



UNIVERSITI PUTRA MALAYSIA

***EFFECTS OF HIGH PRESSURE PROCESSING (HPP) ON
MICROSTRUCTURE AND RHEOLOGICAL PROPERTIES OF GOAT MILK***

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182489

**A THESIS SUBMITTED IN PARTIALLY FULFILLMENT OF THE
REQUIREMENT FOR THE DEGREE OF
BACHELOR OF ENGINEERING (PROCESS AND FOOD)**

DEPARTMENT OF PROCESS AND FOOD ENGINEERING

FACULTY OF ENGINEERING

UNIVERSITI PUTRA MALAYSIA

2019

ACKNOWLEDGEMENT

First and foremost, I would like to express my greatest gratitude to my supervisor, Assoc. Prof. Ir. Dr. Siti Mazlina Mustapa Kamal, lecturer of Department of Process and Food Engineering, Universiti Putra Malaysia. Without her assistance and dedicated involvement in every step throughout the process, this thesis would have never been accomplished. I would like to thank you very much for your support, guidance and understanding over these past four years.

My sincere thanks also goes to my seniors, Nurfatimah Binti Mohd Thani whom I had asked for help and guideline throughout this research. Besides, I would like to thank to En. Raman Morat and Pn. Siti Hajar Zakaria, the assistant engineers in our laboratory who help to solve my problems when I was completing my laboratory works.

In addition, I have many, many people to thank for listening to and, at times, having to tolerate me over the past four years. I cannot begin to express my gratitude and appreciation for their friendship. Tan Jin Tian, Tan Sok Fern and Ng Khai Yee have been unwavering in their personal and professional support during the time I spent at the University.

Most importantly, none of this could have happened without my family. Thanks to my parents Lee Koh Wah and Heng Sok Wyang for supporting me spiritually in every moment and stage of my life.

ABSTRACT

Goat milk was subjected to high pressure processing (HPP) at 200, 400 and 600 MPa for 5, 10 and 15 minutes. The effects of pressure and holding time on the microstructure and rheological properties were determined. Goat milk treated with 600 MPa increased the number of small size fat globules. Smallest mean diameter (5.215 μm) of milk fat globules was obtained at the treatment of 600 MPa and 10 minutes. Fission of fat globules occurred when the goat milk was treated with 400 MPa for 15 minutes. In terms of rheological properties, both treated and untreated goat milk exhibited as Newtonian fluids. Besides, a Central Composite Design (CCD) of Response Surface Methodology (RSM) was used to optimize the pressure applied and holding time during high pressure processing. pH, lactose and total carbohydrate of goat milk were set as responses. The predicted optimum condition was obtained at 400 MPa, 5 minutes of HPP treatment and the predicted value for each response was 6.66, 2.5532 g/100ml and 31.08 % respectively.

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CHAPTER 1

INTRODUCTION

1.1 OVERVIEW

Goats with scientific name of *Capra hircus* were among the first domesticated animals. According to the archaeological evidence, goats and human have been in a symbiotic relationship for up to ten thousand years. They can adapt to harsh climates which make them suitable for landless and marginal farmers (Zenebe et al., 2014). Due to the rapidly increase of human population in the developing countries, the demand for milk and products made from milk is on the rising trend (Bhattarai, 2014). There are nearly 300 breeds of goats and about 600-700 million of dairy goats are present in the world. However, only a small number of them are generally raised for their milk purpose (Hirst, 2018). Some consumers are hard to accept the products made from goat milk due to the sensory aspects of goat products, such as a more intense flavour and aroma.

Goat milk contains protein, lipid, carbohydrate, vitamin and mineral. The effective digestibility of goat milk, the proper composition of fatty acids and its content of bioactive compounds seem to give properties good for curing or preventing certain medical conditions. One of the most important contributions of goat milk to human nutrition is the calcium and phosphate that it supplies. In one litre of goat milk, it contains about 1.2 g calcium and 1 g phosphate (Getaneh et al., 2016). Major role of milk proteins

is to supply amino acids and nitrogen to the children and constitute an important part of dietary proteins for the adult. In addition, milk proteins have physiological importance, they facilitate uptake of several important nutrients such as trace elements and vitamins and contain a group of proteins which perform a protective function (Zenebe et al., 2014). Producing high quality with extend shelf life of raw milk is very important for successful production and marketing of dairy goat products. Raw goat milk must be safe to consume and free of any pathogenic bacteria, antibiotic, insecticide and herbicide compounds (Park, 2010).

Nowadays, in the emerging field of functional foods, minimal processed foods have increased in popularity together with organic foods. The non-thermal processing technologies are gaining importance since the consumers are more concern to food safety and health aspect in this era of minimally processed foods. One of the promising technology which could serve as an alternative method for food preservation is the application of high pressure processing (HPP). High pressure processing (HPP) is non-thermal processing method in which the food is subjected to a very high pressure ranging from 200 to 600 MPa (Okpala et al., 2009). High pressure treatment can be used to process both liquid and solid food with high moisture content such as goat milk. It does not break the covalent bonds of food and has a minimal effect on food chemistry (Muntean et al., 2016).

High pressure processing improves food safety by destroying the bacteria that can cause food borne illness and spoilage, but keep the food fresh at the same time. This type of foods can be kept for a longer period under better condition (Naik et al., 2013). Other than that, HPP provides a means to retain quality of food without the need for excessive

thermal treatment or chemical preservatives. High pressure processing is also known as high hydrostatic pressure processing (HHP) or ultra-high pressure processing (UHP) (Muntean et al., 2016).

1.2 PROBLEM STATEMENTS

Nowadays, there is an increasing interest in goat milk both in Malaysia and in the world, resulting with increasing the production of goat's milk and its products. Therefore, more information is needed on production and processing of goat milk. There is no study that has investigated the effects of high pressure processing on the microstructure and rheological properties of goat milk. Microstructure and rheological properties are important monitors of quality control in dairy processing and in scientific research. 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 goat milk production with HPP is a practical option. So, these are important factors that need to be considered in this research.

1.3 OBJECTIVES

The general objective of this study is to improve the quality of goat milk by high pressure processing (HPP). In order to achieve the general objective, the goat milk that subjected to HPP is compared with the control goat milk with the following specific objectives:-

- 1) To investigate the effect of process parameters of HPP on microstructure and rheological properties of goat milk.
- 2) To optimize the HPP parameter conditions (pressure and time).

1.4 SCOPE OF WORKS

In this study, the focus is given on the high pressure processing of goat milk. The fresh goat milk obtained from local farm was subjected to high pressure processing with the pressure of 200 MPa, 400 MPa and 600 MPa for 5 mins, 10 mins and 15 mins. After high pressure treatment, the goat milk was analysed with rheological properties and examined with the microstructure. Proximate analysis was also carried out to determine the quality of goat milk after high pressure processing. Besides, optimization was done to find the best parameter conditions for high pressure treatment in terms of pH, lactose content and total carbohydrate of goat milk.

1.5 THESIS STRUCTURE

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 analysis involved and the equipment used throughout this research. Chapter 4 is the results obtained and the discussions based on the results obtained. The final chapter provides general conclusions on the research.

CHAPTER 2

LITERATURE REVIEW

2.1 GOAT MILK

Goat milk is the most complete food known which is highly compatible and nourishing natural food. It contains very high nutritional value that can even serve as a substitute for a meal. It is also preferred due to its low fat content and its capability to neutralize the acids and toxins present in the body (Getaneh et al., 2016). According to Yanmış & Coşkun (2018), goat milk products have some properties that provide technological advantages such as a smoother texture, lower amount of α -casein, smoother gel products, higher water retention capacity and lower viscosity compared to cow milk.

Gerosa & Skoet (2012) reported that from year 2006 to 2009, goat milk contributed only 2.1 percent of global milk production which was far more less than the production of cow milk that contributed about 85.4 percent. Among the five different types of milk obtained from sheep, goat, cow, camel and buffalo, goat milk comes in third in terms of global milk production after cow milk and buffalo milk.

In recent years, goat milk consumption and production increases globally as people recognize the advantages and benefits of goat milk especially in the developed countries. It is well known that goat milk has high nutritional value than other species of

animals. Since goats are mainly raised in rangelands in semi deserts and sub tropic conditions where there is adequate browse and water supply, thus goats have the ability to produce milk of good composition and quality for human consumption. Fat, protein, ash, vitamins, lactose and enzymes are the example of these useful compositions obtained from milk.

2.2 COMPOSITION OF MILK

From Table 1, it shows that the composition of goat milk is almost similar with cow milk. Goat milk has more fat, protein, and ash and less lactose than cow milk. Goat milk contains less lactose than cow milk, which may make it a better option for those with mild lactose sensitivities.

Table 1: Basic Composition of Goat and Cow Milk (Per 100g) (Adapted from Arora et al., 2013)

Constituent (g)	Goat	Cow
Fat	3.8	3.6
Protein	3.5	3.3
Lactose	4.1	4.6
Ash	0.8	0.7
Total Solids	12.2	12.3

The gross milk composition of goat and cow milk is similar, but from a nutritional points of view, goat milk has many advantages over cow milk. The most significant differences include smaller milk fat globules and casein micelles which can allow for better digestion. The fat of goat milk is more digestible than that of cow milk because the fat globules of goat milk are smaller and have a greater surface area (Arora et al., 2013). The average size of goat milk fat globules is about 2 micrometers, as compared to 21/2 -

31/2 micrometers for cow milk fat. The smaller sized fat globules of goat milk provide a better dispersion, and a more homogeneous mixture of fat in the milk (Getaneh et al., 2016).

Besides, goat milk has a higher proportion of fatty acids with health benefits; casein composition closer to human casein composition associated with lower allergenicity, better availability and utilization of major (calcium) and minor (iron) minerals; and much higher concentration of various minor components with health-promoting properties (Silanikove et al., 2016). Due to the low allergenicity of goat milk, it has been recommended as a substitute for patients allergic or lactose intolerance to cow milk (Getaneh et al., 2016).

2.2.1 Lactose Content in Milk

Lactose is the major free carbohydrate that has been identified in goat milk, which is about 0.2-0.5% less than in cow milk. Lactose is a disaccharide made up of a glucose and a galactose molecule and is synthesized in the mammary gland. Lactose in milk content may play an important role for human especially during infancy in the absorption of calcium and other minerals such as copper and zinc. Moreover, it may be used by the intestinal microbiota (the microorganism population that lives in the digestive tract) as a nutrient (prebiotic) if not digested in the small intestine.

There are several studies reported that the lactose content of goat milk is lower than other types of milk such as cow milk, buffalo milk and sheep milk (Arora et al. 2013; Rasheed et al., 2016). The lower lactose content does not represent a problem in cheese

making or other dairy processing industry as enough lactose is available to ensure lactic acid fermentation (Alichanidis et al., 2016).

2.2.2 Fat Globules in Milk

Fat globules geometrical parameters measured is most beneficial for dairy manufacturers, as they enable them to predict the properties of the final product and through manipulating those parameters, an improvement could be achieved in the dairy industry. Setting those parameters provides a chance to choose the best fitting type of milk to good quality products (El-Zeini, 2006).

According to the same author, cow milk had the biggest diameter of milk fat globules (3.95 μm), then followed by sheep milk (3.78 μm) and goat milk (3.20 μm) as shown in Figure 1, 2 and 3 respectively. In cheese making, small fat globules were found to improve stretching and elasticity, compared to large fat globules. Small fat globule-formed yoghurts had lower shear stress and appeared to possess smoother and finer texture (Truong et al., 2016).

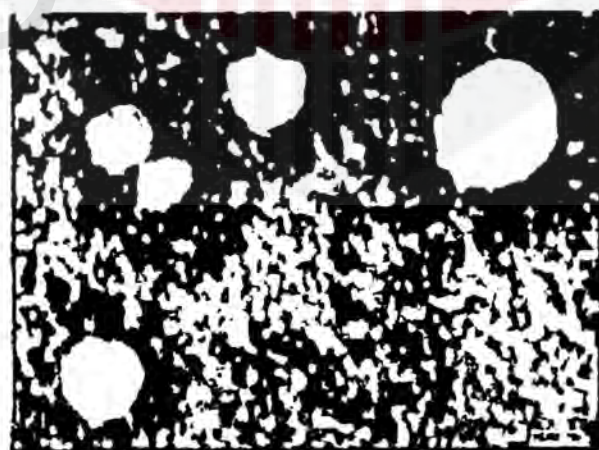


Figure 1: Microstructure of Cow Milk

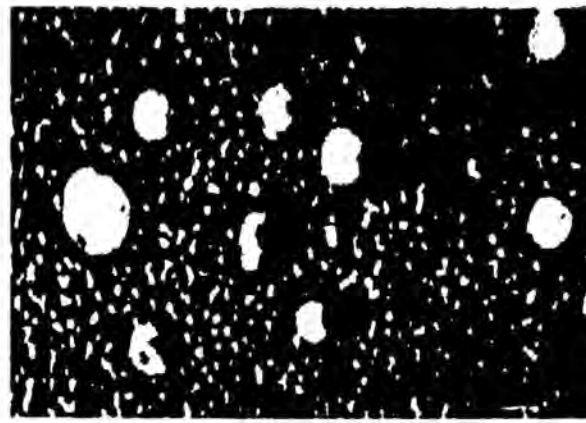


Figure 2: Microstructure of Sheep Milk

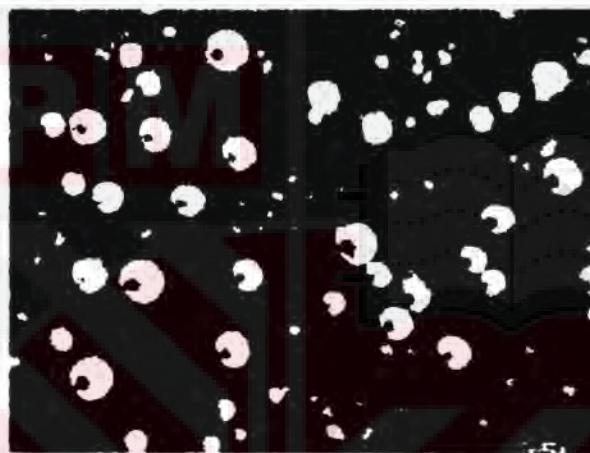


Figure 3: Microstructure of Goat Milk

On the other hand, Table 2 shows the geometrical properties of fat globules from different milk species. Goat's milk fat globules had the best spherical structure ($S = 0.33$) compared to those of the other milk species. Sphericity describes how close a shape is to perfect shape, for a perfect sphere, $S = 0$. Sphericity of cow's milk fat globules and sheep's milk fat globules are 0.91 and 0.89 respectively.

