COMPARISON OF THREE DNA EXTRACTION METHODS FROM RAW COW'S MILK SAMPLE

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COMPARISON OF THREE DNA EXTRACTION METHODS FROM RAW COW'S MILK SAMPLE

by

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Thesis submitted in partial fulfilment of the requirements
for the degree of
Master of Science (Forensic Science)

September 2020

CERTIFICATE

This is to certify that this dissertation, “COMPARISON OF THREE DNA EXTRACTION METHODS FROM RAW COW’S MILK SAMPLE” is bona fide record of research work done by MS SYAHIRAH OMAR during the period February 2020 to September 2020 under my supervision. I have read this dissertation and that in my point of view it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation to be submitted in partial fulfillment for the Master of Science (Forensic Science).

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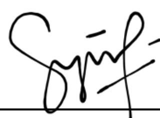
DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except where otherwise stated and duly acknowledge. I also declare that it has not been previously for concurrently submitted as a whole for any other degrees at Universiti Sains Malaysia or other institutions. I grant Universiti Sains Malaysia the right to use the dissertation for teaching, research and promotional purposes.



(SYAHIRAH BINTI OMAR)

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LIST OF ABBREVIATIONS AND SYMBOLS

°C	Degree celcius
Bp	Base pair
DNA	Deoxyribonucleic acid
g	Gram
Kbp	Kilobase pair
mg	Milligram
M	Molar
mL	Millilitre
mM	Millimolar
μL	Microlitre
ng	Nanogram
PCR	Polymerase Chain Reaction
PPE	Personal Protection Equipment
RNA	Ribonucleic acid
UV	Ultraviolet
rpm	Revolutions Per Minute
TE	Tris EDTA
TBE	Tris Borate EDTA

PERBANDINGAN TIGA KAEDAH PENGEKSTRAKAN DNA DARIPADA SAMPEL SUSU LEMBU MENTAH

ABSTRAK

Perkembangan dan kemajuan dalam pengekstrakan asid deoksiribonukleik (DNA) telah banyak berlaku sejak awal penemuannya pada tahun 1986. Pengekstrakan DNA menjadi langkah pertama yang diperlukan sebelum melakukan analisis DNA yang lainnya. Dalam kajian ini, susu lembu mentah telah didedahkan kepada tiga kaedah pengekstrakan yang berbeza untuk menentukan kaedah yang paling baik dalam penghasilan DNA. DNA genomik yang diekstrak daripada sampel susu lembu mentah dengan tiga kaedah telah dinilai daripada sudut ketulenan dan kuantiti dengan menggunakan spektrofotometri dan gel elektroforesis. Secara keseluruhannya, ketiga-tiga kaedah tidak menunjukkan perbezaan yang ketara secara statistik pada hasil akhir kepekatan asid nukleik, namun begitu, daripada segi kos, kaedah konvensional iaitu kaedah fenol-kloroform dan kaedah TENS lebih menjimatkan berbanding dengan kit komersial. Keputusan hasil kajian ini telah menunjukkan bahawa kaedah fenol-kloroform mempunyai penghasilan DNA terbaik berdasarkan pengukuran spektrofotometer Nanodrop, diikuti dengan kaedah TENS dan kit komersial.

COMPARISON OF THREE DNA EXTRACTION METHODS FROM RAW COW MILK SAMPLE

ABSTRACT

The progress and advancement in deoxyribonucleic acid (DNA) extraction has greatly evolved since it was initally performed in year 1986. DNA extraction has always be the first step required prior to any DNA analysis. In this study, raw cow's milk has been subjected to three different extraction methods for determining the method that perform best in terms of DNA yield. Genomic DNA extracted from raw cow's milk sample by the three methods was evaluated for purity and quantity by spectrophotometry and gel electrophoresis. On average, all three methods statistically showed no significant differences on final results of nucleic acid concentration, however, in terms of cost-effectiveness, conventional methods of phenol-chloroform and TENS method saved more compared to commercial kit. The results of this study showed that phenol-chloroform method had the best DNA yield based on the Nanodrop spectrophotometer measurement, followed by TENS method and commercial kit.

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Milk can be defined as the secreted fluid of the mammary glands of female mammals and contains nearly all the nutrients necessary to sustain life (Belitz *et al.*, 2009). Generally, the world's milk is predominantly cow's milk, followed by buffalo milk. Commercially, the term "milk" nowadays correspondent with cow's milk and the milk of other animals is usually spelled out (i.e., sheep milk or goat milk). The wide availability of the cow's and other animal's milk as well as the many benefits of its consumption has made the consumption of milk to be a routine among many children across the world, and is frequently recommended in food-based dietary guidelines by the expert (Wiley, 2017).

