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PULSED ELECTRIC FIELD (PEF) INACTIVATION OF ALKALINE PHOSPHATASE (ALP) AND EFFECT ON THE FAT GLOBULES OF GOAT MILK

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ABSTRACT

Goat milk demand has increased nowadays due to its nutritional benefits that are superior to cow milk. Most dairy industries use pasteurization for milk treatment because it is able to increase the milk shelf-life and reduce harmful microbes. However, these conventional treatments have been found to cause an adverse effect on milk quality and nutritional value. Hence, this study was done to investigate the impact of electric field strength (EFS) and pulse time on alkaline phosphatase (ALP) inactivation of goat milk and to further study the effect of EFS and treatment time on the milk fat globule (MFG) of goat milk. Goat milk was treated with a pulsed electric field (PEF), with parameters, EFS of 10 kV/cm, 20 kV/cm and 30 kV/cm and treatment time of 5 μs and 10 μs, whereas pasteurization; high-temperature short time (HTST) with parameter, 67°C, 72°C and 77°C for 15 s and low-temperature long time (LTLT) with parameters, 58°C, 63°C and 68°C for 30 minutes. The ALP in goat milk was analyzed using Phosphatesmo test kit. The PEF treatment in this study was not able to reduce the amount of ALP that is safe for consumption in comparison to the LTST pasteurization (63°C and 68°C for 30 minutes) method, as the Phosphatesmo stripes for the PEF-treated showed similar yellow-like stripe in raw milk while the LTST stripe showed white-like stripe. Further proves that the PEF treatment was unable to inactivate the ALP, while LTLT was able to do so. The MFG size of PEF-treated and pasteurized goat milk was found to be lower than the raw goat milk in terms of d(0.1), d(0.5), d(0.9), D[4,3] and D[3,2]. In terms of SSA, the MFG size of PEF-treated and pasteurized goat milk was higher than the raw goat milk. The MFG size for PEF-treated goat milk showed less reduction compared to pasteurization which indicated less damage to goat milk globules. All the treatment times for PEF and pasteurization did not significantly change ($p > 0.05$) the goat milk globules in comparison to EFS and temperature ($p < 0.05$). The R-value

for pasteurization temperature (R^2 = -0.9734, -0.9736, -0.9746, 0.9788) showed greater correlation in reducing goat milk MFGs compare to EFS of PEF (R^2 = -0.6814, -0.6841, -0.6722, 0.7054) while treatment time for PEF (R^2 = -0.7496, -0.7504, -0.7546, 0.7462) has stronger correlation in goat milk MFGs reduction compare to pasteurization time (R^2 = -0.3583, -0.3568, -0.3486, 0.3298). Thus, it is recommended that the EFS and treatment time should be increased to ensure PEF is able to reduce ALP, similar to the effect of the pasteurization method with minimal damage to goat milk particle size.

ABSTRAK

Permintaan susu kambing semakin meningkat pada masa kini kerana faedah pengambilannya lebih baik daripada susu lembu. Sebahagian besar industri tenusu menggunakan kaedah pasteurisasi untuk merawat susu kerana cara ini dapat memanjangkan jangka hayat susu dan mengurangkan mikrob yang berbahaya. Walau bagaimanapun, cara konvensional ini menyebabkan kesan buruk terhadap kualiti dan nilai nutrisi susu. Oleh itu, kajian ini dijalankan untuk mengkaji kesan kekuatan medan elektrik (EFS) dan tempoh rawatan terhadap nyahaktifkan enzim alkali fosfatase (ALP) dan untuk mengkaji lebih lanjut kesan EFS dan tempoh rawatan terhadap globul lemak susu (MFG) kambing. Susu kambing dirawat PEF dengan parameter, EFS; 10 kV/cm, 20 kV/cm dan 30 kV/cm dalam tempoh masa 5 μs dan 10 μs, manakala pasteurisasi; suhu tinggi dan waktu singkat, (HTST) dengan parameter, 67°C, 72°C dan 77°C untuk 15s dan suhu rendah dan waktu lama (LTLT) dengan parameter, 58°C, 63°C dan 68°C selama 30 minit. ALP dalam susu kambing dianalisis menggunakan kit ujian Phosphatesmo. Rawatan PEF dalam eksperimen ini tidak dapat mengurangkan jumlah ALP dalam susu kambing yang selamat diminum berbanding dengan kaedah pasteurisasi LTST (63°C dan 68°C selama 30 minit) kerana jalur Phosphatesmo yang dirawat menggunakan PEF menunjukkan jalur kuning sama dengan Phosphatesmo yang direndam ke dalam susu mentah manakala jalur Phosphatesmo yang dirawat menggunakan LTST menunjukkan jalur putih. Saiz MFG susu kambing yang dirawat menggunakan PEF dan pasteurisasi didapati lebih rendah daripada susu kambing mentah dari segi d(0.1), d(0.5), d (0.9), D[4,3] dan D[3,2]. Dari segi SSA, pengiraan MFG susu kambing yang dirawat menggunakan PEF dan pasteurisasi lebih tinggi daripada susu kambing mentah. Ukuran MFG untuk susu kambing yang dirawat menggunakan PEF menunjukkan pengurangan yang lebih sedikit berbanding dengan kaedah

pasteurisasi yang menyimpulkan bahawa lebih sedikit kerosakan pada globul susu kambing menggunakan PEF. Kedua-dua tempoh masa rawatan untuk PEF dan pasteurisasi menunjukkan perubahan tidak ketara ($p > 0.05$) terhadap perubahan saiz globul susu kambing berbanding dengan EFS PEF dan suhu pasteurisasi ($p < 0.05$). Nilai R^2 untuk suhu pasteurisasi (R^2 = -0.9734, -0.9736, - 0.9746, 0.9788) menunjukkan kolerasi yang lebih besar untuk mengurangkan MFG susu kambing jika dibandingkan dengan EFS PEF (R^2 = -0.6814, -0.6841, -0.6722, 0.7054) sementara tempoh rawatan untuk PEF (R^2 = -0.7496, -0.7504, -0.7546, 0.7462) mempunyai kolerasi yang lebih besar dalam pengurangan MFG berbanding dengan tempoh pasteurisasi. EFS dan tempoh rawatannya seharusnya dinaikkan nilainya untuk mengurangkan kuantiti ALP dalam susu kambing, sama seperti rawatan menggunakan kaedah pasteurisasi tetapi dengan kerosakan yang minimum pada saiz zarah susu kambing.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ALP	Alkaline Phosphatase
EFS	Electric field strength
HTST	High-temperature short time
LTST	Low-temperature short time
MFG	Milk fat globule
MFGM	Milk fat globule membrane
PEF	Pulsed Electric Field
SSA	Specific surface area

CHAPTER 1

INTRODUCTION

1.1 Research background

Increased goat milk consumption is a result of its health benefits for the human. With that, the goat's population in Malaysia has also increased over the years. According to the Department of Veterinary Services, Ministry of Agriculture Malaysia, the goat population was 312,571 in 2019, 324,355 in 2020, and 328,944 in 2021 (DVS, 2021). Goat milk production worldwide is predicted to rise as the number of small-scale, non-commercial producers grows. Goat milk's high nutritional content has led to its widespread use around the world. (Mohamad et al., 2020).

Goat milk is a great source of vitamin A and has a higher concentration of a group of B vitamins, including thiamine (B1), riboflavin (B2), niacin (B3), and pantothenic acid (B5) (Mohamad et al., 2021). Goat milk is also a popular alternative to bovine milk due to its superior nutritional characteristics, which is especially beneficial for nursing moms and newborns suffering from milk allergies and other gastrointestinal disorders (Mohamad et al., 2020). Furthermore, goat milk has a higher concentration of whey protein and short and medium-chain fatty acids (Zhao et al., 2021). A high concentration of medium fatty acids chain (C6:0, C8:0, and C10:0) also gives goat milk a unique flavour (Jaafar et al., 2018). It is also known that these medium-chain fatty acids can impede development and dissolve cholesterol deposits that are highly beneficial to human health (Jaafar et al., 2018). However, due to its high nutritional content, goat milk may be the optimal substrate for bacterial development, providing an optimal setting for pathogen intervention (Mohamad et al., 2020).

To ensure the safety of the goat milk, the goat milk enzyme, alkaline phosphatase (ALP) is measured. ALP is a naturally occurring enzyme found in all milk that serve as a marker for adequate milk pasteurization (Ahmad Punoo, 2018). It is an endogenous enzyme found in milk that is more resistant to heat than the harmful pathogen and is used to determine the effectiveness of pasteurization (Shamsi et al., 2008). Because ALP has higher thermal stability than bacteria that may be present in milk, it acts as a signal of milk safety from harmful microbe (Shamsi et al., 2008). ALP is a membrane-bound glycoprotein containing the sugar sialic acid and a phospho-monoesterase enzyme that catalyzes the hydrolysis of phosphoric acid monoesters (at alkaline pH), generating phosphate and the corresponding alcohol (Rankin et al., 2010).

Most industries process dairy milk use conventional heat treatment, pasteurization. Pasteurization is critical to drastically reducing the microbial load of goat milk and subsequently extending its shelf-life. Pasteurization, on the other hand, alters the organoleptic qualities of milk, frequently imparting a cooked flavour as a result of the removal of sulphydryl groups in sulphur-containing amino acids (Mohamad et al., 2020). It may increase the shelf life of milk but degrades its nutritional and sensory characteristics (Shabbir et al., 2021). Heat treatment affects milk components that are labile and alters the functional characteristics of protein milk (Sharma et al., 2014). The size of the milk fat globule membrane (MFGM) structure will change, causing the whey proteins to denatured (Sharma et al., 2014). Additionally, the unfavourable effect of thermal pasteurization is frequently dependent on the process's intensity and the surrounding environment, and extreme thermal pasteurization may dephosphorylate, hydrolyze, or agglomerate milk casein (Mohamad et al., 2021). According to Lopes et al., (2015), heat treatment at 73°C for 30 seconds denatures whey proteins, degrades labile vitamins, and alters the taste of

milk. In addition, Lopes et al. (2015), discovered that heat treatment at 85°C for 15 minutes alters the size of casein micelles size, denatures whey proteins and changes the viscosity of milk.

Due to the quality and sensory reduction of goat milk by heat treatment, non-thermal treatment is introduced. There are many types of non-thermal treatments for goat milk. PEF is one type of non-heat treatment method. Shabbir et al. (2021) stated that PEF is able of removing pathogenic and spoilage-causing bacteria, as well as enzymes associated with product quality degradation, without compromising on customer demand. Additionally, PEF is able to minimize colour modification, flavour degradation, and nutritional loss (Shamsi et al., 2008). PEF is also environmentally friendly, cost-effective, and processes food faster than the standard thermal treatment method, pasteurization (Kumar et al., 2021). This type of food preparation employs extremely brief, high-voltage EFS (Kumar et al., 2021). The primary process parameters for PEF are the electric field strength, the form of the pulses, frequency, the number of pulses and duration of treatment (Sharma et al., 2014). All these parameters may affect the efficacy of PEF treatment. Due to the presence of charged molecules in the liquid food sample, current flows into it and is transmitted to each site when the electric field is introduced (Shabbir et al., 2021). When a cell is subjected to a high voltage or strong EFS, the lipid bilayer and proteins in the membranes cell are weakened (Shabbir et al., 2021). The pulses enhance cellular permeability, protein channels opening and forming hydrophilic pores (Kumar et al., 2021). This action on a biological cell affect by PEF is referred to as "electroporation". The electroporation impact compromises the cell membrane's structural integrity and functioning (Raso et al., 2014).

1.2 Problem Statement

For now, most dairy industries use heat treatment for milk treatment because it may increase the milk shelf life and reduce harmful microbes. However, it may have an adverse effects on milk quality and nutritional value. There was a study by Lopes et al. (2015) that treat milk with pasteurization treatment (73°C, 30s) which results in differing in flavor due to sulfhydryl groups formation and labile vitamins degradation and whey proteins denaturation while pasteurization treatment (85°C, 15min) results in casein micelle size alteration and whey proteins denaturation. Furthermore, the heat treatment process required more amount of energy (Mohamad et al., 2020). Hence, the alternative for this problem is non-thermal treatment, PEF treatment is proposed for the treatment of goat milk.

There are only a few studies on PEF treatment especially on the ALP of goat milk as there are many studies focused on cow milk compared to goat milk, heat treatment pasteurization and microbial activity study. Hence, in order to find the best combination for ALP inactivation from Food and Drug Administration (FDA) requirement, the EFS and treatment time of PEF treatment need to be studied.

Goat milk is composed of milk fat globules (MFG) encased in a phospholipid membrane that maintains the emulsion's stability and suppresses coalescence (Sharma et al., 2014). According to Sharma et al. (2014), the milk fat globule membrane (MFGM) is made of phospholipids an inner monolayer and an outer bilayer that encloses the MFG. MFGM's main physical function is to shield the triacylglycerol (TAG) core from lipolysis by endogenous or exogenous lipases and maintain the colloidal structure. The MFG may be harmed during milk pumping in a commercial processing setting because of the shear and temperature conditions

(Sharma et al., 2014). Reduced fat globule size increases the surface area of the overall emulsion, resulting in a change in the composition of the MFGM due to the whey protein and casein micelles adsorbed onto the surface of MFG (Sharma et al., 2014). Due to the limitation of information regarding PEF treatment on goat milk components and functionality, the MFG of goat milk was studied to analyze the particle size of goat milk after the PEF treatment.

Lastly, to the best of our knowledge, there are limited studies on the relationship between ALP and MFG after undergoing PEF or any other heat treatment which means this relationship has yet to be studied to link the relationship.

1.3 Objectives

The following are the objectives for this study;

1. To study the impact of EFS and pulse time towards ALP inactivation of goat milk.
2. To investigate the effect of EFS and pulse time on the fat globule of goat milk.

1.4 Thesis Description

1.4.1 Scope of Limitations

The scope of the study is limited to goat milk where it was processed from a farm located in Seremban, Malaysia. The PEF treatment machine used in this study is a model from Agro-Biotechnology Institute Malaysia which some of its parameters have been fixed, i.e. flowrate input.

