UNIVERSITI PUTRA MALAYSIA

EFFECTS OF HIGH PRESSURE PROCESSING (HPP) ON PHYSICOCHEMICAL PROPERTIES AND SAFETY OF COCONUT MILK

OU KEL LON

EFFECTS OF HIGH PRESSURE PROCESSING (HPP) ON PHYSICOCHEMICAL PROPERTIES AND SAFETY OF COCONUT MILK

OU KEL LON

176490

PROJECT REPORT SUBMITTED IN PARTIALLY FULFILLMENT OF THE REQUIREMENT FOR THE

BACHELOR OF ENGINEERING (PROCESS AND FOOD)

DEPARTMENT OF PROCESS AND FOOD ENGINEERING

FACULTY OF ENGINEEERING

UNIVERSITI PUTRA MALAYSIA

2018

ACKNOWEDGEMENT

Firstly, I would like to express my great thanks and gratitude to my supervisor, Assoc. Prof., Ir., Dr. Siti Mazlina Mustapa Kamal, lecturer of Department of Food and Process Engineering, Universiti Putra Malaysia for her support, patience, knowledge, and guidance both in my project and in writing this thesis.

Extreme thanks to my parents, Ou Kewai Chow and Chew Yuh Ling, for their support in every moment and stage of my life. Furthermore, my deepest thanks to my sisters and brothers.

Special thanks also to my seniors, Daniel Ng Liong Nam and Nurfatimah Binti Mohd Thani whom I had asked for help and guide line throughout this research. Also, I would like to thank my friend Amy Lim Jia Hui, who has worked with me in the same lab and we shared every moment together and she gave me a lot of support and advice.

Finally, I would like to express my deepest appreciation to our coursemates for giving such valuable comments and suggestions which gave me an inspiration to improve my research project.

ABSTRACT

Coconut milk is the liquid obtained by mechanical extraction of grated coconut meat. In the following, a comparative study was performed to evaluate the physical properties (viscosity, total soluble solid) of coconut milk, investigate the chemical properties (fat content, free fatty acid, pH) of coconut milk and analyse the safety (total plate count, water activity) of coconut milk treated with HPP. There are two parameters for HHP treatment which are pressure (400MPa, 500MPa and 600MPa) and holding time (1, 3 and 5 minutes). The results showed that treatment using HPP had slightly effects on physicochemical properties such as viscosity, total soluble solid (TSS), pH, fat content and free fatty acid content of coconut milk which were closed to fresh coconut milk. Furthermore, a Central Composite Design (CCD) of Response Surface Methodology (RSM) was used to optimize HPP parameter conditions (pressure and time) of the coconut milk. The predicted optimum condition at maximum desirability index of 0.903 (90.30%) was obtained as 600MPa, 3 minutes of HPP treatment. The shelf-life of coconut milk which is treated by optimum condition of HPP and being stored under refrigerated condition for more than 7 days was improved compared to non-treated control which exceeds the maximum limit of total bacteria in coconut milk which is 1×10^4 CFU /g starting from the first day of storage. In conclusion, HPP treatment is a suitable approach to ensure adequate pathogen reduction whilst retaining nutrients content of the beverage.

ABSTRAK

Santan merupakan cecair yang diperolehi dari ekstraksi mekanikal isi kelapa parut. Dalam kajian ini, kajian komparatif dilakukan untuk menilai sifat-sifat fizikal (kelikatan, jumlah larut pepejal) santan, menyiasat sifat kimia (kandungan lemak, asid lemak bebas, pH) santan dan menganalisis keselamatan (jumlah bilangan plat, aktiviti air) santan yang dirawat dengan HPP. Terdapat dua parameter untuk rawatan HHP iaitu tekanan (400MPa, 500MPa dan 600MPa) dan masa (1, 3 dan 5 minit). Hasil kajian menunjukkan bahawa rawatan menggunakan HPP mempunyai kesan yang sedikit terhadap sifat fizikokimia seperti kelikatan, jumlah larut pepejal (TSS), pH, kandungan lemak dan kandungan asid lemak bebas santan dimana ia lebih kurang sama dengan santan segar. Selain itu, Reka Bentuk Komposit Sentral (CCD) dari 'Response Surface methodology' digunakan untuk mengoptimumkan parameter HPP (tekanan dan masa) bagi santan. Keadaan optimum yang diramalkan pada indeks 'desirability' maksimum ialah 0.903 (90.30%) telah diperolehi pada 600MPa, 3 minit rawatan HPP. Bukan itu sahaja, jangka hayat santan yang dirawat dengan keadaan optimum HPP dan disimpan dalam peti sejuk selama lebih dari 7 hari telah dipertingkatkan berbanding dengan santan tanpa rawatan HPP, dimana ia melebihi had maksimum jumlah bakteria pada santan iaitu $1x10^4$ CFU/g bermula dari hari pertama penyimpanan. Sebagai kesimpulan, rawatan HPP

adalah pendekatan yang sesuai untuk memastikan pengurangan patogen yang mencukupi sambil mengekalkan kandungan nutrien minuman.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

The coconut tree (*Cocos nucifera*) which belongs to the family of Arecaceae or known as palm family is the only species of the genus *Cocos*. It is regarded as perfect diet because it contains almost all essential nutrients needed by the human body. It contains high oil content and the protein which is high quality. Also, it contains all amino acids that are important for the growth and maintenance of the body. It is rich in minerals such as K, Na, Mg and S. Besides, it is nourishing, strengthening and fattening food (Sciences, 2004).

Coconut palm (Cocos nucifera L.) is found throughout the tropics as it is one of the most important sources of vegetable oil. It is grown in abundance in Malaysia and southern Asia. It contains two distinct endosperms which are nut water and kernel (Manivannan et al., 2016). It is commonly used as an important source of coconut oil, milk and cream products. Furthermore, it can be eaten fresh or processed into desiccated coconut flesh, coconut water and coconut milk (Alyaqoubi et al., 2015).

Coconut milk which is the milky white oil in water emulsion extracted from the gratings of coconut with or without addition of water is suitable for people of all ages. It plays an important role in many tropical cuisines of Southeast Asia, as well as Brazilian, Indian and Polynesian cuisines. The consistency of this milk varies considerably depending on quantity of water added during the process (Sciences, 2004).

Coconut milk becomes an increasingly important raw material in the preparation of a wide variety of food products such as curry, desserts, coconut jam, spread, coconut syrup, coconut cheese, bakery products and beverages as it adds creamy taste, smooth, and aromatic flavour to these delicacies. Furthermore, it can also be used as a substitute for milk in some desserts such as chocolate which are flavoured with coconut milk as its nutritional content is higher compared to cow milk. Besides that, coconut milk also serves as an excellent source of raw material for the development of dairy-like products such as yogurt. Coconut milk contains about 73.57% moisture, 10.99% solid non-fat and 15.44% fat, and is high in minerals and vitamin content (Alyaqoubi et al., 2015). It is also rich in proteins such as albumin, globulin, prolamin and glutenin. Therefore, coconut milk also provides health benefits. Coconut milk fat has been reported to improve digestion and bowel function, support tissue repair and immune system functions, protect the body from breast colon and other cancers, improve the cholesterol ratio, reduce the risk of heart disease and increase the metabolic rate of body fat. Furthermore, the fats present in coconuts are less likely to clog arteries because the body does not store coconut fats which makes coconut milk a healthy alternative to cow's milk to preserve heart's health (Ephraim Edem, 2016).

The coconut milk which is primarily the extract obtained from the coconut endosperm is a rich medium to support the growth of many spoilage microorganisms. In addition, it is highly susceptible to chemical deterioration by lipid auto-oxidation and lipolysis which results in off odors and flavors due to the presence of peroxides and MDA which is the end products of lipid oxidation ("Extension of shelf life of coconut milk," n.d.). Therefore, there are few thermal processing methods which are applied to preserve the quality and extend the storage life of coconut milk such as sterilization, ultrahigh temperature (UHT) and treatment pasteurization. Different storage condition and product life usually caused by different methods (Khuenpet, et. al, 2016). In this study, High Pressure Processing (HPP) is used to extend the shelf life of coconut milk.

Thermal processing methods affect the nutritional contents, flavors and taste of food. Therefore, food industry practitioners are attracted to non-thermal processing technology. There are numerous conferences to discuss high pressure, pulsed electric field, pulsed light, electron beam, plasma and modified atmosphere packaging on non-thermal processing technologies which are held each year worldwide. However, the most successfully commercialized non-thermal processing technology is high pressure processing (HPP). During HPP, the food which is sealed in a flexible container is treated by high pressure from 100 MPa up to 900 MPa at room temperature. Water is used as the pressure transfer medium which subjects even pressure on the internal parts and surface of the food to achieve pasteurization.

HPP technology is known as a non-thermal processing technology that ensures both flavor and safety of food. This is because HPP destroys the microbes in food without adding any chemicals such as preservatives by maintaining the nutritional value and original flavors of the food products. In addition, packaging form and volume of the food do not have effects on HPP. Therefore, food of different volumes can be processed in the same batch as the pasteurization effect. Besides that, HPP reduces energy consumption for heating and subsequent cooling as it is performed at room temperature. Also, the pressure transfer medium which is water can be recycled after HPP processing. Furthermore, the secondary contamination of food after pasteurization can be avoided as the food which is in packaged form when treated by HPP does not contact directly with the processing devices. Therefore, HPP is recognized as an eco-friendly processing technology with the low energy consumption and contamination risk (Huang, et. al, 2017).

There is no study that has investigated the effects of HPP on the physicochemical properties and shelf life of coconut milk. As the quality of food is a major driving force behind the products that the consumers buy, we must investigate these parameters to determine if coconut milk production with HPP is a practical option. Thus, the overall aim of this study is to determine a suitable HPP parameter that would maintain the quality of coconut milk and prolong its shelf life.

The objectives of this research are as follows:

1. To investigate the effects of process parameters of HPP in terms of physical properties (viscosity, total soluble solid) and chemical properties (fat content, free fatty acid, pH) of coconut milk.
2. To optimize the HPP parameter conditions (pressure and time).
3. To analyse the safety analysis (Total Plate Count (TPC), water activity) of coconut milk treated with HPP.

This thesis starts with a general introduction that recognizes the research problem and covers the thesis organization. Chapter 2 is the literature review which contains background information on the research problem. Chapter 3 is the methodology for this research which consists of the analyses involved and list of equipments used throughout this research. Chapter 4 is the results obtained and Chapter 5 is the discussions on the results obtained. The final chapter provides general conclusions on the research followed by acknowledgements.

CHAPTER 2

LITERATURE REVIEW

2.1 COCONUT

Coconut (*Cocosnucifera L.*) is classified as a fruit and frequently confused for being a nut. However, the coconut is actually a one-seeded drupe (Onsaard et al., 2006). Coconut fruit is required for living because nearly all parts of it can be used because even the husks and leaves are used as materials in furnishings and decoration. Palm trees produce coconuts up to 13 times a year. Although it takes a year for the coconuts to mature, a fully blossomed tree can produce between 60-180 coconuts in a single harvest. Therefore, coconut palm is an economic plant which is cultivated in most tropical countries. It is grown in more than 93 countries. Indonesia, Philippines and India are the major producers and account for about 75% of world production. In Malaysia, the coconut palm which is known as *kalpa* is the fourth important industrial crop after oil palm, rubber and paddy in terms of total planted area. It is one of the oldest agro-base industries (Alyaqoubi et al., 2015).

Coconut plays an important role in the socio-economic position of the

Malaysian rural population as there are coconut production for domestic consumption and export and industrial processing. The domestic demand for coconut products takes in the form of fresh coconut, tender coconut, coconut oil and processed cream powders. On the other hand, in terms of exports, the country has seen an increase in the export of end-products of coconut such as desiccated coconut, coconut milk powder and activated carbon (Sivapragasam, 2008).

2.2 COCONUT MILK

Coconut endosperm contains a white coconut kernel, liquid portion and a thin brown outer skin of coconut kernel which is known as coconut testa. In addition to the method of extraction, the components of the endosperm may also play an important role in determining the final phenol content of coconut oil that are mainly responsible for the antioxidant properties of coconut milk (Alyaqoubi et al., 2015).

In Malaysia, coconut milk which is called *Santan* in Malay language is the milk obtained after adding water to freshly grated coconut flakes and giving it a good press or squeeze, whereas *Pati Santan* is produced without addition of water. Malaysian traditional dishes such as curries and deserts which represent culinary diversity from multi-ethnic such as Malay, Chinese, Indian and Nyonya contain coconut milk as the main ingredient. For generations, coconut milk has been used in Malaysian cuisines for the source of thickener and for the perfect harmonies blend of flavor and aroma. Furthermore, there is an increase in research of product developments whereby coconut milk is used to substitute dairy milk (cow milk) in dairy products such as cheese, yogurt, chocolate and frozen dessert. This is because dairy milk is originated from animals whereas coconut milk is perceived to be healthier as it is originated from plant source. In addition, coconut milk would be a better option for vegetarians and some people are allergic or have lactose intolerance to dairy milk which limits their dairy products intake. Thus, coconut milk is said to be one of the most suitable replacer for dairy milk (Marina and Nurul, 2014).

2.2.1 COCONUT MILK COMPOSITION

According to Malaysian Food Act 1983 and Regulations 1985, coconut milk is the emulsion extracted from fresh and ripe kernel of the fruit of *Cocosnucifera*. Consequently, the physicochemical characteristics of coconut milk such as protein, fat, pH, moisture, ash in Malaysia have been investigated and compare to other tropical countries. Table 1 shows the mean values of the physiochemical parameters of the coconut milk samples in Malaysia with comparison with Philippine, Thailand and Sri Lanka (Alyaqoubi et al., 2015).

Table 1: Physiochemical parameters of the coconut milk samples in Malaysia with comparison with Philippine, Thailand and Sri Lanka. (Adopted from Alyaqoubi et al., 2015)

Country	Malaysia	Thailand	Philippine	Sri Lanka
Fat	15.44	20.00	38.00	35.80
Protein	3.40	2.06	3.50	3.10
Moisture	73.57	74.60	52.00	57.00
Ash	0.71	0.64	0.90	0.84
Carbohydrate	6.88	2.70	5.60	3.26

The results show that fat content in the Malaysian coconut milk samples was 15.44% which had significantly lower content than the other tropical coconut milk samples. By contrast Philippine coconut milk was the higher content of fat with percentage of 38%. Coconut milk samples showed variance in total protein content between the different countries. Protein content in Malaysian coconut milk samples was higher 3.4% compared to other samples except for the sample from Philippine 3.5%. This result demonstrates that Malaysian fresh coconut milk is a rich source of protein. Milk samples from Thailand were found to have highest moisture content which is 74.60% followed by Malaysia, Sri Lanka and Philippine which are 73.57%, 57.00% and 52.00% respectively. From the table, there are significant difference amounts of ash among all the comparative coconut milk samples. Philippine's coconut milk had the highest ash content value of 0.90% whereas Thailand's had the lowest at 0.64%.

