmacia Biotech, UK). The standard curve was established using gallic acid. The total phenolic content was expressed as gallic acid equivalent in mg/L.

## **CHAPTER 4**

### **RESULTS & DISCUSSIONS**

#### **4.1 Phase 1: Enzymatic Pre-treatment of Raw Banana Juice**

##### **4.1.1 Data Analysis**

The results obtained on the enzymatic pre-treatment for banana juice in terms of viscosity and color characteristics are displayed in Table 5 and will further discussed in the subsequent sections.




**Table 5: Data analysis of colorimeter and viscometer**


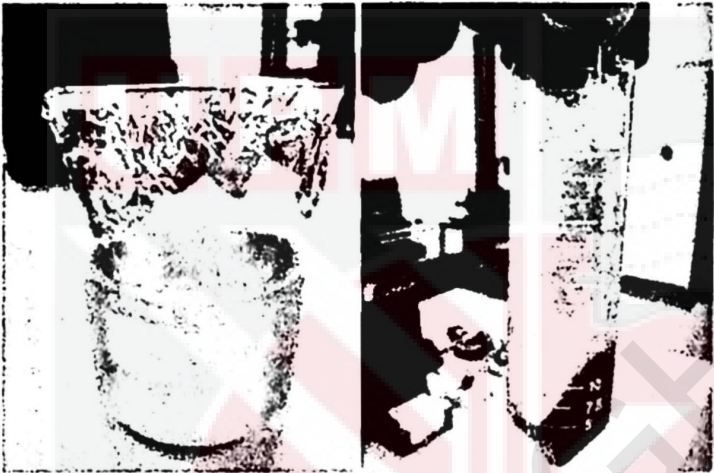

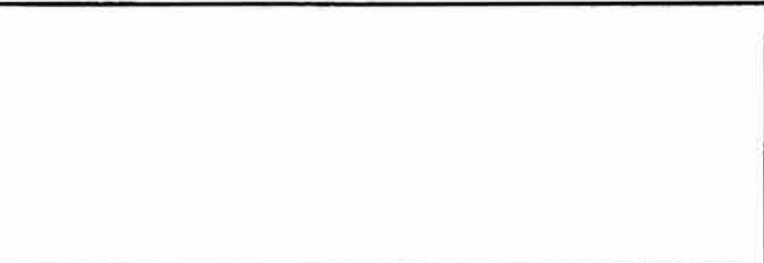
Sample	Color					Viscosity (cP)
	L*	a*	b*	Hue Angle, h (°)	Chroma	
1	47.89	5.68	11.74	64.20	13.04	224.5
2	30.50	-0.03	0.81	-87.90	0.81	43.6
3	28.34	-0.19	0.35	-61.50	0.40	37.0
4	25.65	-0.34	1.74	-78.90	1.77	18.4
5	25.97	-0.27	2.80	-84.50	2.81	17.6
6	26.61	-0.29	2.50	-83.40	2.52	16.6
7	27.40	-0.50	1.88	-75.10	1.95	18.8
8	28.06	-0.25	2.74	-85.80	2.75	18.8

#### 4.1.2 Color Changes Observation

Table 6 showed the color changes of enzymatic pre-treatment of banana juice which is subjected to different enzyme concentrations. All samples that had treated with enzyme having color changes and become less turbid.

**Table 6:** Color changes of pre-treated banana juice that subjected to different enzyme concentrations

	<b>Color before Centrifuge</b>	<b>Description</b>
<b>Raw banana juice</b>		The juice contains fibers, suspended solids and high molecular weight carbohydrates.
<b>Sample</b>	<b>Color after Centrifuge</b>	
<b>1</b>		The juice is darker and turbid.
<b>2</b>		The juice is less dark and slightly turbid.

<p>3</p>		<p>The juice is lighter and a little cloudy.</p>
<p>4</p>		<p>The juice is less clear.</p>
<p>5</p>		<p>The juice is clear.</p>
<p>6</p>		<p>The juice is very clear.</p>



7



The juice is very clear.

8

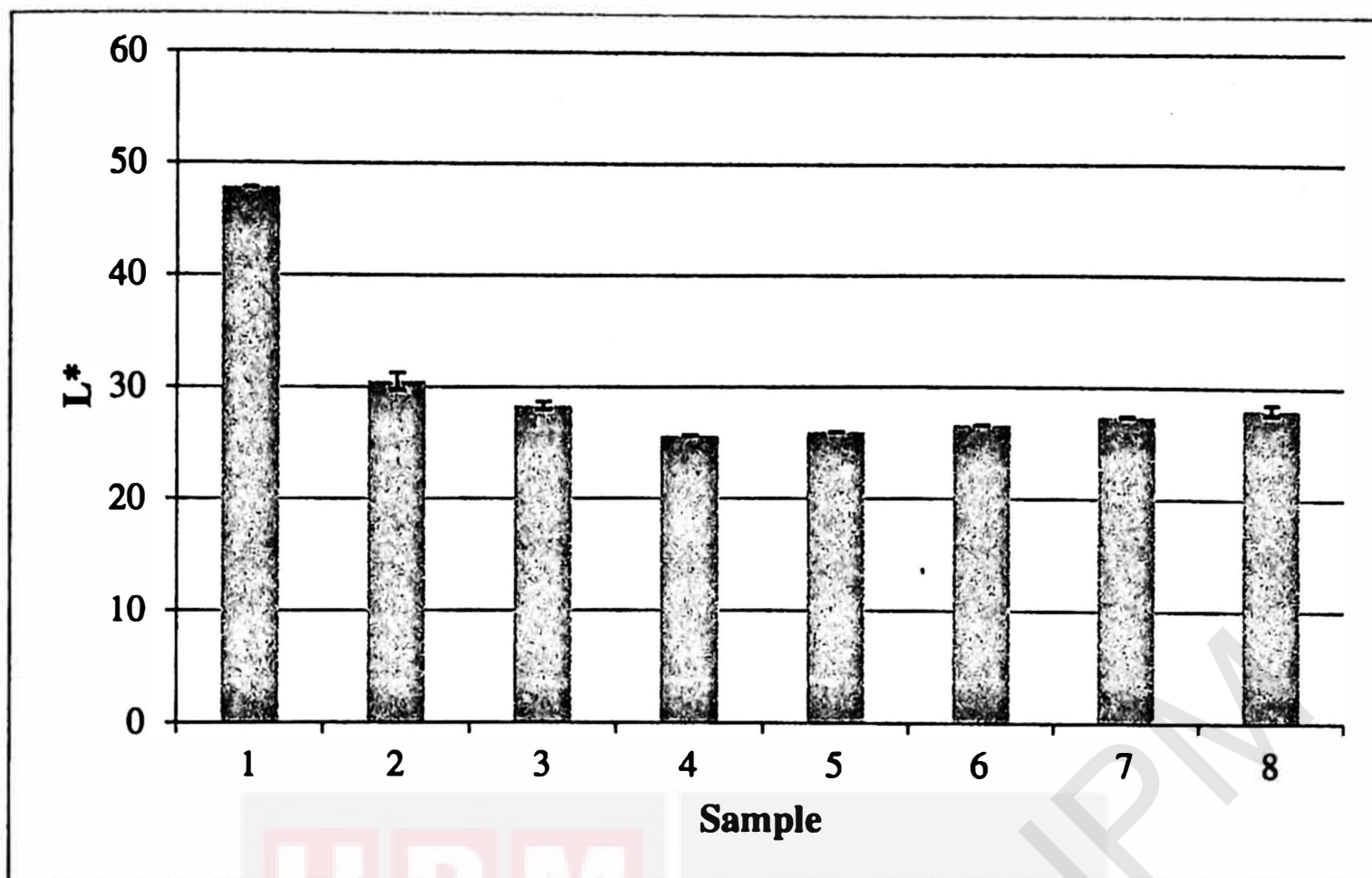


The juice is very clear.