The scope of the study will be focused on EFS and pulse time as the PEF machine used can only adjust these two parameters. Other parameters are fixed. There also will be no heat treatment applied to the goat milk before and after treatment as this study is to investigate only the PEF treatment method. Then, with the analysis of ALP, there will be no microbial study of the goat milk because the ALP test alone can be used as a safety measure for goat milk. The component and functionality of goat milk are analyzed by MFG because MFG may affect the texture and colour of goat milk.

1.4.2 Thesis Contribution

This study contributes to the field of food engineering by proposing the non-thermal treatment, PEF treatment to treat goat milk from harmful microbe and reduce the nutritional and sensory loss during the treatment in comparison to heat treatment pasteurization that denatures whey proteins, change of flavor due to sulfhydryl groups formation and labile vitamins degradation. This study also can be used by milk manufacturers to design a PEF machine to treat milk which they can find the optimum combination of EFS and treatment time that is best for

goat milk treatment. This study can also be used to analyze how PEF can affect the MFG which subsequently may affect the texture of milk after PEF treatment.

1.4.3 Thesis Structure

Chapter 1, Introduction chapter introduced the overview of the topic being studied. The main aspects are background information, problem statements and objectives of the thesis. Then, Chapter 2, Literature Review indicated previous research on the topics and presented in a structured manner. Here, the gaps between the research are known. Chapter 3, Methodology showed the method used in this study to obtain the result analysis of the experiment. Next, Chapter 4, Results is to analyzes the result and justifies the results in the discussion section. Graphs, figures and tables are provided in this chapter to visualize the results. Finally, the conclusion chapter (Chapter 5) includes a summarized analysis and recommendations for research in the future.

CHAPTER 2

LITERATURE REVIEW

2.1 Goat milk

2.1.1 Nutritional content

The general chemical composition of goat milk was same to that of cow milk. However, there were some notable changes in the components of individual, as listed below Table 2.1.1. Among all the milks, goat milk can be considered contains the greatest levels of all components.

Table 2.1.1: Goat, human and cow milk basic composition from Posati & Orr (1976)

Constituents	Food energy (kcal)	Total solid (g/100g)	Fat (g/100g)	Total protein (g/100g)	Lactose (g/100g)	Minerals (g/100g)
Goat	69	13.2	4.5	3.6	4.3	0.8
Human	70	12.4	3.8	1.0	7.0	0.2
Cow	61	12.6	3.7	3.4	4.7	0.7

Turkmen (2017) stated that goat milk and cow milk have different milk fat compositions and structures. Both types of milk include globules of fat between 1 and 10 μm, while over 80% of goat milk has globules smaller than 5 μm, compared to 60% of cow milk. Additionally, he discovered that the globules of fat in cow milk ranged from 0.92 to 15.75 μm (3.51 μm average),

but those in goat milk between 0.73 to 8.58 μm (2.76 μm average). Goat milk contains high amounts of conjugated linoleic acids (CLAs) and essential fatty acids (EFAs). CLAs have been demonstrated to have antiatherogenic, antioxidant, anti-inflammatory, anti-adipogenic, antihypertensive, antiobesogenic and anticarcinogenic properties in animal models. According to the author, goat milk contains medium-chain triglycerides (MCTs), which are primarily saturated fatty acids with a chain length of 6 to 10 carbons. This demonstrated that goat milk delivers a notable amount of energy that is ideal for growing children.

In general, goat milk has a higher amount of protein and nonprotein nitrogen content, while cow milk's casein nitrogen concentration is lower (Deeth, 2022b). Goat milk has a greater value of buffering capacity (BC) because these components influence the BC together with phosphate which is good for the treatment of ulcers (Park, 1992). Goat milk proteins digestion was also easier compared to proteins of cow milk (Deeth, 2022b). Almaas et al. (2006) found that goat milk can be digested faster compared to bovine milk. The most significant finding was that just (~23%) of goat milk β-lactoglobulin stayed undigested, whereas (~83%) of the β-lactoglobulin in bovine milk remained undigested. It had been found that people who suffer cow milk allergies (CMA) can consume goat milk (Silanikove et al., 2010). The goat milk hypoallergenic activity was directly linked to the high genetic αs1-casein polymorphism that decrease the goat milk casein level.

Milk consists some lactose-derived products such as lactulose, galactooligosaccharides and various other oligosaccharides (Deeth, 2022b). These products played a vital role as prebiotics to enhance the probiotic growth bacteria in the gastrointestinal tract (Saarela et al., 2003). These products also increase the rate of magnesium and calcium intestinal absorption (Schaafsma, 2008). Besides, oligosaccharides of milk stimulate the nervous system and brain as

they have tolerable antigenic properties Deeth (2022). Goat milk was closely same to human milk when comparing the composition of oligosaccharide, which is higher compared to cow milk (Martinez-Ferez et al., 2006).

Goat milk has a better minerals content compared to human and cow milk because it has higher calcium, phosphorus, potassium, magnesium and chlorine value than cow milk as shown in the Table 2.1.2.

Table 2.1.2: Mineral content in milk from Turkmen (2017)

Minerals (mg/100g)	**Goat**	**Human**	**Cow**
Calcium	134	32	119
Chlorine	150	60	100
Copper	0.05	0.06	0.06
Iodine	0.022	0.007	0.021
Iron	0.05	0.03	0.05
Magnesium	14	3	13
Manganese	0.032	0.07	0.02
Phosphorus	111	14	93
Potassium	204	51	151

Selenium	1.33	1.52	0.96
Sulfur	28	14	32
Sodium	50	17	49
Zinc	0.30	0.17	0.38

Vitamins are biochemical, physiological and metabolically organic compounds contained in milk (Park, 2009). From Table 2.1.3 below, it can be said that goat milk is better than cow milk as it has more content of vitamins.

Table 2.1.3: Content of milk vitamin in goat, human and cow milk in 100g from Park et al. (2007)

Vitamin	Goat	Human	Cow
Ascorbic acid (mg)	1.29	5.00	0.94
Folic acid (μg)	1.00	5.00	5.00
Thiamine (mg)	0.048	0.014	0.038
Riboflavin (mg)	0.138	0.036	0.162
Niacin (mg)	0.277	0.177	0.084

Pantothenic acid (mg)	0.310	0.223	0.314
Vitamin A (IU)	185	241	126
Vitamin B_6 (mg)	0.046	0.011	0.042
Vitamin B_7 (µg)	1.5	0.4	2.0
Vitamin B_{12} (µg)	0.065	0.045	0.357
Vitamin D (IU)	2.3	1.4	2.0

2.1.2 Malaysia's production, demand and market value

Based on the Department of Veterinary Services, Ministry of Agriculture Malaysia, the goat population was 312,571 in 2019, 324,355 in 2020, and 328,944 in 2021 (DVS, 2021). High prices, strong demand, technical innovation, and government backing contributed to the expansion of the local goat population. Global goat milk output is expected to increase due to a greater number of small-scale non-commercial goat milk producers (Zhao et al., 2021). The demand for goat milk from the dairy industry is expected to increase by 3.2% from 1.4 billion litres to 1.9 billion litres worldwide (Yusof et al., 2021). For now, Muslims in Malaysia tend to consume goat milk as they want to follow Prophet Muhammad's Sunnah (Aimi Fadzirul, 2019).

2.2 Treatment of goat milk

2.2.1 Conventional thermal treatment

Most dairy industries use heat treatments like pasteurization to treat milk. As stated in Table 2.2.1 and 2.2.2, there are various types of this treatment. For now, pasteurization has been the number one choice for milk manufacturers to treat milk.

Table 2.2.1: Function of each type of heat treatment from Deeth (2022b)

Type of heat treatment	Major function
Thermization	Able to increase the shelf-life of raw milk before more severe heat treatments such as pasteurization, and before the manufacture of some cheeses
Pasteurization	Able to delay spoilage and ensure the milk safety
Extended-shelf-life (ESL)/ higher pasteurization processing	For pasteurization but to further delay spoilage
Ultra-high-temperature (UHT) processing	Able to produce milk that stable at room temperature for 9 to 12 months
In-container sterilization	Able to produce milk that stable at room temperature for up to 9 months

Table 2.2.2: Heat treatments that were used in milk production industry Deeth (2022b)

Heat treatments	**Function**	**Bacteria killed**	**Chemical effects**	**Remarks**
Thermization (57–68°C/30-5s)	Extending shelf-life of milk prior to further pathogens and drinking as some pathogens processing	Psychrotrophic spoilage bacteria and some non-spore-forming pathogens	No significant effects	Some pathogen remains viable
Pasteurization (72–80°C/15 30s or 63–65°C /15–30 min)	Drinking milk with a shelf life of more than 20 days; chilled	Psychrotrophic spoilage bacteria and some non-spore-forming pathogens	Denaturation of 5%-10% whey protein, minor effect on vitamins, inactivation of ALP and lipoprotein lipase	-

Extended-shelf-life (ESL) processing (125–140°C /1–10 s)	Drinking milk with a shelf life of more than 20 days; chilled	Most psychrotrophic and mesophilic and all non-spore-forming bacteria	Significant but variable whey protein denaturation (25-28% of β-lactoglobulin)	Minimal alteration of flavour at higher temperatures for a shorter time
Ultra-high temperature (UHT) processing (135–150°C /1–10s)	Drinking milk with a shelf life of 6 to 9 months	All spores except highly resistant spores and all non-spore-forming bacteria	High level of denaturation of whey protein (70-95% of β-lactoglobulin)	Cause chemical changes during storage
In-container sterilization (110–120°C /10–20 min or 125°C /5 min)	Condensed or evaporated milk with a shelf life up to 1 year; stored at ambient temperature	All spores except highly resistant spores and all non-spore-forming bacteria	Complete whey protein denaturation	Light brown discolouration and great cooked flavour

Whereas milk subjected to thermization is not considered a safe product, pasteurized milk is considered safe because the heating conditions used are designed to inactivate the most heat-resistant pathogenic non-spore forming organisms. The minimum condition for pasteurization is 63°C C for 30 min (batch) or 72°C for 15 s high-temperature short time (HTST). According to Pearce et al. (2012), pasteurized 65°C for 15s caused 6.8 log reduction of *E. coli* 0157:H42 while 66.5°C for 15s caused 6.7 log reduction of *Staphylococcus aureus*. However, increasing the temperature of pasteurization by several degrees above 72°C has been shown by several researchers to decrease the keeping quality of milk (Deeth, 2022a).

A study by Sosnowski et al. (2016) showed ALP activity below 350 mU/L, mainly between 100 and 200 mU/L in goat milk by UHT (135°C for 5s) which is properly pasteurized. Higher enzyme activity was demonstrated in UHT than in pasteurized milk, but these differences were not statistically significant ($p > 0.05$).

2.2.2 Non-thermal Treatment

2.2.2.1 High-pressure processing (HPP)

Over the past few years, high-pressure processing (HPP) has been extensively investigated. The detection and characterization of HPP-induced alterations in milk components following its treatment to dairy products have advanced significantly (Sánchez et al., 2020). HPP has been shown to modify the properties of dietary proteins. However, this change is dependent on the power used, temperature and treatment time which produces irreversible alterations in structure of protein (secondary, tertiary and quaternary) by mainly disrupting covalent bonds (Dhakal et al., 2014).

Harte et al. (2003) found that HPP lowered the milk L-value by disintegrating casein micelles, resulting in a decrease in milk turbidity. When milk was subjected to 200 MPa pressure, a modest effect on L-value was detected, however at 250-450 MPa pressure resulted significant decrease in L-value. Skim milk's L-value decreased from 78 to 42 after 30 minutes at 600 MPa, and it has a translucent or semi-transparent colour (Shabbir et al., 2021). HPP reduces the time necessary for the induction of fat crystallization because the value of milk fat at high-pressure liquid/solid transition temperature rises. According to Shabbir et al. (2021), the temperature of melting and crystallization of milk fat increased at 15.5 °C at the same pressure during HPP treatment (100 MPa, 16.3 °C). Moreover, the MFGM did not deteriorate after HPP treatment at 800 MPa. The mean diameter of MFG showed no change and there was no increase in lipolysis, but some whey proteins were absorbed into the MFGM and the membrane remains undamaged after HPP treatments (Harte et al., 2003). HPP treatment on skim milk for 84°C and 300 MPa causes a 0.67-log reduction of *Clostridium sporogenes* PA3679 and *Bacillus stearothermophilus* ATCC 7953 (Pinho et al., 2011). In human milk, *L. monocytogenes* ATCC 19115 and *Staphylococcus aureus* ATCC 25923 were reduced by 6-log and 8-log under HPP treatment (21 to 31 °C, 400 MPa for 50 min) (Shabbir et al., 2021). Similarly, according to Amador Espejo et al. (2014), *Bacillus* spores reduction of 5 CFU/mL occurred when HPP at 75°C to 85°C and 300 MPa was applied to commercial sterile milk.

2.2.2.2 Ultraviolet (UV)

Ultraviolet (UV) is one of the non-thermal treatment methods for dairy products. UV radiation technology was being used effectively for the microbial decontamination of water, surfaces and air (Guneser & Karagul Yuceer, 2012). The main disadvantage of employing this technique is that it has little penetrating power and its usefulness in liquid samples is reduced by suspended particulates (30% loss due to 254 nm UV radiation; in intensity in 10% sucrose solution, below 5 cm surface) (Falguera et al., 2011). UV light has three areas in the electromagnetic spectrum. The UV-A, UV-B, and UV-C spectrums are 315-400 nm, 280-315 nm, and 200-280 nm, respectively, with the UV-C region having germicidal characteristics. UV-C light damages the DNA of pathogenic and spoilage-causing microbes (viruses and protozoa), preventing transcription and replication and resulting in cell death (Shabbir et al., 2021). The optical and flow characteristics of the product, length of radiation path, device geometric configuration, power, UV source physical arrangement, wavelength and microbial load influence the effect of UV-C radiation (Guneser & Karagul Yuceer, 2012).