The production of milk-based product has been expanding steadily all across the world and it is estimated that the world production of milk reaches 730 million tons per year (Hemme and Otté, 2010) with the leading producers including Asia with 30% production and followed by Europe with 28% production of dairy products (Burke *et al.*, 2018). Most milk is manufactured into a variety of more stable dairy products of worldwide commerce, such as cheese, dried milks, ice cream, butter and condensed milk in order to meet the consumers' need as well as satisfaction. Availability of a variety of dairy products also intended to resolve problems related to lactose intolerant individuals so that these individuals may not need to completely eliminate dairy products from their diet, as both yogurt and hard cheese are well tolerated (Rozenberg *et al.*, 2015).

In forensic science, DNA plays an important role in investigation that can lead to individualisation of criminals. Thus, to solve of any investigations related to human crime especially, obtaining of good DNA through DNA extraction method is considered as a superior step in order to obtain good genotype profile. Every extraction method that has been developed, whether manual or automated, has its own basis and principles as well as benefits in different ways. For example, traditional methods such as phenol-chloroform and Chelex are still applicable for a routine DNA extraction in laboratory to extract particular sample types although these methods have been developed long time ago (Lee and Shewale, 2017).

Milk is found to be a good source of genomic DNA. There are many sampling method reported the need to utilise milk sample with volume more than 50 mL for the purpose of DNA extraction (Liu *et al*., 2014). However, a study by Pokorska *et al*. (2016) reported that a minimum volume of 10 mL of raw cow's milk has already sufficient to obtain a quality DNA that suitable for PCR analysis. In addition, milk sample is commercially available compared to blood sample, thus, it is a perfect sample to perform DNA analysis related to that particular animal.

There are many studies reporting different nucleic acid extraction method from different types of biological sample such as from blood and tissue sample (Singh *et al.,* 2018), sperm sample (Griffin, 2012) and nasal swab sample (Foley *et al*., 2011). With revolution in molecular technology, modification on extraction method happens from time to time (Figure 1.1) for the purpose of overcoming limitations in prior studies and introducing new ways for improvement in many targeted aspects. In many occasions, yield and purity of DNA are often evaluated to

determine whether the extraction method was an optimal choice for a particular sample.

