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EFFECTS OF STORAGE CONDITIONS ON ORGANIC ACIDS CONTENT AND SENSORY PROPERTIES OF COTTAGE CHEESE

MSc Thesis

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DECLARATION AND APPROVAL

I declare that this thesis is my original work and affirm to the best of my knowledge that, this has not been submitted for research work, award of a degree to this or in any other university. Where peoples work has been used, this has properly been acknowledged and referenced in accordance with the University of Nairobi requirements.

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DEDICATION

I dedicate this thesis to my family, my beloved wife Mrs. Ann Wanjiku , my two daughters Leeze Woki and Linnet Muthoni and my son Lenny Waweru together with my parents Mr. Samuel Waweru and Mrs. Elizabeth Waweru for their full support during my studies.

This thesis is also dedicated to my brothers and sisters for their unwavering support throughout my studies.

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ABBREVIATIONS AND ACRONYMS

B. cereus	Bacillus cereus
B. subtitis	Bacillus subtitis
B. nivea	Byssochlamys nivea
CO_2	Carbon dioxide
˚C	Degrees centigrade
DNA	Deoxynucleic acid
E. coli	Escherichia coli
HACCP	Hazard analysis and critical control points
H_2SO_4	Sulphuric acid
HPLC	High Performance Liquid Chromatography
KDB	Kenya Dairy Board
LAB	Lactic acid bacteria
PPE	Personal Protective Equipment
RNA	Ribonucleic acid
SHE	Safety, Health and Environment
UHT	Ultra high temperature
VOC	Volatile Organic Compounds

UNITS OF MEASUREMENTS

$cfu.m^{-3}$	Colony forming units per cubic meter
$cfu.g^{-1}$	Colony forming units per gram
$cfu.m^{-2}$	colony forming units per square meter
$cfu.ml^{-1}$	Colony forming units per milliliter
$mg.ml^{-1}$	milligram per milliliter
$mg.kg^{-1}$	milligram per kilogram
µl	micro litre
m.s-1	meters per second
ɳm	nanometer

ABSTRACT

In order to conserve milk proteins and extend its shelf life, production of different cheeses is essential. Cottage cheese is grouped under soft cheeses. Soft cheeses are those cheeses that do not undergo maturation and are characterized with high moisture contents, low fats and mild pH. This characteristic increases their chances of microbial contamination which results in off- odors, discoloration and slime formation thereby shortening their shelf life. The objective of the present study was to evaluate acceptance of cottage cheese and effects of its quality changes as result of different storage conditions thereby improving its shelf life. Cottage cheese was prepared by collecting fresh pasteurized milk from a local processing joint (Fresha Diaries) located in Githunguri area (Kenya). In the laboratory the pH of the milk was determined then boiled. Vinegar was added resulting in curd formation. The curd was then heated to drain whey, cut, collected and stored inside polyvinylchloride mould, kept at room temperature 25℃. Samples of cottage cheese were separated into 8 lots, each of which contained 25 samples which were packed under different conditions as follows; Fresh cottage sample (FR) (control sample) under normal conditions, Open sample (OP) under room conditions, Vacuum sample (V) under normal conditions, Frozen sample (F) at-18℃, Refrigerated sample (Ref) at 4℃, 10% honey sample (HON 1) at room temperature, 20% honey sample (HON 2) at room temperature, and 30% honey sample (HON 3) at room temperature. The samples were analyzed using High performance liquid chromatography (HPLC) method daily in the first week and once in subsequent weeks for a period of nine weeks. A standard HPLC methodology was developed and chromatographic conditions optimized with an isocratic elution done using 0.05N H_2SO_4 as mobile phase at a flow rate of 1mL/min and a column temperature of 60℃ at Coffee Research Foundation Ruiru. Basing on retention time of the organic acid standard, it was observed that there were a number of organic acid present in cottage cheese in the range of lactic acid> citric acid >Malic acid >Oxalic acid >Acetic acid. Levels of organic acid depended on storage conditions which in turn led to changes in organoleptic properties of cheese. A nine-point hedonic scale was used to evaluate the attributes for consumer's acceptance varying from liked extremely (9) to dislike extremely (1). The sensory attributes evaluated were color, appearance, taste flavor, smell/odor and texture/mouth feel. Tests were applied to cheese stored under different conditions of 1 week and 4 weeks. The tests were carried out in individual sensory evaluation scores during 30 days of storage. The results showed that the first week received highest scores. However sensorial was not significant during storage of deep freeze and Vacuum ($p>0.005$) but the difference in sensory of cheese stored in normal fridge, 10% honey, 20% honey and 30% honey were significantly difference ($p<0.005$). Despite its nutrition values it is evident that cottage cheese has a small shelf life hence the shelf life can be improved by employing a combination of different physical conditions (Hurdle technology) most probably according to our study modified vacuum packaging and use of honey.

CHAPTER ONE
INTRODUCTION
1.1 Background of the study

In Kenya, dairy industry has become one of the most important sectors of our economy. New and sophisticated investment have been put in place by local and foreign investors in the last few years in order to produce new and long-lasting dairy products.

According to a report from USAID Kenya in 2010, Kenya dairy industry contributes to 14% of agricultural GDP and 6-8% of the country's GDP, with job generation to a tune of 1 million at farm level, while 500,000 jobs are created as employment for many people directly and more than 700,000 jobs indirectly as support to the dairy industry thereby supporting the governments Big Four Agenda items of food and nutrition security and manufacturing (Philosophy *et al*., 2017) .

As such Kenya's dairy sector is an important sector in eradication of poverty both at village level and also in towns thereby contributing to food and financial security to many people. It is estimated that the industry contributes to a tune of USD 2 billion in Kenyans GDP (Philosophy *et al*., 2017).

According to Africa food business magazine released in June 2020, the amount of milk produced in Kenya yearly is about 5 billion liters. This makes Kenya as one of the biggest producers of milk in Africa contributing about 30-40% of the 5% that Africa contributes globally. The country has faced equal number of challenges to reach where it is today, taking third position in Africa after Ethiopia and Sudan. Average milk consumption per capital consumption in Kenya which is at 120 liters exceeds Africa's average of 50 liters and is projected to rise due to milk demand growth rate of 7% per annum to reach 220 liters by 2030

Today, Kenya is in process of becoming self-sufficient in production of dairy products having many industries specializing in processed and packaged milk products such as cheese, yoghurt, butter, ice creams. Cheese consumption is becoming common in Kenya based on influx of many expatriates from cheese producing countries both in Africa e.g., Ethiopia and Europe that are fond of eating cheese. Kenyans are also adopting to foreign foods from different malls that provides more market to dairy products as well as urbanization growth and development. Increased incomes as many Kenyans becomes middle class earners is another reason why cheese is becoming a favorite delicacy to many.

Cottage cheese is a product of milk. The term cottage cheese is an old name with a long span of history. Most olden dairy farmers made the cottage cheese from milk where the milk would be left in warm places for some time and the effect of natural bacteria within the milk would produce acids which could contribute to coagulation of milk thereby forming milk curds. Later this curd was cut and washed with cold water forming a dry curd which had a tangy flavor. With more discoveries the cheese was made tastier by adding cream to it (Gerhard, 2012).

In Kenya cheese making is in small scale and mostly depends with tradition of people and availability of milk (Connor, 1993). The nature of milk used i.e., raw or pasteurized dictates the quality of cheese produced. Due to presence of several enzymes found in raw milk as compared to few enzymes in pasteurized milk, raw milk produces cheese with a shorter ripening period. The most common enzymes in milk are lipases and proteases with their presence in cold milk contributing to milk degradation since proteases degrade proteins and lipase degrades fats (Scott *et al*., 1998) (Kosikowski, 1999).

The preservation of milk and other dairy products in Kenya and other developing countries present a major challenge because of high temperatures and limited refrigeration facilities. Despite milk pasteurization, sometimes we are not able to completely eradicate all the pathogenic microorganisms which results to damage of milk samples during shelf life. This presents more problems in conditions where the cold chain is a restraining factor.

As one of dairy products cottage cheese is highly susceptible to contamination hence should be stored in lower temperature to improve its shelf life (Nelson & Barbano, 2005). Depending on the form in which milk is used in making cheese i.e. whether raw or pasteurized cheeses will have different shelf life. (Nelson & Barbano, 2005) (Gammariello *et al*., 2008).

According to (Melilli *et al*., 2002) cheese spoilage is affected by several factors. They discovered that factors such as the initial bacteria used as the starter culture, the types of microorganism infecting the cheese, temperature, pH, water activity and salt to moisture ratio were some of the factors affecting the spoilage of cheese.

Study of organic acids is important in manipulating organoleptic properties within cottage cheese and proper usage of microbial culture. Analysis of these organic acid profiles is important in determining the spoilage of cottage cheese and its shelf life (Walther *et al*., 2008).

1.2 Statement of the Problem

Several studies have shown that in order to reduce the rate of spoilage of cottage cheese, we can either ferment lactose to lactic acid thereby reducing its pH or lowering the pH by adding some acids, adding some approved preservatives, inhibiting growth of micro flora thereby restricting growth of spoilage microbes by setting good packaging conditions that edges out any oxygen, addition of sugar or salt that reduces water activity, eliminating water and freezing (Modise, 2014). The study focuses more on the need to determine the best storage conditions for cottage cheese by analyzing the organic acids changes at different storage conditions and selected preservation methods. This was to produce optimal storage and preservation conditions that are good for prolonging cottage cheese shelf life within our tropic climates, since majority of documented work are within the temperate regions in most developed countries. Since most cheese producers in the country produce hard cheeses, this will create a platform for advocacy for cottage cheese production.

1.3 Justification of the study

Majority of cottage cheese sold at our markets have a life span of fourteen days. They are stored under refrigeration conditions and once they are out of refrigeration they cannot survive for long.

We embarked on a study to determine the best preservation and storage conditions that do not affect the quality of cottage cheese while prolonging the shelf life. The study focuses on the types of organic acid found in cottage cheese and how they influence spoilage of cheese under different storage conditions. A comparison was also made on sensorial properties of cottage cheese stored at different conditions.

Globally majority of cottage cheese is produced from fresh cow's milk. Cheese production in Kenya has been on an increasing trend. There are few studies on cottage cheese production in Kenya and most information available are on hard cheese. Currently information on the manufacturing of cottage cheese is scanty and less documented therefore there was a need for this study.

1.4 Objectives

1.4.1 Main objective

The main objective of this study was to investigate the effects of different storage, packaging and preservation conditions on organic acids and sensorial properties of cottage cheese so that we can prolong the shelf life of cottage cheese.

1.4.2 Specific objectives

1. To establish the types of the organic acids found in cottage cheese.
2. To compare how the organic acids of cottage cheese changes with time.
3. To evaluate the effects of several storage conditions on concentrations of organic acids in cottage cheese which we use to predict the shelf life under those conditions.
4. Sensory analyses of samples of cottage cheese stored in different conditions.

1.5 Scope and Limitation of the study

One of the easiest cheeses to make with several nutritional benefits is cottage cheese. However, depending on how it is handled during its making process and milk used whether raw or pasteurized this can determine how quickly it can be spoilt. Throughout the study we choose to make our own cottage cheese using low fat (3.5%) pasteurized milk from Githunguri dairy farmers cooperative (Fresha Brands) in the lab and then preserving and storing it at different conditions. HPLC machine was used to analyze the organic acids changes with different samples which was used to determine the best conditions without affecting the quality of cheese.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Milk is rich in nutrient with the highest component being water at almost 88%, followed by milk sugar(lactose) at about 4.8%, milk fats ranges between 3.3 to 3.4% and milk protein such as casein at 3.2% as well as enzymes such as proteases, reductases, phosphates (Scott *et al*., 1998). This makes them attractive to several pathogenic microorganisms which ultimately lead to their spoilage. Fellows of *Pseudomonas, Bacillus and Staphylococcus* are some of the mostly encountered bacterial species found in contaminated milk (Ledenbach and Marshall, 2009).

Cheese production in global level has been rising e.g., in the year 2005, Western Europe produced 8.6 million tons of cheese while United States produced 4.8 million tons as the two leading producers (Ledenbach and Marshall, 2009).

2.2 Main cheese producers in Kenya

From Kenya dairy Board (KDB) analyses released in 2013 comparing growth of dairy industry for ten years as seen from table 2.1, milk production and processing through the formal channel have grown more than 150% in the decade to 2013.

Table 2.1: Milk sold through formal channels in Kenya.

YEAR	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
QUANTITIES MILLION LTRS	197.3	274.1	339.5	360.2	423.1	398.5	406.5	515.7	549	495.2

Sources: Kenya Dairy Board

Kenya Dairy Board data bank of 2018 reveals that Kenya produces almost 3.8 million liters of milk per day coming from 30 milk dairy processors and 67 mini dairies which are licensed in processing and packaging of milk in Kenya (Wambugu *et al*., 2011). Our dairy market is controlled by four big milk processors namely; Brookside, New KCC, Sameer agriculture and Livestock limited (SALL) and Githunguri Dairy Cooperative Society. In combination, the four milk processors are able to generate 85% of total milk produced in Kenya. However, there are other milk processors that are contributing to a substantial intake such as Kinangop Dairy, Meru Dairy cooperative society, Upland premium Dairies, Bio Food products and Kabianga Dairy. Smaller specialists dairies include Bio Food Products, Raka Cheese, Brown Cheese, Happy cows Kenya and Alpha diaries (Wambugu *et al*., 2011).

In Kenya production of cheese is limited to some few companies. In 2016 for example, the leading producer was Browns Cheese which contributed 22% of all cheese produced in the country, followed by Raka Milk processors with 19% value share and Happy cows Kenya taking position three with a share valued at 15%. Eldoville Dairies and New Kenya Co-operative Creameries topped the first five companies with 12% and 10% value shares, respectively. Cheese industries are growing tremendously being supported by increased fast food restaurants such as KFC, Galitos, Pizza Hut and more.

2.3 Cottage cheese production

When raw milk is left at room temperature for about ten hours it converts to sour milk. This is due to bacteria activities such as Lactic acid bacteria that are present in raw milk which will first utilize dissolved oxygen for aerobic respiration. Once the oxygen is over the bacteria multiply by converting lactose in milk through fermentation process resulting to production of lactic acid which in turn triggers casein (milk protein), to coagulate at a pH value of around 4.6 a process referred as fermentation (Paul *et al*., 2002).

Cheese may be grouped into three categories i.e., hard, medium and soft. Cottage cheese is in the bracket of soft cheese. Soft cheeses are ones that do not undergo ripening and are characterized by having high water content, acidic with a short lifespan (Scott *et al*., 1998) (Kosikowski, 1999) (Cogan, 2004).

Cottage cheese is made from milk curds after coagulation when vinegar or enzymes are added to fresh milk at room conditions. The process of making cottage cheese on both small scale and large scale is quite similar and the only difference being the proportion and size of milk used to obtain cheese.

For cheese manufacturing involving enzymes we need to use good quality bacteria's such as lactic acid bacteria (LAB) which converts milk sugar (lactose) into lactic acid. Failure to properly convert lactose will produce off flavors. A good example of LAB includes the genera *Lactococcus, Lactobacillus, Leuconostoc, Streptococcus* and *Pediococcus*. These bacteria's will also reduce the growth of spoilage pathogens and other microbes in milk by producing some chemicals such as hydrogen peroxide, bacteriocins and diacetyl which reduces their activities (Scott *et al*., 1998) (El-Garhi *et al*., 2018).

There are several steps involved in making of cheese as shown in Figure 2.1. First pasteurized milk is heated and then cooled to 32 $^{\circ}$C since starter bacteria will do well at this temperature, and lactic acid bacterium (LAB) is then added. Inoculation of non-starter and starter bacteria initiates bacteria growth increasing acidity of the milk causing the milk to coagulate into a solid gel like milk and fermentation starts thereby forming the curd. The curd can be heated further to a temperature of about 60˚C for one hour to release all the moisture and cooled/ chilled by submerging them in cold water to the final cottage cheese. In order to produce a final creamed cottage cheese, we can opt to add cream, salt, herbs and fruits which in turn improves the texture. Soft cheeses such as cottage cheese and cream cheese are typically consumed fresh (Paul Ross *et al.*, 2002).

Most cheese in the market are the hard cheese which takes a long ripening period but have a long shelf life. These cheeses are produced in a slightly different process than that of soft cheese. To begin with we start by having the milk undergo a mild lactic acid fermentation that does not coagulate the casein. The Mesophilic culture helps to acidify thereby allowing cheese flavor to develop. The coagulation of milk is brought by addition of rennin (chymosin), an enzyme mostly found in calf stomachs but today produced by genetically engineered microorganisms which splits a certain bond in the casein molecule. The casein solubility is altered hence start to coagulate at a pH value of 5.5. The rennet helps to coagulate milk and forms curd from milk proteins. The curd is then separated from the rest of the whey and finally undergoes maturation process that finally results in the various types of cheese (Fox *et al.*, 2004).

The nature of cottage cheese which is soft, mildly flavored and highly perishable makes it a good medium for increasing of many spoilage microorganisms (Kilic *et al.*, 2004). Cheese shelf life is known to increase when microbial growth within it are inhibited by addition of sodium chloride and sugar content (Fox and McSweeney, 2004). For texture development, ripening and milk fermentation, milk proteins such as casein, play an important role (Scott *et al.*, 1998).

Steps involved in production of cheese

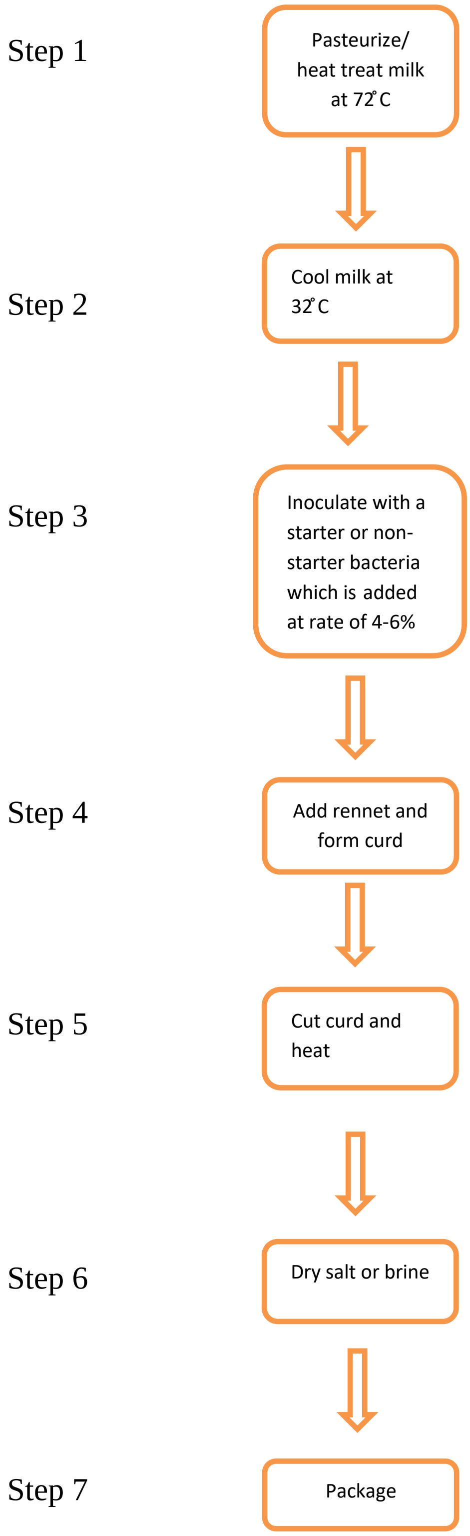

Figure 2.1: General flow diagram for cottage cheese manufacturing (Fox *et al.*, 2004)

different kinds of cheeses include: Mozzarella, Dutch Gouda, Swiss, and Romano (Whetstine *et al*., 2003) and are classified according to soft, semi-soft, semi-hard and hard cheeses (Table 2.2).

Table 2.2: Different types of cheese

Soft cheese	Semi-soft cheese	Semi-hard	Hard cheese
Brie	Feta	Cheedar	Asiago
Camembert	Dutch	Colby	Blue
Cottage	Gouda	Swiss	Mozarella
Cream	Havarti	Ricotta	Pamesan

Source: Gregerson, 2009

2.4 Benefits of cottage cheese

100g of cottage cheese contains; energy (98kcal), carbohydrates (3.38g), fat (4.5g), sugar (2.6g), protein (11.12g), vitamin A (37µg), and vitamin D (21µg). It also includes trace elements such as calcium, iron, magnesium, phosphorus, potassium, sodium, selenium and zinc. Due to these various nutrients cottage cheese has been found to have several health benefits.(Nagdeve, 2021.) (Buriti *et al*., 2005).

2.4.1 Easy to make

Cottage cheese is one of the easiest cheeses that one can make. It is made by boiling milk in presence of an acidic substance such as vinegar or lime. When milk boils casein which is one of milk protein reacts with the acid leading to formation of curds which is then separated by filtration from whey (watery liquid) which can be heated to produce a lump of curd (Nagdeve, 2021).

2.4.2 Rich in Protein

Cottage cheese is beneficial to several people owing to its great content of casein a milk protein that is known to be slow in digestion thereby releasing energy slowly. As a result, the cottage cheese will be useful by sports lovers such as athletes, sprinters, and body builders. The amount of proteins in Cottage cheese is high and necessary for vegetarians (Nagdeve, 2021).

2.4.3 Fights diseases

(Shin, 2002), conducted study over a period of 16 years at Harvard school of public health, and discovered that for premenopausal women, cottage cheese and other dairy products produces a lot of calcium and vitamin D that reduces the risk of breast cancer. Potassium found in cottage cheese is a key component in balancing body fluid as well as play a major function in neural activities of the muscle and brain. This helps in reducing stress levels and anxiety. Daily intake of potassium prevents the risk of brain stroke.

2.4.4 Improves body internal functions

Cottage cheese contains zinc which has proportions of 4% of daily recommended value. Zinc is a trace element mostly located in the brain, muscles, bones, kidneys, prostate, liver and eyes. Zinc aids in ribonucleic acid (RNA) and Deoxynucleic acid (DNA) metabolism. Cottage cheese therefore helps us to improve our immune system and this is known in controlling digestion and diabetes Zinc also helps in reducing stress and anxiety as well as curing night blindness (Nagdeve, 2021)

Despite several benefits associated with cottage cheese it is highly prone to contamination (Nelson and Barbano, 2005). Milk and milk products such as cheese, yoghurt among others is known to have a neutral pH but contain a lot of water and other nutrients. As a result of this they are known to attract several microorganisms in them which lead to their spoilage. Some of these microorganisms are harmful to milk and milk products and will also lead to production of organic acids. Also, during fermentation of milk to produce cheese and other milk products there is formation of several organic acids like lactic acid together with other acids which form the bases of our study. As it is expected cottage cheese organic acid concentration will remain unaffected for at least some few days in both refrigerated and room temperatures conditions. Since the lactic acid producing bacteria are known to be active during cheese formation it is expected that lactic acids will increase while the rest of acids will remain unchanged. Changes in acid profile will be an indication of microbial contamination during storage and these formed the bases for this study as this will be the cause for the spoilage of the cheese.

The large number of dairy products have several numbers of spoilage microorganism associated with them. According to a survey conducted in United States it was shown that

Dairy product consumers are likely to consume more dairy product if they last fresh longer (Buriti *et al*., 2005)

2.5 Factors influencing growth of microorganism in cottage cheese

Cottage cheese is spoiled by yeasts, molds and bacteria. The microbial growth in cheese is dependent on both intrinsic and extrinsic factors.

2.5.1 Intrinsic factors-

This are physical and chemical properties of food such as nutrient contents, pH and water activity (a_w).

