UNIVERSITI PUTRA MALAYSIA

THE EFFECT OF HIGH PRESSURE PROCESSING AND THERMAL PROCESSING ON THE QUALITY OF SOYMILK

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ABSTRACT

Soymilk can be easily contaminated because of its nutritional profile which this can lead to the short shelf life. The objectives of this study are to investigate the change of quality, colour, viscosity, pH values, brix value also microbial count in soymilk samples which were stored in 0, 1, 5, 15, 20, 25 and 30 days at two conditions, room temperature and 4° C. In this study, the methods used in preservation of soymilk are thermal processing (60°C, 70°C, 80°C) and high-pressure processing (200 MPa, 400 MPa, 600 MPa). The result showed that the pH value decreased along with the storage time from 6.8 to 5.9 in HPP treatment. The viscosity increased from 21 to 33 Pa.s. In addition, the number of total bacteria increase from 4.0 to 8.0 log CFU/ml unit. HPP at 400 MPa, 5 min show ND while thermal at 70°C, 10 min not fully inactivate microbial population.

The study of preservation techniques in of soymilk is very important to improve the shelf life of the nutritious drink and to encourage people to take soymilk as another alternative to replace dairy milk.

ABSTRAK

Susu soya akan mudah tercemar kerana profil nutrisi di mana akan membawa kepada jangka hidup yang pendek. Objektif kajian adalah untuk menyiasat perubahan kualiti, warna, kelikatan, nilai pH, nilai brix dan juga bilangan mikrob dalam sampel susu soya di mana di simpan selama 0, 1, 5, 10, 15, 20, 25 dan 30 hari pada dua keadaan, suhu bilik dan 4°C. Dalam kajian ini, kaedah yang digunakan dalam pemeliharaan susu soya adalah rawatan haba (60°C, 70°C, 80°C) dan pemprosesan tekanan tinggi (200 MPa, 400 MPa, 600 MPa). Hasil menunjukkan bahawa, nilai pH berkurang dengan masa penyimpanan dari 6.8 ke 5.9. Kelikatan bertambah dari 21 ke 33 Pa.s. Tambahan pula, bilangan bakteria bertambah dari 4.0 ke 8.0 log CFU/ml unit. Pemprosesan tekanan tinggi pada 400MPa, 5 minit menunjukkan tidak dikesan manakala rawatan haba pada 70°C, 10 minit populasi mikrob tidak sepenuhnya tak aktif.

Kajian dalam teknik pemeliharaan susu soya adalah sangat penting untuk meningkatkan jangka hayat minuman yang berkhasiat and untuk menggalakkan manusia untuk mengambil susu soya sebagai salah satu alternative untuk menggantikan susu.

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CHAPTER 1 INTRODUCTION

1.1 Soybean

The soybean plant (Glycine max) belong to the legume family (Joseph, 2011). As with most of the plants, soybeans are grown from its seed. The soybean seeds normally are matured soybeans from its previous crop. The soybean is grown as one of the commercial crops over 35 countries as the major oilseed. The fruits of soybean are simple or alike the shape of crescent pod of the length of about 3-7 cm, including to 1 or 2 seeds which mass of 1000 seeds take out 115-280g. on the fodder designed the seeds in mass about 180-200g. Unripe seeds are green in color and mature seeds of modern cultivars have spherical shape, and the yellow and green color is the most desirable (Figure 1). The soybean products are used in food industry on whole world. (Teresa, 2004). It is able the nitrogen of the air through the action of bacteria on its roots. The protein content of the seed is about 40%. After the hulls and the oil are removed, the remaining defatted flake, which is the starting material for most commercial protein ingredients, has a protein content of approximately 50%. (Joseph, 2011).

Figure 1 Soybean plant

Farmers and animal feeders worldwide determine the economic growth of the soybean seed. These two components have benefitted from the expansion of soybean production, as people around the world strive to improve their diets. This is being accomplished through increase animal productivity and usage of soy protein directly in human consumption. Availability of large supplies of soy oil helped the food industry develop and market many few new food products. (Duane et. al., 2003).

However, there is no commercial cultivation of soybeans in Malaysia. Most of the food beans are brought via container primarily from Canada, the United States and China (Malaysia Oilseeds and Product Annual Report 2009). Import of whole soybeans to Southeast Asia has remained largely unchanged from 4.084 MMT in the year of 2001 and 2002 to 4.025 (million metric tons) MMT in year 2009 and 2010. The countries with a largest soybean import in year 2009 and 2010 in MMT are Thailand 1.85, Indonesia 1.60, Malaysia 0.61, Vietnam 0.22, Philippines 0.125 and Singapore 0.02. (William et. al., 2010).

1.2 Soymilk

Soymilk is a traditional Asian food product, which is more and more consumed in Western countries as well. The increasing consumption of soymilk is probably related to the consumer interest in the positive health aspects of soy protein products. In 1999, the FDA approved that the use of soy protein health claim in the United States stating the consumption of 25g of soy protein a day as part of a diet low in saturated fat and cholesterol may reduce the risk of heart disease (Kin-Char Kwok et.al, 2005). It is a soy-based drinks that constitute one of the shining stars of the food industry with 31% growth between 2003 and 2004 (Sloan 2005).

Soymilks productions have several thermal treatments which can negatively affect its nutritional quality, color and sensory attributes (Kwok and Niranjan, 1995) and modify isoflavone distribution. Isoflavone functions in lowering cholesterol levels, preventing both prostate and breast cancers and attenuating bone loss in postmenopausal women, and alleviating menopausal symptom (Hendrich & Murphy, 2007; Liu 2004). Daidzin, genistin and glycitin are the three isoflavone glucosides existing in soybeans and soy-based food.

During the past few decades' alternative food preservation methods have been developed, inspired by the desire to produce more fresh-like product that meet the microbial safety status of pasteurized or sterilized foods. Thermal processing is known as a reliable method for maintaining the physio-chemical properties and modification in food. But, the use of heat can destroy nutrients such as heat labile vitamins and also component related to product flavor and taste (Deliza et. al. 2005). As the technical and scientific knowledge about the technology improved significantly, high pressure processing (HPP) is one of the techniques that most significant alternative to thermal food processing. It can be applied for the inactivation of microorganisms, enzymes and for the unfolding of proteins. It is also unaffected and minimally altered in food characteristic such as flavor, texture, color and nutrients. High pressure processing (HPP), newly developed food technology could be an alternative to thermal processing for soymilk production (Kajiyama, et.al, 1995; Lakshmanan, et.al, 2005). The unique effects of HPP are due to the effect of pressure on non-covalent bonds while leaving the covalent bonds of the food intact, in contrast to the changes occurring during thermal processing. HPP food preservation technique could have a large food industry impact due to the benefits it can bring to the products if these benefits are well communicated with consumers.

1.3 Problem Statement

Soymilk is a liquid extract, obtained by mixing and grounding soybeans with water, resulting in a product with a similar appearance to cow milk, although with different composition. Consumers also demand high quality food products, which mean that they have guarantee good nutritional quality, long shelf life and high colloidal stability. Hence, physic-chemical properties like color, rheological properties, microbial activity and storage are important quality characteristics of fruits and vegetables and major factors affecting sensory perception and consumer acceptance of foods. Various processing methods are used not only to increase the edibility and palatability of foods but also to prolog their shelf life.

Currently, thermal treatment is commonly used in food industry to ensure food safety and extended shelf life and inactivates undesirable biologically active compound (Liener 1981; Kumar and others 2003). However, indicated that thermal treatment adversely affects the quality of soymilk, including its flavor, color and in some cases nutritional quality (Kwok and Niranjan 1995; Chauhan and others 1998; Sancho and others 1999). Therefore, high pressure processing (HPP) has been a great interest to researches and food companies as alternative to thermal processing whereas it has potential to retain food nutritional content by preserving vitamins, and to inactive vegetative microorganisms and some spoilage enzymes while either maintaining fresh-product properties or providing products with unique texture and functional properties (Estrada-Giron and others 2005).

1.4 Objectives

The focus of this research is to investigate the effectiveness of thermal treatment and high-pressure processing on soymilk. The specific objectives are;

1) To compare the effect between the thermal and high-pressure processing in quality of soymilk and microbiological safety of soymilk after both treatment.
2) To determine the storage effect on soymilk at room temperature and 4 °C.

1.5 Significant of Study

Nowadays, research in soymilk has been varies in order to produce a milk that have similar appearance with cow milk although with different composition. The important of this research is for vegetarian people that avoid animal's milk as well as people who allergic with dairy product or lactose tolerant, soymilk is a good alternative. Moreover, one of the problem that have been face by companies is producing the fresh and health food that meet the microbial safety status of pasteurized and sterilized food. It is crucial to study the effect of high pressure processing in production of soymilk that can be applied to the industries. High pressure processing can be an opportunity to the non-thermal process, hence producing a fresh-like product with premium quality (Norton & Sun 2008).

CHAPTER 2 LITERATURE REVIEW

In this chapter, it focuses on literature review of the past studies that have been done by the other researchers in order to provide the foundation background and basic for the research. It shows that theories supporting the research and served as guidance.

2.1 Soy Milk from Soybeans

Soybeans are greatly valued as a source of oil and high-quality protein meal. Soy derived products on market have been increasing steadily, ranging from soy ingredients (soy flour, concentrate, isolate and textured protein) to soy foods (soymilk, tofu, tofu spreads, soy butter, candy bars, yogurt and frozen dessert). Soybeans is the most important legume in the traditional oriental countries' diet that can provide inexpensive high-quality proteins and essential amino acid particularly lysine. It is also rich in unsaturated fatty acid and isoflavones where containing no cholesterol and lactose. However, disagreeable beany flavour and indigestible oligosaccharides such as raffinose limited the consumption of soybean and food products.

Soymilk is the liquid extract of the soybean that can be used in the preparation of tofu, or as a nutritious beverage. Jooyandeh, 2011 stated that the utilization of soybean is very important as it is widely used for high cholesterol, high blood pressure and preventing diseases of the heart and blood vessels. It is also used for type 2 diabetes, asthma, lung cancer, endometrial cancer, prostate cancer and thyroid cancer, as well as preventing weak bones (osteoporosis), and slowing the progression of kidney disease.

During the fermentation process, useful active substances and pleasant flavour are released through metabolic processes of microorganisms, which further provide additional health benefits. The characteristic aroma and flavour of fermented soybeans food are partially generated by lactic acid bacteria (LAB) (Chou and Hou 2000). LAB play an important role as natural preservatives and flavour enhancer because of the organic acid including lactic acid and acetic acid that been produced by bacteria. Soymilk, the water extract from soybeans is considered as a colloid which contains particulate protein, water, soluble protein and minerals. Soymilk is usually used as a suitable culture medium for the growth and biochemical activities of various LAB (Fanworth et. al. 2007; Tran and Rousseau, 2013). Soymilk fermented by LAB also provides texture properties of soymilk and modulated and improved, such as water holding and apparent viscosity (Champagne, Green-Johnson, Raymond, Barrette & Buckley 2009; Yeo & Liong 2010).

Soymilk is the highest consumed product among soy foods, is the aqueous suspension of soluble solids extracted from ground, soaked beans (Munoz and Florez 1998). The traditional soymilk process has been extensively improved leading to the elimination of the beany flavour, which has facilitated its emergence in the western market (Wang et. al. 2001; Huang et. al. 2004). Soymilk is composed of 94% moisture, 3.0% protein, 1.5% fat, 1.5% carbohydrate and ash (de Man et. al. 1987). The conventional process of making soymilk involves using intense heat for the extraction of soybeans and to destroy trypsin inhibitor. But, it denatures protein, degradation of amino acid and other deteriorative reactions. Moreover, certain flavours, colors, vitamins and nutrients can also be affected by heat treatment (Adam 1991). Therefore, by decreasing thermal processing can avoid quality loss of soymilk.

