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NUTRITIVE SWEETENER PROFILES OF DAIRY-BASED BEVERAGES COMMERCIALLY AVAILABLE IN KOTA BHARU, KELANTAN

Dissertation submitted in partial fulfillment for the
Degree of Bachelor of Science (Hons.) in Forensic Science

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ROSZAMILAHWATI BINTI RAMLI

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LIST OF ABBREVIATION AND SYMBOL

ADP	Adenosine-5'-diphosphate
ATP	Adenosine-5'-triphosphate
$Cu(NH_3)_4^{26}$	Copper (II) sulfate and ammonia complex
F-6-P	D-fructose-6-phosphate
G-6-P	D-glucose-6-phosphate
G6P-DH	Glucose-6-phosphate dehydrogenase
Gal-GH	β-galactose dehydrogenase
GC	Gas Chromatography
GC-MS	Gas chromatography Mass spectrometry
HK	Hexokinase enzyme
HPLC	High Performance Liquid Chromatography
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
IDF WP	International Diabetic Federation Western Pacific
$K_4[Fe(CN)_6]$	Potassium hexacyanoferrate (II)(ferro cyanide)
NAD	Nicotinamide-adenine dinucleotide
NADP	Nicotinamide-adenine dinucleotide phosphate
NADPH	Reduced nicotinamide-adenine dinucleotide phosphate
NaOH	Sodium hydroxide
PGI	Phosphoglucose isomerase
PS1M	Program Susu 1 Malaysia

PSS	Program Susu Sekolah
RI	Refractive Index
RTD	Ready-to-drink
TLC	Thin layer chromatography
UHT	Ultra-high Temperature
UV-Vis Spectrophotometry	Ultra-Violet and Visible light spectrophotometry
WHO	World Health Organization
$ZnSO_4$	Zinc sulphate

ABSTRACT

Enzymatic technique was an alternative method applied in the sugar analysis of dairy-based beverages locally available in Malaysia. The technique was simple, using non-toxic chemicals, react rapidly, highly specific, and also sensitive to low concentration. The technique used to analyze several types of common sugars such as glucose, fructose, sucrose, lactose, and maltose. The measurement of the sugar concentration has been observed using the UV-Vis Spectrophotometry (Varian Cary 100) at 340nm wavelength. Based on the analysis which has been conducted, the dairy-based beverages majorly contained the sucrose and lactose sugar about 50% and 42% from the overall sugar. However, the amount of the total sugar does not in high level, therefore an individual can take other food or drinks to consume and control their sugar intake daily in order to prevent from exceeding the amount recommended by WHO.

ABSTRAK

Teknik enzimatik merupakan teknik alternatif yang diaplikasikan ke dalam analisis gula pada minuman berasaskan tenusu di Malaysia. Teknik ini mudah, menuggunakan bahan kima yang tidak bertoksik, bertindak dengan pantas, sangat khusus, dan sensitif pada kepekatan yang rendah. Teknik tersebut digunapakai untuk menganalisa beberapa jenis gula yang biasa dimakan seperti glukosa, fructosa, sukrosa, laktosa, dan maltosa. Pengukuran kepekatan gula dilakukan menggunakan UV-Vis Spektrophotometri (Varian Cary 100) pada 340nm panjang gelombang. Berdasarkan analisis yang telah dibuat, minuman berasaskan tenusu mengandungi sukrosa dan laktosa secara majoriti sebanyak 50% dan 42% daripada jumlah keseluruhan gula. Walau bagaimanapun, jumlah keseluruhan gula tidak berada di paras tinggi. Oleh sebab itu seorang individu boleh mengambil makanan atau minuman lain dan mengawal kadar pengambilan gula harian bagi mengelakkan pengambilan gula melebihi had yang disarankan oleh WHO.

CHAPTER 1

INTRODUCTION

1.1 Dairy-Based Beverages

Milk and other dairy products known as nutrient-rich; consists of essential nutrients such as calcium, protein, potassium, niacin, phosphorus, vitamin A, vitamin B2, vitamin B12, and vitamin D. It gives a balanced diet which being consumed by children and adolescents in the world from recent decades. Those dairy products provide energy, protein, micronutrients, and also bioactive compounds which help in supporting the development and growth of an individual, especially for children, and give an ideal figure of body composition.

According to (Hazen, 2010), dairy-based beverages provide a naturally nutritious food items which give a healthy drink to the thirsty consumers. Nowadays, the healthy drink has been improvised with an appealing nutrients, healthy bacteria, colors, flavors, and etc in order to make it more appeal to attract consumers. This healthy drink not just only consumed by children, but also being taken by adults which help them in maintaining their health status.

Chocolate flavored drink has been on the top rank in the list of type of milk favored by consumers (Susan Niekrasz, 2010). The vanilla flavored place the second rank and followed by the strawberry flavored drink. In addition, banana flavored drink is rarely consumed by consumers.

According to (Özer and Kirmaci, 2010), the dairy product can be categorized into three main groups which are:

I. Basic milk products, it is a product that include the classical dairy products such as yoghurt, cheese, butter, and also ice cream.

II. Added-value products, it is a dairy products which includes low-lactose or lactose-free products and being enriched with calcium, vitamin, and mineral. It also designed as hypo-allergenic formulae.

III. Functional dairy product, as it is a product that gives a healthy benefit beyond its nutritional value, besides enriched with functional components that originated from dairy or non-dairy sources. For example, probiotic dairy-based beverage.