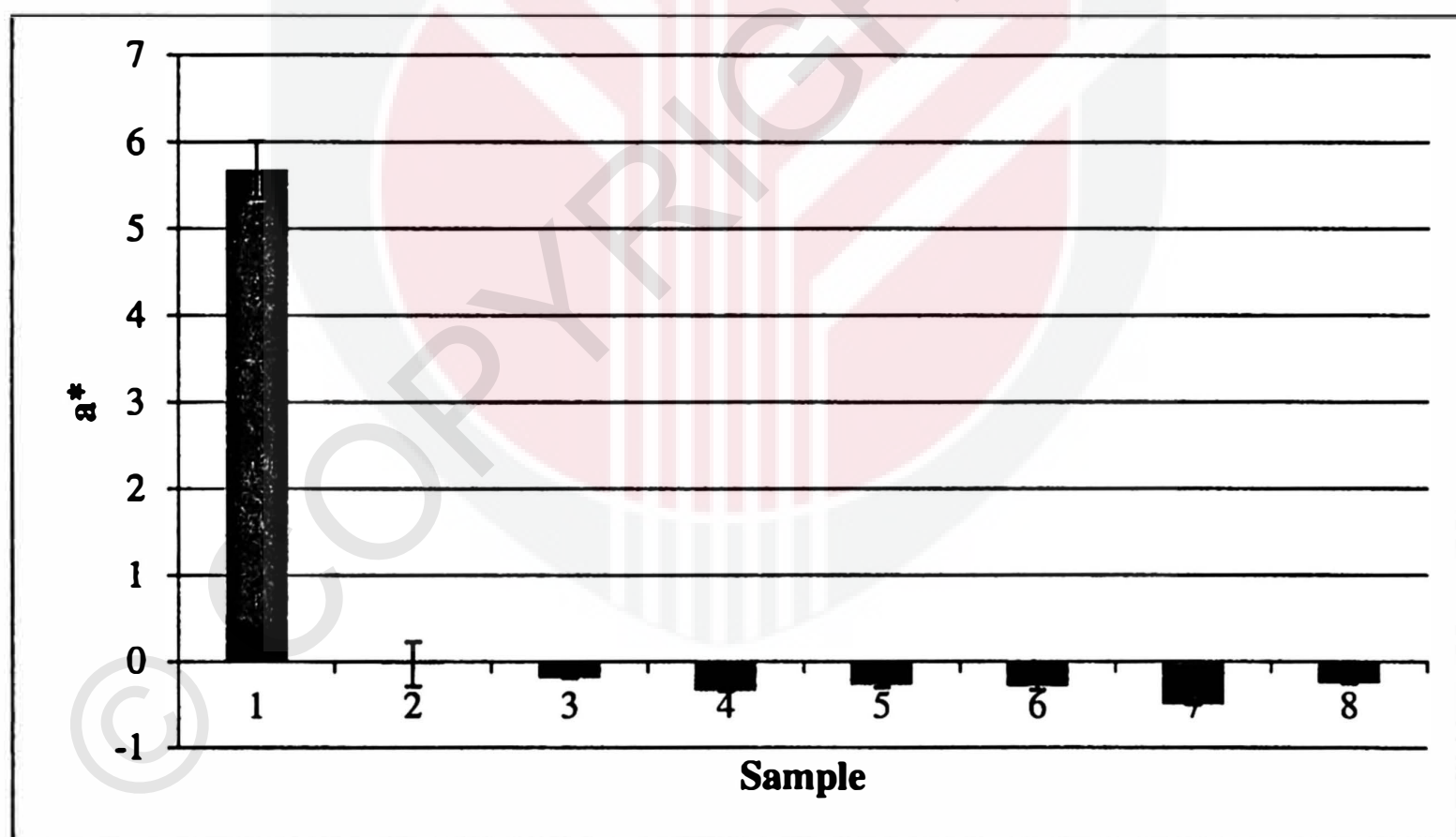
### **4.1.3 Color Properties**

Color is one of the important sensory attribute in the fruit juice production. Consumers are normally attracted and appealed to a bright color fruit juices. A turbid and darker color fruit juice indicated that the deterioration and spoilage. The color of the banana juice after enzymatic treatment is being evaluated with tristimulus color system CIELab to obtain the C.I.E-L\*, a\* and b\* color coordinates. The L\*, a\* and b\* denoted lightness/darkness, greenness/redness and yellowness and blueness (Abid, Yaich, Hidouri, Attia, & Ayadi, 2018).

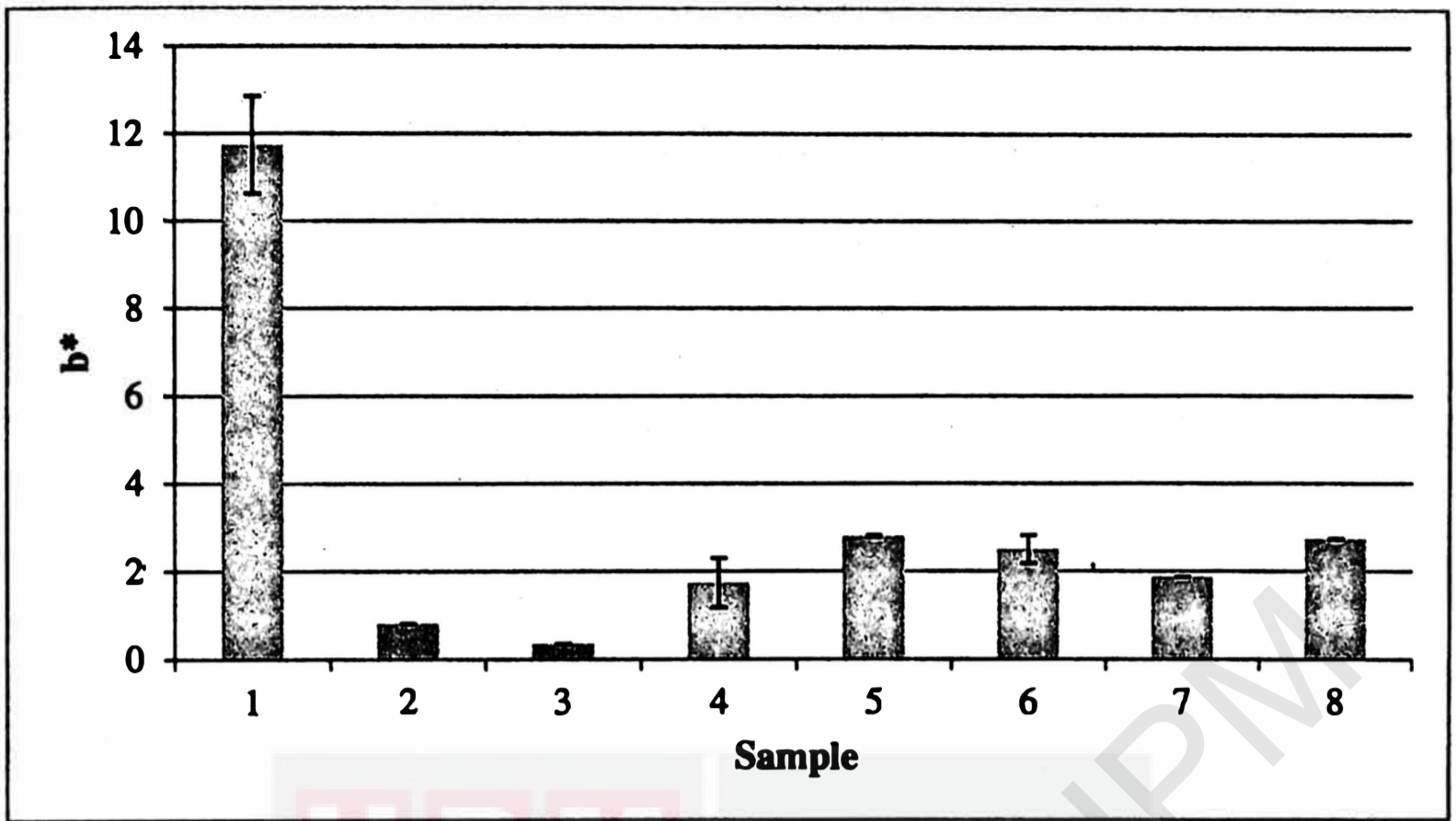
The value of L\*, a\* and b\* for each sample were evaluated on Table 5. It is observed that as the increasing in the enzyme concentration, the L\* value increases too. Sample 8 showed the highest L\* value among the samples that treated with pectinase enzyme. Figure 4 showed the effect of enzymatic treatment of banana juice on the L\* value. It was observed that the increased of enzyme concentration increases the L\* value. This is due to the increased agglomeration of floc as more pectin was degraded by higher enzyme concentration. Figure 5 and 6 showed the effects of enzymatic treatment of banana juice on a\* and b\* value.



**Figure 4: Graph of L\* versus sample**



**Figure 5: Graph of a\* versus sample**



**Figure 6: Graph of b\* versus sample**

#### **4.1.4 Juice Viscosity**

Viscosity is one of the most important parameters in the physical properties of fruit juice industry. As it may become a constraint in the flow rate requirement. According to Table 3, the viscosity of the samples does not showed significant difference. Sample 1, 2 and 3 is set as the control where sample 1 is without addition of enzyme and centrifugation. While sample 2 is being controlled without the addition of enzyme and centrifuged at 6500rpm. Sample 3 is being controlled without the addition of enzyme and centrifuged at 9000rpm.

It is clearly showed that there is no significance difference among all the enzyme pre-treated samples. In contrast, without any enzyme activity, the juice cannot perform any clarification process by itself. The high viscosity and cloudy of the banana juice is due to the presence of fibres, suspended solids, high molecular weight carbohydrates and total soluble solids. The enzyme activity reached its maximum and doesn't conduct any effect on the clarification of banana juice.