2.2 Content of Soymilk

Soy-based beverages provide an alternative protein source for consumers who have given up part or all of the consumption of animal-based proteins. In 1999, the Food and Drug Administration allowed food suppliers to advertise soy-based foods with a heart-healthy claim after studies showed that eating soy might help lower the risk of heart disease. Soymilk is essentially a water extract of soybeans and it is a high protein, iron-rich milky liquid produced from pressing ground, cooked soybeans. Not only is it higher in protein and iron content, but it is cholesterol-free, low fat and low sodium. The basic steps of preparation of soymilk are: selection of soybeans, adding water, wet grinding, extraction and separation of soymilk from fiber (okara), cooking to inactivate lipoxygenase and trypsin inhibitors, formulation and fortification and packaging of the soymilk. Nowadays, food industries try to commercialize their soymilk products by further processing of soymilk into soy-based dairy alternative. Beside beverages, the soy-based dairy product produce by further processing of soymilk extraction are tofu, cheese, dessert, yogurt, ice cream and so on.

Soymilk is a stable emulsion of oil, water and protein. Soymilk is a good source of protein, vitamin A, vitamin B12, potassium, isoflavones, calcium and vitamin D (Table 1). Also, a source of protein because it contains no lactose. Lactose is disaccharide sugar composed of galactose and glucose that is found in milk. Moreover, it is naturally free of cholesterol and low in saturated fat because it comes from plants, isoflavones which is a class of phytochemical, which are compound found from a plant. It is always found in soybeans, chick peas and other legumes. According to the U.S. Food and Drug's administration definition, 8 glass of soymilk contains 7 to 12 grams of protein. This is because, unlike the most other plant-based protein, soy contains all the essential amino acid that making it a complete source of nutrients as cow's milk substitute or on its own. Soymilk and cow's milk provide similar amount of protein but soymilk contain 3 grams of fiber that has fewer calories, less fat and cholesterol. It fortified with nutrients and provide alternative to cow's milk for those who want to avoid animal's protein.

Other than that, soymilk also contains oligosaccharides which is a saccharide polymer containing a small number of simple sugar (monosaccharide). However, the oligosaccharide contains in soymilk such as raffinose and stachyose cannot digest in human body and can cause flatulence. Soymilk is also good source of vitamins and contains 7.36 and 0.33 mg/100 ml of riboflavin and thiamin, respectively (Hou and others 2000).

Besides these favorable features, soybeans are also known to contain antinutritional factors such as trypsin inhibitors, lectin and phytate. The presence of trypsin inhibitor in animal feed has been associated with growth suppression and pancreatic hypertrophy. On the other hand, trypsin inhibitor has been reported to have an anticarcinogenic effect. Although the nutritional effect of trypsin inhibitor in human body are not fully clear, reduction of trypsin inhibitor such as heat and fractionation are generally applied to soy products. Soybeans contain two types of trypsin inhibitor which is Kunitz trypsin inhibitor (KTI) and the Bowman-Birk inhibitor (BBI). Both of these inhibitors are rather heat stable, which is caused by the presence of the several disulphide bridges. Soybean trypsin inhibitors required long treatment times that can affect other properties of soymilk such as the taste due to the browning reactions, protein quality as a result of amino reactions and vitamin content. According to Kwok and co-workers, to reduce the heat treatment times and heat damage, soymilk can be treated by using ultra high temperature (UHT) that can reduce trypsin inhibitor by 90%. This treatment can retain thiamin, one of the vitamins in soymilk at 90% and minimizes sensory deterioration.

Table 1 Nutrition facts of soymilk per 100g

Nutrition	% Daily value
Total fat 1.8 g	2%
Saturated fat 0.2 g	1%
Polyunsaturated fat 1 g	
Monounsaturated fat 0.4 g	
Cholesterol 0 mg	0%
Sodium 51 mg	2%
Potassium 118 mg	3%
Total carbohydrate 6 g	2%
Dietary fiber 0.6 g	2%
Sugar 4 g	
Protein 3.3 g	
Vitamin A 0%	Vitamin C 0%
Calcium 2%	Iron 3%
Vitamin D 0%	Vitamin B-6 5%
Vitamin B-12 0%	Magnesium 6%

2.3 Thermal Processing of Soymilk

Heating condition are the most important variables in the processing of soymilk. It occurs during extraction, cooking and subsequent pasteurization or sterilization that can influence the yields and nutritive quality of solids and proteins, destruction of spoilage microorganisms and the colour and flavour of the milk. Excess heating generally can destroy the amino acid and vitamins, browning and the development of cooked flavour. In addition to the heating process mentioned above, in commercial soymilk production, soymilk is further heated before or after packaging to extend its shelf life. Three basic types of heat treatment are carried out; pasteurization, in container sterilization and ultra-high temperature treatment.

Pasteurization is referring to the heat treatment that normally below 100 °C., which is just adequate to destroy pathogenic microorganisms. This process also inactivates some enzyme and greatly reduces the total microbial count. For destroying pathogenic microorganisms, time and temperature combination used for dairy milk typically is 75 °C for 15s. It also can use for pasteurized soymilk. Pasteurized soymilk in Pure Pak or bottle containers requires refrigeration at 4 °C to maintain shelf life about 1 week. In container sterilization is a severe heat treatment which aims to kill all microorganisms. In container sterilization of soymilk requires heating 121 °C for 15-20 min to achieve commercial sterility, which may be defined as that degree of sterility at which all pathogenic and toxin-forming organisms have been destroyed. Commercially sterilization of soymilk in tin or glass bottle is shelf stable without refrigeration. UHT processing requires high temperature (135-150 °C) for short time (a few seconds) to achieve a product which is commercially sterile. The process is designed to inflict maximum lethal effect on bacteria but at the same time cause only those chemical changes which are necessary. UHT processing of soymilk can be done by using direct or indirect heating. Direct heating involves direct contact of soymilk and steam. Indirect heating carried out in plate or tubular heat exchanger.

Thermal treatment is a conventional processing method used to kill or inactive microbiological contaminants (Ramasvamy, 2006). It is also intended to inactive significant enzymes with respect of quality and vegetative forms of microorganisms that can be found in food products. However, thermal pasteurization has some disadvantages due to its thermal energy and processing time.

Thermal processing of soymilk reduces or eliminates the microbial flora and extends its shelf life. Proper heat treatment of soymilk also improves the nutritive quality by destroying various anti nutritional factor and modifying protein, permitting more complete digestibility and utilization of the growth limiting Sulphur containing amino acid. Heating also eliminate flavors by inactivating lipoxygenase in beans before or during grinding with water (Liener 1981; Kumar and others 2003). Excess heating generally can destruct the vitamins and amino acid, browning and development of the foods (Sancho and others 1999).

2.4 High Pressure Processing (HPP)

High pressure processing is a cold pasteurization technique by which product, already sealed in its final package, are introduced into a vessel and subjected to a high level of isostatic pressure. The objective of this method is to maintain its freshness throughout the shelf life. HPP treatment of foods can be used to create new products (new texture or new taste) or to obtain analogue products with minimal effect on flavor, color and nutritional value and without any thermal degradation. It has potential for food preservation purposes because it can inactivate microorganisms and enzymes. The ability of high pressure to inactivate microorganisms and food quality enzymes and to leave other quality attributes intact has encouraged Japanese food industries and also American Food Company to introduce high pressure preserved food on market (Oey, et.al, 2005).

This non-thermal food processing has the potential to retain food nutritional content by preserving vitamins, and to inactivate vegetative microorganisms and some spoilage enzyme while either maintaining fresh product properties or providing products with unique texture and functional properties (Estrada Giron and others 2005). Furthermore, high pressure processing could preserve nutritional value (Oey, et.al, 2007) and the delicate sensory properties of fruits and vegetables due to its limited effect on the covalent bonds of low molecular mass compounds such as colour and flavour compounds. During high pressure processing, different pressure and temperature combination can be used to achieve desired effects on texture, colour and flavour of foods. The quality of the HP processed food can be change during storage due to coexisting chemical reaction such as oxidation, and biochemical reaction when endogenous enzymes or microorganisms are incompletely inactivated.

An advantage of this new technology, the food quality characteristic such as flavour and vitamins are unaffected or only minimally altered by high pressure processing at room temperature. On the other hand, enzyme related to food quality can be deactivated by pressure. The pressure needed strongly depend on the enzyme. Some enzyme can be deactivated in room temperature by a few hundred mPa while others can withstand 1000 mPa. Because of that, the combination of pressure and temperature might be necessary for enzyme inactivation. However, in few cases, enzyme activation due to the pressure treatment alone has been observed.

The ability of high pressure processing to inactivated microorganisms and food quality enzyme and to leave other quality attributes intact has encouraged Japanese and American food companies to introduce high pressure preserved food in market. In addition to food preservation, high pressure processing opens up the possibility of producing foods with novel texture. The modification of food protein functionality by high pressure has recently been reviewed by Messens et. al. 1997.

2.5 The Effect of Physio-Chemical on Soymilk

Colour, flavour and texture are important characteristic of fruits and vegetables and major affecting sensory perception and consumer acceptance of foods. Various processing methods are used not only to increase the edibility and palatability of fruits and vegetables but also to prolong shelf life while the original sensory and nutritional properties are maintained as high as possible within the constraints put forward by microbial safety.

2.5.1 Color Measurement

Colour measurement systems are used to measure a broad range of food products. These include fresh and processed fruits and vegetables, formulated foods, dairy products, meat products, spices and flavours, cereals and grains, oils, syrups, sugar, and beverages. Visible light is found between 380 and 780 nm in the electromagnetic spectrum. It is bordered by ultraviolet light on the low end and infrared light on the upper end. When light strikes an object, it is reflected, absorbed, or transmitted. Because reflected light determines the colour of a material, the appearance can change depending on amount of light, the light source, the observer's angle of view, size, and background differences.

The HunterLab L*, a*, b* and the modified CIE system called CIELAB colour scales are opponent-type systems commonly used in the food industry. The systems measure the degree of lightness (L), the degree of redness or greenness (+/-a), and the degree of yellowness or blueness (+/-b). Every colour has three qualities or attributes: hue, value, and chroma. The color is then identified by its hue, value, and chroma.

In thermal processing, the colour changes mainly due to the Maillard reaction that occurs in soymilk following heat treatment. Quantitative evaluation of brown compound may be considered an indicator of the severity of heat treatments (Mauron, 1981). Van Buren et. al., 1964 used the degree of browning as a quality index to estimate protein damage in soymilk. As mentioned earlier, they measured the L* value of soymilks processed at 93° C and 121° C for various lengths of time and soymilk spray-dried at different drying temperatures. The L* values decreased within the time heating indicating that browning of the soymilk was induced by heat (Table 2). The color change in the soymilk obtained was influenced by the heat effects as well as by the yield of solids in the soymilk fraction, which has been shown to be temperature and time dependent (Johnson et. al., 1981). Therefore, the data obtained by these investigators do not represent the effect of heat alone, and the kinetics of colour change is complicated by the change of the solids yield in the heating process.