Dairy-based beverages are beneficial sources for major minerals contents influenced by several factors. For examples, stage of lactation, environmental influences, and genetics. About 99% of the calcium in our body found in teeth and bone where they undergo cycle process of removing and replacing with more calcium. Consumption of sufficient amount of calcium may able to protects individual from hypertension, cancers, osteoporosis, kidney stones, and weight management (Miller *et al.*, 2006).

1.2 Sugar Intake

Sugar needed in our body as it helps in stimulating the same pleasure centers of the brain which respond to heroin and cocaine when being injected into the bloodstream (Cohen, 2013). According to (Flora and Polenick, 2013), high level intake of sugar or glucose very helpful in improvising athletic, academic, and cognitive performance, and also help in enhancing self-control.

Child's early experience begins from the infant feeding methods such as breast feeding or using bottle. Therefore, they develop the food-acceptance patterns later in life which then affect their adult food selection (Birch, 1999). Normally, an individual prefer to take foods with sweetness or saltiness taste and rejecting foods that are bitter and sour in taste (Health, 2013).

Nowadays, sugars are mostly being added into the food and drinks due to its biological, sensory, physically and chemically properties. Besides, sugar added in the modern food such as fast foods, soft drinks, sweets, cakes, and also chocolates which increase sugar intake in individual life. In brief, sugar is being regularly consumed nowadays and commonly found in every foods and beverages taken (Sigman-Grant and Morita, 2003).

1.3 Sugar Definition

Sweeteners can be categorized into nutritive sweetener and non-nutritive sweetener. The nutritive sweetener contains of carbohydrates and few vitamins or minerals in the food or beverages which good for our diet, whereas the non-nutritive sweetener is in contrast. All sugar having 4-5 grams of carbohydrates per level teaspoon and contributes 4 calories for gram of it mass weight.

Sugar can be classified into monosaccharides and disaccharides such as sucrose, glucose, and also fructose. Monosaccharides consist of 3-7 carbon atoms per monomer and basic primary sugars are glucose, fructose, and galactose. Whereas, disaccharides consist of two monomers of monosaccharides which being joined together and basic primary sugar are sucrose, lactose, and maltose.

1.3.1 Monosaccharide

All monosaccharide sugars share the same molecular formula which is $C_6H_{12}O_6$ but their arrangement of atoms differs in each case. Glucose is a sugar that normally found in blood which act as immediate source of energy for cellular respiration, and also known as dextrose which glucose produced from corn. Biochemically they are identical/isomer.

Fructose is a simple sugar which can be found mostly in fruit and plants. The sugar being accompanied by the nutrients and also fibers, as for example fructose sugar can be found in honey. Whereas, galactose is a reducing sugar that can be found in milk or yogurt samples.

1.3.2 Disaccharide

The molecular formula of the disaccharide sugars is 2 $C_6H_{12}O_6$. Sucrose is known as a double sugar (table sugar) which containing two parts of sugar, glucose and fructose, which chemically joined together. When consumed by human, an enzyme inside the intestine will efficiently and quickly splitting the sucrose into its basic sugars and absorbed as single sugar. Similar to lactose, combination of glucose and galactose, it is a major sugar that can be found in milk. The bonding that joining the monosaccharide sugars in both sucrose and lactose is known as "glycosidic bond".

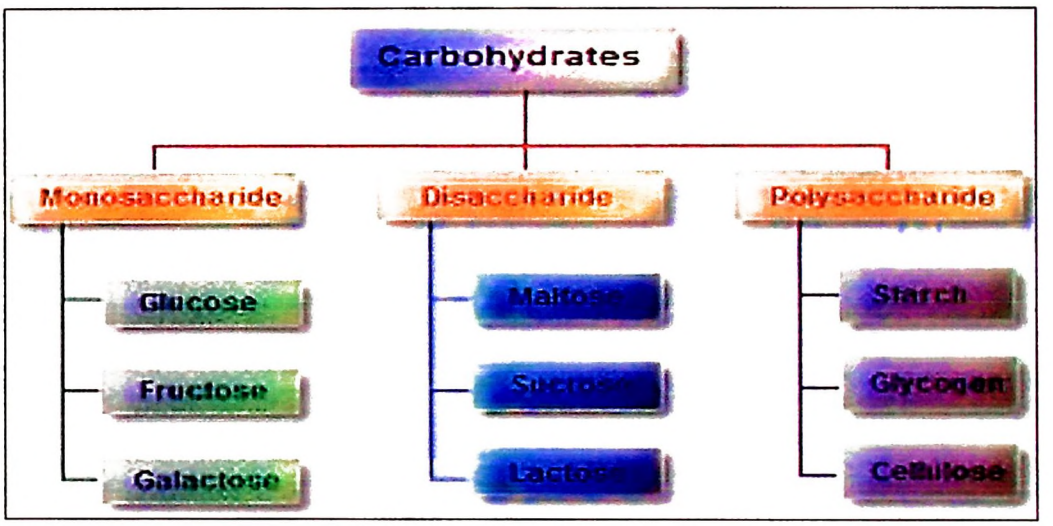

Fig 1.1: Classification of sugar

However, in this research, we only focused on five types of basic sugars which are glucose, sucrose, fructose, lactose, and also maltose. Figure 1.2 below shows the chemical structure of those sugars.