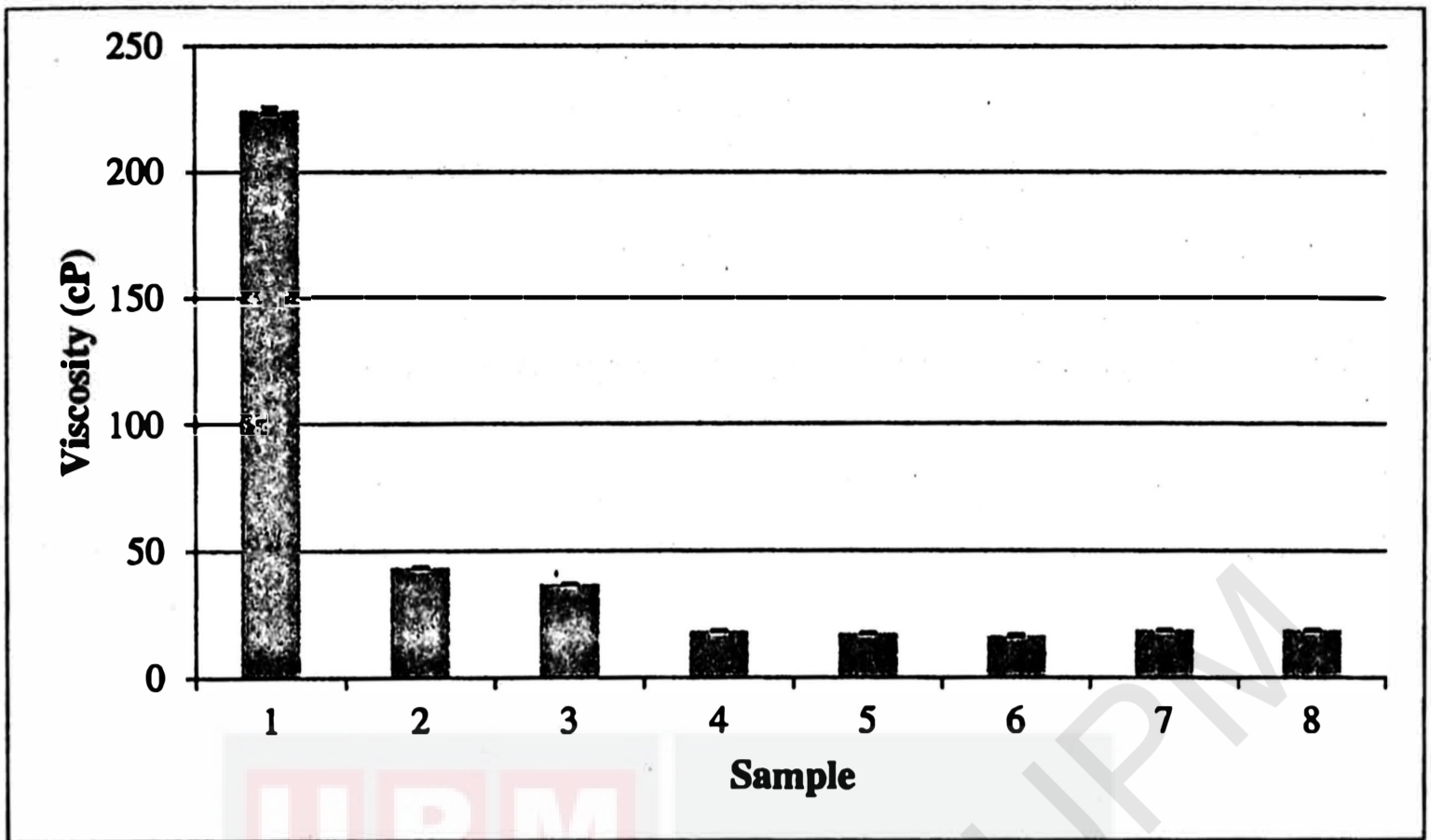
Figure 7 showed the enzymatic hydrolysis of the banana juice and the effects on the viscosity reduction. Compare to the control set, the viscosity for sample 4 – 8 are showed greatly reduction in the viscosity and turbidity of banana juice. Indeed, pectinase had showed its effectiveness toward the juice clarification. As pectin is responsible for the viscosity, application of pectinase was more effective in decreasing the viscosity of the juice.

When there is addition of enzymes, the viscosity of the juice was reduced. This clearly showed that pectinase had played a significant role and effect on banana juice clarification. Pectinase helps to degrade pectin content and release the juice from the cells of the fruits. The degradation of pectin aids the separation of juice

from the pulp. Polygalacturonases and pectin lyase of the pectinases group are the most particular element in the pectin hydrolysis that leads to flocculation of pectin-pectin complex (Sandri, Lorenzoni, Fontana, & da Silveira, 2013).

As the pectin begins to degrade, it leads to the reduction of water holding capacity of the juice. Thus, free water was released to the system and resulted in the viscosity reduction. Commercial fruit juices are normally treated with enzyme before filtration process. High viscosity of the juice may increase the fouling on the filtration membrane and reduce the yield of the process. Some journal had showed that high viscosity fruit juice may cause significant problems during filtration process (Vaillant, Pérez, Acosta, & Domier, 2008).

According to the results obtained based on color and juice viscosity, we selected that 0.1% enzyme to proceed to UF process. As there is clearly no significant difference on the results among all the samples. There is a study is reported that 0.1% is the optimum enzyme concentration for enzymatic pre-treatment of banana juice (Ninga et al., 2018). Thereby, 0.1% is sufficient in the enzyme pre-treatment on banana juice.



**Figure 7: Graph of viscosity (cp) versus sample**

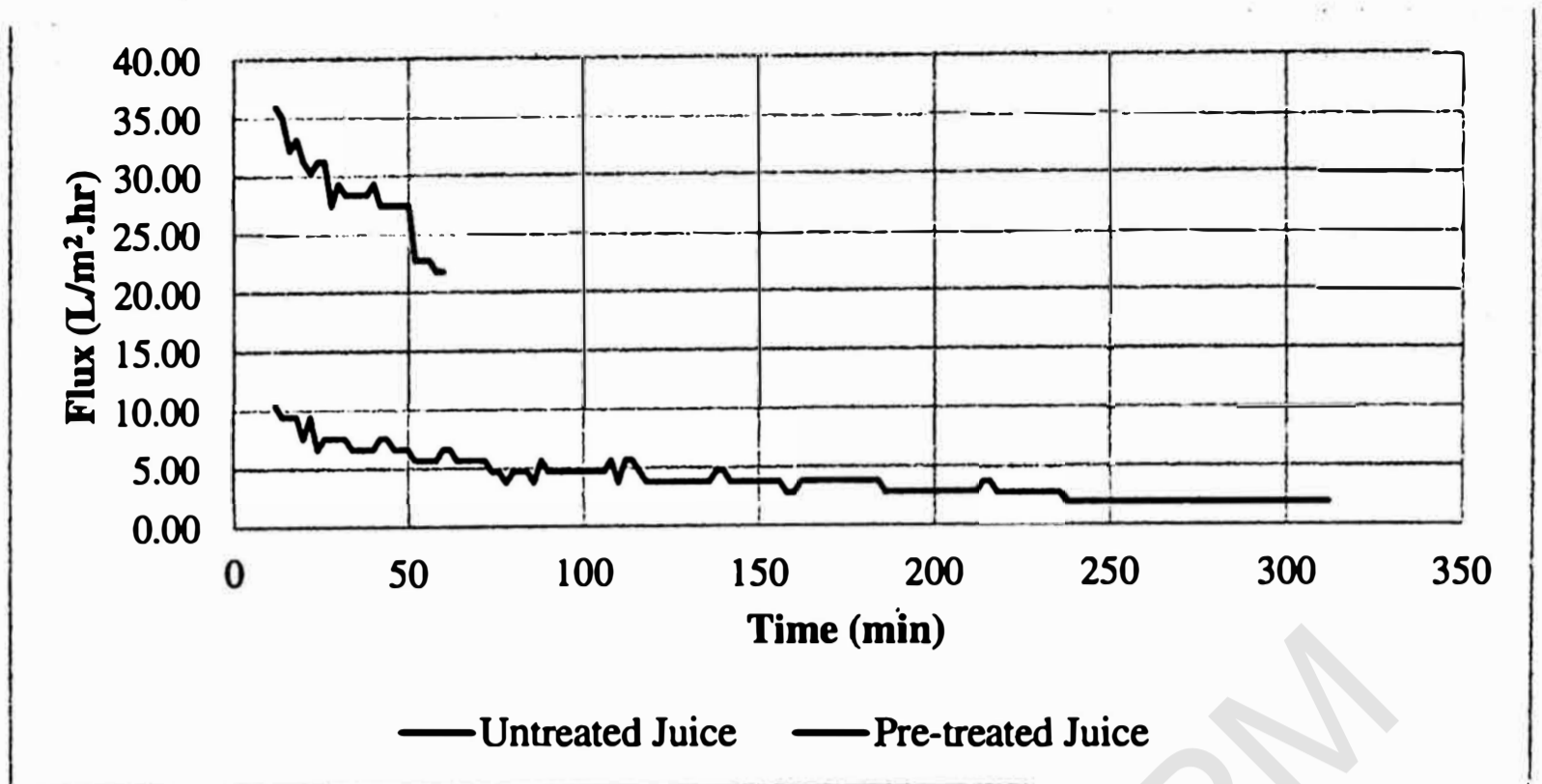
## **4.2 Phase 2: Banana Juice Clarification by Ultrafiltration**

### **4.2.1 Evaluation of Flux Behavior**

Permeate flux behavior during UF of untreated and pre-treated banana juice on 2.0 bar TMP is showed in Figure 8. As overall, both samples showed a similar flux decreasing trend from rapid decline to a slower decrement before achieving stable values towards the end of the filtration process.

According to the Figure 8, it showed that the flux behaviour between untreated and pre-treated banana juice at 2.0 bar TMP. As observed the figure, permeate flux for both untreated and treated juice is decrease continuously with time of operation. The reducing of flux is because of the increasing of solute concentration on the surface of membrane. Thus, leads to the continuous development of gel type of layer over the membrane surface which causes gradual increase in gel resistance. Subsequently, the permeate flux declines gradually. The decreasing of the permeate flux cause fouling is due to the pore blocking and cake formation over the membrane surface.

Based on the graph, it clearly showed that the pre-treated juice has a better permeate flux compare to the untreated juice. Untreated juice required longer time in order to undergo UF process. Untreated juice experienced a quite lower and steady flux while pre-treated juice showed noticeable higher permeate flux. This results indicate that permeate flux is depends on the amount of total pectin present in the banana juice.



**Figure 8:** Comparison of flux behaviour between untreated and pre-treated banana juice at 2.0 bar TMP

The pre-treated juice had undergone enzymatic pre-treatment which aids in enhancing clarification and filtration process by maintaining higher flux. As enzymatic treatment helps to reduce the juice viscosity and pectic materials. Moreover, the pre-treated juice is able to absorb colloidal species and build up concentration polarization layer quickly. Thus, resulting in a higher flux performance.