2.2.2 PHYSICAL PROPERTIES OF COCONUT MILK

2.2.2.1 Viscosity

Based on the research done by Simuang et. al. (2004), the effect of heat treatment at 70, 80 and 90 °C and fat concentration of 15, 20, 25 and 30% on the viscosity of coconut milk was investigated and the results are tabulated in the Table 2 as follows:

Table 2: Apparent viscosity at 300 s^{-1} for coconut milk samples at different fat contents and temperatures. (Adopted from Simuang et. al., 2004)

Fat content (%)	Apparent Viscosity (Pa s)			
	Temperature (°C)			
	25	70	80	90
15	8.13×10^{-3}	4.58×10^{-3}	3.44×10^{-3}	2.43×10^{-3}
20	1.16×10^{-2}	5.62×10^{-3}	4.45×10^{-3}	4.25×10^{-3}
25	1.39×10^{-2}	7.69×10^{-3}	7.33×10^{-3}	6.16×10^{-3}
30	1.72×10^{-2}	9.60×10^{-3}	8.96×10^{-3}	8.31×10^{-3}

These results showed that heat treatment had significant effect on the viscosity of coconut milk as when the temperature increases, the apparent viscosity of coconut milk decreases. Besides, the apparent viscosity of the emulsion system increases at higher fat concentration as the resistance to flow is increased by the presence of a large number of fat globules.

2.2.2.2 Total Soluble Solid (TSS)

Total Soluble Solid (TSS) or known as Brix is important in studying the physicochemical compositions in coconut milk as nutritional value attribute. Table 3 shows the Total Soluble Solid (TSS) of the coconut milk samples in Malaysia with comparison with Philippine, Thailand and Sri Lanka (Alyaqoubi et al., 2015).

Table 3: Total Soluble Solid or Brix of the coconut milk samples in Malaysia with comparison with Philippine, Thailand and Sri Lanka. (Adopted from Alyaqoubi et al., 2015)

Country	**Brix (°)**
Malaysia	7.50
Thailand	5.40
Philippine	9.00
Sri Lanka	9.00

From the table, the Brix value on Malaysian coconut is 7.50° was also within the concentration range of comparative samples from 5.40° to 9.00°.

2.2.3 CHEMICAL PROPERTIES OF COCONUT MILK

2.2.3.1 pH

The pH of the coconut milk is one of the important factors in order to determine the quality of coconut milk. Table 4 shows the mean values of the pH of the coconut milk samples in Malaysia with comparison with Philippine, Thailand and Sri Lanka (Alyaqoubi et al., 2015).

Table 4: pH of the coconut milk samples in Malaysia with comparison with Philippine, Thailand and Sri Lanka. (Adopted from Alyaqoubi et al., 2015)

Country	**pH**
Malaysia	5.60
Thailand	5.80
Philippine	6.00
Sri Lanka	6.30

The pH of the coconut milk samples that were obtained from different sellers in Malaysia was measured at the same sampling day and the mean value was 5.60 which lies within the pH values range of comparative samples from 5.60 to 6.30.

2.2.3.2 Free Fatty Acid (FFA)

Based on the research done by Raghavendra and Raghavarao (2010), fatty acid compositions for coconut oil obtained by the combination of enzyme treatment at 37 °C followed by thawing and chilling of coconut milk emulsion are evaluated as shown in the Table 5:

Table 5: Fatty acid compositions are evaluated for coconut oil. (Adopted from Raghavendra & Raghavarao, 2010)

Fatty acids	Fatty acid compositions of coconut oil (%)
$C_{8:0}$ (caprylic)	9.4
$C_{10:0}$ (capric)	6.3
$C_{12:0}$ (lauric)	50.7
$C_{14:0}$ (myristic)	18.9
$C_{16:0}$ (palmitic)	6.7
$C_{18:0}$ (stearic)	2.3
$C_{18:1}$ (oleic)	3.9
$C_{18:2}$ (linoleic)	0.5

Based on the Table 5, the coconut oil obtained from coconut milk has the highest amount of 50.7% lauric acid. $C_{16:0}$, $C_{18:0}$, $C_{18:1}$ and $C_{18:2}$ which are long chain fatty acids are 6.7%, 2.3%, 3.9% and 0.5% respectively in the coconut oil obtained from coconut milk.

2.3 THERMAL TREATMENT ON COCONUT MILK

Coconut milk is the liquid obtained by mechanical extraction of grated coconut meat (Law, et. al., 2009). In general, thermal treatments such as pasteurization and UHT processing are given to food fluid to kill pathogenic micro-organisms and degrading enzyme in order to increase shelf life, product quality and product safety. The indirect plate heat exchangers with preheating, heating and cooling sections are normally used for heating process. After passing through heat treatment process, the coconut milk is filled in cans, boxes, soft plastic bags or processed powder forms.

Pasteurization process has been found to be a short-term preservation process in which the coconut milk is heated to pasteurization temperature of 72°C for 20 min. Normally, coconut milk pasteurized in soft plastic bags has been found to be fresher and more convenient for cooking with a shelf life of not more than 5 days (Pichitvittayakarn, et. al., 2006). On the other hand, the UHT processing would be preferred to be used to extend shelf life of longer than 6 months due to its ability to kill microorganisms and maintain the product quality (Khuenpet et al., 2016).

At high temperature, some components will lose their rheological properties and denature to form a fouling deposit which is the deposition of soils or unwanted material on the heat transfer surfaces. Therefore, coconut milk fouling deposit can affect greatly the performance of the process. In food processing, fouling causes increase in pressure drop through the plant, lowers the effectiveness of heat transfer, limits the operational time of the plant and most importantly it endangers plant and process sterility (Law et al., 2009).

2.3.1 EFFECTS OF THERMAL TREATMENT ON MILK PRODUCTS

2.3.1.1 Effect of Thermal Treatment on physicochemical properties of milk products

The cow milk, camel milk, sheep milk, goat milk and coconut milk were pasteurized at 72°C for 20 minutes (Eibaid et. al., 2017). Table 6 shows the physicochemical properties of different milk product treated by thermal treatment.

Table 6: Physicochemical properties of different milk product treated by thermal treatment.

Types of milk	Physicochemical properties			References
	pH	Brix (°)	Fat content (%)	
Cow	6.66	4.57	3.81	Eibaid et. al. (2017)
Camel	6.62	4.32	2.70	Eibaid et. al. (2017)
Sheep	6.71	4.01	8.69	Eibaid et. al. (2017)
Goat	6.89	4.00	3.10	Eibaid et. al. (2017)
Coconut	6.10	9.00	35.8	Yalegama et. al. (2008)

Based on the results, the effect of heat treatment on physic-chemical properties of milk samples of cow, camel, sheep, goat and coconut showed varied results depending on milk type.

The pH value of raw milk samples were 6.90, 6.86, 6.66 and 6.61 from goat, sheep, cow and camel mike samples respectively (Eibaid et. al., 2017). For cow and camel milk, pH values are 6.66 and 6.62 respectively which were not affected by heat treatment. For sheep milk, heat treatments decreased the pH values significantly to 6.71 from 6.86. For goat milk heat treatments decreased pH values but not significantly to 6.89 from 6.90. The pH value of coconut milk treated by heat which is 6.10 lies in the range of pH value of fresh coconut milk at 5.6 – 6.3.

Furthermore, the Brix (°) of raw milks was found to be 4.585, 4.365, 4.320 and 4.180 in cow, camel, goat and sheep respectively (Eibaid et. al., 2017). Based on the results, it is found that heat treatments decreased the Brix (°) of cow, camel, goat and sheep milks significantly to 4.57°, 4.32°, 4.01° and 4.00° respectively. Moreover, the heat treatment increased the Brix (%) of coconut milk from 7.5° to 9°.

The fat content (%) of raw milk was found to be 8.790, 3.795, 3.120 and 2.705 in sheep, cow, goat and camel milk samples respectively (Eibaid et. al., 2017). The results show that the heat treatment has not significantly affected fat percentage in sheep, cow, goat and camel milks. On the other hand, the heat treatment increased the fat content (%) of coconut milk from 15.44% to 35.8%.

2.3.1.2 Effect of Thermal Treatment on Microbial Analysis of coconut milk

Table 7 shows the effect of pasteurization on reducing the microbial load. The results are obtained by pasteurization of the coconut milk at 72°C for 10, 20 and 30 minutes and the non-pasteurized sample as control (Yalegama et. al., 2008).

Table 7: Total Plate Count (colonies/ml) of coconut milk stored for three weeks (Adopted from Yalegama et al., 2008).

Days	**Colonies/ml**			
	Control	**72°C, 10 mins**	**72°C, 20 mins**	**72°C, 30 mins**
0	2.03×10^5	2.23×10^5	4.32×10^3	3.60×10^3
7	4.30×10^6	1.02×10^6	1.62×10^5	1.05×10^5
14	5.42×10^6	1.49×10^6	1.74×10^5	3.20×10^5
21	1.21×10^7	2.23×10^6	6.15×10^5	4.20×10^5

From Table 7, the initial microbial count of the control treatment and the treatment of heating 10 minutes show higher values than the other treatments. The initial value increased during the storage and the microbial count decreased with increase of the heating time.

2.4 HIGH PRESSURE PROCESSING (HPP)

Recently, demands for better food quality with improved nutritional value, food safety, flavors and freshness with the increasing of health-conscious consumers (Zemser, 2015). Therefore, this has increased the development of non-thermal processing methods which can reduce additives while maintaining natural flavors and food quality. High Pressure Processing (HPP) is a non-thermal processing treatment used in the food production industry. It utilizes 100-800 MPa that all parts of the product are exposed to equal pressure all at the same time with the temperature being from 0-100°C from a few seconds to 20 minutes (Rawson et al., 2005).

The statement of bacteria can be eliminated by pressurization was reported by the earliest HPP study that was conducted in the United States in 1885. Accordingly, the potential application of high pressure to extend the storage life of milk in the food industry had been found by the researchers. Then, to establish a common technical standard for food pasteurization, many studies on HPP technology using lab-scale HPP equipment were carried out by governmental departments, research institutions, and universities. However, since HPP technology is a new processing method for the food industry, economic value and the health and safety aspects of HPP should be evaluated in detail before commercialized application. HPP jam in Japan is the earliest commercial product of HPP. Subsequently, Europe and North America had launched other HPP food products (Huang et al., 2017).

2.4.1 CURRENT STATUS AND FUTURE TREND OF HPP

The development of the capacity to produce HPP equipment was carried out by the manufacturers in America, Spain, U.K, China and Japan due to the gradual maturation of HPP equipment technology in recent years. There are two types of HPP devices which are the horizontal and vertical types. Marketsandmarkets (2013) stated that the horizontal type is the common devices used in commercial applications to facilitate the loading and unloading of containers in the production line. Nowadays, HPP technology has been widely utilized in the production of vegetable and fruit products, meat products, aquatic products, dairy products and various beverage products (Visiongain, 2015).

2.4.2 HPP EQUIPMENTS

HPP is carried out using batch equipment for high pressure food processing especially for packaged foods (Koutchma, 2014a). Food is prepared and aseptically filled and sealed in plastic containers. Then it is placed in a pressure chamber for pressurizing and the chamber is then decompressed (A. Elbrhami, 2016). According to Hogan et. al. (2005), the main components of a high pressure processing system are as follows and as shown in Figure 1:

1. A cylindrical pressure vessel which operates in vertical, horizontal or tilting

mode.

2. A low pressure pump.
3. Two end closures for sealing the vessel which bring the vessel to pressure process conditions, decompress the vessel and remove the product.
4. A restraining device for the end closures including yoke, threads, and pin.
5. An intensifier pump that uses liquid to generate high pressure process fluid for system compression (direct piston type compression and indirect compression method) (Dalai & Sahu, 2010).
6. A system for controlling and monitoring pressure and temperature.
7. A product-handling system

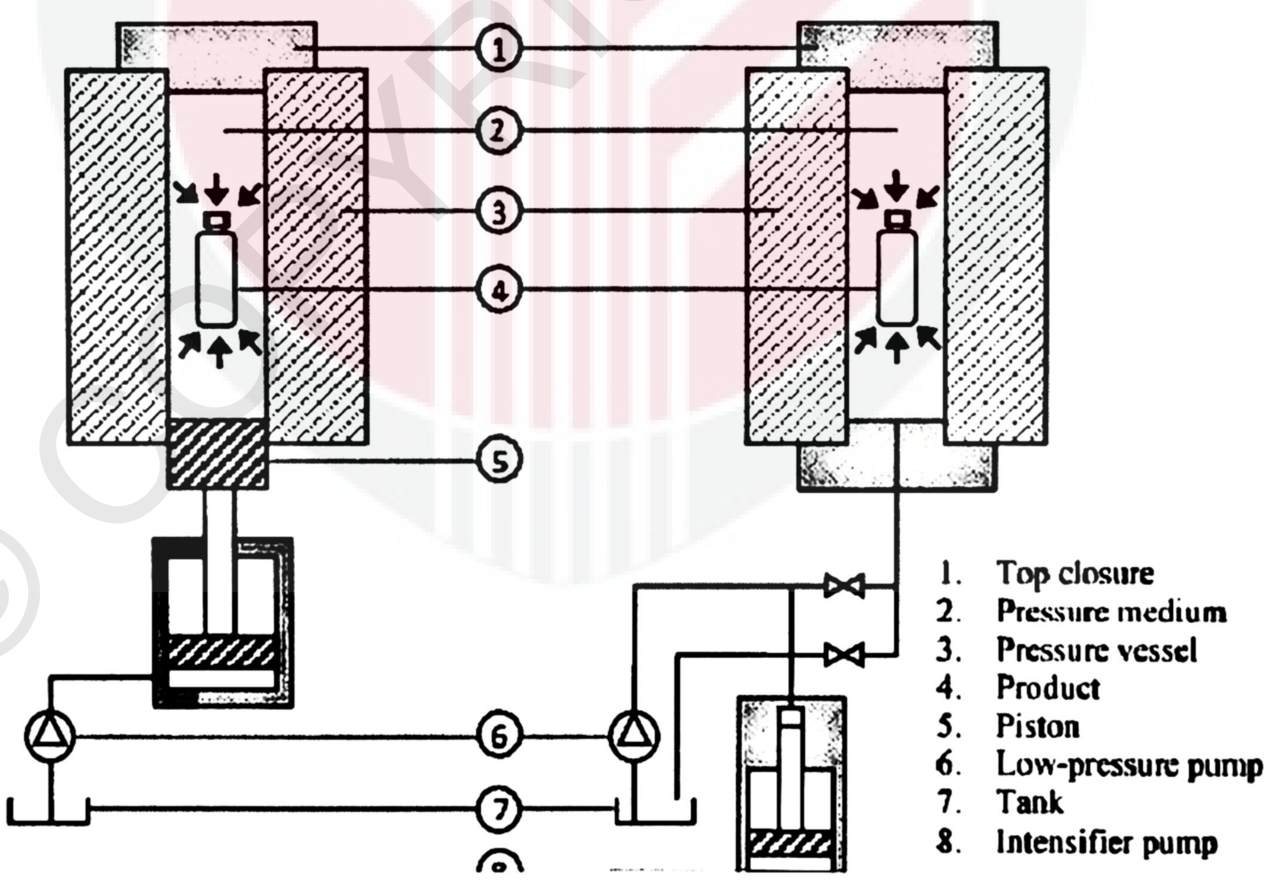

Figure 1: Schematics of High-Pressure Food Processing Techniques (Adopted from Balasubramanium, 2008)

For batch operation, packaged food is placed into the pressure vessel, the vessel is sealed and water is pumped into the vessel to remove any air. The pressure relief valve is closed after the vessel is full and water is pumped into the vessel until the process pressure is reached (Fellows, 2009; USFDA, 2014).