Few investigations on the impact of UV processing on whole milk quality found no discernible change in milk pH, viscosity, colour or soluble solid contents. The UV-treated whole milk (UV dose of 10 mJ/cm^2 for 720 to 14,100 s) caused viscosity was on average 2.00 ± 0.01 (mPa.s), pH range was 6.66 to 6.70, soluble solid contents was 12.78 ± 0.10 (% g/g) and change of colour ΔE* was in between 0 to 0.5 (Shabbir et al., 2021). Raw cow milk that was treated with UV dose (1.5 J m/L by 1 and 2 pure reactors version) caused natural microflora a 3-log reduction. (D.J. Reinemann et al., 2013). According to Shabbir et al. (2021), goat milk that treated with UV dose 15.8 ± 16 mJ/cm^2 for 18s reduced *Listeria* by 5 log units.

2.2.2.3 Pulsed electric field (PEF)

The basic principle of the PEF technology is the application of short pulses of high electric fields with the duration of microseconds micro to milliseconds and intensity in the order of 10 to 80 kV/cm (Shabbir et al., 2021). Electroporation and dielectric breakdown of the cell membrane are considered to be responsible for the PEF-induced inactivation of some enzymes and pathogens (Huang et al., 2014). Due to the presence of charged molecules, current flows into the liquid food sample when the electric field is applied (E.A. & Amer Eiss, 2012). Cold storage and aseptic packaging are required after the treatment in order to sustain prolonged shelf claims. (Pal, 2017).

PEF treatments on fresh skim milk at 15°C with field strengths of 28, 31, 34, and 37 kV/cm resulted in 24, 25, 31, and 42 ALP reduction (Shamsi et al., 2008). While there was no notable change in ALP inactivation percentages between 28 and 31 kV/cm and 34 and 37 kV/cm ($p > 0.05$), no significant difference in inactivation percentages between 28 or 31 and 34 and 37 kV/cm ($p < 0.05$). There was a study on ALP activity reduction (1 to 2 mg/ml) by PEF in SMUF or heat-pasteurized milk consisting 0.2 or 4% fat (Castro et al., 2001b). They found that the settings (batch Kodak GeneZapper, 70 exponential decay pulses, 100 l BioRad cuvette, $\tau = 400$ µs, EFS = 18.8 kg/cm and $f = 0{:}07$ Hz) induced 65% inactivation of ALP in skim milk and 59% in 2 or 4% fat milk. ALP dissolved in SMUF was inactivated by 33% to 43% after 70 pulses ($\tau =$ 780 µs, ~5.3 J/pulse, 23 kV/cm, $f = 0{:}07$ Hz, $T_{max} = 44$°C). Using the PEF system of gene electroporator (Kodak), exponential decay wave form, $f = 0.06667$ Hz and an electrode gap of 0.1 cm with no temperature control, Castro et al. (1994) reported that ALP was reduced by 65% in SMUF at a voltage of 22 kV/cm after 70 pulses and by 59% in raw milk and 2% milk at a voltage of 18.8 kV/cm after 70 pulses. Less than 5% of ALP inactivation was observed at 21.5

kV/cm for 20 pulses, with energy input of 400 kJ/L (Grahl and Markl, 1996). Enzyme activity was reduced by 5% in a study by Ho et al. (1997) using the PEF system (enzyme solution in buffer solution, pulse count = 30, no cooling system, pulse width = 0.74 s, EFS = 73.3 kV/cm). When using the PEF with EFS of 10 kV/cm, of 1–40 μs, the number of pulses of 200, the frequency of 1 Hz, and T_{max} of 70°C, ALP activity in bovine milk is decreased by 26%. ALP activity is reduced by 35% in milk treated with EFS = 18 kV/cm, pulse width = 400 μs, number of pulses = 70 and T_{max} = 43.9°C according to Castro et al. (2001b). They also recorded that 70.45 ms pulses of EFS 14.8 kV/cm treated to 2% milk resulted in the inactivation of ALP activity by 43%, 70.40 ms pulses of EFS 18.8 kV/cm applied to 2% milk resulted in the inactivation of ALP by 59%, 70.74 ms pulses of a field EFS 22.0 kV/cm applied to 2 mg/mL ALP dissolved in modified simulated milk ultrafiltrate (MSMUF) reduces the ALP activity by 65% and 70.45 ms pulses of EFS 14.8 kV/cm treat to ultra-high temperature (UHT) pasteurized 2% and 4% milk reduces the ALP activity by 59%. There is no ALP inactivation in milk at the batch operating mode with a square of 2 and EFS of 20 kV/cm and T_{max} of 35°C whereas there is 74% ALP inactivation at the batch operating mode with a square of 40 and EFS of 10 kV/cm and T_{max} of 70°C (Van Loey et al., 2002). Additionally, Sharma et al. (2014) indicated that MFGM when treated with PEF can be adsorbed and repaired by milk proteins. Heat treatment (63°C for 30 minutes and 72°C for 15s) was more disruptive than PEF treatment (20 kV/cm) whole milk, although these structural alterations were determined to be less detrimental.

2.2.2.4 MFG affects by PEF

The consequence of heat and mechanical treatments on milk components such as MFG are well documented; however, less is known about the impacts of PEF. According to Garcia-Amezquita et al. (2009), the MFG distribution size when treated with (36 kV/cm and 42 kV/cm) milk was the same with treatment by heat (63 °C for 30 min) milk, result a greater pulse number or electric field intensity did not affect particle size significantly ($p < 0.05$). Shamsi et al. (2008) stated that regardless of temperature and EFS used, there was no notable ($p > 0.05$) change in MFG average size in PEF-treated skim milk compared to raw milk. The effective volume of the fat globule may be greater by interaction with denatured skim milk serum proteins (Yang et al., 2021). Size of fat globule in sample solutions, like β-lactoglobulin-stabilized oil-in-water emulsions (pH 7.1) and pasteurized whole and skim milk, result no alter substantially ($p > 0.01$) by treatment of PEF for 29-36 kV/cm (Picart & Cheftel, 1997). They also stated that under comparable treatment circumstances, PEF-treated liquid dairy cream had a modest decrease in aggregates; nevertheless, photon microscopic analysis of cream that treated with PEF revealed no changes in size. PEF conditions (EFS = 21-26 kV/cm, 200 exponentially decaying pulses of 0.8-1.6 μs, f = 1 Hz, T_o: 30°C) and treatment media b-lactoglobulin-stabilized model emulsions; partial skim milk (1.5% fat); whole milk (3.5% fat); and cream (35% fat), Picart & Cheftel (1997) recorded no change in partial skim milk and whole milk MFG size and no damaging functional properties affect while for cream, stability index decreased and large MFG dissociated into smaller ones. When raw, skim and whole milk were treated with EFS = 35 and 38 kV/cm, monopolar square waved pulses of 2 μs, f = 1 Hz, flow rate = 60 mL/min T_i: 15 & 45°C and corresponding T_o: 30 & 60°C for 19.2 μs, Shamsi (2008) analyzed that MFG and casein size was unaffected while coagulation and rheological properties result in less change than heat treatments

(97°C for 10 min). Treatment conditions (24-64 and 8-24 exponentially decaying pulses with EFS = 36 & 42 kV/cm, $\tau = 2.6$ μs, flow rate = 0.383 L/min, $T_o < 25$°C) have no obvious influence on distribution of MFG particle size in whole milk. A significant rise in the number of bigger MFGs was detected after natural raw whole milk was processed using PEF techniques (Garcia-Amezquita et al., 2009). Due to the binding or aggregation to other components of certain casein micelles or tiny MFGs, raw milk treated with 16 kV/cm consist virtually greater number of particles of 0.4 – 1.0 μm in diameter, which led certain casein micelles or tiny MFGs to enlarge (Garcia-Amezquita et al., 2009).

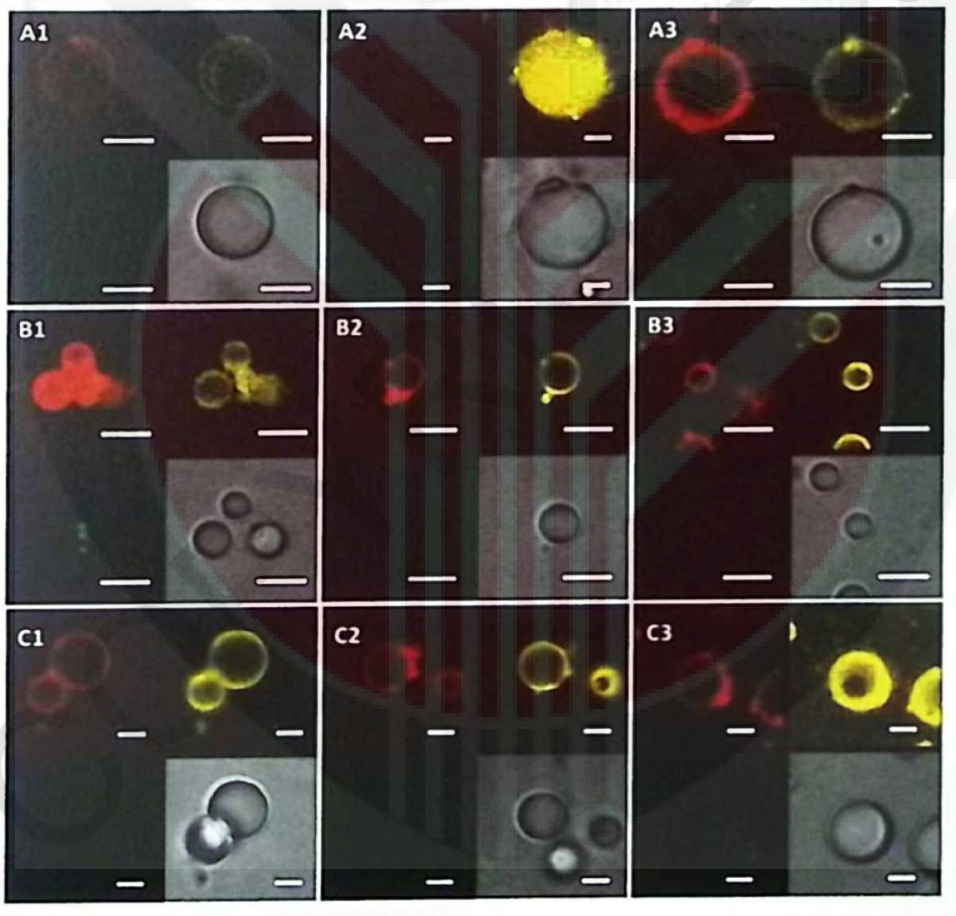

Figure 2.2.1: Confocal micrograph of fat globules (Yang et al., 2021)

Figure 2.2.1 shows confocal micrographs of fat globules. The polar lipids, glycoproteins and proteins in MFGM were labelled with different fluorescent dyes, glycoproteins with WGA-488 (red), phospholipids with Rhod-PE (yellow) & proteins with fast green (green). The grey pictures are the optical micrographs. Confocal laser scanning microscopy (CLSM) micrographs indicate MFGs of A represent raw milk (RM), B represents milk with small fat globules (MS) and C represents milk with large fat globules (ML); The numbers indicate the PEF treatment which 1 (control samples), 2 (treated sample at 9 kV/cm by PEF) & 3 (treated sample at 16 kV/cm by PEF). Scale bars = 5 μm (Yang et al., 2021). The RM that was treated with PEF treatment resulted in some tiny MFGs and phospholipid fragments adsorbing onto the MFG surface (A2 & A3, yellow, red & grey pictures). Certain milk proteins (green images) were also found on the surface of the MFGs sample. Sharma et al. (2014) reported that PEF treatment can cause whey protein and casein micelles to adsorb to the MFGM. PEF-induced membrane electroporation affects a tiny portion of the membrane, which is "repaired" by adsorbing proteins or small MFGs (A3) (Yang et al., 2021). This showed that MFG aggregation was substantially less prominent in the non-PEF treated. By enabling the milk proteins, phospholipid fragment and the tiniest MFG adsorption or reassembling of onto the MFG surface, PEF treatment was able to "repair" some of the damaged regions formed during the treatment (B2, B3, C2 & C3).

CHAPTER 3

METHODOLOGY

3.1 Research design

The ALP of the goat milk sample was treated by using the following method in Figure 3.1.1. Next, ALP and MFG of the treated milk were analyzed by using the following method in Figure 3.1.1. The flowchart in Figure 3.1.1 illustrates the research design used to analyze ALP and MFG of treated goat milk by PEF and pasteurization.