2.5.1.1 Nutrient content

The chemical composition of cottage cheese in 100 gm comprises of energy (98 kcal), fat (4.5 g), Vitamin D (21 µg), carbohydrates (3.38 g), protein (11.12 g), vitamin A (37 µg), and sugar (2.6 g). Cottage cheese also contains several trace elements such as; calcium, iron, magnesium, phosphorous, potassium, sodium, selenium, and zinc (Gilliland, 2005). As a result, this attracts more spoilage microorganisms which utilize these nutrients for their growth.

2.5.1.2 pH and organic acids

Cottage cheese is high in nutrients which allow growth and acid production by many bacteria's that led to fermentation. Decrease in pH due to fermentation encourages growth of yeasts and mold which are more acid tolerant than bacteria. Foods with low pH are more stable microbiologically than neutral food. Some foods have low pH due to production of organic acids while others are due to inherent acidity.

2.5.1.3 Water activities (a_w)

All microorganisms require water for their growth but the exact amount of water needed by each microorganism varies. The water requirements is best expressed in terms of available water activity (a_w) which is the vapor pressure of food substrate / vapor pressure of pure water at the same temperature as per Raoults law. A_w of pure water is 1.0. Yeast and molds can tolerate lower a_w than bacteria. Gram negative bacteria require higher a_w than gram positive bacteria. Most cheese has a_w between 0.85-0.93 while honey has a_w below 0.60. For growth of microorganism each has its lowest a_w values that support its growth as shown in Table 2.3.

Table 2.3: lowest a_w allowing growth of spoilage microorganism

Group of organisms	Lowest aw value
Bacteria	0.91
Yeast	0.88
Mould	0.88

2.5.2 Extrinsic factors

These are properties affecting the storage environment of both foods and their microorganisms. They include temperature of storage and gaseous environment.

2.5.2.1 Gaseous environment

Most aerobic microorganisms require oxygen for metabolism. Since our atmosphere comprises 21% of oxygen cheese is stored in open conditions have a high degree of spoilage compared to the well packed cheese. In some cases, packing involve modified atmosphere storage containing increased amounts of CO_2 of up to about 10% CO_2 which retards growth of aerobic microorganisms.

2.5.2.2 Temperatures

Microbes grow over an extensive range of temperatures as can be seen from the Table 2.4. Microorganism will grow under different temperature conditions e.g.; Mold can grow even at refrigeration temperature unlike bacteria. On the other hand, yeasts cannot grow on thermophilic range but do well on psychotropic and Mesophilic ranges.

Table 2.4: Ideal temperature for growth of different microbes.

Microorganism	**Grow well at Temperature**	**Optimum Temperature**	**Examples**
Psychrotrophs	Below 7℃	20 – 30℃	Genera Pseudomonas, Enterococcus
Mesophiles	20-45℃	30 – 40℃	Enterococcus feacolis

Thermophiles	45℃ and above	55 – 65℃	Genera Bacillus, Clostridium

Adapted from Ecolab 2007

2.5.3 Spoilage microbes in cottage cheese

Quality of different foods are affected by chemical, physical microbiological and biochemical processes. The quality of food is affected by microorganisms which can leads to products that are disadvantageous commercially since they affect the shelf life or lead to quality grievances. It became more serious when infectious or toxicogenic microorganisms are involved because they threaten the health of the consumers. Despite many scientific breakthroughs in food preservatives, food safety problems continue to exist. This has led to several illness as well as deaths worldwide.

Table 2.5 shows the summary of different microorganism associated with spoilage of milk products.

Table 2.5: Types of spoilage microorganisms associated with milk products.

Milk products	Different types of Spoilage microorganism
Raw milk	Several microbes
Pasteurized milk	Psychrotrophs, spore-formers, microbial enzymatic degradation
Dried milk	Several spoilage microbes
Butter	Psychrotrophs, enzymatic degradation
Cottage cheese	Psychrotrophs, Yeasts, molds, Coliforms, microbial enzymatic degradation
Cultured buttermilk, sour cream	Psychrotrophs, yeasts, coliforms, lactic acid bacteria
Yoghurt	Yeasts
Processed cream cheese	Fungi, spore forming bacteria
Soft cheeses	Psychrotrophs, LAB, coliforms, fungi
Hard cheeses	Bacteria that produces spores, fungi and LAB

Source: (Buriti *et al*., 2005)

2.6 Factors affecting spoilage of cheese

Spoilage of cheeses depends on the following factors i.e. pH(acidic concentration of the cheese), water activity, temperature, the ratio of salt to moisture , nature of the bacteria's in the starter culture, types of microorganisms contaminating the cheese and nature of residual enzymes (Ledenbach and Marshall, 2009).

With several factors affecting spoilage of cheese, it can be shown that cheeses spoilage varies widely depending on their characteristics. Cottage cheese among other soft cheeses tend to have high pH and their salt to water ratio which are low make them susceptible to spoilage quickly as compared to other ripened or aged cheeses which have low pH, water activity and redox potential thus stay longer before they spoil (Melilli *et al*., 2002)

Melili *et al*. (2004) was able to observe that higher brining temperature (18˚C) and low initial salt supported growth of coliforms, leading to cheese gassiness. According to Brocklehurst and Lund, 1988 they found out that the leading factors affecting growth of microorganism in cottage cheese were storage temperatures and higher pH. They noted that some spoilage microorganisms could grow fairly at low pH values (4.6-4.7) at a temperature of 7˚C and when temperature were adjusted to 20˚C they grew at pH 3.6.

2.7 Prevention and Control Measures factors affecting spoilage of cheese

2.7.1 Heating Milk

Heating of milk to a temperature of about 62˚C for 30 min or to a temperature of 72˚C for 15 seconds kills most of pathogenic bacteria which are of significance value in milk. In today's world, fluid milk and other milk products are required to retain acceptability for a number of days, supposedly 14-21 days.

When all vegetative bacterial cells are killed due to adequate heating these protect the milk from recontamination thereby prolonging the shelf life to 21 days and beyond. Although Ultra-high-temperature (UHT) treatment mostly kills majority of spores in milk, some of them such as B. *stearothermophilus* have been known to survive afterwards.

2.7.2 Addition of CO_2

McCarney, (1995) showed out that treating cheese with carbon dioxide could be a good treatment of cheese. Dagostin *et al*., (2013) verified that treatment with CO_2 at a temperature of 6.1˚C and a pressure of 689 kPa produced a considerable decrease in bacteria counts.

2.7.3 Washing of the Curd

Despite that cooking of the curd helps in destroying most of the bacteria capable of spoiling cottage cheese, the way one's handle and treat the curd after cooking matters a lot as these could introduce quite a number of spoilage microorganisms. Many researchers working in this field have concluded that in order to reduce solids crumps in cottage cheese the cheese should be washed with acidified alkaline waters (Kosikowski, 1999).

2.7.4 Addition of lactic acid bacteria

Ultra heating of milk during pasteurization reduces the number of spoilage microorganism in cheese, and also reduces the gassiness in cheese. Apart from this, cheese made by adding a large number of lactic acid bacteria to raw milk reduces the growth of several microorganisms such as Psychrotrophic microbes (Melilli *et al*., 2002). For improved food safety bio preservation methods such as use of LAB has been so much support (Reis *et al.,* 2012).

The preservation effects of these bacteria is as a results of production of a wide variety of antagonistic primary and secondary metabolites (Paul *et al*., 2002) ,(Reis *et al., 2012*).

2.8 Organic acid profile

2.8.1 Role of organic acids in cheese

Organic acids are produced in animals during metabolism of proteins, fat and lactose. These acids are very important especially in flavoring of these milk products thereby affecting the quality of cheese (Seçkin and Esmer, 2011). Several researchers have used organic acids as an indication on growth of bacteria and indicators of starter cultures during cheese ripening. Different cheese have different organic acids profiles which produces specific flavors (Seçkin and Esmer, 2011). It is not in doubt that organic acids have been used and studied in length as an indicator of fermentation of many foods. Fermentation has been pointed as one of the best preservation method for milk products (Paul *et al*., 2002).

Organic acids have been widely studied and known to have two functional properties, i.e., they act as natural preservatives and also improve the physical characteristics of dairy products. These acids appear in dairy products as a result of normal biochemical metabolism (oxalic, malic and citric) and bacteria growth (lactic, acetic and propionic acids). Microorganisms display different tolerance to acids. Most organic acids are as a result of bacteria activities. LAB is one of most occurring bacteria in milk and it will start its action immediately milk is stored. Because LAB doesn't possess functional respiratory systems, they get their energy through substrate level phosphorylation. In case of hexoses sugars two fermentative pathways are followed i.e., homofermentative and heterofermentative pathways. Homofermentative pathway produces only lactic acids while heterofermentative pathway produces lactic acids, carbon dioxide and ethanol or acetate as can be seen from the Figure 2.2.

Fermentation is the process where we generate ATP energy molecule in the absence of oxygen in a process referred as glycolysis. During glycolysis process two molecules of pyruvate are produced from one glucose molecule thereby having two ATP and two NADH molecules as can be seen in Figure 2.2 and the two subsequent equations 2.1 and 2.2 below.

$$\text{Glucose} + 2\text{NAD}^{+} + 2\text{ADP} + 2\text{Pi} \longrightarrow 2\text{Pyruvate} + 2\ \text{NADH} + 2\text{ATP} \qquad (2.1)$$

(GLYCOLYSIS)

$$\text{Pyruvate} + \text{NADH} \longrightarrow \text{Lactic acid} + \text{NAD}^{+} \qquad (2.2)$$

(FERMENTATION)

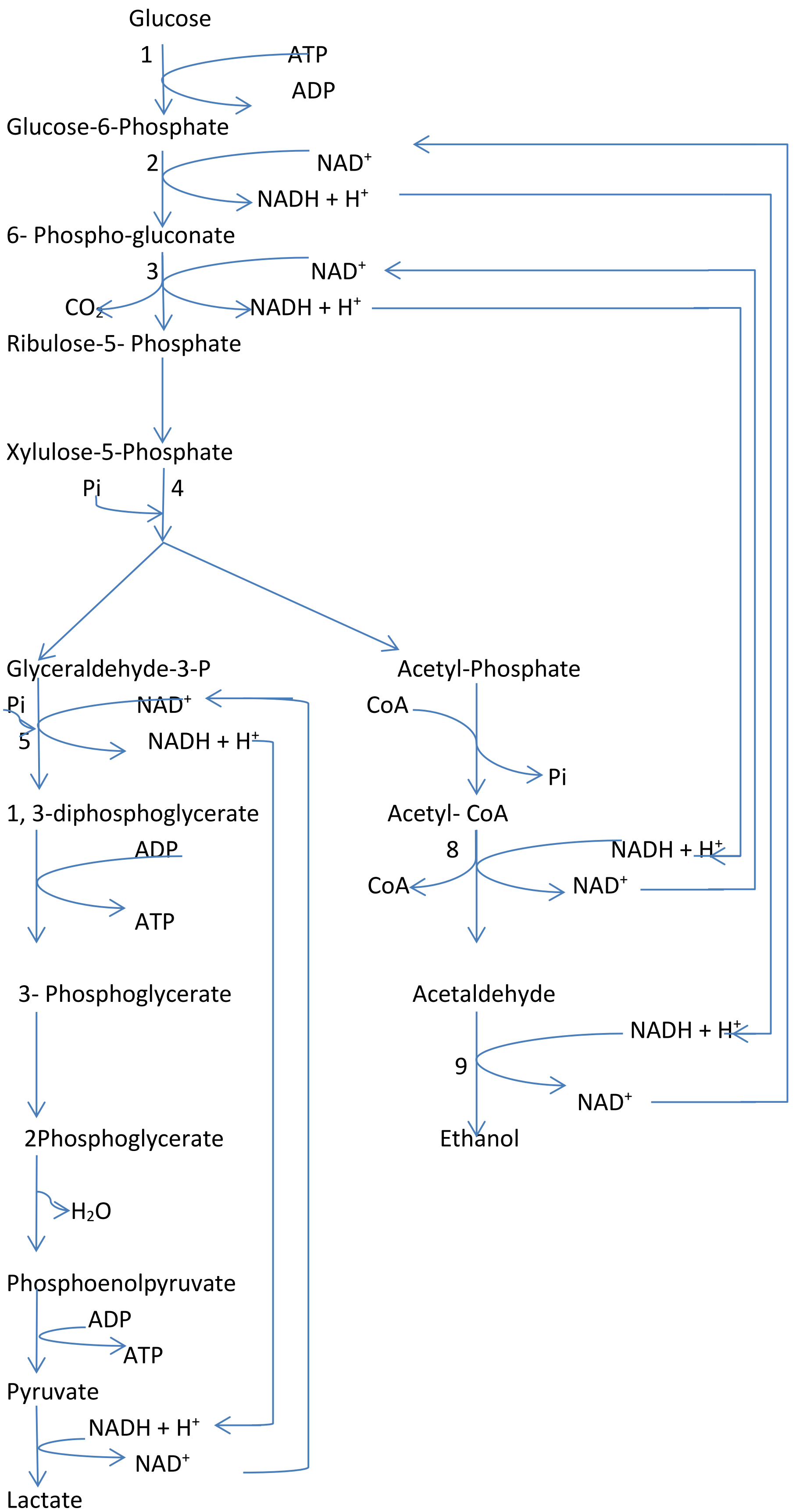

Figure 2.2: Heterolactic fermentation of 6-phosphogluconate or phosphoketolase pathway.

2.9 Role of Pyruvate in organic acids formation.

Pyruvate plays a vital role in fermentation pathways by forming lactic acid through accepting an electron thereby maintaining the oxidation reduction balance in the cell. But several pathways do exists depending on the type of LAB strain and specific growth conditions as can be seen summarized in the Figure 2.3.

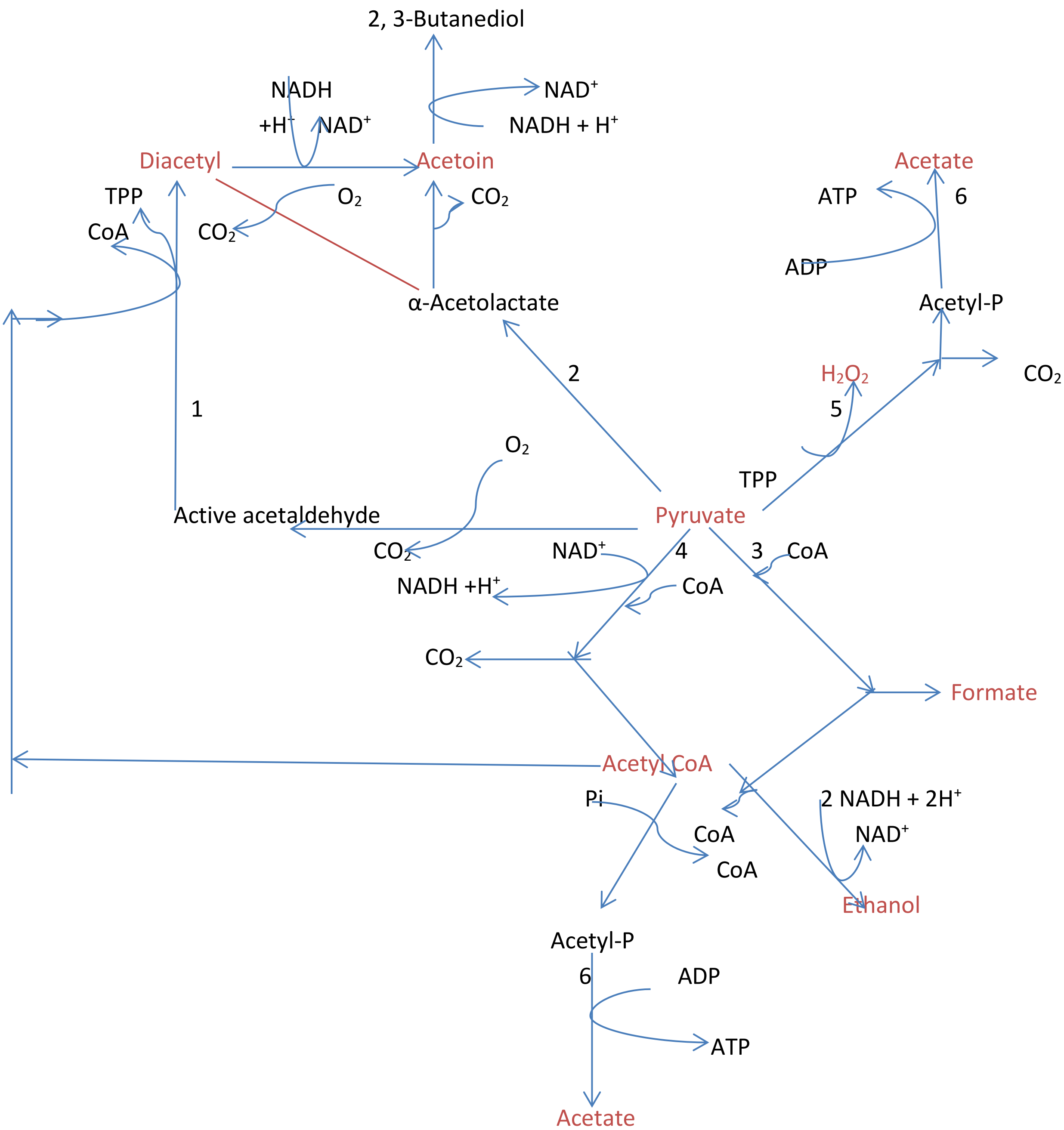

Figure 2.3: Pathways for the alternative fates of pyruvate.

2.9.1 Lactic acids (2-Hydroxypropanoic acid)

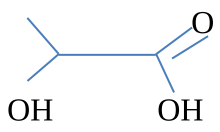

Figure 2.4: shape of lactic acid

Presence of lactic acid in many foods comes as a natural product or as a result of microbial fermentation. Lactic acid is used in many processed foods as an acidulate, flavor, pH-buffering agent and inhibitor of bacterial spoilage. The common bacteria that are known to produce lactic acids include, *lactobacillus, streptococcus, leuconstoc and Entrecoccus* species. Lactic acid inhibits growth of microorganisms in many ways. One the nondissociated molecule dissolves in microorganism cytoplasm via its cytoplasm membrane causing acidification of cytoplasm thereby influencing the trans membrane pH gradient and reduces the amount of available energy of cells to grow (Reis *et al., 2012*).

2.9.2 Acetic acid (Ethanoic Acid)

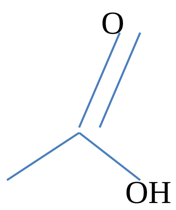

Figure 2.5 shape of acetic acid

Acetic acid is known to be as a result of many biochemical pathways, that includes fermentation of lactate and citrate or amino acids metabolism by bacteria. Acetic acid is known to contribute a lot to the final flavor of cheese. Presence of acetic acids provide an indication of the degree of heterofermentative metabolism that may have taken place in cheese (Seçkin and Esmer, 2011). Acetic acid has a characteristics aroma and is used in different food as Gram –positive and Gram-negative bacteria inhibitor and reduces their viability as well as yeasts and fungi (Reis *et al., 2012*).

2.9.3 Citric acid. (2-Hydroxypropane-1, 2, 3-tricaboxylic acid)

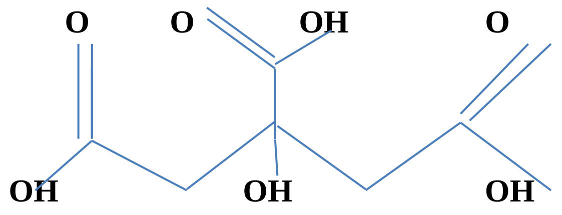

Figure 2.6: shape of citric acid

In many years citric acid has been considered as one of the normal constituents of milk. All aerobic organisms produce citric acid. Used as a flavor agent, and as a preservative. As a preservative it has been considered to be a harmless additive by food regulating agencies all over the world although it has been shown to cause erosive of tooth enamel. In commercial scale citric acid is produced from *Aspergillus niger* which is a black mould that efficiently convert sugars into citric acid.

The citric acid content is predominant in raw milk than in pasteurized milk and decreases during aging in the presence of high developed acidity, the utilization of citrates by certain microorganism reduces the level of citric acid with time as seen in the Krebs cycle.

2.9.4 Oxalic acids. (Ethanedioic acid)

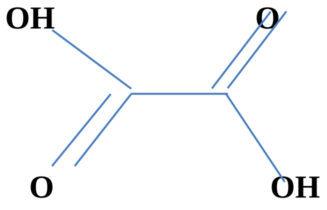

Figure 2.7: shape of Oxalic acid

Are known to come from pyruvate by lactate dehydrogenase or from glycolate-by-glycolate oxidase in plants. The synthesis of oxalic acids is increased by the mediums containing high lactose. It can also be traced from pastures on which the cows feed (Güler, 2014)

2.9.5 Malic acid (2-Hydroxylbutanedioic acid)

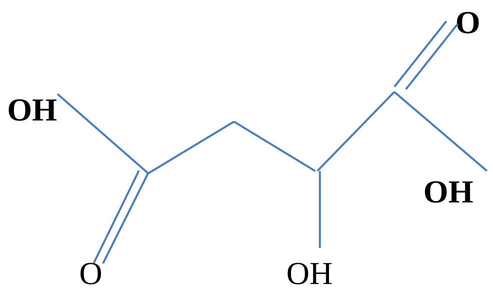

Figure 2.8: shape of malic acid

It is a products of incomplete sugar oxidation and deamination of amino acids (Khanal & Uprety, 2014), Malic acid is found naturally in many fruits such as apples as l-form. Malic and citric acids are products of fermentation after major growth phase as result of lactic acid bacteria enters the stationary phase. Malic acid is decarboxylated to release lactic acids from the cell activates H^+ uptake thereby generating ATP via ATPase. Small proportions of malic acids metabolized directly to lactic acids may also generate reducing energy (NADH). Malic acid is a dicarboxylic acid with two acid carbonyl groups and a double bond in α, β positions L-malic acids are produced by the fermentation of fumaric acids which is produced as a result of fermentation of glucose. Malic acid lowers pH thereby used as a pH adjustor and inhibits growth of bacteria.

2.10 Effects of organic acid on growth of bacteria.

Organic acids are known to inhibit the growth of bacteria. Most of spoilage bacteria are known to be neutrophilic but their cytoplasm pH is affected by partial dissociation of organic acids in their cytoplasm. In order to help them keep their internal pH slightly higher than that of the medium, they use a lot of their energy in trying to remove protons inside their cytoplasm as a result of pH gradient which causes protons to flow through the membrane bound ATPase's, thus producing the cell energy. The process consumes energy since it is an active process. Increasing acidity outside the bacteria cell consequently forces the bacterium to spend larger amounts of energy to eliminate the greater number of incoming protons, thereby affecting their growth (Ouattara *et al., 1997*). This is different from organic acids such as hydrochloric acids on inhibition of bacteria growth (Ouattara *et al., 1997*)

Different bacteria will show different results when subjected to various organic acids. At similar concentration, citric and lactic acids tend to decrease the pH of the growth medium more than acetic and propionic acids. Due to its high pKa, acetic acids are almost undissociated and have the longest retention time, so it could easily be separated from the other organic acids (Niu *et al., 2012*).

2.11 Use of food preservation technology for cottage cheese

As per definition food preservation is referred as measures taken to preserve spoilage of food. In other terms it means those processes taken either in biochemical or microbial manner against food pathogens. This means that existing technologies focus on making microorganism inactive or by preventing their growth. What is mostly considered is processes that affects the growth or multiplication of microorganism.

A term known as hurdle have been crafted to explain factors used for food preservation. In 1995 Leistner described a combination of different preservation factors and processes that reduced or inhibited microbial growth and called the process hurdle technology. The principle hurdle employed in food safety are temperature (higher or lower), water activity, pH, redox potential, chemical preservatives, vacuum packaging, modified atmosphere and competitive microbiota, such as LAB producing antimicrobial compounds (Leistner, 1995) as shown in the Table 2.6.

