THE EFFECTS OF LACTOSE AND INCUBATION TEMPERATURES ON SIMILAC 1 AND SIMILAC LF MILK SAMPLES

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ABSTRACT

Milk spoilage can be determined through chemical and physical analyses. The chemical analysis includes the analysis on the lactic acid content while the physical analysis uses the sensory tests to determine the spoiled milk. Most spoilage microbes such as *Bacillus, Proteus, Staphylococcus* and *Lactobacilus* grow easily in milk using the available nutrients such as lactose to produce lactic acid as the main end-product. Thus, the aim of this research was to study the effects of the lactose in the lactose-containing milk (Similac 1) and lactose-free milk (Similac LF) and the incubation temperature of 4°C, 25°C, 37°C and 60°C on the lactic acid content (g/100 mL) and the CFU/mL. The incubated milk was also analyzed by observing the curd formation, mold growth and the colour change of whey. The lactic acid content of the incubated milk was determined using the titration test with 0.1M NaOH. The CFU/mL was determined using the pour plate procedure and lastly, gram staining was done to identify the gram reaction of the spoilage microbes isolated from the pour plate method. The Analysis of Variance (ANOVA) indicated that the lactose in the milk and the incubation temperatures affected the lactic acid concentration as well as the CFU/mL of the incubated milk samples. In addition, the amount of the lactic acid also affected the CFU/mL of the incubated milk samples as the acidic condition probably inhibited the growth of acid-labile bacterial cells. Gram staining reactions revealed more gram-positive bacteria in the milk samples compared to gram-negative bacteria. The preliminary chi-square analysis of the physical observation indicated that only the incubation temperature affected some of the physical characteristics of the milk

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LIST OF ABBREVIATIONS

α	Significance level
ANOVA	Analysis of variance
°C	Degrees Celsius
CFU/g	Colony forming unit per gram
CFU/mL	Colony forming unit per milliliter
CO_2	Carbon dioxide gas
df	Degree of freedom
E. coli	*Escherichia coli*
°F	Fahrenheit
FFA	Free fatty acid
G	Gram
g/100 mL	Gram per hundred milliliter
GLM	General linear model
H_0	Null hypothesis
H_1	Alternative hypothesis
H_2	Hydrogen
LC	Lactose-containing
LF	Lactose-free
M	Mole
mL	Milliliter
MS	Mean square
µL	Microliter
n	Number of replicates
NaOH	Sodium hydroxide

SS	Sum of squares
UHT	Ultra-high temperature
UP	Ultrapasteurization
V/V	Volume to volume

CHAPTER 1

INTRODUCTION

Milk is a nutritious food (Singh, Kaushal, Tyagi & Sharma, 2011) that is important for all including expectant mothers and growing children (Srujana, Rajender, Krishna & Ram, 2011). Milk contains fats, proteins, carbohydrates, vitamins and minerals and high water content (Singh et al., 2011; Al-Shammary, 2013). This makes milk a suitable growth medium for microorganisms (Al-Shammary, 2013). There are two main groups of microorganisms found in milk; the spoilage and the pathogenic microorganisms. The spoilage microorganisms utilize the milk components such as lactose to synthesize compounds such as, lactic acid (Singh et al., 2011; Al-Shammary, 2013). Meanwhile, the pathogens will produce toxins or harmful substances leading to milk-borne diseases such as typhoid fever (Singh et al., 2011; Al-Shammary, 2013). In some cases, bacteria such as *Bacillus cereus* can be both pathogenic and spoilage microbes (Al-Shammary, 2013).

There are various factors that cause spoilage of milk. One of the main factors is the high moisture level and the availability of dissolved oxygen that enables the growth of both aerobic and facultative anaerobic microorganisms (Lu et al., 2013). Besides that, improper storage temperature of milk will also cause the milk to spoil faster. Studies show that temperatures between 20-30°C to increase the metabolic rate of the microbes causing them to grow faster in milk (Ranken, Christopher & Kill, 1997; Samarzija, Zamberlin & Pogacic 2012). Besides that, unsterilized milking machines and non-pasteurized milk will also cause the milk to spoil faster (Samarzija et al., 2012).

Microbial growth and the metabolites produced changes the appearance, taste and odor of milk. The spoiled milk can turn bitter, rancid, sour and malty in taste (Duyvesteyn, Shimoni & Labuza, 2011; Al-Shammary, 2013). It is hard to measure milk spoilage accurately (Lu et al., 2013). However, there are certain methods that can be used to detect milk spoilage with higher accuracy such as pH testing, methylene blue reduction with amperometric sensor, megnetoelastic, gas-sensor array, infrared spectroscopy and protein or fat count (Lu et al., 2013; Al-Qadiri, Al-Holy, Cavinato & Rasco, 2007). The infrared spectroscopy method is the most accurate and it has broad spectrum of detection with high sensitivity. The results of the spoiled milk can be obtained within 4 minutes. However, the cost for buying this infrared spectroscopy is high and the running cost for the infrared test is low (Lu et al., 2013).