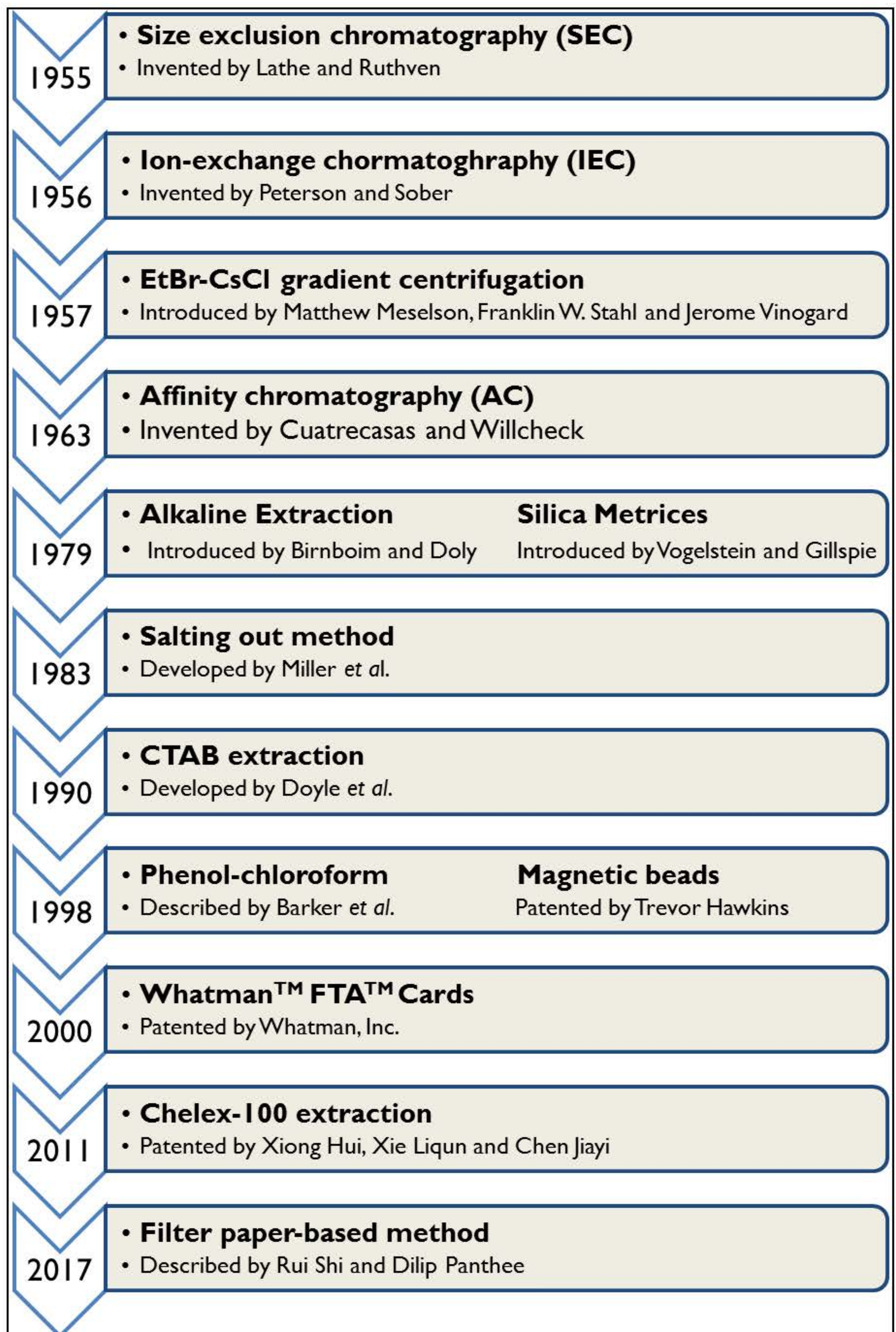

Figure 1.1 Evolution of DNA extraction method (Preetha and Mariyam, 2020)

1.2 Problem statement

The advancement in DNA extraction method had grown significantly over the years along with the development in science and technologies. In general, different types of samples may exhibit different characteristics because they originated from different sources. To perform DNA extraction, it is crucial to primarily be familiarised with the nature of the desired sample to be extracted, only then, selection of extraction method can be done. Thus, DNA extraction method often needs some modification and optimisation according to different types of samples. Currently, many studies have been reported on comparison between varieties of DNA extraction methods to extract different samples including animal's milk. The evaluation of different methods for DNA extraction from milk has been reported from the studies by Jeršek *et al*. (2014), Psifidi *et al*. (2010), Quigley *et al*., (2012), Usman *et al*. (2014), etc. There are several studies that consume large volumes of sample for extraction of DNA materials. Therefore, a comparison study that utilise small amount of raw cow's milk sample subjected to three different DNA extraction methods involving conventional methods and commercial kit was carried out in order to find the suitable and best method that could achieve good DNA yields.

1.3 Significance of Study

DNA extraction method is considered as the crucial step prior to any DNA analysis. An optimised extraction method to extract DNA from the desired sample must be achieved in order to produce good yields. Sample pre-treatment as well as the methods for the DNA extraction must be selected based on the nature of the sample that can further aid to achieve accurate and consistent result on the next steps. The methods of DNA extraction selected in this study for the extraction of raw cow's milk are seek to discover which methods that can meet the requirements that are sought in DNA extraction method, including high recovery of DNA and removal of impurities and inhibitors.

1.4 Objectives of the Study

General Objective:

To compare three different methods of DNA extraction on raw cow's milk.

Specific Objectives:

1) To perform three different DNA extraction methods namely phenol-chloroform, TENS and commercial kit on raw cow's milk sample.

2) To evaluate the extracted DNA obtained from three different DNA extraction methods by using NanoDrop^{TM} spectrophotometer.

3) To assess all the three different DNA extraction methods through statistical analysis.

CHAPTER 2

LITERATURE REVIEW

2.1 Deoxyribonucleic Acid (DNA)

Deoxyribonucleic acid or also known as DNA is a long molecule that contains hereditary material found in humans and almost in all other organisms. A DNA molecule consists of two long polynucleotide chains composed of four types of nucleotide subunits (Alberts *et al.*, 2002). Each of these chains is known as a DNA chain, or a DNA strand. Identical strands of DNA are formed in every cell in the body as a basis to determine the structure and function of the cell. Thus, overall appearance, health, and actions of the entire animal were determined by this so-called genetic code, transmitted from the parents to its offspring.

The discovery of DNA and its importance has occurred a long time ago. According to Dahm (2005), the discovery of DNA as hereditary materials was discovered by Avery and his colleagues in the year of 1944 which then followed by the decipherment of its structure later by Watson and Crick (Figure 1.1) which is after 10 years of experimenting. The leading light of genetic research has actually begun since 1986 where a Swiss physiological chemist Friedrich Miescher discovered so-called "nuclein" from human white blood cell sample (Pray, 2008).

2.1.1 DNA in Modern World

The discovery of DNA is said to be the most significant biological discovery of the 20th century which is seen to have had an immense impact on both science and medicine in this modern world. Not only in modern medicine and genetic

research, DNA discovery has been proven to be one of the most powerful tools in criminal investigation since no two people can have the same DNA profile, excluding identical twins (Artur, 2011). In addition to that, de Boer *et al*. (2018) in their study stated that DNA profiling is considered as one of the most effective and efficient ways of distinguishing individuals or different parts of the body in disaster victim identification (DVI).