Table 2.6: Most important hurdles for food preservation

Parameter	**Symbol**	**Application**
High temperature	F	Heating
Low temperature	T	Chilling , freezing
Reduced water activity	a_w	Drying, curing, conserving
Increased acidity	pH	Acid addition or formation
Reduced redox potential	Eh	Removal of oxygen or addition of ascorbate
Preservatives	Pres.	Sorbate, sulfite, nitrite
Competitive flora	c.f.	Microbial fermentation.

Adapted from (Leistner, 1995)

Over the recent past, about 50 hurdles have been used in food preservation. They include: ultra-high temperature (UHT), mano-thermo-sonication, photodynamic inactivation, modified atmosphere packaging of both non respiring and respiring products, edible coatings, ethanol, Maillard reaction products and bacteriocins (Table 2.7).

Table 2.7 Different hurdles used to preserve food.

Types of Hurdle	**Examples**
Physical hurdles	• Aseptic packaging, • electromagnetic energy (microwave, radio frequency, pulsed magnetic fields, high electric fields), • high temperatures (blanching, pasteurization, sterilization, evaporation, extrusion, baking, frying) • ionic radiation • low temperature (chilling freezing) • modified atmospheres, • Packaging films (including active packaging, edible coatings) • photodynamic inactivation • ultra-high pressures • ultra sonication, ultraviolet radiation
Physico-chemical hurdles	• Carbon dioxide, • ethanol, • lactic acid, • lactoperoxidases, • low pH, • low redox potential, • low water activity, • Maillard reaction products, • organic acids, • oxygen/ ozone, • phenols, phosphates, • salt, smoking, • Sodium nitrite/nitrate, sodium or potassium sulphite, • spices and herbs, • surface treatment agents
Microbioally derived hurdles	• Antibiotics, • bacteriocins, • competitive flora, • protective cultures

Source Adapted from (Ohlsson and Bengtsson, 2002)

2.12 Mechanisms of Hurdle technology.

The combination of several food preservation methods (Table 2.6) has been in use for many years. For example, in the past people used to preserve smoked meat for future use. In this case several hurdles were in play. They involved heat treatment, reduction in moisture content and sometimes smoke could produce chemicals that had antimicrobial elements that were deposited on the food surface. Sometimes the smoked food was initially soaked in brine before smoking to fill the flesh with salts and thus add other preservative mechanisms.

In recent years the concepts of combining different factors with the goal of not only making food safe and stable but to improve the microbial quality of the food has been in use. For example, milk pasteurization, use of defined starter and ripening cultures. Hurdle technology employs different hurdles to achieve multi-target, mild but reliable preservation effects. The importance of this technology to the consumers are : Healthier foods that retain the original nutritional properties, ready to eat food that do not require further processing and natural food with fewer chemical preservatives (Irlinger and Mounier, 2009).

2.13 Protective cultures

It is worth mentioning that in today's society very few protected cultures are in the market due to several restrictions on GMOs and players such as European market that impedes the development of more protective cultures (Irlinger and Mounier, 2009). The recent development of cheese cultures with protective functionalities and the limit of their utilization have been reviewed and are shown in the (Table 2.8) below.

Table 2.8 Inventory of microbial interaction in cheese

Interaction types	Microorganism involved	Effects in cheese and /or microorganism involved	Microbiological phenomena observed
Commensalism	Yeast, ripening bacteria	Cheese surface deacidification: Lactic acid utilization Alkaline metabolites (NH_2) production	Growth of acid sensitive bacteria
Amensalism	LAB, spoilage and pathogenic bacteria	Curd acidification Organic acids (i.e., lactate)	Inhibition of acid sensitive bacteria
Competition	Siderophores containing bacteria, auxotrophic bacteria	Harvest of iron	Reduced colnization capacity of auxotrophic strains

	Microbiota of wooden shelves, listeria monocytogenes	Jameson effect	Limited colonization of L. monocytogenes
Parasitism	Phage, bacteria	Failure of fermentation	Inactivation of dominant strains

Source: Irlinger and Mounier, 2009

2.14 Honey as a preservative

Honey has been found to contain inhibitory activities due to its peroxide component and non–peroxide component. The main antibacterial component in honey being the hydrogen peroxide. The non-peroxide factor contributing to the anti-bacteria property of honey includes lysozomes, phenolic acids, flavonoids, and a number of aromatic acids with syringic and phenyl lactic acids being the abundant ones. Honey has other phenolic compounds known to prevent growth of several bacteria. Investigation on honey preservative on milk and milk products has arrived to a conclusion that it is safe and effective preservative method. The lactoperoxidases (LP) system is naturally occurring antimicrobial system in milk which is active against both gram positive and gram-negative microbes to varying extents. The antibacterial has the abilities to reduce bacterial growth by damaging the cell membranes and inhibiting the activity of many cytoplasmic enzymes (Krushna *et al*., 2007).

2.15 HPLC.

According to Marsili *et al.,* 1981, Organic acids take the first position among the compounds that have been studied using HPLC due to their significance in cheese industry. Knowledge about organic acid is important since it enable us to understand the metabolism and quality traits of milk and products. HPLC separates compounds based on their solubility in particular solvents. Many researchers have taken organic acid profile and content as an important quality indicators, using HPLC as the reference method for organic acids determination (Hu, 2012).

2.16 Determination of organic acids using HPLC

Nollet, 2000 noted that HPLC is the reference method for determining various components in food analysis. In dairy products the information about organic acids is extremely valuable in understanding the quality traits (Bevilacqua and Califano, 2006). To better understand the organic acid profile in cottage cheese and confirm causes of spoilage of the cheese HPLC analysis were performed in different samples of cheese under different storage conditions.

According to (Chinnici *et al*., 2002) either the Sulphuric acid or phosphoric acid can be used as the mobile phase to analyze organic acids in Aminex HPX-87H columns. In this study we used Sulphuric acid as eluent due to its higher resolution and safety of the chromatographic column.

The effects of column temperature were also under test where we used a temperature of 60 ° C. this is because retention time is affected by temperature changes. High temperature can optimize the efficiency of the chromatographic.

CHAPTER THREE
METHODOLOGY

3.1 Research Design

Samples stored at different storage and preservation conditions were analyzed using a HPLC machine. Duplicate control analyses were done where sample were weighed using same weights, aliquots taken and similar reagents used. Operation done within similar time frames where HPLC instrument was operated with the same parameters and operations performed identically. The chromatogram generated are easy to read and interpret since the peak height or peak area were plotted verses the concentration of the organic acid. Standard solutions of organic acids of known concentration were injected first into HPLC in order to generates peak and compared with peaks produced by different samples of cottage cheese. Concentration of organic acids were then calculated as per section 3.9.3.

3.2 Samples and Sampling Procedures

Two types of fresh samples were used for this experiment. Cottage cheese sample from Happy cow industries in Nakuru that was bought from retail markets specifically Tusky supermarket in Nairobi. Manufacturing dates were checked to ensure a fresh sample was picked for analysis. The cheese as to other dairy products was stored under refrigerated conditions in the supermarket and measures were taken during transportation in order to retain the freshness. The sample was transported in an ice box for analyses in the laboratory. The second sample was a homemade cheese that was prepared as per (Felfoul *et al.*, 2017.) guidelines with little modifications. Fresh pasteurized milk was sourced from Githunguri dairy processing factory and cottage cheese made from it as explained in 3.6.

Two hundred samples of cottage cheese were analyzed in this study. Cottage cheese samples meant to assess the storage conditions such as refrigerated conditions, deep freeze conditions, vacuum conditions and influence of natural preservatives such as honey were collected once, divided into 1gm samples and stored under these conditions. The first and second week of analysis show fresh samples being collected daily. After the third week the fresh sample were collected once on a weekly basis for nine repeated weeks. Care was taken in transportation of all samples to the laboratory for analyses since all samples were transported on ice cool box containing ice cubes.

Studies on preservation of honey were done to check whether honey alone can help in extending cottage cheese shelf life. Selected honey from Woodland Company Limited from Kitui was used. Fresh cottage cheese had been observed to spoil within three days of storage under normal conditions. To three samples of cottage cheese, different concentration of honey was added in ratios in order to obtain 10%, 20%, and 30% w/v cheese /honey sample. Samples were then sub-divided into 5 gms samples and marked depending on the day of analysis and left in open conditions. Each sample of cheese mixed with honey that were prepared once and stored; together with pure honey sample as well as fresh cottage sample were analyzed with the HPLC machine daily for the first seven days and in subsequent weeks. All the samples were analyzed and their organic acid profiles compared with organic acid profiles of the standards.

3.3 Instruments:

The instrument used in these experiments was;

HPLC equipped with online solvent degasser, S1000A and B Knaeur liquid chromatography pump with smart line RI detector (A46014 Germany). Ultrasonic homogenizer mixer (ISCO) at a bath temperature of 60^o C, (Ramton RF/223) Refrigerator for storage of samples both at 4^o C and -18^o C, a centrifuge (Mckesson 6) and a blender (Nunix PB 1).

3.4 Reagents and Chemicals

Organic acid standards associated with natural milk and cheeses (acetic, oxalic, propionic, citric, formic, lactic and malic), ultra-pure water and other chemicals were from Sigma – Aldrich Germany sourced from Kobian Kenya limited. All reagent used were of analytical grade with initial purity not less than 99.9%.

3.5 Standards and quantification

In order to get the actual organic acids of cottage cheese several studies of organic acid profiles of different cheese previously done was studied giving the likely organic acids to encounter in cottage cheese. Standards solution of lactic, acetic, citric, oxalic and malic acids associated with cottage cheese were prepared as follow:

Weighing 1 gm of Oxalic acid, 1 gm of citric acid, 1 ml of lactic acid (density 1.2 gm/cm^3), 1 ml of malic acid (1 molar) and 1 ml of acetic acid. The mixture of acids standards was dissolved in 100 ml volumetric flasks up to the mark by adding 0.05N H_2SO_4 making 10,000 ppm. 10ml of this sample was mixed with 10ml of the mobile phase and further serial dilution resulted to 20 µl sample which was then injected to the HPLC machine to provides standard lines based on the peak for each organic acids and the linear regression curve based peak areas were calculated for individual organic acids plotted in Table 3.1. All samples were analyzed in duplicate. The working solutions of standards were prepared at ten different concentrations. The coefficient of determination (**R^2**) obtained were 0.99 considered adequate to verify linearity of regression lines for analytical methods. The acid profiles of these standards were then compared with those of the cheese and several acids identified.

Table 3.1 Regression equations for the standard samples and analysis of linearity.

Acids	**Regression equation (y= ax + b)**	**R^2**
Oxalic	y= 10635X - 43071	0.9957
Citric	y= 10566X - 1E+06	0.9958
Malic	y= 6173.5X + 2E+06	0.991
Lactic	y= 9012X + 5E+06	0.9986
Acetic	y= 6573.5X + 808381	0.9966

Where y: concentration; a: slope; X: the response; b: intercept; R^2: coefficient of determination

3.6 Cottage cheese preparation procedure

Fresh cheese sourced from supermarket mostly from Happy cows' industry (control sample) and another fresh sample which was homemade were selected for the study. For the homemade cottage cheese, it was prepared by little modifications done earlier by (Gomes *et al.,* 2011). Pasteurized packed milk (3.5% butterfat) from Githunguri dairy farmer's processor was boiled and vinegar was added to form curd. After 30 minutes the curds were cut, squeezed and cooked to remove whey and then cooling followed. The homemade sample was repacked and stored in a plastic container which was thoroughly cleaned and rinsed and kept in an ice box for transportation to the laboratory (5± 0.5℃). Analysis was done two to three hours after obtaining the sample thus it was considered fresh. Apart from fresh cheese that was prepared daily in the first week and once in the subsequent weeks, the other sample of cheese was produced in one batch to minimize variability between cheese samples (Akalin *et al.*, 2002).

To the manufactured cheese from Happy cow industry, we started by removing the rind of 5g layer of the cheese in order to get cheese as it is consumed. The sample was then mixed thoroughly using a blender. The analytical sample was then transferred to an air tight container awaiting analysis which was carried after grinding. Precautions was taken to ensure proper preservation of the samples and to prevent condensation of moisture on the inside surface of the air tight container.

Comparison of homemade sample with purchased sample on organic acid profile was done. In the laboratory fresh sample from both homemade and manufactured samples were analyzed and gave similar acid profiles. The fresh samples were then separated and samples of 5gm stored under different storage conditions awaiting analysis in different timelines. Samples of cottage cheese were packed in readiness to be used for Physico-chemical, sensory analyses and shelf-life determination.

Samples of cottage cheese were separated into 8 lots each of which contained 25 samples which were packed under different conditions as follows; Fresh cottage sample (FR) (control sample) under normal conditions, Open sample (OP) under room conditions, vacuum sample (V) under normal conditions, frozen sample (F) at -18℃, refrigerated sample (Ref) at 4℃, 10% honey sample (HON 1) at room temperature, 20% honey sample (HON 2) at room temperature, and 30% honey sample (HON 3) at room temperature. The determination off physic-chemical, sensory and organic acid profiles were determined in each day of the first week and repeated every Wednesday on the subsequent weeks till the ninth week in duplicate.

3.7 Physico-chemical analysis

3.7.1 Moisture content/ total solid content

Total solids content was determined by gravimetric method (Güler, 2014). Glass petri dish were taken and preheated to remove moisture and then cooled inside a desiccator and weighed. Six grams of each sample were kept in the petri dish and weighed. The dish was then dried at 80℃ in hot air oven to avoid volatile evaporation for 24 hours, then the weight of flask and sample were cooled down in a desiccator and the remaining materials were weighed. Three samples were selected randomly for each sample during analysis i.e., done in triplicate. The percentage of total solid content was calculated using the formula 3.1 and 3.2:

$$\% \text{ moisture} = \frac{\text{loss in weight} \times 100}{\text{Weight of sample}} \quad (3.1)$$

% Solid = 100 - % moisture (3.2)

3.7.2 pH and titratable acidity

The pH was determined through Gerber method where a sample was determined by weighing 10gm of the sample and then homogenizing it with sterile distilled water (100ml) in a ratio of 1:10, followed by shaking for 5 minutes. The pH of the cheese was then measured using a globe probe digital pH meter. The pH meter was calibrated with fresh pH 4.0 and 7.0 standard buffers. The measurement of pH was carried out in triplicate (NO, 2012)

3.8 Sensorial Analysis

A round table discussion with a twelve-member panel staffs from coffee research foundation selected from their willingness to participate was conducted to identify the descriptive flavor and textures for the cheeses (Karagul-Yuceer *et al*., 2007).

A nine-point hedonic scale was used to evaluate the attributes for consumer acceptance, varying from liked extremely (9) to dislike extremely (1). The sensory attributes evaluated were color, appearance, Taste Flavor, Smell/Odor and Texture/Mouth feel. Tests were applied to cheeses stored under different conditions of 1week and 4 weeks. The cheeses were cut into cubes (2.0 x 2.0 x 2.0 cm) and offered in a monadic way on a plastic dish coded with three random digits. Water and plain crackers were provided to clean the palate between samples (Cruz *et al*., 2010), (Karagul-Yuceer *et al*., 2007).

3.9 Extraction of Organic Acid

During each analysis samples from different storage conditions were analyzed within six (6) hours of preparation. Organic acids were extracted from these cottage cheese samples. Our extraction method did not require a clean-up step because HPLC columns are not susceptible to contamination by co-extractants. The method for extraction of the acids performed followed the procedures described by Fernandez-Garcia and McGregor with little modifications (Güler, 2014). Extraction of organic acids were done using 0.05N H_2SO_4 which was then centrifuged for five (5) minutes at a temperature of 5℃ at 7000 x g. Two faces of filtration were done in order to remove the upper layer starting with filtration through whatman No. 1 filter paper, and then through 0.45 µm syringe filters x (Ministart single use filter unit non pyrogenic). This solution was then injected directly into the chromatography with Sulphuric acid as the mobile phase.

For fresh samples we started by checking the organoleptic properties such as smell and texture in order to confirm its freshness. The samples were then divided into two aliquots each weighing approximately 5 gm. 0.05 N Sulphuric acid was added and mixed thoroughly and homogenization was done using ultrasonic bath at 6℃ for 3 minutes. The upper layer was then filtered through whatman No. 1 filter paper, and the filtrate was then filtered again using 0.45 µm syringe filters x (Ministart single use filter unit non pyrogenic). This solution was then injected directly into the chromatography with Sulphuric acid as the mobile phase and acid profiles were determined. The organic acids were compared to the standards acid profiles for identification.

The sample preparation method for analyses for other samples were similar to that of fresh sample. Changes in organoleptic conditions were also observed during analyses.

3.9.1 Determination of acid from profiles

Residue = PA(s)/PA (STD) * C (STD) * V(s)/W(s) (3.3)

Where:

PA(s) = peak area recorded for sample (cm^2)

PA (STD) = peak area recorded for reference standard (cm^2)

C (STD) = concentration of standards

V (STD) = volume of the solvent of extraction (ml)

W(s) = weight of the sample extracted (ml)

3.10 HPLC sample preparation and operation conditions.

Five grams of cottage cheese from each sample was taken and each mixed with 20 ml of 0.05 N H_2SO_4 as the mobile phase. The mixture was then homogenized thoroughly using an ultra sound mixer at a temperature of 60℃ for five minutes and then filtered through 0.45 µm (Ministart single use filter unit non pyrogenic) disposable syringe membrane filter. The filtrate was then ready for HPLC measurements.

Separation of organic acids in cottage cheese in order to produce profiles was done using HPLC system that was equipped with a Knaeur online solvent degasser, S1000 A and B Knaeur liquid chromatography pump, Knaeur smart line column oven 4050, smartline RI detector 2300 fitted with 20 µl injection loop and vscom communication box. All separations were carried out in a Knaeur C621181H Eurokat H^+, 300 ×8 mm ion exchange column at a constant temperature of 60℃. Isocratic elution was done using 0.05 N H_2SO_4 (mobile phase). The mobile phase was prepared by diluting reagent grade sulfuric acid with HPLC grade water and filtered through a 0.45 µm membrane filter (Ministart single use filter unit non pyrogenic) which was ready for experimental use and was recommended for use within one week. The sample eluted at a rate of 1 $ml.min^{-1}$ for 30 minutes total run time with an injection volume of 20 µl. Each sample produced chromatograms that were then compared to those of the organic acid standards. The probable organic acids in cottage cheese from previous researchers had been identified and their standard calibration curves generated from running pure acids in HPLC and were used to positively identify organic acids in the cottage cheese samples.

3.11 Statistical analysis

The results of all analytical experiments for the different samples of cottage cheese were presented as a mean of the obtained values with the standard deviation. The data was analyzed statistically using ANOVA test and subjected to relative standard deviation (RSD) which is the ratio of standard deviation to average value expressed as percentage.

CHAPTER FOUR
RESULTS AND DISCUSSION

4.1 Introduction

Two hundred different samples were analyzed in duplicate for lactic acid, acetic acid, citric acid, malic acid and oxalic acid. Different samples which were preserved in different storage conditions for different timelines for a period of nine weeks were analyzed and the results are presented in this chapter.

4.2 Method Validation

The common acids detected during this analysis were oxalic, citric, malic, lactic, and acetic. Different samples revealed different forms of organic acids depending with conditions they were stored in. First samples of a freshly prepared cheese were injected into A HPLC machine in order to compare the chromatogram of organic acids with standards organic acids of oxalic, citric, malic, lactic, acetic and propionic acids as from literature review. Five chromatogram peaks were discovered which were identified as seen in figure 4.1.

Peak one was Malic acids. Malic acid being a product of fermentation was not high in freshly prepared cottage cheeses. As per Güler, (2014) Malic acid is known to occur after major growth phase as result of lactic acid bacteria entering the stationery phase.

As per observation the most predominant acid at peak two was identified as Lactic acid. Lactic acid is a major product of lactose catabolism by lactic acid bacteria (Güler, 2014). Lactic acid is high in milk due to presence of high lactose. Once vinegar is added to milk to form curd the milk sugars degenerate to form the lactic acid.

Peak three was citric acid. Citric acid is a product of normal body metabolism and occurs in all living. Cottage cheese being an acidic cheese prepared by treating fresh milk with an acid coagulant such as vinegar tends to have higher value of citric acid. This result was similar to (Fox *et al*., 2017) for acid type of cheese.

Peak four was Oxalic acid. Oxalic acid was low in cottage cheese. Being a product of pyruvate by action of lactate dehydrogenase or similarly produced from glycolate-by-glycolate oxidase in plants it is mostly dominant in mediums containing high lactose as is the case of cottage cheese. This is similar to a study done by (Güler, 2014) in surk cheese though the presence of his oxalic acid was attributed to spices used.

Peak five was found to be that of acetic acid. Presence of acetic acid in cottage cheese could be due to vinegar used during the making process. Vinegar is known to contain 5% to 8% of acetic acid. Acetic acid is also considered as a product of several biochemical pathways, such as fermentation of lactate and citrate or metabolism of amino acids by bacteria (Jessica *et al*., 2016). Acetic acids may provide an indication of the degree of heterofermentative metabolism that may have taken place in cheese (Seçkin and Esmer, 2011), similarly its low production could be an indication of decrease in heterofermentation of lactic acid bacteria (Güler, 2014).

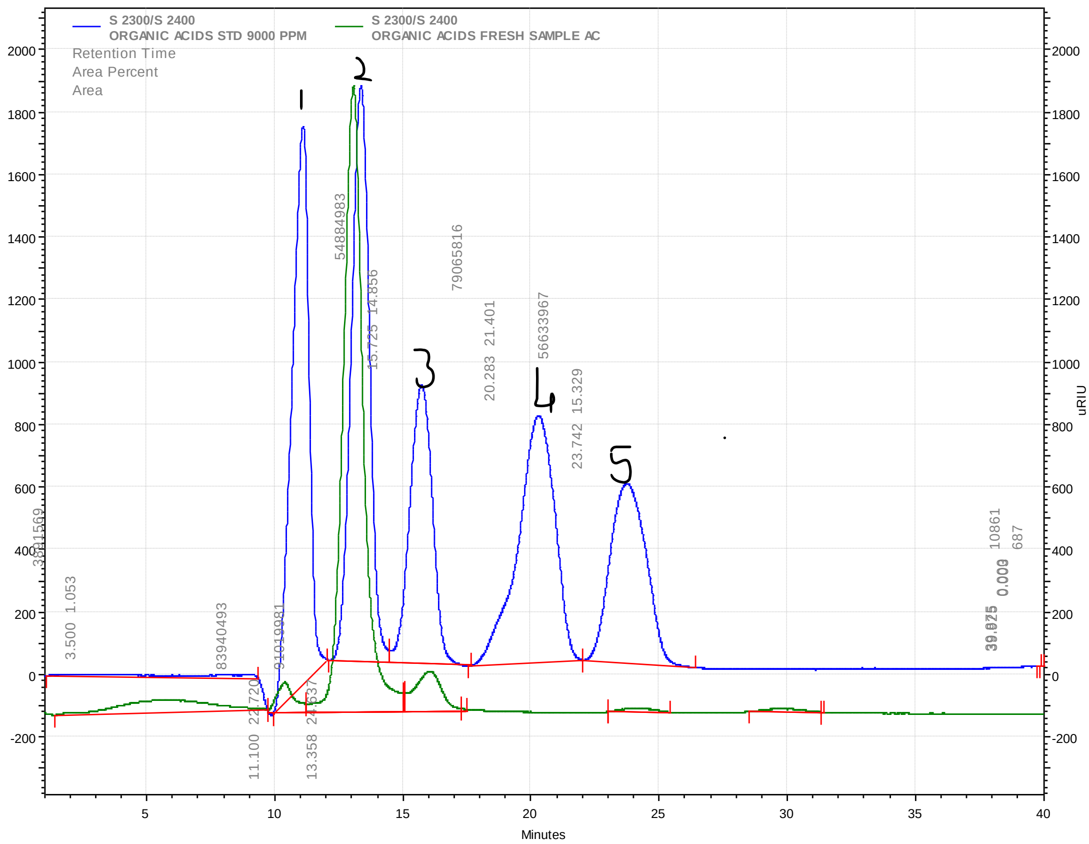

Figure 4.1: Chromatogram of standard organic acids mixture and fresh cottage cheese sample. (in blue) Standard mixture, (in green) fresh cottage cheese sample. Peaks as, 1. Malic acid, 2. Lactic acid, 3. Citric acid, 4. Oxalic acid, 5. Acetic acid.