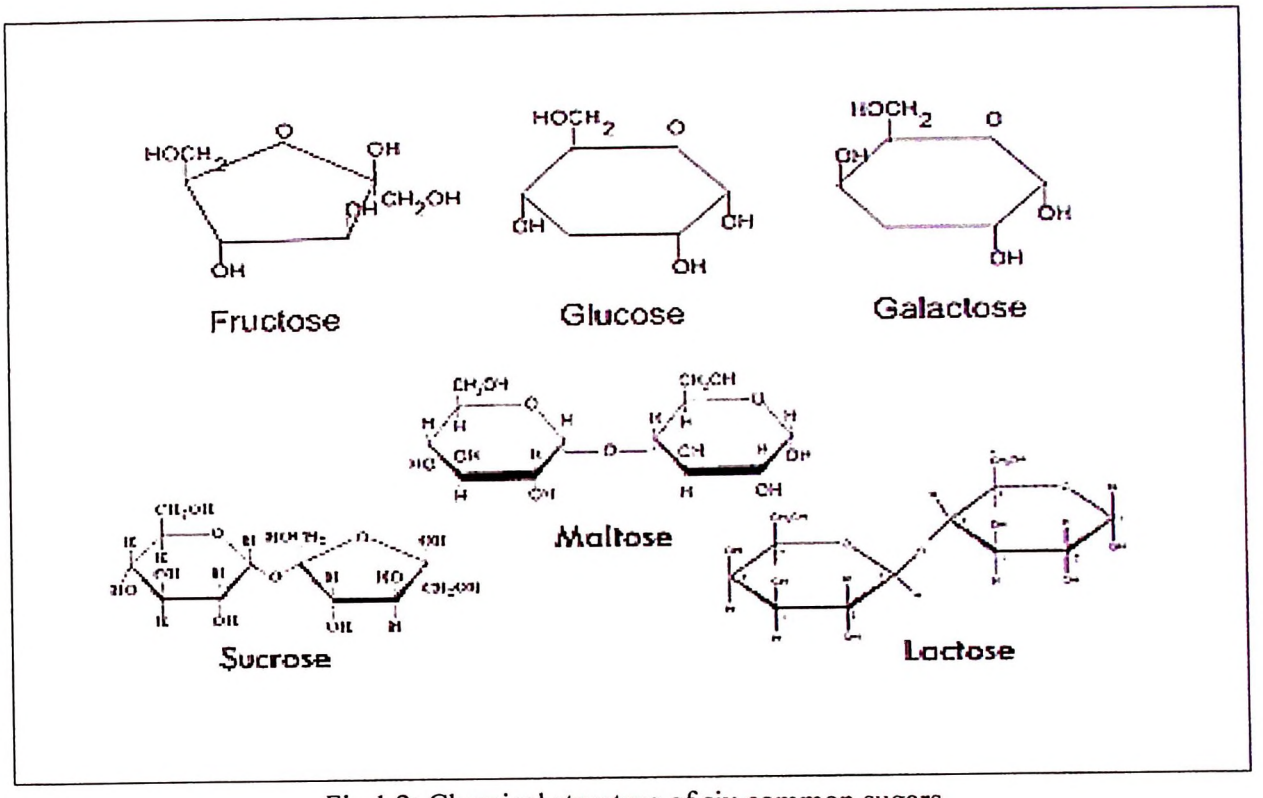

Fig 1.2: Chemical structure of six common sugars

1.4 Problem Statement

In 1972, Yudkin pronounced sugar as "pure, white and deadly" substances and relate it to health problems such as obesity, heart disease, hyperactivity, diabetes, and also dental caries. According to (Health, 2013), the numbers of people with diabetes which includes several low- and middle-income countries world-wide increase from 143 million in 1997 to 300 million in

2025., The number of individuals that predicted to have diabetes in 2003 are about 194 million and will rise to 333 million in 2025 (Federation, 2014).

About 3.6 million adults being affected by diabetes in Malaysia has been reported recently (Chong, 2013 in (Online, 2013). Due to the high number of prevalence of diabetes, Malaysia is ranked number one in diabetics among Asian countries and ranked number sixth among western pacific region. Malaysia is one of the 23 countries and territories of the IDF WP region. Almost 138 million people have diabetes in that region which will rise to 202 million in 2035. Besides, about 3.2 million cases of diabetes that happen in 2014 in Malaysia has been reported (Federation, 2014).

Table 1.1: Statistical report on the prevalence of diabetes

Total adult population (1000s) (20-79 years)	19,422	Number of deaths in adults due to diabetes	34,422
Prevalence of diabetes in adults (20-79 years) (%)	16.6	Cost per person with diabetes (USD)	565.4
Total cases of adults (20-79 years) with diabetes (1000s)	3,225.2	Number of cases of diabetes in adults that are undiagnosed (1000s)	1,717.4

Source: (Federation, 2014)