Table 2: Geometrical Properties of Fat Globules from Different Milk Species (Adapted from El-Zeini, 2006)

Property	Cow Milk	Sheep Milk	Goat Milk
Sphere Diameter (μm)	3.95	3.78	3.20
Sphericity (Perfect Sphere = 0)	0.91	0.89	0.33

2.3 RHEOLOGY OF MILK

Properties of both solid and liquid foods can be represented by rheology where texture is the rheology related to solid food and viscosity is the rheology of fluid food (Park, 2007). For milk, viscosity is affected by state and concentrations of fat and protein, temperature and pH. Viscosity is a physical property of a fluid to resist its flow or pour. It depends on internal friction within a liquid and the relation between kinetic motion and free surface (Jennes & Patton, 1976).

Under most conditions, milk behaves as a Newtonian liquid for which the shear stress is directly proportional to the shear rate (dv/dx) (Walstra et al., 1999). According to Arora & Jha (1984), the viscosity of goat milk (1.186 cp) at 30°C was reported to be lower than cow milk (1.236 cp) and buffalo milk (1.318 cp).

2.4 FREEZE-DRYING ON DAIRY PRODUCT

Freeze-drying or lyophilization is a process in which a solvent is removed from a frozen solution by sublimation. With freeze-drying, moisture content of the food products can be reduced to as low as 2%. Being a cold process, it minimizes the degradation reactions and maintains adequate physical, chemical, and biological stability of the product (Ibrahim & Khalifa, 2015). This process is very useful for heat sensitive food products during long term storage at ambient temperature.

From Table 3, it showed that the chemical composition of camel milk has been changed after freeze-drying process. Freeze-drying causes the moisture content of the camel milk to decrease from 87.87% to 3.33% but increase the percentage of the other chemical composition such as fat, protein and ash. The fat content of camel milk increased

from 3.20% to 25.63% after freeze-drying. Protein and ash content also increased from 3.33% and 0.93% to 26.13% and 7.20% respectively.

Table 3: Chemical Composition of Fresh and Freeze-Dried Camel Milk (Adapted from Ibrahim & Khalifa, 2015)

Constituents (%)	Fresh Milk	Freeze-Dried Milk
Moisture Content	87.87	3.33
Fat	3.20	25.63
Protein	3.33	26.13
Ash	0.93	7.20

2.5 THERMAL TREATMENT ON GOAT MILK

Thermal treatments such as pasteurization, long temperature long time (LTLT), high temperature short time (HTST) and ultra-high temperature (UHT) processes are the methods that applied to food fluid to kill pathogenic micro-organisms and degrading enzyme in order to increase shelf life, product quality and product safety (Elhasan et al., 2017). Milk obtained from milking process normally will be subjected to thermal treatment either pasteurization or ultra-high temperature process to kill the pathogenic bacteria and thus increase the shelf life.

Pasteurization of raw goat milk either at 63°C for 30 mins (LTLT) or 72°C for 15s (HTST) within one day of milking ensures a better tasting product both initially and during storage at temperature below 4°C for 6 weeks than if the raw milk is aged for several days at 4°C before being pasteurized (Guy et al., 1985). Ultra-high temperature process takes place when the milk is subjected to 140°C for 3 s (Pestana et al., 2015). In general, milk subjected to ultra-high temperature has longer shelf life than pasteurized milk.

2.5.1 Effect of Thermal Treatment on Different Source of Milk

Based on the results reported by Elhasan et al. (2017) which is shown in Table 4, the effect of pasteurization on chemical properties of milk samples of cow, camel, sheep and goat showed varied results depending on milk type. It was found that lactose content of all types of milk decreased after heat treatment. After heat treatment, lactose content of goat milk decreased significantly from 4.320 % to 3.995 %.

For cow milk, pH of raw milk and pasteurized milk was the same and it was not affected by heat treatment. For sheep milk, heat treatment decreased the pH value significantly from 6.86 to 6.71. Heat treatments decreased pH values of goat milk but not significantly to 6.89 from 6.90 whereas pH of camel milk increased but not significantly from 6.61 to 6.62.

In addition, fat content of cow milk increased from 3.795 % to 3.810 % while its protein content decreased from 3.235 % to 3.095 % after pasteurization. Both protein and fat content of camel and goat milk decreased after heat treatment. For sheep milk, fat content decreased but protein content increased after treatment.

Table 4: Effects of Pasteurization on Milk Samples (Adapted from Elhasan et al., 2017)

Treatment	Source of Milk	Chemical Properties			
		pH	Lactose (%)	Fat (%)	Protein (%)
Raw Milk	Cow	6.66	4.585	3.795	3.235
	Camel	6.61	4.365	2.705	2.610
	Sheep	6.86	4.180	8.790	5.750
	Goat	6.90	4.320	3.120	2.680
Pasteurized Milk	Cow	6.66	4.570	3.810	3.095
	Camel	6.62	4.320	2.695	2.440
	Sheep	6.71	4.010	8.685	5.760
	Goat	6.89	3.995	3.100	2.575

2.6 HIGH PRESSURE PROCESSING

High pressure processing (HPP) is an example of non-thermal processing where the food is subjected to a very high pressure range from 100 MPa to 800 MPa (Naik et al., 2013; Okpala et al., 2009). It can be carried out at ambient temperature. The idea of using high pressure in food processing industry has been known for more than a century. The first report of high pressure being used as a food preservation method was by Hite in 1899. Hite (1899) reported that milk can keep for longer after a pressure treatment of 600 MPa for 1 hour at room temperature. HPP does not break covalent bonds, it can retain food quality and natural freshness while extending microbiological shelf-life (Balasubramaniam & Farkas, 2008; Muntean et al., 2016).

High-pressure processing of food is of great interest to the food industry, especially for inactivating microorganisms and extending the shelf life of foods, while causing minimal changes in food quality (Koca et al., 2011). High pressure processed foods have better texture and colour compared to that of heat processed foods. However, the structure of casein micelles is disrupted and the whey proteins, α -lactalbumin and β -lactoglobulin, are denatured after high pressure treatment (Chopde et al., 2014). According to Naik et al. (2013), any solid or liquid foods can be subjected to high pressure processing as long as they have high moisture content.

2.6.1 Principles of High Pressure Processing

After the food is treated with high pressure, the food will not return to its original shape and size because of the pressure difference between the compressibility of air and water. According to Le Chatelier's principle, any phenomenon such as phase transition,

change in molecular configuration and chemical reaction leading to a decrease in volume is enhanced by pressure. During HPP, the pressure is applied to food in all direction uniformly and simultaneously and it is known as isostatic pressure. The food will not be damaged during the treatment (Muntean et al., 2016). The basic components of a HPP system are a pressure vessel, a pressure-transmitting fluid, a material handling pressurizing system and supporting units such as heating or cooling components (Naik et al., 2013) as shown in Figure 4.

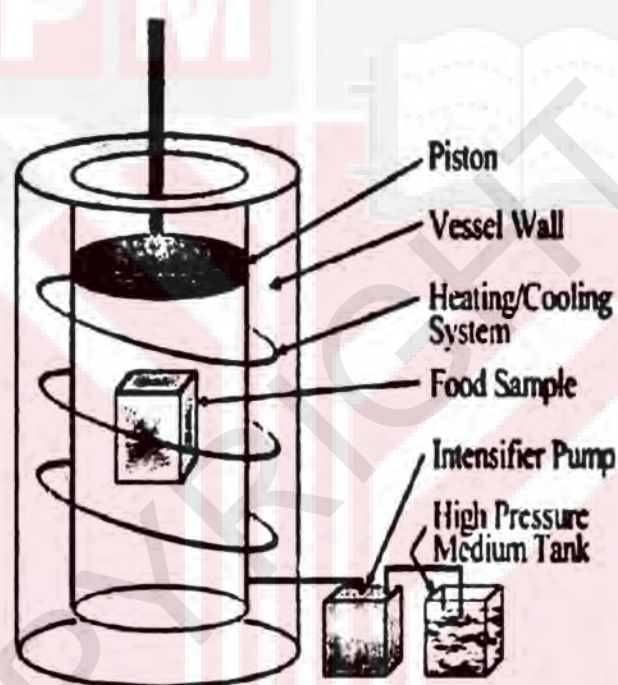


Figure 4: Components of HPP (Naik et al., 2013)

Once loaded and closed, the vessel is filled with a pressure-transmitting medium. The most common transmitting fluids are water, food-grade glycol-water solutions, silicone oil, sodium benzoate solutions, ethanol solutions and castor oil (Balasubramaniam et al., 2015). Air is removed from the vessel with an automatic deaeration valve by means of a low-pressure fastfill-and-drain pump, and high hydrostatic pressure is then generated by direct or indirect compression or by heating the pressure medium.

The flow sheet of high pressure processing is shown in Figure 5. Batch operation of high pressure processing starts with the packaging of untreated products in sterilized containers or vacuum bags and followed by loading them into a high-pressure chamber filled with water to exert the pressure. The vessel is sealed and pressure applied by forcing additional water into the sealed vessel until the desired processing pressure is achieved. The pressure is held for a specific time and then released, then the processed products are removed (Balasubramaniam & Farkas, 2008).

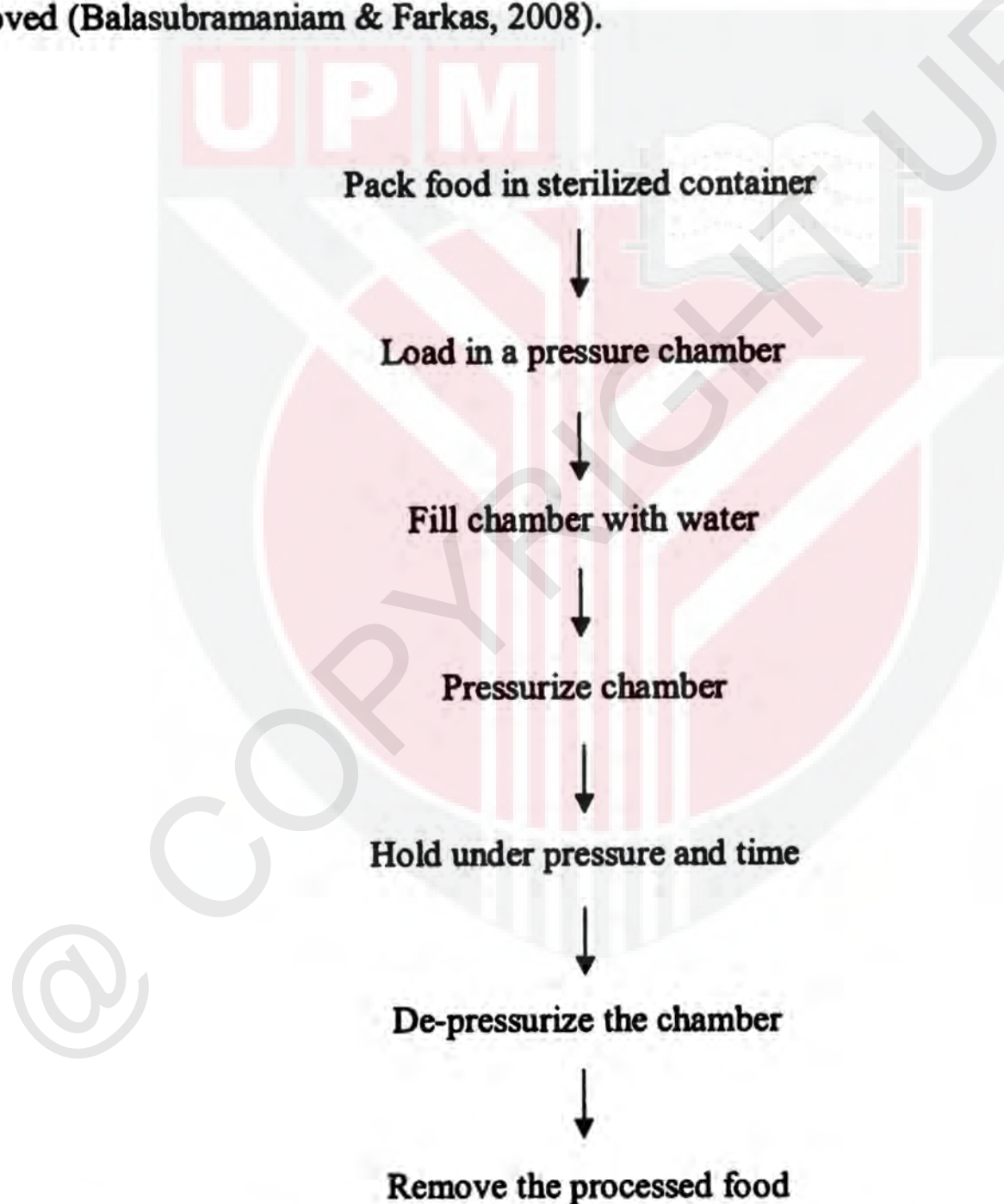


Figure 5: Flow Chart of High Pressure Processing (Chawla et al., 2011)

2.6.2 Application of High Pressure Processing

According to Naik et al. (2013), there are many advantages of using high pressure processing and the main benefits of HPP in dairy food processing include inactivation of microorganisms, structural modification of proteins and depression of freezing point of water. These could be used advantageously in several segments of food industry including seafood, meat and dairy industry. Some of the advantages of HPP are listed below:

- Retains natural antimicrobial systems without changing the sensory and nutritional quality of foods and extend shelf life up to 2-3 folds.
- Minimize thermal degradation of food components as no heating is required.
- Inactivation of microorganisms, spores and enzymes.
- High retention of flavour, colour and nutritional value.
- Pressure is transmitted uniformly and instant so that food product retains its shape.
- Process time is less dependence of product shape and size.