Table 2 Browning of soymilk heated at 93 °C and 121 °C

Temperature (°C)	Time heated (min)	ΔL value	Reference
93	0	83	Van Buren et. al. 1964
	30	78	
	60	78	
	120	77	
	240	73	
	360	74	
121	0	70	
	5	70	
	10	70	
	20	62	
	41	62	
	64	57	
	121	52	

Effect of high pressure generally marked as retention of colour, flavour and fresh appearance as a result of the mild processing character. Colour and flavour deterioration of pressure process food during storage has been attributed to residual enzyme activity (Cano, et.al, 1997). During storage, enzymes such as peroxidase can induce negative changes in colour and flavour of vegetables. Peroxidase is a heat stable enzyme (Guenes & Bayindirli, 1993) as well as a pressure stable enzyme in green beans (Knorr, 1995). Based on Table 3, The L* values obtained for our control soymilk at range 81 to 84, which agreed with the result reported by Kwok and Niranjan (1995) for untreated soymilk. Overall there was no drastic change in lightness (L*), redness (a*), and yellowness (b*) of the HPP and heat treatment samples when compare to control sample. Decreasing of L* values can occur due to the formation of brown pigment as a result of the Maillard reaction and was observed when the soymilk was heated from 80 to 140 °C for 0.5 to 180 min (Kwok and others 1999). High pressure treatment at ambient and moderate temperatures results in limited colour changes of green vegetables. In many cases, the green colour of vegetables becomes even more intense (decrease in L*, a* and b* values). This might be caused by cell disruption during high pressure treatment resulting in the leakage of chlorophyll into the intercellular space yielding a more intense bright green colour on the vegetable surface. However, at elevated temperature, the green colour shifted visibly to olive-green with a concomitant increase in the a* value. (Krebbers, et.al, 2002).

Table 3 Effect of high pressure processing on soymilk in color properties

pH	Treatment	L*	a*	b*	ΔE
6.0	Control	83.6	-1.5	17.8	
	500 MPa	83.5	-1.5	17.8	0.2
	600 MPa	82.8	-1.7	17.2	1.1
	Heat treatment	83.3	-1.5	15.9	1.9
7.0	Control	81.5	-1.8	17.3	
	500 MPa	83.1	-1.6	16.1	2.0
	600 MPa	82.9	-1.8	15.9	2.0
	Heat treatment	82.7	-1.7	16.2	1.6

2.5.2 Rheological Properties (Viscosity)

Viscosity is an important property of fluid foods. It is defined as the internal friction of a liquid or its ability to resist flow. The internal friction in a fluid can be easily demonstrated by observing a liquid that has been vigorously stirred to create a vortex. Once the stirring has stopped the speed of the vortex is gradually reduced and rotation of the liquid eventually stops. This happens as a result of the frictional force within the liquid and this force has to be overcome in order for the liquid to flow. Viscosity is also a characteristic of the texture of food. This means that the viscosity of a product must be controlled and measured in production so that each batch is consistent from day to day.

Table 4 shows the viscosities of soymilk heated at different conditions. Comparing to one step heating and two step heating significantly increase the soymilk viscosity. The soymilk viscosity was not significantly affected by one step heating time up to 10 min. The thermal treatment of soymilk causes protein to unfold as a result of denaturation and formation of aggregates through disulphide bond and hydrophobic interaction, which results in higher water binding capacity (Wagner and others 1992). When heat treatment was applied, the changes in shape and interactions between particles did not promote as much water binding that can increased viscosity.

With regard to the potential commercialization of pressurized soymilk, its visual appearance characteristic is viscosity that paramount importance. In HP treatment, based on Ramamoorthi, et.al. (2006), the viscosity decreased with the increasing of the pressure treatment.

Table 4 Effect of heating method on soymilk viscosity physical properties

Heating method	Heating condition	Soymilk viscosity	Reference
One-step	95 °C, 5 min	38±2.1	Liu et.al. (2004)
	95 °C, 7 min	38±2.5	
	95 °C, 10 min	38±1.4	
Two-step	75 °C, 5 min	96±2.8	
	95 °C, 5 min		

The apparent viscosity of the untreated soymilk did not vary with pH (Table 5). This result is unexpected because when the pH of protein is increasing from the isoelectric point to higher basic value, the number of negative charged group COO- gradually increase, which promotes the electrostatic repulsion between proteins. This change in protein charge balance can cause the opening of the protein that binds more water molecules, increasing the viscosity. A lack of difference in viscosity after pH adjustment was previously observed by Bau and others (1985).

Table 5 Apparent viscosity, n and K value of high pressure processing

Ph	Treatment	Viscosity	n value	K value	Reference
6.0	500 mPa	7.9±0.2	0.49±0.1	428.4±21.1	Ramamoorthi,
	600 mPa	7.6±2.1	0.45±0.0	454.3±24.6	et. al. (2006)
7.0	500 mPa	2.2±0.1	0.91±0.0	4.0±0.0	
	600 mPa	2.2±0.1	0.92±0.0	3.9±0.8	

2.5.2 Microbiological Activity

Food spoilage may be defined as a process or change which renders a product undesirable or unacceptable for consumption. This complex ecological phenomenon is the outcome of the biochemical activity of microbial chemical processes which will eventually dominate according to the prevailing ecological determinants. To ensure the safety and quality of foods and beverages, the effective monitoring of the chill chain through production, transportation, distribution and storage in retail cabinets and home refrigerators is essential. Currently, a variety of different methodologies are used for assessing food spoilage, in which microbiological methods play a decisive role. Recently, the relationship between microbial growth and the chemical changes occurring during food storage has been recognized as a potential indicator which may be useful for monitoring freshness and safety. For this purpose, interesting analytical approaches have been developed for rapid and quantitative assessment of food spoilage.

Soymilk is an excellent growth medium for many kinds of microorganisms, being high in moisture, nearly neutral in pH and rich in nitrogenous compounds, fat, sugar, minerals and vitamins. Yan (1927) reported that organisms that proliferated in soymilk were acid formers, gas formers and putrefies. At room temperature, soymilk undergoes acid curdling with a rapid drop in pH accompanied by separations of curd and whey. Thus, type of spoilage usually occurs after standing for 24 hours. (Lo et. al.1968).

Table 6 Effect of heating method on soymilk microbial activity

Process	Temperature	Time	Storage	Result	Reference
Pasteurization soymilk	60 ℃	30 min	3 days	No significant spoilage	Tan (1958)
	120 ℃	5 min	1 year		
Mesophilic anaerobic bacteria	121 ℃	3 min		Produce sterility	Bouno et. al. (1989)
	121 ℃	4-5 min		Sterility with additional safety factor	

Based on the Table 6, Tan (1958) studied on the effect of pasteurization and sterilization. Bouno et. al. (1989) investigated the effect of different heat treatments on the destruction of indigenous Bacillus spores in soymilk which was used as a substrate for soy yogurt fermentation. Sterilization requires a more severe heat treatment which practically destroyed all microorganisms, including the spores of mesophilic anaerobic bacteria such as Clostridium botulinum. It was found that heating in an agitated retort at

121 °C for 3 min was adequate to produce sterility. However, they recommended a heat treatment for 4-5 min at 121 °C, which would result in sterility with an additional safety factor that can improved the protein efficiency ratio of the soymilk. In addition of the mesophilic spore-forming bacteria, there are also thermophilic spore-forming organisms which are extremely heat resistance. It is designated to kill all thermophilic spore-forming bacteria may also result in a product overheated and degraded in nutritional and sensory qualities. This thermophilic spore may still survive after commercial sterilization process, but they do not multiply and cause spoilage if the product is stored at room temperature or lower.

2.6 Storage of Soymilk

Soy products are well appreciated for their nutritional and potential health benefits. Soy beverages consumption is increasing among North American consumers because of the improvements in soy beverages quality and processing technologies. It could be an excellent carrier for functional and nutritive ingredients such as vitamins, minerals and omega 3. However, addition of such ingredients may affect the stability of the product and required high storage stability. In storage of soymilk, we are investigating the effect of soymilk in colour and viscosity of soymilk in three different temperatures.

The colour of the soymilk decreased significantly during the first month of the storage and then remained stable over time (Figure 2). Colour and the viscosity of the soymilk were affected by both the soymilk composition and soymilk treatment. In general, the lower the ΔE, the whiter the colour of the soymilk sample (A. Achouri, et.al, 2007).

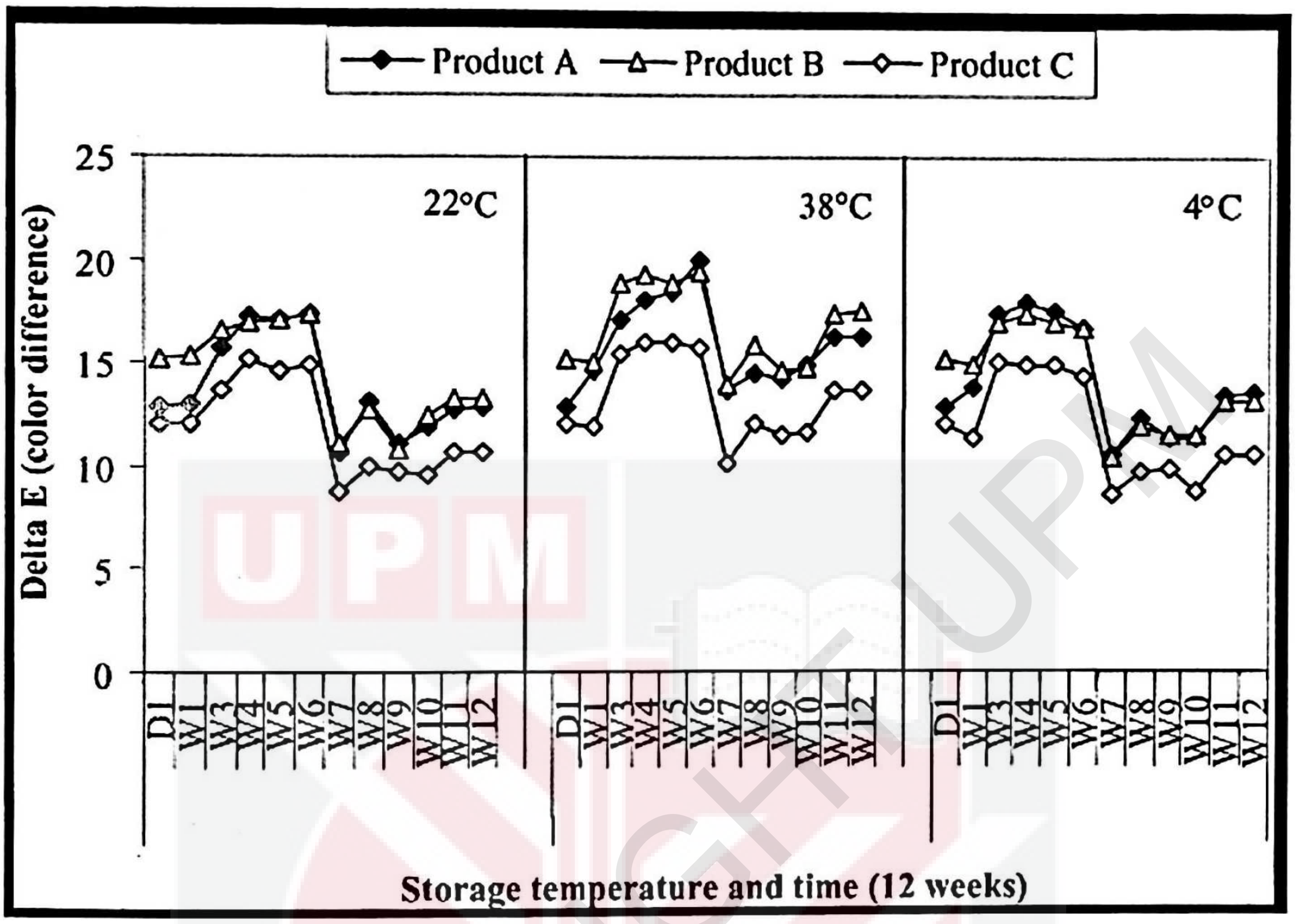

Figure 2 Color measurement of the formulated soymilk products in different temperature

HP treatment (at low and moderate temperature) has a limited effect on pigments (e.g.; chlorophyll, carotenoids, etc.) responsible for the colour of fruits and vegetables. The colour compounds of HP processing fruits and vegetables can, however, change during the storage due to incomplete inactivation of enzymes and microorganisms, which can result in undesired chemical reaction (both enzymatic and non-enzymatic) in the food matrix. Matser, et.al, 2004 also reported that the chlorophyll degradation of green beans and spinach due to the HP processing at elevated temperatures, even for a short exposure time (two pulses of 90°C/ 700 MPa/ 1 min).