As observed from figure 4.1 and table 4.1 lactic acid was high in 30% (v/v) honey/cheese sample at 77.56±2.66 mg/kg and lowest at the fresh sample (42.73±2.22 mg/kg). The second most abundant organic acid was citric acid which ranged from 8.17±0.54 mg/kg in fresh cheese to 32.53±1.26 mg/kg in 30% (v/v) honey/cheese sample. This is closely followed by Malic acids with a value range of 9.62±0.34 mg/kg under open room conditions to 21.39±0.43 mg/kg in 30%(v/v) honey/cheese sample. The fourth abundant acid was Oxalic acid which ranged from 1.27±0.02 mg/kg under vacuum conditions to 3.58±0.03 mg/kg in

30% (v/v) honey/cheese sample. The last most abundant organic acid found in cottage cheese was acetic acid. It ranged from 0.21±0.0 mg/kg in fresh sample to 0.65±0.01 mg/kg in 30%(v/v) honey/cheese sample.

Table 4.1: Organic acid concentration under different storage conditions mg/kg, n=9

	Fresh Samples	**Room conditions**	**Deep freeze**	**vacuum**	**Refrigerated**	**10% honey**	**20% honey**	**30% honey**
Citric	8.17±0.54	16.16±0.14	11.64±0.4	9.06d±0.6	17.32±0.7	14.56±1.04	25.8±1.32	32.53±1.26
Lactic	42.73±2.22	44.11±2.31	42.21±2.34	42.77±2.22	46.73±2.43	52.20±2.67	59.50±2.34	77.56±2.66
Malic	10.25±0.48	9.62±0.34	12.43±0.44	16.14±b	15.65±0.42	11.62±0.44	13.71±0.46	21.39±0.43
Oxalic	1.85±0.03	1.75±0.003	1.57±0.02	1.27±0.02	1.64±0.02	1.55±0.02	2.32±0.03	3.58±0.03
Acetic	0.21±0.0	0.40±0.0	0.49±0.01	0.40±0.01	0.40±0.01	0.24±0.0	0.40±0.01	0.65±0.01

4.3 How organic acids changes with storage conditions.

4.3.1 Lactic acid

Lactic acid is the most abundant acids among other organic acids with up to levels of 42.73 ±2.22 mg/kg in fresh sample as seen from the figure 4.2. Lactic acid is produced by free aerobic bacteria found in milk where they break lactose (main sugar component of milk) to generate energy. Lactic acid of fresh samples did not differ significantly (P>0.005) within the nine weeks of analysis.

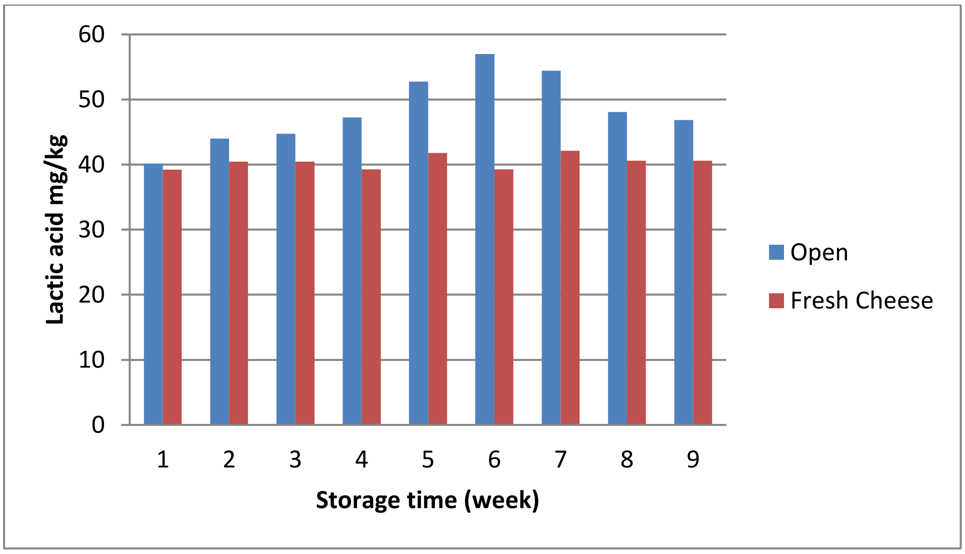

Figure 4.2 Lactic acid changes in fresh sample compared with cheese sample stored at open/room condition (without preservation)

However, there were significant differences of lactic acid ($P< 0.005$) under open conditions. Lactic acid level under open conditions was at 40.17 mg/kg at week one of storage. During storage this change increases significantly with the highest concentration observed in sixth week at 57. mg/kg as seen from figure 4.2 Increase in lactic acids is an indication of bacteria growth activities and a sign of a short shelf life of cottage cheese. This study concur with several other researchers such as (Seçkin and Esmer, 2011) who observed lactic acid as a dominant acid in goat cheese.

When cottage cheese was stored under refrigerated, vacuum and freeze conditions the level of lactic acids in the first week did not differ significantly. Lactic acid levels of refrigerated, vacuum and deep freeze cheese sample in the first week of storage was at 41 mg/kg, 43 mg/kg and 40 mg/kg. During storage time the amount of lactic acid under refrigerated conditions increased from week one to week four with the highest concentration observed in week four at 47 mg/kg as seen from the figure 4.3. After forth week the concentration of lactic acid in refrigerated conditions decreases with lowest level observed at 40 mg/kg.

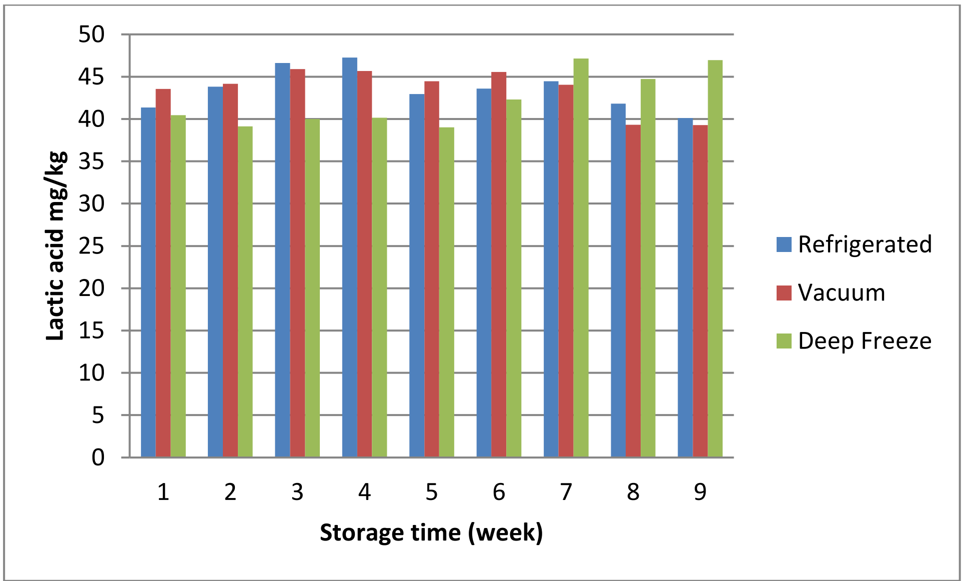

Figure 4.3 Lactic acid changes in refrigerated, vacuum and under deep-freeze conditions

Increase of lactic acid in refrigerated samples is a proof of bacteria growth an indication that refrigeration alone does not prolong shelf life of the sample. This conforms to other researchers such as (Seçkin and Esmer, 2011) and (Jessica *et al*., 2016) who observed increase of lactic acids in their samples when cheese was stored in fridge.at a temperature of 8℃.

The concentration of lactic acid under vacuum conditions do not differ significantly (P>0.005) within the first seven week of storage with the first week having a level of 43 mg/kg and seventh week at 44 mg/kg thereafter the level decreasing in the eighth and ninth week at 39 mg/kg in both weeks. Vacuum conditions proof to be a good storage method for cottage cheese. This is similar to other researchers such as (Felfoul *et al*., 2017) Who observed that cheese packed under vacuum conditions allowed shelf life extensions of 46 days at 4℃.

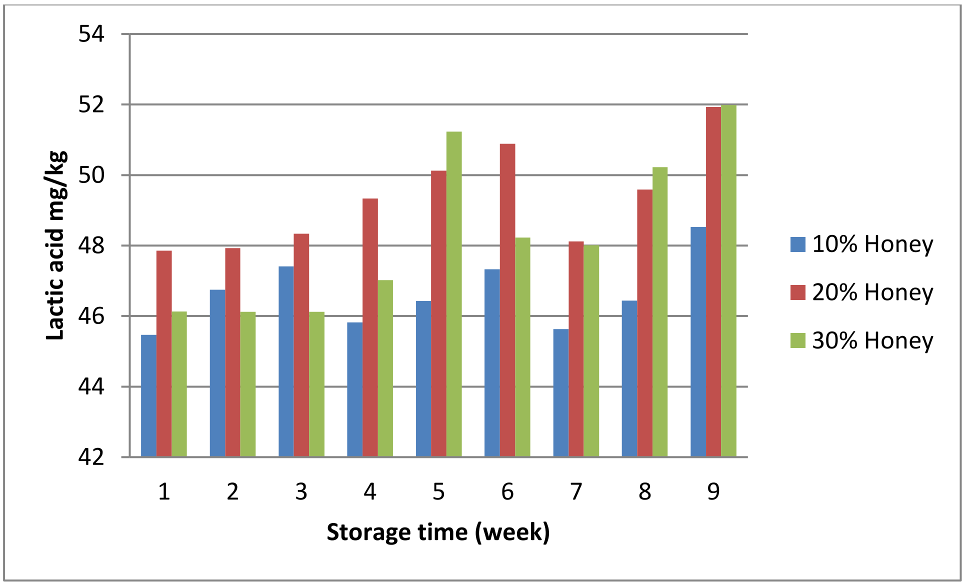

Figure 4.4 Lactic acid changes in cheese mixed with 10%, 20% and 30% honey

Honey has been proved to be a natural preservative to milk and milk products by several researchers. (Krushna *et al*., 2007) showed that honey preservation is as a result of production of hydrogen peroxide that initiates lactoperoxidases process in milk thereby producing antibacterial activates that inhibit growth of bacteria. Other researchers such as (Lusby *et al.*, 2005) and (Mandal and Mandal, 2011) have shown antibacterial effect of non- peroxide honey (manuka honey) due to low pH and high sugar content (high osmolarity). This conforms with our study as can be seen from figure 4.4 that show inhibition of lactic acid production within nine weeks of storage an indication of slowed bacteria growth. When cheese sample was mixed with 10 %, 20 % and 30 % honey (v/v), the initial concentration of lactic acid at start of storage period at 10 % honey v/v was 45 mg/kg with a sign of little increase at 46 mg/kg in the second week showing little significant difference $p<0.005$. This is followed with increase in the level of lactic acid in the subsequent weeks with the third week having the highest concentration at 47.4 mg/kg. At 20 % honey v/v the concentration of lactic acid in the sample remained in the same level with that in the second week at 47 mg/kg an indication of bacteria inhibition. However, as the storage increases a significant difference $p>0.005$ is observed at week four, with the highest concentration observed at sixth week at 50.8 mg/kg. At 30 % v/v the concentration of lactic acid in the sample remained the same for the first three weeks at 46 mg/kg with a slight increase in the subsequent weeks. There is a slight increase in the concentration of lactic acid in the fourth week at 47 mg/kg. However, there is an increase in lactic acid level after forth week with the fifth week recording a concentration of 51 mg/kg. This proofs that Cheese mixed with honey has an extended shelf life and in this case the concentration of honey at 30% proof to be the best preservative. However, honey alone does not preserve the cottage cheese in totality but can extend the shelf life of cottage cheese to weeks.

4.3.2 Citric Acid

Citric acid is an organic acid that is a component of all aerobic living organisms. It is found in fresh milk and is more dominant in raw milk than in pasteurized milk. Cottage cheese is formed by coagulation of fresh milk with rennet or acid material such as vinegar to form milk curds with little maturation.

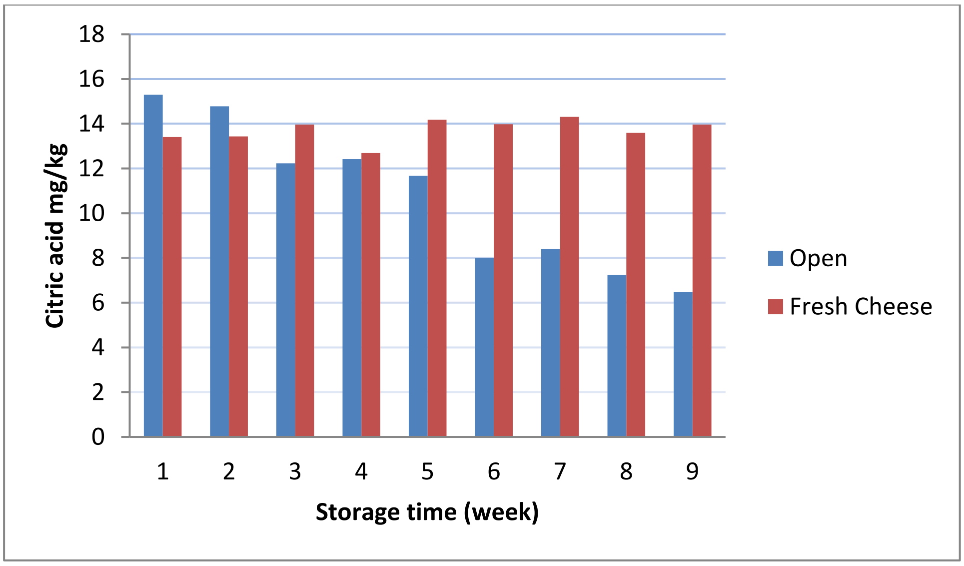

Figure 4.5 Citric acid changes in fresh sample compared with cheese sample at open/room condition.

Comparison of citric acid in fresh samples over the weeks was done with a sample of cottage cheese that was stored under room conditions without any preservative as seen from figure 4.5. It was evident that at the start of storage period the level of citric acid in open sample was at its highest level at 15 mg/kg. Citric acid is formed after glycolysis and most probably is not an indication of bacteria activities. However, the changes in its level are an indication of lactic acid bacteria activities. When lactic acid bacteria in milk metabolize citrates they produce flavor compounds such as acetate, acetaldehyde and diacetyl (Seçkin and Esmer., 2011).

With time the acid reduces as is evident in our study. As the storage period increased the level of citric acid decreased significantly $P<0.005$ to such low level of 6 mg/kg at week nine.

This is probably because the citric acid content in milk decreases during aging in the presence of high developed acidity as proven by (Supplee, 1921).

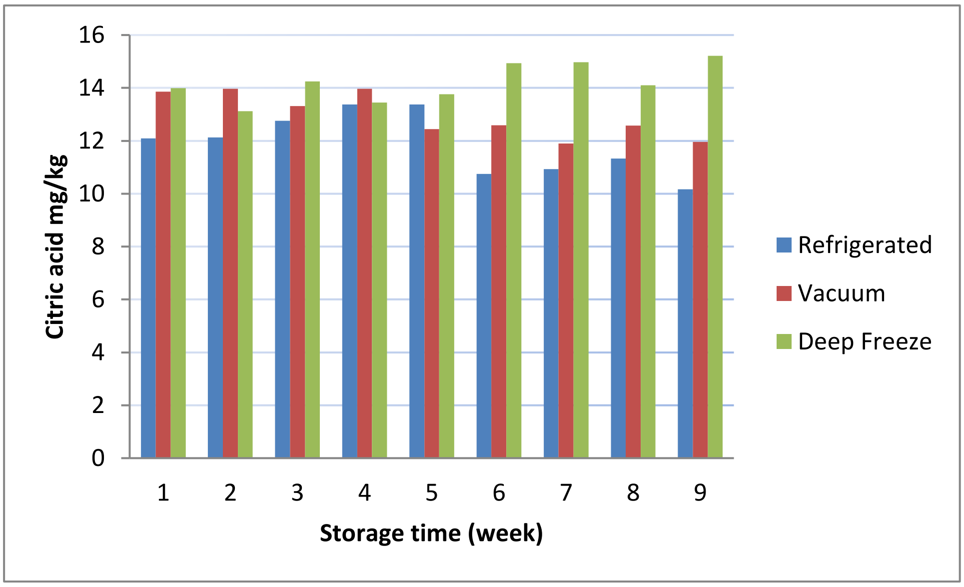

Figure 4.6 Citric acid changes in Refrigerated, vacuum and under deep-freeze conditions

When cottage cheese samples were stored under refrigerated, Deep freeze and vacuum conditions different results were observed. In refrigerated conditions the concentration of citric acid in week one and two was at 12 mg/kg, with a slight increase to 13 mg/kg at week three and four. However, as storage period increased the level dropped significantly $p>0.005$ to 10 mg/kg at week six. This research concurs with other researchers such as (Güler, 2014) who observed that Citric acid catabolize to produce acetic acid and they are inversely proportional to each other.

Storage conditions effected level of citric acid as similarly observed by (Seçkin and Esmer, 2011) on goat cheese with vacuum and deep freeze showing less effects. However as seen from figure 4.6, citric acid contents of vacuum conditions did not differ significantly ($P>0.005$), instead it was at its maximum content level at Week two (13.9 mg/kg). The level of citric acid under vacuum showed an irregular pattern with its lowest value decreasing with storage period to 11.9 mg/kg at week nine. In overall the trend of citric acid in vacuum conditions showed a decrease in its level as had been observed by other previous researchers.

In a general perspective the citric acid level in deep freeze conditions were higher than in vacuum conditions. For frozen sample citric acid level showed a significant difference at week three ($P>0.005$) with a level of 14.25 mg/kg against 13.11 mg/kg in week two. There was a drop of citric acid level between week one and two from 13.99 mg/kg to 13.11 mg /kg respectively. The level of citric acids seems to increase with storage time with a high of 15.2 mg/kg at week nine and indication of effects of deep freeze on the cheese. This differ with (Seçkin and Esmer, 2011) in goat cheese who observed that deep freeze did not effects the level of citric acids until sixth week of storage. Citric acid being one of the acids generated as a result of normal biochemical metabolism in milk (Güler, 2014), its presence is not an indication of bacteria growth.

When sample of cheese were mixed with different concentration of honey i.e. 10%, 20%, 30% honey in order to check the effectiveness of honey on preservation of cottage cheese,

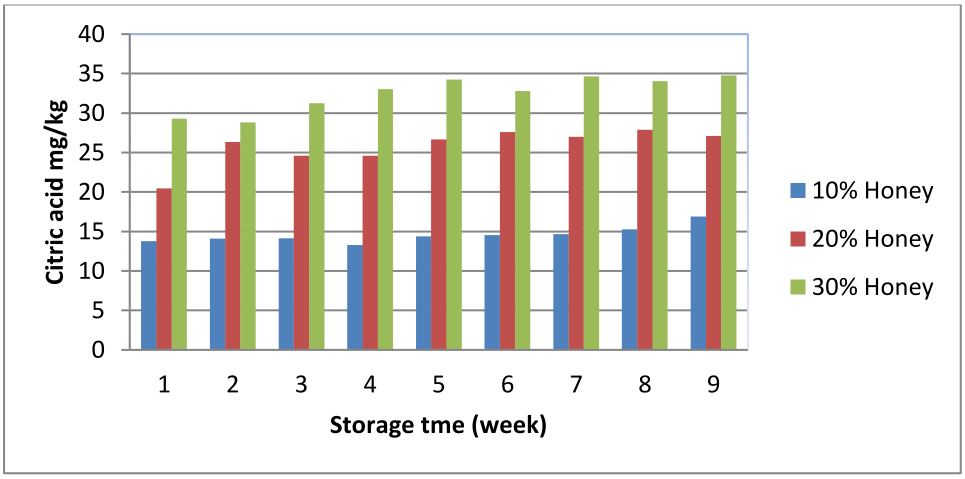

Figure 4.7 Citric acid changes in cheese mixed with 10%, 20% and 30% honey

As seen from figure 4.7, the highest level of citric acid was observed at 30% v/v honey/cheese sample with values ranging from 29.28 mg/kg in week one to 34.77 mg/kg in week nine. The level of citric acid in all samples with honey shows an irregular pattern though citric acid increased with storage time. Probably honey could stimulate bacteria metabolism in cottage cheese thereby producing citric acid.

4.3.3 Malic acid

When cottage cheese was stored under open condition at room temperature was evaluated, the level of malic acid showed a decrease in trends over the period. Malic acid was at 10.24 mg/kg at the beginning of storage and dropped significantly ($p<0.005$) to 0.32 mg/kg at week eight. Malic acid levels of fresh samples apart from second week did not show a significant difference over the weeks as can be seen from figure 4.8

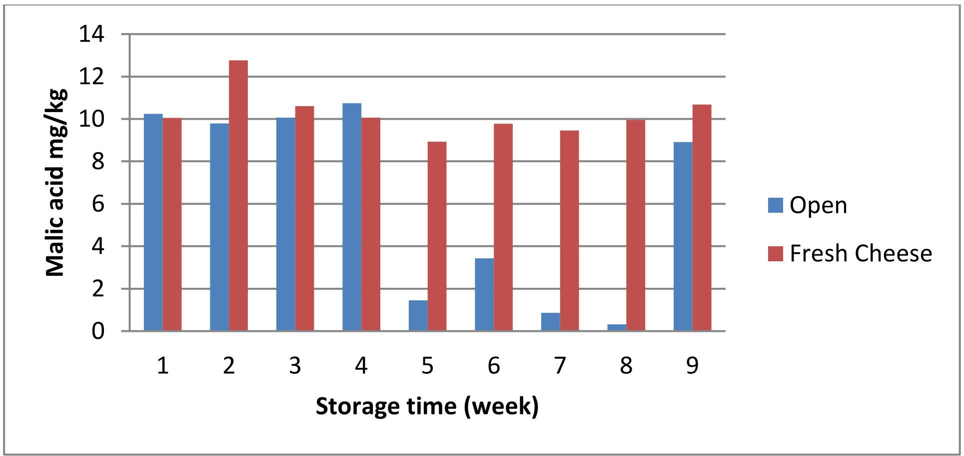

Figure 4.8 Malic acid changes in fresh sample compared with cheese sample at open/room condition.

Storage period in open condition effected the level of malic acid ($p<0.005$) in cottage cheese since malic acid is affected by bacteria activity a proof of bacteria growth. This study concurs with other researchers such as (Seçkin and Esmer, 2011) who observed a decrease trend of malic acid in goat cheese.

When Cottage cheese was stored under refrigerated, deep freeze and vacuum conditions, different results were observed. Under refrigeration conditions the level of malic acid at the beginning of storage period was 19 mg/kg with a slight significant difference $p<0.005$ at week two having a concentration level of 17.59 mg/kg. There was a significant difference $p<0.005$ between week two and three with a level of 14.5 mg/kg observed in week three against 17.5 mg/kg in week two. A decrease in trend of malic acid levels is observed with the lowest concentration levels observed at week eight at 11.2 mg/kg. (Park and Lee, 2006) observed that malic acid for refrigerated goat cheese increased with storage period and this shows a contrast with our research though it agree with (Seçkin and Esmer, 2011) who observed a decrease in refrigerated sample.