However, there are also some limiting aspects of high pressure processing such as the equipment of HPP is very expensive with high installation cost and only the food with high moisture content can be subjected to it as the whole phenomenon is based on compression. Table 5 shows that high pressure processing that applied in dairy industry are mostly between 200 MPa to 600 MPa.

Table 5: Application of High Pressure Processing in Dairy Industry

Conditions	Products and Results	References
100-400 MPa / 10-60 mins	Improved the microbiological quality of milk without modifying the activity of native milk enzymes such as lactoperoxidase or plasmin.	Lopez-Fandiño et al., (1996)
400-600 MPa	Main effects of HPP in milk appeared to involve dissociation of casein micelles from the colloidal to the soluble phase. Suggests that milk treated at 500 MPa or 550 MPa for 15 min may be beneficial for improving the coagulation properties of milk.	Liepa et al., (2017)
400 MPa / 5 mins	HP treatment did not significantly effect composition, pH, proteolysis, rheological properties of the unheated cheese or flowability and stretchability of the heated reduced-fat mozzarella cheese.	Sheehan et al., (2005)
200-500 MPa / 60 mins, 20°C	Pressure treatment of cheese milk increased the yield of low fat cheese by improving protein and moisture retention. Pressurization of pasteurized milk improved its coagulation properties. The protein degradation and the development of texture and flavour was also rapid and the product had lower hardness and cohesiveness and higher sensory scores.	Molina et al., (2000)

2.6.3 Effect of High Pressure Processing on Dairy Product

Effect of HPP on Rheological Properties of Glycomacropptide

Viscosity increases with increasing pressure as shown in Figure 6 because the amount of free volume in the internal structure decreases due to compression (Muhammed, 2017).

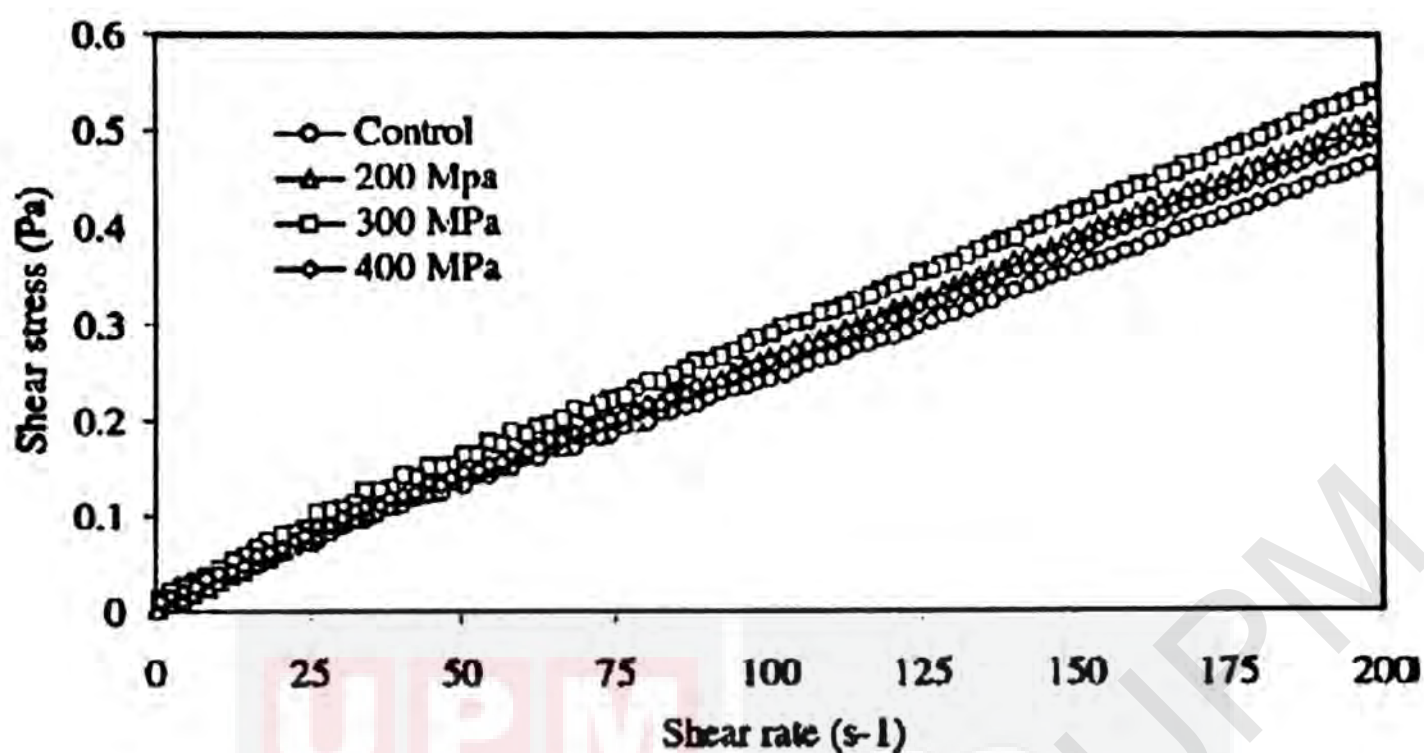


Figure 6: Effect of pressure on rheology of glycomacropptide (Ahmed & Ramaswamy, 2003)

Shear stress increased with pressure at a constant shear rate up to 300 MPa and decreased at 400 MPa. The coagulation of protein observed at pressure level of 400 MPa, which was reflected through decrease in magnitudes of shear stress, consistency coefficient and apparent viscosity.

Effect of HPP on size of milk fat globules (Microstructure)

High pressure up to 500 MPa produces some modifications on size and distribution of milk fat globules of ewe milk. HP treatments at 200 MPa and 300 MPa showed a tendency to increase the number of small fat globules with the range of 1 - 2 μm and decrease the number of MFG between the range 2 to 10 μm . The average diameter of pressurized MFG hardly changed between 100 and 500 MPa in comparison with the average size of unpressurized MFG (Gervilla et al., 2001).

Effect of HPP on pH of Bovine Milk

High pressure treatment due to casein micelle disaggregation alters the distribution of minerals and raises the concentration of ionic calcium in milk. As a result of an increase in the concentration of phosphate in the milk serum, an increase in bovine milk pH occurs (Zobrist et al., 2005). Therefore, high pressure increased the pH value of milk and caused the milk to be less acidity.

Effect of HPP on Lactose of Bovine Milk

Lactose in milk and milk products may isomerize to lactulose by heating and then degrade to form acids and other sugars. No changes in lactose have been observed after high pressure processing (100 - 400 MPa for 10 - 60 min at 25°C) suggesting no Maillard or lactose isomerization reaction occurs in milk after pressure treatment (Lopez-Fandiño et al., 1996).

CHAPTER 3

METHODOLOGY

3.1 EXPERIMENTAL DESIGN

The flow chart of the experimental work is shown in Figure 7. The fresh goat milk was purchased from local farm packed in a quantity of 100 ml per pouch. These samples were subjected to high pressure treatment at the pressure range from 200 MPa to 600 MPa for 15 minutes with the interval of 5 minutes at constant room temperature. After high pressure treatment, proximate analysis, pH, viscosity, lactose content and microstructure of the goat milk samples were being tested. Proximate analysis and microstructure were analysed in powder form where the goat milk samples treated with high pressure processing were subjected to freeze-drying first. After conducted all the analysis, statistical analysis was carried out to analyse the data obtained. Then, Central Composite Design (CCD) of Response Surface Methodology (RSM) was used to optimize HPP parameter conditions (pressure and time) of the goat milk.

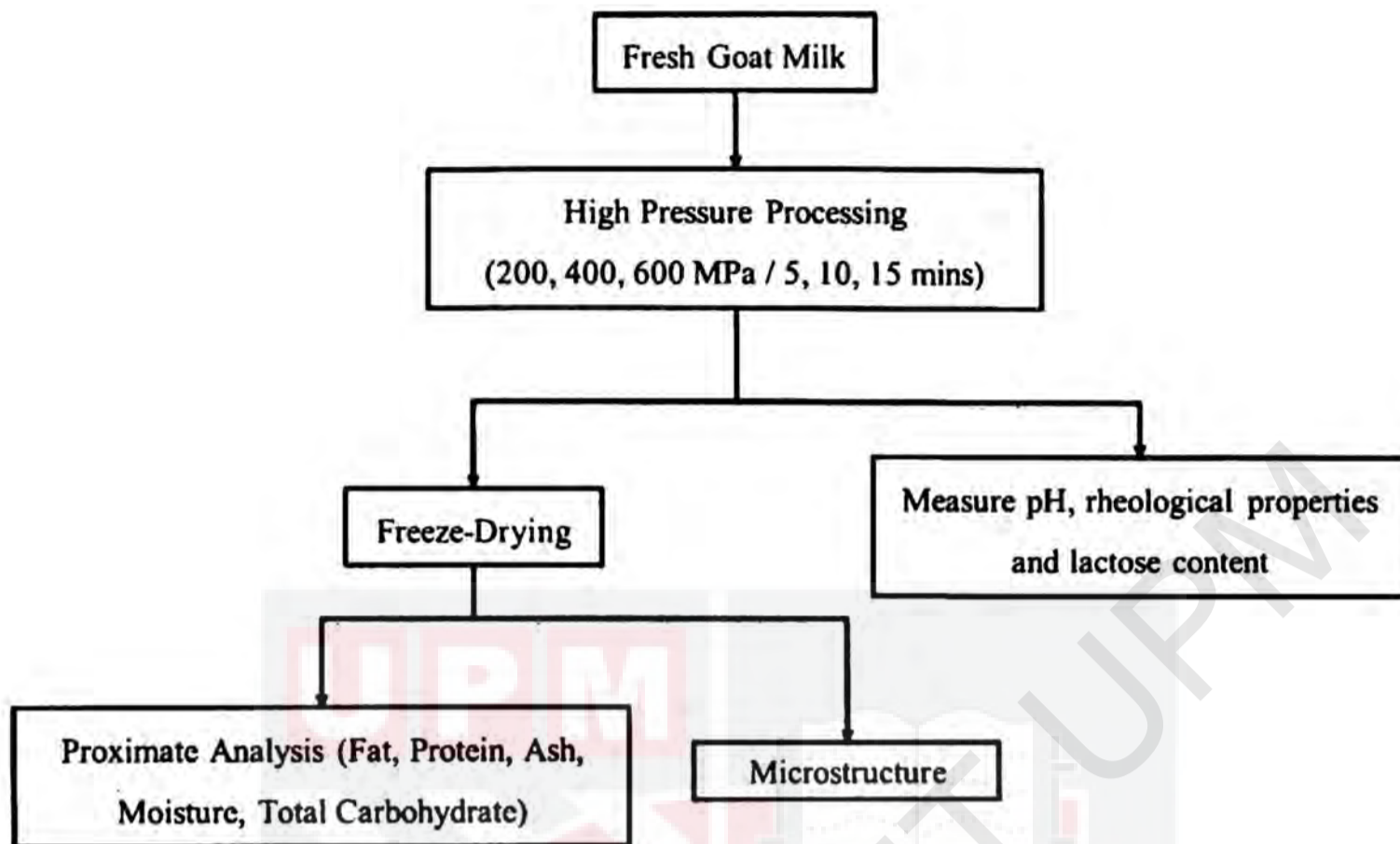


Figure 7: Flow of Experimental Design

3.2 SAMPLE PREPARATION

Fresh raw goat milk was obtained from MFRS Agrofarm at Kajang, Selangor. The fresh goat milk was filled in bottles without being processed and stored at chilled condition (4°C) prior to delivery. Control samples were kept refrigerated at 4°C while test samples were being pressure treated. Milk samples obtained were filled in food grade Quiware vacuum bag (embossed) composed of polyethylene with outer layer of nylon for added strength and rigidity. These vacuum bag can withstand high temperatures and pressure and it is suitable for high pressure applications. 100 ml of goat milk sample was packed in vacuum bag for HPP treatment. Packed milk samples were treated for each processing condition (pressure and time) and the properties of milk were analyzed.

3.3 HIGH PRESSURE PROCESSING (HPP) OF GOAT MILK

The vacuum packed goat milk samples were subjected to high pressure treatment at 200 MPa, 400 MPa and 600 MPa at 5, 10 and 15 minutes. Temperature for each treatment was set at 25°C. The three different extreme pressures were selected to observe the effect of high pressure processing on the quality of goat milk. The vacuum packed goat milk samples were introduced into the High Pressure Processing Machine (Figure 8) using distilled water as the pressure-transmitting medium in the pressure temperature. The temperature was indicated by the thermocouple which was equipped with the HPP chamber during the HPP cycle.