Soymilk viscosity

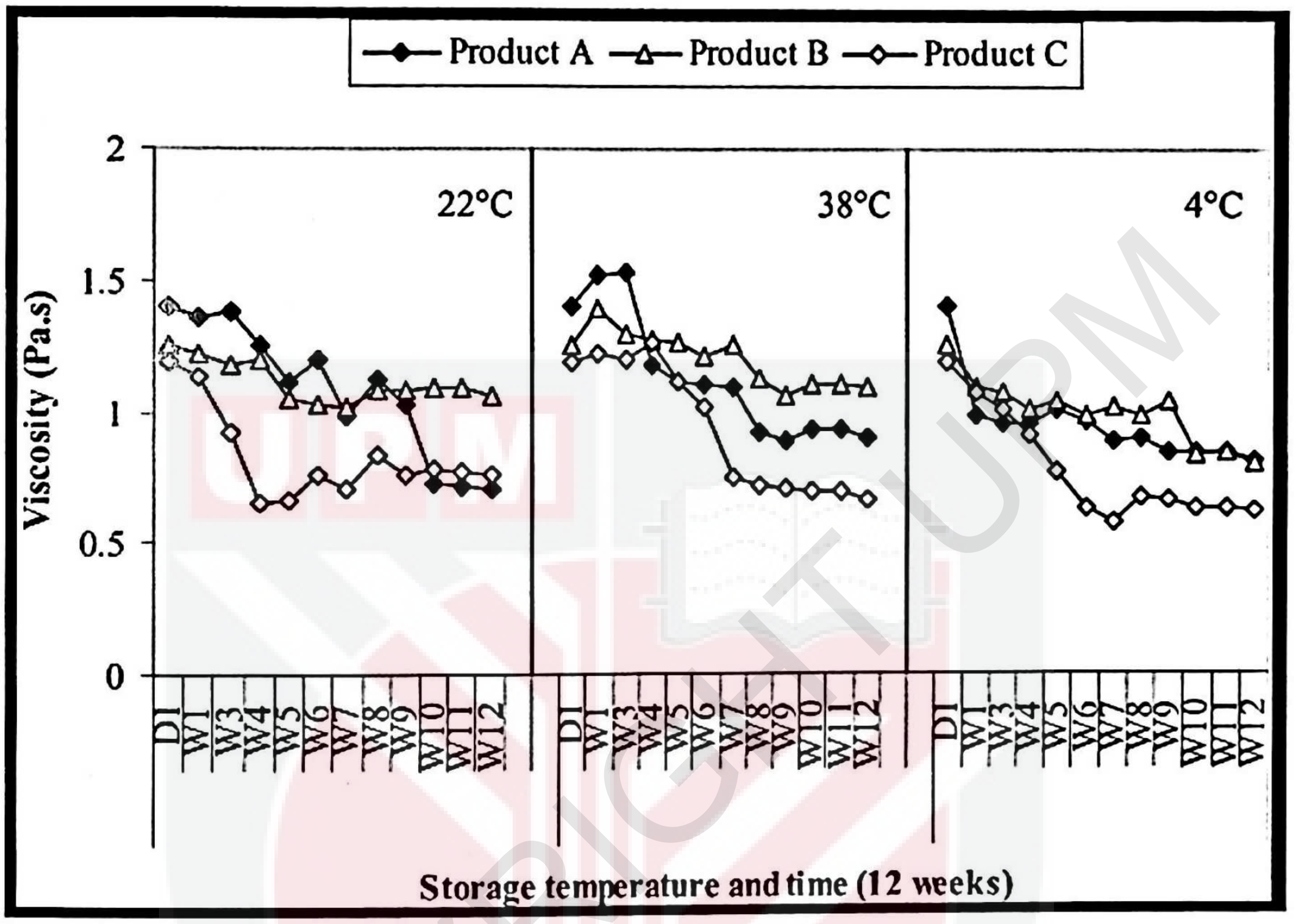

Figure 3 Change in viscosity of soymilk product in different temperature

Changes in the viscosities of the soymilk products (A, B, C) during storage are shown in Figure 3. All the soymilk products showed a decreasing trend in viscosity with the increasing storage time. Variation in viscosities were also observed between the soymilk products, where products A and C consistently showed the highest initial viscosity compared with products B. Beyond the seventh week of storage, the viscosity of product C (high fat, high starch) remained stable. The sweetened soymilk sample (product B) seemed to be the most stable over time. This stability may have been due to the formation of stronger network structures as a result of the sugar-protein interactions, and merits further investigation.

The result further showed that among three different temperature treatments, storage at 38°C resulted in an initial increase in viscosities of the three soymilk products during the first three weeks. Beyond this point, their viscosities decreased and remained stable after 8 weeks of storage. The denaturation of protein by heating could have increased the surface hydrophobicity and exposed more sites for hydrophobic interactions with other soymilk components, which in turn may have increased the viscosity (Sorgentini et. al., 1995).

Microorganism in Soymilk

Tan 1958 studied the effect of pasteurization and sterilization on soymilk. Pasteurized soymilk, heated for 30 min at 60° C, could be stored for 3 days with no significant spoilage. heating for 5 min at 120°C was adequate for one-year storage. Buono et. al., 1989 investigated that the effect of different heat treatments on the destruction on indigenous Bacillus spores in soymilk which was used as a substrate for soy yogurt fermentation. They found that the microbial load in raw soymilk was reduced from 2.2 X 10^3 colony forming units (CFU) ml^{-1} to 33 CFU ml^{-1} after boiling for 1 min in a microwave oven; while no organisms were detected in soymilk after autoclaving at 121° C for 15 min. Evidently, sterilization requires a more severe heat treatment which practically destroy all microorganisms, including the spores of mesophilic aerobic bacteria such as Clostridium Botulinum and Clostridium Sporogenes.

CHAPTER 3: METHODOLOGY

This chapter explains in details on how this project was carried out. In this section present the methods used to produce soymilk and all the psycho-chemical test with all the equipment used in this research. Then there was brief explanation about the method and technique used to analyze all the food samples.

3.1 Preparation of Soymilk

600 grams of soybeans was washed to remove dirt and soaked for 8 hours at room temperature in tap water. The drained soybeans were blend with 2 liters of water in a laboratory blender. Finally, the slurry was squeezed manually to separate the insoluble residue, okara, using cloth filter. The soy milk (pH 6.6, Brix 6.5) yield was calculated as the weight (g) of soymilk obtained from 600 grams of soybeans.

Soymilk was placed in glass bottle (Duran bottle) temporarily before the packing process. Next, 100 ml of soymilk were sealed in 100 mm x 150 mm pouches. This bag was 1 mm thick with low oxygen transmission rate and could withstand temperature up to 130 °C and very suitable for thermal and high-pressure processing. The packed sample were stored at -20 °C and thawed in laboratory refrigerator for a night before treatment.

3.2 Thermal processing of Soymilk

For thermal pasteurization treatment, the packed samples were placed in thermostatic water bath (Sul Supplies (M) Sdn. Bhd. Malaysia). According to (S.Jung et al Chemistry Book, 2008), soymilk are pasteurize at 95 °C for 15 min. In this treatment, the thermal treatment was performed at 60 °C, 70 °C and 80 °C for 5 min, 10 min and 15 min. The samples were immediately cooled in an ice water bath before enzyme extraction. All the samples were checked their pH, Brix, color, viscosity and microbial. After that, the high quality will be chosen.

3.3 High Pressure Processing of Soymilk

Soymilk and hydrated soybeans in water was vacuum packed in polyester bag and was treated using Avure 2L-700 HPP Laboratory Food Processing System. The HPP consists of computerized control and treatment chambers. Distilled water was used as pressure medium in treatment chamber. The HPP chamber composed with thermocouple to register the temperature during HPP cycle. The packed samples were processed at 200 MPa, 400 MPa and 600MPa for varying time from 5 min to 15 min. The processed samples were stored in lab refrigerator before the enzyme extraction.

3.4 Color Detection Technique

Soymilk was taken for the measurement of color using lab scan XE spectrocolorimeter in terms of CIE 'L' (lightness and yellowness) 'a' (redness and greenness) and 'b' (blueness), Yi (Yellowness index) and Wi (Whiteness index) following the method of Chantrapornchai et al. (1998). ΔE (color difference) values were calculated using the following formula:

$$\Delta E = [(\Delta L)^2+(\Delta a)^2+(\Delta b)^2]^{½}$$

Where ΔL, Δa and Δb are the differences in the specified tristimulus coordinate between the test soymilk and tofu with commercial soymilk and tofu (ΔE).

3.5 Rheological properties of Soymilk

Apparent Viscosity

Rheological measurements of 200 mL of soymilk sample were performed using a Haake RS150 Rheometer (viscometer) equipped with a DG41 sensor system. The system consisted of a cup and bell-shaped rotor. The inner and outer cylinder had an outer diameter of 36.0 and 43.3 mm respectively. The shear rate was increased from 10 to 1,500 s^{-1} within 7.5 min. Apparent viscosity was determined at 1,500 s^{-1}. Sample was tested minimum for three times.

3.6 Microbial Activity Measurement

Soymilk samples were undergoing sterility test that were incubated at 36 °C for 24 hours. Microbiological quality of soymilk also was assessed by following microorganisms; mesophilic aerobic bacteria were counted on PSA medium incubated for 24 hours at 36 °C. Before the experiment conduct, all the materials and apparatus must be autoclave at 121 °C for 3 hours. The experiment was conducted in laminar flow and the dilutions of peptone water and distilled water are used. There are five dilutions concentration are used for first trial and then three dilutions used. The colony will be calculated in log CFU/ml unit.

Microbial count=log [(no. of colony X dilution factor)/aliquot plated]

3.7 Storage

After the high quality of soy milk was chosen from thermal processing and high-pressure processing, it undergoes storage effect within 0 day to 30 days. Within the days, pH, Brix, color, viscosity and microbial were tested. The experiment will be conducted in two conditions; room temperature and at 4°C. The storage will be continuing until the soymilk have been spoiled.