After storing cottage cheese under deep freeze conditions, the level of malic acids was determined over the storage period. The frozen sample showed a decrease trend with a small significant difference ($p<0.005$). At week one malic acid level was at 10.5 mg/kg with almost similarly concentrations to week four. At week five the level drop slightly to 9 mg/kg as recorded to be the lowest concentration level for frozen sample. (Park and Lee, 2006), (Seçkin and Esmer, 2011) observed similar results in their studies with Park observing decrease in malic acid concentration on frozen goat cheese.

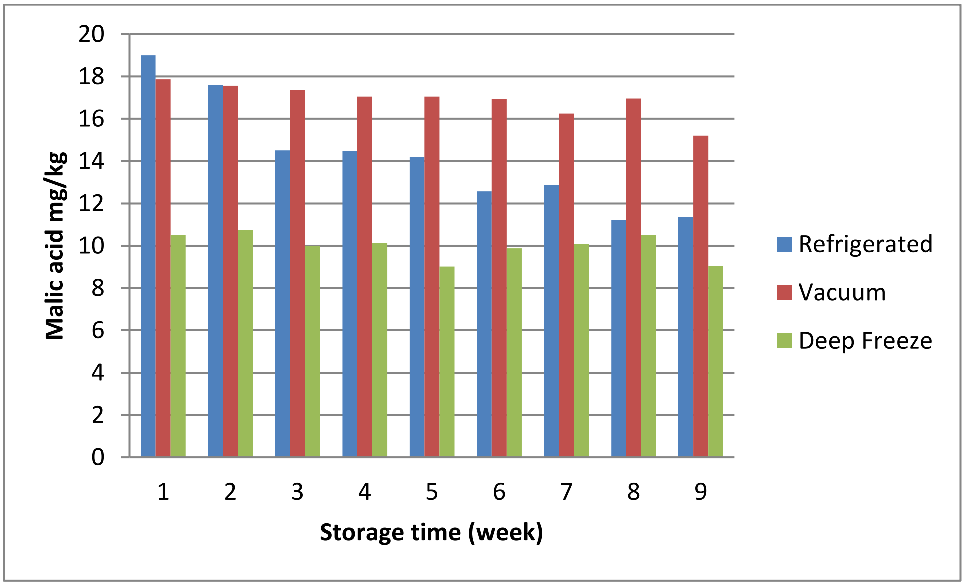

Figure 4.9 Malic acid changes in refrigerated, vacuum and under deep-freeze conditions

The Malic acid level in deep freeze seems to have the lowest concentration compared to vacuum and refrigerated samples. But for vacuum sample the trend in malic acid concentration do not differ significantly ($P>0.005$), with the highest concentration observed in week one at 17.8 mg/kg and the lowest concentration at week nine recorder as 15.2 mg/kg. Vacuum conditions proofs to be a good preservation method for cottage cheese.

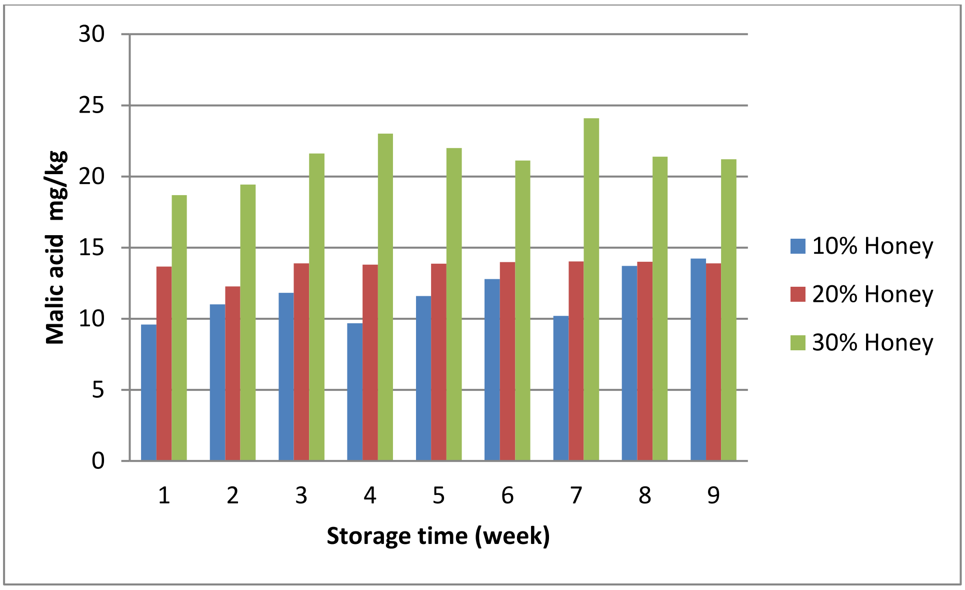

Figure 4.10 Malic acid changes in cheese mixed with 10%, 20% and 30% honey

Effects of honey of different concentration on malic acids showed an irregular pattern especially for 10%, and 30% (v/v) honey/cheese sample. The highest malic acid levels at 30% (v/v) honey/cheese sample were observed as 24mg/kg in week seven and the lowest malic acid level at 28mg/kg in week one. For 20% (v/v) honey/cheese sample a slight significant difference is observed ($p<0.005$), with week one recording malic acid levels of 13.65 mg/kg and week nine recording 13.8 mg/kg. 10% honey also affected malic acid levels with week one recording 9.5 mg/kg as the lowest malic acid level under this condition and week nine recording 14.2 mg/kg as the highest malic acid level. There was an increase trend at 10% v/v honey an indication of bacteria growth at this concentration. Honey being antimicrobial agents has been proved to be a good preservative against many food pathogens. (Krushna *et al*., 2007) as evident in our study. This results on malic acid resembles (Park and Lee, 2006) and (Seçkin and Esmer, 2011) among many other researchers.

4.3.4 Oxalic Acid

At the beginning of storage of cottage cheese stored in open and room conditions the level of oxalic acid was at 1.2 mg/kg. An irregular pattern was observed within the storage period with the highest level observed at week seven as 2.24 mg/kg. The oxalic acid levels in fresh samples (test sample) did not differ significantly within the period as observed from figure 4.11.

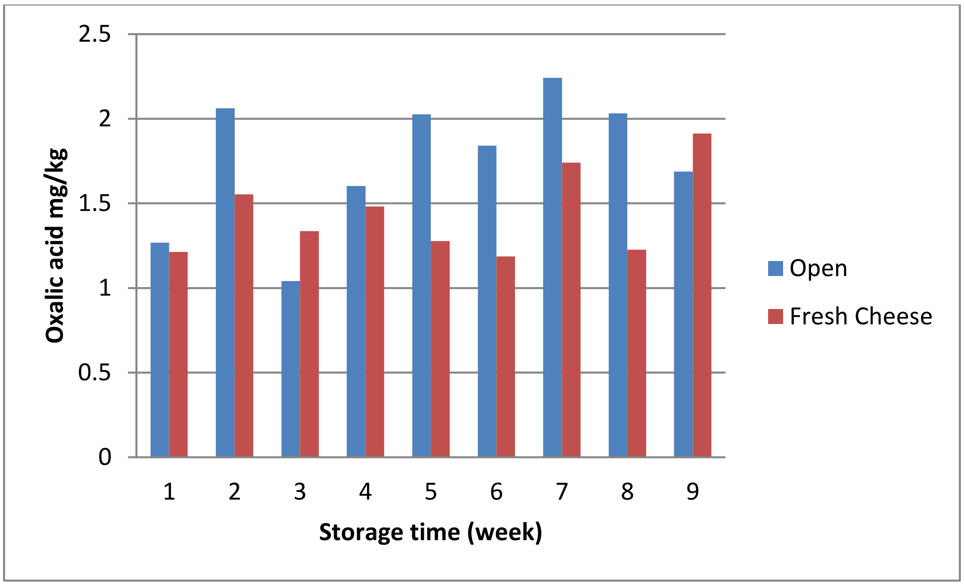

Figure 4.11 Oxalic acid changes in fresh sample compared with cheese sample at open/room condition.

Presence of oxalic acid can either be due to action of glycolate oxidase from glycolate in plants or by lactate dehydrogenase of pyruvate in animals (Güler, 2014). Oxalic acid tends to be high in medium containing high lactose such as whey or soft cheese. The oxalic acid level recorded in our study could be attributed to be as a result of high lactose in cottage cheese.

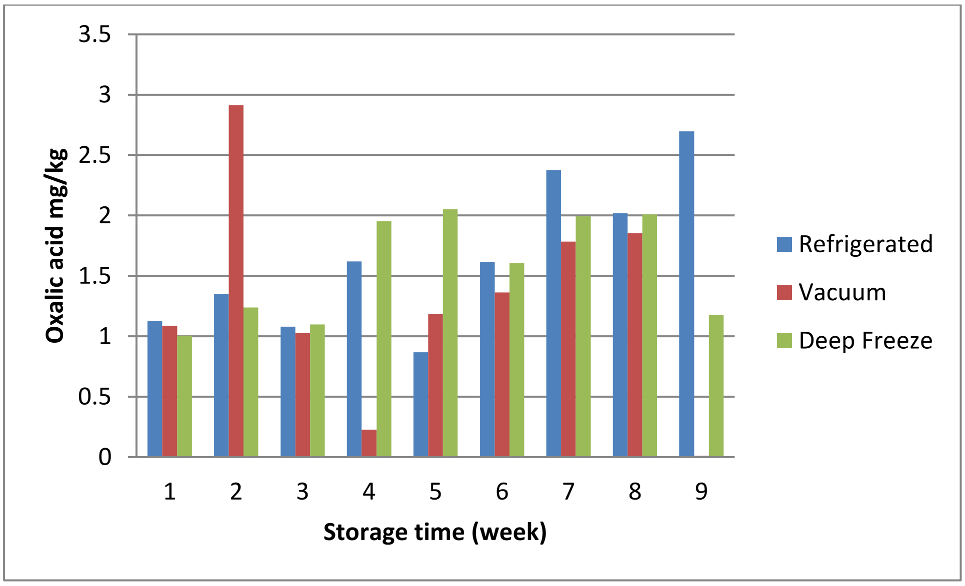

Figure 4.12 Oxalic acid changes in Refrigerated, vacuum and under deep-freeze condition

In refrigerated sample the oxalic acid levels at week one was 1.12 mg/kg with a slight increase in subsequent weeks to a high of 1.62 mg/kg in week four. An irregular trend is observed as seen in figure 4.12 though there is no significant difference p<0.005. An irregular trend is recorded for both frozen and vacuum sample with no significant difference p<0.005. The highest oxalic acid level under vacuum conditions is 2.9 mg/kg observed at week two and the lowest level 0.01 mg/kg observed at week nine. Frozen sample is at 1 mg/kg in week one (lowest concentration) and has it highest concentration 2.05 mg/kg at week five. This study resembles (Güler, 2014) of surk cheese though he attributed his oxalic acid on spices used.

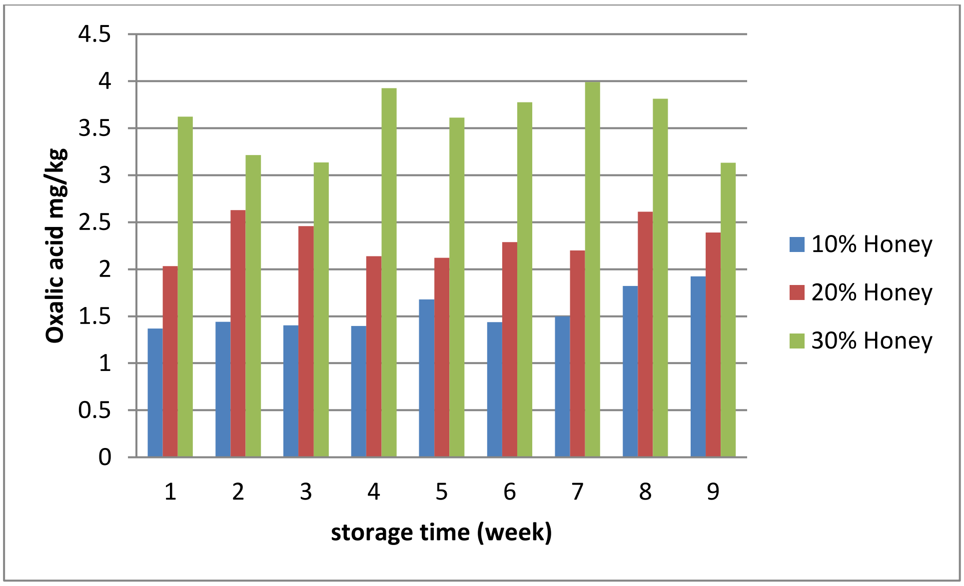

Figure 4.13 Oxalic acid changes in cheese mixed with 10%, 20% and 30% honey

The effect of honey on oxalic acids levels as observed on figure 4.13. 10% honey had no significance difference (p<0.005) over the storage period with week one recording 1.36 mg/kg as the lowest oxalic acid level and week five recording 1.67 mg/kg as the highest oxalic acid level. The level of oxalic acid in 20% honey was higher than that of 10% with week one recording 2.0 mg/kg as the lowest value and week two recording 2.62 mg/kg as the highest value. There was no significant difference (p<0.005) in oxalic acid level within storage period an indication of bacteria growth inhibition. High oxalic acid levels were recorded in a sample with 30% honey compared to 10% and 20% honey concentrations. At week one the oxalic acid level at 30% honey was recorded as 3.62 mg/kg though not the lowest with the lowest value 3.13 mg/kg observed in week three and nine. There was an irregular pattern though no significant difference (p<0.005).

4.3.5 Acetic acid

When lactate and citrates are fermented or amino acids are metabolized by bacteria in several biochemical pathways this results in production of acetic acid (Seçkin and Esmer, 2011). Presence of acetic acid in our sample is an indication of the degree of heterofermentative metabolism (Ogier *et al*., 2008).

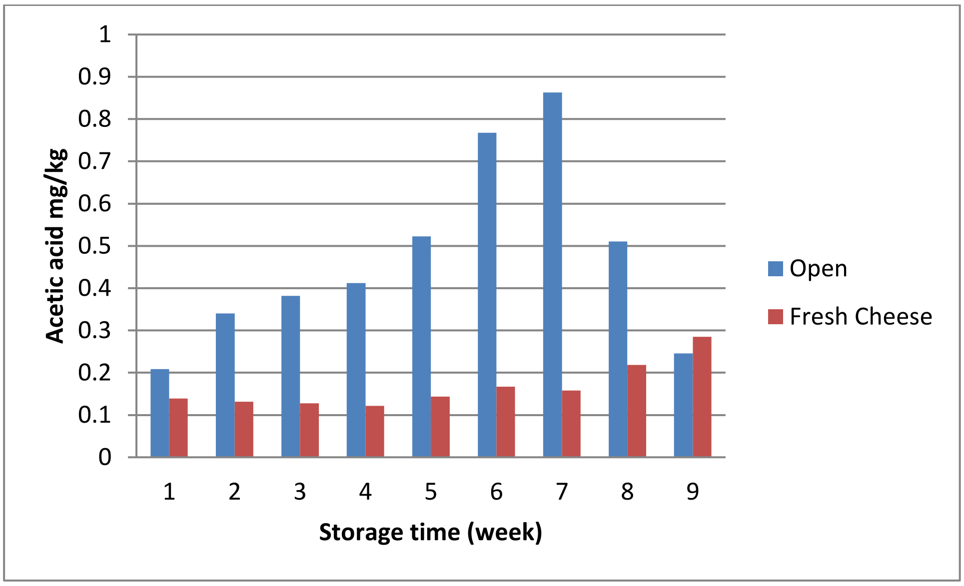

Figure 4.14 Acetic acid changes in fresh sample compared with cheese sample at open/room condition.

Acetic acid level in cottage cheese sample under open conditions shows an increase trend as observed in the figure 4.14, with the lowest level 0.2 mg/kg at week one and the highest concentration on week seven at 0.86 mg/kg. The acetic acid in fresh sample (control sample) shows no significant difference within storage period.

When a comparison of acetic acid level was done in cheese stored under refrigerated, frozen and vacuum conditions as seen from figure 4.15, it was observed that, under refrigerated conditions a regular increase pattern was observed. The lowest concentration was recorded as 0.32 mg/kg at week one and the highest concentration was 0.51 mg/kg at week five. The acetic acid levels in deep freeze shows an irregular pattern with the highest level recorded as 0.49 mg/kg at week seven and the lowest level as 0.21 mg/kg at week four. Sample stored under vacuum conditions do not show a significant difference $P<0.005$ with the highest concentration as 0.36 mg/kg at week eight and the lowest level as 0.22 mg/kg at week four.

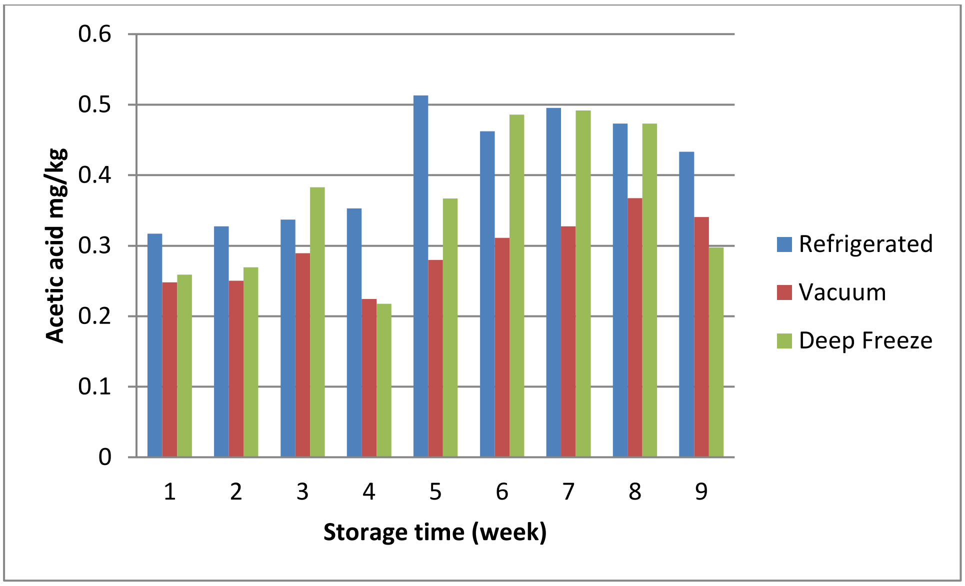

Figure 4.15 Acetic acid changes in refrigerated, vacuum and under deep-freeze conditions

Sample with 10% honey had the lowest concentration of acetic acid as compared to other samples with honey. The level of acetic acid in 10% v/v honey/cheese sample did not differ significantly ($p<0.005$).

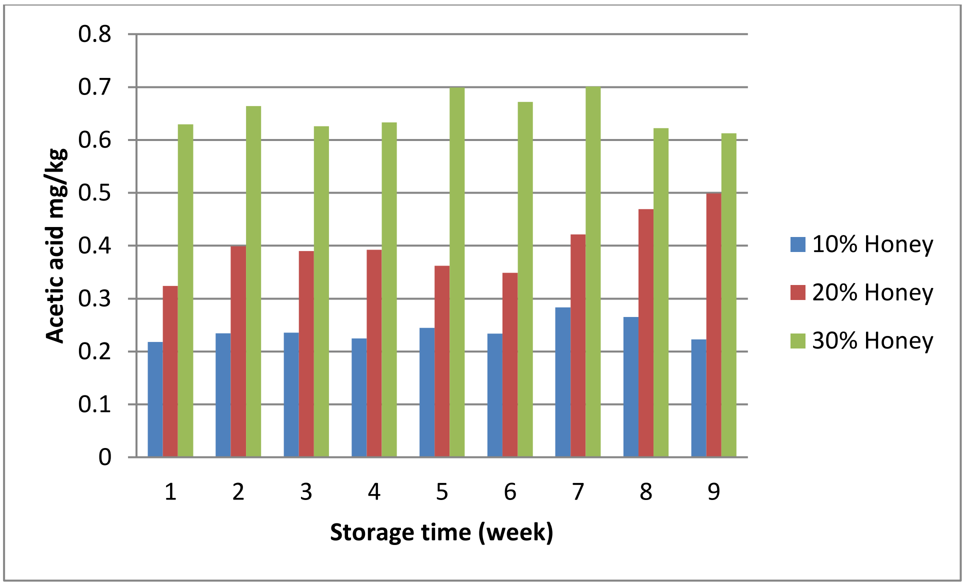

Figure 4.16 Acetic acid changes in cheese mixed with 10%, 20% and 30% honey

The highest acetic acid level was 0.28 mg/kg at week seven and the lowest acetic acid level was at 0.21 mg/kg at week one. At 20% v/v honey/cheese sample the level of acetic acid was higher compared to that of 10% honey but lower than in 30% honey. Acetic acid level was highest at 20% honey in week nine at 0.49 mg/kg and lowest at 0.32 mg/kg in week one. A regular pattern is observed even with 30% v/v honey/cheese sample. The highest value is observed as 0.7 mg/kg in week seven with lowest level observed in week nine at 0.61 mg/kg as observed in figure 4.16.

Acetic acid were effected by both storage conditions and time and this concurs with other researchers e.g. (Seçkin and Esmer, 2011).

4.4 Effects of Several Storage condition on organic acids.

4.4.1 Fresh samples of cheese.

As evident from (Fig 4.1) the chromatogram of a fresh prepared cottage cheese in comparison to standard organic acids used for calibration, the acid profile shows lactic acid as the most abundant organic acid in cottage cheese. Lactic acid levels throughout the storage period ranged from 39.26 mg/kg (lowest) to 47.71 mg/kg (highest) with a small significant difference ($p>0.005$). This could be attributed to procedures of making fresh cheese. This was in an agreement with other researchers e.g. (Seçkin and Esmer, 2011) in his research on goat cheese and (Güler, 2014) on surk cheese among many other researchers.

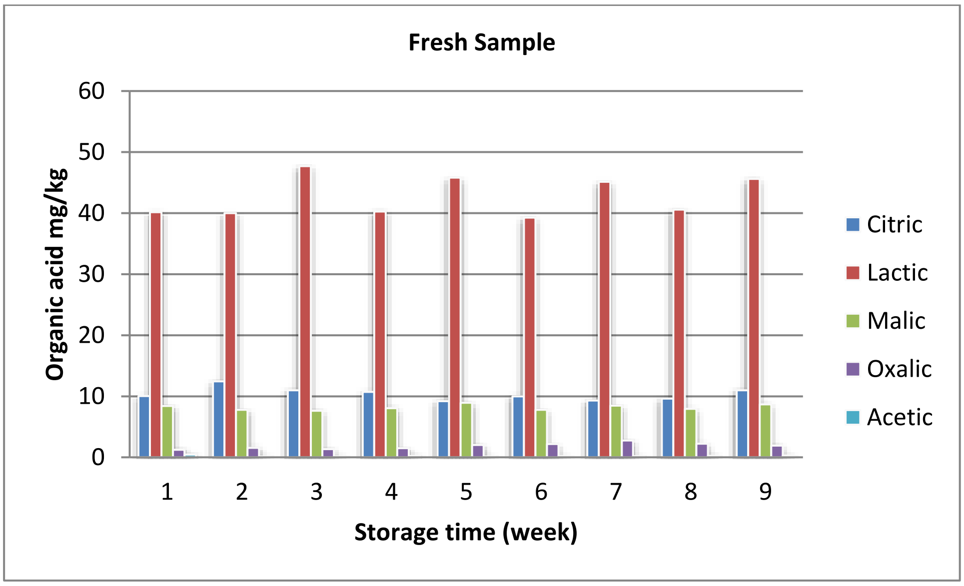

Figure 4.17 Comparison of organic acids in a fresh cheese sample within nine weeks

Citric acid was the second abundant organic acid observed. Its lowest value was recorded in week five as 9.1 mg/kg and has a high of 10.96 mg/kg in week nine showing little significant difference ($p<0.005$) Since cottage cheese is made from fresh milk that has not fermented but rather coagulated to produce the milk curds, presence of substantial high level of citric acid was not a surprise. Citric acid is a major constituent of fresh milk. Malic acid and oxalic acid are products of biochemical metabolism found in fresh milk. Malic acid was recorded with its highest value as 8.92 mg/kg in week five and the lowest value as 7.59 mg/kg in week three with no significant difference ($p<0.005$). There was low level of oxalic acid with its highest value recorded as 2.74 mg/kg in week seven and the lowest value as 1.21 mg/kg in week one with no significant differences ($p<0.005$). No significant difference ($p<0.005$) for acetic acid a product of bacteria growth since no fermentation has taken place in fresh cottage cheese. This shows that a fresh prepared cheese has less of organic acids and accumulation or increase of more acids is an indication of bacteria growth that results to spoilage. This was used as control sample throughout the analysis to evaluate changes in organic acids of other samples stored under different conditions.