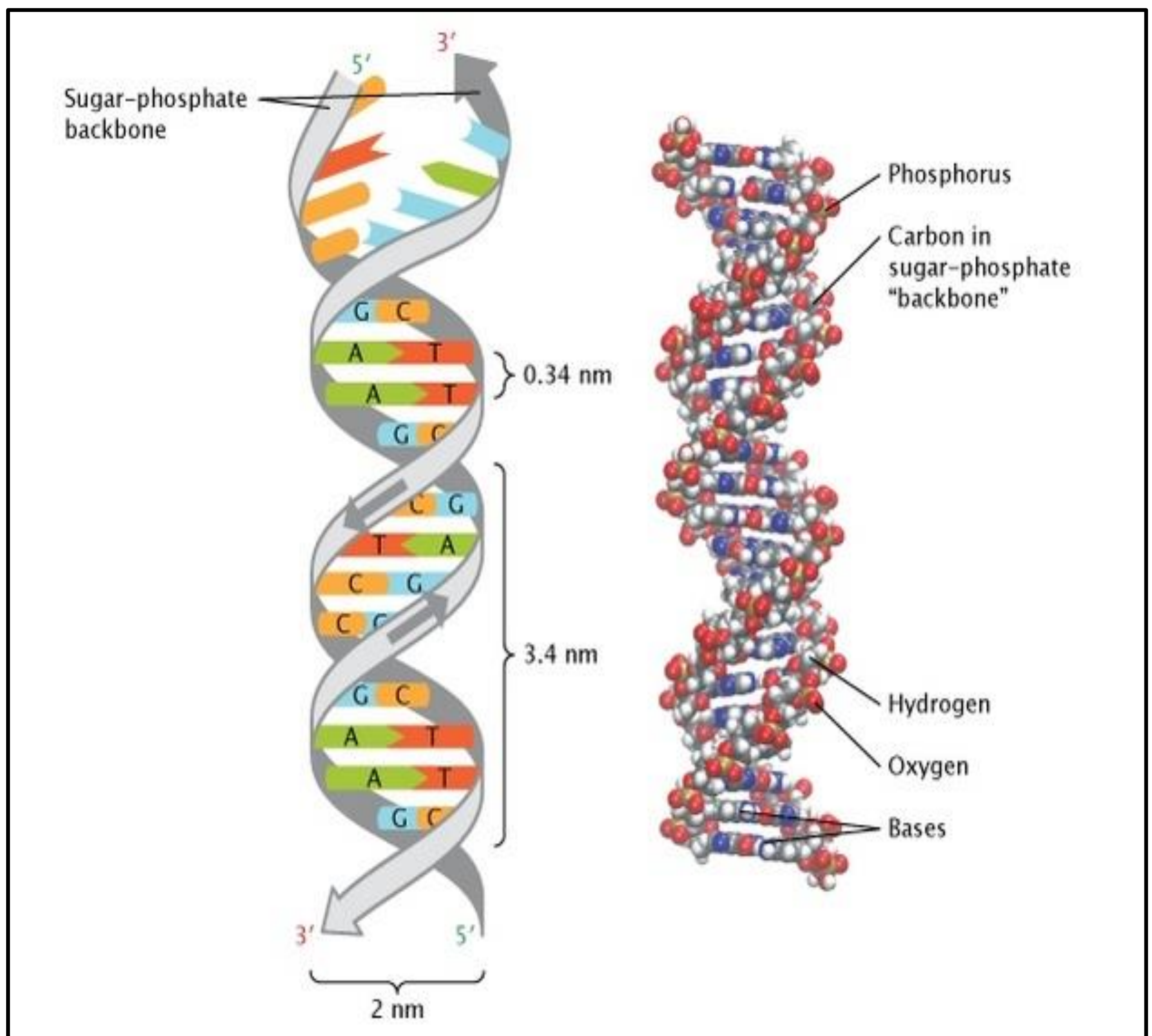

Figure 2.1 The double-helical structure of DNA elucidated by James Watson and Francis Crick (Pray, 2008)

DNA analysis has also advancing into the development of DNA barcoding. Letchuman (2018) defined DNA barcoding as a taxonomic method where the genetic marker is used as a tool to identify the DNA of an organism or a particular species that can later be classed to the group it belongs to. In general, barcoding provided a way of differentiating and identifying species with a short, standardised gene sequence (Hebert *et al.*, 2003). DNA barcoding has been employed in many applications including genetic analysis of animals. Other than for determining extended lineage reconstruction and kinship analysis (Cassidy and Gonzalez, 2005), DNA barcoding has been widely adopted as one potent molecular tool in species identification for tracing adulteration in food samples as well as for analysing samples from suspected wildlife crime incident (Staats *et al.*, 2016).

Analysis of DNA is significant in food fraud issue as well. Food adulteration is a form of food fraud that includes the addition of undeclared ingredients, for example, the act of mixing cow's milk with buffalo milk in the production of mozzarella cheese or adding some amount of water to frozen food to increase its weight (Primrose, 2019). Adulteration issue happened all across the world, both in developed and undeveloped countries and that explained why different countries have different laws regarding food adulteration in order to overcome the issue. Despite the existence of legislation in most countries, the need for growing of new method to assist investigation on food authenticity issue is still crucial and must be done accordingly. Thus, recent development of DNA-based method which excellent in term of sensitivity, multiplexing ability and cost-efficient is seen to be one powerful tool to counter with adulteration issue (Böhme *et al.*, 2019).