CHAPTER 4: RESULT AND DISCUSSION

4.1 Thermal Treatment

4.1.1 Color Measurement

Below show the demonstrate of the color detection of the soymilk during the heat treatment at temperature 60° C, 70° C and 80° C at 5, 10, and 15 min. Excess heating generally leads to the destruction of amino acid and vitamins, browning and the development of cooked flavor (K-C.Kwok & Niranjan, 1995). From Figure 4, the L* values (lightness and yellowness) where at 70°C the value decrease with time. The value decreased slightly due to the Maillard reaction between the ϵ-amino acid group of lysine and reducing sugar where the formation of brown pigment (Kwok and others 1999). Soymilk and dairy milk have similar compositions but differ in some chemical constituents because soymilk does not contains reducing sugar such as lactose in dairy milk. As reducing substances may cause damage to some amino acid through Maillard reaction, the heat stability of proteins in soymilk and dairy milk may be differ (K.-C.Kwok & K.Niranjan, 1995). Based on Dennis A. Savaiano et.al (1984), dairy products contain same vitamins and minerals as soymilk but they also have added enzyme called lactase, that helps digest any lactose. Soymilk could be processed by direct steam-infusion into the soy flour slurry for about 600s at 121°C, 400s at 132°C, 210s at 143°C or 90s at 154°C before equivalent chemical browning to 60min at 99°C would occur. In this method processing, the soy slurry was heated to get extraction of solid (Johnson et. al. 1981). Therefore, the color of soymilk obtained was influence by the temperature and time dependent.

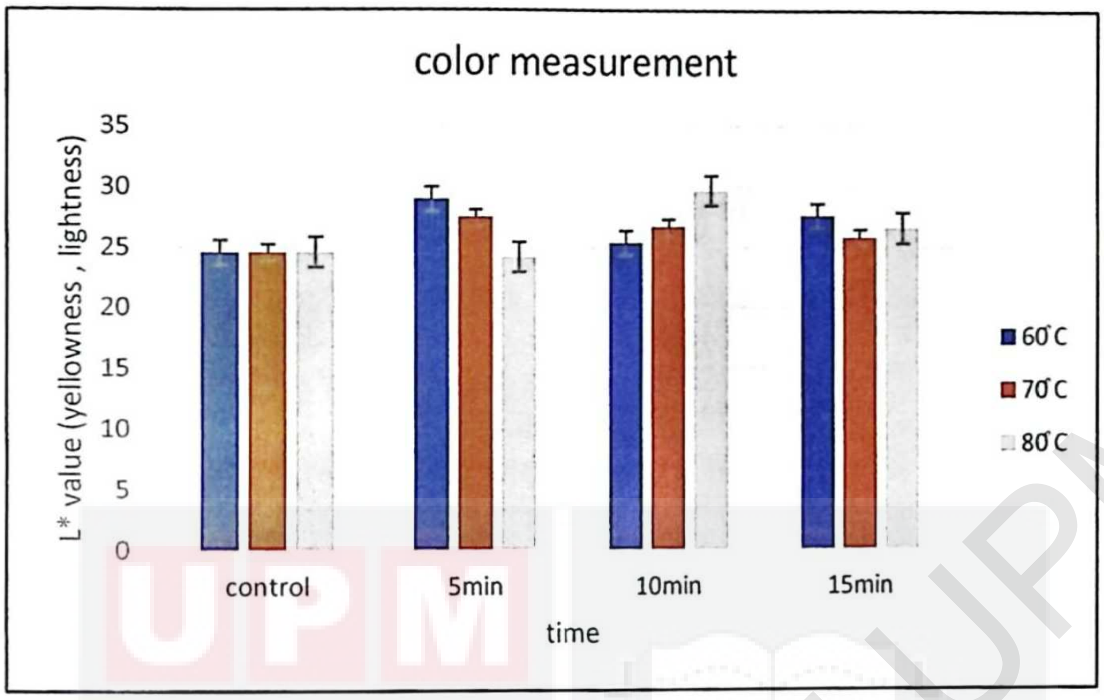

Figure 4 L values against time in varies temperature*

4.1.2 Apparent Viscosity

The apparent viscosity of soymilk did not vary with pH and brix. Brix is a sugar content or concentration of soymilk. As the viscosity increased, the value of brix decreased (Table 8). It may due to the glucose content in soymilk where sucrose has better sweetener than glucose (C.S. Favaro et.al. 2001). Based on Figure 7, the viscosity increases with the increasing of temperature and time but overall there is no slightly difference between it. Liu et.al. (2004) stated that the viscosity increases with the increasing of temperature and time. The increasing viscosity due to the formation of protein. The thermal treatment of soymilk causes protein to unfold as a result of denaturation and formation of aggregates through disulfide bond and hydrophobic interaction, which results in higher water binding capacity (Wagner and others 1992). When heat treatment was applied, the changes in shape and interactions between particles did not promote as much water binding that can increased viscosity. It is also known that the viscosity changes resulting from heat treatments are related to heat-induced denaturation and subsequent aggregation of proteins (Jeurnink & de Kruif, 1993). The changes in soymilk viscosity indicate that the selective thermal denaturation modified the status of the aggregations of denatured soybean proteins. The changes in protein aggregation might be related to the interactions between glycinin and b-conglycinin.

From Figure 6, soymilk became more acidity as the temperature increased. At 70°C, the pH of soymilk increased slightly may due to isoelectric points, the number of negative charge in protein which promotes the electrostatic repulsion between proteins, while the pH value decreased may due to sol formation (Ramamootrhi Lakshamanan, et.al. 2006).

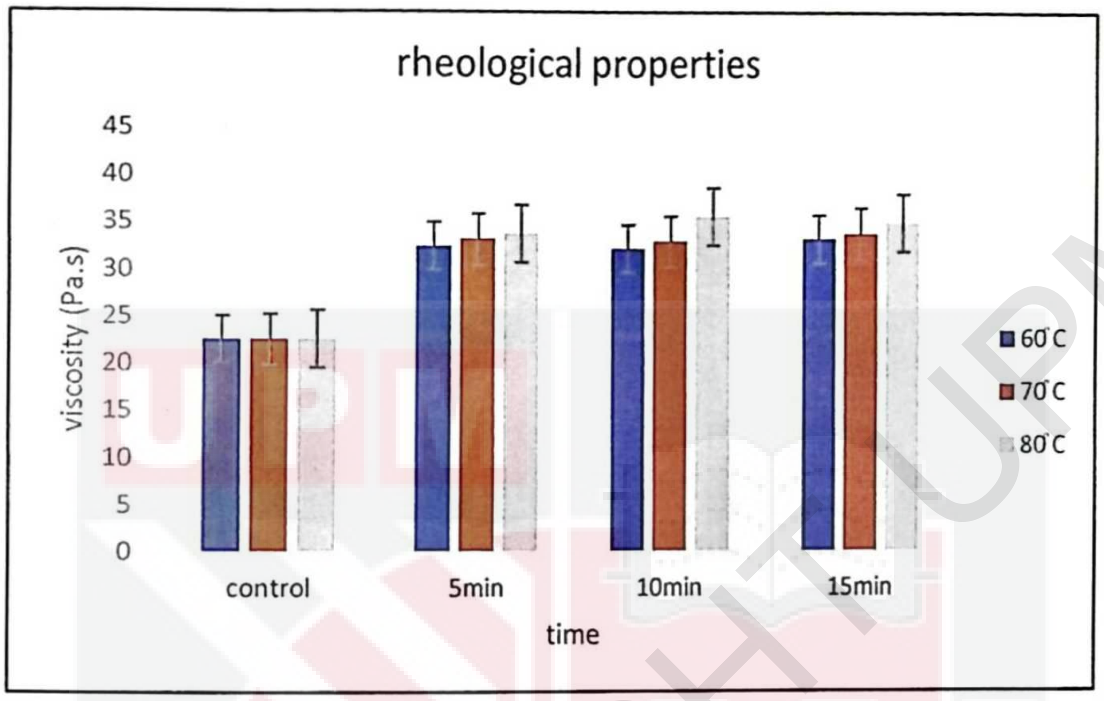

Figure 5 Viscosity against time in varies temperature

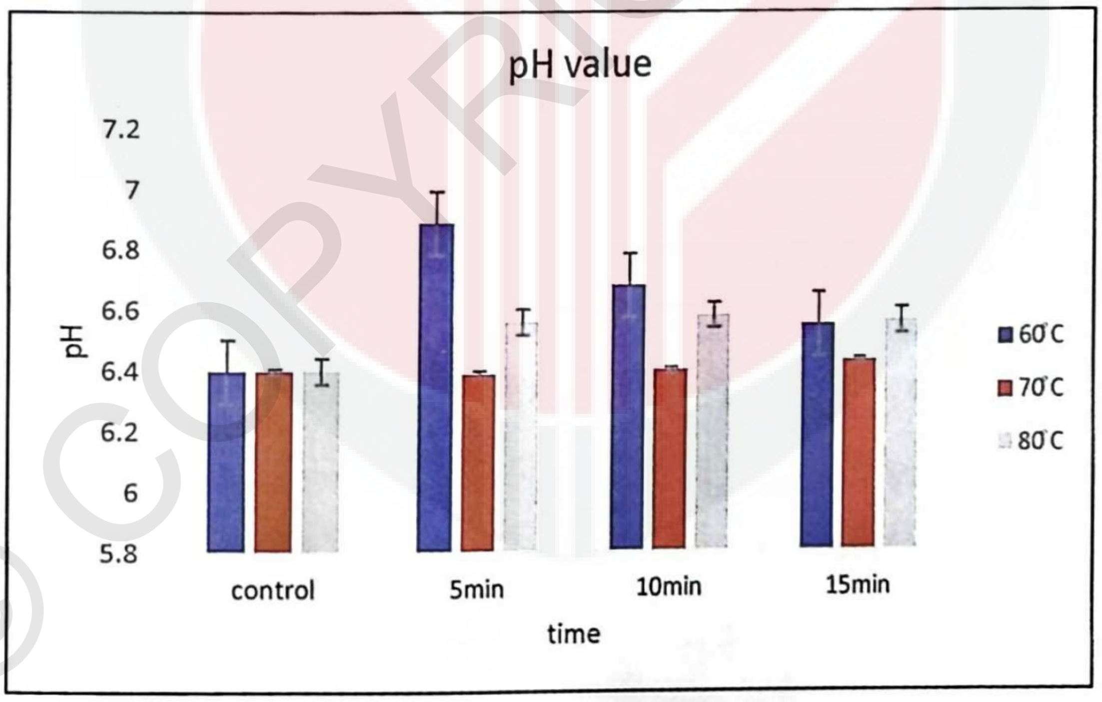

Figure 6 pH values against time in varies temperature

4.1.3 Microbial activity

For the microbial test on soymilk using plate count agar (PCA), from the experiment the microbial count for untreated soymilk is in range 4.0 to 6.0 log CFU/ml supported by K.Smith et. al. Food Microbiology (2006).