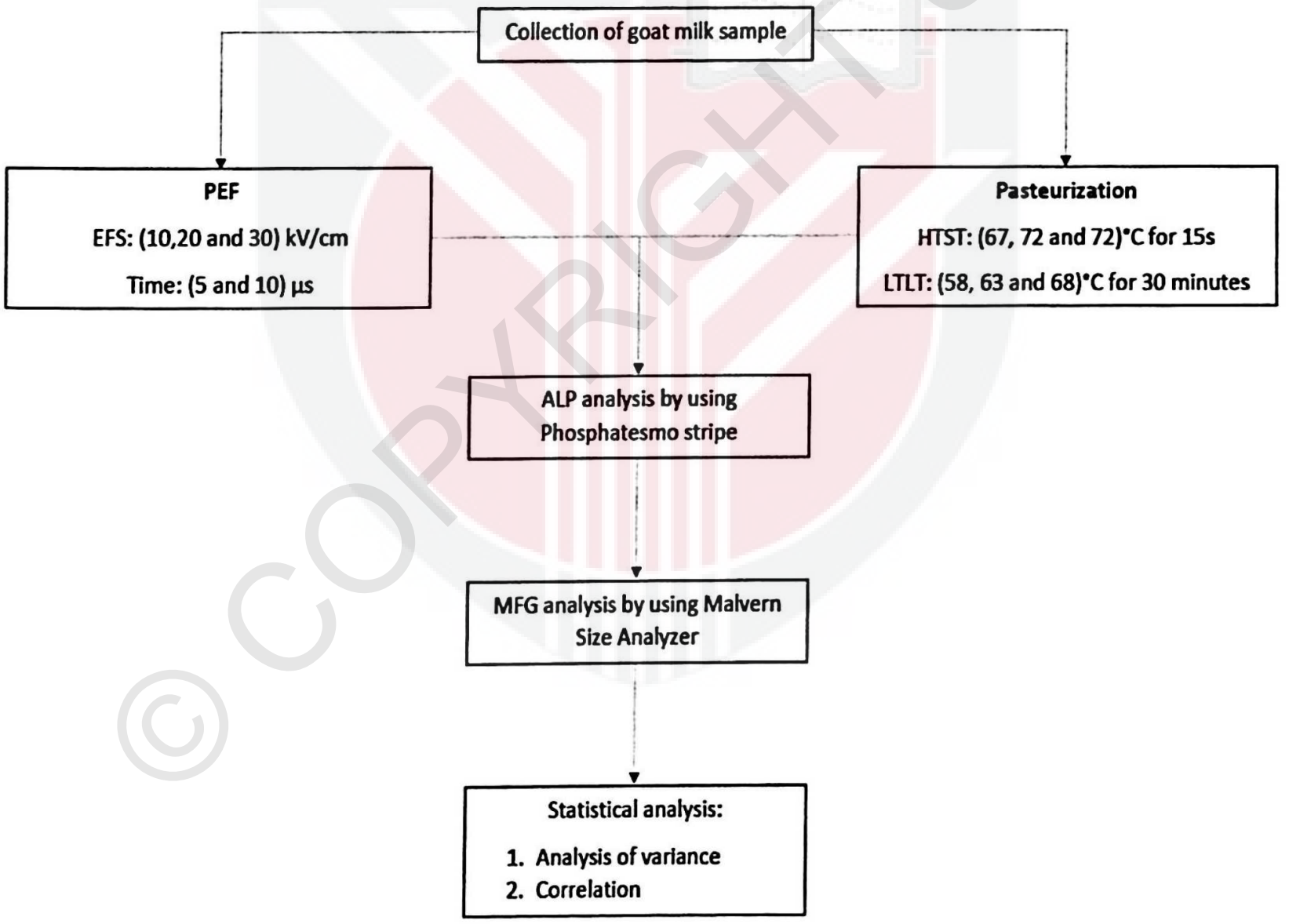

Figure 3.1.1: Flow chart of the research

3.2 Goat milk sample preparation

Samples of raw goat milk were bought from local dairy farms, Aeriel Agro and Food Processing located in Sepang, Selangor and delivered within 2 hours. The samples were transported at 4 ± 1 °C to Agro-Biotechnology Institute, Serdang, Selangor and, packed in low-density polyethylene (LDPE) bags. Then, the goat milk samples were stored at 4 ± 1 °C prior to PEF and after PEF treatment in the chiller of the Food and Process Engineering lab prior to further analysis.

3.3 Goat milk treatment by PEF

A lab-scale PEF system, the PowerMod 25 kW model made by Diversified Technologies, Inc. in Bedford, Massachusetts, was used to process PEF in a continuous mode. The system had two electrodes and a single co-field flow chamber. The parameters of PEF processing were a monopolar square wave pulse, a pulse repetition rate of 1 kHz, a fixed pulse width of 8 μs, a gap distance of electrode 4.7 mm, a chamber diameter of 3.5 mm, and a flow rate of 2.5 L/min. The PEF Unit's oscilloscope showed the shapes of the voltage and current waves. Raw goat milk was processed at EFS; 10, 20 and 30 kV/cm for 5 and 10 μs. 40 kV/cm is the maximum limit for the equipment. Right after the PEF was processed, samples were put in sterile 300 ml glass bottles and kept at 4 ± 2°C.

3.4 Goat milk treatment by pasteurization

The goat milk was treated by two pasteurization methods which are HTST and LTLT. The HTST used was 72°C for 15 s. (Huang et al., 2014). For LTLT used was 63°C for 30 minutes (Qi et al., 2015). To differentiate the change of test field colour, the temperature will be plus-minus by 5°C. The pasteurization was at (67, 72, 77)°C for 15 s and (58, 63, 68)°C for 30 minutes.

3.5 ALP analysis

The ALP analysis was referred from Suebsiri et al. (2019). The analysis was done by using test stripe, The Macherey-Nagel™ Phosphatesmo MI Qualitative Test Paper (Macherey-Nagel™, model Macherey-Nagel™ 90612, Germany) that was purchased from CSQ Analytic for the determination of ALP in milk. Firstly, the test stripe was dipped briefly into the treated milk and the excess milk at the test stripe was shaken. Secondly, the test stripe was placed in an enclosed PE bag that has been provided to prevent the test stripe from drying out. Next, the test stripe needs to be incubated at 36°C for 1 hour before visualizing the results. In the presence of ALP, the test field will turn yellow. A yellow colouration indicates that goat milk was not sufficiently treated. No colouration indicates that the goat milk was correctly completed the treatment.

3.6 MFG analysis

The procedure for MFG analysis was referred from Mohamad et al. (2020) approach was made while determining particle size distribution. A particle size analyzer linked with a Hydro 2000MU Masterizer 2000 (Malvern Instrument, UK) linked with a Hydro 2000MU dispersion unit was utilized with a sample with dispersed phase refractive indices of 1.33 and 1.4 (Mohamad et al., 2020). The instrument's small volume presentation unit already held 120 mL of deionized water, thus, 0.01 L of the sample was added. A 2000 rpm stirrer pumped the sample into the optical cell. Malvern's software was used to automatically measure the volume fractions vs particle size (size distributions).

3.7 Statistical analysis

The statistical analysis, two-way analysis of variance (ANOVA) was carried out to investigate how independent variable (EFS, temperature and treatment time) may affect the dependent variable which was d(0.1), d(0.5), d(0.9) and SSA using Microsoft Excel software. The level for statistical significance value was set at ($p = 0.05$). ($p > 0.05$) showed that the change is not significant while $p < 0.05$ showed that the change is significant. The statistical analysis, correlation was also carried out to study the existence of a potential linear connection between two continuous variables by using Microsoft Excel software. It is straightforward, both in terms of calculation and interpretation where it calculates the degree to which two variables are related to one another by finding the R^2 value. The value ranges from -1 (perfect negative correlation) to +1 (perfect positive correlation).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 ALP analysis for PEF

Figure 4.1.1 below shows the Phosphatesmo test stripe before the experiment started and when it was dipped into the raw goat milk. When the stripe is dipped into the raw goat milk, the white field turns yellow.

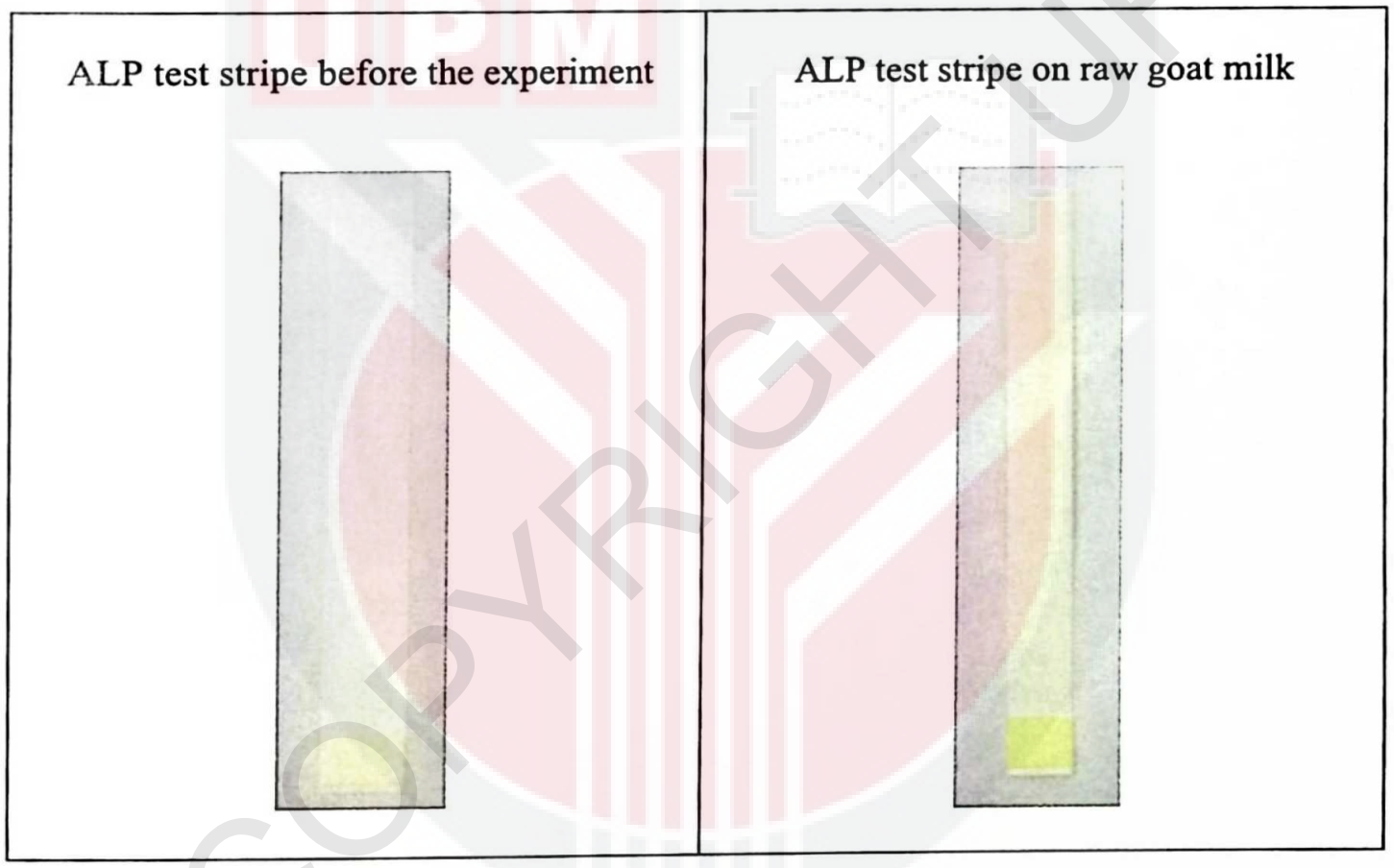

Figure 4.1.1: Phosphatesmo before the experiment and after dipped in raw goat milk

Table 4.1.1: Table of Phosphatesmo for PEF-treatment goat milk

Time (μs) / EFS (kV/cm)	5	10
10		
20		

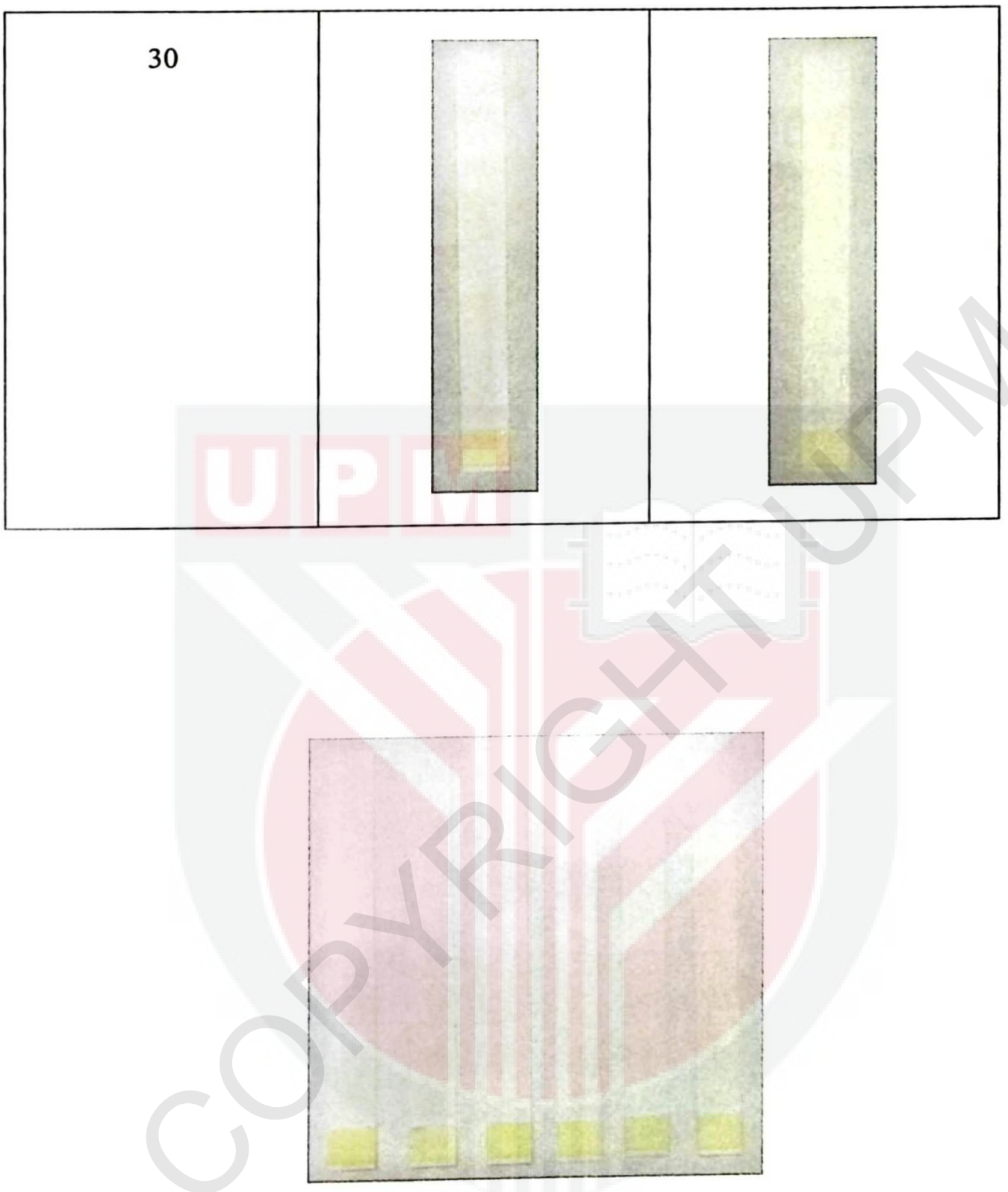

Figure 4.1.2: ALP test stripes under PEF treatment. From left, (10 kV/cm, 5 μs), (10 kV/cm,10 μs), (20 kV/cm, 5 μs), (20 kV/cm, 10 μs), (30 kV/cm, 5 μs) and (30 kV/cm,10 μs).