2.4.3 MECHANISM OF HPP

USFDA (2014) states that HPP mechanisms of inactivation can be categorized into four groups which are biochemical aspects, cell envelope-related effects, effects on genetic mechanisms and pressure-induced cellular changes. Pressure can change the cellular morphology and by increasing pressure application at pressures of 100-300 MPa, its division can be slow down. Besides, high pressure treatments can cause damage to the cell membrane by activating reactions that decrease volume and inhibiting reactions that increase volume. In addition, HPP changes the intermolecular structures of enzymes, causing conformational changes at the active site and leads to enzyme inactivation.

2.4.4 APPLICATIONS OF HPP

Studies showed that the HPP products increases each year as commercial applications of HPP were developed by the manufacturers in North America, Japan and Europe. Every year, ready-to-eat meat products like burger patties which are the largest application among about 500,000 tons of HPP products circulated in global market. In addition, the development of HPP juices such as coconut water has been boosted to become a famous topic in the field of HPP technology by the clean label trend in developed countries. This causes the estimation of juice products will hold a higher market share than meat products in future HPP food market (Huang et al., 2017).

There are many advantages of HPP technology compared to traditional thermal pasteurization. Therefore, the innovative values created by HPP technology for the food industry have been accepted gradually by the consumers. For instant, meat products treated by HPP such as bacon, vacuum-packaged ham and ready-to-eat products which have the same texture and shape as original products proved that HPP extends their shelf life and eliminates the secondary contamination without changing the original process conditions by maintaining the freshness of the meat products effectively as HPP increases enzyme activity such as protease. Furthermore, HPP also adds special flavors and texture to meat products by accelerating the aging and tenderization of meat products so that the meat products can be digested easily by the consumers (Huang et al., 2017).

Previous studies reported that HPP shortens the period of wine fermentation by maintaining the quality such as color, pH, acerbity, and turbidity of wine that has been aged for many years. In addition, HPP can reduce the microbial spoilage by inhibiting the growth of yeast and lactobacillus to produce natural and healthy foods (Buzrul, 2012).

2.4.5 EFFECTS OF HPP

2.4.5.1 Effect of HPP on Physicochemical Properties and Composition of Tiger Nut Milk

The effects of HHP treatment on the physicochemical properties and composition of tiger nuts milk represented in Table 8.

Table 8: Effects of HHP treatment on the physicochemical properties and composition of tiger nuts milk (Adopted from A. Elbrhami, 2016)

Physicochemical properties	HPP Treatment						
	Control	500 MPa			600 MPa		
		90 s	120 s	180 s	90 s	120 s	180 s
Total Soluble Solid (TSS) / Brix	4.5	4.5	4.5	4.5	4.5	4.5	4.5
Viscosity (cP)	10.4	11.1	11.9	12.6	13.6	14.7	12.2
pH	6.5	6.5	6.4	6.4	6.4	6.5	6.5

Based on the results obtained, there were no significant changes in the pH and total solids following high pressure treatment except treatment at 500 MPa. There was significant increase in the viscosity of tiger nut milk treated at 600 MPa for 120s.

2.4.5.2 Effect of HPP on Shelf Life of Tiger Nut Milk.

The effect of HHP on tiger nut milk treated at 600MPa for 180s then stored at 4°C for 8 days was determined in Table 9 (A. Elbrhami, 2016).

Table 9: Effect of HHP Treatment on Tiger Nut Milk Storage 8 Days at 4°C

Days	**Total Aerobic Count (log10 CFU/ml)**	
	Control	**600 MPa/180s**
0	4.94	4.24
4	0.8	3.74
8	6.6	4.8

According to the table above, the total aerobic count was decreased by HHP treatment to 4.8 log CFU during 72 storage at 4°C for 8 days. The final total aerobic count was significantly lower compared to non-treated control.

CHAPTER 3

METHODOLOGY

3.1 OVERVIEW OF RESEARCH FRAMEWORK

First of all, the samples of fresh coconut milk were subjected to freeze-drier for 3 days to produce coconut milk in powder for their composition and physicochemical properties test. Then, the samples are treated under 400 MPa, 500 MPa and 600 MPa for 3 different time durations which are 1, 3 and 5 minutes which is being modified from the journal by Keefe et. al (2013). After that, the physicochemical properties tests are carried out. A Central Composite Design (CCD) of Response Surface Methodology (RSM) was used to optimize HPP parameter conditions (pressure and time) of the coconut milk. Finally, samples of coconut milk with treatment of HPP under optimum conditions and coconut milk without any treatment) underwent storage at temperature 4°C (located in chiller) with various storage durations of 1, 2, 3, 4, 5, 6, 7, 14, 21 and 28 days to evaluate the shelf-life.

The overview of this research framework is shown in the Figure 2 as follow:

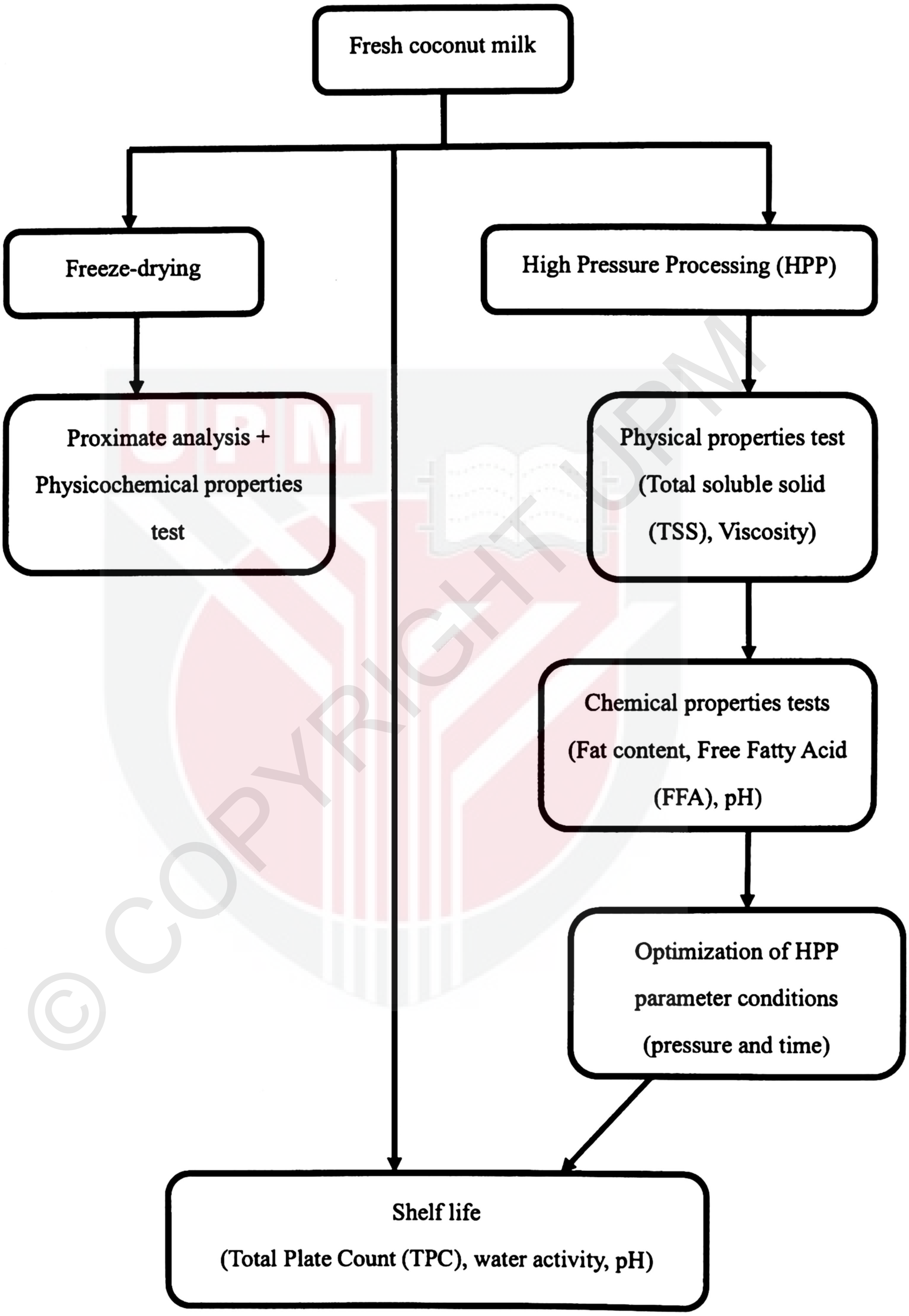

Figure 2: Overview of research framework

3.2 SAMPLE PREPARATION

The freshly grated coconut milk was purchased from fresh coconut milk seller in local wet market. The coconut milk sample was distributed evenly into plastic containers and stored in the freezer for freeze-drying whereas another coconut milk sample is stored in chiller for physicochemical analysis before and after treated by HPP.

3.3 FREEZE-DRYING

The samples of fresh coconut milk and the coconut milk which treated using HPP were subjected to freeze-drier for 3 days to produce coconut milk in powder. After freeze-drying, the coconut milk powder was collected and stored in freezer. The coconut milk powder obtained from freeze-drying was analyzed for their composition and physicochemical properties.

3.4 PROXIMATE ANALYSIS

The samples which are the coconut milk powder produced by freeze-drying were analyzed for their composition according to standard procedures. Data analysis was performed in duplicate.

3.4.1 Fat

A Soxtec System was used. The coconut milk powder was placed in an extraction thimble and the oil in coconut milk is being extracted with 90 ml of *n*-hexane for 60 min. Then, the thimble was raised to the rinse position for another 60 min. After the extraction, the sample undergo the clean-up procedure (Sporring, et. al, 2005).

3.4.2 Protein

To establish raw protein from coconut milk powder, the classical method of determination "Kjeldahl method" was used. The laboratory of the object called Nutrition is endowed with equipment used for the determination of raw protein, consists of mineralizator, distiller and also the necessary utensils and reagents (Petruł, et. al, 2008). A blank (all reagents and no sample) was digested and distilled to obtain the blank titre value. A known quantity of sample was digested using concentrated H_2SO_4 in a digestion flask until a clear solution was formed. The acid hydrolysate was neutralized with NaOH and steam distilled. The distillate was collected in 2% Boric acid with two drops of mixed indicator which consists of methyl red and bromocresol green. The distillate was titrated against 0.01N HCl until a colourless solution was formed. The percentage of protein in coconut milk powder values were displayed on the screen (Naik et al., 2017).

3.4.3 Moisture content

Moisture content was evaluated by drying the coconut milk samples at 105°C overnight in a Memmert Oven (Germany) according to AOAC (Alyaqoubi et al., 2015).

3.4.4 Ash

Ash content in coconut milk powders was evaluated according to AOAC. A known quantity of sample was placed in crucibles and burned on hot plate until there is no fume produced. The crucibles were then placed in furnace at 550°C overnight. After cooling, the ash left was weighed. Ash content was calculated as the ratio of weight of ash and weight of sample (Naik et al., 2017).

3.4.5 Carbohydrate

For coconut milk powder samples, the balance of subtracting fat, ash, moisture and protein contents which was expressed as % was total carbohydrate content (Naik et al., 2017).

3.5 PHYSICAL PROPERTIES TESTS

These tests involve the samples of coconut milk before and after the treatment using HPP.

3.5.1 Viscosity

The viscosity of coconut milk samples were measured using a rotational, concentric cylinder viscometer using spindle 3 at speed 100 rpm. Shear rate was adjusted to desired value (Peamprasart & Chiewchan, 2006).

3.5.2 Total Soluble Solid (TSS)

Total Soluble Solids (TSS) was measured with a refractometer at 20°C (Alyaqoubi et al., 2015). Two to three drops of coconut milk were placed onto the prism and the reading was recorded.

3.6 CHEMICAL PROPERTIES TESTS

These tests involve the samples of coconut milk before and after the treatment using HPP.

3.6.1 pH

The pH of the fresh coconut milk sample was determined using a digital pH meter at room temperature (Alyaqoubi et al., 2015). The pH meter was calibrated using pH 4 and pH 7 standard solutions on each test day.

3.6.2 Free Fatty Acid (FFA)

The FFA content was determined as recommended by the standard Association of Official Analytical Chemists method (AOAC, 1984). 25ml of 95% alcohol was neutralized using standardized 0.0598M NaOH using Phenolphthalein as the indicator. The solution was added to 25ml of coconut milk and mixed well. 25ml of diethyl ether was added to the resulting solution and mixed well. The solution was titrated with 0.0598N NaOH with Phenolphthalein as the indicator, till the pink color persisted. The FFA was calculated in terms of percentage of Lauric acid per 100ml of coconut milk ("Extension of shelf life of coconut milk," n.d.).

3.6.3 Total Fat Content

A Soxtec System was used. The coconut milk powder was placed in an extraction thimble and the oil in coconut milk is being extracted with 90 ml of *n*-hexane for 60 min. Then, the thimble was raised to the rinse position for another 60 min. After the extraction, the sample undergo the clean-up procedure (Sporring, et. al, & Bj, 2005).

3.7 HIGH PRESSURE PROCESSING (HPP)

The coconut milk was dispensed into sterile plastic pouches and was being vacuum sealed. Tightly tied samples were loaded into the pressure chamber of HPP unit and the treatments were performed at 400 MPa, 500 MPa and 600 MPa for 3 different time durations (Keefe et al., 2013). Three samples are treated for each conditions of treatment. After the HPP treatment, the samples were stored at 4 °C until further analysis.

Figure 3: HPP machine at Agro- Biotechnology Institute Malaysia, MARDI, Serdang, Selangor.

3.8 OPTIMIZATION OF HPP PARAMETER CONDITIONS (PRESSURE AND TIME)

Box–Behnken of Response Surface Methodology (RSM) in Design Expert software version 11.0 was used to optimize HPP parameter conditions (pressure and time) of the coconut milk. All experiments were performed in duplicates. The experimental data were fitted to a quadratic model to express the response variables as a function of the independent variables using equation. The analysis of variance (ANOVA) was discussed and the effect and regression coefficients of individual linear, quadratic and interaction terms were determined (Ephraim Edem, 2016).

There were 2 independent variables which are pressure and time of HPP treatment. Each variable had 3 different coded levels which is shown in Table 10 from low (-1), to medium (0) and high (+1). Dependent variables such as pH, Brix, viscosity, total fat content and free fatty acids (FFA) of coconut milk were evaluated as responses for the factors studied.

Table 10: Experimental range and levels of the independent variables.

Independent Variable	Coded Levels		
	-1	**0**	**1**
Pressure (MPa)	400	500	600
Time (min)	1	3	5

3.9 SHELF LIFE STUDY

Samples (fresh coconut milk without any treatment and coconut milk after treated using HPP under optimum conditions) underwent storage at temperature 4°C (located in chiller) with various storage durations of 1, 2, 3, 4, 5, 6, 7, 14, 21 and 28 days to evaluate the shelf-life according to their water activity and microbial count. The results were reported as an average of two replicates.

3.9.1 Total Plate Count (TPC)

The plate count agar was prepared by suspending 23.5 grams of media powder in 1000 ml boiling distilled water to dissolve the medium completely. Autoclave is used to sterilize the medium at 15 lbs pressure (121°C) for 15 minutes. Finally, the media was mixed well and poured into sterile petri plates (Wehr and Frank, 2004).