After UF process run for a certain time, there was cake formation on the surface of the membrane which caused fouling. This phenomenon is due to the formation of high molecular weight compound on the membrane surface. The untreated juice does not undergo any of pre-treatment process before UF. Since banana is a pulpy fruit and therefore, the juice consists of macromolecule or colloidal species which caused fouling occurs at the beginning of the UF process that resulted in declining of permeate flux as compared to the pre-treated juice.

A study had reported that cake can be formed within 5 minutes after the microfiltration of raw pomegranate juice (Mirsaeedghazi, Emam-Djomeh, Mousavi,

Aroujalian, & Navidbakhsh, 2010). So, it is possible that the flux could be decrease so rapidly particularly for the untreated juice sample. The rate of permeate flux remains constant over time is due to the effect of fouling on the concentration polarization.

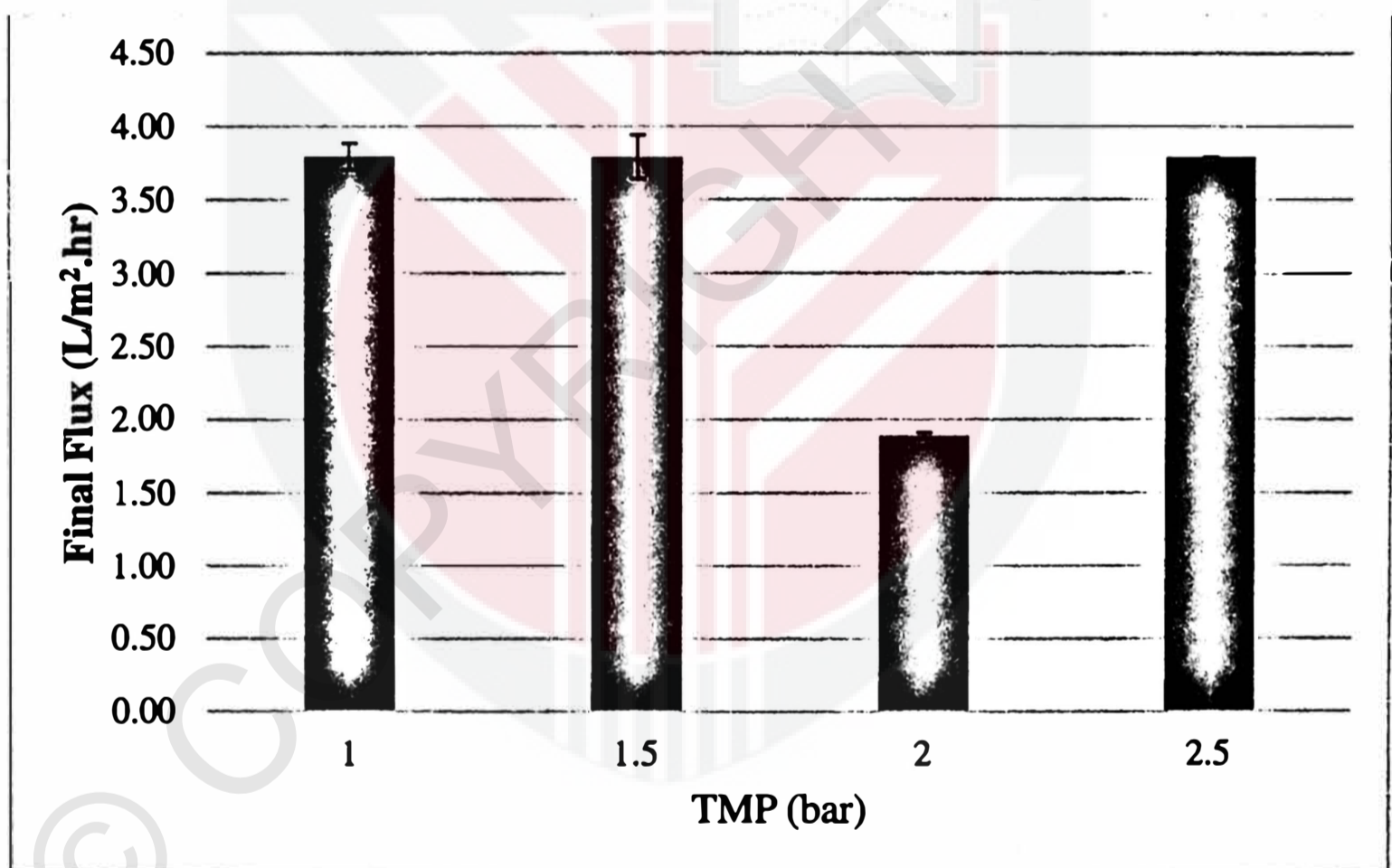
Enzymatic treatment had been widely used as pre-treatment in juice processing industries (Bhattacharjee et al., 2017). Enzymatic pre-treatment able to hydrolyse polysaccharides such as pectin, starch, cellulose, and hemicellulose, thereby improving the membrane performance by increase the permeate flux (Bhattacharjee et al., 2017). Besides, it also can improve the clarity of juice in which it does not alter the total soluble solids content (Domingues et al., 2014; Nandi et al., 2009).

The decreasing rate in clarification process is due to the high concentration of retentate. Generally, the pressure-driven membrane process is dependent on the physiochemical composition of the extracts, some membrane characteristics such as MWCO, configuration, membrane material, hydrophobicity, surface roughness, surface charge, permeability, fouling phenomenon, as well as solute-membrane interactions (Boussu, Vandecasteele, & Van der Bruggen, 2008).

The performance of pressure-driven membrane process can be influenced by the operating parameters during UF such as TMP, cross-flow velocity and temperature. Operating at high pressure does not enhance the permeate flux. On the other hand, it will increase the thickness and density of retained material at the membrane surface layer (Porter, 1972).

Figure 9 and 10 showed the final flux for untreated and pre-treated juice at different TMP. The final flux values were obtained during the last 5 minutes of each

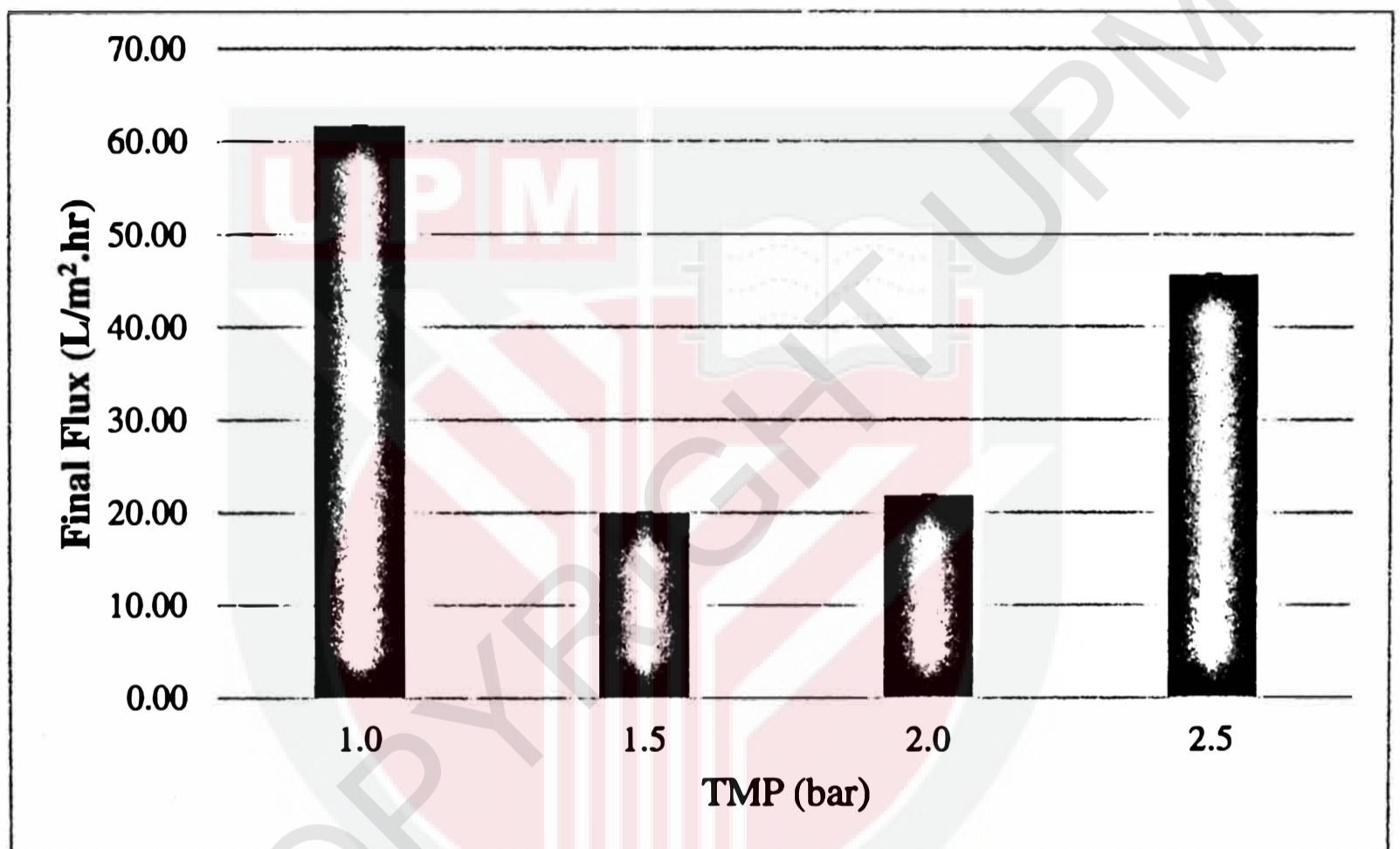
filtration process. In general, the higher the TMP, the higher flux value will be obtained. However, the final flux for the untreated juices obtained in this study was the same ( $3.79\text{L}/\text{m}^2\cdot\text{hr}$ ) when the filtration process was performed at TMP of 1.0, 1.5 and 2.5; and at  $1.89\text{L}/\text{m}^2\cdot\text{hr}$  when the juice was filtered at 2.0 bar. The inconsistent trend might be due to the uncontrolled fouling effect during the process. At a certain stage of filtration, TMP is no longer a dominant factor that will affect the flux performance as other factors such as absorption rate and fouling thickness may play a bigger role.