2.2 The Cow's Milk

Carbohydrate, protein, fat, minerals, vitamins and water are the elements composed in all milk produced by the animals, with water as the major component contained in the milk (Table 2.1). The nutritional values and properties of processed milk and dairy products are greatly dependent on the chemical composition of raw milk (Mourad *et al*., 2014). In addition, aside from blood, milk is also one of DNA source. d'Angelo *et al*. (2007) mentioned in his paper that apart from less expensive sample, milk can be obtained easily from animals when compared to blood because it does not require capture, handling, and venipuncture procedure that may issue stress to the animals.

Table 2.1 Gross composition of cow's milk (Walstra and Jenness, 1984)

Component	Average Content Percentage (w/w)	Range Percentage (w/w)	Average Percentage of Dry Matter (%)
Water	87.3	85.5-88.7	
Solids not fat	8.8	7.9-10.0	69
Lactose	4.6	3.8-5.3	36
Fat	3.9	2.4-5.5	31
Protein	3.25	2.3-4.4	26
Casein	2.6	1.7-3.5	20
Material substances	0.65	0.53-0.80	5.1
Organic acids	0.18	--	1.4
Miscellaneous	0.14	--	1.1

The somatic cells in bovine milk are predominantly leukocyte with a small proportion of epithelial cells that functioned to facilitate in obtaining the DNA samples of the cow (Lipkin *et al.*, 1993). The authors also mentioned that the status of the cow, whether parity, season, stage of lactation or health, will affect the number of somatic cells contain in the milk. However, extraction of DNA from the somatic cells might exhibit some limitations. The fat and proteins contain in milk might act as inhibitors that makes the extraction of high amount of quality DNA hard to be achieved (Usman *et al.*, 2014). Generally, the purity of the extracted DNA as well as its quantity is some of the crucial parameters monitored in any extraction process that has been performed. Thus, experimenting different methods of DNA extraction on the milk served as a medium to determine the most suitable and appropriate procedures to monitor those particular parameters.

2.3 DNA Extraction

A routine procedure performed to isolate DNA from the nucleus of the cell is called as the DNA extraction method. The technique for extracting DNA is greatly dependent on the nature of the sample itself. For instance, extracting DNA from plant-typed sample is different from blood sample. This is because plant has different structure from blood cell. Apart from that, the existing conventional DNA extraction methods when compared to that of newly advanced commercially available kits differ in certain ways including in their degree of homogenisation, mode of cell lysis, whether by enzymatic and/or mechanical, and nucleic acid recovery principle (Quigley *et al.*, 2012).

Generally, there are several requirements that must be fulfilled in every DNA extraction method. Surzycki (2000), in his paper listed a few important requirements

for good extraction methods which are the method should be efficient and can yield DNA without major contaminants, as well as should be able to maintain the integrity of the DNA sample which means low probability rate by which the DNA molecules being altered physically or chemically using that particular method. In addition, as mentioned by Chacon-Cortes *et al*. (2014), apart from monitoring the quality and quantity of extracted nucleic acid prior to any downstream application, other factors need to be considered during optimisation of DNA extraction method such as time, cost, laboratory materials and apparatus, sample amount as well as expertise requirements. Basic essential steps in DNA extraction composed of the following in Figure 2.2, primarily begun with the disruption of cell nuclear membrane and cytoplasmic, followed by separation and purification of DNA from other component such as protein and lipid, then proceeding to concentration and purification step for DNA (Ali *et al*., 2017).

Challenges in performing nucleic acid extraction can be considered as a catalyst in development of new improved extraction methods. There are several challenges in DNA extraction, as stated by Sajali *et al*., (2018), in order to select an appropriate extraction method, many factors must be taken into account such as time, cost and toxicity of the chemicals employed. Dealing with hazardous chemical not only provides risk in contaminating the DNA but also exposing the analyst with serious health hazards especially in longer terms (Yue and Orban, 2001).

Apart from that, researchers usually dealt with challenging sample that contained inhibitors, for instance, protein in milk that may decrease the solubility of pellet from extracted sample (Rijpens *et al*., 1996) which will affect the yield of DNA at the end of the process. Processed food can also be categorised under

challenging sample, where Şakalar *et al*., (2012) mentioned that degradation of DNA fragments may occur with longer duration of extreme heat treatment as well as increased temperature. This can be further supported by a study by Şakalar *et al*., (2012) on effect of heat processing on DNA quantification of meat species where they found that there was a decline in detectable copy numbers of specific genes on variety of extracted meat samples (i.e., beef, pork, and chicken) that has been exposed to the procedures of boiling and baking with varying heat time and degree. From the study, the authors have concluded that temperature and duration of the heat treatment affect the species determination and quantification using real-time PCR.