Next, for microbial analysis, the count was multiplied by their dilution factor and the colony growth on the plate agar was counted using a colony counter (Insausti et al., 2001). Samples were done in triplicate and they were incubated for 48 hours at 37°C (Mohd Thani et. al., 2016).

3.9.2 Water Activity (Aw)

Water activity meter was calibrated using distilled water. Samples were then inserted into water activity meter and values of water activity were read and recorded from the meter display. (Mohd Thani et al., 2016).

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 PROXIMATE ANALYSIS

Coconut milk is a complex biological fluid which consists of fat, protein, carbohydrates, and moisture as a milky white oil-in-water emulsion. The results on the compositions of fresh coconut milk are shown in Table 11. Except moisture, other parameters are given as on dry weight basis.

Table 11: Composition of coconut milk samples

Contents	Percentage (%)
Fat	23.950
Protein	1.533
Moisture	70.110
Ash	0.550
Carbohydrate	3.857
Total	**100**

Based on the table above, the moisture content in the coconut milk samples is the highest which is 70.11%, followed by the fat content which is 23.95%. On the other hand, the ash content is the lowest in coconut milk which is 0.55%. The protein and carbohydrate contents are 1.533% and 3.857% respectively.

4.2 HPP TREATMENT

4.2.1 HPP Effects on Physical Properties of Coconut Milk

4.2.1.1 Viscosity

The viscosity of coconut milk treated by different conditions of HPP is shown in Figure 4.

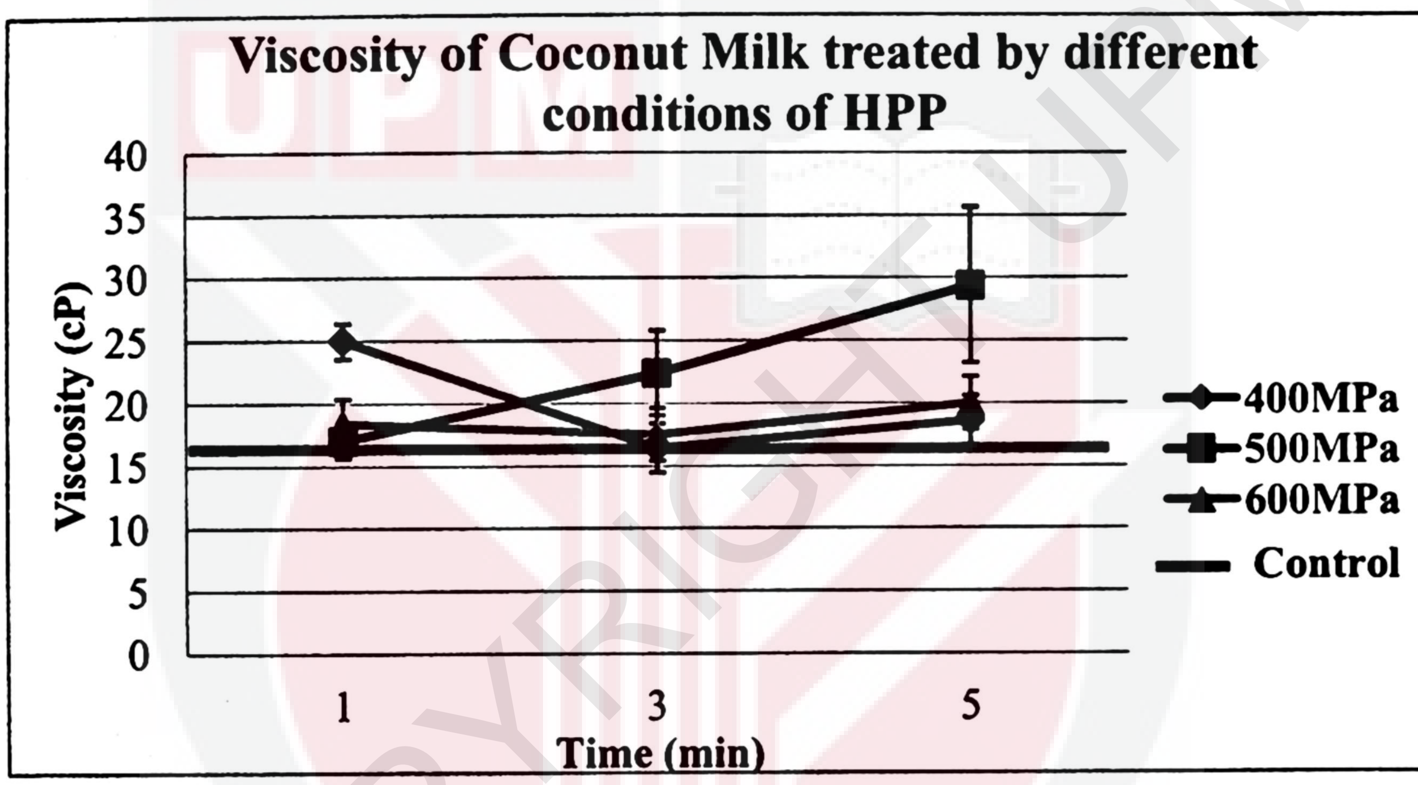

Figure 4: Viscosity of Coconut Milk treated by different conditions of HPP

By comparing with the viscosity of fresh coconut milk without HPP treatment which is 16 cP as orange line shown in Figure 4, there was no significant decrease in the viscosity of coconut milk treated by HPP. In contrast, there was increase in the viscosity of coconut milk treated by HPP at 400MPa for 1 minute, 500MPa for 3 minutes and 500MPa for 5 minutes which are 25 cP, 22.4 cP and 29 cP respectively. The increase in the viscosity of coconut milk is caused by the decrease in amount of free volume in the internal structure in the coconut milk due to compression during HPP treatment (Muhammed, 2017).

4.2.1.2 Total Soluble Solid (TSS)

The total soluble solid (TSS) of coconut milk treated by 9 different conditions of HPP is reported in Figure 5.

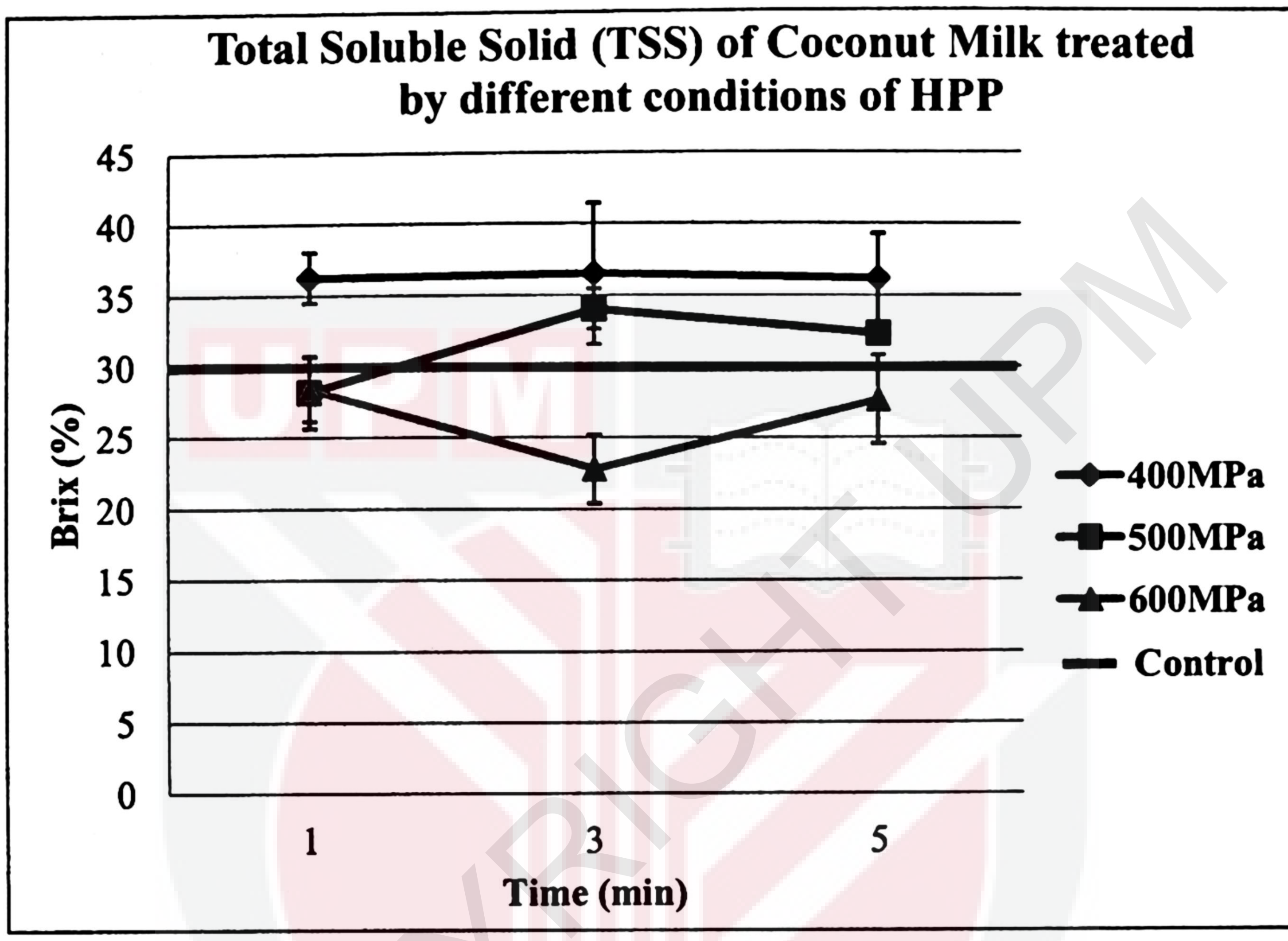

Figure 5: Total Soluble Solid (TSS) of Coconut Milk treated by different conditions of HPP

Based on the figure, the total soluble solid (TSS) of coconut milk treated by HPP at 600MPa for 3 minutes decreases to 22.8% compared to the total soluble solid (TSS) of fresh coconut milk without HPP treatment which is 30% as orange line shown in Figure 5. Moreover, the coconut milk samples which are treated at 400 MPa shows slightly increase in the total soluble solid (TSS). For 1 minute, 3 minutes and 5 minutes of holding time at this pressure, the total soluble solid (TSS) of coconut milk is 36.25%, 36.5% and 36.2% respectively.

Moreover, it is found that the coconut milk treated by HPP at 400MPa for 3 minutes has the highest sugar content whereas the coconut milk treated by HPP at 600MPa for 3 minutes has the lowest Brix. After treated by HPP at 400 and 500MPa, the Brix of the coconut milk increases which is caused by increasing pressure significantly reduced liquid compressibility due to loss of secondary and tertiary structure of protein (Min et. al., 2010). However, after treated by HPP at 600MPa, the Brix of the coconut milk decreases. In my opinion, this may due to the breakdown of polysaccharide chain in coconut milk.

4.2.2 HPP Effects on Chemical Properties of Coconut Milk

4.2.2.1 pH

The pH values of the coconut milk samples that were treated by different conditions of HPP which were measured at the same treatment day are presented in Figure 6.

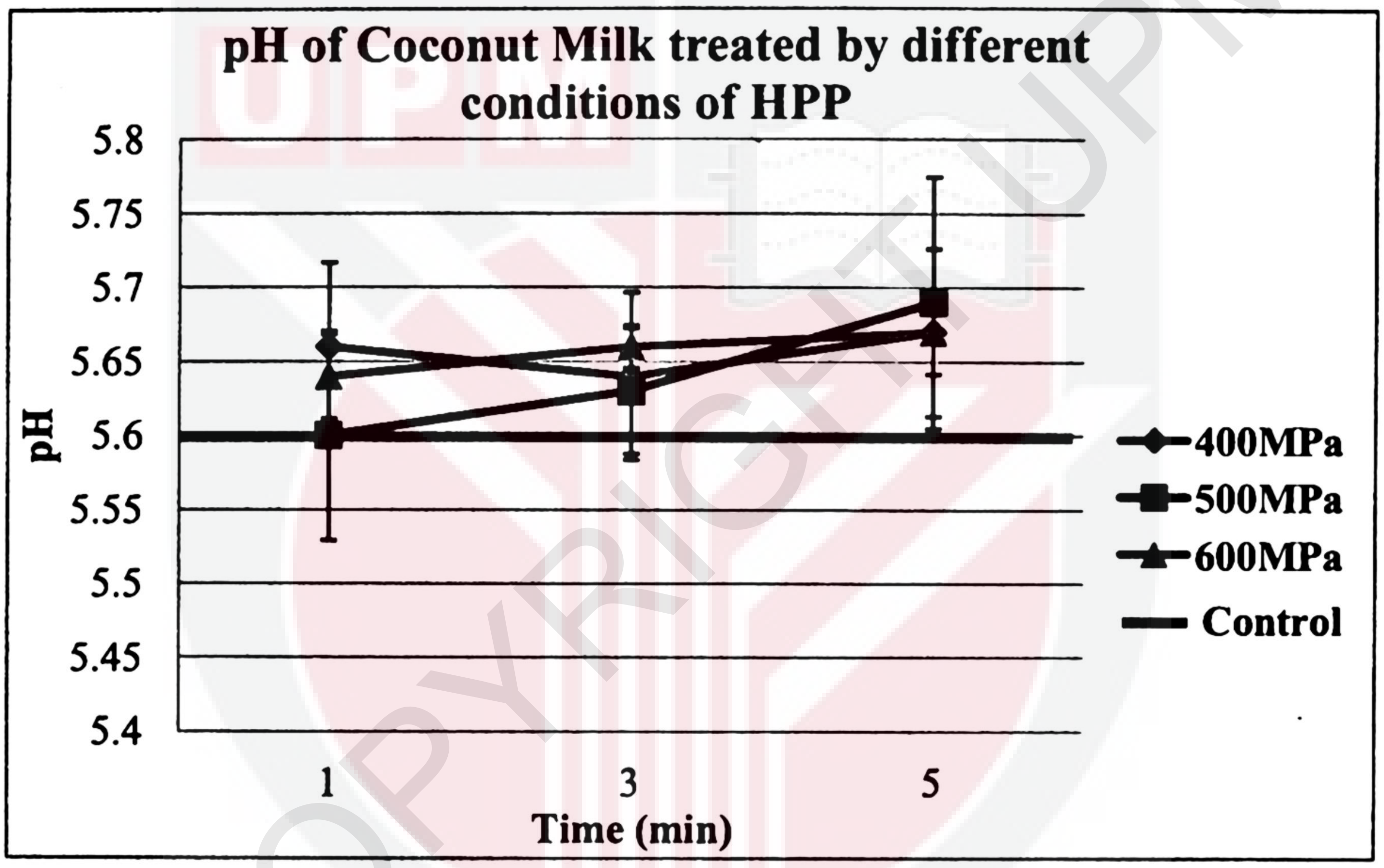

Figure 6: pH of Coconut Milk treated by different conditions of HPP

According to the result, the coconut milk treated by HPP at 500MPa for 1 minute has the lowest pH which is 5.60 whereas the coconut milk treated by HPP at 500MPa for 5 minutes has the highest pH which is 5.69. This shows that all of the pH values obtained are acceptable which lay within the pH values of fresh coconut milk from 5.60 (orange line in Figure 6) to 6.30, even though the pH values of the coconut milk samples that were treated by HPP is lower than that of the fresh coconut milk samples without treatment of HPP which is at pH 5.9.