**Figure 9:** Graph of final flux versus TMP for untreated juice

As for the pre-treated samples, the final flux for all the process was significantly higher as compared with the untreated juice (Figure 10). Since the fouling effect has been reduced in the treated samples, it is expected that high TMP would lead higher final flux of the processes. However, this study found unexpected

finding where the highest final flux values were obtained when the process was performed at 1 bar. This might be due to some error or inconsistency of readings. Nevertheless, as indicate in Figure 10, the final flux values showed some increasing trend from 1.5 to 2.5 bar, proving the significant effect of TMP in improving the flux behaviour, particularly for the enzymatic treated juice.



**Figure 10:** Graph of final flux versus TMP for pre-treated juice

#### 4.2.2 Evaluation of Quality Attributes of Banana Juice

The results of analyses for the typical characteristics of permeate and retentate for raw, untreated and pre-treated banana juice were present in the Table 7. Details will be further discussed in the subsequent sub-sections.

**Table 7: Physical analyses for permeate and retentate for raw, untreated and pre-treated banana juice.**

Index	Pressure (bar)	Clarity (%A <sub>660</sub> )	Turbidity (NTU)	pH	Total Soluble Solids (°Brix)	Color				
						L*	a*	b*	Hue Angle (°)	Chroma
<b>Raw Juice</b>		2.918 ± 0.025	798.67 ± 13.868	4.85 ± 0.023	10.9 ± 0.173	26.6 ± 0.557	6.3 ± 0.651	23.0 ± 0.252	74.68	23.85
<b>Untreated Juice</b>										
<b>Filtrate</b>	1.0	0.132 ± 0.001	1.74 ± 0.384	4.91 ± 0.021	8.3 ± 0.351	29.4 ± 0.557	3.7 ± 1.217	16.6 ± 0.416	77.43	17
	1.5	0.810 ± 0.041	38.27 ± 1.150	4.90 ± 0.007	7.3 ± 0.751	29.5 ± 1.704	3.1 ± 0.874	16.7 ± 3.953	79.48	17
	2	0.200 ± 0.002	2.87 ± 1.464	4.30 ± 0.007	9.4 ± 1.050	31.4 ± 1.301	3.9 ± 1.308	18.4 ± 3.172	78.03	18.81
	2.5	0.080 ± 0.001	20.86 ± 1.179	4.90 ± 0.000	8.5 ± 0.058	31.7 ± 0.751	2.7 ± 1.124	16.6 ± 1.582	80.76	16.82

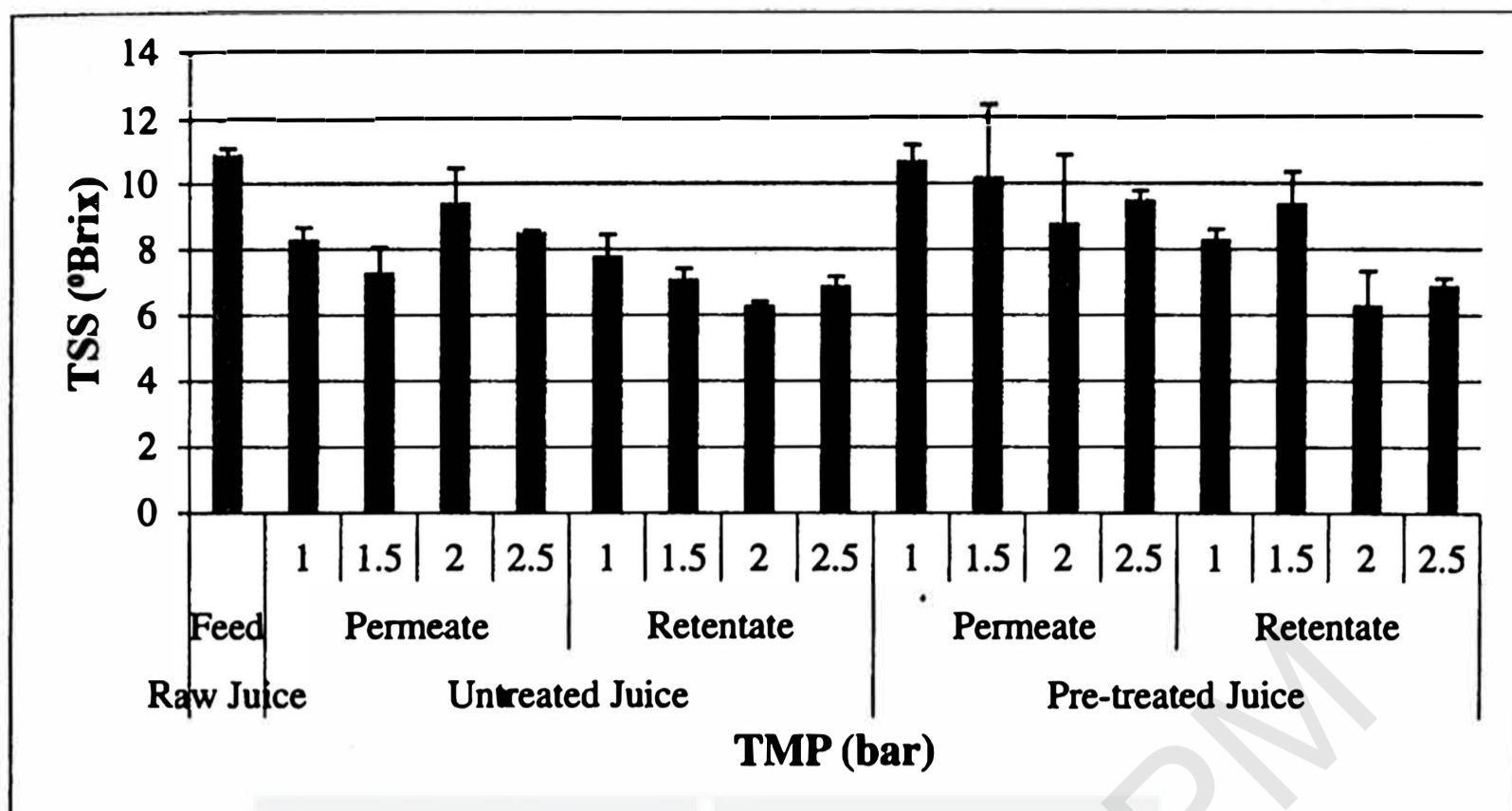
<b>Retentate</b>	1.0	0.980 ± 0.062	470.67 ± 102.500	4.86 ± 0.006	7.8 ± 0.643	29.5 ± 1.153	4.4 ± 0.265	21.6 ± 0.702	78.49	22.04	
	1.5	1.300 ± 0.026	657.00 ± 32.450	4.90 ± 0.006	7.1 ± 0.306	27.0 ± 0.954	5.3 ± 0.404	23.0 ± 0.173	77.02	23.6	
	2	0.583 ± 0.065	854.00 ± 122.854	4.6 ± 0.000	6.3 ± 0.100	29.1 ± 1.320	3.9 ± 2.254	21.7 ± 2.082	79.81	22.05	
	2.5	1.150 ± 0.050	655.33 ± 17.673	4.90 ± 0.007	6.9 ± 0.265	29.4 ± 1.002	3.9 ± 1.966	20.1 ± 2.413	79.02	20.47	
<b><u>Pre-treated Juice</u></b>	<b>Filtrate</b>	1.0	0.096 ± 0.001	0.74 ± 0.112	4.22 ± 0.010	10.7 ± 0.462	30.7 ± 1.474	3.0 ± 1.973	19.1 ± 1.153	81.07	19.33
		1.5	0.115 ± 0.000	0.80 ± 0.103	4.18 ± 0.020	10.2 ± 2.201	29.9 ± 1.153	3.2 ± 0.493	19.3 ± 1.963	80.59	19.56
		2	0.123 ± 0.001	6.52 ± 4.726	4.33 ± 0.006	8.8 ± 1.060	28.2 ± 0.850	4.0 ± 2.483	23.7 ± 4.362	80.42	24.04
		2.5	0.123 ± 0.001	1.07 ± 0.070	4.13 ± 0.010	9.5 ± 0.265	36.8 ± 3.342	3.0 ± 0.586	8.1 ± 1.595	69.68	8.54
<b>Retentate</b>	1.0	0.314 ± 0.012	271.00 ± 9.165	4.21 ± 0.017	8.3 ± 0.306	31.0 ± 0.656	5.1 ± 0.458	16.3 ± 4.912	72.63	17.08	
	1.5	0.231 ± 0.034	87.80 ± 16.929	4.23 ± 0.015	9.4 ± 0.950	29.4 ± 1.102	2.7 ± 1.026	19.5 ± 3.821	82.12	19.69	
	2	0.583 ± 0.065	854.00 ± 122.854	4.60 ± 0.000	6.3 ± 0.100	29.1 ± 1.320	3.9 ± 2.254	21.7 ± 2.082	79.81	22.05	
	2.5	1.150 ± 0.050	655.33 ± 17.673	4.9 ± 0.007	6.9 ± 0.265	29.4 ± 1.002	3.9 ± 1.966	20.1 ± 2.413	79.02	20.47	

#### **4.2.2.1 pH and Total Soluble Solids (TSS)**

With regards to the TSS, UF did caused a slightly reduction in the permeate and retentate of the untreated and pre-treated banana juice which showed in Figure 10. This noticeable results is probably due to the high suspended solids content that exists in the pulpy juice in which can interfere with the measurement of refractive index (Mady, Fabrice, Ana, Manuel, & Max, 2005; Vaillant et al., 1999). Thus, this indicated that the TSS content was not well preserved during UF.