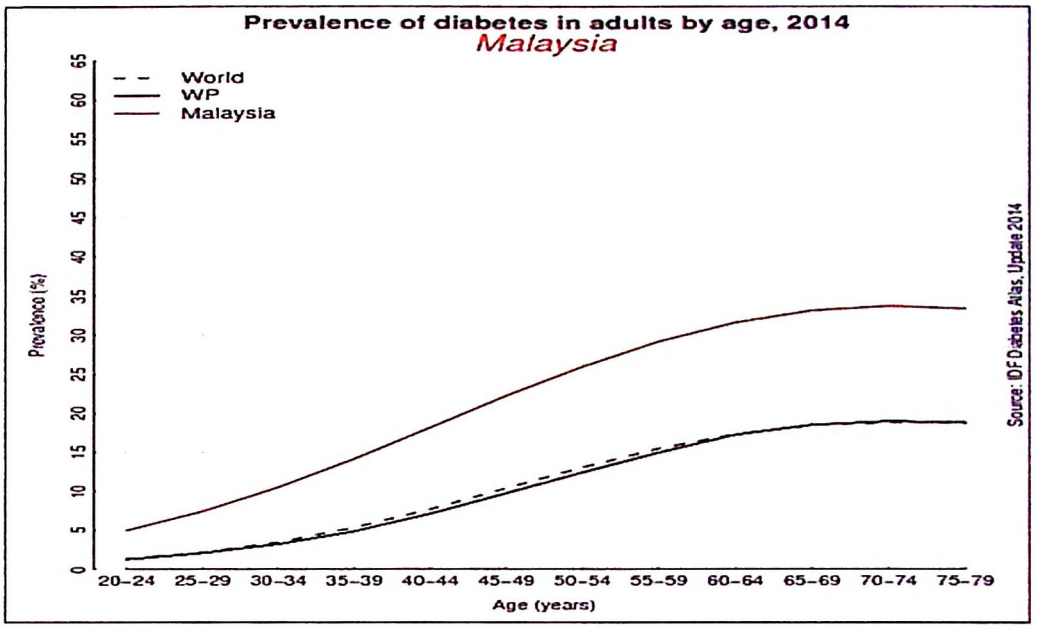

Fig 1.3: Malaysia vs World prevalence of diabetes

Sugar is cheap, taste goods, and available everywhere. However, highly consumption of sugar can be linked to the abuse of health status. Besides, it stimulates the brain for subsequent intake, similar to tobacco and alcohol, which give signals hunger to the brain. This results in obesity of individual due to the high consumption of sugar (Lustig *et al.*, 2012).

In the beverages, nutritive sugar has been switched to sugar substitute as highly intake of sugar that can cause problem in teeth and waistline. However, the sugar substitute also can cause health problem such as weight gain and obesity (Tandel, 2011). According to Ogden & Statistics (2011), the intake of sugar drinks lead to poor diet quality, weight gain, obesity, and type 2 diabetes in adults. Sugar should be consumed in a moderate amount in order to convert it into energy. However our body will converted it into lipid and store as fats if we consume in excessive amount. Thus, it will resulting in weight gaining, obesity and increased in waistline circumference of the individuals.

In the previous study, High-Performance Liquid Chromatography (HPLC) is the most common instrument applies in the sugar profiling. However, HPLC deals with the time taken in column equilibration, high cost in machine maintenance, and practice acquired of toxic reagents. In this research, the enzymatic technique is applied to analyzed sugar profiles in the ready to drink dairy-based beverages locally available in Malaysia.

1.5 Significance of Study

This study contributes to the analysis of the type of sugar and its composition present in the dairy-based beverage. This analysis is important for health conscious consumers that include the diabetic individuals in order to manage and plan their daily dietary intake. As if they know how much sugar in their drink that normally they take, thus they will minimize or consumed other food that contain either high or low sugar level which then not exceed the recommended daily intake of sugar (50g).

Besides, this study also helps in providing the nutritional knowledge to the communities regarding the types of sugar present in the dairy-based beverages and it roles in our body towards the improvements and sustainability of their health status via regularly consumption of dairy-based beverages.

1.6 Objectives of the study

The general objective of this research is to investigate nutritive sweetener profiles of dairy-based beverages commercially available in Kota Bharu, Kelantan by using the enzymatic technique. The specific objective of the study is to elucidate types of sugars present in commercially available dairy- based beverages in Kota Bharu, Kelantan, Malaysia.

CHAPTER 2

LITERATURE REVIEW

2.1 Dairy-based Beverages and Milk

Consumption of dairy-based beverages and milks has increased during the last decades as people became more concern about their nutritive diet and health protection. This is because of the medical costs that needed to be spent much higher than buying any healthy foods. The healthy foods much cheaper and can effectively help them in protecting their health. Presently, consumers are more likely to consume functional foods and functional beverages which help them protecting their health and enhancing their life span, without relying on any intake of pharmaceuticals (White *et al.*, 2015).

Dairy-based products act as transferring the probiotics bacteria to the human through drinking or eating. The probiotic bacteria help in alleviate lactose intolerance symptom, lower the cholesterol level, curing the antibiotic-associated diarrhea, preventing any infection in the intestine, and etc. Besides, vitamins and minerals inserted into the beverages compensate for any loss of vitamins and minerals during the process (Özer and Kirmaci, 2010)

Milk contains lactose which low in sweetness power and solubility, is important during the production of dairy-based products. The participation of lactose in the process leads to the formation of lactulose. Lactulose is disaccharide that contains glucose and galactose, formed by isomerization of lactose in small quantities during the heat treatment of milk which usually known as UHT milk (Spreer, 1998).

2.1.1 Milk Processing

There are varieties of milk products that available nowadays in the markets that can match consumer preferences and fit every age and lifestyles. They are whole milk, low-fat milk, fat-free milk, and high-calcium milk. Mostly they undergo several vital steps of milk processing before marketing and selling. There are three primary processing steps involved such as pasteurization, homogenization, and vitamin fortification. Milk undergoes pasteurization by heating it at specific time and temperature to destroy harmful microorganisms and thus prolonging the shelf life. The normal pasteurization helps in maintaining valuable nutrients. Whereas, Ultra-high temperature (UHT) milk, where milk being pasteurized at higher temperature than normal to make it sterile as it can be kept into special container safely without being refrigerated.

The second step is homogenization. The purpose of homogenization is to prevent the separation of milk fat from the milk fluid, besides it creates smooth and uniform texture. Then the fortification step is performed where the nutrients lost during the processing by adding some other vitamins. For example, Vitamin D being added to most milk produced in United States in order to facilitate the absorption of calcium, whereas Vitamin A require for reduced-fat, low-fat, and fat-free milks and it also promotes normal vision (Dairy Council of California, 2015).