4.4.2 Comparison of acid profiles stored under open normal conditions in comparison with fresh cheese sample in the first week of analysis.

It was evident that cottage cheese stored under open conditions spoilt within the first week of storage after day three. The first two days under these conditions, cottage cheese sample showed similar acid profiles to that of fresh cheese samples with lactic and citric acids as most dominant acids. After day three, the acid profiles deviate from the fresh sample profiles with more acids detected. This is an indication of bacteria activities within the sample and a significant difference was observed $p<0.005$.

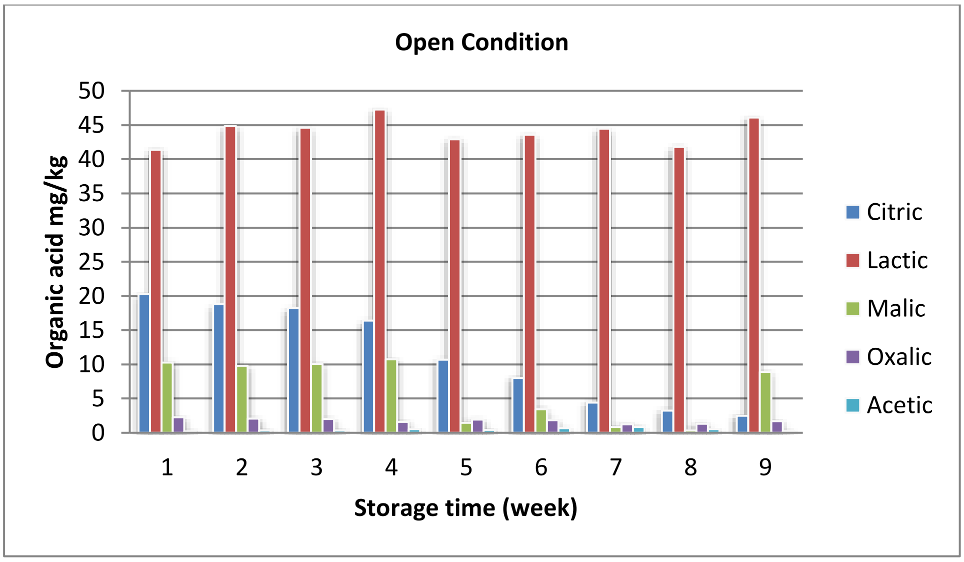

Figure 4.18 comparison of organic acids in open condition

In this experiment the analysis was done in two folds. First the behavior of cheese stored for one week's was investigated every day and later the acid profiles of cheese stored under open conditions was analyzed once every week for nine weeks. Figure 4.18 shows the comparison of acid profile of cottage cheese in nine weeks of storage. Week one results were a mean product of each day analysis. Lactic acid was the dominant acid with a value of 41.36 mg/kg in week one. Increase in storage period was directly proportional to increase in lactic acid value up to week four where the highest value recorded was 47.27 mg/kg. Lactic acid increase is an indication of bacteria growth in the cheese sample and its drop in value from week five is an indication of declining activities of bacteria as result of death. Citric acid was high at the beginning of storage period with a record of 20.28 mg/kg in first week and a low of 2.49 mg/kg in week nine. Citric acid value was found to be inversely proportional to acetic acid value. Acetic acid was found to be high where citric acid was low and vice versa. This study was similar to what (Güler, 2014) recorded when he studied surk cheese. This is because acetic acid is considered as a product of several biochemical pathways, such as fermentation of lactate and citrate or metabolism of amino acids by bacteria and is considered as an indication of the degree of heterofermentative metabolism that may have taken place in cheese (Seçkin and Esmer, 2011). Acetic acid had a value of 0.2 mg/kg in week one and 0.86 mg/kg in week seven as the highest value recorded with no significant difference $P<0.005$. Acetic acids are critical to the metabolism of lactobacilli which means increase in acetic acids favors production of lactic acids

Similarly, we can observe that oxalic acids and citric acids reduce with increase in storage time. This is probably because the citric acid content in milk decreases during aging in the presence of high developed acidity and is more rapid in raw milk than in pasteurized milk (Supplee, 1921). Malic acid was not detected in day three and four, but was detected in day five of analysis. However, there was significant difference in the first week of storage ($p>0.005$). However, in subsequent week's malic acid show an irregular trend with the highest value as 10.73 mg/kg at week four and the lowest value of 0.32 mg/kg at week eight of storage. The variation of acids is an indication of bacteria activities within samples. It was also evident that during this analysis organoleptic properties of cheese as discussed later was also observed. Both texture and smell of the sample had changed significantly with production of a very strong smell. But as time went by in the fifth and sixth week of analysis the activities of bacteria were almost over to the sample at sixth week almost producing peaks nearer to that of a fresh sample an indication of reduction or death of bacteria. As evident from fig 4.8 the amount of oxalic, citric and malic acids has decreased significantly with progressive storage. Lactic acid and Acetic acid seem to be the only acids increasing with storage. Acetic acid increase is an indication of heterofermentive activities of bacteria within the cheese. As more of lactose decreases due to breakdown by more bacteria the level of lactic acid increases up to week four of storage and from week five start to decrease. Diminishing sugar content necessary for energy productions in bacteria could have led to the death of bacteria. This leads to end of bacteria activities.

4.4.3 Cheese stored under vacuum conditions at room temperatures without any preservative.

Samples stored under vacuum conditions at room temperature revealed an increase in cottage cheese shelf life. The profiles of acids in week one of storage showed presence of few acids with almost a similar resemblance to that of fresh cheese sample.

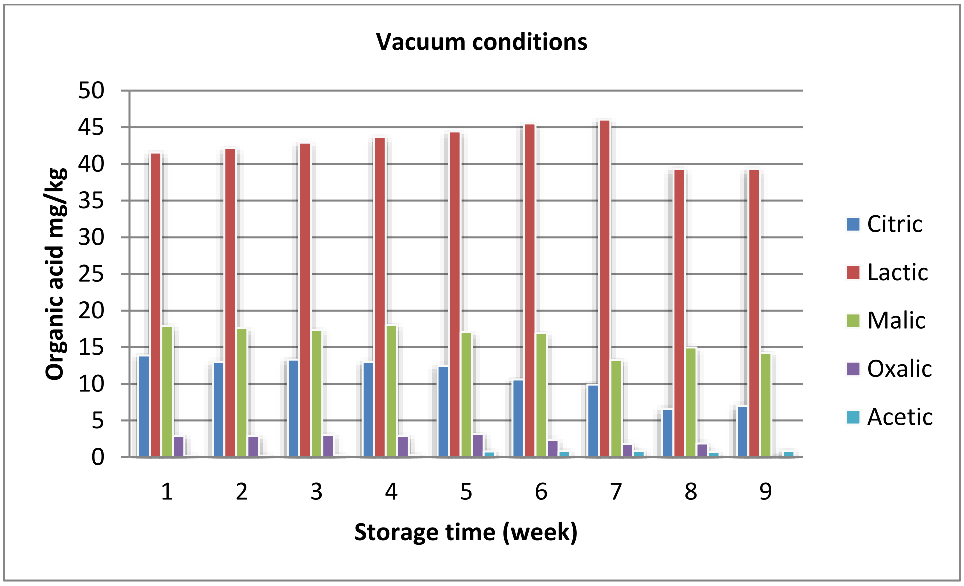

Figure 4.19; comparison of organic acid under vacuum conditions

Citric acid was high at the beginning of storage with week one recording a value of 13.85 mg/kg. There was no significant difference in citric acid concentration in the first five weeks but as storage time increased a significant difference was observed from week six to week nine with ninth week recording a value of 6.95 mg/kg. Decrease in citric acid after week five is an indication of metabolism of citrate in the cheese sample by heterofermentative bacteria (Seçkin and Esmer, 2011). When the level of citric acid was compared to that of acetic acid an inverse proportional relationship is observed. Acetic acid increase with storage period with a significant difference observed at week five. Lactic acid remains the most abundant acid in the cheese sample with a value of 41.53 mg/kg in week one and increases with storage period to 46.03 mg/kg in week seven showing a significant difference ($P<0.005$) as can be seen from Figure 4.19 and chromatogram at appendices 2. There was a significant difference in Oxalic acid $p>0.005$ within the storage period with an irregular trend being witnessed. Similar results were obtained by Mulin and Emmons for various cheeses. In this study acetic acid was likely a product of catabolism of citric acid (Güler, 2014).

4.4.4 Cheese stored under refrigeration temperatures (4°C)

Normal refrigeration temperatures were not a reason enough to stop spoilage of cheese. Several significant differences of many acids were observed. There was significant difference for citric acid between week three of storage and week four. The first Three weeks had no significant difference on citric acid an indication of less bacteria activities within the sample. This means cottage cheese can be stored without spoiling for three weeks under refrigerated conditions. Level of lactic acid in the refrigerated remained high as compared with organic acid with increase in storage period experienced. The highest value recorded was 57.85 mg/kg in the ninth week. Presence of lactic acid with a significant difference is a proof of bacteria activities within the sample though in small quantities as can be seen from Figures 4.10.

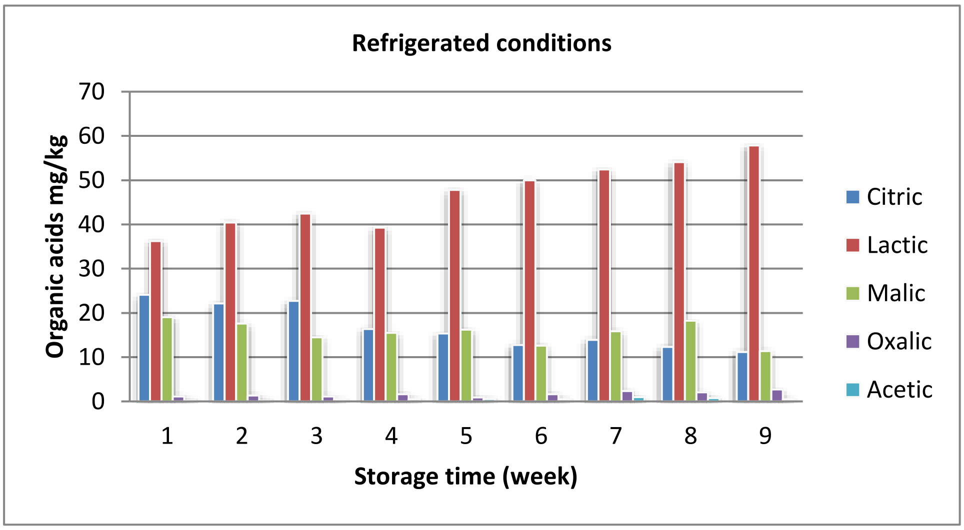

Figure 4.20 Comparison of organic acid under Refrigerated conditions

It is noticeable that the level of acetic acids within the storage period is small an indication of little bacteria growth. However, as storage period increases a significant difference $p<0.005$ between week six and seven is observed. The value of acetic acid in sixth week is 0.46 mg/kg and in the seventh week it is 0.95 mg/kg. Acetic acid is an indication of catabolic break down of lactate and citrate an indication of bacteria activity within the sample.

4.4.5 Cheese stored under deep freeze conditions (-18°C)

Cheese stored under deep freeze showed little changes in acid profile for the period of analysis. Under extreme low temperature conditions most of microorganisms that spoil cheese are inactivated thereby leading to little or no growth. As a result of this the acid profiles of cheese under deep freeze almost resembles that one of fresh cheese as can be seen from graph 4.11 and appendices 2. However significant differences were observed during storage.

There is a significant difference in citric acid, oxalic acid, lactic acid, and acetic acid, during the storage under deep freeze conditions though a similarity is observed and almost similar differences. Malic acid shows no significant difference

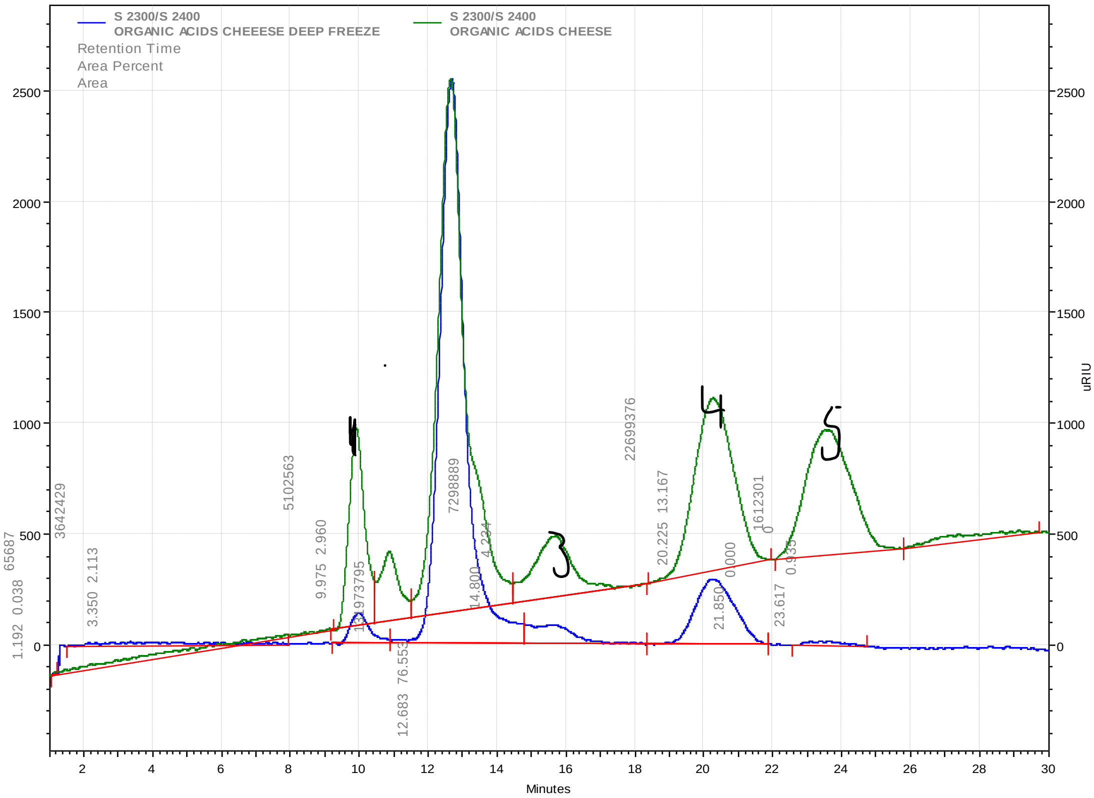

Figure 4.1 Chromatogram of standard organic acids mixture and frozen cheese sample

(in blue) Standard mixture, (in green) frozen cottage cheese sample. Peaks as, 1. Malic acid, 2. Lactic acid, 3. Citric acid, 4. Oxalic acid, 5. Acetic acid

As time goes by the acids in the sample shows little variations in terms of quantity. The main acids observed include lactic acid, citric acids, malic and some amount of oxalic acids. The amount of oxalic acids seen throughout the storage period is very little and does not vary much with its highest value being 2.05 mg/kg in fifth week and the lowest values as 1.00mg/kg in first week of storage. There is no significant difference $p<0.005$ as can be seen from Figure 4.13. The most abundant of acids seen in these samples across the weeks were lactic acid though there was a high concentration of citric acids, this confirms with earlier research work done by Kunz et al which indicated citric acids will decrease gradually with aging of milk as a result of increase in milk acidity (Supplee, 1921). Citric acid is an organic acid that is a component of all aerobic living organisms. It is formed after glycolysis and hence not a sign of bacteria activities. With time the acid reduces but to a small extent as shown in Figures 4.12 and 4.13. Increase in lactic acid is observed in week seven with the rest of weeks showing almost same level of lactic acid. Deep freeze can be used to prolong cottage cheese shelf life though tropic temperatures where the study is being conducted are high and not many people could be able to afford freezer (Modise, 2014).

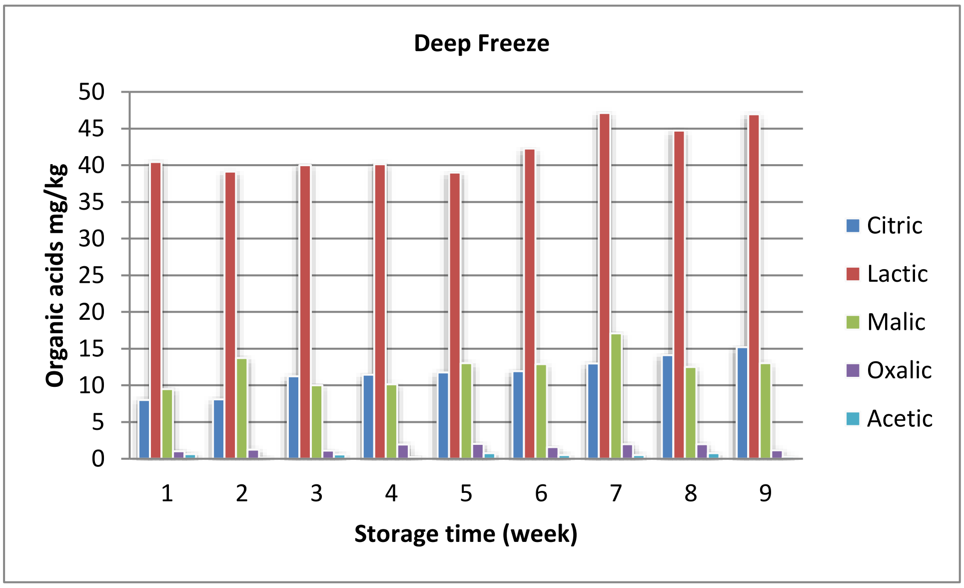

Figure 4.21 variations of acids in cheese stored under deep freeze conditions within nine weeks of storage

The level of malic acid within the sample was high with an irregular pattern across the storage period. Since malic acid is a naturally occurring acid as a result of metabolic reactions within living things and a product of kreb and glycoxylate cycle (Williams and O'Neill, 2018) the highest level of malic acid in the beginning as an indication of normal activities within the cottage cheese sample. But as time proceeds the variation in malic acids within the sample indicates bacteria growth within the sample though no significant difference. This study agrees with previous researchers (Felfoul *et al*., 2017) and (Seçkin and Esmer, 2011).

4.4.6 Cheese mixed with different concentration of honey

(Krushna *et al*., 2007) observed that addition of honey at a final concentration of 50 mg/ml had a considerable inhibitory effect (50-55% inhibition) on the growth of bacteria in comparison to milk sample devoid of honey as can be seen in the table 4.1 below. The table clearly shows the turbidity tests of samples in each day of storage. Turbidity refers to the amount of cloudiness in liquid which can be caused by bacteria, germs and other microorganism that cannot be seen by the naked eyes.

Table 4.2 Turbidity assay (550nm) of stored milk samples with honey

No of days of storage	With honey	without honey	Inhibition of growth (%)
0	0.62	0.75	82.6
3	0.78	1.41	55.3
4	0.82	1.56	52.5
5	0.89	1.73	51.4
6	0.94	1.84	51

Adapted from Krushna *et al* 2007.

When different concentration of honey at 10%, 20% and 30% w/v was mixed with our cottage cheese sample and then stored under room conditions, the predominant acids observed within the nine week of storage was lactic acid. Lactic acid is high in milk due to metabolism of lactate (milk sugar) by lactic acid bacteria. When the acid changes from the initial concentration of 45.4 mg/kg in week one to high value of 58.52 mg/kg in week nine at 10% honey w/v, 57.85 mg/kg in week one to 61.92 mg/kg in week nine for 20% w/v honey.

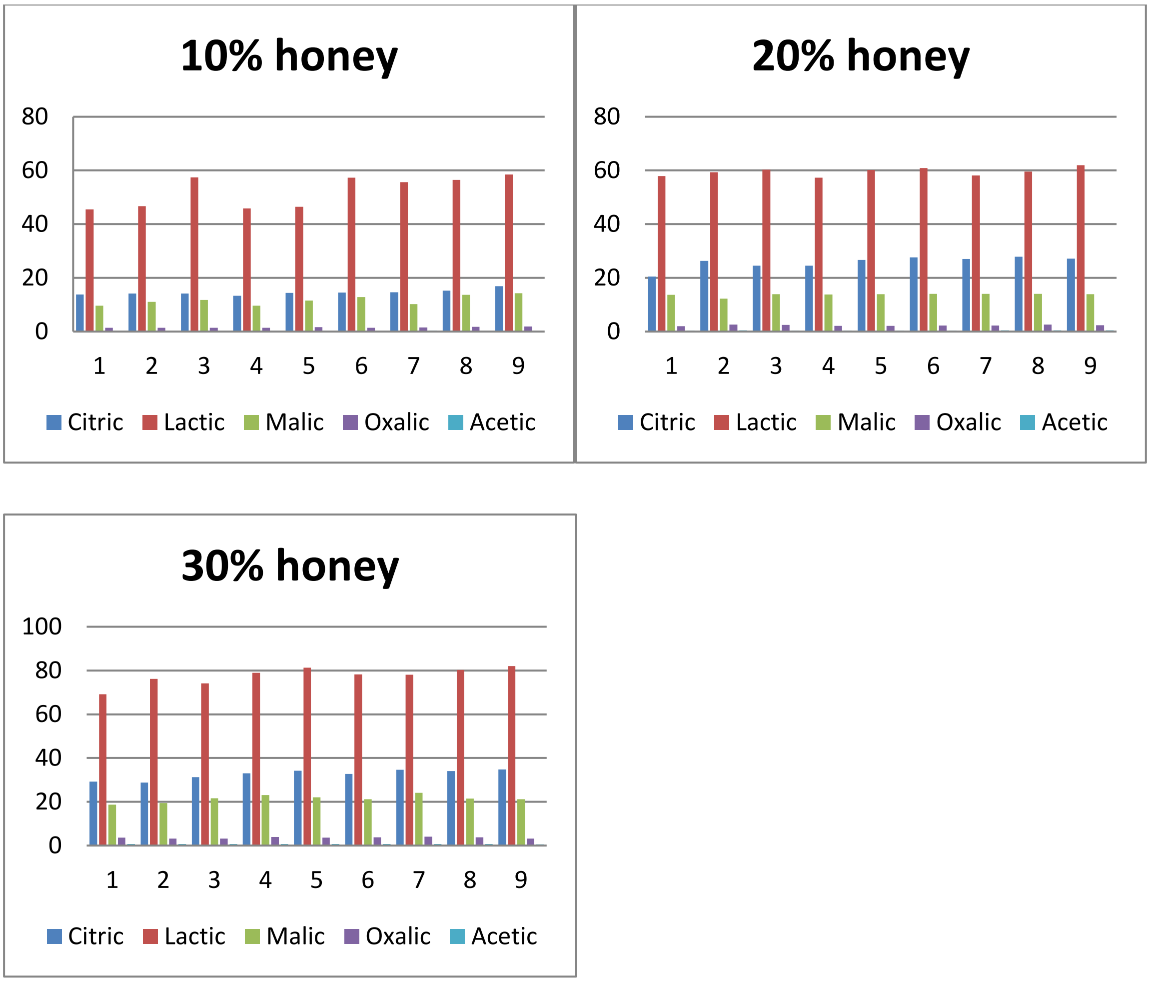

Figure 4.22 Organic acids and sugars in cheese mixed with different concentrations of honey in different days of storage under open conditions.