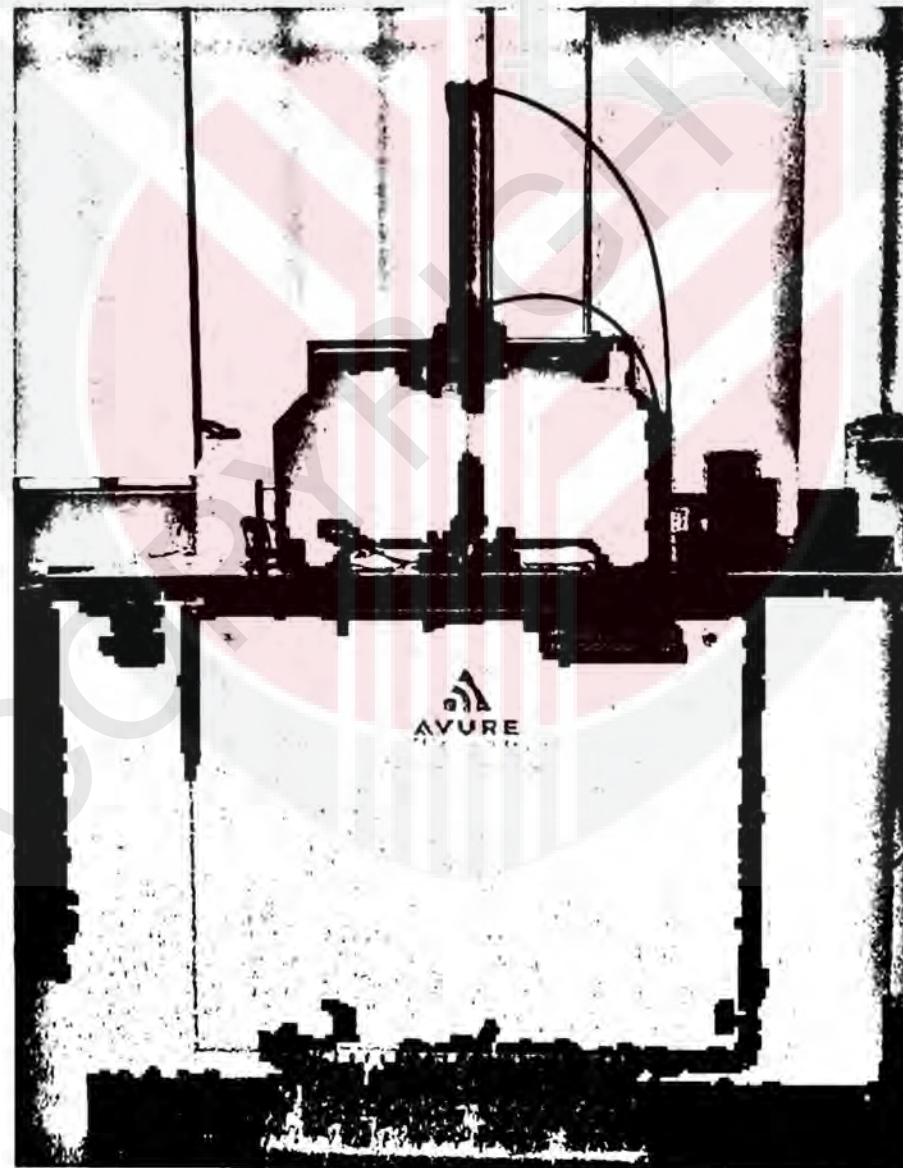


Figure 8: High Pressure Processing Machine at Agro-Biotechnology Institute, MARDI, Serdang, Selangor

3.4 FREEZE-DRYING

The goat milk samples treated with HPP that were frozen in freezer at -20°C for 24h (Hazirah et al., 2018) were subjected to freeze-dryer for 3 days to produce goat milk in solid form. The goat milk powder obtained was used for examination of microstructure and proximate analysis.

3.5 MICROSTRUCTURE

Microscopy observations of goat milk powder control and samples were using Scanning Electron Microscope (SEM) (S-3400N, Hitachi, Japan). The samples were coated with carbon prior to examination to avoid charging of specimens. The fat globules present in the goat milk powder were analysed from the image generated with the magnification of 3000x. In scanning electron microscopy an image is obtained of the surface of the specimen. ImageJ (1.52a, NIH, United States) was used to analyse the geometrical properties of the milk fat globules.

3.6 RHEOLOGICAL PROPERTIES

Rheological measurements were carried out in an AR-G2 rheometer (TA Instruments, Delaware) using cone and plate sensor geometry (cone angle 1° , 60mm diameter) under controlled stress and temperature (Gabas et al., 2012). The thin layer of goat milk in contact with temperature-controlled metal base plate enables measurements to be made at high rates of shear without the need to compensate for the heating effect of the high shear rate (Mohammed, 2010). Shear rate was set to range from 0.01 to 1000 s^{-1} and the gap between top plate and the Peltier plate was set to be 100 micro meters. The

viscosity values were obtained as the slope from the graph of shear stress against shear rate.

3.7 LACTOSE CONTENT

High performance liquid chromatography (HPLC) was used to determine the lactose content in goat milk. This is a rapid, simple and specific technique for the determination of sugars in food (Pirisino, 1983). Degassed HPLC grade water was used as the mobile phase. The lactose content was detected using evaporative light scattering detector (ELSD).

3.8 pH

The pH of the goat milk sample was determined using pH meter. The pH was calibrated with standard buffers 4 and 7 before measuring the pH of the goat milk sample (Al-Abdulkarim et al., 2013).

3.9 PROXIMATE ANALYSIS

The goat milk powder samples produced after freeze-drying were analyzed for their composition according to standard procedures. Data analysis was performed in duplicate.

3.9.1 Fat

The determination of fat contents of the goat milk samples (before and after HPP) was carried out using hexane as solvent for Soxtec extraction. A FOSS Soxtec™ 2050

was used. One gram of goat milk powder sample was accurately weighed, transferred to thimble and extracted with 90ml of hexane at 155°C for one and half hour. After extraction, the solvent was evaporated to dryness in an oven at 105°C weighed and then the percentage of the oil was calculated (Al-Abdulkarim et al., 2013).

3.9.2 Protein

This was determined by the Kjeldahl method using Kjeltec™ model 2300. The method involved digestion of the goat milk sample at 420°C for 1 h to liberate the organically bound nitrogen in the form of ammonium sulphate. The ammonia in the digest (ammonium sulphate) was then distilled off into a boric acid receiver solution, and then titrated with standard hydrochloric acid (Oladeji et al., 2016). For dairy product, a conversion factor of 6.38 was used to convert from total nitrogen to percentage crude protein (Gomaa et al., 2014).

3.9.3 Moisture Content

The moisture content of goat milk powder was analysed according to AOAC method. The goat milk sample was dried at 100 - 105°C for 24 hours in a Memmert Oven (Alyaqoubi et al., 2015).

3.9.4 Ash Content

Gravimetric method was used to determine ash content of goat milk sample. The sample was incinerated with a furnace at 550°C as described by AOAC 2000 (Alyaqoubi et al., 2015).

3.9.5 Total Carbohydrate

Proximate carbohydrate content in the milk sample was determined by difference. It was calculated by deducting the total amount of moisture, ash, protein and fat with 100% (Lai et al., 2016).

3.10 OPTIMIZATION OF HPP PARAMETER CONDITIONS (PRESSURE AND TIME)

Response Surface Methodology (RSM) based on Central Composite Design (CCD) in Design Expert (Stat-Ease Inc., Minneapolis, MN, USA) software version 11.0 was employed to optimize HPP parameter conditions (pressure and time) of the goat 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 (Edem & Elijah, 2016).

Pressure and time of HPP treatment were the 2 independent variables in the optimization process. There were three coded factor levels, -1, 0 and +1 (where -1 corresponded to the low level of each factor, 0 to the middle level, and +1 to the high level) as shown in Table 6. Dependent variables such as pH, lactose and total carbohydrate of goat milk were evaluated as responses for the factors studied.

Table 6: Experimental Range and Levels of Independent Variables

Independent Variables	Coded Levels		
	-1	0	1
Pressure (MPa)	200	400	600
Time	5	10	15

3.11 STATISTICAL ANALYSIS

Each sample was analysed in duplicate and the means of two data was presented. The data were assessed by analysis of variance (ANOVA) by one way using Minitab 18 (Version 18, Minitab Statistical Software, United States). Evaluations were based on a 5% significance level ($P < 0.05$).

CHAPTER 4

RESULT AND DISCUSSION

4.1 EFFECT OF HIGH PRESSURE ON MICROSTRUCTURE

The microstructure of fresh goat milk and pressure treated goat milk are shown at the Figures 9, 10, 11 and 12. Table 7 shows the average geometrical parameter values of goat's milk fat globules analysed using ImageJ (1.52a, NIH, United States) from the micrographs obtained. As shown in Figure 9, the milk fat globules of raw milk was remained intact, no burst or cleavage had been observed. The mean diameter of it was $6.126 \pm 0.609 \mu\text{m}$ with the mean area of $33.908 \pm 4.619 \mu\text{m}^2$. Raw milk's fat globule had the best spherical structure with the roundness of 0.877 ± 0.099 although some of the fat globules were coalesced.

Table 7: Average Geometrical Parameter Values of Goat's Milk Fat Globules

Pressure (MPa)	Time (mins)	Geometrical Properties		
		Diameter (μm)	Roundness	Area (μm^2)
Control		6.126 ± 0.609	0.877 ± 0.099	33.908 ± 4.619
200	5	7.136 ± 2.175	0.798 ± 0.051	49.206 ± 22.551
	10	6.176 ± 2.255	0.864 ± 0.090	44.044 ± 31.135
	15	5.894 ± 1.634	0.890 ± 0.049	43.512 ± 35.412
400	5	8.300 ± 2.830	0.830 ± 0.061	64.181 ± 34.428
	10	6.805 ± 1.226	0.814 ± 0.071	43.321 ± 14.188
	15	10.066 ± 1.624	0.694 ± 0.022	74.779 ± 21.307

600	5	5.397 ± 0.750	0.859 ± 0.091	24.627 ± 8.168
	10	5.215 ± 1.070	0.876 ± 0.109	22.310 ± 14.084
	15	5.651 ± 2.228	0.879 ± 0.119	25.032 ± 19.808



Figure 9: Microstructure of Fresh Goat Milk

The microstructure of goat milk when treated with 200 MPa for 5 mins, 10 mins and 15 mins are shown in Figure 10. The average diameter of MFG decreased when the holding time increased with the value of $7.136 \pm 2.175 \mu\text{m}$, $6.176 \pm 2.255 \mu\text{m}$ and $5.894 \pm 1.624 \mu\text{m}$ respectively. More small size fat globules were observed when goat milk were treated with 200 MPa for 10 and 15 minutes. No much differences were observed for the mean area of three treated samples with different holding time as all of them consisted of big and small size fat globules. The goat's milk fat globules after subjected to 200 MPa for 5 mins was more to oval shape with the average roundness of 0.798 ± 0.051 . Distortion of fat globules was found when the goat milk was treated with 200 MPa for 10 mins.



Figure 10: Microstructure of Goat Milk when Treated with 200 MPa for (a) 5mins, (b) 10mins, (c) 15mins

The fat globules of goat milk when treated with 400 MPa for 5 mins (Figure 11a), 10 mins (Figure 11b) and 15 mins (Figure 11c) showed very much differences in terms of shape and size. The average diameter of fat globules increased from $8.300 \pm 2.830 \mu\text{m}$ for goat milk treated with 400 MPa for 5 mins to $10.066 \pm 1.624 \mu\text{m}$ for goat milk treated with 400 MPa for 15 minutes due to the fission of fat globules was observed for the latter one. Same reason can be applied to the increasing of mean area and decreasing of roundness of fat globules.



Figure 11: Microstructure of Goat Milk when Treated with 400 MPa for (a) 5mins, (b) 10mins, (c) 15mins

Figure 12 shows the microstructure of goat milk when treated with 600 MPa. The average diameter of fat globules of goat milk when 600 MPa was applied for 5 mins, 10 mins and 15 mins was $5.397 \pm 0.750 \mu\text{m}$, $5.215 \pm 1.070 \mu\text{m}$ and $5.651 \pm 2.228 \mu\text{m}$ respectively (Table 7). It seemed that high pressure hardened the MFG membrane and therefore no coalesce or fission of fat globules was found when the goat milk was treated with 600 MPa. There was more number of small size fat globules compared with the size of milk fat globules when the goat milk were treated with 200 MPa and 400 MPa. Similar results was obtained by Gervilla et al. (2001).



Figure 12: Microstructure of Goat Milk when Treated with 600 MPa for (a) 5mins, (b) 10mins, (c) 15mins

The smallest diameter of goat's milk fat globules ($5.215 \mu\text{m}$) was observed when the goat milk was treated with 600 MPa for 10 minutes while goat milk that subjected to pressure treatment of 400 MPa for 10 minutes had the largest diameter ($10.066 \mu\text{m}$) of goat's milk fat globules. According to Jandal (1996), the size of goat's milk fat globules varied from 0.1 to $10 \mu\text{m}$. In cheese making, small fat globules were found to improve stretching and elasticity, compared to large fat globules (Truong et al., 2016).

4.2 EFFECT OF HIGH PRESSURE PROCESSING ON RHEOLOGY OF GOAT MILK

Both fresh goat milk and high pressure processing goat milk are Newtonian fluid where the graph of shear stress is directly proportional to shear rate. The slope from the graph of shear stress versus shear rate represents the viscosity of the goat milk. The viscosity of goat milk increases when the pressure and holding time increase. Untreated goat milk had lowest viscosity compared to all the high pressure treated goat milk.

Based on the graph obtained in Figure 13, fresh goat milk had lowest viscosity while goat milk that treated with 200 MPa for 10 minutes had highest viscosity. At 200 MPa, goat milk exhibits as Newtonian fluid for all 3 different holding times as the graph of shear stress against shear rate showed a linear line. Goat milk treated with 200 MPa

for 10 minutes had the highest viscosity among all the goat milk sample that treated with 200 MPa. This can be explained by the high lactose content found in the goat milk sample when it was treated for 200 MPa with the holding time of 10 minutes (Table 9 in Section 4.4.1).