2.2 Sugar profiling analysis

This research involves the use of analytical analysis technique. In the past research analysis, they used different separation procedure such as the reverse phase, ion-exclusion, and ion chromatography. Besides, samples were detected by different detectors, for example the UV absorption amperometric and also by the refractive index (RI). The common methods used in the qualitative and quantitative analysis were Thin-Layer Chromatography (TLC), Gas Chromatography (GC) and High Performance Liquid Chromatography (HPLC) (Chinnici *et al.*, 2005).

2.2.1 Analysis using Thin Layer Chromatography (TLC)

The analysis of carbohydrates done by using immersion method where the carbohydrates was separated on silica gel impregnated with copper (II) sulfate and ammonia complex, $Cu(NH_3)_4$[26] as the pH is at pH5 and pH8. Research done based on the principle of polarity as the carbohydrates required the chromatographic systems that composed of polar solvents and sorbents of low activity. However, the application of metal sulfate salt limits the access of the monosaccharides to the active center of an absorbent. In addition, there was limitation in the interaction of hydrogen-bridge type of carbohydrate molecules with the silanol groups due to presence of impregnating agent on the absorbent surface (Szumiło and Flieger, 2000).

The application of TLC in the carbohydrates separation has much less widespread due to the poor separation of some sugars and low capacity of the chromatoplates. The advantages that been identified are the fast separation and sensitive, using the TLC plate compared to paper (De Stefanis and Ponte Jr, 1968). This method suitable in separating the sugar from the other

components for the qualitative methods, however the results were difficult to be quantitated (Cegla and Bell, 1977).

2.2.2 Analysis using Gas Chromatography

Gas Chromatography equipped with Mass Spectrometer (GC-MS) was an excellent technique in carbohydrates analysis; however preparation of an adequate derivative is needed. The preparation consist of several difficulties such as due to the high number of functional groups in a molecule, due to the presence of different tautometric forms in solution which the caused complex chromatograms, and due to the ability of some molecules in certain cases (Ruiz-Matute *et al.*, 2011).

2.2.3 Analysis using High Performance Liquid Chromatography (HPLC)

Another method that stated to be used was High Performance Liquid Chromatography (HPLC), to identify the sugar contents in the popular sugar-sweetened beverages. The analysis is done in analytical laboratory and the researcher focused the study on the identification of the fructose contents. Therefore, the HPLC method has become popular method in order to analyze sugar and to measure the percentage of the sugar contents in the samples. The method used is not only provides the qualitative analysis but also the quantitative analysis of reducing sugars and disaccharides.

According to (Cegla and Bell, 1977), HPLC benefits in providing rapid analysis of a large spectrum for saccharides with a minimum sample preparation is required. Besides, it removes

some of the limitations that carry out by other methods for the analysis. Thus, HPLC has been found to be an acceptable method in determining the carbohydrate contents.

The main systems applied for the separation of underivatized carbohydrates includes the anion-exchange column with water containing bases as the eluent, cation-exchange column with water as the eluent, alkyl-bonded silica gel column with water as the eluent, and amine-bonded silica gel column with water–acetonitrile as the eluent which most favor by the researcher. However, the carbohydrate separation using that column is not always quantitative due to the possible interaction between reducing carbohydrates and the amino group of the ligate and to a self-hydrolysis of the basic material (Wei and Ding, 2000).

Another research has been conducted on the analysis of sugar in ice cream and lactose in whole milk and nonfat dry milk. The sugar being extracted with 80% ethanol, 20% water and analyzed using carbohydrate column and refractive index (RI) detector. Based on the research 98% lactose recover from nonfat dry milk and 97% from whole milk with coefficient of variation in range of 0.7-5.8% which show that HPLC was a useful method for sugar analysis (Warthesen and Kramer, 1979).

The analysis using HPLC method was simple and reproducible in qualitative and quantitative analysis of the sugar with requirement of refractive index (RI) detection. In the recent study, HPLC most favor to be used due to its accuracy, separation abilities and also its rapidity. HPLC-RI basically being used in quantifying various types of carbohydrates compounds which can be found in juices, fruits, cereals, food (meat) and beverages (Chávez-Servín *et al.*, 2004).

Based on (Chinnici *et al.*, 2005), both separation methods, GC and HPLC, being used for simultaneous quantification of both acids and sugars. The advantages of using GC method were

the availability to analyze fruit or fruit juice samples by giving an excellent separation spike and it have high sensitivity. However, the GC methods required time-consuming derivative steps, needed to use the toxic derivatization agents, and should have high temperature for analysis process. These advantages lead to sample decomposition and resulting to the usage of HPLC method latter.

2.2.4 Enzymatic Analysis Method

Enzymatic analysis nowadays more widely used as analytical tools in the analysis of the food productions such as fruit juices, wine, beer, dairy products, egg, meat, and also probiotics drinks. However, the application of this enzymatic method analysis is considered new in Malaysia. Basically, the enzymatic test kits were required for the determination of sugars, acids, alcohols, and other foods components. This kit is ready-to-use-reagents which include in time saving aspects, high degree in safety, and using the safe reagents compared to other analytical method.

The term "enzymatic analysis" is defined as analysis with the aid of enzymes generally. The practice of using enzyme in food chemistry has been applied for several decades based on the assay of enzyme activity in organs and biological fluids. In fact, all metabolic reactions of living cells were catalyzed by enzymes, but the determination of the compound by living cells is part of microbiological assays. Enzymatic analysis involves reactions which are equilibrium. To be simple, the result is easily calculated by means of a known constant of the substances, if the substrate is virtually completely consumed (Bergmeyer, 2012).