To date, there isn't a concrete research to study the relation of the lactose content and the incubation temperatures on the incubated milk. Hence, this study was aimed to provide a better insight on the effects of the lactose containing milk and the lactose free milk to the milk by looking at the lactic acid content of the milk samples incubated at 4 temperatures, 4°C, 25°C, 37°C and 60°C. The effects were determined using titration (determining the lactic acid content), plate counting and physical observation based on the colour of the whey, the curd formation and mold growth. Gram-staining was done on the bacterial colonies to identify the gram reaction of the spoilage bacteria found in the milk.

There were two main hypotheses in this study which were:

H_0: There were no significant effects of the lactose (the type of milk) and the incubation temperatures on the lactic acid content (g/100 mL) and CFU/mL

H_1: There were significant effects of the lactose (the type of milk) and the incubation temperatures on the lactic acid content (g/100 mL) and CFU/mL

CHAPTER 2

LITERATURE REVIEW

2.1. MILK

Milk is produced in the mammary glands of mammals (Codex, 2011). The colour of milk varies from white to yellow. The difference in the colour is determined by the content of carotene in milk fat (Bylund, 1995). Milk is a very nutritious as it consists of nutrients which are needed by our body such as high protein content, riboflavin, magnesium, vitamin B12, selenium, pantothenic acid and calcium (FAO, 2013). Milk also contains 87.3 - 88.1 % (V/V) of water (FAO, 2013). It is either directly consumed or processed before consumption, or processed into different products (Codex, 2011).

Milk can be categorized into several classes. It can either be classified according to processing procedures, for examples the ultra-high-temperature treated milk (UHT-milk), pasteurized milk or sterilized milk. It can also be classified by its fat content such as low-fat milk, full-cream milk or skimmed milk. In addition, milk can be classified according to the sugar content for instance lactose-containing milk (the usual milk), and the lactose-free milk. Lactose content is generally around 4.8 – 5.2% in milk. In fact, lactose is the major carbohydrate found in milk (Choi, Lee & Won, 2007).

Lactose-free milk is produced for the consumption of lactose intolerant individuals. This is because these individuals are unable to digest lactose as they produce insufficient lactase, the enzyme responsible for digesting lactose (Dairy Council of California, 2015). Lactose-free milk is usually produced by the process of enzyme hydrolysis. In this process, the milk is treated by microbial β-galactosidase so that the lactose content in the milk can be broken down into glucose and galactose (FDA, 2011). The mixture of glucose and galactose causes the lactose-free milk to taste sweeter compared to the normal lactose-containing milk (Choi, Lee & Won, 2007). Due to the sweet taste, Tossavainen and Sahlstein (2003) used ultrafiltration technique to remove permeate, which consists of milk lactose and milk salt, from the milk. The milk salt in the permeate was recovered again through reverse osmosis and nanofiltration and added back into the reconstituted milk which is the milk without permeate. The reconstituted milk with low lactose content is then treated with β-galactosidase in order to produce less-sweet lactose-free milk (Tossavainen and Sahlstein, 2003).

2.2. MILK SPOILAGE

Milk spoilage refers to the changes or deterioration of the physical appearance, volatility, taste and odor (Kim, Hardy, Novak, Ramet & Weber, 1983; Al-Shammary, 2013) of the milk. These changes are caused by the growth of microbes in the milk (Kim et al., 1983). Some of the common pathogens found in milk samples include species of *Bacillus, Proteus, Staphylococcus, Salmonella* as well as *E. coli* (Singh et al., 2011). Both the nutritional composition of the milk as well as the storage temperature encourage these microbes to grow and spoil the milk.

2.2.1. The Four Stages of Milk Spoilage

There are four stages of milk decay which are rancid, curdling, coagulation and dry (F&N Magnolia, 2013). Rancidity refers to the slight souring of milk. Curdling refers to the formation of curd (F&N Magnolia, 2013; Antunes et al., 2014) that is caused by the renin-like enzyme secreted by certain thermophiles such as *Proteus* and *Bacillus* (Jay, Loessner & Golden, 2005; Al-Shammary, 2013). In both the rancid and curdling phases, the milk is still consumable but it is not advisable (F&N Magnolia, 2013). However, the milk is no longer consumable if it is coagulated as mold is usually noted to grow at this phase. The milk can become dehydrated and turns chalky and hard when it comes to the dry phase due to the vaporization of water in the milk.

There are also other characteristics in milk that indicates milk spoilage, for example, gas production in milk. Production of gas such as carbon dioxide (CO_2) and hydrogen (H_2) by the lactic acid bacteria will cause clot formation and foaming (Al-Shammary, 2013). The appearance of ropy texture (viscous-slimy texture) also indicates spoilage of milk. The formation of ropy texture is caused by the production