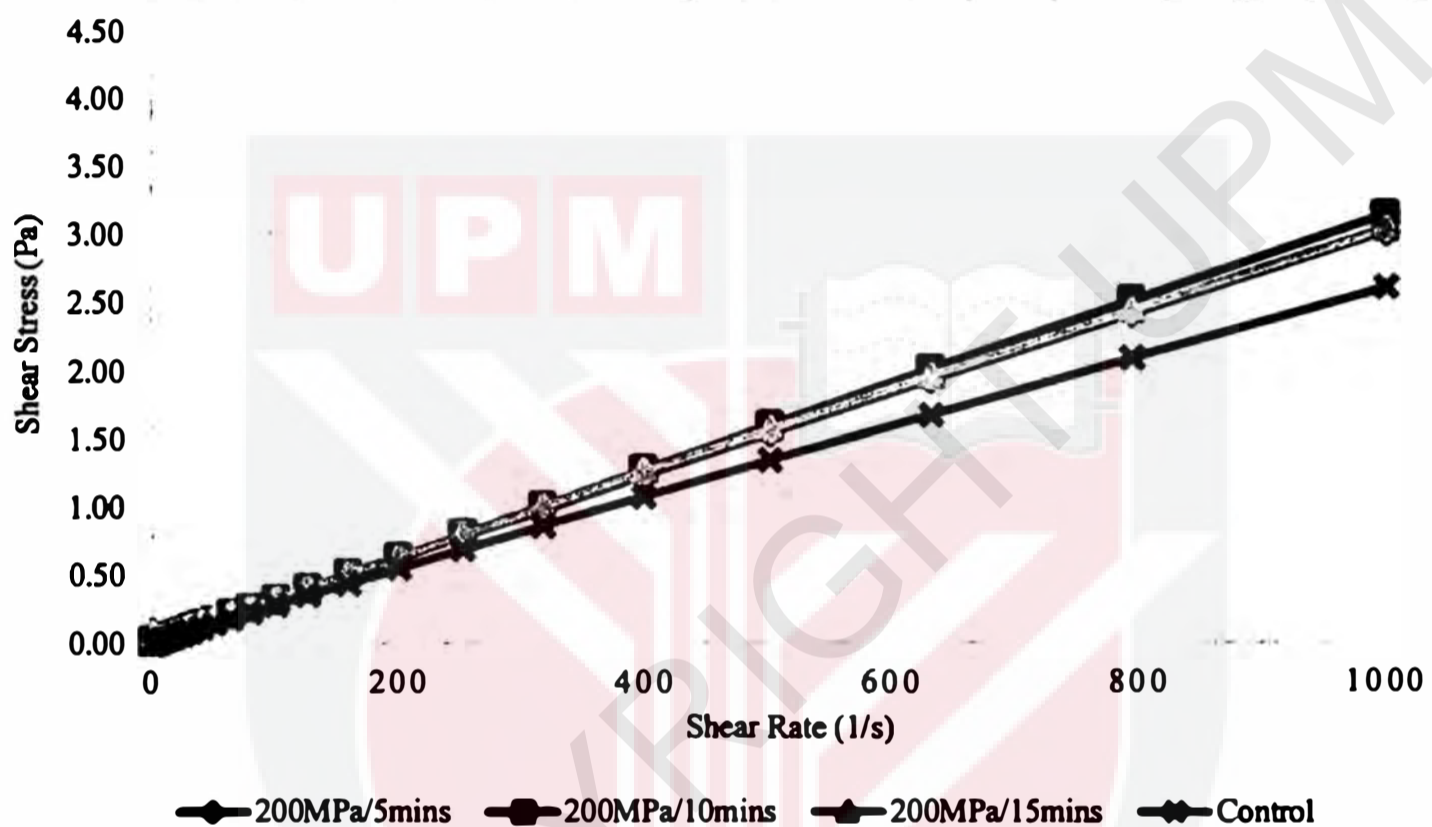


Figure 13: Graph of Shear Stress vs Shear Rate of Goat Milk Treated at 200 MPa for Different Holding Times

The viscosity of goat milk increases with holding time when the goat milk was treated with 400 MPa and 600 MPa which are shown in Figure 14 and Figure 15 respectively. It was found that when the holding time during pressure treatment increased, the viscosity of goat milk also increased.

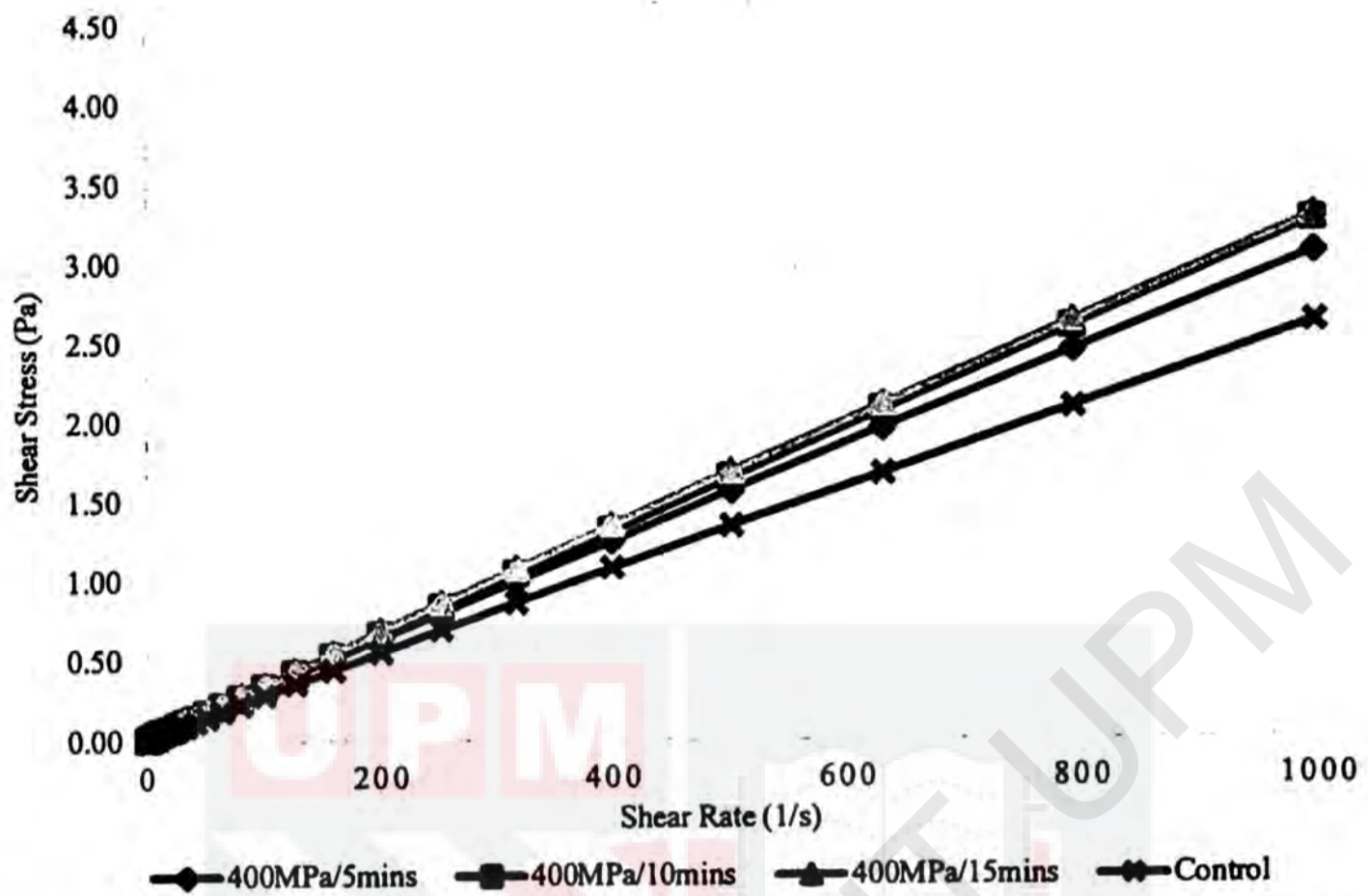


Figure 14: Graph of Shear Stress vs Shear Rate of Goat Milk Treated at 400 MPa for Different Holding Times

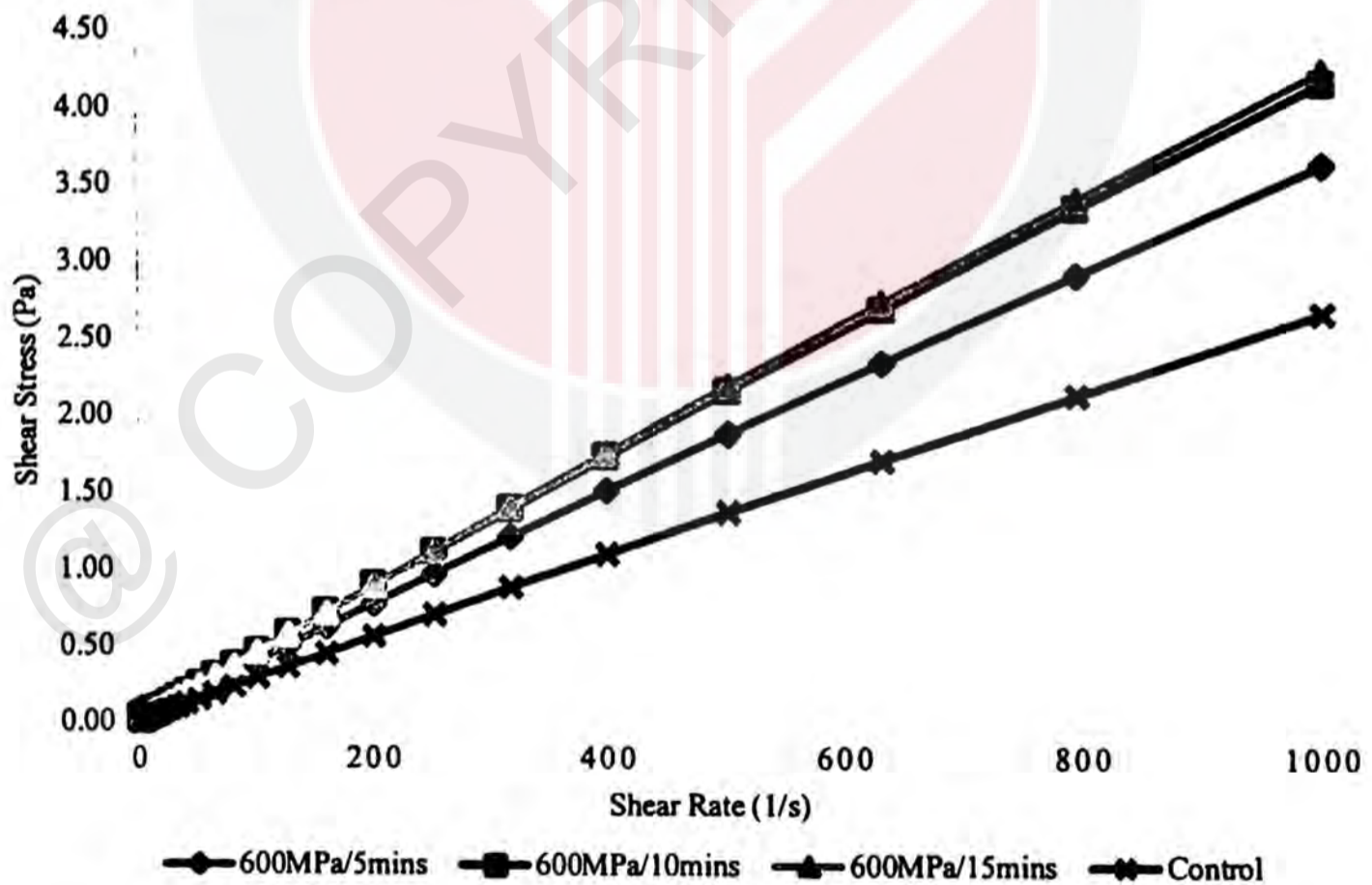


Figure 15: Graph of Shear Stress vs Shear Rate of Goat Milk Treated at 600 MPa for Different Holding Times

For the goat milk that treated with same holding time but different pressure, the viscosity also showed an increasing trend. On Figure 16, 17 and 18, goat milk treated with 600 MPa had highest viscosity for all holding time (5 mins, 10 mins and 15 mins). For each holding time, the viscosity of goat milk increased as the subjected pressure increased.

According to Mussa & Ramaswamy (1997), increased in pressure during ultra high pressure treatment increased the viscosity of milk. When milk is subjected to HP, the casein micelles disintegrate into smaller particles (Huppertz et al., 2002) can cause an increase of viscosity of the milk (Kim et al., 2008). Besides, the increasing of viscosity with pressure and time can be correlated with the increasing of lactose content as discussed in Section 4.4.1.

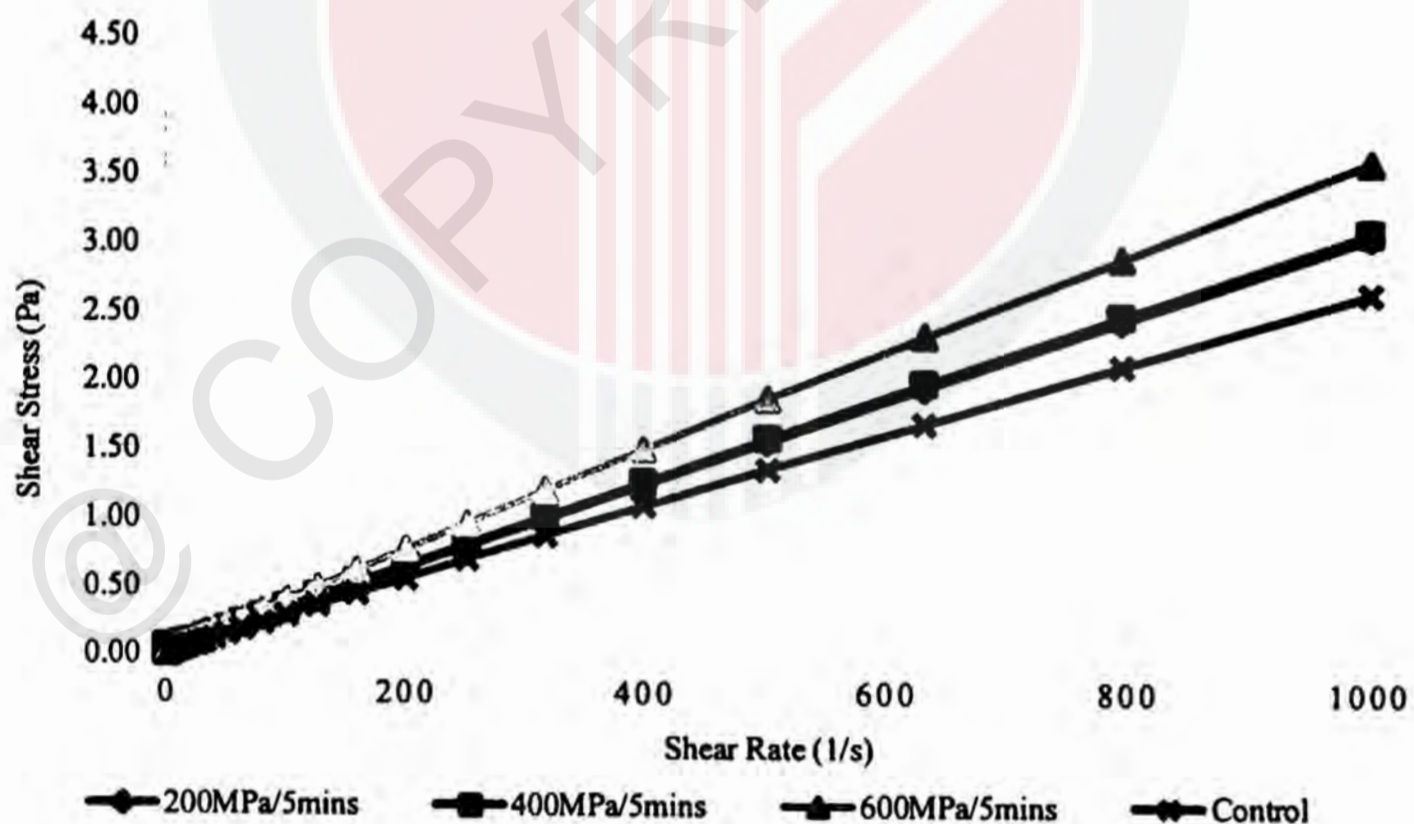


Figure 16: Graph of Shear Stress vs Shear Rate of Goat Milk Treated at Different Pressures for 5 minutes