CHAPTER 3

METHODOLOGY

3.1 Sample selection

The selected samples for this research are ready-to-drink (RTD) dairy-based beverages which commonly available in Kota Bharu, Kelantan, Malaysia. The selected beverage samples were purchased for sugar profile analyses which focus on common sugars such as glucose, fructose, sucrose, lactose, and maltose. About 20 types of RTD dairy-based beverages have been chosen for this research.

The development of the sample selection is by undergoing survey in several local hypermarkets located in Kota Bharu district, Kelantan, Malaysia. A list is made by identifying the milk products that available in the hypermarkets and any commonly available of the products will take into consideration during sample selection. The dairy-based products includes the flavored milk drinks, fresh and full cream milk, low fat and high calcium milk, and also a healthy milk drink.

Most of the sample can be categorized as shown in Table 3.1 below.

Table 3.1: Category of milk chosen in the present study

Category of Milk	Number of sample
Sterilized recombined flavored milk	6 samples (which include fresh milk, full cream, chocolate, grape, vanilla)
Ultra-high Temperature (UHT) recombined milk	10 samples (which include low fat and high calcium milk, chocolate, vanilla, coffee, and strawberry)
Sterilized, pasteurized and homogenized milk	2 samples (homogenized milk and strawberry flavour)
Homogenized and pasteurized milk	2 samples (fresh milk, chocolate flavour)

3.2 Sample Preparation

Sample preparation is important to produce sample with clear, colorless and practically neutral liquid and have pH 7.5 - 8.5. Sample needs to be processed to remove fat and protein contents, and any turbid solution need to be filtered. Carrez clarification is the recommended technique in the milk sample preparation in order to remove unnecessary nutrients prior to sugar profile analysis.

In the Carrez clarification, the solution used are Carrez I solution; potassium hexacyanoferrate (II)(ferro cyanide) –3.6 g/100 mL of $K_4[Fe(CN)_6]$, and Carrez II solution; Zinc sulphate – 7.2 g/

100 mL of $ZnSO_4$. In order to adjust the pH level, addition of 0.1M sodium hydroxide (0.4 g/ 100 mL of NaOH) is needed. Brief steps for Carrez clarification procedures are shown below:

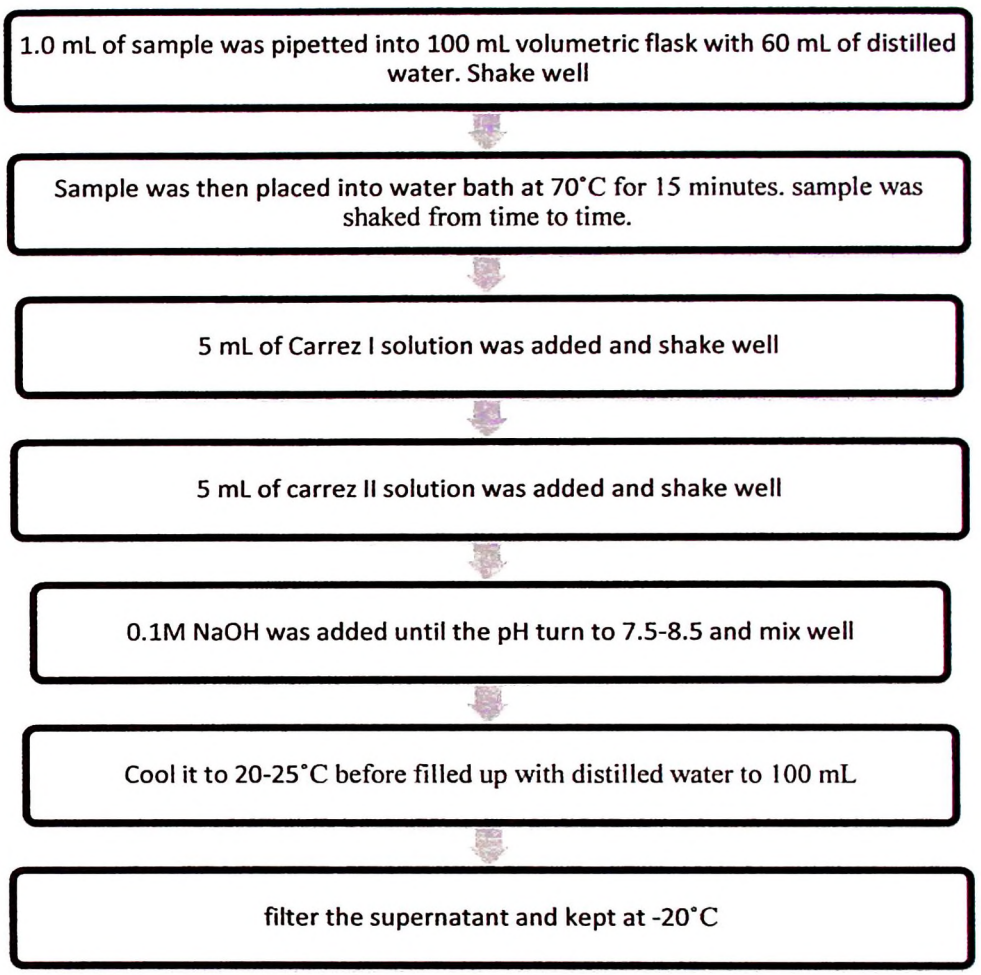

Fig 3.1 : Steps on sample preparation using Carrez clarification

In this preparation, hot water is used to extract the sample that containing fat. The extraction temperature should be higher than the melting point of the fat involved. Whereas, cooling down step of the sample is required in order to undergo fat separation before make up to the mark with distilled water. Figure 3.1 above show the steps in sample preparation using Carrez clarification.