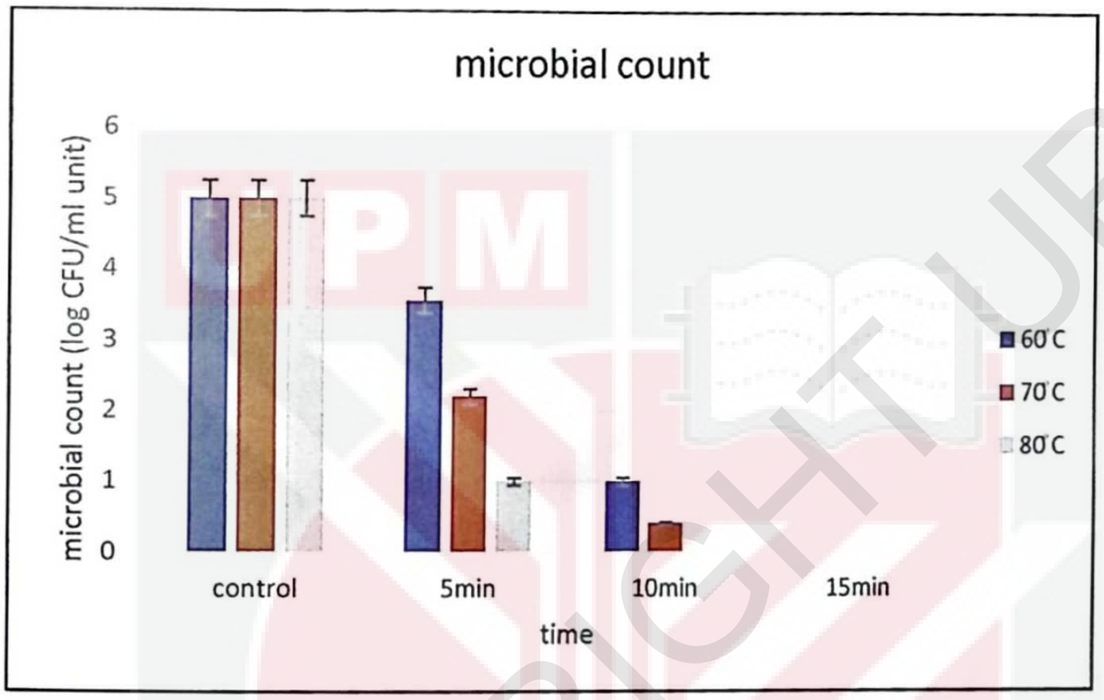

Figure 7 Microbial count against time in varies temperature

From Figure 7, it shows that the higher the temperature can lower the microbial count with increasing time. The microbial count for untreated soymilk high, it shows that soymilk is a good medium for the microbial growth. Such high number of microorganisms in the soymilk indicates problem with the production method or because of the hygiene. High microorganisms can affect the shelf life as well as potential safety factor. The decreasing of the microbial count because of the present of the heating treatment. Based on T.G.Rehberger, L.A.Wilson & B.A.Glatz (1983) state that 121°C for 2 to 3 min to avoid the nutritional loss of the soymilk. Bouno et. al. (1989) also investigated the effect of different heat treatments on the destruction of indigenous Bacillus spores in soymilk which was used as a substrate for soy yogurt fermentation.

Sterilization requires a more severe heat treatment which practically destroyed all microorganisms, including the spores of mesophilic anaerobic bacteria such as Clostridium botulinum.

4.2 High Pressure processing (HPP)

4.2.1 Color Measurement

Based on Figure 8, with regard to potential commercialization of pressurized soymilk, its visual appearance characteristics, that is, color and viscosity are of paramount importance.

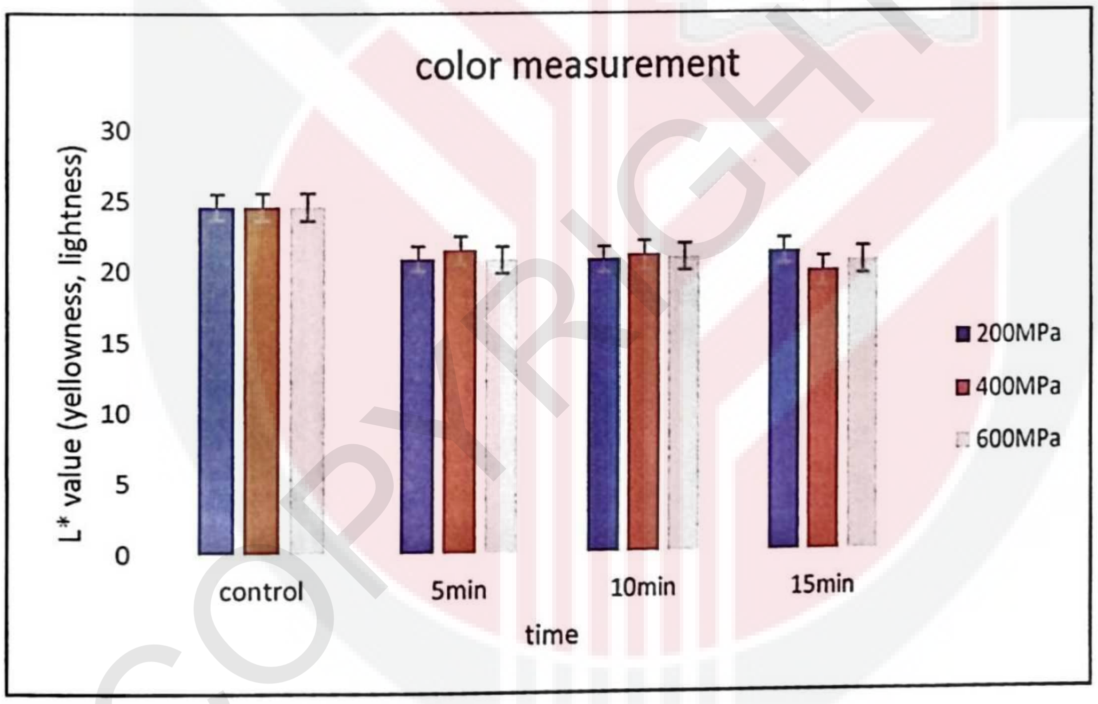

Figure 8 Effect of HPP on appearance of soymilk

Overall there is no drastic change in the lightness (L*), redness (a*), and yellowness (b*) of the HPP compare to the control sample. There is no significant difference between the pressure. Effect of high pressure generally marked as retention of color, flavor and fresh appearance as a result of the mild processing character. Color and flavor deterioration of pressure process food during storage has been attributed to residual enzyme activity (Cano, Hernández, & De Ancos, 1997). It is also because HP treatment has effect on the pigments such as chlorophyll and carotenoids. Chlorophyll is a green compound found in green stems of plants. The higher the pressure accelerates the degradation of the chlorophyll because HP processing affect their stability (Butz, Edenharder, Fernandez Garcia, Fister, Merkel &Tauscher, 2002). Carotenoids are important for the red appearance of fruits and vegetables. HP pressure increase the extraction yields of carotenes from the plant matrix. Therefore, from Figure 8, the value of L* increase with the increasing of pressure. But, for soymilk the suitable pressure treatment is between 200 mPa and 400 mPa. High pressure is not recommended because high pressure-induced tofu gels could be formed that had gel strength and a cross-linked network microstructure (Hongkang Zhang, Lite Li, Eizo Tatsumi & Seiichiro Isobe, 2005).

4.2.2 Apparent viscosity

High pressure effects on proteins are primarily related to the rupture of non-covalent interactions within protein molecules and to the subsequent re-formation of intra and intermolecular bonds within or between protein molecules. The apparent viscosity of soymilk did not vary with pH and brix. Based on Figure 9, the viscosity increases with the increasing of temperature and time but overall there is no slightly difference between it. The increasing viscosity due to the formation of protein. When the pH of protein is increasing from the isoelectric point to higher basic value, the number of negative charged groups COO- gradually increase, which promotes the electrostatic repulsion between proteins. This change in protein charge balance can cause the opening of the proteins that bind more water molecules that resulted the increasing of viscosity. At 400MPa at 15min, we can see that the value of viscosity increased rapidly the fall at 600MPa at 5min. This may due that at this time, soymilk had achieved the maximum value of coagulating at this pressure because high pressure does not recommend in pasteurized soymilk because high pressure can have formed gel and cross-linked network microstructure (Hongkang Zhang et.al. 2005).

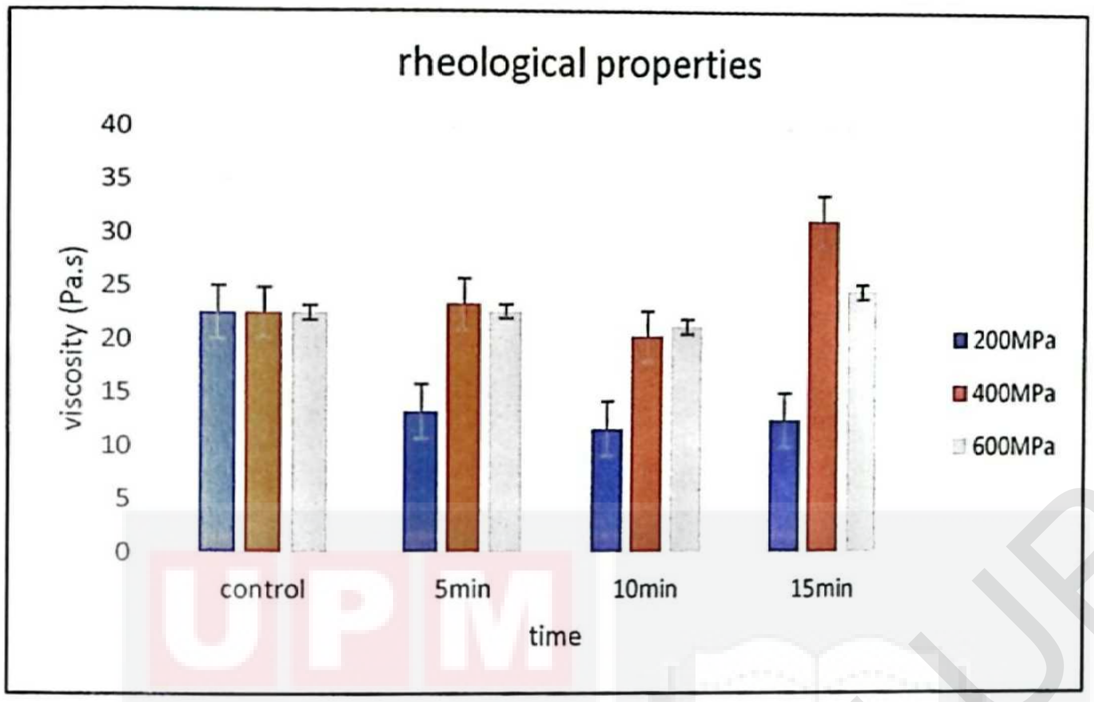

Figure 9 Effect of HPP on viscosity of soymilk

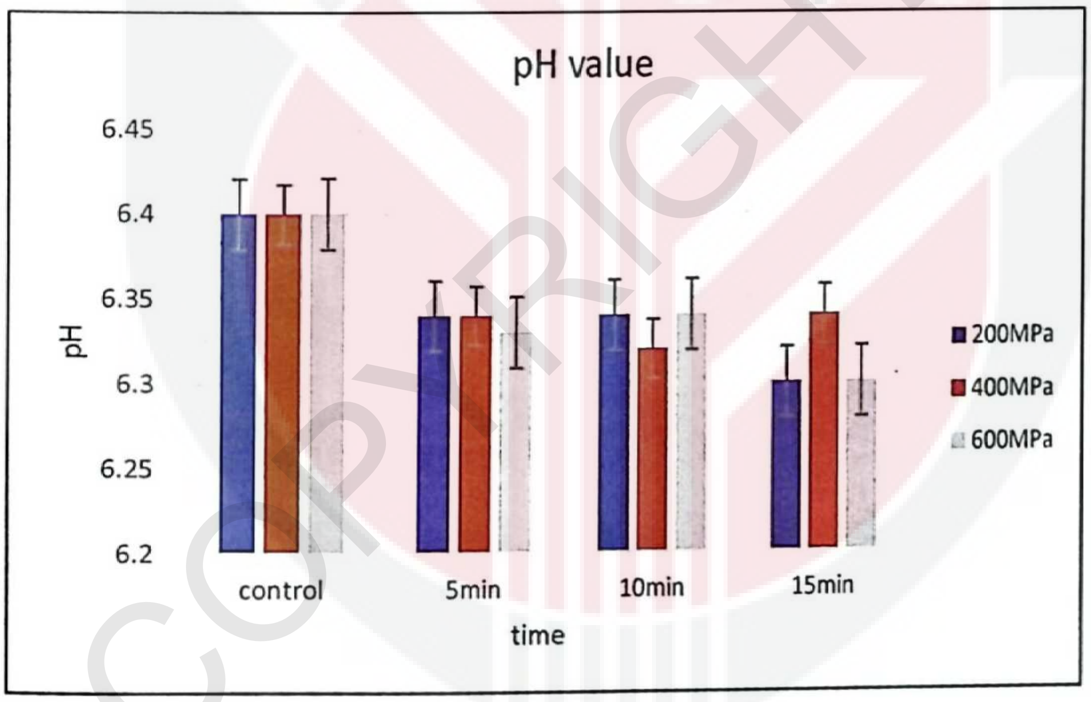

Figure 10 Effect of HPP on pH of soymilk

4.2.3 Microbial activity

From Figure 11, the microbial count for 200 MPa decrease with the increasing of time. Pressure at 200 MPa, 5 min the microbial count is 3.11 log CFU/ml and decrease to 2.3 log CFU/ml at 10 min. The higher the pressure the lower the microbial count. The reduction in microbial count was pressure dependent, showing better inactivation as pressure increased (Hayes et.al. 2005; Thiebaud et.al. 2003).