The reduced in TSS is caused by the removal of suspended solids by UF. TSS is depends on the low molecular weight compounds such as sugars (sucrose, glucose and fructose), mineral salts and some organic acids (Castro-mu, Fila, & Barrag, 2017). Commonly, the variability of TSS is depends upon the degree of maturity of the fruits. Variation can be reduced to 10% once the parameter is kept homogenous (de Barros et al., 2003).

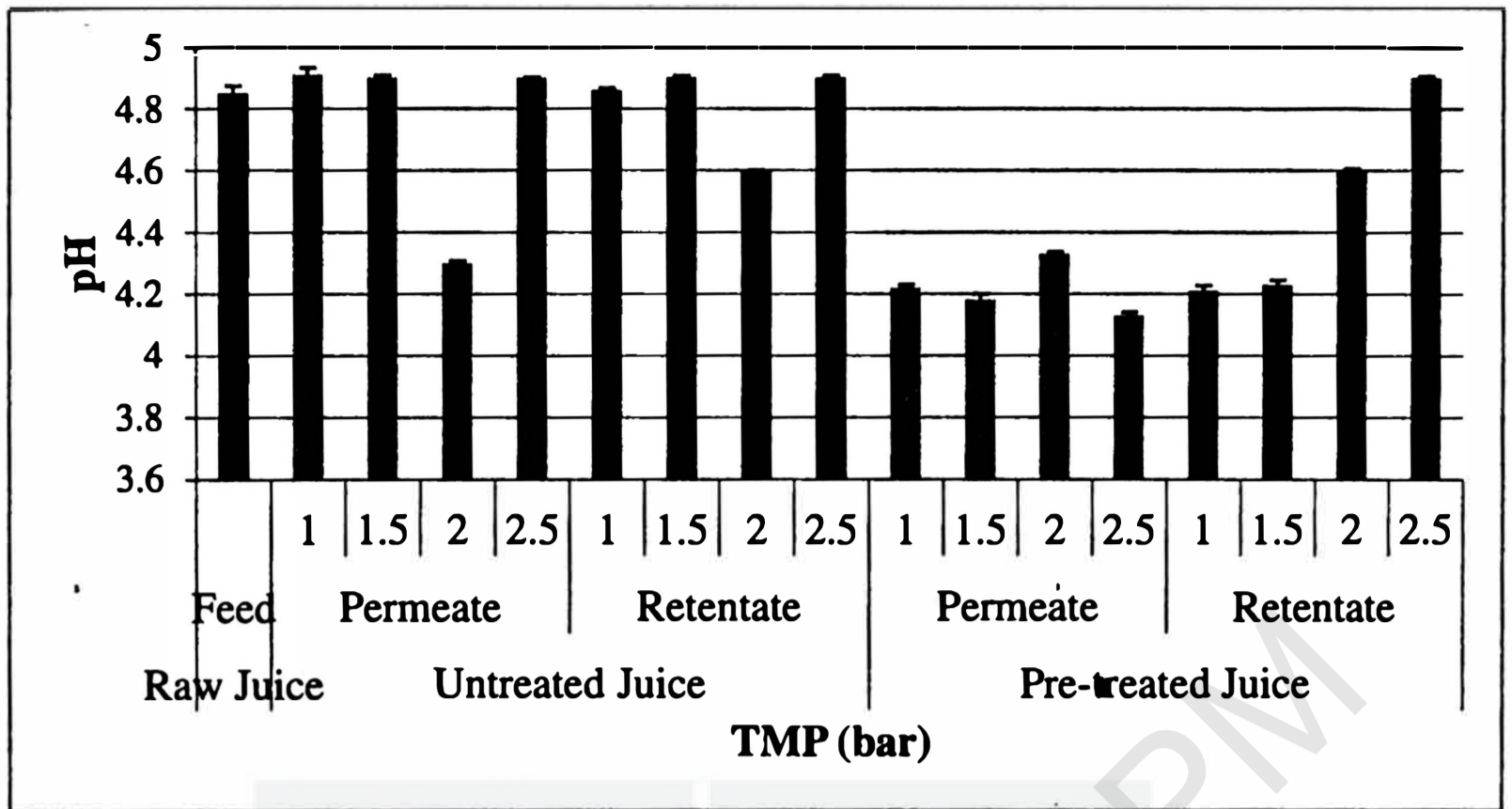
The TSS of untreated and treated juice are reduced initially and increases at the end stage when increasing TMP is applied. The fouling formation over the membrane surface and thus increase the impurity content at the end stage. Figure 11 showed the effect of TSS on samples at different TMP.



**Figure 11: Graph of TSS on samples at different TMP**

Even so, the pH of the untreated and pre-treated juice does not have significant difference as compared to the raw juice. UF process does not cause acidity to the juice as it does not altered the pH. The pH of both permeate and retentate for pre-treated juice is slightly lower than the untreated juice. This is because the pre-treated juice had undergone enzymatic pre-treatment by pectinase enzyme.

There is no significant effects are shown of the untreated and pre-treated juice at different TMP. Thus, decreasing or increasing of TMP does not affect the pH of the juice when undergo UF process. Figure 12 showed the effect of pH on samples at different TMP.



**Figure 12: Graph of pH on samples at different TMP**

#### 4.2.2.2 Clarity and Turbidity

According to the results obtained, the pre-treated banana juice did caused notable reductions in turbidity compare to the raw juice with the application of UF. Subsequently, it achieved a high level of clarity. The results in Table 7 showed that the suspended solids were removed as the turbidity was significantly reduced. UF filtrates and removes all the macromolecules that cause the juice to become turbid.

The turbidity of the banana juice is decreases initially and then increases at the end stage with increasing TMP. The increasing of the turbidity at higher TMP is governed by solutes diffusion across fouling layer and membrane. The formation of denser fouling layer that caused higher solute retention over the membrane surface. Figure 13 showed the effect of turbidity banana juice sample at different TMP.

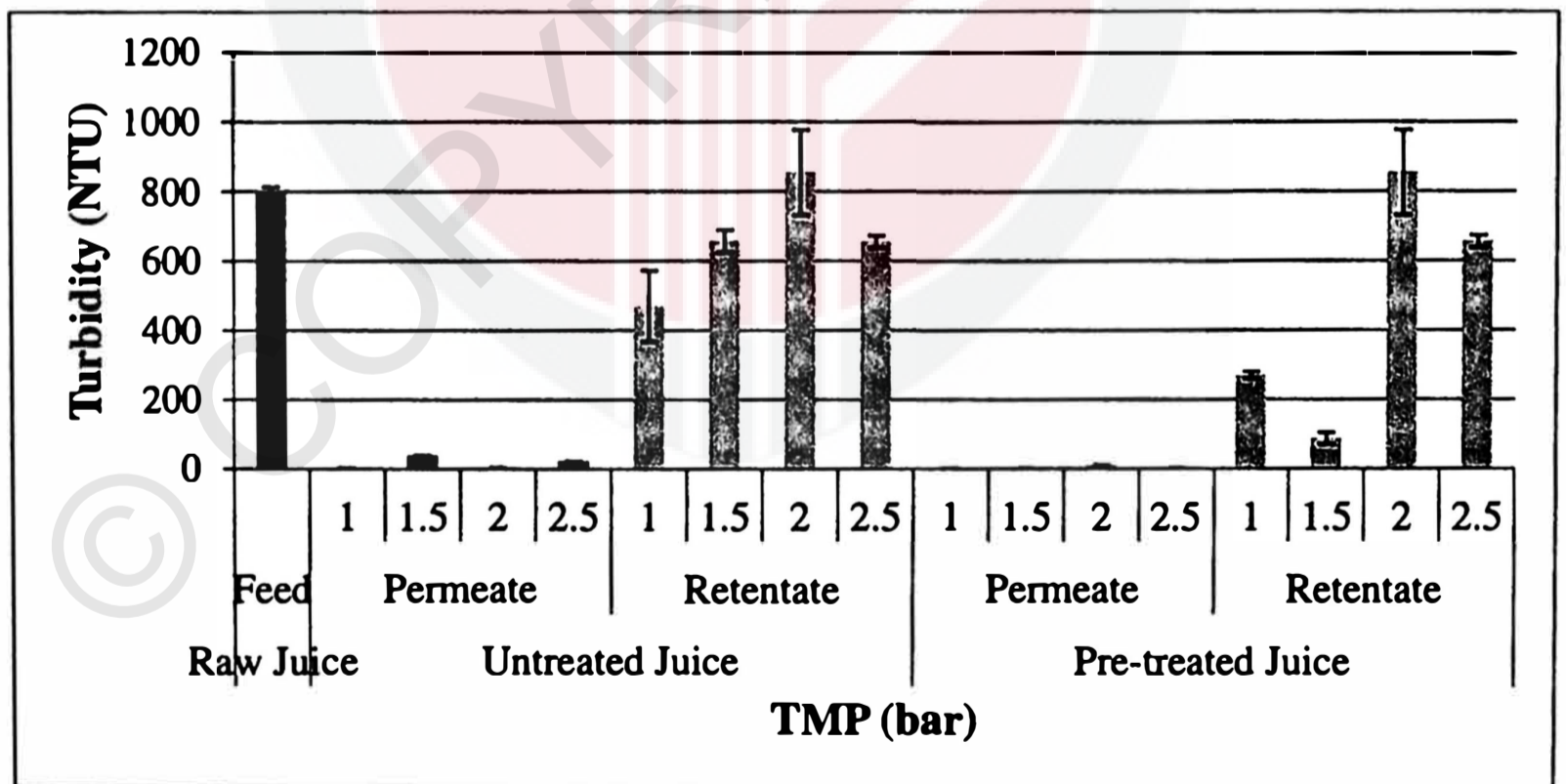


Figure 13: Graph of turbidity of samples at different TMP

In terms of clarity, there is a minimal change on the retentate for both untreated and pre-treated juice. The cloudiness of the retentate is caused by the hydrolysis of pectinase enzyme and degradation of pectin. The decreasing in clarity of permeate for untreated and pre-treated juice contributes to a clear and limpid juice.