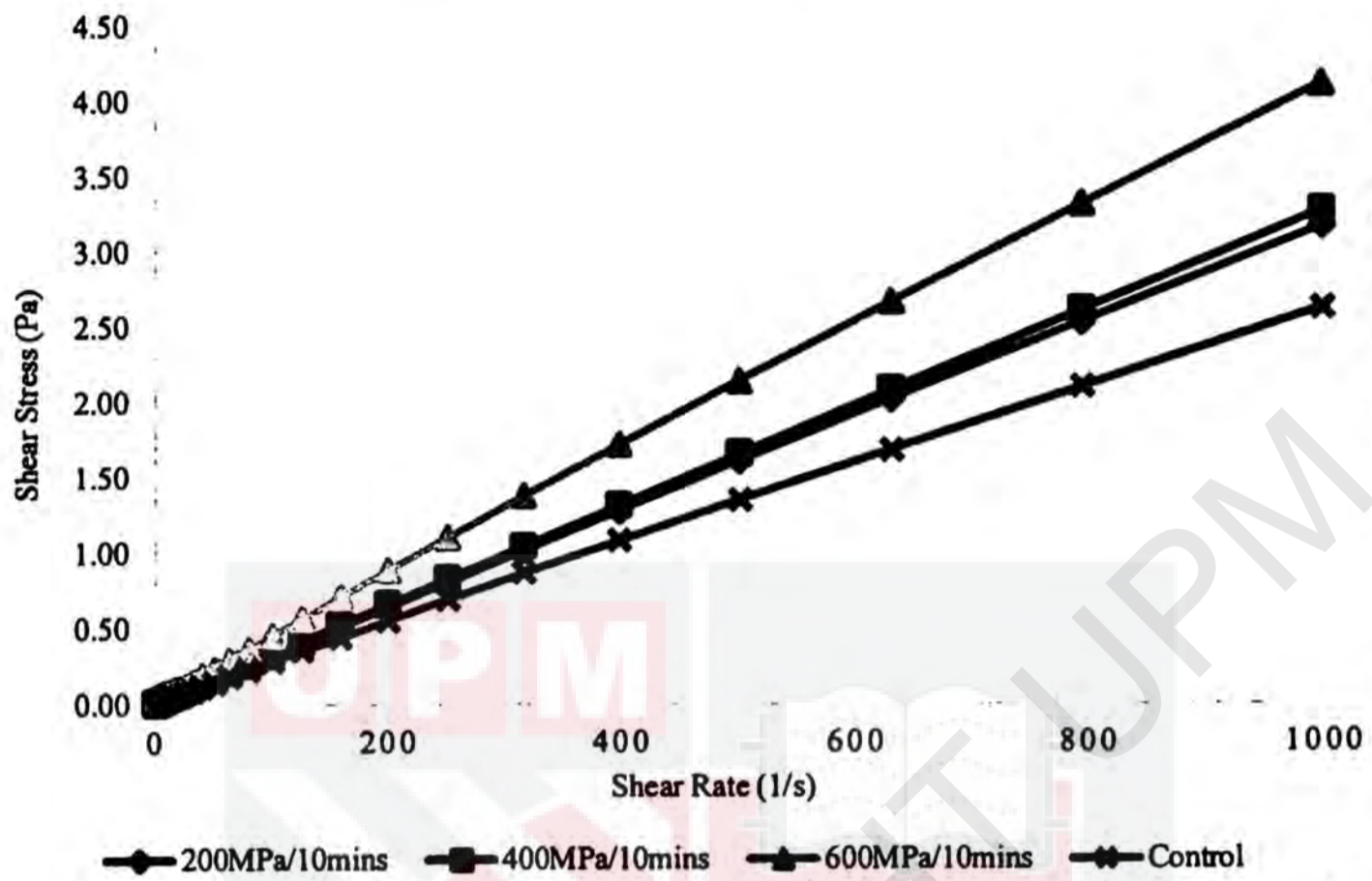


Figure 17: Graph of Shear Stress vs Shear Rate of Goat Milk Treated at Different Pressures for 10 minutes

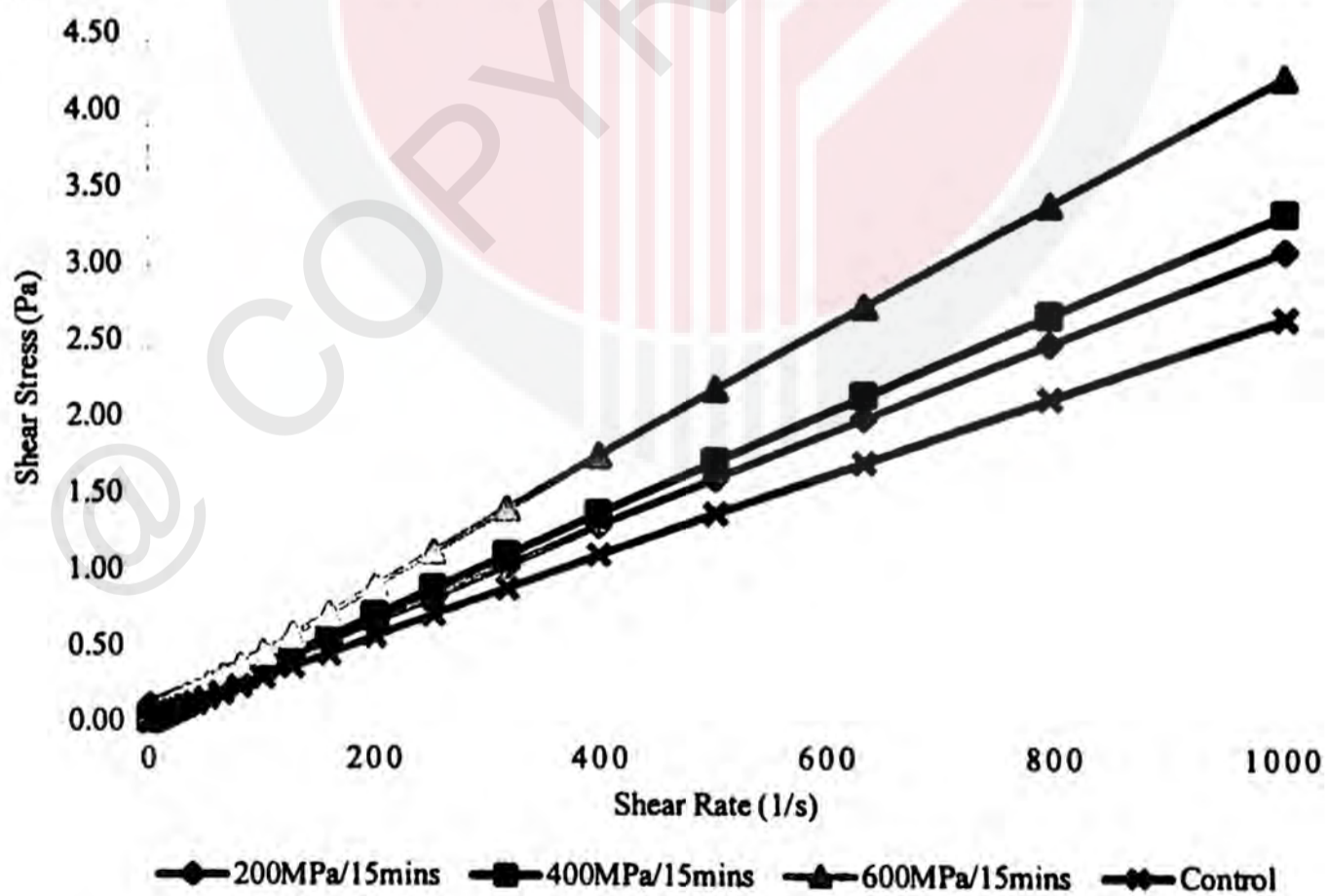


Figure 18: Graph of Shear Stress vs Shear Rate of Goat Milk Treated at Different Pressures for 15 minutes

4.3 PROXIMATE ANALYSIS

The proximate analysis were performed after freeze-drying process. The moisture content became lower after freeze-drying since the liquid milk was transformed to solid milk powder. The percentage of total carbohydrate was determined by difference. It was calculated by deducting the total amount of fat, protein, ash and moisture content with 100%.

In general, the results showed that the fat, protein, ash, moisture content and total carbohydrate of goat milk were not influenced by the high pressure treatment (Table 8). The values were changed slightly but statistically insignificant ($P>0.05$) when compared with the control sample. Similar result was reported by Koca et al. (2011).

Table 8: Composition of Goat Milk

Pressure (MPa)	Time (mins)	Composition (Percentage %)				
		Fat	Protein	Ash	Moisture Content	Total Carbohydrate
Control		29.35 ± 0.19 ^a	30.38 ± 0.19 ^{abcd}	5.52 ± 0.81 ^a	1.33 ± 0.03 ^a	33.43 ± 1.22 ^a
200	5	32.78 ± 0.50 ^a	28.78 ± 0.67 ^{bcd}	5.55 ± 0.66 ^a	1.43 ± 0.02 ^a	31.46 ± 0.48 ^a
	10	32.57 ± 1.99 ^a	29.14 ± 0.68 ^{abcd}	6.04 ± 0.07 ^a	1.38 ± 0.01 ^a	30.87 ± 1.22 ^a
	15	33.80 ± 0.93 ^a	28.64 ± 0.02 ^{cd}	5.88 ± 0.06 ^a	1.42 ± 0.11 ^a	30.27 ± 0.86 ^a
400	5	33.57 ± 1.25 ^a	28.52 ± 0.59 ^d	6.00 ± 0.04 ^a	1.42 ± 0.01 ^a	30.50 ± 1.88 ^a
	10	31.30 ± 1.79 ^a	30.79 ± 0.26 ^a	6.06 ± 0.12 ^a	1.39 ± 0.04 ^a	30.46 ± 1.61 ^a
	15	30.80 ± 1.99 ^a	30.57 ± 0.07 ^a	5.59 ± 0.69 ^a	1.40 ± 0.08 ^a	31.63 ± 1.46 ^a
600	5	30.13 ± 1.88 ^a	31.06 ± 0.60 ^a	5.74 ± 0.36 ^a	1.35 ± 0.03 ^a	31.73 ± 0.88 ^a

10	30.35 ± 2.49 ^a	30.56 ± 0.09 ^{ab}	5.55 ± 0.77 ^a	1.34 ± 0.05 ^a	32.19 ± 1.76 ^a
15	31.98 ± 2.88 ^a	30.51 ± 0.78 ^{abc}	5.51 ± 0.75 ^a	1.36 ± 0.04 ^a	30.64 ± 1.39 ^a

Means followed by different letter (a-d) in the same column are significantly different among treatments (P<0.05)

4.4 EFFECTS OF HIGH PRESSURE PROCESSING

4.4.1 Effect of High Pressure Processing on Lactose Content of Goat Milk

The lactose content and viscosity of goat milk treated by different conditions of high pressure is shown in Table 9. Viscosity was obtained from the gradient of the graph in Section 4.2. Based on the result obtained, the differences in lactose content of pressure treated goat milk were significant (P<0.05) as compared with the control sample. Lactose content of goat milk increased by double after treated with high pressure.

Goat milk treated with three different pressure for 5 minutes also showed a significant different (P<0.05), compared with the sample treated with same pressure but different holding time. For the holding time of 10 minutes and 15 minutes, the results showed no significant different (P>0.05) at the same pressure. The highest lactose content among high pressure treated milk sample was the goat milk treated with 600 MPa for 15 minutes with lactose content of 2.9856 ± 0.01 g/100ml while the lowest lactose content of 2.5606 ± 0.01 g/100ml was obtained when the goat milk was treated with 200 MPa for 5 minutes. The increasing of lactose content might be due to the breakdown of polysaccharides into the disaccharides (lactose) during high pressure treatment.

The studies reported by Wang et al. (2015) and Marti et al. (n.d.) had proven that the lactose concentration influenced the viscosity. Viscosity increases as lactose content

increases. Therefore, the increasing of viscosity as discussed in Section 4.2 can be related the increasing of lactose content with the pressure and holding time during high pressure treatment.

Table 9: Effect of High Pressure Treatment on Lactose and Viscosity of Goat Milk

Pressure (MPa)	Time (mins)	Lactose (g/100ml)	Viscosity (Pa.s)
Control		1.2528 ± 0.01 ^c	0.0026
200	5	2.5606 ± 0.01 ^d	0.0030
	10	2.7795 ± 0.04 ^b	0.0032
	15	2.7687 ± 0.03 ^{bc}	0.0031
400	5	2.5399 ± 0.02 ^d	0.0030
	10	2.7969 ± 0.00 ^b	0.0033
	15	2.7969 ± 0.02 ^b	0.0033
600	5	2.6795 ± 0.02 ^c	0.0036
	10	2.8948 ± 0.03 ^a	0.0041
	15	2.9856 ± 0.01 ^a	0.0042

Means followed by different letter (a-e) in the same column are significantly different among treatments (P<0.05)

4.4.2 Effect of High Pressure Processing on pH of Goat Milk

The pH value of goat milk treated by different conditions of high pressure is shown in Table 10. pH of goat milk increases for every condition treated by high pressure as compare to the fresh goat milk without HPP treatment. The differences in the pH were not significant (P>0.05) among all high pressure treated milk samples. However, high pressure treatment affected pH of milk considerably (P<0.05) compared with a control sample.