Table 4.1.2: Table of Phosphatesmo for pasteurization-method goat milk

Time	Temperature (°C)	Phosphatesmo
15s	67	
	72	

	77	
30 min	58	
	63	

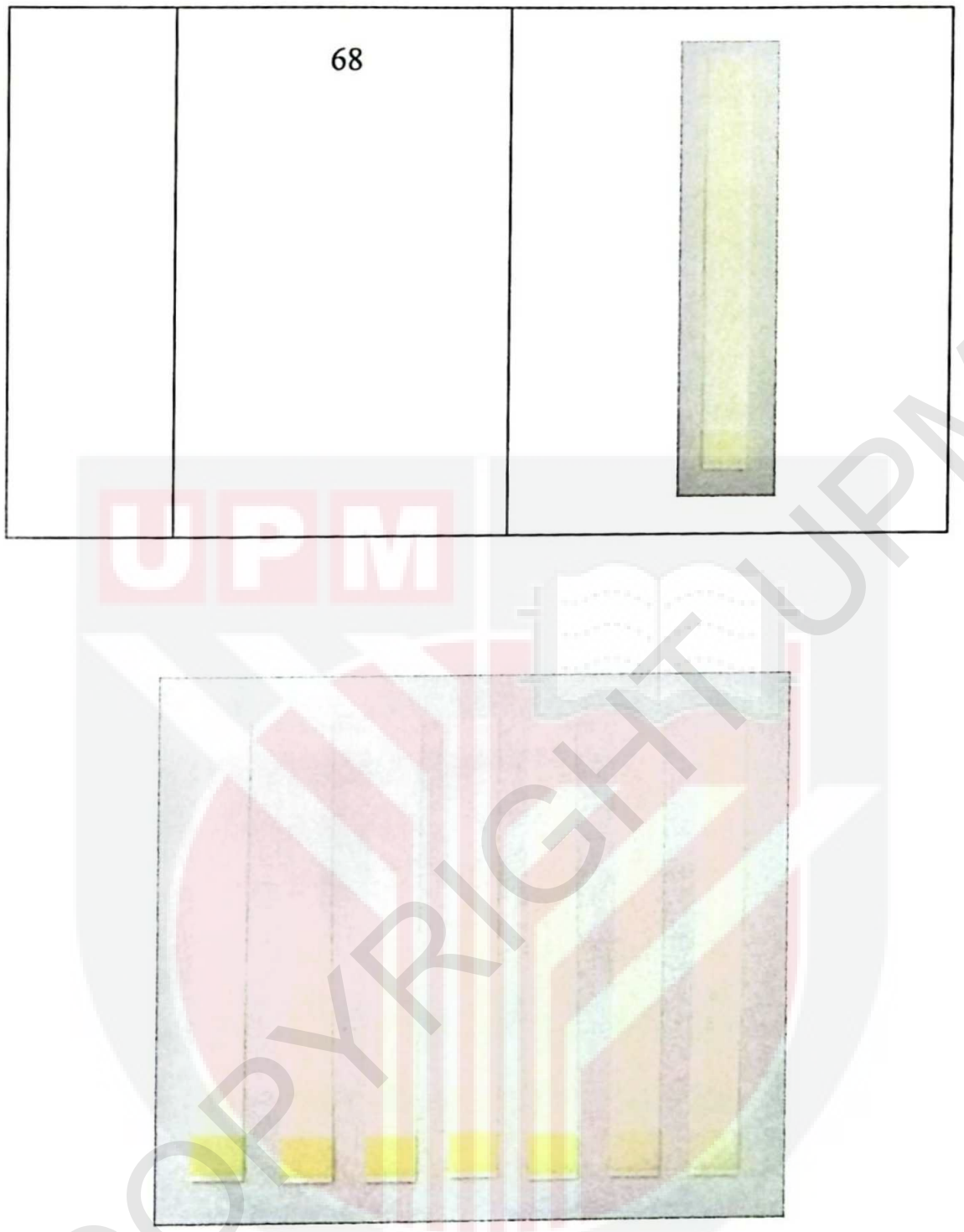

Figure 4.1.3: ALP test stripe under pasteurization treatment. From left, raw goat milk, HTST (67°C, 15s), HTST (72°C, 15s), HTST (77°C, 15s), LTLT (58°C, 30 min), LTLT (63°C, 30 min) and LTLT (68°C, 30 min)

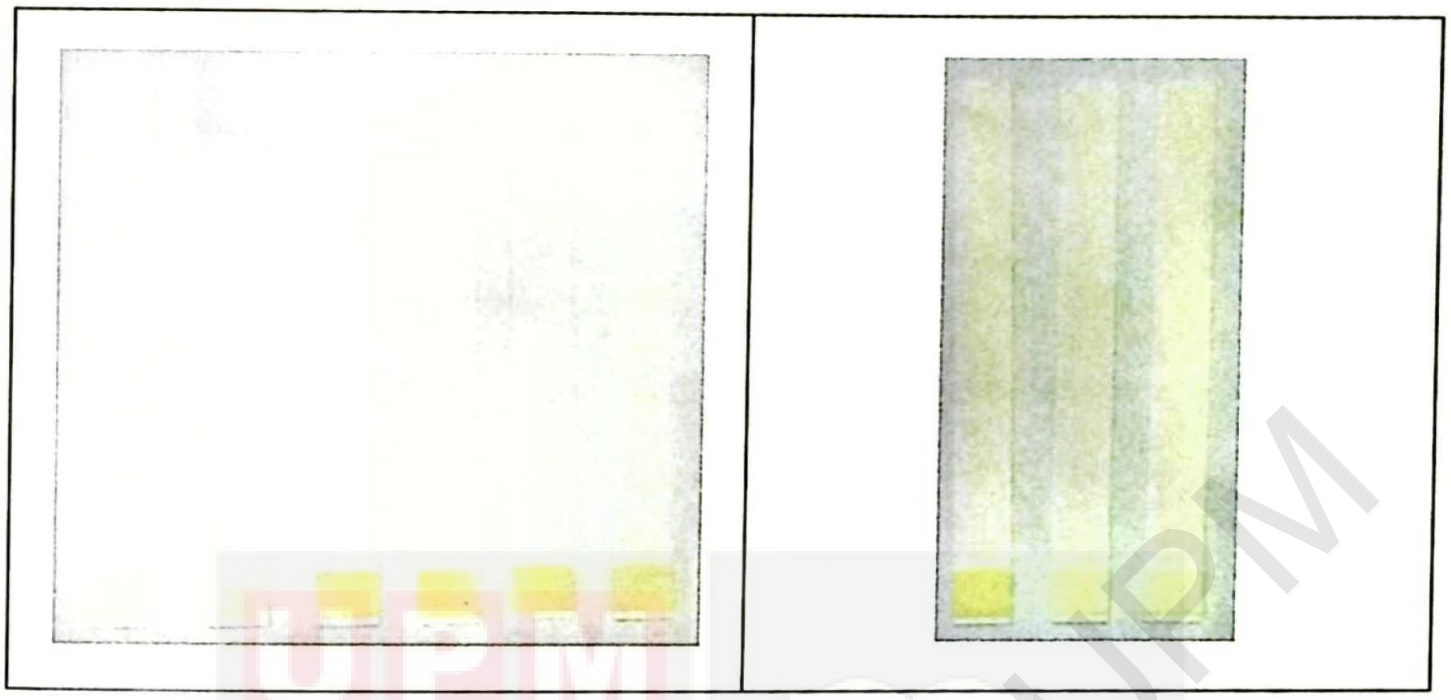

Figure 4.1.4: ALP test stripes under PEF and pasteurization treatment. From left, PEF: (10 kV/cm, 5 μs), (10 kV/cm,10 μs), (20 kV/cm, 5 μs), (20 kV/cm, 10 μs), (30 kV/cm, 5 μs) and (30 kV/cm,10 μs). LTLT: (58°C, 15s), (63°C, 15s) and (68°C, 15s)

From Table 4.1.1 and Figure 4.1.2, all test stripes' fields change to yellow and do not remain white like the test field before the experiment started. From Table 4.1.2, only LTST at 63°C and 68°C for 30 min was able to maintain the white colour of test field compared to all HTST and LTLT at 58°C. These indicated that for this experiment, only LTLT-treated milk at 63°C and 68°C for 30 min safe for human consumption which had been used by industry and recommended by FDA. These showed also that there is still the amount of ALP contained in the PEF-treated goat milk, which indicates that the PEF-treated goat milk is still not safe to consume. This is also related to research by Mohamad et al. (2021) which found that goat milk that PEF-treated goat milk with maximum EFS, 40 kV/cm and time, 13 μs still not safe for human consumption because the *E.coli* reduction did not reach the requirements by FDA. However, the PEF test stripe for ALP cannot be compared with pasteurization for 15s test stripe because all the temperature for this duration turns the test field to yellow which showed the goat milk still contain

the amount of ALP that is still not safe for human consumption. Lombardi et al. (2000) claimed that ALP is extremely sensitive to heat than the electric field. They also found that 98% ALP inactivation by pasteurization; LTST and HTST compared to PEF only able inactive ALP maximum by 67% by 35 kV/cm 19.6 μs with pre-heat, 15°C and post-PEF, 60°C. Then, UHT treatment on goat milk was able to reduce ALP activity in goat milk below 350 mU/L which was safe for a human to consume and in terms of bacterial pathogen, it also can be considered safe.

4.2 MFG analysis for PEF and pasteurization

Table 4.4.1 and 4.4.2 show the particle size distribution based on d(0.1), d(0.5), d(0.9), SSA, D[3,2] and D[4,3]. d(0.1) signify 10% of the particles have the same size distribution at the D value (diameter). d(0.5) signify that the median of the particles, where half of the populations reside below the D value and the other half of the population resides above the D value. d(0.9) signify that 90% of the sample population of particles resides above the D value. SSA means specific surface area. D[3,2] means surface-weighted mean and D[4,3] means volume-weighted mean.

Table 4.2.1: Effects of PEF on the particle size distribution of goat milk

PEF time (μs)	EFS (kV)	Diameter (μm),			SSA (m^2/g)	D[3,2] (μm)	D[4,3] (μm)
		d(0.1)	d(0.5)	d(0.9)			
Untreated	Untreated	5.022	26.845	89.085	0.896	6.681	38.795
5	10	0.833	2.587	12.891	3.15	1.904	5.443

	20	0.825	2.458	15.296	3.20	1.877	6.776
	30	0.823	2.248	12.359	3.32	1.808	5.668
10	10	0.840	2.404	11.581	3.21	1.868	5.140
	20	0.818	2.402	14.236	3.24	1.850	6.072
	30	0.828	2.449	13.370	3.20	1.873	5.835

Table 4.2.2: Effects of pasteurization on the particle size distribution of goat milk

Pasteurization time	**Temperature (°C)**	**Diameter (µm)**			**SSA (m^2/g)**	**D[3,2] (µm)**	**D[4,3] (µm)**
		d(0.1)	**d(0.5)**	**d(0.9)**			
15s	67	0.831	1.961	7.121	3.57	1.681	3.090
	72	0.832	2.041	7.634	3.49	1.718	3.296
	77	0.805	1.806	6.939	3.75	1.599	2.939
30 minutes	58	0.824	1.934	7.489	3.59	1.674	3.188
	63	0.823	2.031	8.657	3.48	1.722	4.119
	68	0.825	1.962	7.547	3.56	1.684	3.221