The clarity of the juice increases with the increasing TMP during UF. The clarity of the juice tends to improve when applied at higher TMP. This can be related to the increases of color when TMP is increase. Figure 14 showed the effect of clarity of banana juice sample at different TMP.

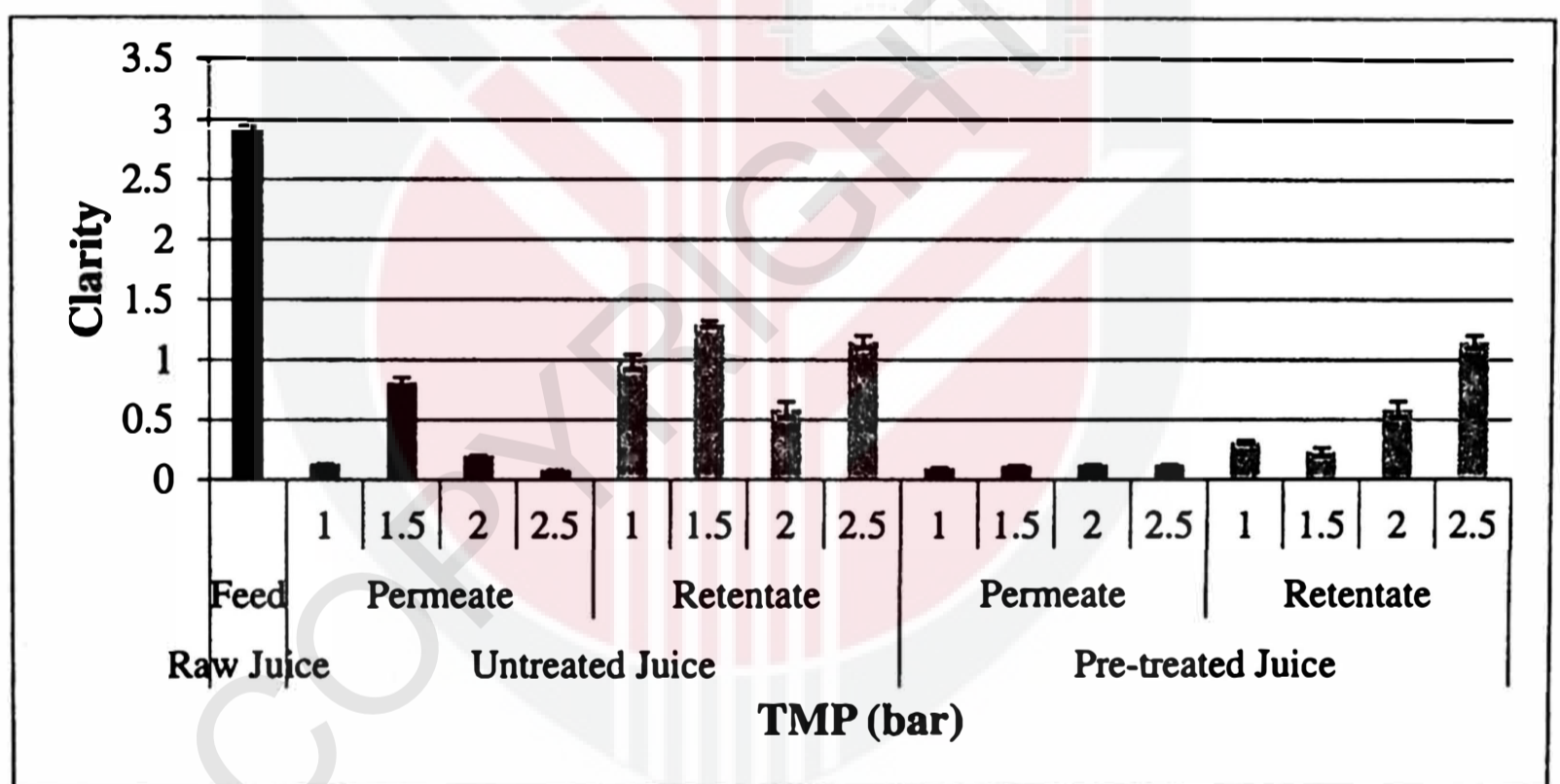


Figure 14: Graph of Clarity ( $A_{660}$ ) on samples at different TMP

Turbidity of juice tends to increase with the storage temperature and time (Lee & Yusof, 2006). It also indicates the browning of the juice and caused the juice become cloudy. Generally, relationship can be made between turbidity and TSS. Turbidity of juice implied the existing of fibers, suspended solids, high molecular weight carbohydrates and TSS (Castro-mu et al., 2017).

**The reduction in the turbidity and clarity of banana juice resulting in visually clean product. Clear juice from banana juice can be more attractive and pleasant for the human consumption. Furthermore, the removal of turbidity of juice denoted a remarkable changes that able to be observed on the color properties of processed fruit juice.**



### **4.2.2.3 Color Characteristics**

The color characteristics of raw juice and pre-treated banana juice were determined with  $L^*$ ,  $a^*$  and  $b^*$  parameter by CIELAB colorimeter system. The obtained  $L$ ,  $a$  and  $b$  value was used to calculate for the hue angle and chroma and displayed in Table 7. The lightness value ( $L^*$ ) and yellowness value ( $b^*$ ) are conserved for the retentate for untreated and pre-treated juice. There is a slight decrease in the redness value ( $a^*$ ) for both permeate and retentate for untreated and pre-treated juice.

The  $L^*$  value is higher for the permeate of untreated and pre-treated juice as compare to the raw juice. While for the redness value ( $a^*$ ) and yellowness value ( $b^*$ ) is higher for the retentate of untreated and pre-treated juice. The recorded high value of positive  $a^*$  and  $b^*$  is due to the color of retentate was in dark brown as the present of macromolecules and browning of the juice. In contrast to the pre-treated juice, it was translucent and clear which contributes to a higher lightness value ( $L^*$ ).

The brightness (chroma values) of the permeate for both untreated and pre-treated juice resulted on a minimal changes which indicated UF is successfully clarify the banana juice. Whilst, there is no significant difference of the brightness of retentate for untreated and pre-treated juice.

In terms of the effect of TMP for the untreated and pre-treated juice, there is no significance difference of the color value for  $L^*$ ,  $a^*$  and  $b^*$ . Since the results showed the variation of color for untreated and pre-treated juice remains almost invariant for the operating conditions of TMP.

#### 4.2.2.4 Total Phenolic Content

The total phenolic content from feed, permeate and retentate for both filtered and pre-clarified juice was determined using Folin-Ciocalteu colorimetric method by manipulating the regression equation of gallic acid calibration curve ( $y = 0.0012x + 0.0023$ ,  $R^2 = 0.9897$ ). The results obtained from the assay were expressed as means  $\pm$  standard deviation of triplicate analyses and are presented in Table 9.

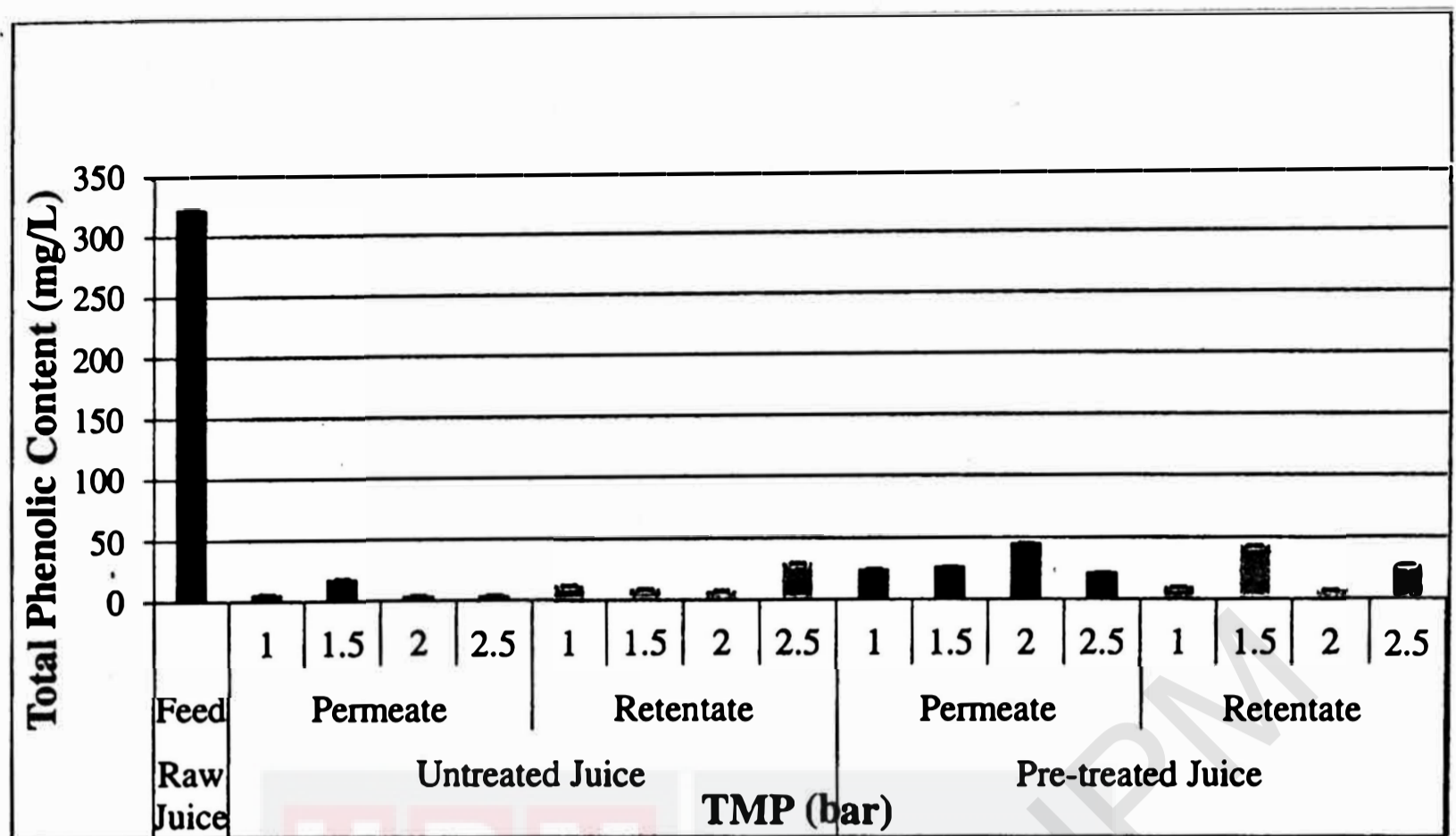
**Table 8: Total phenolic content of banana juice at different TMP**