Besides, Figure 6 shows that the pH values of coconut milk increase with increasing the processing time of HPP at 500 MPa and 600 MPa. This is because longer processing time for higher pressure treatment generally decreases the acidity of the coconut milk with decreasing the hydrogen ion (H^+) which can inactivate the microorganisms in the coconut milk (Patterson, 2005).

4.2.2.2 Free Fatty Acid (FFA)

Figure 7 shows the Free Fatty Acid (FFA) content in coconut milk treated by different conditions of HPP in terms of lauric acid which is the major component of coconut oil and palm kernel fat.

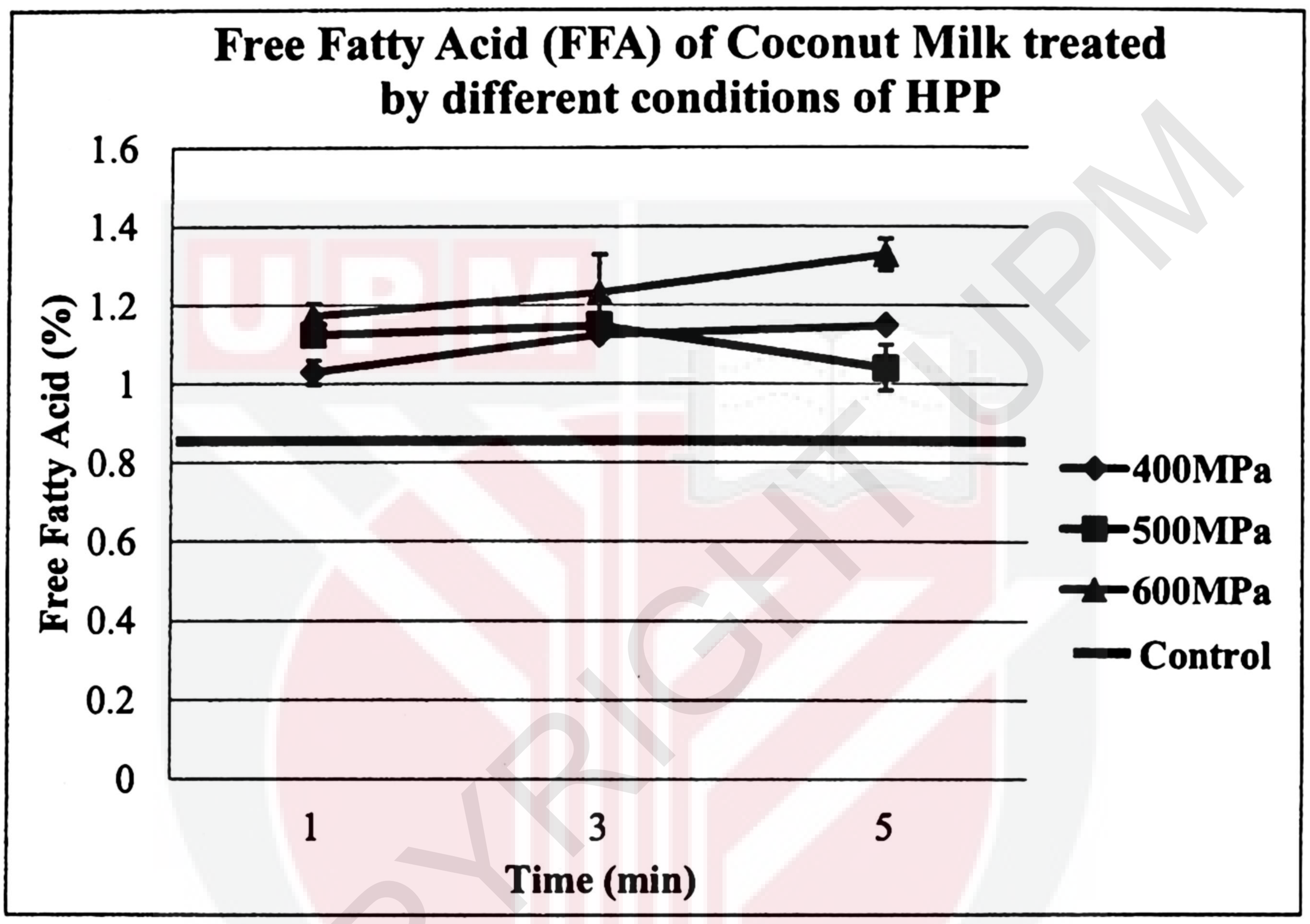

Figure 7: Free Fatty Acid (FFA) of Coconut Milk treated by different conditions of HPP

The result shows there was increase in the FFA content in coconut milk samples from with 0.8611% of FFA without HPP treatment as orange line shown in Figure 7 to 1.0286% and 1.3276% of FFA after HPP treatment at 400 and 600 MPa respectively. This shows that the higher the pressure of HPP treatment, the more triglycerides being hydrolyzed into free fatty acids (Alenezi et. al. , n.d.).

4.2.2.3 Total Fat Content

The total fat content of coconut milk treated by 9 different conditions of HPP is reported in Figure 8.

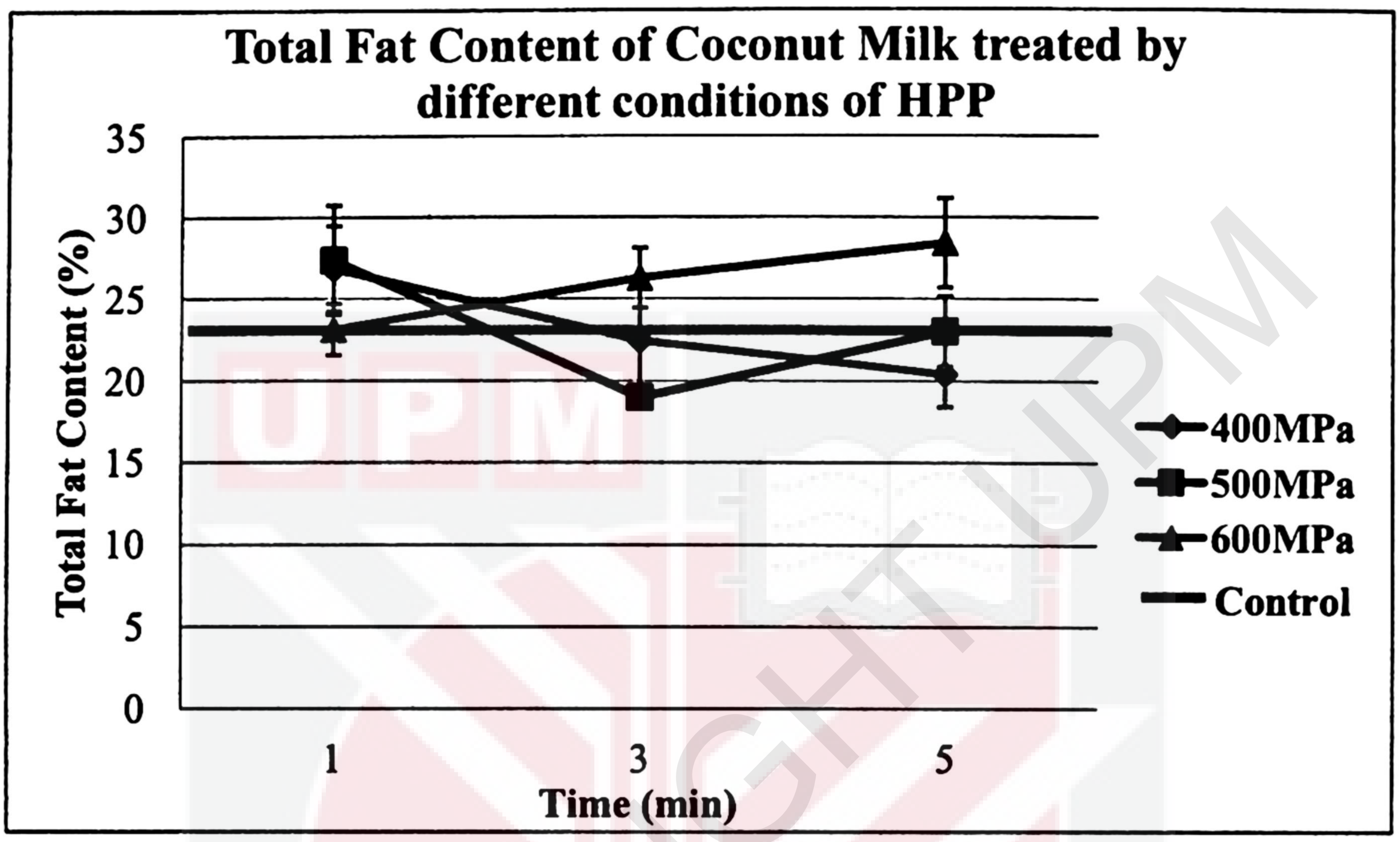

Figure 8: Total Fat Content of Coconut Milk treated by different conditions of HPP

From the figure above, it can be observed that there was significant decrease from 23.9% without HPP treatment as orange line shown in Figure 8 to 22.44%, 20.4% and 19% after treated by HPP at 400 MPa for 3 and 5 minutes and 500MPa at 3 minutes respectively. On the other hand, the coconut milk treated at 600MPa for 5 minutes gives the highest fat content which is 28.43%. High amount of fat content in coconut milk is good for human body as coconut milk consists of unsaturated fatty acids which can protect body from infections and viruses.

4.3 OPTIMIZATION OF HPP PARAMETER CONDITIONS (PRESSURE AND TIME)

4.3.1 Model Fitting

The experimental results of the effect of HPP pressure and time on coconut milk pH, Brix, viscosity, total fat content and free fatty acids (FFA) are presented in Table 12. These experimental data were used in RSM program to create the best predicted model and its statistical analysis.

Table 12: Design matrix of central composite design obtained from RSM and experimental values of the responses for coconut milk treated by HPP.

Run	Actual variable		Responses				
	Pressure (MPa)	Time (minute)	pH	Brix (%)	Viscosity (cP)	Total Fat (%)	FFA (%)
1	600	1	5.64	28.40	18.4	23.12	1.1721
2	400	3	5.64	36.50	16.4	22.44	1.1242
3	500	1	5.60	28.20	17.0	27.37	1.1242
4	500	5	5.69	32.30	29.4	23.02	1.0405
5	500	3	5.63	34.00	22.4	19.00	1.1482
6	500	3	5.63	34.00	22.4	19.00	1.1482
7	400	5	5.67	36.20	18.6	20.40	1.1482
8	500	3	5.63	34.00	22.4	19.00	1.1482
9	500	3	5.63	34.00	22.4	19.00	1.1482
10	600	3	5.66	22.80	17.5	26.29	1.2319
11	500	3	5.63	34.00	22.4	19.00	1.1482
12	600	5	5.67	27.70	20.0	28.43	1.3276
13	400	1	5.66	36.25	25.0	26.86	1.0286
14	600	3	5.66	22.80	17.5	26.29	1.2319
15	400	3	5.64	36.50	16.4	22.44	1.1242
16	500	5	5.69	32.30	29.4	23.02	1.0405
17	500	1	5.60	28.20	17.0	27.37	1.1242

Based on the results of analysis of variance (ANOVA), the fitness of the final predicted polynomial model was considered.

Table 13: Analysis of variance (ANOVA) for the fitted polynomial model of the dependent variables.

Response	Source	Sum of square	Mean square	F-value	P-value	Significant / Not significant
Viscosity	Model	154.60	30.92	2.74	<0.0001	Significant
	Residual	124.33	11.30			
	Lack of fit	124.33	41.44			
	Pure error	0	0			
Brix	Model	240.76	120.38	21.47	0.0763	Not significant
	Residual	78.49	5.61			
	Lack of fit	78.49	13.08			
	Pure error	0	0			
pH	Model	0				Not significant
	Residual	0.0112	0.0007			
	Lack of fit	0.0112	0.0014			
	Pure error	0	0			
Total Fat	Model	146.87	29.37	7.23	0.0032	Significant
	Residual	44.69	4.06			
	Lack of fit	44.69	14.90			
	Pure error	0	0			
FFA content	Model	0.0570	0.0114	4.28	0.0209	Significant
	Residual	0.0293	0.0027			
	Lack of fit	0.0293	0.0098			
	Pure error	0	0			

*significant at $p < 0.05$

Result in Table 13 showed that the probability value (p-value) of the response models was less than 0.05, suggesting that the models for the responses were statistically correct and effective with insignificant lack of fit for pH. In addition, the goodness-of-fit of the model was also ascertained by the coefficient of determination (R^2) shown in Table 14 as follows.

Table 14: Estimated regression coefficients of the fitted polynomial model representing the relationship between responses and process variables at the design response surface.

Source	Viscosity		Brix		pH		Total Fat		FFA content	
	Coefficient	p - value	Coefficient	p - value	Coefficient	p - value	Coefficient	p - value	Coefficient	p - value
A - Pressure	-0.3750	0.7583	-5.41	0.0763	-	-	1.50	0.0593	0.0673	0.0036
B - Time	2.50	0.0593	0.9313	0.2847	-	-	-1.23	0.1120	0.0135	0.4763
AB	2.00	0.2592	-	-	-	-	2.94	0.0139	0.0090	0.7347
A^2	-4.15	0.0277	-	-	-	-	2.59	0.0230	0.0568	0.0450
B^2	2.10	0.2251	-	-	-	-	3.42	0.0051	-0.0389	0.1500
R^2	0.5543		0.7541		0		0.7667		0.6604	
Adjusted R^2	0.3517		0.7190		0		0.6606		0.5060	
Predicted R^2	-1.6442		0.6395		-0.1289		-0.0329		-0.9789	
C.V. %	0.4692		7.49		16.12		8.74		4.51	
Adeq. Prec.	5.7691		12.7431		-		7.6999		5.8006	

R^2 = Coefficient of determination, **C.V. %** = Coefficient of variation, **Adeq prec** = Adequate precision

The best R^2 value for a good model fitting was estimated between 0.8 and 1.0 (Jusoh et al, 2013). On the other hand, Gupta et al. (2014) reported that $R^2 < 0.80$ indicates a fair fit of the model but reliable in making predictions. Consequently, based on Table 14, R^2 of 0.5543, 0.7541, 0.7667 and 0.6604 for viscosity, Brix, total fat content and FFA content respectively indicated fair fit of the models. This implied that only 55.43%, 75.41%, 76.67% and 66.04% of variations in viscosity, Brix, total fat content and FFA content respectively.

Firatiligil-Durmus and Evranuz (2010) states that CV is a measure of deviation from the mean values. It shows the reliability of the experiment and describes the extent to which the data were dispersed as well as the reproducibility and repeatability of the model. CV > 10% indicated that the experiment was less precise but reliable (Gupta et al., 2014). This implies that the fitted models of responses were highly reliable and can be considered reasonably reproducible. The results in Table 14 revealed that viscosity had low coefficient of variation (CV) values of 0.4692% which is less than 3.5%.

The predicted R^2 was in reasonable agreement with the adjusted R^2. This is because the difference between them was less than 0.2, which illustrated that there were excellent correlations between the independent variables in predicting the responses. Adequate precision is a comparative measure between the predicted values and the mean predicted error and measures the signal to noise ratio. A ratio greater than 4 is desirable. The results in Table 14 showed that adequate precision values were greater than 4. Therefore, the respective models can be used to navigate the design space.