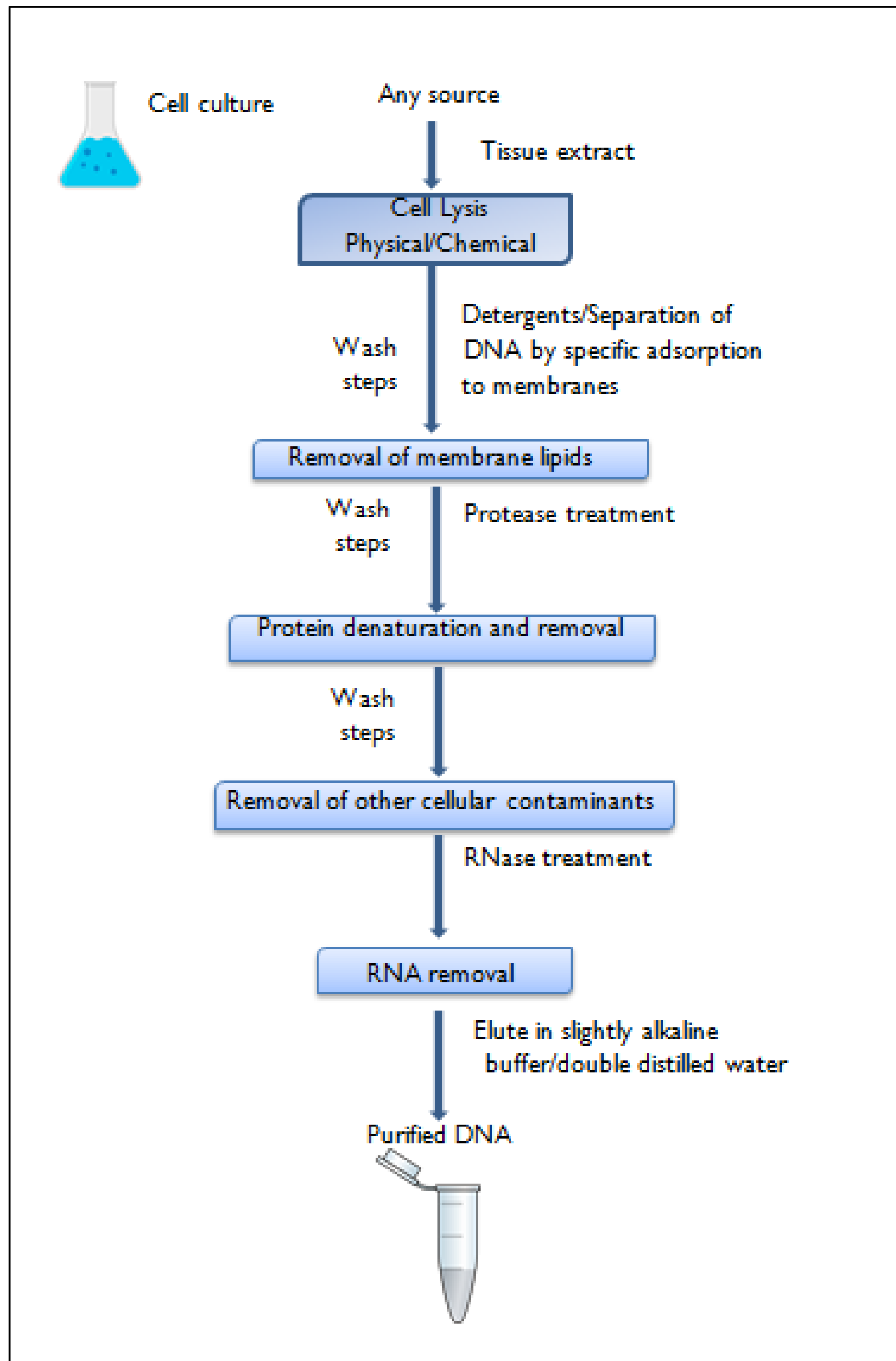

Figure 2.2 Basic steps involved in all DNA extraction methods (Dhaliwal, 2013)

2.3.1 Conventional Method

Phenol-chloroform extraction is one of the earliest conventional DNA extraction methods introduced by Barker *et al.* in 1998 (Elkins, 2013). Phenol-chloroform is a liquid-liquid DNA extraction method that works on the basis of solubility of DNA material in aqueous solution. In order to prevent the degradation of DNA materials by phenol, chloroform is added as a medium to preserve the DNA (Ebeling *et al.*, 1974), where it functioned to increase the density of organic phase which later preventing phenol solution from mixing with the aqueous phase. Green and Sambrook (2017) revealed that the procedure of using phenol and chloroform together to remove protein from nucleic acid solutions is more efficient rather than using one organic solvent. In general, phenol-chloroform organic extraction is considered to be a gold-standard method where it is applicable to extract DNA materials from different types of sample such as blood, tissue homogenate and suspension culture (Preetha and Mariyam, 2020).

The advantages from choosing organic phenol-chloroform extraction method is that this method provide a high yield of nucleic acid concentration and relatively a cost-effective method (Peterson and Sober, 1956) when compared to more advance method of extraction, for example, a commercial kits. This can be supported from a study by Yahya *et al.* (2017) on comparison of different extraction methods on raw and boiled bovine milk for PCR amplification where they found that conventional phenol-chloroform method produced the highest DNA yield compared to other extraction methods which are alkali-based method, buffer-only method and commercial DNA extraction kit.