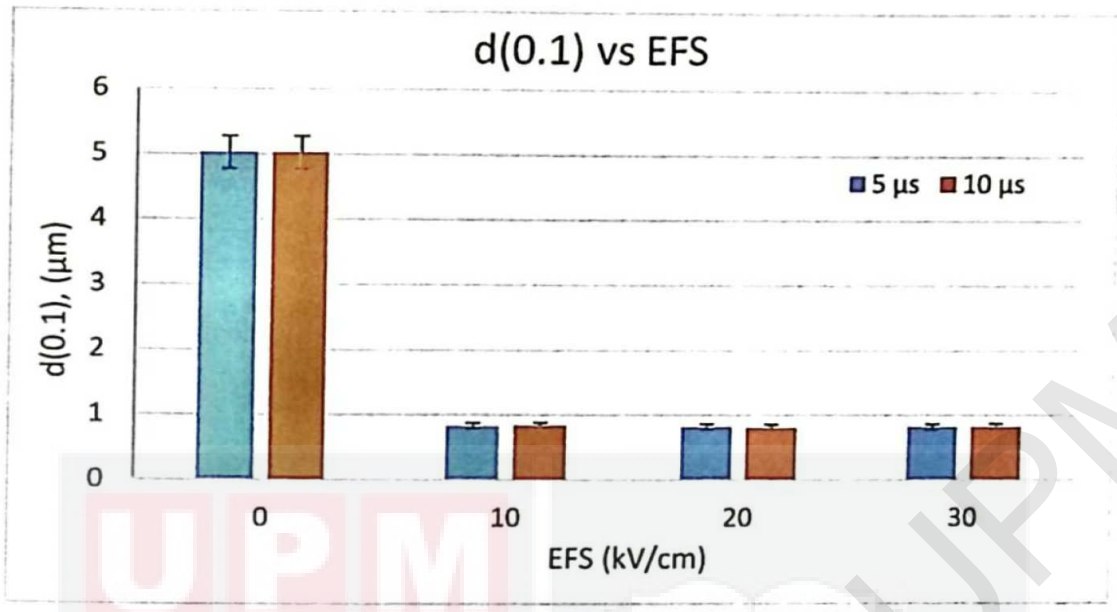

Figure 4.2.1: d(0.1) vs EFS bar chart

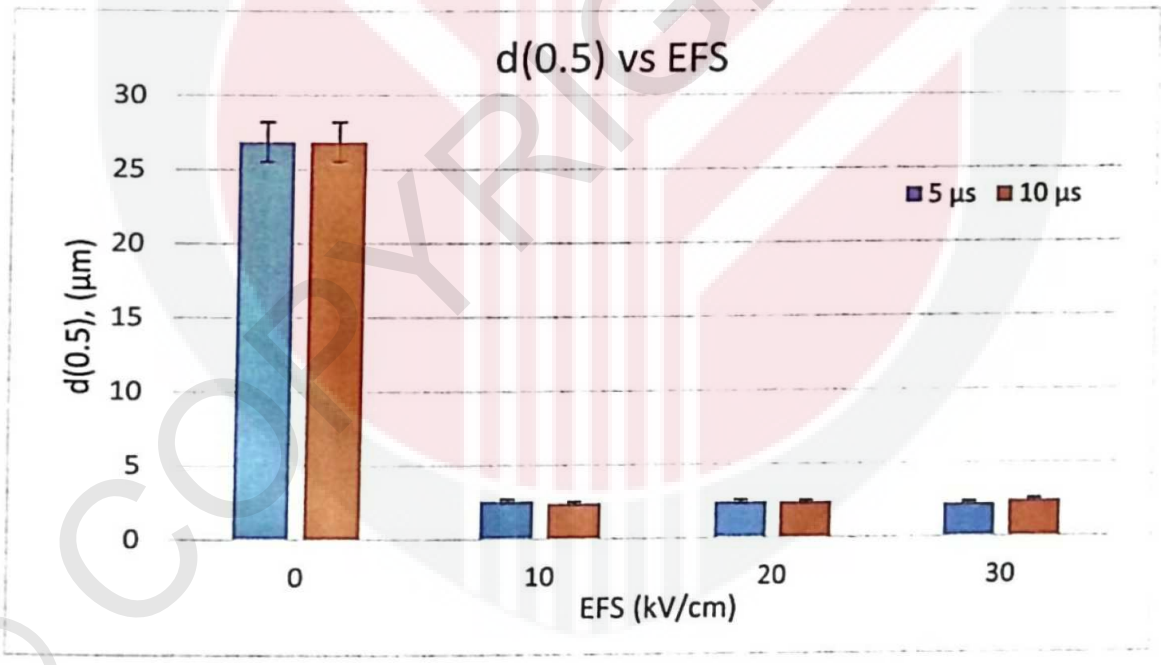

Figure 4.2.2: d(0.5) vs EFS bar chart

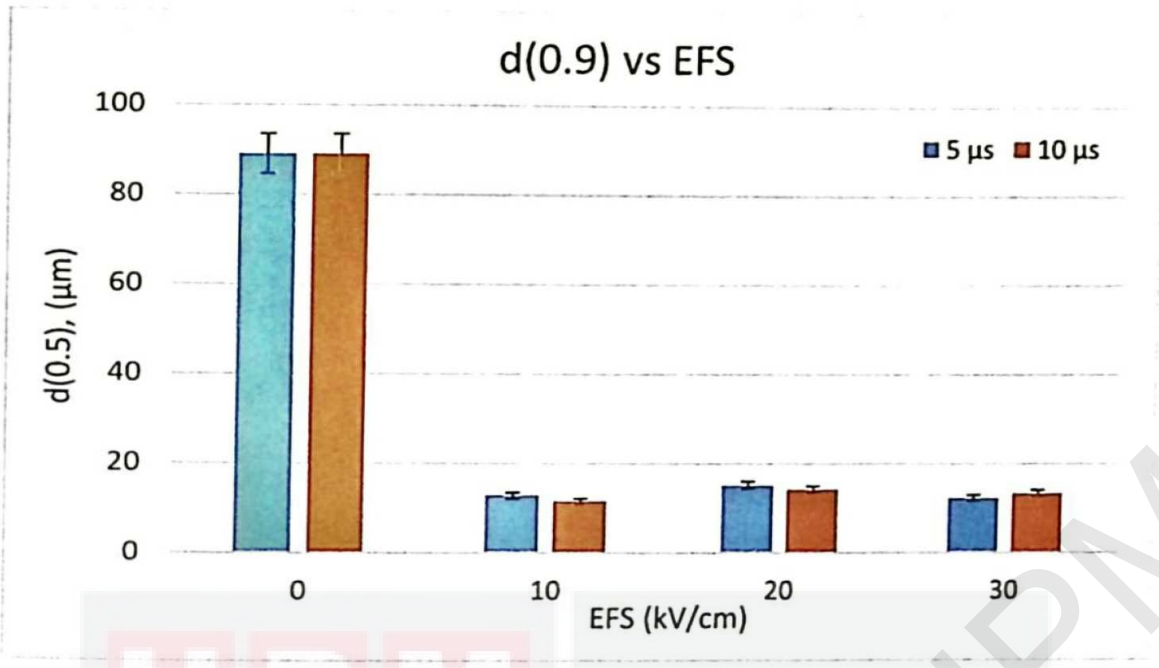

Figure 4.2.3: d(0.9) vs EFS bar chart

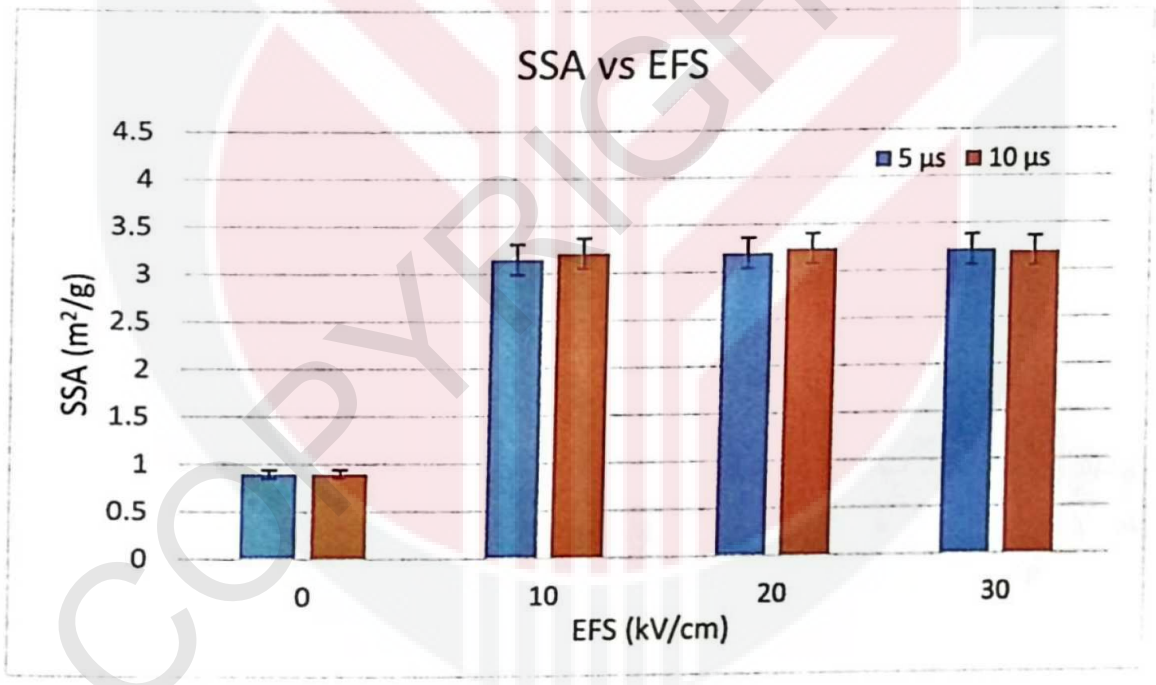

Figure 4.2.4: SSA vs EFS bar chart

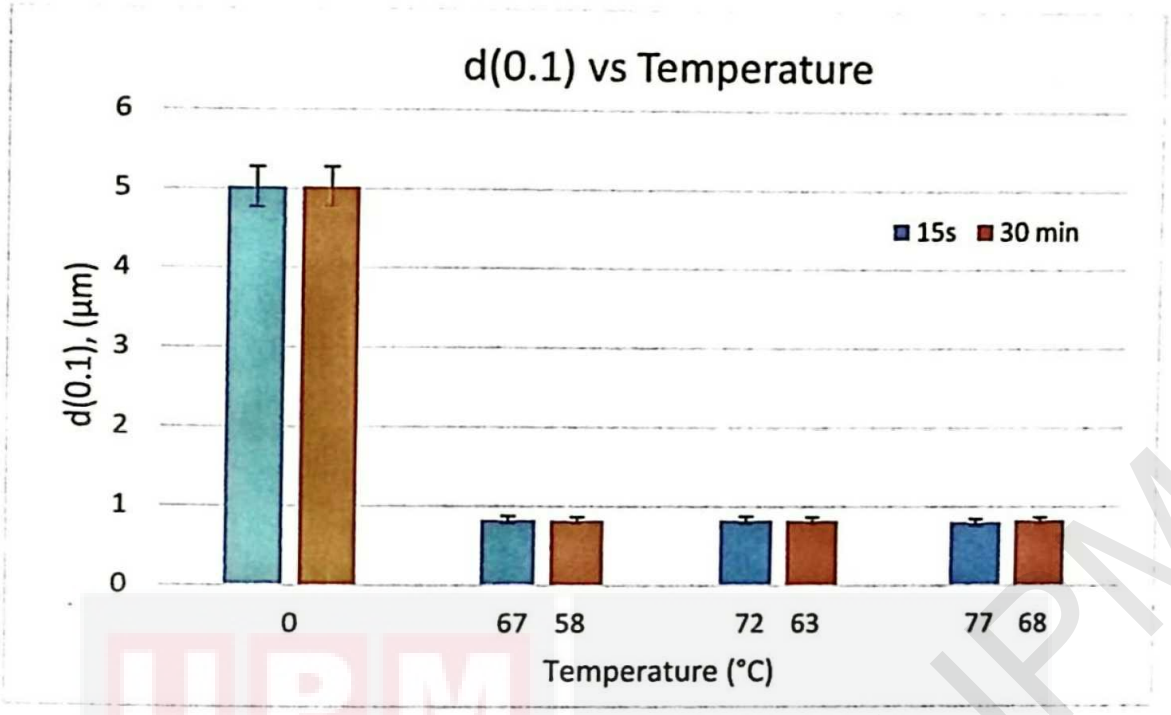

Figure 4.2.5: d(0.1) vs Temperature bar chart

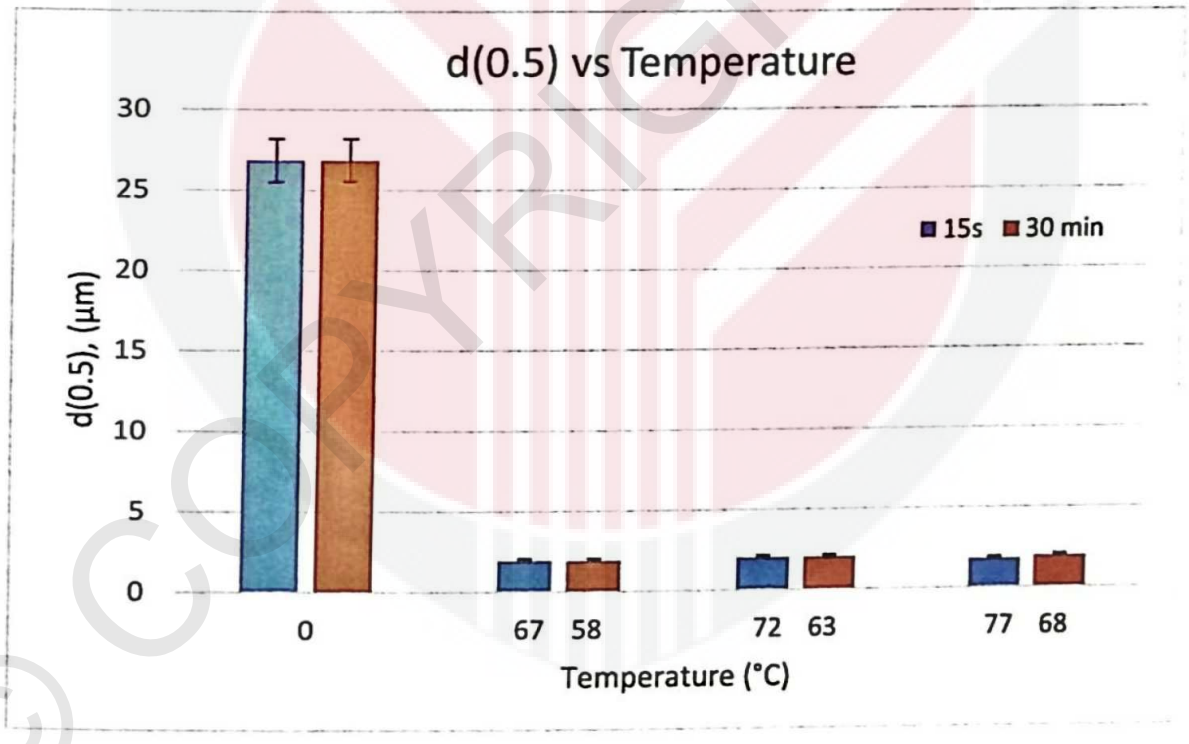

Figure 4.2.6: d(0.5) vs Temperature bar chart

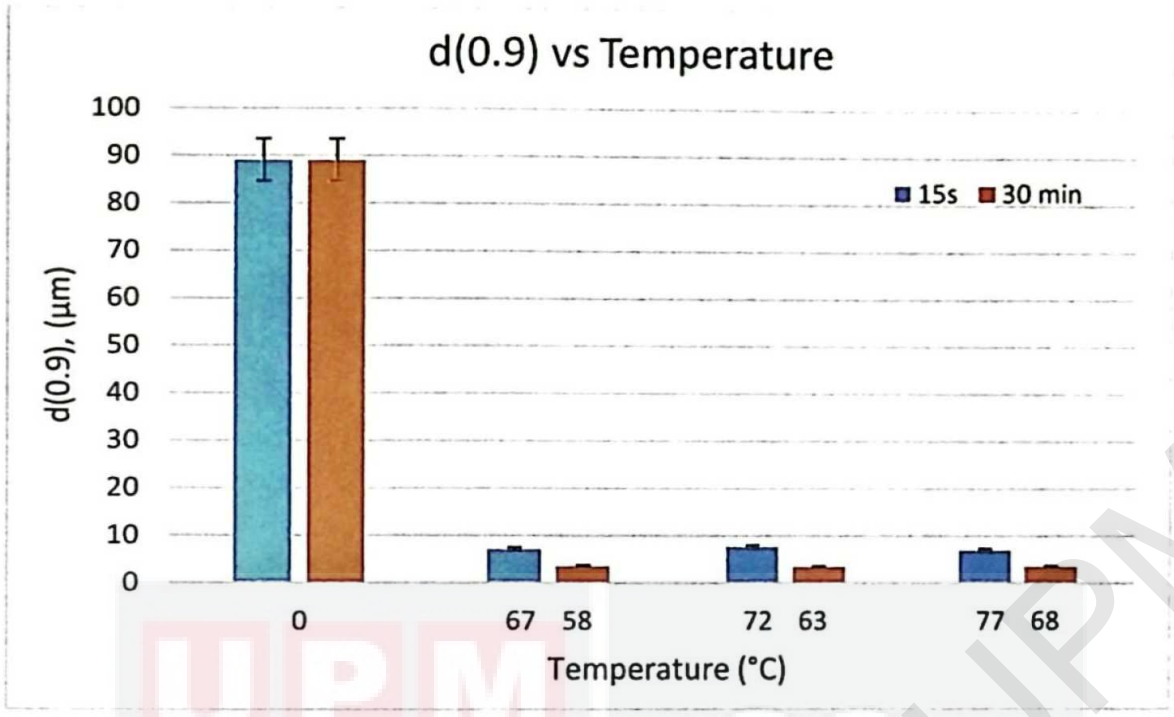

Figure 4.2.7: d(0.5) vs Temperature bar chart

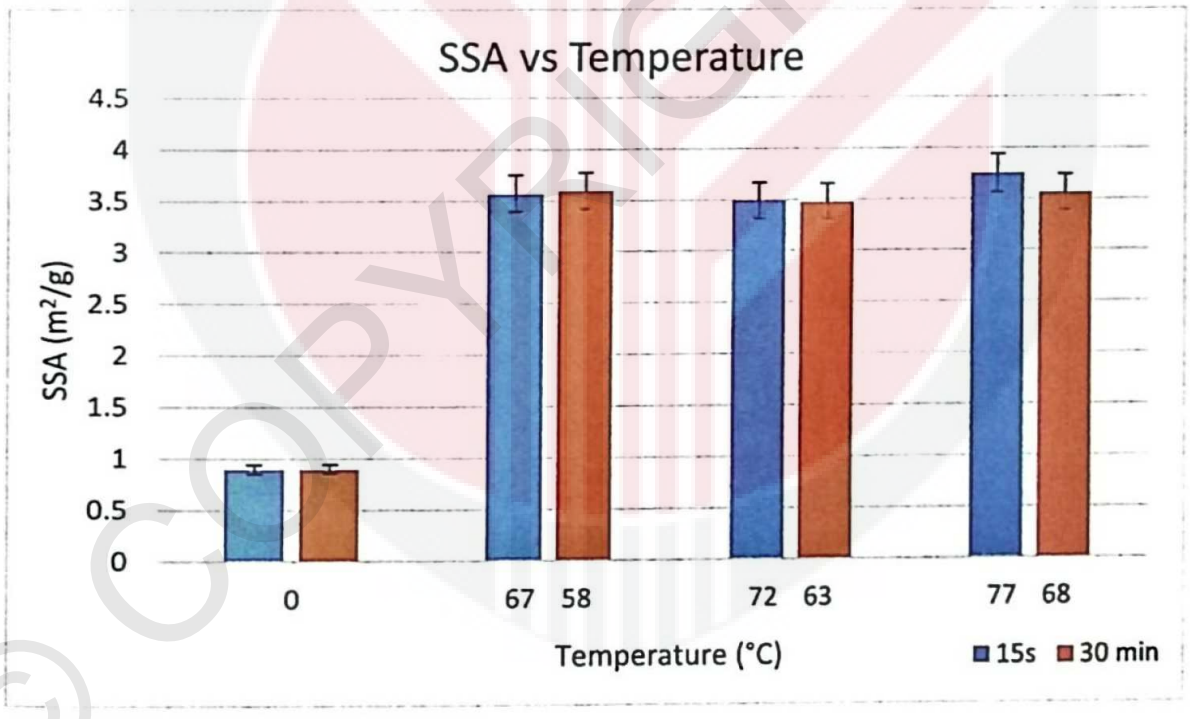

Figure 4.2.8: SSA vs Temperature bar chart

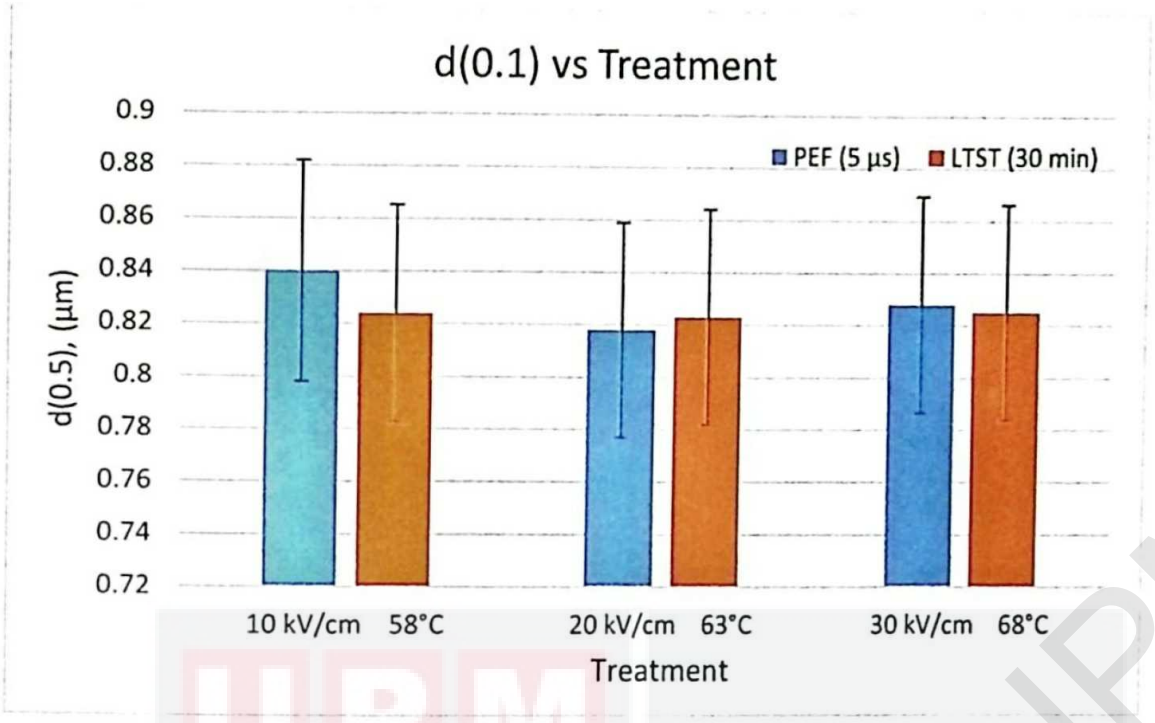

Figure 4.2.9: d(0.1) vs Treatment bar chart

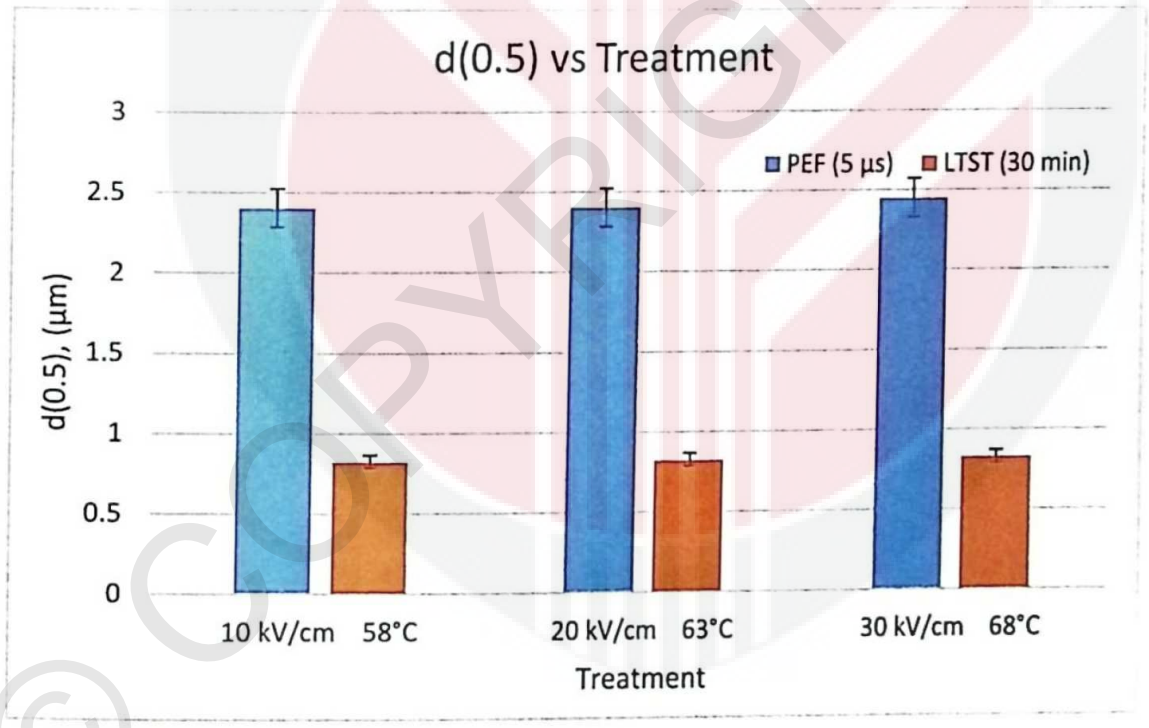

Figure 4.2.10: d(0.5) vs Treatment bar chart

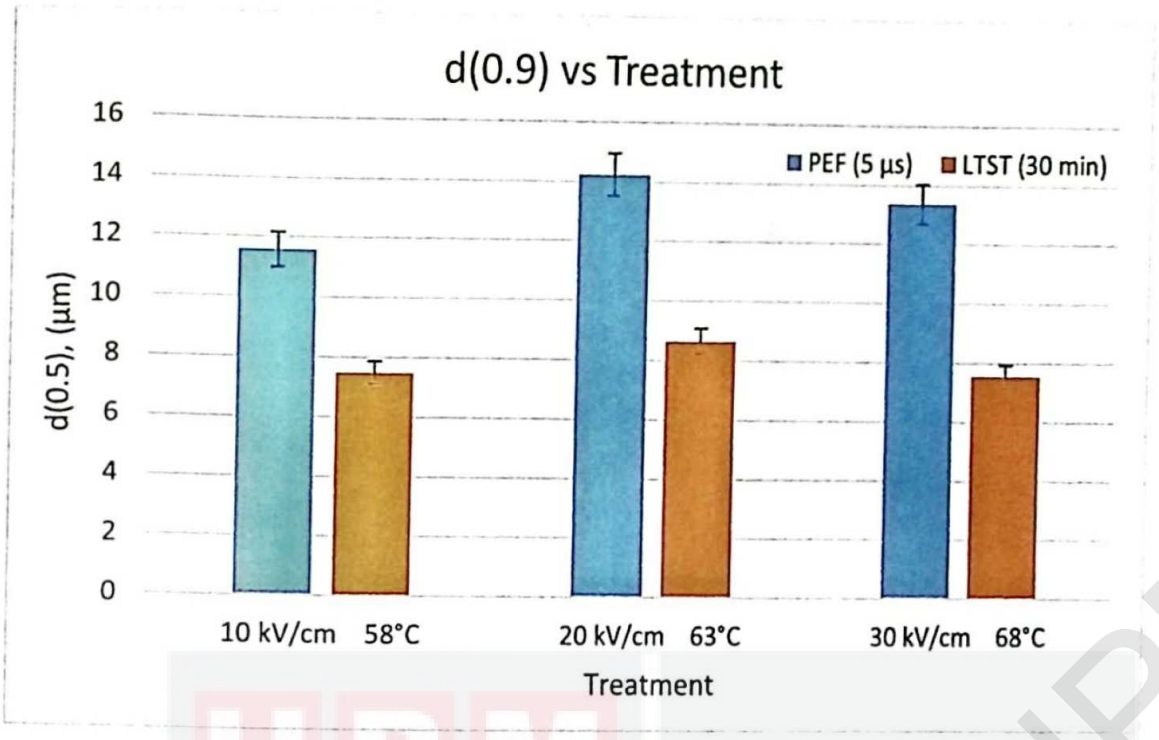

Figure 4.2.11: d(0.9) vs Treatment bar chart

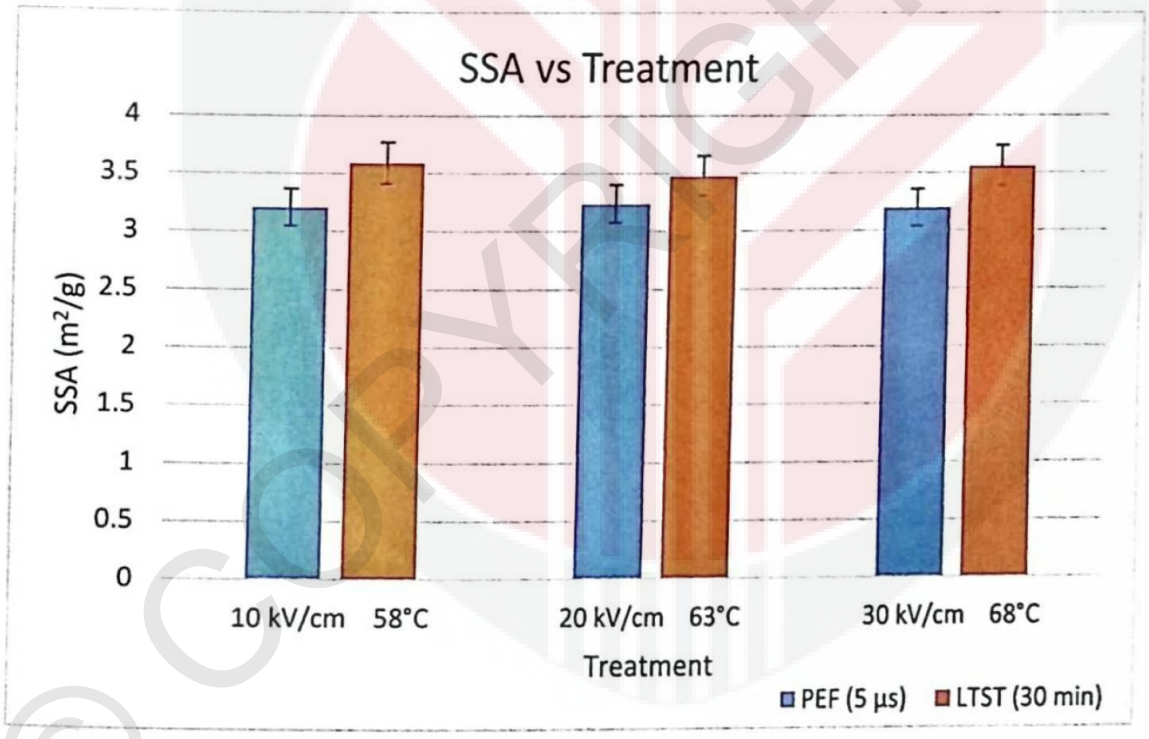

Figure 4.2.12: SSA vs Treatment bar chart

Based on Figure 4.2.1 to 4.2.8, d(0.1), d(0.5) and d(0.9) for treated goat milk, all decrease from the value of untreated goat milk while SSA for treated goat milk, all increase from the value of untreated goat milk value. This was caused by the functional properties of milk had been altered that stated by Sharma et al. (2015) and there was the shear force exerted during pumping that damaged the properties of milk stated by Sharma et al. (2014). For d(0.1) in Figure 4.2.5, the value in PEF treated and pasteurization was not too different. This correlates with studies from Garcia-Amezquita et al. (2009) observed that there was no difference in MFGs size distribution in pasteurization at 63°C for 30 minutes and in PEF-treated at 36 and 42 kV/cm for 2.6 μs. Based on Figure 4.2.4, 4.2.8 and 4.2.12, pasteurization has a higher value for SSA as this treatment has more particle size reduction compared to the particle size of untreated milk and PEF-treated milk. The reduction size of fat globule size increases the value of SSA as casein micelles and whey proteins have been adsorbed onto the MFG surface, changing the MFGM's chemical components (Garcia-Amezquita et al., 2009) In variable of time, the particle size of goat milk for PEF is varied with each other based on Figure 4.2.1 to 4.2.8. From Figure 4.2.9 to 4.212, d(0.1), d(0.5) and d(0.9) for pasteurization were lower compared to PEF and SSA for pasteurization was greater than PEF. This was consistent with findings by Sharma et al. (2015) who found that pasteurized milk at 73°C for 15 seconds and 63°C for 30 minutes results in more damage to MFG size than PEF-treated milk (20 and 26 kV/cm for 2.6 μs). The heat was also the main important factor that affects milk droplet sizes (Qi et al., 2015). This has been stated by Kilic-Akyilmaz et al. (2022), heat treatment alters MFGM by promoting interaction between its components and native plasma proteins. Mechanical elements during treatment which are pumping, agitation and high shear also affect the composition of MFGM (Kilic-Akyilmaz et al., 2022). This is also due to casein micelles adsorb in their natural state without interacting with other plasma proteins on the MFGM surface (Michalski et al., 2002). According to Sharma et al. (2015), casein micelle structure is impacted