The fitted regression models, in terms of coded factors are shown using polynomial equations as follows:

$$Viscosity = -4.15\,{X_1}^2$$

$$Brix = -5.41\,X_1$$

$$Total\ Fat\ Content = 2.94\,X_1X_2 + 2.59\,{X_1}^2 + 3.42\,{X_2}^2$$

$$FFA\ Content = 0.0673\,X_1 + 0.0568\,{X_1}^2$$

4.3.2 Effect of pressure and time on Viscosity

Figure 9 shows the 3-Dimensional plot of RSM of the effect of HPP pressure and time on viscosity of coconut milk.

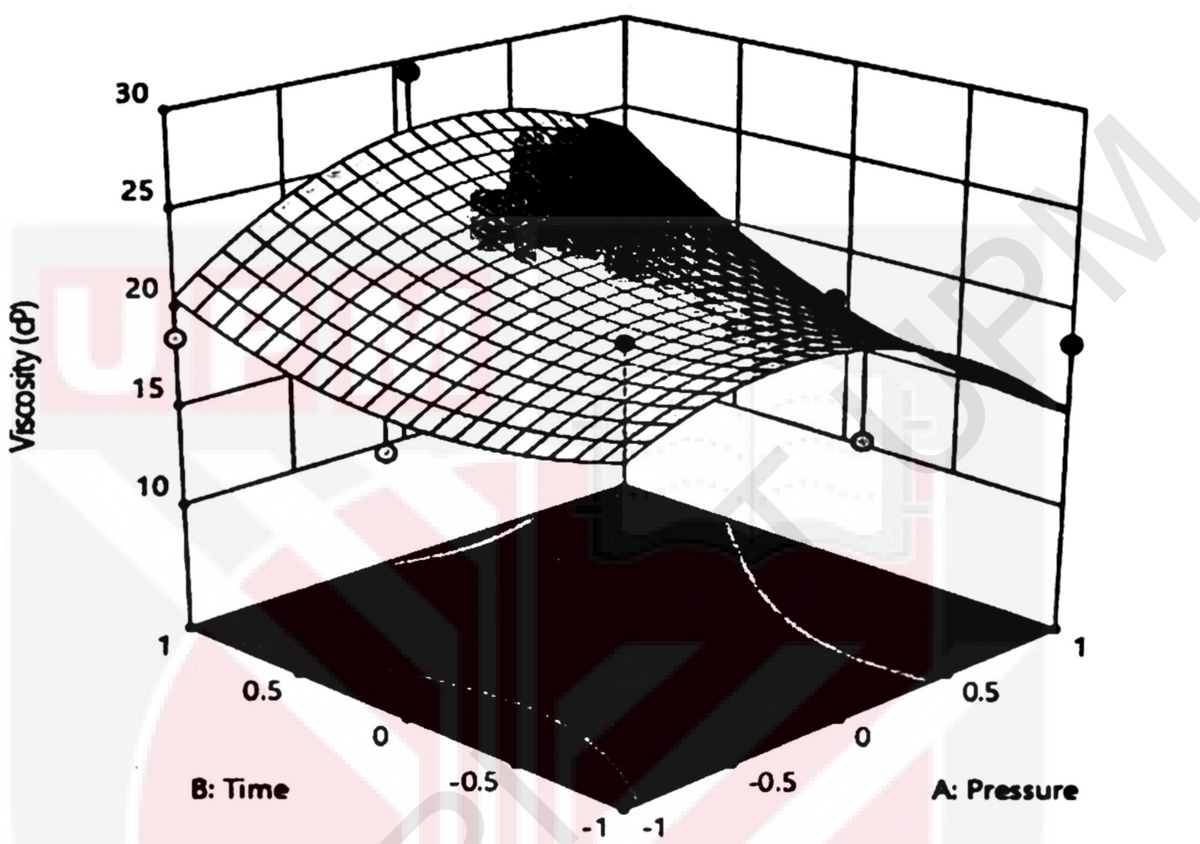

Figure 9: Effect of HPP pressure and time on Viscosity

The viscosity of coconut milk ranged from 16.4 to 29.4 cP. ANOVA showed that viscosity was significantly affected by pressure of HPP, whereas holding time of HPP had no significant effect on the viscosity of coconut milk. The 3-D plot of RSM in Figure 9 shows that the viscosity of the coconut milk is the highest at pressure of 500 MPa. The negative coefficient of HPP pressure indicated that HPP pressure exerted an antagonistic (negative) effect on viscosity. This implied that viscosity of coconut milk is a function of quadratic effect of HPP pressure.

4.3.3 Effect of pressure and time on Brix

Figure 10 shows the 3-Dimensional plot of RSM of the effect of HPP pressure and time on Brix of coconut milk.

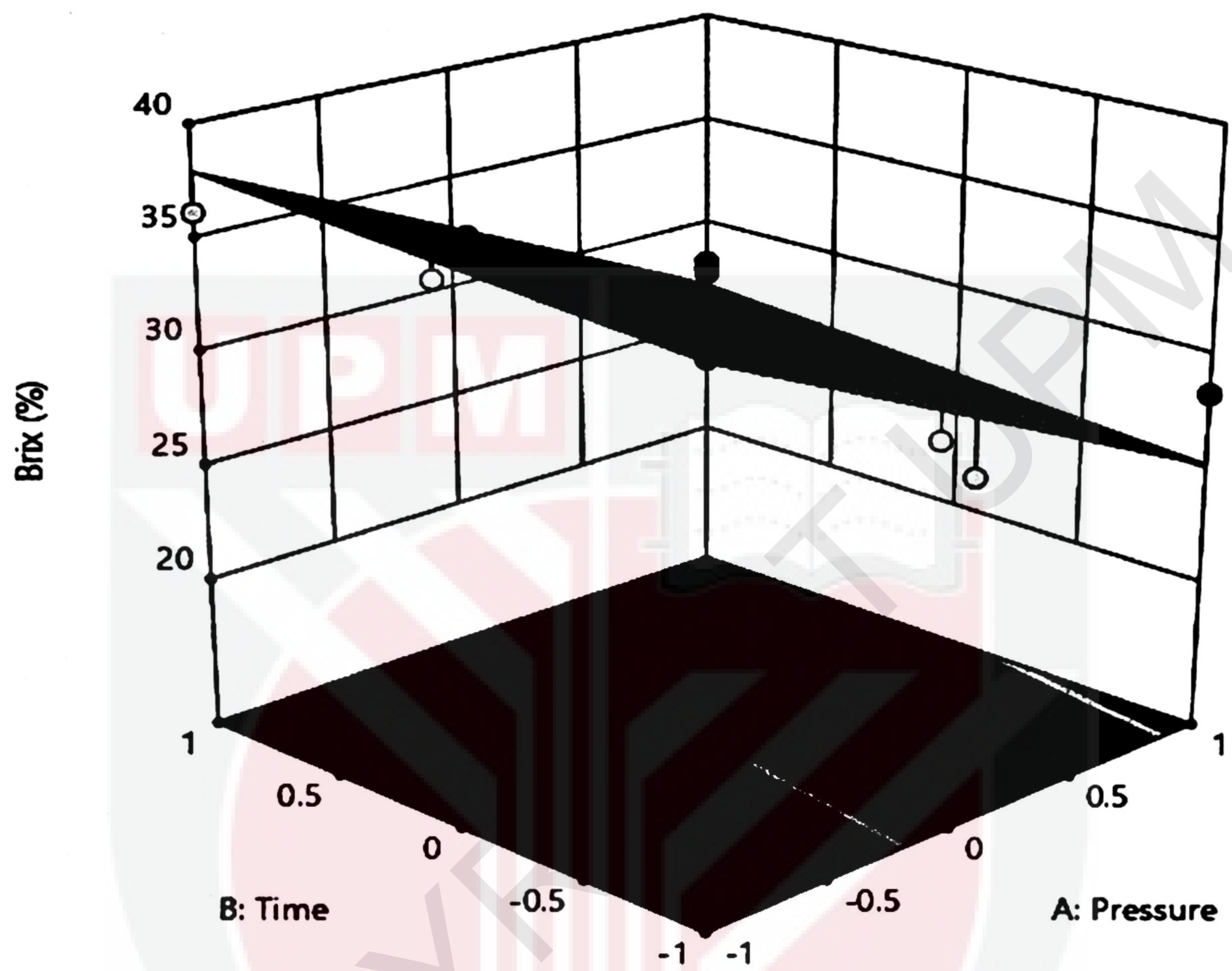

Figure 10: Effect of HPP pressure and time on Brix

The Brix of coconut milk ranged from 22.8 to 36.25. ANOVA showed that the Model F-value of 21.47 implies the linear model is significant. As the P-value is less than 0.05 indicating model terms are significant, in this case, the pressure of HPP is a significant model term. Figure 10 shows that the increase in pressure led to sharp decrease of Brix of coconut milk. The result showed that Brix was found to be a function of linear terms of HPP pressure and time, with a mean Brix of 31.63.

4.3.4 Effect of pressure and time on FFA

Figure 11 shows the 3-Dimensional plot of RSM of the effect of HPP pressure and time on Free Fatty Acid (FFA) content of coconut milk.

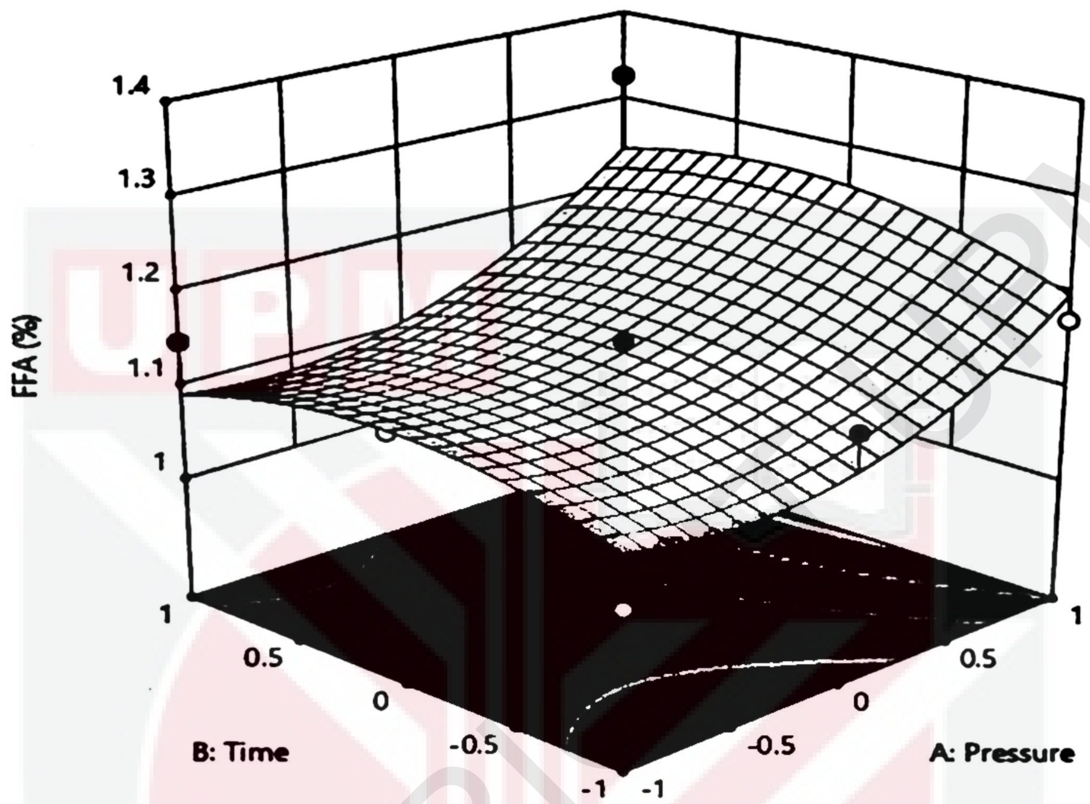

Figure 11: Effect of HPP pressure and time on Free Fatty Acid (FFA) Content

The free fatty acids (FFA) content of coconut milk ranged from 1.0286% to 1.3276%. ANOVA showed that free fatty acids (FFA) content was significantly affected by pressure of HPP, whereas holding time of HPP had no significant effect on the free fatty acids (FFA) content of coconut milk. The 3-D plot of RSM in Figure 11 shows that the higher the HPP pressure, the higher free fatty acids (FFA) content in coconut milk. The result showed that free fatty acids (FFA) content was found to be a function of quadratic terms of HPP pressure, with a mean free fatty acids (FFA) content of 1.14.

4.3.5 Effect of pressure and time on Total Fat

Figure 12 shows the 3-Dimensional plot of RSM of the effect of HPP pressure and time on Total Fat Content (%) of coconut milk.

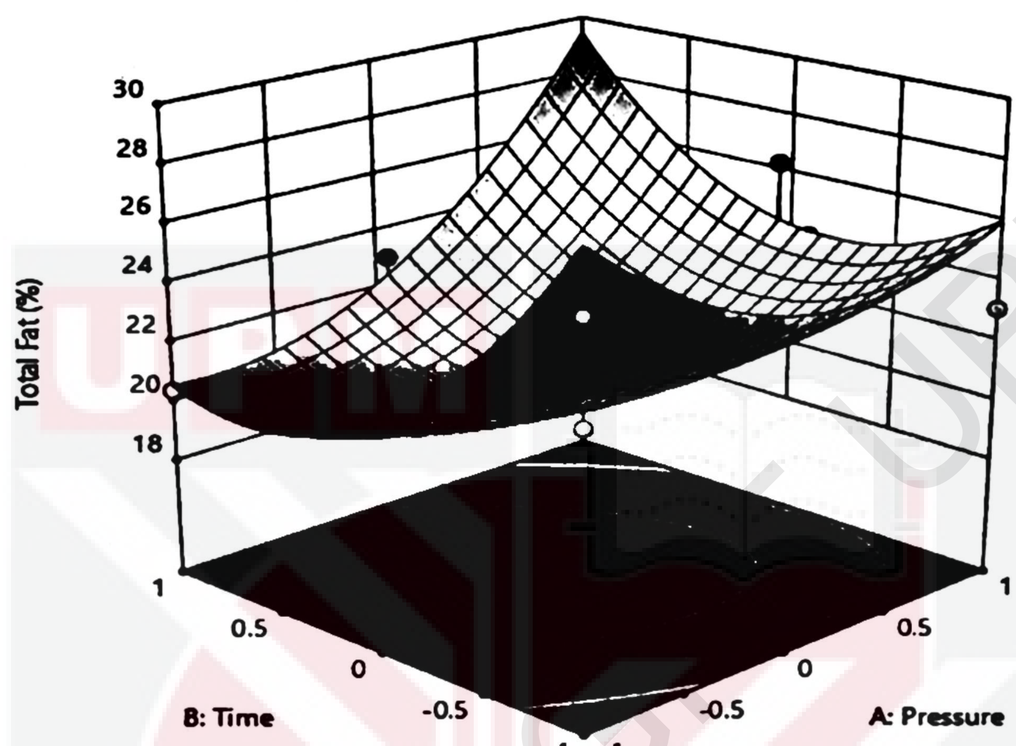

Figure 12: Effect of HPP pressure and time on Total Fat Content

The total fat content of coconut milk ranged from 19% to 28.43%. ANOVA showed that the Model F-value of 7.23 implies the quadratic model is significant. As the P-value is less than 0.05 indicating model terms are significant, in this case, both of the pressure and time of HPP are significant model terms. Figure 12 shows that when the HPP processing time increases, the total fat content increases, however when the HPP pressure increases, the total fat content decreases. The result showed that total fat content of coconut milk was found to be a function of quadratic terms of HPP pressure and time, with a mean total fat content of coconut milk of 23.06.