However, there are a few disadvantages that need to be considered from using this method. Yahya *et al*. (2017) in their study and Butler (2012) in book of "Advanced Topics in Forensic DNA Typing: Methodology" mentioned that this extraction method is not time-effective, labour-intensive, besides involving the handling of toxic reagents and exposure to high contamination resulting from procedure of transferring the samples to multiple tubes repeatedly. It is compulsory to perform the experiment in fume hood with appropriate personal protection equipment (PPE). Djurkin Kušec *et al*. (2015) in their study revealed that conventional phenol-chloroform method did not show good absorbance ratio at A260/280 on the extracted sausages sample with values lower than 1.8 which indicates the contamination from protein, however, they suggested that this issue can be overcome by performing diethyl ether extraction or reprecipitation of the genomic DNA.

Apart from phenol-chloroform, salting out method is an alternative method to extract DNA using non-toxic reagent. Doyle (1991) in his study claimed that salting-out method is better than phenol-chloroform method in term of DNA yield quality, time and cost consumption and most importantly the usage of safe, non-hazardous reagent in extraction process. The basis of DNA salting-out extraction method is that protein will be precipitated out by high salt concentration and DNA will be extracted from the sample (Preetha and Mariyam, 2020). Morover, d'Angelo *et al*. (2007) in their study consume a salting out method protocol to perform DNA extraction from milk somatic cell which successfully yield appropriate amount of DNA suitable for downstream PCR-RFLP application without involving procedure for sample enrichment. Thus, salting out can also be considered as a reliable conventional

method apart from phenol-chloroform, that is not just inexpensive but also consume toxic-solvent free reagent.

2.3.2 Commercial Kit

Many commercial kits for DNA extraction has been developed and being used in the lab. Commercial kits employ spin column that composed of silica resin to selectively bind DNA and RNA in the sample. Study by Jeršek (2014) to evaluate different methods for extraction of milk sample, three commercial kits namely QIAprep Spin Miniprep, DNeasy Blood and Tissue kit, and SmartHelix First DNAid were used to evaluate in terms of the time requirements for DNA extraction process, the cost-effectiveness and labour-intensiveness along with the quality of extracted DNA and they found that a good results of real-time PCR was showed by a commercial kits compared to non-commercial methods, including phenol-chloroform.

In spite of that, in one of a study paper by Kopecka, (2014) stated that conventional phenol-chloroform method showing sufficient stability and purity on extracted DNA on yeast strain whereas commercial kit has evidenced a decline integrity on extracted sample after 6 months-long sample storage as instructed by manufacturer. The author has mentioned that when comparing traditional phenol-chloroform method with commercial kit used in this study, phenol-chloroform method, despite needing more laborious and time consuming from commercial kit, however generated minimal damage and more stable extracted DNA material after months of storage compared to commercial kit.

Djurkin *et al.* (2015) in their paper generated satisfactory results from extraction made on pork in dry or fermented sausages by using four commercially available DNA extraction kits and traditional phenol-chloroform method. These authors mentioned that one kit named as DNeasy Mericon Food Kit generated expected result as the kit is designed specifically for extraction on processed food that exposed to high degradation of DNA during processing. In addition, a comparison study on five commercially available DNA extraction kits for the extraction of bacterial genomic DNA from whole-blood samples performed by Smith *et al.* (2003) that aimed to determine the sensitivity, specificity, ease of automation, and overall efficiency of each kit, found that only two out of five DNA extraction kits excel in all measured parameters. Other than that Pivariu *et al.* (2013) has performed a study on the identification of cow DNA in sheep and goat traditional dairy products found that the two kits selected, namely Fast ID isolation kit and Isolate II (BIOLINE) DNA extraction kit, for the extraction of DNA materials from different sort of cheese products has shown a satisfying results on DNA yield and purity. Therefore, the studies have evidenced that the selection of kits is dependent on the nature of desired sample to be extracted because most of the kits are not specifically designed to extract a particular sample.

CHAPTER 3

METHODOLOGY

3.1 Material

3.1.1 Chemicals and Reagents

The chemicals, commercial kits, consumables and reagents used in this study were listed in Table 3.1.

3.1.2 Equipment and Instruments

All laboratory equipment and scientific instruments utilised in this study were listed in Table 3.2.