Generally, high pressure treatment can cause the increase of concentration of calcium ion in milk (Zobrist et al., 2005). Therefore, high pressure increased the pH value of milk and caused the milk to be less acidity. However, the pH values of the fresh and

pressure treated milks were in the standard range of fresh milk, which is 6.6 to 6.8 (Kim et al., 2008). The highest pH value of 6.68 ± 0.00 was showed when the goat milk was treated with 400MPa for 10 minutes while the goat milk treated with 200MPa for 10 minutes showed a lowest pH value of 6.64 ± 0.01 . This result indicated that high pressure processing did not affect the pH level in the milk samples.

Table 10: Effect of High Pressure Treatment on pH of Goat Milk

Pressure (MPa)	Time (mins)	pH
Control		6.61 ± 0.01^b
200	5	6.67 ± 0.02^a
	10	6.64 ± 0.01^{ab}
	15	6.67 ± 0.01^a
400	5	6.65 ± 0.00^{ab}
	10	6.68 ± 0.00^a
	15	6.65 ± 0.01^{ab}
600	5	6.66 ± 0.00^a
	10	6.65 ± 0.01^{ab}
	15	6.65 ± 0.01^{ab}

Means followed by different letter (a-b) in the same column are significantly different among treatments ($P < 0.05$)

4.5 OPTIMIZATION OF HPP PARAMETER CONDITIONS (PRESSURE AND TIME)

4.5.1 Model Fitting

The experimental results of the effect of HPP pressure and time on pH, lactose and carbohydrate of goat milk are presented in Table 11. These experimental data were used in RSM program to create the best predicted model and its statistical analysis.

Table 11: Design Matrix of Central Composite Design Obtained from RSM and Experimental Values of the Responses for Goat Milk Treated by HPP

Run	Actual Variables		Responses		
	Pressure (MPa)	Time (mins)	pH	Lactose (g/100ml)	Total Carbohydrate (%)
1	400	10	6.68	2.7969	30.46
2	400	15	6.65	2.7969	31.63
3	400	10	6.68	2.7969	30.46
4	400	5	6.65	2.5399	30.50
5	400	10	6.68	2.7969	30.46
6	200	10	6.64	2.7795	30.87
7	600	10	6.65	2.8948	32.19
8	600	5	6.66	2.6776	31.73
9	400	10	6.68	2.7969	30.46
10	200	5	6.67	2.5607	31.46
11	200	15	6.67	2.7687	30.27
12	600	15	6.65	2.9856	30.64
13	400	10	6.68	2.7969	30.46

Based on the results of analysis of variance (ANOVA), the fitness of the final predicted polynomial model was considered. Fit summary statistics was used to choose a suitable model for a response comparing the models based on P-values. A model is considered significant, if the P-value is less than 0.05 or at least less than 0.1. ANOVA is an important tool for the evaluation of significance and goodness of fit of regression model and significance of individual model coefficients (Fentie et al., 2014).

Result in Table 12 showed that the probability value (P-value) of the response models of lactose was less than 0.05, suggesting that the model for the response was statistically correct and effective. Results of effect of process variables on total carbohydrate indicated that, the P-value was more than 0.05 for the model. Thus, the model had a non-significant effect on the response, total carbohydrate.

The results for the effect of process variables on pH indicated that, there was no P-value or F-value for the model. Thus, the terms in the model had neither a significant

effect nor a non-significant effect on the response. Lack of fit is a measure of fitness of the model. Thus Model F-value of total carbohydrate of 1.11 implied the model was not significant relative to the noise. There is a 36.76% chance that an F-value could occur due to noise. While for lactose, the model F-value of 121.03 implied the model was significant.

Table 12: 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
pH	Model	0.0000	-			Not significant
	Residual	0.0027	0.0002			
	Lack of Fit	0.0027	0.0003			
	Pure Error	0.0000	0.0000			
Lactose	Model	0.1698	0.0340	121.03	<0.0001	Significant
	Residual	0.0020	0.0003			
	Lack of Fit	0.0020	0.0007			
	Pure Error	0.0000	0.0000			
Total Carbohydrate	Model	0.8702	0.4351	1.11	0.3676	Not significant
	Residual	3.9300	0.3928			
	Lack of Fit	3.9300	0.6546			
	Pure Error	0.0000	0.0000			

Significant at P<0.05

Regression Coefficient, R^2 is a measure of the amount of variation around the mean explained by the model, which also known as a degree of fit measurement that is beneficial for measuring the proportion of total variability explained by the model. The best R^2 value for a good model fitting was estimated between 0.8 and 1.0 had been reported by Jusoh et al. (2013). However, Gupta et al. (2014) reported that $R^2 < 0.80$ indicating a fair fit of the model but reliable in making predictions. Hence, R^2 value of

0.9886 for lactose suggesting that it was a good model fitting while total carbohydrate indicated fair fit of the model with R^2 value of 0.1814 (Table 13).

The C.V. describes the extent to which the data are dispersed. C.V.>10% indicated that the experiment was less precise. The lower C.V. value for pH, lactose and total carbohydrate indicated that the result was precise and reliable.

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

$$\text{Lactose} = 0.0336X_1 + 0.0996X_2 + 0.0025X_1X_2 + 0.0097X_1^2 + 0.0331X_2^2$$

Table 13: Estimated Regression Coefficients of the Fitted Polynomial Model Representing the Relationship between Responses and Process Variables at the Design Response Surface

Source	pH		Lactose		Total Carbohydrate	
	Coefficient	P-value	Coefficient	P-value	Coefficient	P-value
A – Pressure	-	-	0.0336	< 0.0001	0.6483	0.2278
B – Time	-	-	0.0996	< 0.0001	0.2220	0.4695
AB	-	-	0.0025	0.0204	-	-
A ²	-	-	0.0097	0.0006	-	-
B ²	-	-	0.0331	< 0.0001	-	-
R ²	0.0000		0.9886		0.1814	
Adjusted R ²	0.0000		0.9804		0.0177	
Predicted R ²	-0.1736		0.9047		-0.4995	
C.V. %	0.2260		0.6051		2.0300	
Adeq. Prec.	-		36.6312		3.4613	

R²: Regression Coefficient ; C.V.: Coefficient of Variation ; Adeq. Prec.: Adequate Precision

4.5.2 Effect of Process Condition on Lactose of Goat Milk

The 3-Dimensional plot of RSM of the effect of HPP pressure and time on lactose content of goat milk is shown in Figure 19. Lactose content of goat milk ranged from 2.5399 to 2.9856 g/100ml. ANOVA showed that lactose content of goat milk was significantly affected by both pressure and time of HPP. The highest value of lactose content was shown when goat milk was treated with 600 MPa and 15 minutes. The result showed that lactose content was found to be a function of quadratic terms of HPP pressure and time, with a mean lactose content of 2.7700 g/100ml.

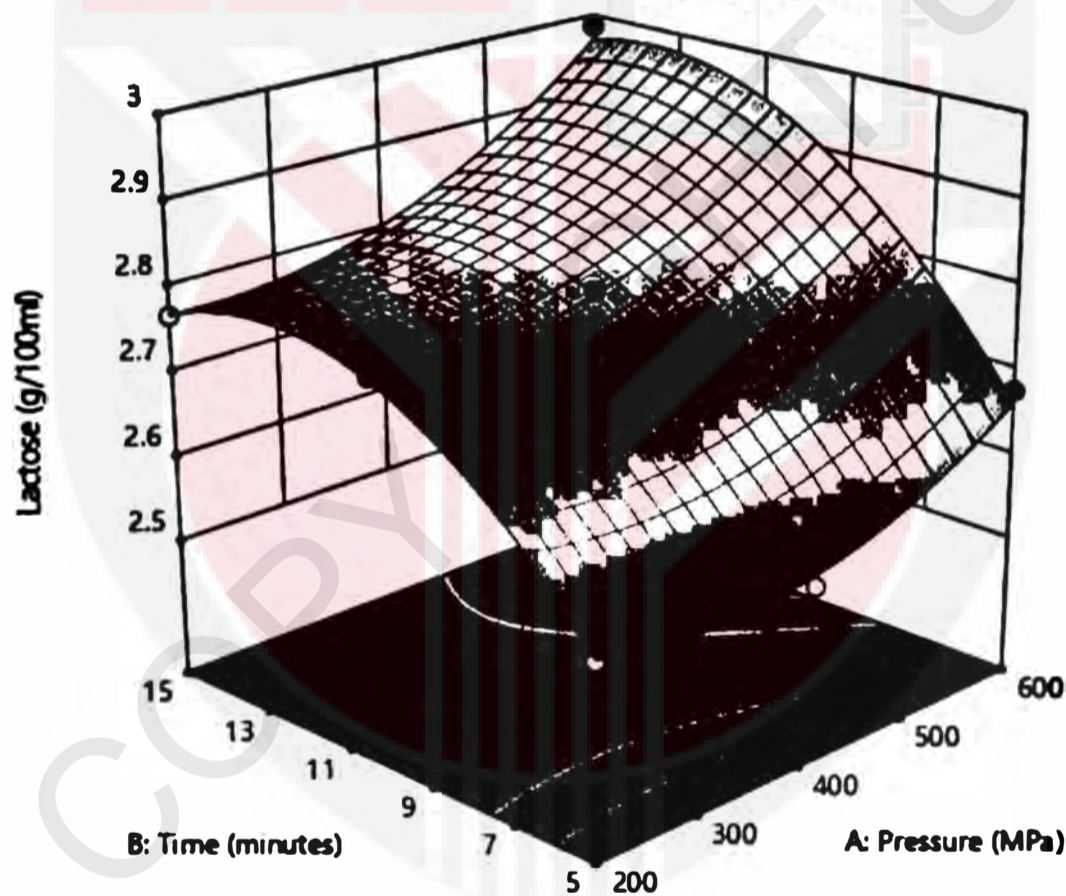


Figure 19: Effect of HPP Pressure and Time on Lactose Content

4.5.3 Effect of Process Condition on Total Carbohydrate of Goat Milk

The 3-Dimensional plot of RSM of the effect of HPP pressure and time on total carbohydrate of goat milk is shown in Figure 20. ANOVA showed that total carbohydrate of goat milk was insignificantly affected by both pressure and time of HPP.

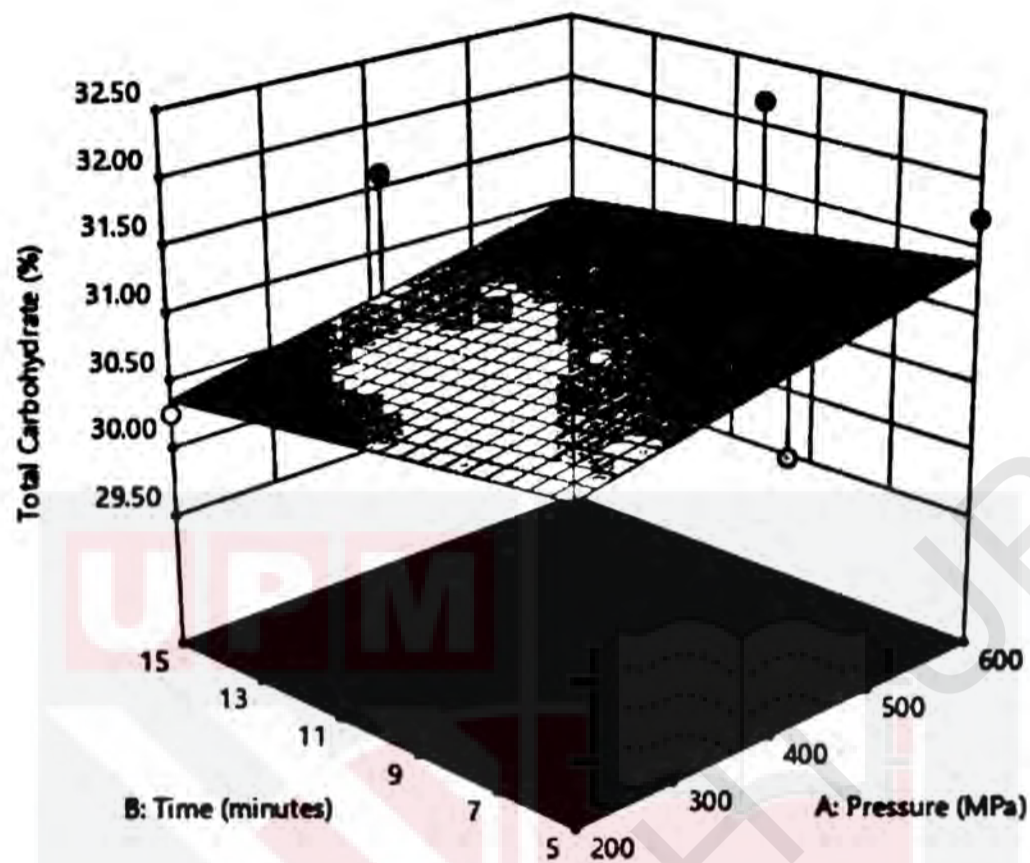


Figure 20: Effect of HPP Pressure and Time on Total Carbohydrate

4.5.4 Optimization of HPP Treatment on Goat Milk

Numerical optimization option was employed. The desirability function was generated after limiting the preferred goal of extraction variables and responses, such as minimizing lactose content while HPP pressure, HPP time, pH and total carbohydrate of goat milk were allowed to be in range. 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 0.986 (98.60%) was obtained as 400 MPa, 5 minutes of HPP treatment. The predicted pH, lactose content and total carbohydrate of goat milk were estimated to be 6.66, 2.5532 g/100ml and 31.08% respectively as shown in Table 14.

4.5.5 Validation of Optimization of HPP Treatment on Goat Milk

To validate optimization of HPP pressure and holding time, the experimental data obtained when goat milk was treated with 400 MPa and 5 minutes was compared with the predicted value. The predicted and experimental values are presented in Table 14. The result showed that there were no significant difference (less than 2.00 %) on the corresponding experimental values between the predicted (simulated) and actual properties of goat milk. This result attests to the effectiveness of this framework for optimum and effective HPP treatment on goat milk.