by the PEF treatment's intensity and temperature, which also changes other casein attributes like gelation rate, gel strength, thermal stability, aggregation, rennetability, emulsion stability and hydrophobicity.

4.3 Statistical analysis

Table 4.3.1: Analysis of Variance of treatment time and EFS to goat milk particle size

Results	Independent variable	P-value	Significance
d(0.1)	Treatment time	$p > 0.05$	Non-significant
	EFS	$p < 0.05$	Significant
d(0.5)	Treatment time	$p > 0.05$	Non-significant
	EFS	$p < 0.05$	Significant
d(0.9)	Treatment time	$p > 0.05$	Non-significant
	EFS	$p < 0.05$	Significant
SSA	Treatment time	$p > 0.05$	Non-significant
	EFS	$p < 0.05$	Significant

Table 4.3.2: Analysis of Variance of treatment time and temperature to goat milk particle size

Results	Independent variable	P-value	Significance
d(0.1)	Treatment time	$p > 0.05$	Non-significant
	Temperature	$p < 0.05$	Significant
d(0.5)	Treatment time	$p > 0.05$	Non-significant
	Temperature	$p < 0.05$	Significant
d(0.9)	Treatment time	$p > 0.05$	Non-significant
	Temperature	$p < 0.05$	Significant
SSA	Treatment time	$p > 0.05$	Non-significant
	Temperature	$p < 0.05$	Significant

Table 4.3.3: Correlation coefficient R^2 of PEF-treated goat milk to goat milk particle size

	Time (µs)	EFS (kV/cm)	d(0.1) (µm)	d(0.5) (µm)	d(0.9) (µm)	SSA (m^2/g)
Time (µs)	1.0000	-	-	-	-	-
EFS (kV/cm)	0.5095	1.0000	-	-	-	-
d(0.1) (µm)	-0.7496	-0.6814	1.0000	-	-	-
d(0.5) (µm)	-0.7504	-0.6841	0.9999	1.0000	-	-
d(0.9) (µm)	-0.7546	-0.6722	0.9990	0.9992	1.0000	-
SSA (m^2/g)	0.7462	0.7054	-0.9984	-0.9988	-0.9978	1.0000

Table 4.3.4: Correlation coefficient R^2 of pasteurization-treated goat milk to goat milk particle size

	Time (min)	**Temperature (°C)**	**d(0.1) (µm)**	**d(0.5) (µm)**	**d(0.9) (µm)**	**SSA (m^2/g)**
Time (min)	1.0000	-	-	-	-	-
Temperature (°C)	0.1888	1.0000	-	-	-	-
d(0.1) (µm)	-0.3583	-0.9734	1.0000	-	-	-
d(0.5) (µm)	-0.3568	-0.9736	1.0000	1.0000	-	-
d(0.9) (µm)	-0.3486	-0.9746	0.9999	0.9999	1.0000	-
SSA (m^2/g)	0.3298	0.9788	-0.9966	-0.9969	-0.9972	1.0000

Based on Table 4.3.1 and 4.3.2, EFS and temperature showed significant changes ($p < 0.05$) to d(0.1), d(0.5), d(0.9) and SSA. However, both treatment time for PEF and pasteurization did not show significant relationship ($p > 0.05$) to d(0.1), d(0.5), d(0.9) and SSA. For SSA, it correlated with results from Mohamad et al. (2020) that found a significant relationship between SSA and EFS; the SSA value increased from 29.30 ± 0.25 m^2/g (untreated) to 29.67 ± 0.21 (5 µs, 20 kV/cm;) to 30.65 ± 0.39 m^2/g (10 µs, 40 kV/cm). However, for d(0.1), d(0.5) and d(0.9), Mohamad et al. (2020) found there was non-significant relationship with EFS. These were different from this paper's findings.