4.3.6 Optimization of HPP treatment on coconut milk

Numerical optimization option was employed. The preferred goal of extraction variables and responses, such as minimizing Brix and viscosity while HPP pressure, HPP time, pH, total fat content and free fatty acid (FFA) content were allowed to be in range is limited to generate desirability function. According to the desirability function, the software generated several solutions of process variables with the predicted values of responses. The predicted optimum condition at maximum desirability index of 0.903 (90.30%) was obtained as 600MPa, 3 minutes of HPP treatment. The predicted pH, viscosity, Brix, total fat content and free fatty acid (FFA) content were estimated to be 5.65, 25.29, 14.90cP, 26.03% and 1.20% respectively as shown in Table 15.

4.3.7 Validation of Optimization of HPP treatment on coconut milk

To validate optimization of HPP pressure and holding time, two experiments were carried out under the suggested optimum condition at 600MPa for 3 minutes. The predicted and experimental values are presented in Table 15. The result showed that there were no significant difference on the corresponding experimental values between the predicted (simulated) and actual properties of coconut milk. This result attests to the effectiveness of this framework for optimum and effective HPP treatment on coconut milk.

Table 15: Predicted and actual responses for optimized process.

Response	Predicted values	Experimental values
pH	5.65	5.62
Brix (%)	25.29	24.6
Viscosity (cP)	14.90	15.1
Total Fat (%)	26.03	25.6
Free Fatty Acids (%)	1.20	1.3398

4.4 SHELF LIFE STUDY

The coconut milk treated by optimum condition at 600MPa for 3 minutes determined by RSM as mention earlier and the coconut milk without any treatment (control) underwent storage at temperature 4°C (located in chiller) with various storage durations of 1, 2, 3, 4, 5, 6, 7, 14, 21 and 28 days to evaluate the shelf-life

4.4.1 Total Plate Count (TPC)

The calculation of colonies based on data from each sample is only calculated dilution with the number of colonies between 30 to 300. This aims to minimize the probability of error in the calculation. As the experiment was done twice (Duplo), the data from both repetitions by taking the average of the two data is calculated. The analysis of total plate count in terms of log10 CFU/g on the coconut milk treated by optimum condition at 600MPa for 3 minutes and the coconut milk without any treatment (control) for storage of 28 days is shown in Figure 13. The maximum limit of total bacteria in coconut milk which is 1×10^4 CFU/g is being converted into 4 log10 CFU/g and drawn in Figure 13 as follows.

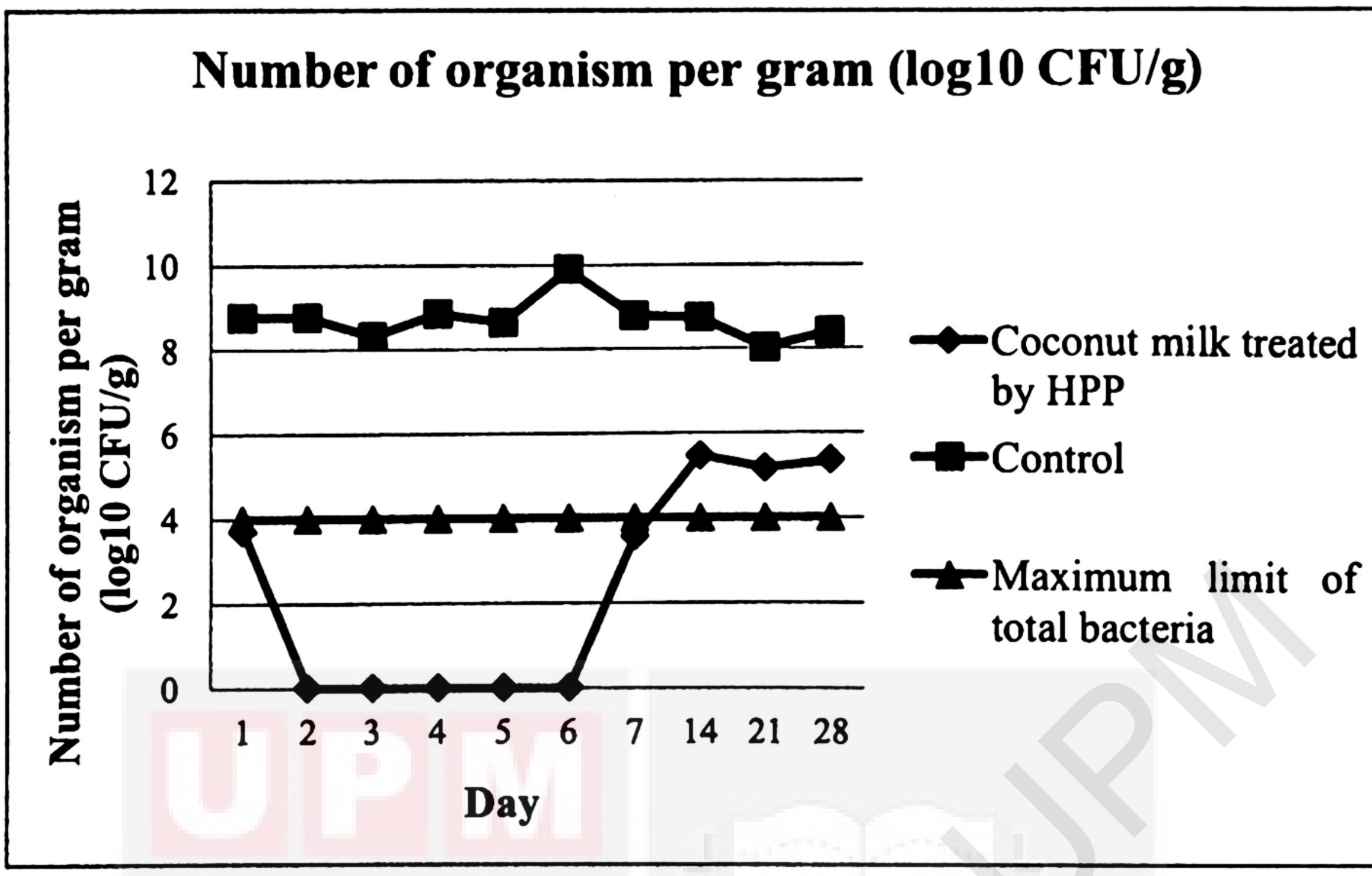

Figure 13: Number of organism per gram (log10 CFU/g) of the coconut milk treated by optimum condition at 600MPa for 3 minutes and the coconut milk without any treatment (control) for storage of 28 days.

Based on the results of TPC analysis in figure above, it can be seen that Sample A has a total value ranging from 3.65×10^3 to 2.935×10^5 CFU /g. This indicates that the coconut milk treated by optimum condition at 600MPa for 3 minutes is acceptable microbiological until Day 7. From Day 14, it exceeds the maximum limit of total bacteria in coconut milk which is 1×10^4 CFU /g as shown in Figure 13.

Moreover, the coconut milk without any treatment (control) has a total value ranging from 1.06×10^8 to 7.8×10^9 CFU /g starting from the first day of storage. This indicates that the control exceeds the maximum limit of total bacteria in coconut milk which is 1×10^4 CFU /g starting from the first day of storage.

The coconut milk treated by optimum condition at 600MPa for 3 minutes has longer shelf life of less than 14 days than the control. This is because High Pressure Processing (HPP) is a non-thermal processing treatment that all parts of the product are exposed to equal pressure all at the same time which can cause chemical and enzymatic reactions, such as browning to accelerate, slow down, or stop with retention of flavors, nutrients and colors of food products.

4.4.2 Water Activity (Aw)

The following discussion focus on storage days when coconut milk started to spoil based on the water activity of coconut milk treated by HPP at 600MPa for 3 minutes and coconut milk without any treatment (control) with various storage durations of 1, 2, 3, 4, 5, 6, 7, 14, 21 and 28 days as shown in Figure 14.

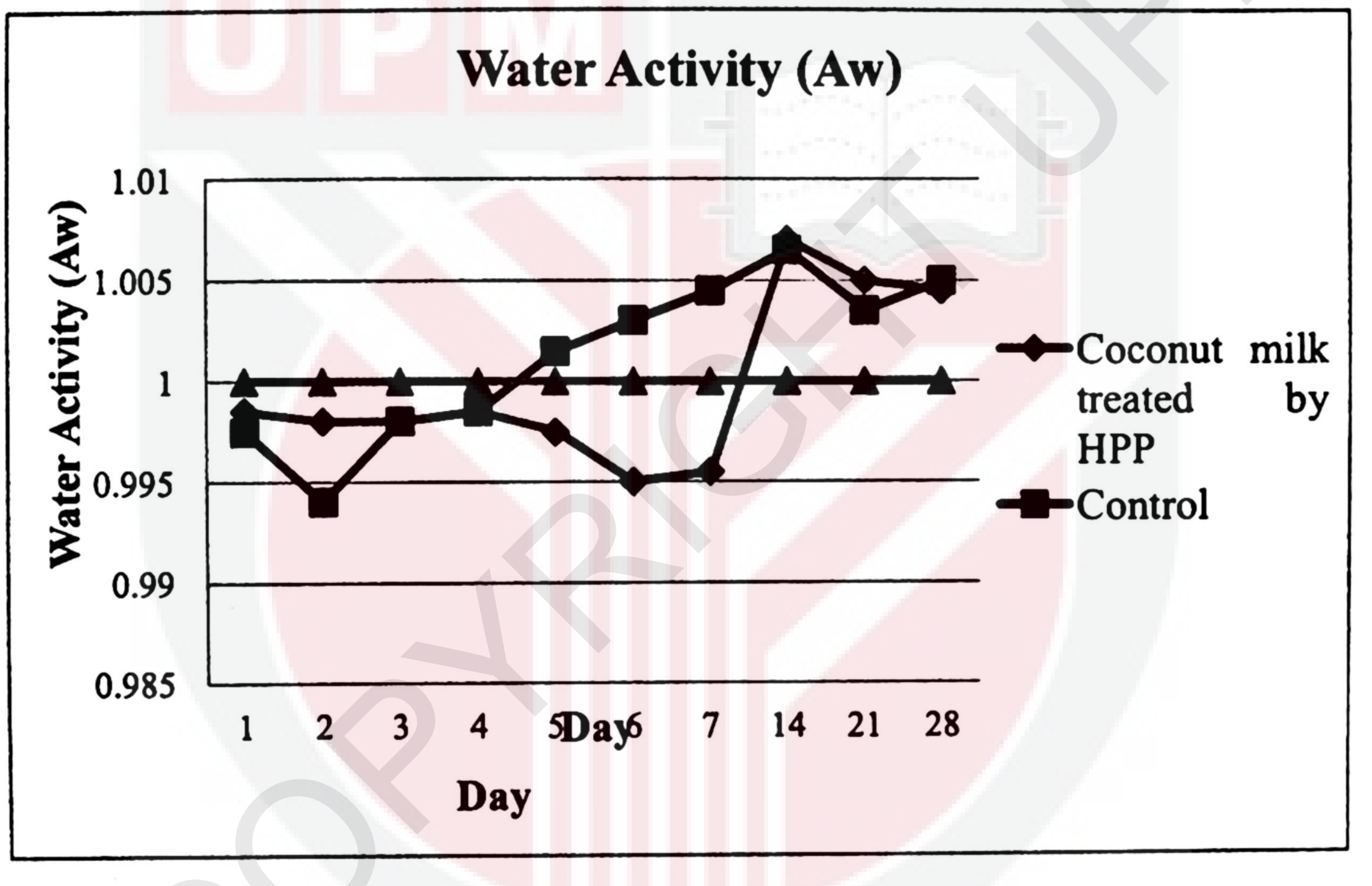

Figure 14: Water Activity of Sample A and Sample B for storage of 28 days.

For the coconut milk treated by HPP at 600MPa for 3 minutes, the water activity (aw) obtained from Day 1 until Day 7 lies between 0.995 to 0.998. On Day 14, the coconut milk treated by HPP at 600MPa for 3 minutes with water activity of 1.007, exceeded the maximum aw (1.00) which indicates the samples was spoiled and no longer safe to be consumed.

On the other hand, the water activity (aw) of coconut milk without any treatment (control) obtained from Day 1 until Day 4 lies between 0.995 to 0.998. During Day 5, the control with water activity of 1.0015 which exceeded maximum aw of 1.00, indicating the food was spoilt and not safe for consumption upon Day 4.

The coconut milk treated by HPP at 600MPa for 3 minutes has a longer shelf-life than the control as the coconut milk treated by HPP took longer time to reach the maximum water activity of 1.000 compare to the control which was spoilt after Day 4. This result shows that using HPP, it can preserve the coconut milk without additional of other substances especially chemical substance such as sodium benzoate, sodium nitrite and others. Treatment by HPP can inactivate vegetative microorganisms in the coconut milk.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

Nowadays, consumers are willing to purchase healthy foods with high prices due to their concerns on global aging issue and healthy lifestyles. This creates a great environment for the development of healthy foods.

In this research study, coconut milk was subjected to non-thermal technologies which is high hydrostatic pressure to demonstrate the effectiveness of this method to extend the storage life or preserve food quality and at the same time maintain the flavors, taste and nutritional value of food. With that, it is able to increase the target markets of the product.

The study concluded that HPP treatment is an effective non-thermal technology to preserve the coconut milk without additional of chemical substances. HPP treatment had slightly effects on physicochemical properties which were closed to fresh coconut milk.

In term of shelf-life extension, HHP treatment at 600 MPa for 3 minutes was an effective method to extend shelf life of coconut milk for storage at 4°C for more than 7 days. This shows that HPP treatment is an effective method in terms of microbial inactivation.

Overall, HPP is an appropriate method for preservation of the coconut milk with no further damage of flavor, tastes, or colors happens at a higher pressure, but there seems to be a higher reduction in both pathogens and activity of enzymes. Another reduction method such as adding antimicrobials, changes in pH, acidification or a minimal thermal treatment along with HPP is recommended if producer is trying to achieve an extended shelf life of coconut milk.

In short, future research to determine the aspects that produce the required chemical composition for a specific intention of the nutritional value should be conducted. For instant, in order to produce coconut milk enriched with specific chemical compounds, breeding studies can be carried out. Furthermore, future studies should be focused on any possible unknown solutes which contribute to its special biological effects although coconut milk is already well studied on its chemical content.

Furthermore, for future studies, there needs to be more testing in between the large gaps of pressures. There needs to be a test of 425, 450, and 475 MPa when trying to achieve the desired log reduction. This will save time and money if the machine can do the same log reductions and not have to get all the way up to 500 MPa and just use 450 MPa. More time selections need to be tested. Besides, testing should also be considered for viruses like Hepatitis A. There is a lot of research to be done in HPP, and benefits could be had for both the

producers and the consumers when it comes to new HPP products coming out on the market.

REFERENCES

A. Elbrhami (2016). A Comparative Study of the Effects of High Hydrostatic Pressure and Ultraviolet Light on Stability , Health Related Constituents and Quality Parameters of Tiger Nut Milk.

Alenezi, R., Baig, M. N., Santos, R. C. D., & Leeke, G. A. (n.d.). Continuous Flow Hydrolysis of Sunflower Oil Using Sub-critical Water.