Table 14: Predicted and Actual Responses for Optimized Process

Responses	Predicted Values	Experimental Values	Percentage Difference (%)
pH	6.66	6.65	0.15
Lactose (g/100ml)	2.5532	2.5399	0.52
Total Carbohydrate (%)	31.08	30.50	1.87

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

In conclusion, the objectives of this research have been achieved which are investigating the effect of process parameters of high pressure processing to the microstructure and rheological properties of goat milk and optimizing the pressure and holding time applied during high pressure processing.

In terms of microstructure, the fission of milk fat globules occurred when the goat milk was treated with 400 MPa and 15 minutes. The number of small size milk fat globules increased when 600 MPa was applied. Besides, goat milk exhibited as Newtonian fluid before and after high pressure treatment. The highest viscosity was obtained when the goat milk was treated with 600 MPa for 15 minutes.

The optimum parameter for high pressure treatment was obtained with maximum desirability index of 0.986 at 400 MPa with the holding time of 5 minutes where pH, lactose content and total carbohydrate of goat milk were set as the responses. The predicted value for each response was 6.66, 2.5532 g/100ml and 31.08 % respectively.

High pressure processing (HPP) is a non-thermal processing that can be applied in dairy industries such as cheese and yogurt making. Size reduction of milk fat globules has the advantage in cheese or other dairy product making industries to possess smoother

and finer texture. However, HPP will increase the lactose content of goat milk. Therefore, separation of lactose into its simple sugar such as glucose and galactose is recommended for the patient that suffered from lactose intolerance.



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APPENDICES

A.1 PROXIMATE ANALYSIS

A.1.1 Fat Content in Goat Milk

Pressure (MPa)	Time (mins)	Weight of Sample (g)	Weight of Empty Cup (g)	Weight of Fat + Cup (g)	Weight of Fat (g)	Fat Content (%)	Average (%)
Control		1.0044	44.7928	45.0890	0.2962	29.49	29.35
		1.0463	46.2805	46.5862	0.3057	29.22	
200	5	1.0082	44.7187	45.0528	0.3341	33.14	32.78
		1.0337	44.8797	45.2149	0.3352	32.43	
	10	1.0076	44.0193	44.3617	0.3424	33.98	32.57
		1.0095	44.9388	45.2534	0.3146	31.16	
	15	1.0047	44.9391	45.2721	0.3330	33.14	33.80
		1.0471	46.3689	46.7297	0.3608	34.46	
400	5	1.0050	46.2844	46.6306	0.3462	34.45	33.57
		1.0029	45.0880	45.4158	0.3278	32.69	
	10	1.0061	44.8708	45.1730	0.3022	30.04	31.30
		1.0085	44.8040	45.1324	0.3284	32.56	
	15	1.0085	44.9303	45.2551	0.3248	32.21	30.80
		1.0056	46.2740	46.5696	0.2956	29.40	
600	5	1.0087	46.3657	46.683	0.3173	31.46	30.13
		1.0082	44.8685	45.1589	0.2904	28.80	
	10	1.0013	46.2625	46.5841	0.3216	32.12	30.35
		1.0073	44.9307	45.2187	0.2880	28.59	
	15	1.0031	46.3699	46.7111	0.3412	34.01	31.98
		1.0061	46.3646	46.6659	0.3013	29.95	

For 1st Control Sample:

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

$$= \frac{0.2962\text{g}}{1.0044\text{g}} \times 100\%$$

$$= 29.49\%$$

For 2nd Control Sample:

$$\begin{aligned} \text{Fat Content (\%)} &= \frac{\text{Weight of Fat}}{\text{Weight of Sample}} \times 100\% \\ &= \frac{0.3057\text{g}}{1.0463\text{g}} \times 100\% \\ &= 29.22\% \end{aligned}$$

Average

$$\begin{aligned} \text{Average Fat Content (\%)} &= \frac{29.49 + 29.22}{2} \\ &= 29.35\% \end{aligned}$$

A.1.2 Protein Content in Goat Milk

Pressure (MPa)	Time (mins)	Protein Content (%)	Average (%)
Control		30.5078	30.38
		30.2462	
200	5	28.3061	28.78
		29.2457	
	10	28.6554	29.14
		29.6231	
	15	28.6547	28.64
		28.6256	
400	5	28.9342	28.52
		28.1019	
	10	30.9669	30.79
		30.6054	
	15	30.6225	30.57
		30.5199	
600	5	30.6290	31.06
		31.4820	
	10	30.6247	30.56
		30.5033	
	15	29.9583	30.51
		31.0658	

For 1st Control Sample:

Protein Content (%) = 30.5078%

For 2nd Control Sample:

Protein Content (%) = 30.2462%

Average

$$\text{Average Protein Content (\%)} = \frac{30.5078 + 30.2462}{2} = 30.38\%$$

A.1.3 Moisture Content of Goat Milk

Pressure (MPa)	Time (mins)	Weight of Empty Dish (g)	Weight of Sample (g)	Final Weight after Drying (g)	Weight of Dried Sample (g)	Moisture Content (%)	Average (%)
Control		42.9539	2.0024	44.9293	1.9754	1.35	1.33
		43.4309	2.0078	45.4125	1.9816	1.30	
200	5	31.3508	2.0036	33.3260	1.9752	1.42	1.43
		31.7350	2.0017	33.7079	1.9729	1.44	
	10	43.4791	2.0052	45.4567	1.9776	1.38	1.38
		43.2422	2.0037	45.2180	1.9758	1.39	
	15	31.5968	2.0023	33.5692	1.9724	1.49	1.42
		31.8067	2.0076	33.7874	1.9807	1.34	
400	5	31.8104	2.0063	33.7881	1.9777	1.43	1.42
		31.5490	2.0051	33.5257	1.9767	1.42	
	10	31.7372	2.0004	33.7091	1.9719	1.42	1.39
		31.5628	2.0011	33.5366	1.9738	1.36	
	15	32.1202	2.0034	34.0943	1.9741	1.46	1.40
		31.9834	2.0051	33.9615	1.9781	1.35	
600	5	31.5343	2.0081	33.5158	1.9815	1.32	1.35
		31.9565	2.0051	33.9341	1.9776	1.37	
	10	32.3663	2.0058	34.3460	1.9797	1.30	1.34
		31.5577	2.0097	33.5397	1.982	1.38	
	15	31.6602	2.0053	33.6376	1.9774	1.39	1.36
		32.1869	2.0056	34.1658	1.9789	1.33	

For 1st Control Sample:

$$\begin{aligned}\text{Moisture Content (\%)} &= \frac{\text{Weight of Sample} - \text{Weight of Dried Sample}}{\text{Weight of Sample}} \times 100\% \\ &= \frac{2.0024\text{g} - 1.9754\text{g}}{2.0024\text{g}} \times 100\% \\ &= 1.35\%\end{aligned}$$

For 2nd Control Sample:

$$\begin{aligned}\text{Moisture Content (\%)} &= \frac{\text{Weight of Sample} - \text{Weight of Dried Sample}}{\text{Weight of Sample}} \times 100\% \\ &= \frac{2.0078\text{g} - 1.9816\text{g}}{2.0078\text{g}} \times 100\% \\ &= 1.30\%\end{aligned}$$

Average

$$\begin{aligned}\text{Average Moisture Content (\%)} &= \frac{1.35 + 1.30}{2} \\ &= 1.33\%\end{aligned}$$

A.1.4 Ash Content of Goat Milk

Pressure (MPa)	Time (mins)	Weight of Empty Crucible (g)	Weight of Sample (g)	Weight of Ash + Crucible (g)	Weight of Ash (g)	Ash Content (%)	Average (%)
Control		40.6512	2.0065	40.7734	0.1222	6.09	5.52
		39.5536	2.0026	39.6526	0.0990	4.94	
200	5	39.3685	2.0079	39.4894	0.1209	6.02	5.55
		41.0891	2.0032	41.1910	0.1019	5.09	
	10	40.1633	2.0078	40.2835	0.1202	5.99	6.04
		38.8216	2.0049	38.9437	0.1221	6.09	
	15	38.8245	2.0062	38.9415	0.1170	5.83	5.88
		40.1622	2.0046	40.2809	0.1187	5.92	
400	5	40.0408	2.0039	40.1615	0.1207	6.02	6.00
		40.0397	2.0034	40.1593	0.1196	5.97	
	10	41.6295	2.0055	41.7492	0.1197	5.97	6.06
		39.3690	2.0058	39.4922	0.1232	6.14	
	15	38.7831	2.0047	38.8855	0.1024	5.11	5.59
		41.6304	2.0062	41.7523	0.1219	6.08	
600	5	39.4795	2.0061	39.5895	0.1100	5.48	5.74
		40.6525	2.0026	40.7724	0.1199	5.99	
	10	39.2753	2.0103	39.3759	0.1006	5.00	5.55
		39.2545	2.0050	39.3766	0.1221	6.09	
	15	40.7671	2.0079	40.8671	0.1000	4.98	5.51
		38.7632	2.0042	38.8842	0.1210	6.04	

For 1st Control Sample:

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

$$= \frac{0.1222\text{g}}{2.0065\text{g}} \times 100\%$$

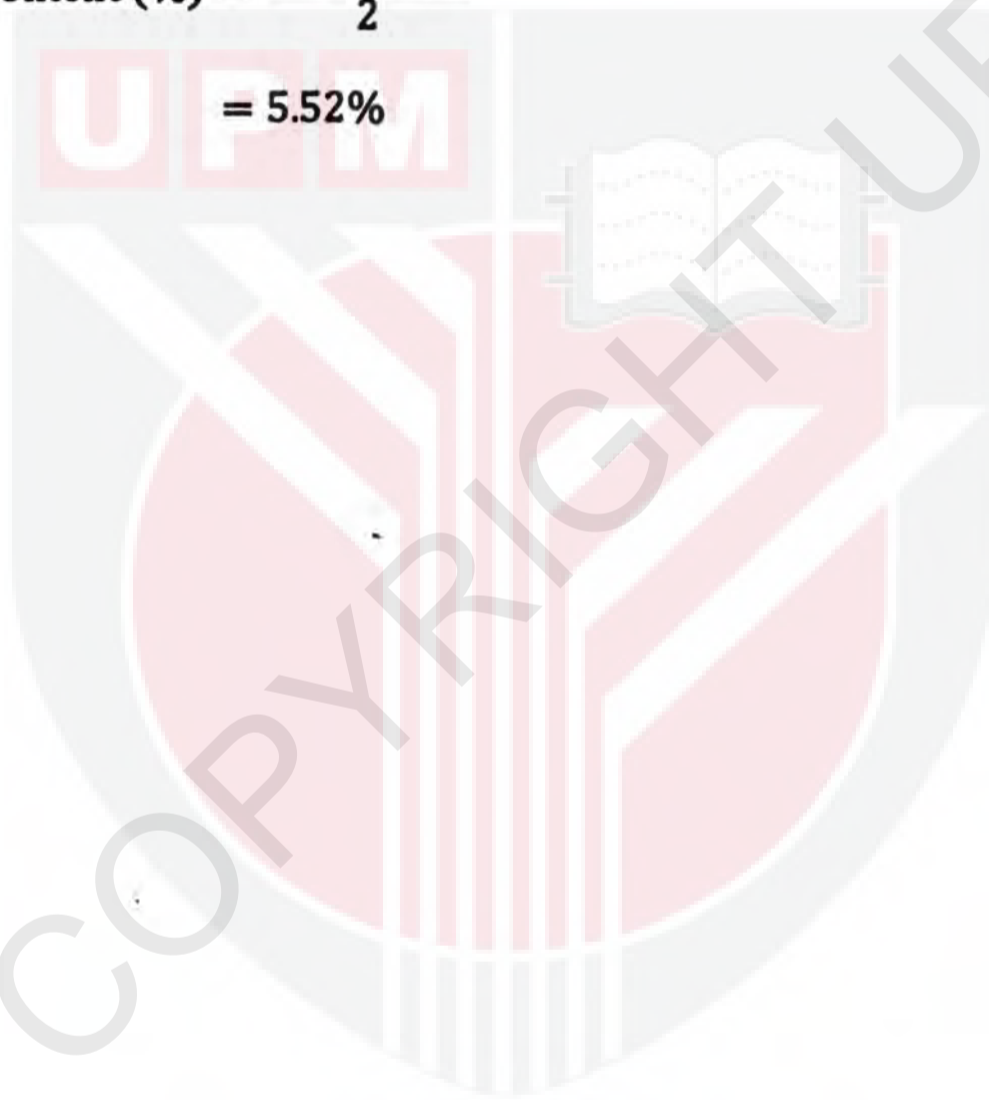
$$= 6.09\%$$

For 2nd Control Sample:

$$\begin{aligned}\text{Ash Content (\%)} &= \frac{\text{Weight of Ash}}{\text{Weight of Sample}} \times 100\% \\ &= \frac{0.0990\text{g}}{2.0026\text{g}} \times 100\% \\ &= 4.94\%\end{aligned}$$

Average

$$\begin{aligned}\text{Average Ash Content (\%)} &= \frac{6.09 + 4.94}{2} \\ &= 5.52\%\end{aligned}$$



A.2 pH

A.2.1: pH of Goat Milk

Pressure (MPa)	Time (mins)	pH	Average
Control		6.60	6.61
		6.62	
200	5	6.68	6.67
		6.65	
	10	6.63	6.64
		6.64	
	15	6.66	6.67
		6.68	
400	5	6.65	6.65
		6.65	
	10	6.68	6.68
		6.68	
	15	6.64	6.65
		6.66	
600	5	6.66	6.66
		6.66	
	10	6.64	6.65
		6.65	
	15	6.64	6.65
		6.66	

A.3 LACTOSE CONTENT

A.3.1: Lactose Content of Goat Milk

Pressure (MPa)	Time (mins)	Lactose (g/100ml)	Average (g/100ml)
Control		1.2458	1.2528
		1.2598	
200	5	2.5506	2.5607
		2.5707	
	10	2.8109	2.7795
		2.7480	
	15	2.7886	2.7687
		2.7488	
400	5	2.5849	2.5699
		2.5548	
	10	2.7976	2.7969
		2.7961	
	15	2.8083	2.7969
		2.7855	
600	5	2.6988	2.6796
		2.6603	
	10	2.8736	2.8948
		2.9160	
	15	2.9915	2.9856
		2.9797	