3.3 Sample Analysis

Enzymatic method is one of the analytical methods which rely on the ability of the enzyme to catalyze specific region of the sample. The methods are rapid, highly specific, and also sensitive at low concentration of analyte. It is an ideal instrument which can be used in this research in order to determine the presence of carbohydrates in the beverages. Simple sample preparation is required before undergo the sugar profiling analysis by using some water and Carrez clarification solutions.

3.3.1 Principle of Enzymatic Analysis

In this research, the enzymatic test kits applied known as "Enzymatic Bio-Analysis and Food Analysis" which manufactured by Roche Diagnostics GmbH (formerly known as BOEHRINGER MANNHEIM). Basically each test comprise of four to five bottles that contain enzymatic and buffer solution. For example, the sucrose/ D-glucose/ D-fructose test kits comprises four bottles of solution (as shown in Figure 3.2).

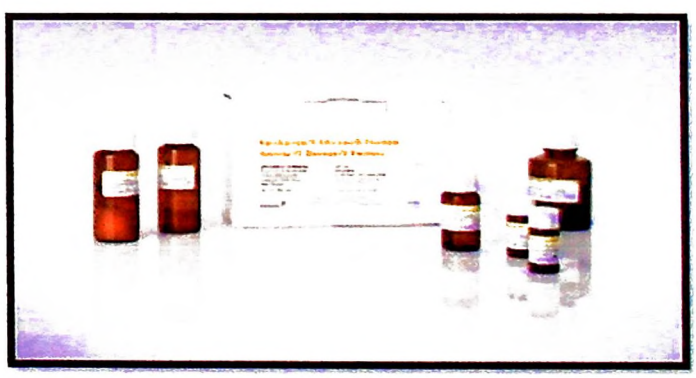

Fig 3.2 : Example of enzymatic test kits

3.3.1.1 Lactose/ D-Galactose

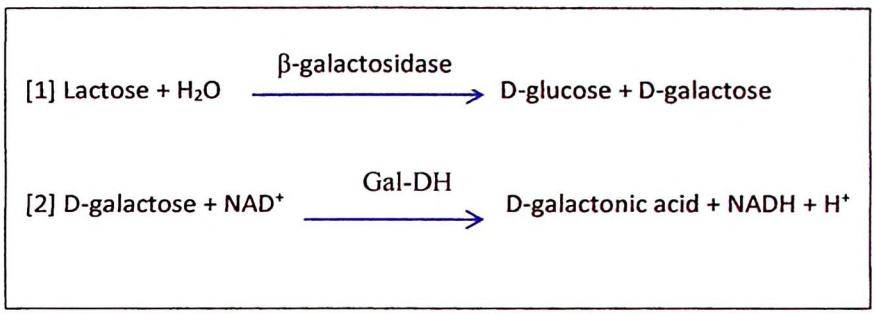

Fig 3.3: The chemical reaction using Lactose/ D-Galactose test kits

Principally, lactose is hydrolyzed to D-glucose and D-galactose at pH 6.6 in the presence of β-galactosidase enzyme and water (Figure 3.3). Then, D-galactose is oxidized at pH 8.6 by addition of nicotinamide-adenine dinucleotide (NAD) to D-galactonic acid in the presence of the enzyme β-galactose dehydrogenase (Gal-DH) to produce NADH. The amount is stoichiometric to the amount of Lactose and D-galactose respectively.