Table 3.1 Lists of chemicals, commercial kits, consumables and reagents

Chemicals/Reagents/Kits	**Company/Supplier**
1 Kb Plus DNA Allelic ladder	Gene DireX, USA
Absolute ethanol	Syne, USA
Agarose powder	1st BASE, Singapore
Boric acid	Merck, USA
Chloroform	Merck, USA
Disodium ethylenediaminetetraacetate dehydrate (Na_2EDTA) powder	1st BASE, Singapore
Disodium phosphate (Na_2HPO_4) powder	Sigma-Aldrich, USA
Ethidium bromide	Sigma-Aldrich, USA

Ethylenediaminetetraacetic acid (EDTA)	Sigma-Aldrich, USA
Exgene^{TM} Tissue SV mini kit	GeneAll Biotechnology Co.,Ltd
Glacial acetic acid, 100%	Merck, USA
Hydrochloric acid (HCl)	Merck, USA
Isopropanol	Merck, USA
Orange G Loading dye	Sigma-Aldrich, USA
Potassium dihydrogen phosphate (KH_2PO_4)	Sigma-Aldrich, USA
Potassium chloride (KCl) powder	Sigma-Aldrich, USA
Proteinase K	Sigma-Aldrich, USA
Sodium acetate powder	Sigma-Aldrich, USA
Sodium chloride (NaCl) powder	Sigma-Aldrich, USA
Sodium Dodecyl Sulphate (SDS)	Bio-Rad, USA
Tris-base powder	1st BASE, Singapore
Tris-buffered phenol	Life Science, USA
Tris EDTA buffer	Invitrogen, USA
Consumables Materials	
1.5 mL Microcentrifuge tube	Bio-Rev, Singapore
Gloves	Teraslab Sdn Bhd
Parafilm	Teraslab Sdn Bhd
Pipette Tips	Greiner Bio One, Austria

Table 3.2 List of equipment and instruments

Instrument/Equipment	**Brand**	**Model**
Pipettes, 10 µL, 20 µL, 100 µL, 200 µL and 1000 µL	Gilson/Eppendoff	P10, P20, P100, P200, P1000
Analytical balance	Sartorius	BSA 224S-CW
DNA Electrophoresis system	Owl, Thermo Scientific	B2
Gel Documentation System	Vilbert Lourmat/Quantum	ST4-1000/20m
Laminar Air Flow Cabinet	ERLA	CFM-4
Microprocessor pH meter	Hanna Instrument	pH 211
Microwave oven	Elba	EMO-1706
Power pack	Bio-Rad	Power-Pac 3000V
Spectrafuge 24D Starter Pack (Microcentrifuge)	Labnet International	C2400
Spectrophotometer	Thermo Scientific	Nanodrop 2000
Vortex Mixer	ERLA	EVM-6000
Water Bath	Memmert	WB 29

3.1.3 Reagent preparations

3.1.3.1 Preparation of Phosphate-buffered saline (PBS), pH 7.4

A total of 0.80 g of NaCl, 0.02 g of KCl, 0.14 g of Na_2HPO_4 and 0.03 g of KH_2PO_4 were weighed and dissolved in 80 mL distilled water in a beaker. The pH of the buffer was adjusted to pH 7.4 by the addition of appropriate amount of HCl. Distilled water was then added to adjust the final volume of the solution to 100 mL. The solution was then autoclaved and stored at room temperature.

3.1.3.2 Preparation of 1 M Tris Hydrochloride (Tris-HCl), pH 8.0

A total amount of 30.285 g of Tris-base powder was weighed and dissolved in 800 mL of distilled water in a beaker. An appropriate amount of 1 M HCl solution was added to adjust the pH of the solution to pH 8.0. The solution was added with distilled water to make up to 250 mL volume solution. The solution was then autoclaved and stored at room temperature.

3.1.3.3 Preparation of 0.5 M Ethylenediaminetetraacetic Acid (EDTA), pH 8.0

A total of 46.525 g of disodium ethylenediaminetetraacetate dehydrate (Na_2EDTA) powder was weighed and dissolved in a beaker of 200 ml distilled water on a hot plate. The pH was adjusted by adding 20 g of sodium hydroxide (NaOH) pellet into the solution. Distilled water was added to adjust the final volume to 250 mL. The solution was then autoclaved and stored at room temperature.

3.1.3.4 Preparation of Tris-EDTA (TE) buffer, pH 8.0

The buffer of Tris-EDTA (TE) was prepared by mixing a volume of 10 mL of 1 M Tris-HCL solution with 2 mL of 0.5 M EDTA solution. Distilled water was added to adjust the final volume to 1000 mL. The solution was sterilised by autoclaving and was kept at room temperature.

3.1.3.5 Preparation of 1 M Tris solution, pH 8.0

A total amount of 6.057 g of Tris- base powder was weighed and dissolved in 50 mL distilled water in a beaker to make 1 M Tris solution. The pH of the solution was then adjusted to pH 8.0 by addition of HCl. The solution was then autoclaved and stored at room temperature.

3.1.3.6 Preparation of 5 M Sodium Chloride (NaCl) solution

This solution was prepared by dissolving 73.05 g of sodium chloride powder in 250 mL of distilled water. The solution was sterilised by autoclaving and was kept at room temperature.

3.1.3.7 Preparation of 0.5% Sodium Dodecyl Sulphate (SDS)

A total of 10 g of SDS powder was weighed and dissolved in 95 mL of distilled water. This procedure was done on the hot plate to aid the dissolution process. An appropriate amount of HCl was added in the solution until the pH was adjusted to pH 7.2. Sterile distilled water was then added to make up to 100 mL solution.