As can be seen from the outcomes above the organic acids observed from the samples are oxalic and citric acid similar to that of a fresh prepared cheese. Their concentration across the seven days of storage does not vary much across different concentration of honey. What looks to differ mostly are concentration of sugars which seem to elute so closely to each other as can be seen from the graphs at the appendices 6, figures a to j. From the literature review it was noted that honey act as a preservative through the mechanism where lactoperoxidases an enzyme naturally present in raw milk catalysis the conversion of hydrogen peroxide in honey to water (Mandal and Mandal, 2011). Presence of hydrogen peroxide and thiocyanate in honey lead to oxidization of thiocyanate by the enzyme together with hydrogen peroxide complex producing, bacteriostatic compounds that inhibit gram negative bacteria such as Escherichia coli, salmonella species and streptococci which in return increases the shelf life of cheese. Lack of significant difference in lactic acids probably can be an indication of less bacteria growth in the sample as observed by (Krushna *et al.*, 2007)

Table 4.3: Mean and standard deviation of cottage cheese sample mixed with different concentration of honey and compared to pure honey and a sample without honey.

ACIDS	Sample without honey	10% honey + sample	20% honey + sample	30% honey + sample	PURE honey
MALIC	13.44 ± 0.91	17.50 ± 1.28	33.30 ± 1.48	28.19 ± 1.28	139.64 ±4.34
CITRIC	1.37 ± 0.07	1.41 ± 0.02	1.08 ± 0.07	1.62 ± 0.06	2.70 ± 0.03
OXALIC	1.36 ± 0.07	1.40 ± 0.02	3.62 ± 0.09	1.61 ± 0.06	2.68 ± 0.02
LACTIC	4.93 ± 0.07	12.62 ± 0.07	22.65 ± 1.32	28.77 ± 1.38	132.00 ±3.51
ACETIC	2.37 ± 0.04	2.12 ± 0.03	1.57 ± 0.01	2.40 ± 0.06	4.24 ± 0.11

Study on preservation of honey was done by comparing changes in organic acids when samples were mixed with different concentration of honey at 10%, 20% and 30% (w/v). This was done within seven days and nine weeks of storage. A comparison with a sample stored without honey and another sample of pure honey was done. Profiles of organic acids were obtained after every 24 hours of analysis with the concentration of each acid shown. The mean and standard deviation of these acids were tabulated as shown in table 4.6 above. Pure honey samples had a high mean residue level than all other samples. Sample of cheese without honey indicates low mean residual levels of all the acids than those with honey. This indicates an increase of bacteria with time and concur with other researchers (Mandal & Mandal, 2011) and (Krushna *et al.,* 2007).

4.5 Sensory Analyses

The tests were carried out in individual sensory analysis where sensorial evaluation scores of cheeses during 30 days of storage are given in table 4.2. The results showed that the first week received highest sensorial scores. However sensorial was not significant during storage of deep freeze and Vacuum ($p>0.05$) but the difference in sensorial of cheese stored in normal room conditions, fridge, 10% honey, 20% honey and 30% honey had a significant difference ($p<0.05$).

Table 4.4 Sensorial attributes of cheese samples at different storage conditions

Fridge	**Week1**	**Week2**	**Week3**	**Week4**
Appearance/ Color	7.67±0.58a	7.67±0.58a	7.00±1.00a	5.33±0.58b
Taste/ Flavor	7.30±0.58a	5.60±0.58b	4.00±1.00c	2.00±1.00d
Smell/ Odor	7.67±0.58a	5.00±1.00b	3.00±1.00c	1.00±0.00d
Texture/ Mouth feel	7.30±0.58a	4.60±0.58b	3.00±1.00c	1.30±0.58d
Vacuum	**Week1**	**Week2**	**Week3**	**Week4**
Appearance/ Color	8.00±1.00a	8.00±1.00a	7.60±0.58a	7.60±0.58a
Taste/ Flavor	8.60±1.00a	8.30±1.00a	7.30±0.58b	7.30±0.58b

Smell/ Odor	8.70±0.58a	8.30±0.58a	8.00±0.00a	8.00±0.00a
Texture/ Mouth feel	8.00±0.00a	8.00±0.00a	7.70±0.00a	7.70±0.00a
Freezer	**Week1**	**Week2**	**Week3**	**Week4**
Appearance/ Color	8.70±0.58a	8.70±0.58a	8.30±0.58a	8.30±0.58a
Taste/ Flavor	8.60±0.58a	8.30±0.58ab	8.00±0.00ab	7.60±0.58b
Smell/ Odor	9.00±0.00a	9.00±0.00a	8.30±0.58a	8.30±0.58a
Texture/ Mouth feel	8.70±0.58a	8.30±0.58a	8.00±0.00a	8.00±0.00a
10%honey	**Week1**	**Week2**	**Week3**	**Week4**
Appearance/ Color	7.30±0.57a	6.70±1.00a	6.30±0.57a	6.30±0.00a
Taste/ Flavor	8.70±0.58a	7.00±1.00b	4.00±0.58c	2.30±0.00d
Smell/ Odor	8.30±0.56a	5.00±1.00b	2.00±1.00c	1.70±0.56c
Texture/ Mouth feel	8.30±0.56a	5.00±1.00b	1.30±0.56c	1.00±1.00c
20%honey	**Week1**	**Week2**	**Week3**	**Week4**
Appearance/ Color	8.00±1.00a	8.00±1.00a	8.00±1.00a	7.60±1.15a
Taste/ Flavor	8.60±0.58a	7.30±0.58b	4.70±0.58c	2.70±0.58d
Smell/ Odor	8.70±0.58a	7.60±0.58a	3.00±1.00b	1.00±0.00c
Texture/ Mouth feel	8.30±0.58a	6.70±0.58b	2.00±1.00c	1.30±0.58c
30%honey	**Week1**	**Week2**	**Week3**	**Week4**
Appearance/ Color	8.00±1.00a	8.00±1.00a	8.00±1.00a	8.00±1.00a
Taste/ Flavor	7.00±1.00a	7.70±0.58ab	6.70±0.58bc	3.00±0.58c
Smell/ Odor	8.30±0.58a	8.00±1.00ab	7.00±1.00b	5.00±1.00c
Texture/ Mouth feel	8.70±0.58a	8.00±1.00a	6.30±1.53b	4.71±0.53c

Mean value of the parameter followed by equal letters in the Column do not differ by the Duncan's test at the 5% probability level

-Mean value of the parameter followed by equal letters in the row do not differ by the Duncan's test at the 5% probability level

-Different letters at different row indicated significantly different ($p<0.05$)

Several sensorial attributes were given by panelists with odor/smell described as fruity, rancid, creamy, cooked, Sulphuric like and whey like. Texture was described as soft, semi-hard, firmness, slippery and sticky. Taste attributes were described as salty, sour, bitter and sweaty while Appearance was described as clumpy, chunky, nonhomogeneous and moldy. This is in accordance with several other researchers (Karagul-Yuceer *et al.*, 2007), (Park and Lee, 2006)

Drake *et al.*, 2001 did a preference mapping of 7 cheddar cheese sample using descriptive analysis and consumer acceptance test. He found out that cheddar cheese consists of aromatics described as whey like, creamy , cooked, sulfur like fruity and rancid (Karagul-

Yuceer *et al*., 2007). (Park and Lee, 2006) discovered that in goat cheese it was found to possess attributes such as waxy, milk fat, sour and salty.

Most of our panelist described the cottage cheese sample in the first week of analysis as creamy, whey like, soft, non-homogeneous, sweet and soupy like. The likeness was high at an average of 7.6 with high likings for fridge sample, vacuum and honeyed samples.

For refrigerated samples it had a mean liking of 7.67 in appearance in the first week with a dominant whitish color dominating. As time progressed the difference was noted in the fourth week where a discoloration was observed. The sample had a sweet taste and creamy smell in the beginning of storage with liking of 7.3 for taste and 7.67 for smell but turned sour as the storage time progressed with a likeness showing a significant difference for both taste and smell across the week. The fourth week of storage indicating a likeness of 2 for taste and 1 for smell. Bad odors are generated to an extent of disliked mostly in the fourth week a clear proof of spoilage.

When a sample of cottage cheese was stored under vacuum conditions in room temperature the following sensorial attributes were found. In terms of appearance the whitish color of cottage was at its highest value at 8 and remained so for two weeks though it changed to 7.6 in the third and fourth week statistically with no significant difference $p<0.005$. Taste and flavor had an initial liking of 8.6 in the first week which reduced to 8.3 in the second week with no significant difference $p>0.005$ but later the liking changed to 7.3 thereby showing a significant difference $p<0.005$ with the same result repeating itself in week four. The sweet flavor had changed in the third week an indication toward spoilage. These were in agreement with previous researchers i.e. Akin *et al* (2003) researched on sensorial properties of white pickled cheeses under different conditions and obtained similar results. For odor attributes the cottage cheese sample in the first week had a liking of 8.7 with its whey like creamy smell dominating. It remained so for the entire four weeks of storage with no significant difference $p>0.005$ within the storage period. The texture of cottage cheese sample did not differ significantly $p>0.005$ across the storage period but rather remained soft and had a soupy feel. Vacuum condition was one of preservation method that prolonged the shelf life of cottage cheese.

For frozen sample that were stored under deep freeze conditions the appearance attributes i.e., non-homogeneous white cheese remained the same in the four weeks of analysis with a liking of 8.7 in the first week and 8.3 in the fourth week showing no significant difference $p>0.005$.

The taste/flavor of cottage cheese sample in the first second and third week of storage did not seem to differ significantly $p>0.005$ with week one having a liking of 8.6, week two a liking of 8.3 and week three a liking of 8. However, toward the fourth week a significant difference was observed with a liking of 7.6. The cheese had salt like taste from its usual sweaty taste. The smell of frozen cottage cheese sample remained the same throughout the storage period with a high liking of 9 in the first week and 8.3 in the fourth week of storage showing no significant difference $p>0.005$. Frozen cottage cheese sample did not differ significantly for texture/ mouth feel attributes across the 30 days of storage with likings of 8.7 in the first week and 8 in the fourth week. It remained soft and soupy like with no changes. Similar results were indicated by (Karagul-Yuceer *et al*., 2007) on ezine cheese analysis.

When a sample was mixed with different concentration of honey at 10%, 20% and 30% w/v the appearance of the sample changed from its usual whitish look to brown like look though the appearance remained the same within the 30 days of storage with no significant difference for all the samples. What seemed to change across the 30 days of storage was taste of the samples. For 10% and 20% w/v honey sample there was a significant difference after every week of storage but for 30% w/v sample the difference is observed in the third week of analysis. Smell attributes for the honeyed samples seem to differ significantly within the storage period with 10% w/v sample showing a significant difference in all the weeks. For 20% and 30% w/v the significant difference is observed in the third week of analysis with sulfur like sour smell appearing towards end of 30 days of storage. The mouth feel/texture of honeyed sample had a high liking in the first week of analysis ranging between 8.3- 8.7 with a soft soupy feel dominating throughout. As time progressed the 10% w/v sample differed significantly to a liking of 1 (dislike completely) at week four showing a chunky i.e. had water layer on top. 20% and 30% sample had a line of 2 and 4 respectively at week four of storage showing a significant difference across the storage period they had developed stickiness to teeth feel from its usual soft but firm texture. Honey though a good preservative could not entirely prevent spoilage of cottage cheese sample but the higher the concentration of honey in the sample the better.

4.6 pH, titratable acidity and organic acid content

Values of pH and total solids content are as explained below. The pH of the cheese varies with the number of storage days and weeks. As cheese is stored there is production of different acids which makes all samples acidic. The variation of pH measurements is as shown in the table 4.2 below.

The pH value of different samples at different condition were measured for week 1,2,3,4,5,6and 7 and ranged from 3.71 - 4.76 with fresh cheese that was acting as a control sample having a pH of between 4.25 – 4.35 in both samples i.e., homemade and purchased samples. Cottage cheese which is known to be mild acidic due to production of acids that causes coagulation of milk as is evident in the table 4.5 below.

Table 4.5. PH measurements for cottage cheese.

pH	**Week1**	**Week2**	**Week3**	**Week4**
Fresh Samples	5.23±0.04a	5.24±0.02a	5.23±0.04a	5.19±0.02a
Deep freeze	5.25±0.02a	5.25±0.01a	5.24±0.01a	5.22±0.02a
Vacuum	5.28±0.01a	5.28±0.01a	5.28±0.01a	5.28±0.01a
Refrigerated	5.22±0.02a	5.14±0.02b	4.92±0.04c	4.28±0.06d
10% honey	5.10±0.01a	4.80±0.01b	4.30±0.04c	3.80±0.01d
20% honey	5.06±0.01a	4.95±0.04b	4.77±0.02c	4.54±0.02d
30% honey	5.03±0.01a	5.01±0.02a	4.92±0.01b	4.88±0.02c

Mean value of the parameter followed by equal letters in the Column do not differ by the Duncan's test at the 5% probability level

-Mean value of the parameter followed by equal letters in the row do not differ by the Duncan's test at the 5% probability level

-Different letters at different row indicated significantly different ($p<0.05$)

For week 1 the average pH value was 5.16. Almost all samples had a similar pH. But as time progressed under different conditions, pH changes were observed indicating organic acids production a proof of increase in bacteria activities resulting to cheese spoilage.

Cheese under deep freeze conditions does not show a significant difference within the four weeks of storage similar to fresh sample cheese (control sample). This is in line with level of organic acids observed in 4.7 an indication that cheese stored under deep freeze has less bacteria activities. Deep freeze can therefore be used to prolong the cottage cheese shelf life though after thawing process has taken place and cheese returned to normal conditions, chances of cheese spoiling quickly was phenomenon.

Vacuum conditions gave a similar result to that of fresh and deep freeze. There was no significant difference within the first 30 days of storage an indicator of less bacteria activities within the sample. Considering the sample is kept at room temperature and only placed under vacuum conditions this is a full proof that vacuum conditions can be used to prolong the shelf life of cottage cheese for more than a month.

Cheese stored at room conditions exhibited a higher pH after week 1. The spoilage was observed within the first three days of storage. This was in line with (Modise, 2014.) Who in her research work observed that when fresh cheese is stored under aerobic conditions in room temperature there is fast decay? Gammariello *et al*., 2009 observed that due to high moisture content of more than 50% in soft cheeses and high pH of around 5% the growth of spoilage bacteria is promoted. Such bacteria are from genera Pseudomonas, flavobacterium and alcaligenes (Dogan and Boor, 2003).

Cheese sample stored at normal refrigerated conditions had a significant difference in the second week of storage. This indicated that refrigerated conditions are not reason enough to prevent spoilage of cottage cheese. Some bacteria in milk will still do well in 4℃ thereby promoting bacteria growth. Previous research done in South Africa by Modise also indicated similar observation.

Effects of honey on cheese according to pH result in table 4.2 seem to differ with other previous researchers who observed extended shelf life. When 10% and 20% w/v of honey was mixed with cheese sample a significant difference was observed between week one and two of storage. However, 30% w/v of honey had a significant difference between week two and three of storage thereby extending the shelf life by one week. Honey is a good natural antibacterial compound which prevents growth of spoilage bacteria in cheese due to its inhibitory properties brought by non-peroxide mediated bacteriostatic/ bactericidal activity. The non-peroxides factors in honey which includes lysozomes, phenolic acids, flavonoids, and organic acids such as syringic and phenyl lactic acids are known to inhibit growth of a wide range of gram-negative and gram-positive bacteria (Krushna *et al*., 2007)

4.7 Moisture content/ solid content analysis.

The moisture content of cottage cheese was calculated as per the formula given as

$$\%\ \text{moisture} = \frac{\text{loss in weight} \times 100}{\text{Weight of sample}} \quad (4.1)$$

$$\%\ \text{Solid} = 100 - \%\ \text{moisture} \quad (4.2)$$

. It was clear that soft cheese have high water content of more than 80% and a small solid content which promotes growth of spoilage bacteria thereby producing off flavor, color defects and sliminess in cottage cheese (Modise, 2014.)

Table 4.6: Moisture content in cottage cheese

	sample 1A(gm)	sample 1B (gm)
Mass of empty glass	95.019	95.967
Mass of the sample	6.376	6.6702
Mass of flask + sample	101.394	102.638
Mass of the flask + sample After 24 hrs.	95.783	87.718

$$\% \text{ moisture} = \frac{5.611 \times 100}{6.376} = 88\%$$

% Solid = 100 – 88 = 12

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

It was observed that there were a number of organic acid present in cottage cheese in the range of lactic acid> citric acid >Malic acid >Oxalic acid >Acetic acid. This includes nonvolatile acids such as lactic and citric and the volatile acids such as acetic acid which contribute to the scent of cottage cheese.

Production of lactic acid within living things such as milk is a metabolic reaction sometimes without necessary use of bacteria. However, increase in lactic acid is an indication of bacteria growth which indicate cheese spoilage. Since most cottage cheese had not undergone fermentation the level of lactic acid was not as high as compared to other studies thereby having a less acidic taste, Mild pH.

The result also observed that storing cottage cheese at 4˚C (refrigeration temperature) for the entire period affected the acid profile in cottage cheese in a minimal way and did not also affect the diacetyl flavors. The organoleptic properties were not affected.

Cottage cheese stored at room temperatures in open conditions resulted to a significant increase in lactic acid, acetic acids concentrations an indication of bacteria growth within the cheese more so after two days of storage.

The moisture content in our cottage sample is at 88% which is high as compared with other researchers. Most of bacteria thrive very well in watery environments and this can be reason enough why most of cottage cheese gets spoilt quickly.

A combination of factors both intrinsic such as pH, water activity and nutrient content and extrinsic factors such as temperature, relative humidity and storage time were found to affect shelf life of cottage cheese. Some storage methods such as vacuum storage and preservation with honey prolonged the shelf life of cottage cheese thus should be considered.

Sensory attributes such as odor, texture, taste and appearance changed with storage time. In early storage the organoleptic properties of cheese remained unchanged. However, some conditions such as Freezing and vacuum storage protected cheese from spoilage up to nine weeks of study. The sensory attributes remained okay.

5.2 RECOMMENDATIONS

Cottage cheese has a lot of nutritive benefits and its uptake should be highly encouraged. However, one of the disadvantages of cottage cheese is its ease of bacterial contamination. The main disease that is associated with bacteria contamination is Listeriosis caused by Listeria monocytogenes bacteria a highly risk disease to pregnant women and their developing foetus. For such reasons the following recommendations are sought:

1. Proper preservation method such as lactoperoxidases of honey and vacuum storage has been found to be quite effective in prolonging shelf life of cottage cheese and can be applied in order to eliminate these challenges. Further studies and research is needed in order to reap the maximum benefits of cottage cheese. Maintaining cottage cheese of good quality with a prolonged shelf life need advanced research.
2. Cheese industries in our country need to device ways through which they can reduce moisture contents as a way of prolonging cheese shelf life. One way to achieve this is by heating the cottage cheese to evaporate more whey during cheese preparation which has proved to be one of preservation method (McIntyre and Hudson, 2009)
3. Cheese processing industries requires use of multiple hurdle effects notably vacuum packaging, cooking of cottage cheese to eliminate water, addition of natural preservatives like honey that inhibit spoilage microorganisms and pathogens. Use of different hurdles in controlling pathogens may not be the only control since despite use of hurdle technology outbreak of foodborne disease have been linked to cheese consumption. Hurdles will reduce the number of pathogens in cheese though they may not fully control growth of bacteria and therefore care must be taken to avoid outbreak of diseases through consumption of contaminated cheese.
4. Several dairy industries in our country need to implement food safety systems such as HACCP. Research on other food safety systems is also encouraged.

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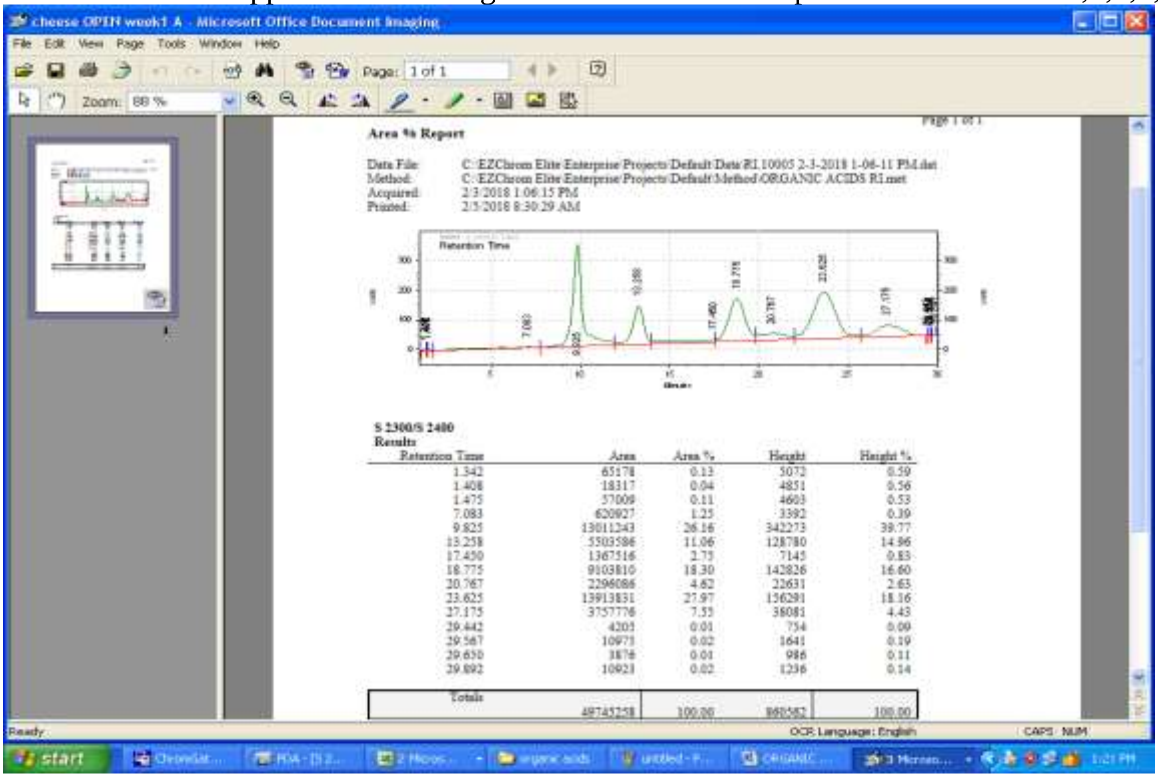
APPENDICES

Appendix 6.1: cottage cheese stored under open conditions week 1,2,3,4,6.