3.3.1.2 Sucrose/ D-Glucose/ D-Fructose

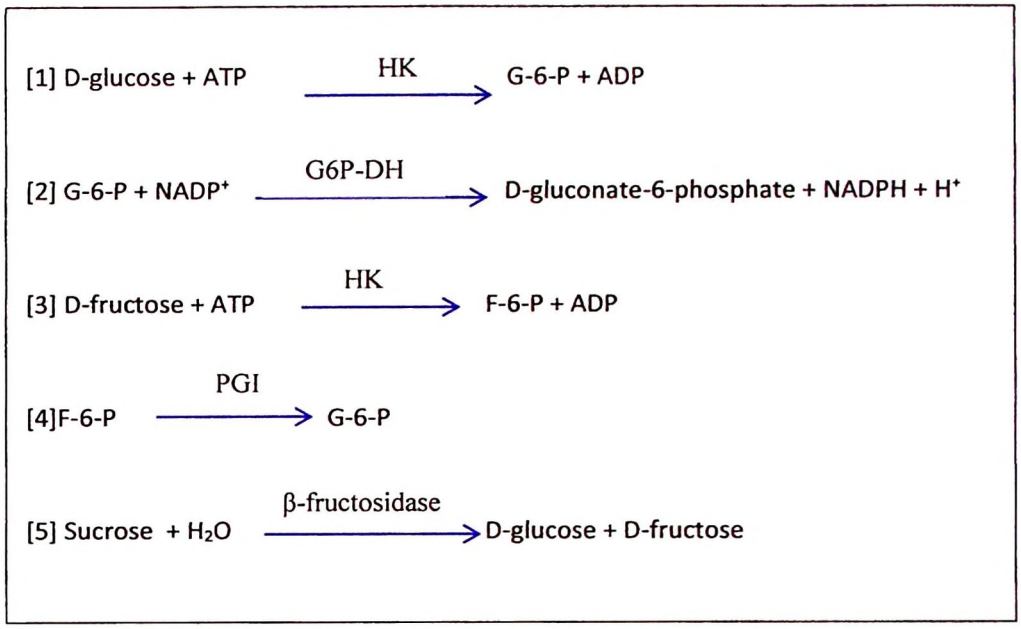

Fig 3.4: The chemical reaction using Sucrose/ D-Glucose/ D-Fructose test kits

D-glucose concentration should be determined before and after the enzymatic hydrolysis of sucrose, whereas the D-fructose determination is subsequently to the determination of D-glucose. Based on Figure 4 above, for equation [1] at pH 7.6, the enzyme hexokinase (HK) catalyzes the phosphorylation of D-glucose by adenosine-5'-triphosphate (ATP) with the simultaneous formation of adenosine-5'-diphosphate (ADP). For equation [2], in the presence of glucose-6-phosphate dehydrogenase (G6P-DH), the D-glucose-6-phosphate (G-6-P) formed specifically oxidized by nicotinamide-adenine dinucleotide phosphate (NADP) to D-gluconate-6-phosphate with the formation of reduced nicotinamide-adenine dinucleotide phosphate (NADPH). The NADPH formed is stoichiometric to the amount of D-glucose.

At equation [3], hexokinase (HK) enzyme also catalyzes the phosphorylation of D-fructose to D-fructose-6-phosphate (F-6-P) with the requirement of ATP. In the completion of it, F-6-P is converted by phosphoglucose isomerase (PGI) to G-6-P as shown in [4]. Then, G-6-P reacts again with NADP with the formation of D-gluconate-6-phosphate and NADPH as [2]. Similar to D-glucose, the amount of NADPH is stoichiometric to the amount of D-fructose. For [5], sucrose is hydrolyzed by the enzyme β-fructosidase (intervase) to D-glucose and D-fructose at pH 4.6. In the sucrose content determination, it is calculated from the differences of the D-glucose concentration before and after the enzymatic inversion.

3.3.1.3 Maltose/ Sucrose/ D-Glucose

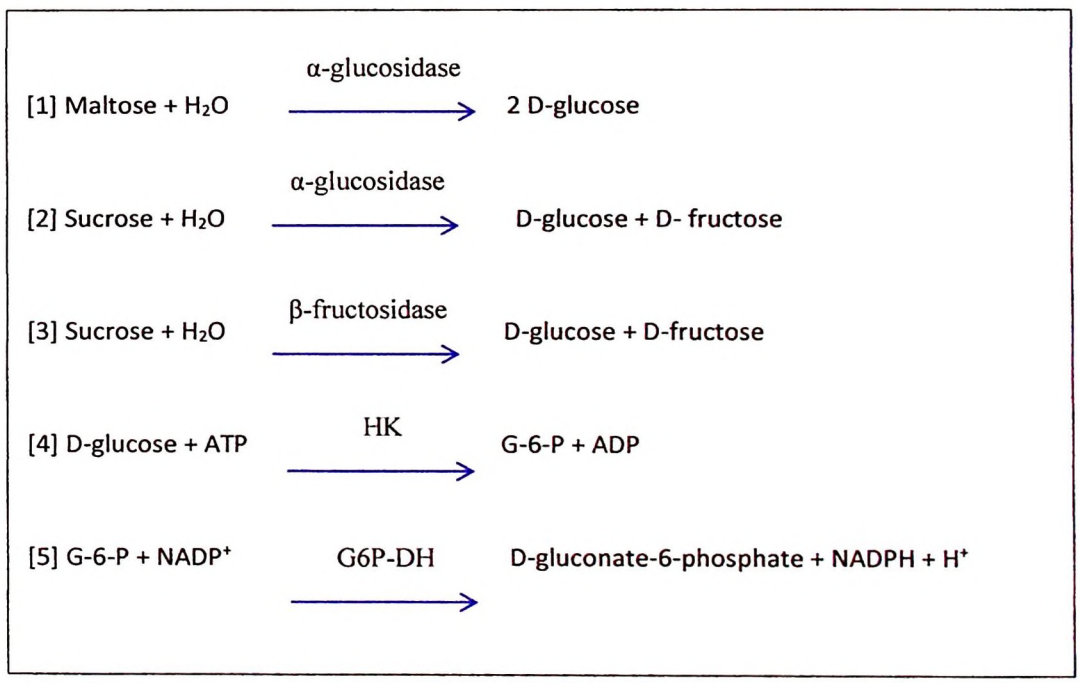

Fig 3.5: The chemical reaction using Maltose/ Sucrose/ D-Glucose test kits

Both equations [1] and [2] show that maltose and sucrose are hydrolyzed in the presence of the α-glucosidase (maltase) enzyme at pH 6.6 to produce two molecules of D-glucose or to produce D-glucose and D-fructose respectively (Figure 3.5). Whereas equation [3], sucrose also can be hydrolyzed by the enzyme β-fructosidase (intervase) at pH 4.6 to produce D-glucose and D-fructose.

At pH 7.6, the enzyme of hexokinase (HK) catalyzes the phosphorylation of D-glucose by adenosine-5'-triphosphate (ATP) under simultaneous formation of adenosine-5'-diphosphate (ADP) [4]. The formation of D-glucose-6-phosphate (G-6-P) is oxidized by nicotinamide-adenine dinucleotide phosphate (NADP) in the presence of glucose-6-phosphate dehydrogenase (G6P-DH) to produce D-gluconate-6-phosphate with the formation of reduced nicotinamide-adenine dinucleotide phosphate (NADPH) [5]. The amount of NADPH produced in the reaction is stoichiometric to the amount of sucrose, D-glucose and half amount of maltose.