Index	Pressure (bar)	Absorbance ( $A_{765}$ )	Total Phenolic Content (mg/L)
Feed		$1.074 \pm 0.071$	322.08
<b><u>Untreated Juice</u></b>			
Filtrate	1.0	$0.009 \pm 0.001$	5.58
	1.5	$0.024 \pm 0.002$	18.08
	2	$0.007 \pm 0.000$	3.92
	2.5	$0.007 \pm 0.001$	3.92
Retentate	1.0	$0.017 \pm 0.002$	12.25
	1.5	$0.013 \pm 0.001$	8.92
	2	$0.011 \pm 0.007$	7.25
	2.5	$0.038 \pm 0.011$	29.75
<b><u>Pre-treated Juice</u></b>			
Filtrate	1.0	$0.106 \pm 0.002$	24.75
	1.5	$0.034 \pm 0.049$	26.42
	2	$0.057 \pm 0.015$	45.58
	2.5	$0.157 \pm 0.001$	21.42
Retentate	1.0	$0.014 \pm 0.004$	9.75
	1.5	$0.054 \pm 0.003$	43.08
	2	$0.011 \pm 0.007$	7.25
	2.5	$0.038 \pm 0.011$	29.75

Based on the table above, it showed that the raw banana juice is high in total phenolic content which is 322.08 mg/L. According to study, the total phenolic content in Cavendish banana is higher compare to the other types of banana (Kuala, Teknik, Kimia, & Aceh, 2012). There is a significant reduction in the permeate and retentate for untreated and pre-treated juice. The great decreasing of total phenolic content is due to the oxidative and hydrolytic enzymes which degrades the phenolic compounds. Additionally, the pre-treated juice had undergo enzymatic treatment and UF. Thus, causing in degradation of large amounts of total phenolic content.

Total phenolic content decreased when the TMP is rose from 1.0 to 2.5 bar. As the higher in TMP, the higher amount of solute flow through the membrane. Therefore, it resulted in the increasing amount of deposited fouling materials on the membrane surface. Consequently, enhance the rejection of total phenolic content.

Formation of fouling at higher TMP can inhibit the solute from crossing the membrane. Thus, lowering the amount of phenolic compounds in the permeate, resulting in a decrease in the astringency while enhance the stability of banana juice. Mirsaeedghazi et al. (2010) discovered that the membrane process able to reduce the phenolic content of pomegranate juice. Figure 15 showed the effect of total phenolic content of banana juice sample at different TMP.



**Figure 15:** Graph of total phenolic content on samples at different TMP

The remarkable reduction in phenolic content is possibly due to the elimination of high molecular weight polyphenols. In another words, the total phenolic content does not retained during the UF process. Banana had been classified as a good source of bioactive phenolic compounds (Youryon & Supapvanich, 2017). The banana pulp and peel contains various phenolic compounds such as gallic acid, catechin, epicatechin, tannins and anthocyanins (Singh et al., 2016a).

Passo Tsamo et al. (2015) stated that heat treatment may degrade most of the phenolic substances in fruits. According to Susanto et al (2009), the studies had revealed that some of the membrane-solute interactions may promote the absorption of polyphenols on the membrane surface (Susanto et al., 2009).

## **CHAPTER 5**

### **CONCLUSION & RECOMMENDATIONS**

#### **5.1 CONCLUSION**

**This study is to determine the effect of pre-treatment using different concentration of pectinase enzyme on the attribution of banana juice. Besides, it also evaluates the effects of different TMP during membrane-based process on the permeate flux performance and the quality attributes of banana juice. 3.8 units/l of pectinase enzyme is sufficient in this study. The enzymatic treated banana juice was optimal at enzyme concentration 3.8units/ml, incubation time 108 minutes and incubation temperature 33°C. The clarification of banana juice was performed by UF using a dead-end membrane. The clarified juice obtained is low in viscosity and tends to have a longer shelf life as some of the suspended solids were removed by UF process. UF with a 100kDa PES membrane able to produce good quality of clarified banana juice with acceptable color, clarity and turbidity values with the**

rejection of pectin starch. In another words, UF process able to achieve a good level of fruit juice clarification. The process had achieved the maximum results with the combination of enzymatic pre-treatment. The enzymatic pre-treatment had successfully to aid in the performance of fruit juice clarification. Higher TMP has better flux behavior. As the potential advantage of UF in the fruit juice processing industry is undeniable. This pressure-driven membrane process is genuinely treated the banana juice which that allows it to become an improved quality in terms of physiochemical, nutritional and functional properties.



## **5.2 RECOMMENDATIONS**

**There is some errors might occur when performing the experiment that caused inaccurate data obtained. There is a few other flux enhancing mechanical techniques can be applied to UF process in order to minimize fouling layer and pore blocking. These include ultrasonic vibration, periodic backwashing with N<sub>2</sub> and backwashing by pulsing flow, use of turbulence promoters. Banana juice contains very high pulp content and is high in viscosity that limits the performance of UF.**

**Besides, we also can apply vibrating and rotating membrane systems to optimize the control of fouling phenomenon by create shear rate at the membrane interface. By this method, the interactions between proteins and other foulants with the membrane can be reduced by using membrane surface modifications.**

**Furthermore, we can replace the 100kDa membrane pore size with a smaller pore size membrane. As narrow pore size distribution can reduce fouling compared to more porous membrane. Hydrophobic membranes are more prone to fouling compared to hydrophilic membranes.**

## CHAPTER 6

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## APPENDICES

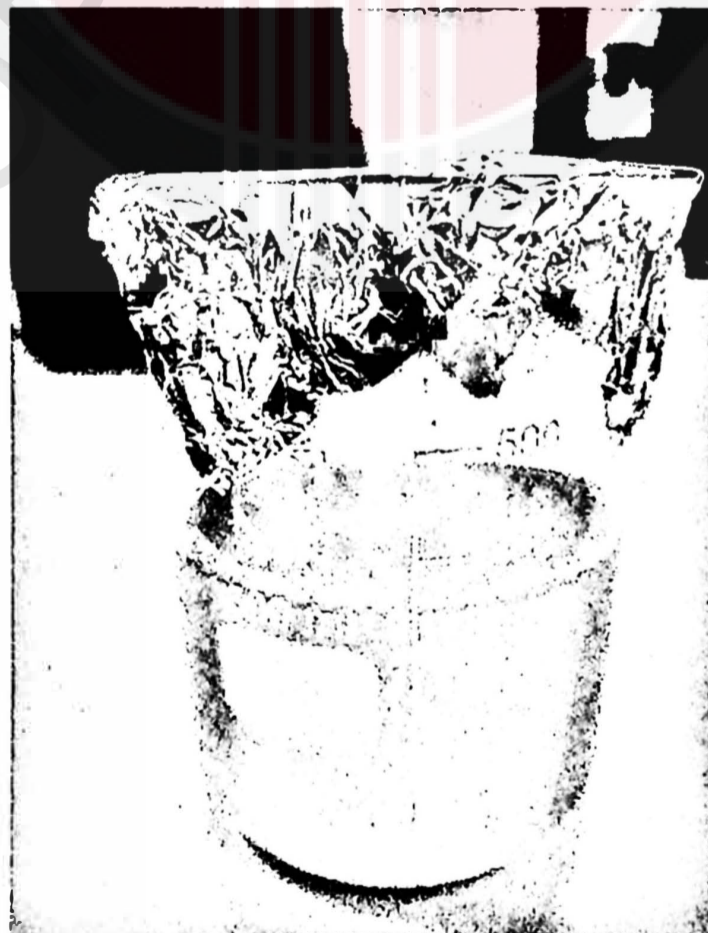
### Appendix A: Enzymatic pre-treatment on banana juice



**Appendix B: Raw banana juice**



**Appendix C: Enzyme pre-treated banana juice**



**Appendix D: Pre-clarified banana juice after UF process**



**Appendix E: Standard curve for gallic acid equivalent**