Alyaqoubi, S., Abdullah, A., Samudi, M., Abdullah, N., Addai, Z. R., & Musa, K. H. (2015). Study of antioxidant activity and physicochemical properties of coconut milk (Pati santan) in Malaysia. *Journal of Chemical and Pharmaceutical Research*, *7*(4), 967–973.

Balasubramaniam, V. M., Farkas, D. & Turek, E. J. (2008). Preserving food through high pressure processing. *Food Technology*, 62(11): 33-38.

Buzrul, S. (2012). High hydrostatic pressure treatment of beer and wine: A review. Innovative Food Science and Emerging Technologies, 13, 1e12.

Eibaid, A. I. A., Farahat, F. H., Ali, E. M. Y., & Sukrab, A. M. A. (2017). Effect of Heat Treatments on Physico-chemical properties of Milk Samples, *6*(3), 40–46.

Ephraim Edem, V. (2016). Optimization of Coconut (<i>Cocos nucifera</i>) Milk Extraction Using Response Surface Methodology. *International Journal of Nutrition and Food Sciences*, *5*(6), 384. https://doi.org/10.11648/j.ijnfs.20160506.13

Extension of shelf life of coconut milk. (n.d.). *Research Report*, 3–6. https://doi.org/10.1016/j.foodchem.2006.02.032

Fellows, P. (2009). *Food processing technology: Principles and Practice* (Third Edition). Woodhead Publishing Limited. CRC Press LLC. P 241-249

Firatiligil-Durmus, E., and Evranuz, O. (2010). Response surface methodology for protein extraction, optimization of red pepper seed (*Capsicum frutescens*). *LWT-Food Science and Technology*, 43 (2): 226-231.

Gupta, K., Verma, M., Jain, P. and Jain, M. (2014). Process optimization for producing cowpea added instant Kheer Mix using Response Surface Methodology. *Journal of Nutritional Health and Food Engineering,* 1 (5): 30-39.

Hogan, E., Kelly, A.L. & Sun, D-W. (2005). High pressure processing of foods: an overview. *Emerging Technologies for Food Processing* (Sun Da Wen, ed.), 3-31. Academic Press.

Huang, H.-W., Wu, S.-J., Lu, J.-K., Shyu, Y.-T., & Wang, C.-Y. (2017). Current status and future trends of high-pressure processing in food industry. *Food Control*, *72*(12), 1–8. https://doi.org/10.1016/j.foodcont.2016.07.019

Insausti, K., Beriain, M., Purroy, A., Alberti, P., Gorraiz, C. and Alzueta, M. (2001). Shelf life of beef from local Spanish cattle breeds stored under modified atmosphere. Meat Science 57(3): 273-281.

Jusoh, J. M., Rashid, N. A. and Omar, Z. (2013). Effect of sterilization process on deterioration of bleachability index (DOBI) of crude palm oil (CPO) extracted from different degree of oil palm ripeness. *International Journal of Bioscience, Biochemistry and Bioinformatics,* 3 (4): 322-327.

Keefe, S. O., Williams, R., & Lukas, A. R. (2013). Use of High Pressure Processing to Reduce Foodborne Pathogens in Coconut Water, 69.

Khuenpet, K., Jittanit, W., Hongha, N., & Pairojkul, S. (2016). UHT Skim Coconut Milk Production and Its Quality. *SHS Web of Conferences*, *23*, 1–15. https://doi.org/10.1051/shsconf/20162303002

Koutchma, T. (2014a). Fundamentals of HPP Technology/ In-Container HPP Principle; *Adapting High Hydrostatic Pressure (HHP) for Food Processing Operations;* pp.5. Guelph, ON, Canada: Agriculture and Agri-Food Canada, Guelph Food Research Center.

Law, H. Y., Ong, C. I., Aziz, N. A., Taip, F. S., & Muda, N. (2009). Preliminary Work on Coconut Milk Fouling Deposits Study, (10).

M. Marina, A., & NurulAzizah, S. (2014). Use of Coconut Versus Dairy Milk Products in Malaysian Dishes: Comparison of Nutritional Composition and Sensory Evaluation. *Journal of Food and Nutrition Research*, *2*(4), 204–208. https://doi.org/10.12691/jfnr-2-4-12

Manivannan, A., Bhardwaj, R., Padmanabhan, S., Suneja, P., Hebbar, K. B., & Kanade, S. R. (2018). Biochemical and nutritional characterization of coconut (Cocos nucifera L.) haustorium. *Food Chemistry*, *238*, 153–159. https://doi.org/10.1016/j.foodchem.2016.10.127

Marketsandmarkets. (2013). HPP (high pressure processing) market by equipment type (orientation, vessel size), application (meat, seafood, beverage, fruit & vegetable), product type (meat & poultry, seafood, juice, ready meal, fruit & vegetable) & geography e Forecast to 2018. Report Code: FB 2151.

Min, S., Sastry, S.K., Balasubramanian, V.M. (2010). Compressibility and density of select liquid and solid food under pressure up to 700MPa. J Food Eng, 96(4), 568-574.

Mohd Thani, N., Mustapa Kamal, S. M., Taip, F. S., & Awang Biak, D. R. (2016). Evaluation on safety and sensory analysis of xylitol substituted dadih. *International Food Research Journal*, *23*(December), S203–S208.

Muhammed, N. (2017). Review in causes of viscosity in fluids, *6*(1), 117–123.

Naik, A., Venu, G. V, Prakash, M., Raghavarao, K. S. M. S., Naik, A., Venu, G. V, … Raghavarao, K. S. M. S. (2017). Dehydration of coconut skim milk and evaluation of functional properties. *CyTA – Journal of Food*, *12*(3), 227–234. https://doi.org/10.1080/19476337.2013.833296

Peamprasart, T., & Chiewchan, N. (2006). Effect of fat content and preheat treatment on the apparent viscosity of coconut milk after homogenization, *77*, 653–658. https://doi.org/10.1016/j.jfoodeng.2005.07.024

Patterson, M. F. (2005). A review: Microbiology of pressure-treated foods. Journal of Applied Microbiology 98 (6): 1400-1409.

Petruł, D., Colibar, O., & Crăiniceanu, E. (2008). ANALYSIS OF PROTEINS FROM SOYA PRODUCTS AND SUBPRODUCTS, *XLI*, 764–767.

Pichitvittayakarn, W., Narataruksa, P., Heggs, P. J., & Tia, S. (2006). Effects of fluid flowrate on coconut milk fouling at pasteurization temperature (70°C - 74.5°C). *Songklanakarin Journal of Science and Technology*, *28*(6), 1275–1288.

Raghavendra, S. N., & Raghavarao, K. S. M. S. (2010). Effect of different treatments for the destabilization of coconut milk emulsion. *Journal of Food Engineering*, *97*(3), 341–347. https://doi.org/10.1016/j.jfoodeng.2009.10.027

Rawson, A., Patras, A., Tiwari, B. K., Noci, F., Koutchma, T., & Brunton, N. (2005). Effect of thermal and non thermal processing technologies on the bioactive content of exotic fruits and their products: Review of recent advances. *Food Research International, 44,* 1875- 1887.

Sciences, S. (2004). Life and Social Sciences Sensory and Nutritional Evaluation of Coconut-Natural Milk Blend, *2*, 104–108.

Simonin, H., Duranton, F., & de Lamballerie, M. (2012). New insights into the high pressure processing of meat and meat products. Comprehensive Reviews in Food Science and Food Safety, 11, 285e306.

Simuang, J., Chiewchan, N., & Tansakul, A. (2004). Effects of fat content and temperature on the apparent viscosity of coconut milk. *Journal of Food Engineering*, *64*(2), 193–197. https://doi.org/10.1016/j.jfoodeng.2003.09.032

Sivapragasam, A. (2008). Coconut in Malaysia: Current developments and potential for re-vitalization. *2nd International Plantation Industry Conference and Exhibition (IPICEX2008)*, (November), 1–9.

Sporring, S., Bøwadt, S., Svensmark, B., & Bj, E. (2005). Comprehensive comparison of classic Soxhlet extraction with Soxtec extraction , ultrasonication extraction , supercritical fluid extraction , microwave assisted extraction and accelerated solvent extraction for the determination of polychlorinated bipheny, *1090*, 1–9. https://doi.org/10.1016/j.chroma.2005.07.008

U.S. Food and Drug Administration FDA. (2014). Kinetics of microbial inactivation for alternative food processing technologies -- High pressure processing. Science & Research (Food)- Safe practices for food processes.

Visiongain. (2015). The food high pressure processing (HPP) technologies market forecast 2015-2015: Pascalization & Bridgmanization.

Wehr, H. and Frank, J. (2004). Standard Methods for the Microbiological Examination of Dairy Products. 17th ed. Washington D.C: APHA Inc.

Yalegama, L. L. W. C., Ambigaipalan, P., & Arampath, P. C. (2008). Physico-chemical and shelf life evaluation of pasteurized coconut milk, (December 2008), 342–349.

Zemser, R. (2015). A clean label challenge for product developers. Food Processing. Mar. 10.

APPENDICES

A.1 PROXIMATE ANALYSIS

A.1. 1: Fat content in fresh coconut milk

	Sample	
	A	**B**
Weight of sample (g)	2.0136	2.0009
Weight of empty aluminium cup (g)	45.0980	44.8240
Weight of fat + aluminium cup (g)	45.5754	45.3079
Weight of fat (g)	0.4774	0.4839
Fat Content (%)	23.71	24.18

<u>For Sample A:</u>

$$\text{Fat Content (\%)} = \frac{\textbf{Weight of fat}}{\textbf{Weight of sample}} \times 100\%$$

$$= \frac{0.4774g}{2.0136g} \times 100\%$$

$$= 23.71\%$$

<u>For Sample B:</u>

$$\text{Fat Content (\%)} = \frac{\textbf{Weight of fat}}{\textbf{Weight of sample}} \times 100\%$$

$$= \frac{0.4839g}{2.0009g} \times 100\%$$

$$= 24.18\%$$

Therefore, the average fat content (%) = $\frac{23.71+24.18}{2}$ = **<u>23.95%</u>**

A.1. 2: Protein content in fresh coconut milk

	Sample	
	A	**B**
Protein Content (%)	1.4994	1.5663

Therefore, the average protein content (%) = $\frac{1.4994+1.5663}{2}$ = **<u>1.533%</u>**

A.1. 3: Moisture content in fresh coconut milk

	Sample	
	A	B
Weight of sample before drying (g)	5.0036	5.0042
Weight of empty crucible (g)	61.5663	51.7565
Weight of sample after drying + crucible (g)	63.0501	53.2648
Weight of sample after drying (g)	1.4838	1.5083
Moisture Content (%)	70.35	69.86

For Sample A:

$$\text{Moisture Content (\%)} = \frac{\text{Weight of sample before drying} - \text{Weight of sample after drying}}{\text{Weight of sample}} \times 100\%$$

$$= \frac{5.0036 - 1.48388g}{5.0036g} \times 100\%$$

$$= 70.35\%$$

For Sample B:

$$\text{Moisture Content (\%)} = \frac{\text{Weight of sample before drying} - \text{Weight of sample after drying}}{\text{Weight of sample}} \times 100\%$$

$$= \frac{5.0042 - 1.5083g}{5.0042g} \times 100\%$$

$$= 69.86\%$$

Therefore, the average moisture content (%) = $\frac{70.35+69.86}{2}$ = **70.11%**

A.1. 4: Ash content in fresh coconut milk

	Sample	
	A	B
Weight of sample (g)	5.0188	5.0578
Weight of empty crucible (g)	57.4838	63.3281
Weight of ash + crucible (g)	57.5155	63.3514
Weight of ash (g)	0.0317	0.0233
Ash Content (%)	0.63	0.46

For Sample A:

$$\text{Ash Content (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100\%$$

$$= \frac{0.0317g}{5.0188g} \times 100\%$$

= 0.63%

For Sample B:

$$\text{Ash Content (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100\%$$

$$= \frac{0.0233g}{5.0578g} \times 100\%$$

$$=0.46\%$$

Therefore, the average ash content (%) = $\frac{0.63+0.46}{2}$ = **0.55%**

A.1. 5: Carbohydrate content in fresh coconut milk

Carbohydrate content (%) = 100 % - fat content – protein content – moisture content – ash content

= (100 – 23.95 – 1.533 – 70.11 – 0.55) %

= **3.857 %**

A.2 PHYSICO-CHEMICAL PROPERTIES TESTS ON COCONUT TREATED BY HPP

Pressure (MPa)	Holding Time (minute)	pH	Brix (%)	Viscosity (cP)	Total Fat (%)	Free Fatty Acids (%)
Control		5.90	30.00	16.0	23.90	0.8611
400	1	5.66	36.25	25.0	26.86	1.0286
	3	5.64	36.50	16.4	22.44	1.1242
	5	5.67	36.20	18.6	20.40	1.1482
500	1	5.60	28.20	17.0	27.37	1.1242
	3	5.63	34.00	22.4	19.00	1.1482
	5	5.69	32.30	29.4	23.02	1.0405
600	1	5.64	28.40	18.4	23.12	1.1721
	3	5.66	22.80	17.5	26.29	1.2319
	5	5.67	27.70	20.0	28.43	1.3276

The free fatty acid (FFA) content is calculated by the following formula:

$$\text{Free fatty acids as lauric, \%} = \frac{\text{ml of alkali} \times N \times 20.0}{\text{weight of sample}}$$

Where: N of NaOH = 0.0598,
Weight of sample = 25g

A.3 SHELF LIFE STUDY

A.3. 1: Total Plate Count (TPC) - Number of organism per gram (CFU/g) of Sample A and Sample B for storage of 28 days.

Day	Number of organism per gram (CFU/g)	
	Samples	
	A	B
1	5.1×10^3	5.85×10^8
2	-	5.95×10^8
3	-	2.09×10^8
4	-	7.3×10^8
5	-	4.35×10^8
6	-	7.8×10^9
7	3.65×10^3	6.15×10^8
14	2.935×10^5	5.6×10^8
21	1.49×10^5	1.06×10^8
28	2.22×10^5	2.6×10^8

A.3. 2: Total Plate Count (TPC) - Number of organism per gram (log10 CFU/g) of Sample A and Sample B for storage of 28 days.

Day	Number of organism per gram (log10 CFU/g)	
	Samples	
	A	B
1	3.71	8.77
2	0	8.77
3	0	8.32
4	0	8.86
5	0	8.64
6	0	9.89
7	3.56	8.79
14	5.47	8.75
21	5.17	8.03
28	5.35	8.41

A.3. 3: Water Activity (Aw) of Sample A and Sample B for storage of 28 days.

Day	Water Activity (Aw)	
	Samples	
	A	B
1	0.9985	0.9975
2	0.998	0.994
3	0.998	0.998
4	0.9985	0.9985
5	0.9975	1.0015
6	0.995	1.003
7	0.9955	1.0045
14	1.007	1.0065
21	1.005	1.0035
28	1.0045	1.005

A.3. 4: pH of Sample A and Sample B for storage of 28 days.

Day	pH	
	Samples	
	A	B
1	6.09	5.92
2	6.24	5.54
3	6.22	5.02
4	5.58	4.61
5	5.57	4.58
6	5.48	4.44
7	5.42	4.3
14	5.59	4.48
21	5.32	4.2
28	5.23	3.98