But there is some another microorganism found other than the colony which is mold or yeast. This can be cause by the inconsistency in processing techniques, sanitation methods and storage conditions. Many cases of inconsistent storage practices at the retail stores were noted during the term of this study. Because of this inconsistent microbiological quality within lots and between lots.

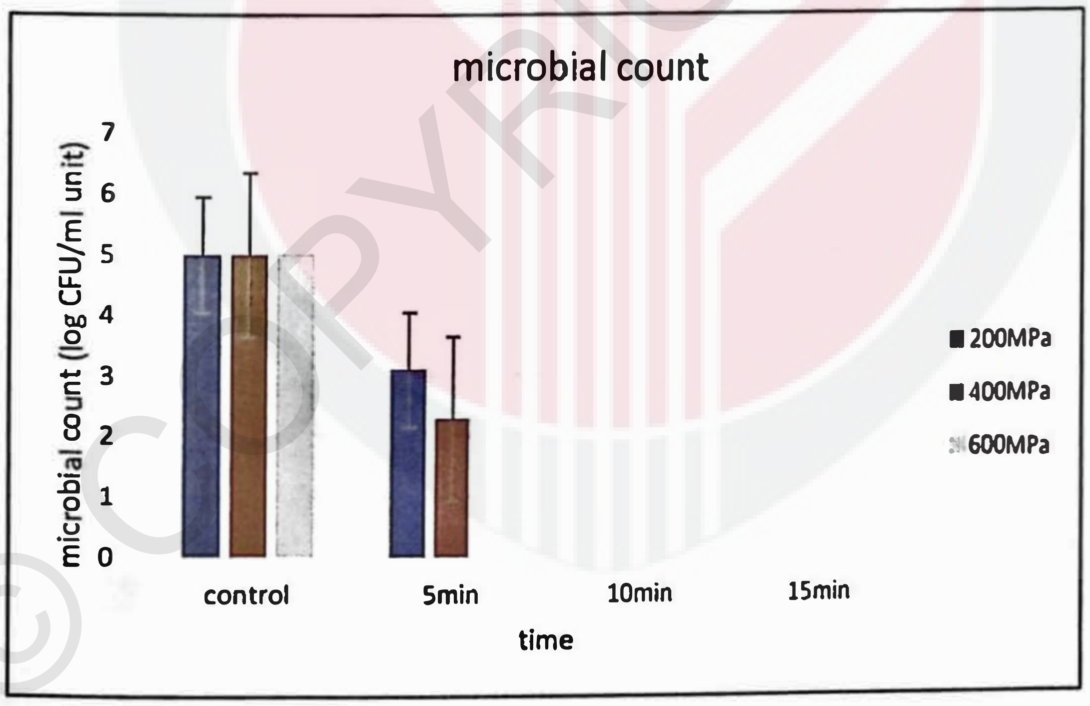

Figure 11 Effect of HPP on microbial count of soymilk

4.3 Storage

Soymilk with two different condition storages were evaluated based on the appearance and physical properties. The conditions that will be experimented are at room temperature and in freezer, 4° C. For thermal treatment, the optimum temperature is 70° C at 10 min and HPP treatment at pressure 400 MPa at 5 min. These values approximately with control sample. The pH and color of the soymilk decrease during the 15 days of storage. Color and viscosity of the soymilk also affected. At room temperature, the soymilk only standing for 24 hours because it undergoes acid curdling with a rapid drop in pH accompanied by separation of curd and whey (Yan, 1927). However, soymilk that take place in 4°C stand within 15 days after thermal and 20 days after HPP treatment. All the data below show the comparison between thermal and HPP treatment along the storage time at 4°C.

Color measurement

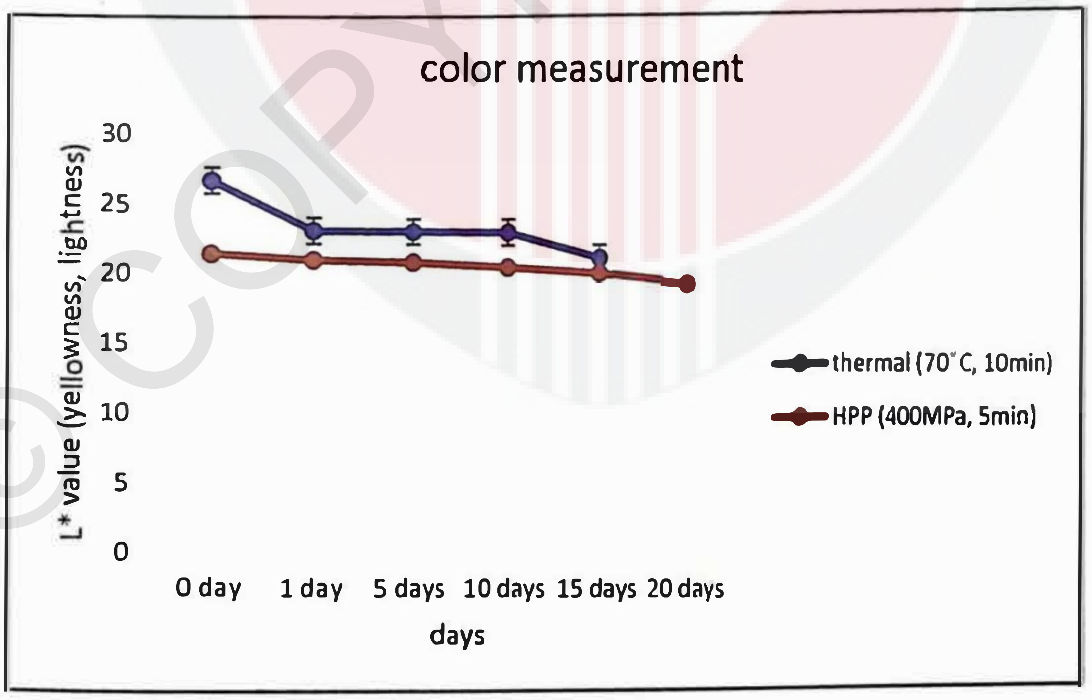

Figure 12 Effect of color on soymilk after thermal and HPP treatment

Figure 12 shows the effect of color along the storage time. In general, the higher the L* values, the whiter the color of the soymilk sample. For thermal treatment, there is a significant decrease in L* value within a day while for HPP treatment the value remained stable for the remaining of storage period. The decreasing of the L* value may due to the Maillard reaction, browning and environment factors.

pH values

Figure 13 shows the pH change under different treatment over the storage period of time. Over time, the pH of soymilk decreased within a day after both treatment and remained stable until 10 days. After that, the pH started to fall again for the remaining of the storage period. This decreased in pH may be an indicator of product acidity, may result from the chemical interactions occurring in soymilk such as lipolysis and proteolysis. It is also probably because of the high fat content in the soymilk (A.Achouri et.al. 2007). Pascall et.al. (2006) also reported that the decreasing of pH over time may due to the chemical reaction that occur from the microbes in soymilk such as presence of fermentable sugar.

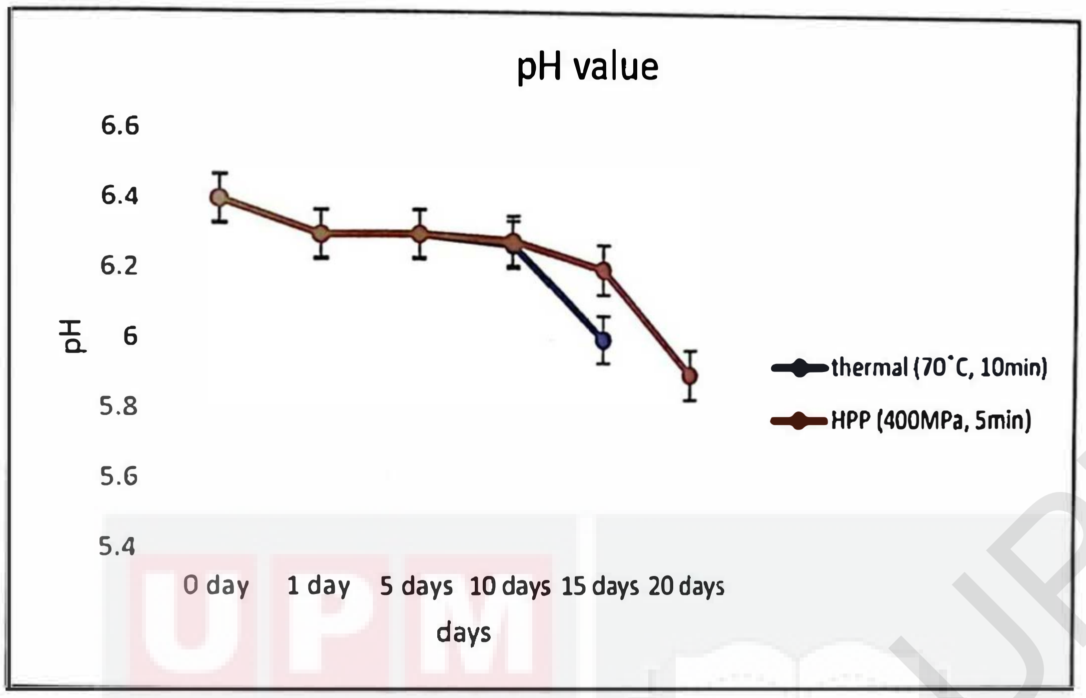

Figure 13 Effect of Ph on soymilk after thermal and HPP treatment

Soymilk viscosity

Change in soymilk viscosity over time after treatment are shown in Figure 14. Soymilk shows increasing in viscosity with the increasing of storage time. The trend of increasing soymilk for both treatment is same. The increasing of soymilk may due to the coagulation and curdling of protein. The denature of protein could have increased the hydrophobicity and exposed more sites for hydrophobic interaction with other soymilk components, which in turn may increase the viscosity (Sorgentini et.al. 1995).