From Table 4.3.3 and 4.3.4, EFS was not strongly correlated with d(0.1), d(0.5), d(0.9) and SSA compared to the temperature which showed the R^2 value nearly approaching 1. This showed that pasteurization reduce more goat milk particle size compared to PEF due to heat being the main important factor that affects milk droplet sizes (Qi et al., 2015). Based on Table 4.3.3, the treatment time has a higher value of R^2 compared to EFS which indicates that increasing of treatment time for PEF may cause more particle size reduction in goat milk than EFS. In contrast, from Table 4.3.4, the temperature has a higher value of R^2 compared to treatment time which showed that increasing temperature cause more particle size reduction in goat milk than the treatment time.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

From this study, it was observed, PEF was able to reduce the amount of certain ALP based on the EFS and treatment time. The greater the EFS and the longer the treatment duration, there will be more ALP can be reduced. However, for this experiment, the amount of ALP reduced during the experiment, was not enough for human safety consumption as all the Phosphatesmo for PEF treatment turned to yellow. The EFS and time should be increased to ensure that ALP can be reduced to the amount that is safe for human consumption. Heat treatment especially pasteurization was still the main method to reduce ALP and bacteria pathogens in milk as LTST at 63 and 68 for 30 minutes maintain their white colour. However, the MFGs of milk get more damaged under the pasteurization method compared to PEF treatment as pasteurization reduced the d(0.1), d(0.5) and d(0.9) value more than PEF and increased the SSA value more than PEF. This causes less damage to PEF MFGs compared to pasteurization. Hence, the physical properties of goat milk can be maintained more due to minimal damage to their MFGs. In conclusion, this study showed that milk treatment by PEF may have a big potential for the treatment of milk in dairy industries instead of traditional treatment, pasteurization.

5.2 Recommendations

PEF treatment on the effect of ALP and MFGs should be further studied to find the right amount of EFS and treatment time to reduce ALP to a desired value recommended by the FDA with minimal damage to MFGs to ensure that goat milk treated by PEF is safe for consumption and maintained the 'originality' of goat milk. Besides that, PEF-processed goat milk should be scaled up to assure PEF technology can be utilized for commercial use. Apart from that, it is very recommended to use fluorometric assay for analysis of ALP in milk as this assay can show the actual amount of ALP in milk and for now, it is the most accurate assay and required a shorter time to measure the amount of ALP in milk.

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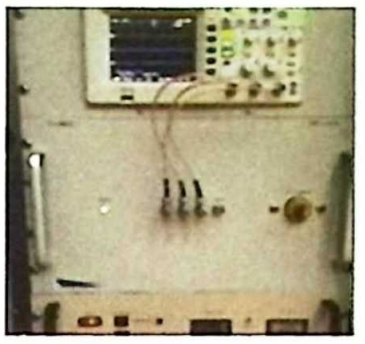
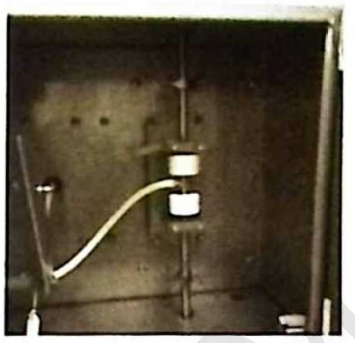
APPENDICES

a) High voltage pulse generator

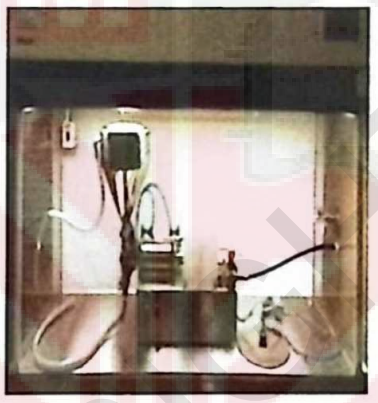


b) Oscilliscope & control system

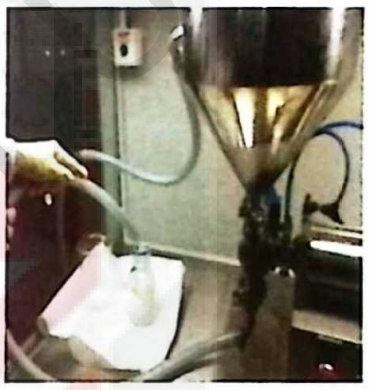


c) Treatment chamber

d) Plate heat exchanger



e) Laminar air flow



f) Dispenser unit

Figure above showed the lab-scale PEF system, the PowerMod 25 kW model (Mohamad et al., 2021)

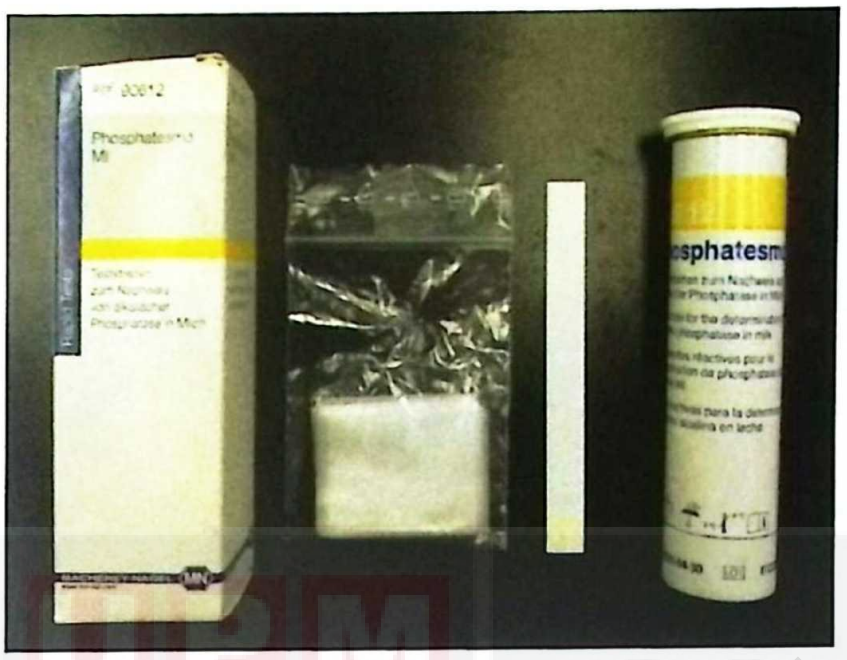


Figure above showed Phosphatesmo MI Qualitative Test Paper

Figure above showed the Malvern Size Analyzer