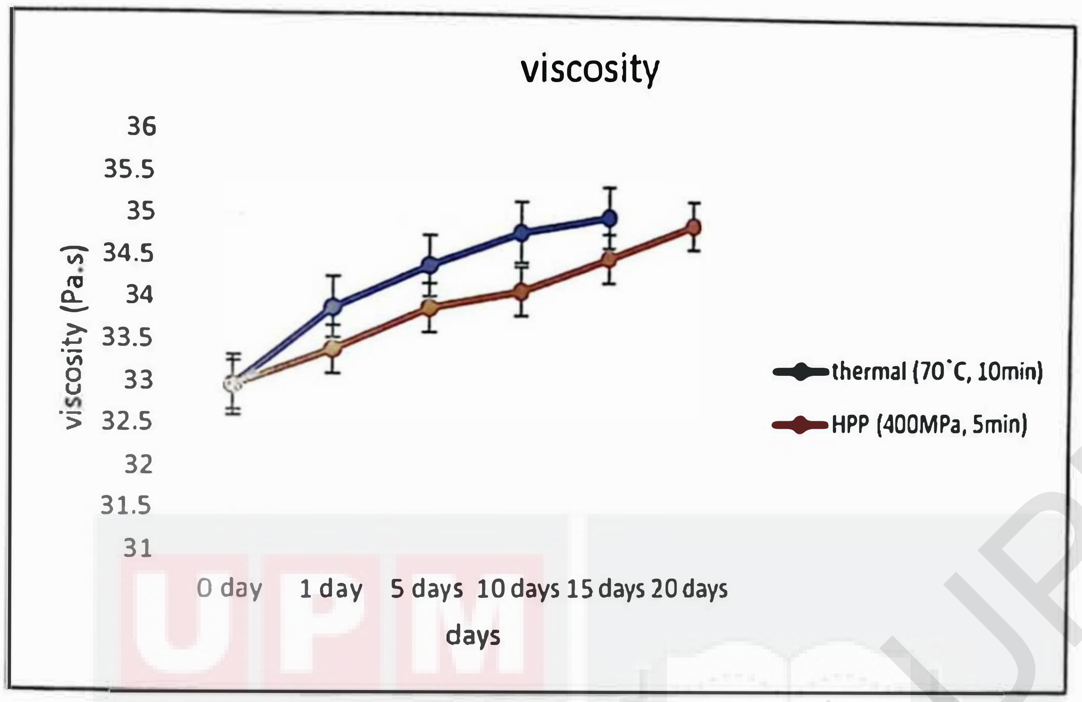

Figure 14 Effect of viscosity on soymilk after thermal and HPP treatment

Microbial count

Microbiological quality of soymilk is an important factor that contributes to the chemical changes in the product during storage. Form Figure 15, microbial count after thermal treatment increase within a day and remained stable until 5 days and continue increased for the remaining storage time. Microbial count after HPP treatment increase gradually until 20 days. The decreasing of the microbial count may due to the contamination between the agar and the environment. Also, may due to the technique processing and sanitation method during the experiment. This is because, there is another colony of bacteria found in the PCA agar such as Escherichia Coli.

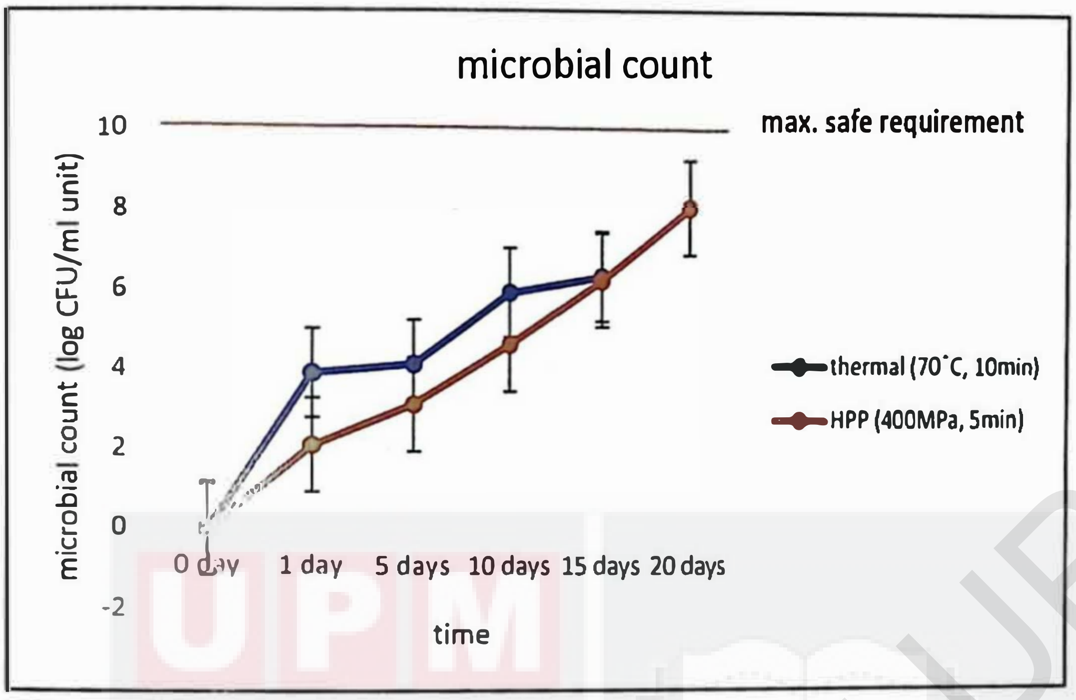

Figure 15 Effect of microbial count on soymilk after thermal and HPP treatment

CHAPTER 5: CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study aimed to employ high pressure processing (HPP) as an alternative way to replace thermal processing in pasteurize liquid foods to extend their shelf life. In this study, the focus that was given is soymilk. In general, HPP treatment are better preservation than thermal processing in term of color, pH, viscosity and microbial count. Impacts of HPP and thermal treatment on the viscosity and microbial count of the soymilk depends on the time and pH values. Combination of these two parameters conducted to either an improvement and non-modification or a decrease of viscosity and microbial count. After thermal treatment, the microbial count decrease from 6 log CFU/ml of colony unit to 4 log CFU/ml and for HPP treatment it decreases to 3 log CFU/ml. This resulted is based on the optimum temperature and pressure chosen for storage.

Soymilk only stands for 24 hours at room temperature and at least 2 weeks at 4° C after undergoes thermal processing. It is evident that heat treatment can influence the quality of soymilk. Soymilk proteins rather stable at lower temperature as well as high pressure processing.

As conclusion, high pressure processing poses a potential treatment for shelf life extension without affect the nutrition content of the soymilk. This study provides a useful data for the commercial production of soymilk.

5.2 Recommendations for Future Work

In order to increase the efficiency of the HPP treatment, we suggested that the combination of the pressure and temperature applied to the soymilk. A shorter exposure time with high temperature can longer the shelf life of the soymilk as well as resulted in sterility with an additional safety factor and also can improved the protein efficiency ratio of the soymilk.

For more accurate and precise data, a specific room must be held for microbial activity because the condition of the laboratory for microbial activity must be in good environment and more hygienic to avoid the cross contamination between the agar and the environment.

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APPENDIXES

Table 7 L values after thermal treatment*

Temp.	Time	L				stdev
		1	2	3	ΔL	
60°C	5 min	28.1	32.7	26.2	29	3.342155
	10 min	23.8	23.5	28.8	25.36667	2.977135
	15 min	28.1	28.4	26.2	27.56667	1.193035
70°C	5 min	28.3	24.1	30.2	27.53333	3.121431
	10 min	29.9	24.8	25.4	26.7	2.787472
	15 min	26.6	25.7	25.1	25.8	0.754983
80°C	5 min	23.8	24.2	24.6	24.2	0.4
	10 min	28.5	30.9	29.6	29.66667	1.201388
	15 min	27.7	26.1	26.1	26.63333	0.92376

Table 8 Apparent viscosity, pH and brix value after thermal treatment

Temp.	Time	viscosity reading				stdev	Ph	brix (%)
		1	2	3	average			
60°C	5 min	32.4	32.5	32.6	32.5	0.1	6.89	6.8
	10 min	32.3	32.2	32.2	32.23333	0.057735	6.68	5.4
	15 min	33.2	33.3	33.2	33.23333	0.057735	6.55	5.5
70°C	5 min	33.1	33.2	33.3	33.2	0.1	6.39	6.3
	10 min	32.9	33	33	32.96667	0.057735	6.4	7.1
	15 min	33.8	33.9	33.8	33.83333	0.057735	6.43	6.1
80°C	5 min	33.7	33.8	33.9	33.8	0.1	6.56	5.4
	10 min	35.6	35.7	35.6	35.63333	0.057735	6.58	6
	15 min	35	35.1	34.7	34.93333	0.208167	6.56	6.1

Table 9 Microbial count on PCA agar after thermal treatment

	control	5min	10min	15min
60°C	5	3.56	1	0
70°C	5	2.2	0.4	0
80°C	5	1	0	0

Table 10 L values after HPP treatment*

Pressure	Time	L				stdev
		1	2	3	ΔL	
200 MPa	5 min	20.8	21.2	20.8	20.93333	0.23094
	10 min	20.1	21.1	21.1	20.76667	0.57735
	15 min	20.9	21.7	21.4	21.33333	0.404145
400 MPa	5 min	20.5	21.9	22	21.46667	0.83865
	10 min	21.4	22.5	19.4	21.1	1.571623
	15 min	19.9	17.9	21.8	19.86667	1.950214
600 MPa	5 min	21.1	20.9	20.5	20.83333	0.305505
	10 min	20.9	21.8	19.9	20.86667	0.950438
	15 min	20.1	19.8	21.9	20.6	1.135782

Table 11 Apparent viscosity, pH and brix value after HPP treatment

Pressure	Time	viscosity reading				stdev	Ph	brix (%)
		1	2	3	average			
200 MPa	5 min	13.7	13.1	13.1	13.3	0.34641	6.34	8.8
	10 min	11.9	11.7	11.6	11.73333	0.152753	6.34	8.5
	15 min	12.5	12.5	12.5	12.5	0	6.3	8.3
400 MPa	5 min	23.4	23.5	23.4	23.43333	0.057735	6.34	7.1
	10 min	20.5	20.4	20.3	20.4	0.1	6.32	6.6
	15 min	31.6	31.3	31.1	31.33333	0.251661	6.34	6.7
600 MPa	5 min	22.4	23.2	22.6	22.73333	0.416333	6.33	8.8
	10 min	21.4	20.9	21.6	21.3	0.360555	6.34	8.5
	15 min	24.6	24.4	24.7	24.56667	0.152753	6.3	8.3

Table 12 Microbial count on PCA agar after HPP treatment

Pressure	control	5min	10min	15min
200MPa	5	3.11	0	0
400MPa	5	2.3	0	0
600MPa	5	0	0	0

Table 13 Effect of color on soymilk after storage

Condition	0 day	1 day	5 days	10 days	15 days	20 days
thermal (70°C, 10min)	26.7	23.1	23	22.9	21	
HPP (400MPa, 5min)	21.47	21	20.8	20.4	19.9	19.1

Table 14 Effect of viscosity on soymilk after storage

Condition	0 day	1 day	5 days	10 days	15 days	20 days
thermal (70°C, 10min)	32.9667	33.9	34.4	34.8	34.989	
HPP (400MPa, 5min)	32.96667	33.4	33.9	34.1	34.5	34.9

Table 15 Effect of pH values in soymilk after storage

Condition	0 day	1 day	5 days	10 days	15 days	20 days
thermal (70°C, 10min)	6.4	6.3	6.3	6.27	6	
HPP (400MPa, 5min)	6.4	6.3	6.3	6.28	6.2	5.9

Table 16 Effect of microbial count on PCA agar of soymilk after storage

Condition	0 day	1 day	5 days	10 days	15 days	20 days
thermal (70°C, 10min)	0	3.9	4.1	5.9	6.3	
HPP (400MPa, 5min)	0	2.1	3.1	4.6	6.2	8