Area % Report

Data File: C:\EZChrom Elite Enterprise\Projects\Default\Data\RI 10005 2-3-2018 1-06-11 PM.dat
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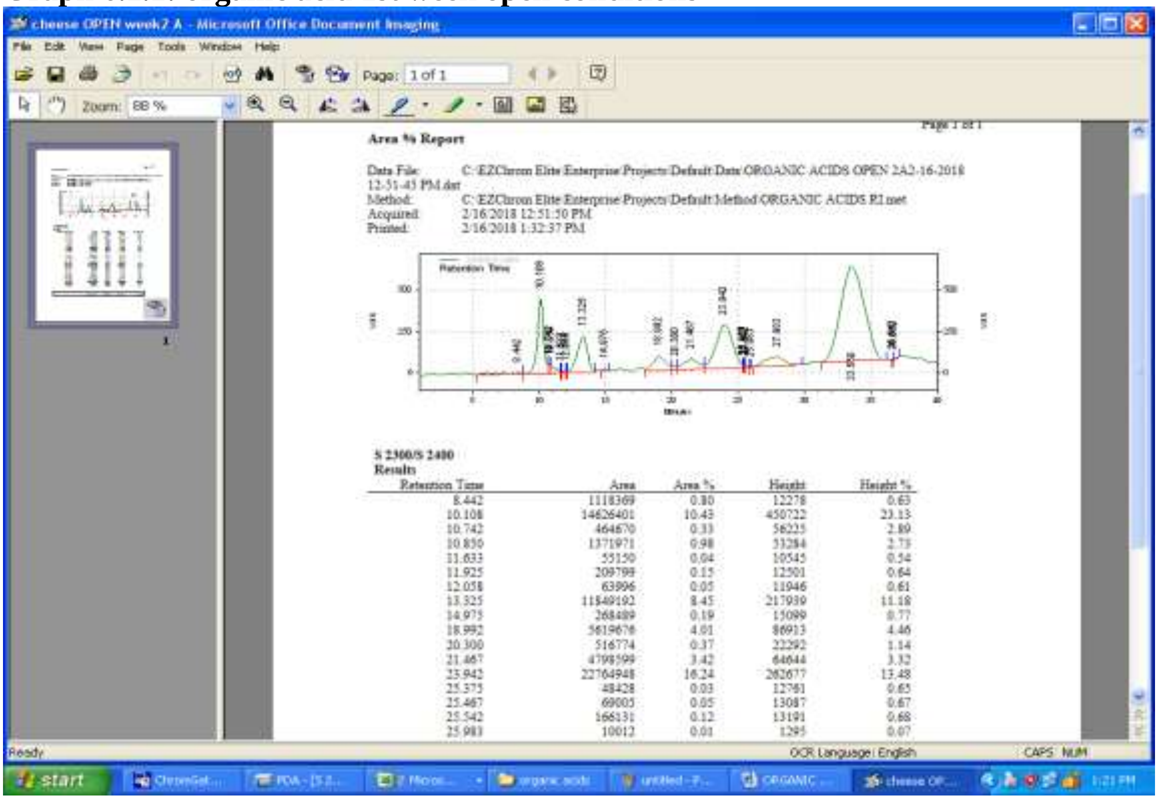
Acquired: 2/3/2018 1:06:15 PM
Printed: 2/5/2018 8:30:29 AM

S 2300/S 2400
Results

Retention Time	Area	Area %	Height	Height %
1.342	65178	0.13	5072	0.59
1.408	18317	0.04	4851	0.56
1.475	57009	0.11	4603	0.53
7.083	620927	1.25	3392	0.39
9.825	13011243	26.16	342273	39.77
13.258	5503586	11.06	128780	14.96
17.450	1367516	2.75	7145	0.83
18.775	9103810	18.30	142826	16.60
20.767	2298086	4.62	22631	2.63
23.625	13913831	27.97	156291	18.16
27.175	3757776	7.55	38081	4.43
29.442	4203	0.01	754	0.09
29.567	10973	0.02	1641	0.19
29.650	3878	0.01	986	0.11
29.892	10923	0.02	1236	0.14
Totals	49745258	100.00	860582	100.00

Graph 6.1.1: organic acid 1st week open conditions



Area % Report

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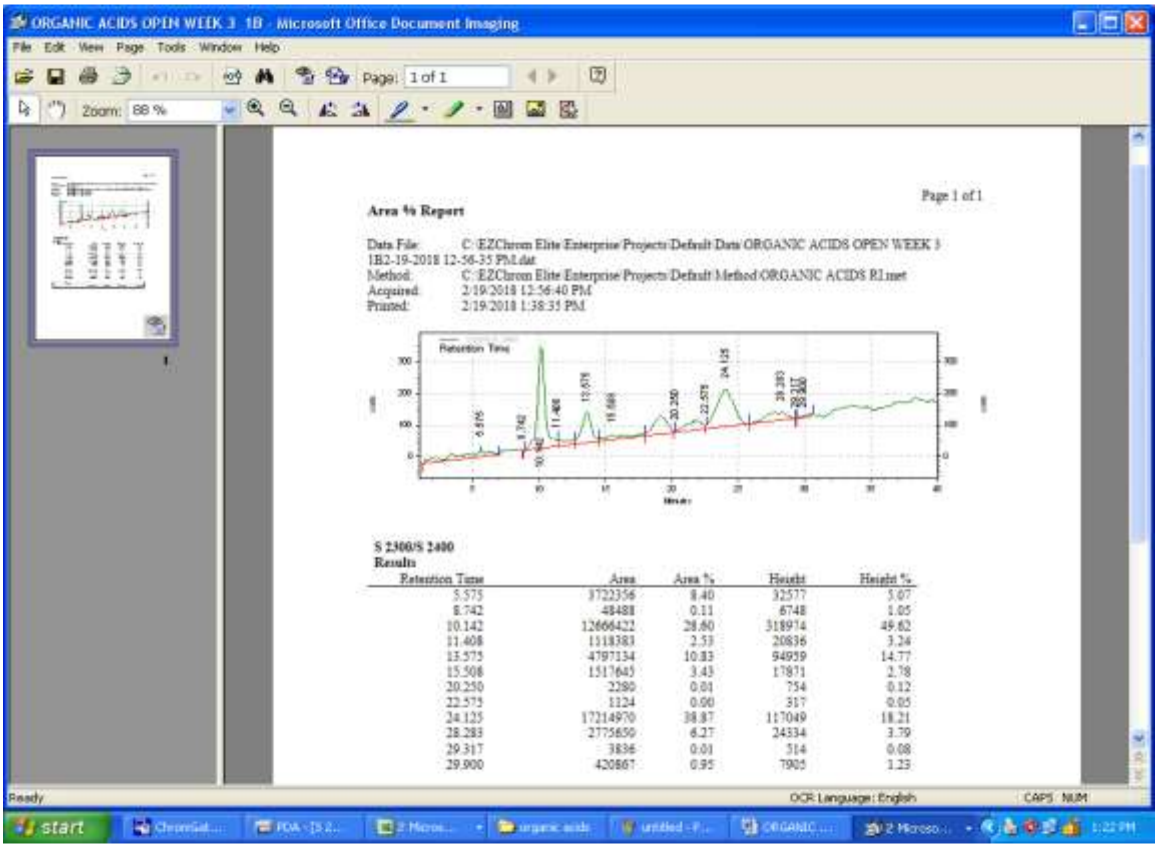
Printed: 2/16/2018 1:32:37 PM

S 2300/S 2400
Results

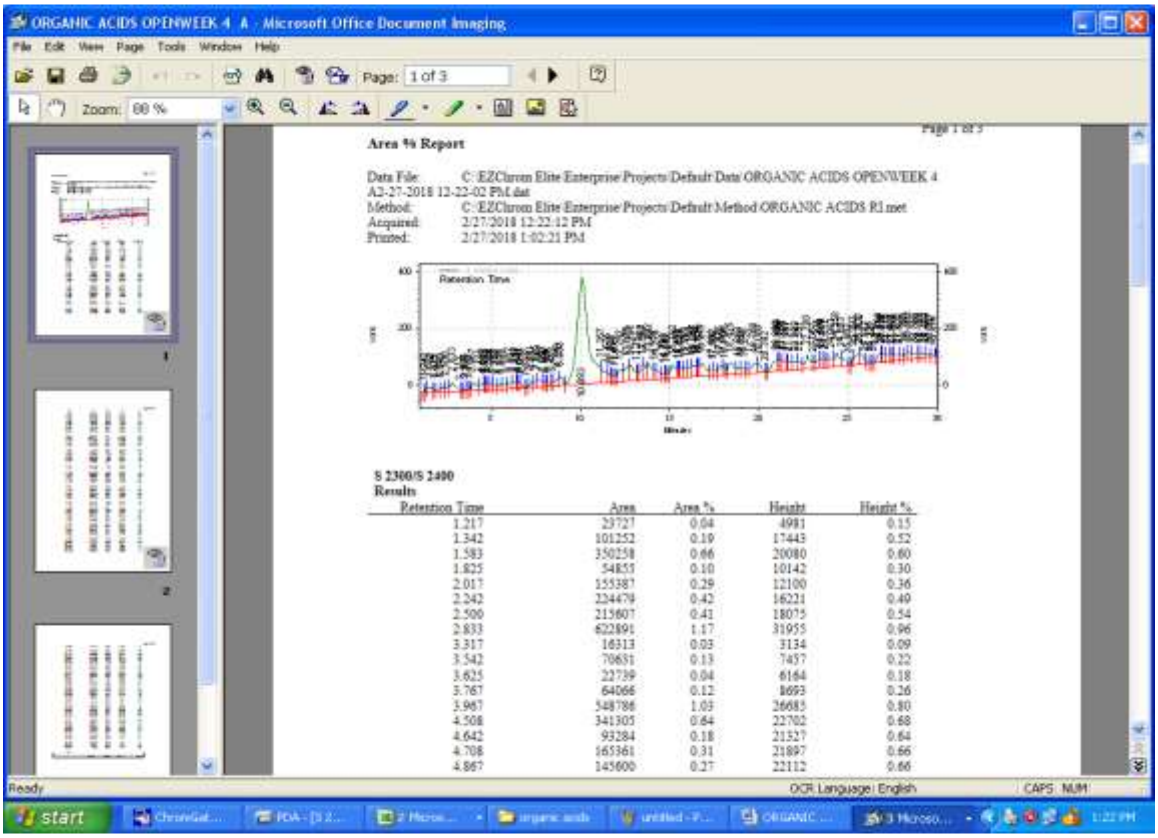
Retention Time	Area	Area %	Height	Height %
8.442	1118369	0.80	12278	0.63
10.108	14626401	10.43	450722	23.13
10.742	464670	0.33	56225	2.89
10.850	1371971	0.98	53284	2.73
11.633	55150	0.04	10545	0.54
11.925	209799	0.15	12501	0.64
12.058	63996	0.05	11946	0.61
13.325	11849192	8.45	217939	11.18
14.975	268489	0.19	15099	0.77
18.992	5619678	4.01	86913	4.46
20.300	516774	0.37	22292	1.14
21.467	4798599	3.42	64644	3.32
23.942	22764948	16.24	262677	13.48
25.375	48428	0.03	12761	0.65
25.467	69005	0.05	13087	0.67
25.542	166131	0.12	13191	0.68
25.983	10012	0.01	1295	0.07

Graph 6.1.2: organic acid 2nd week open conditions



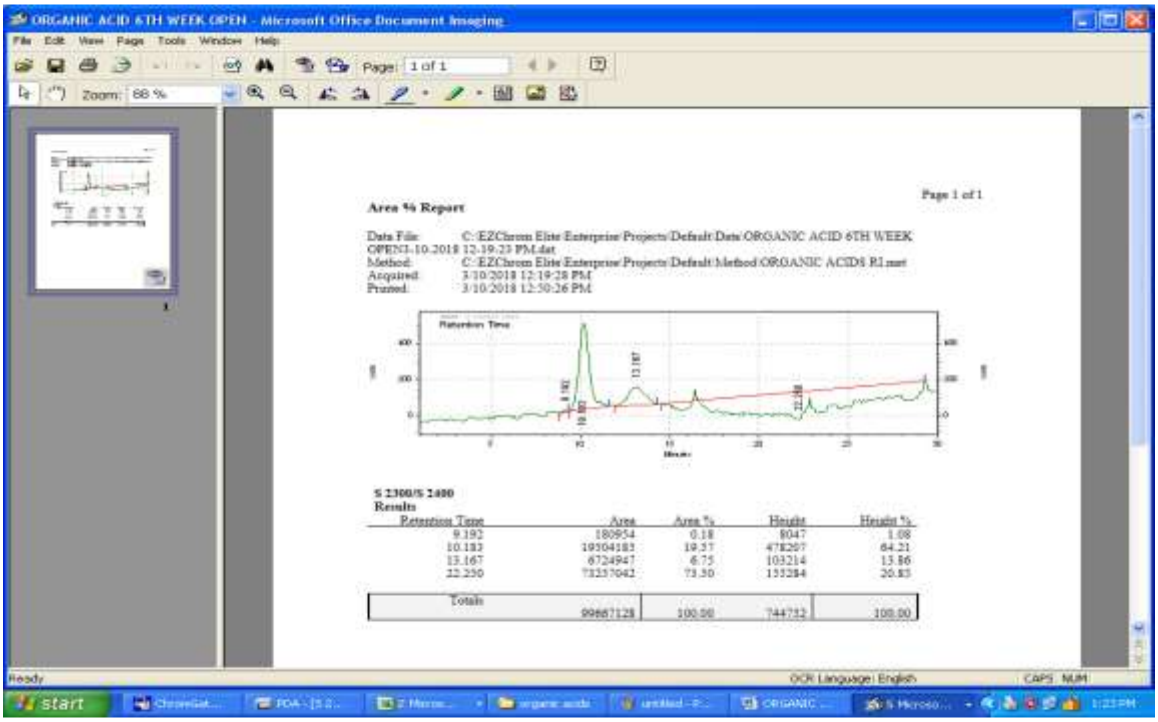
Retention Time	Area	Area %	Height	Height %
5.575	3722356	8.40	32577	5.07
8.742	48488	0.11	6748	1.05
10.142	12666422	28.60	318974	49.62
11.408	1118383	2.53	20836	3.24
13.575	4797134	10.83	94959	14.77
15.508	1517645	3.43	17871	2.78
20.250	2280	0.01	754	0.12
22.575	1124	0.00	317	0.05
24.125	17214970	38.87	117049	18.21
28.283	2775650	6.27	24334	3.79
29.317	3836	0.01	514	0.08
29.900	420867	0.95	7905	1.23

Graph 6.1.3: organic acids 3rd week open conditions



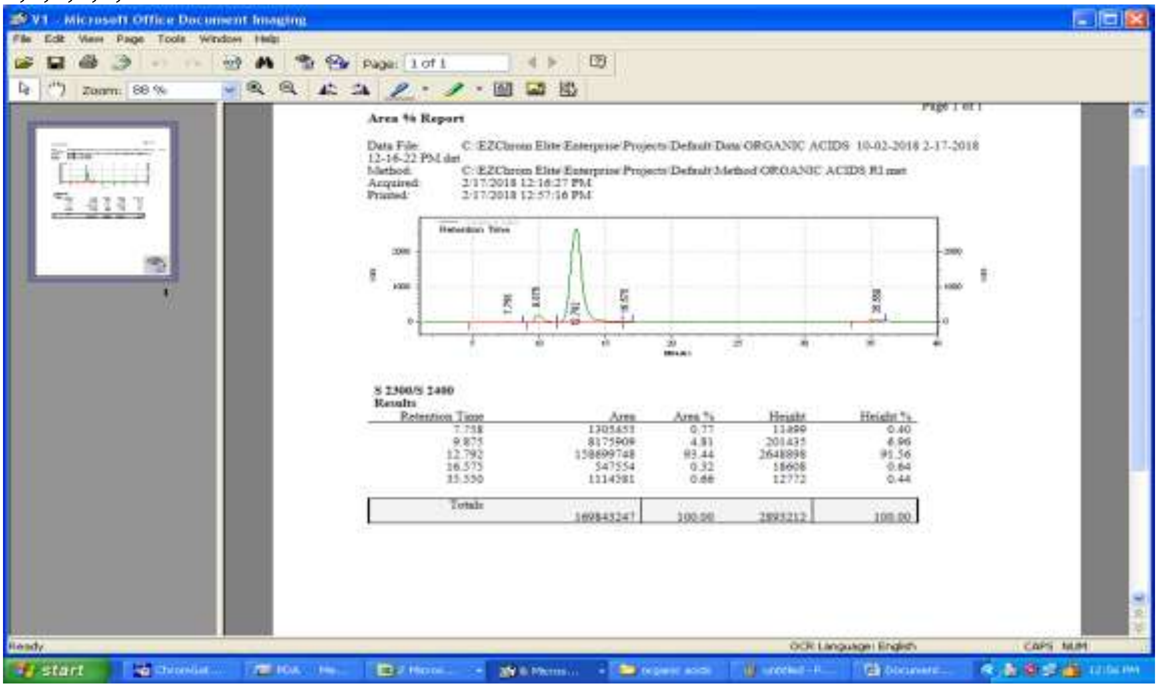
Retention Time	Area	Area %	Height	Height %
1.217	23727	0.04	4981	0.15
1.342	101252	0.19	17443	0.52
1.583	350258	0.66	20080	0.60
1.825	54855	0.10	10142	0.30
2.017	155387	0.29	12100	0.36
2.242	224479	0.42	16221	0.49
2.500	215607	0.41	18075	0.54
2.833	622891	1.17	31955	0.96
3.317	16313	0.03	3134	0.09
3.542	70631	0.13	7457	0.22
3.625	22739	0.04	6164	0.18
3.767	64066	0.12	8693	0.26
3.967	548786	1.03	26683	0.80
4.508	341305	0.64	22702	0.68
4.642	93284	0.18	21527	0.64
4.708	165361	0.31	21897	0.66
4.867	145600	0.27	22112	0.66

Graph 6.1.4: organic acid 4th week open conditions

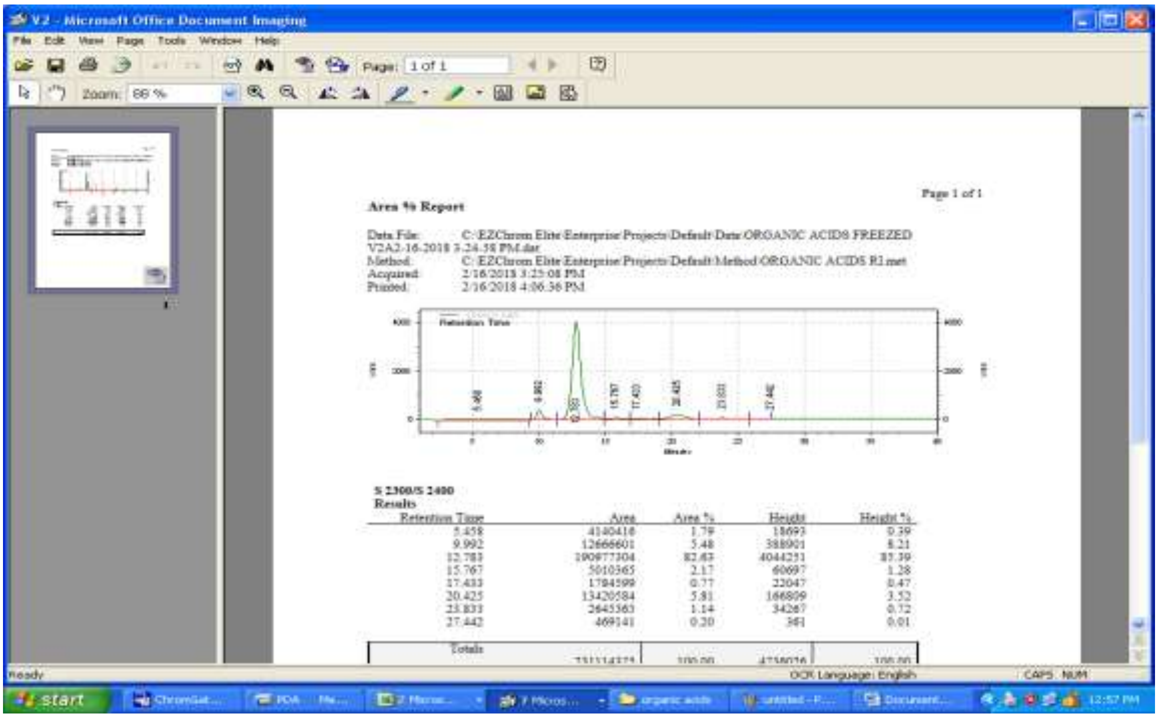


Graph 6.1.5: organic acids 6th week under open conditions

Appendix 6.2: Cottage cheese stored under vacuum conditions at room temperatures for week 1,2,3,4,5,and 6



Graph 6.2.1: organic acid stored under vacuum conditions 1st week



Page 1 of 1

Area % Report

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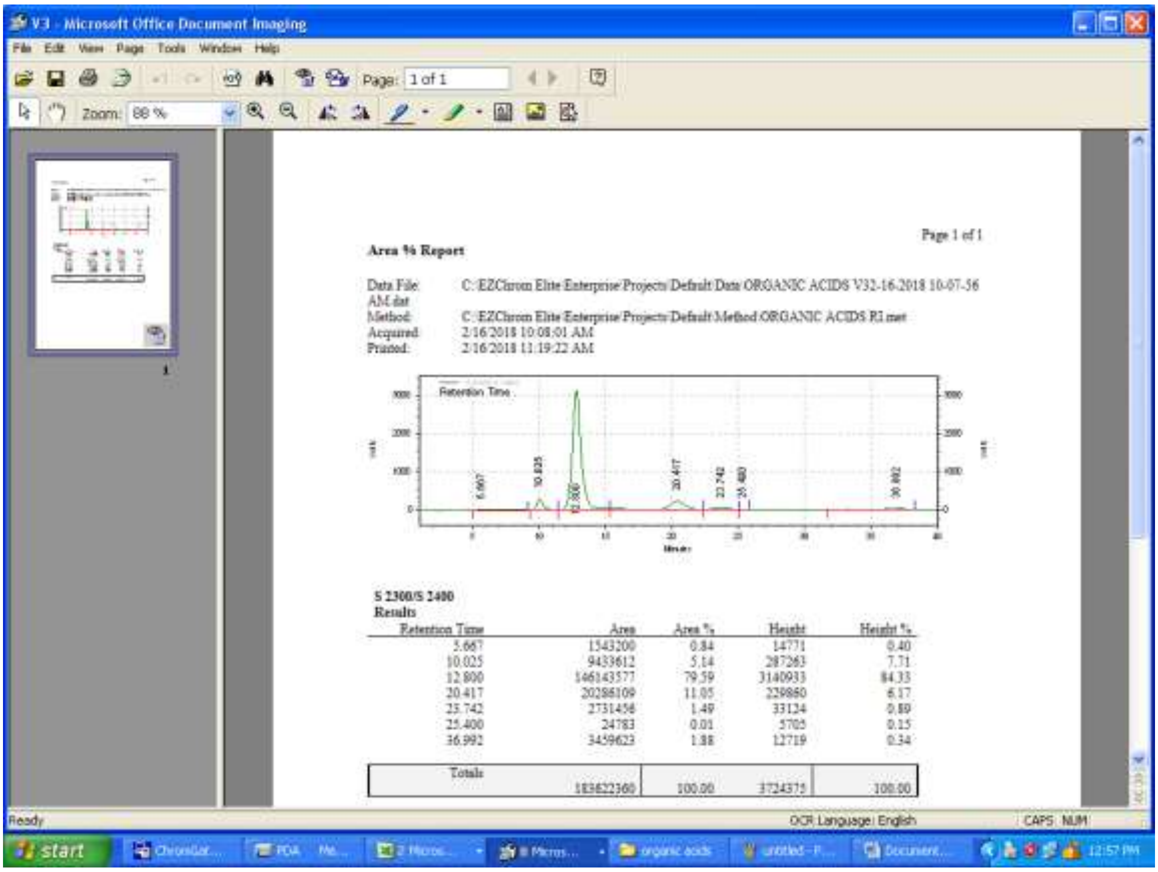
Printed: 2/16/2018 4:06:36 PM

S 2300/S 2400
Results

Retention Time	Area	Area %	Height	Height %
5.458	4140416	1.79	18693	0.39
9.992	12666601	5.48	388901	8.21
12.783	190977304	82.63	4044251	85.39
15.767	5010365	2.17	60697	1.28
17.433	1784599	0.77	22047	0.47
20.425	13420584	5.81	166809	3.52
23.833	2645363	1.14	34267	0.72
27.442	469141	0.20	361	0.01
Totals	231114373	100.00	4736026	100.00

Graph 6.2.2: organic acid stored under vacuum conditions 2nd week



Page 1 of 1

Area % Report

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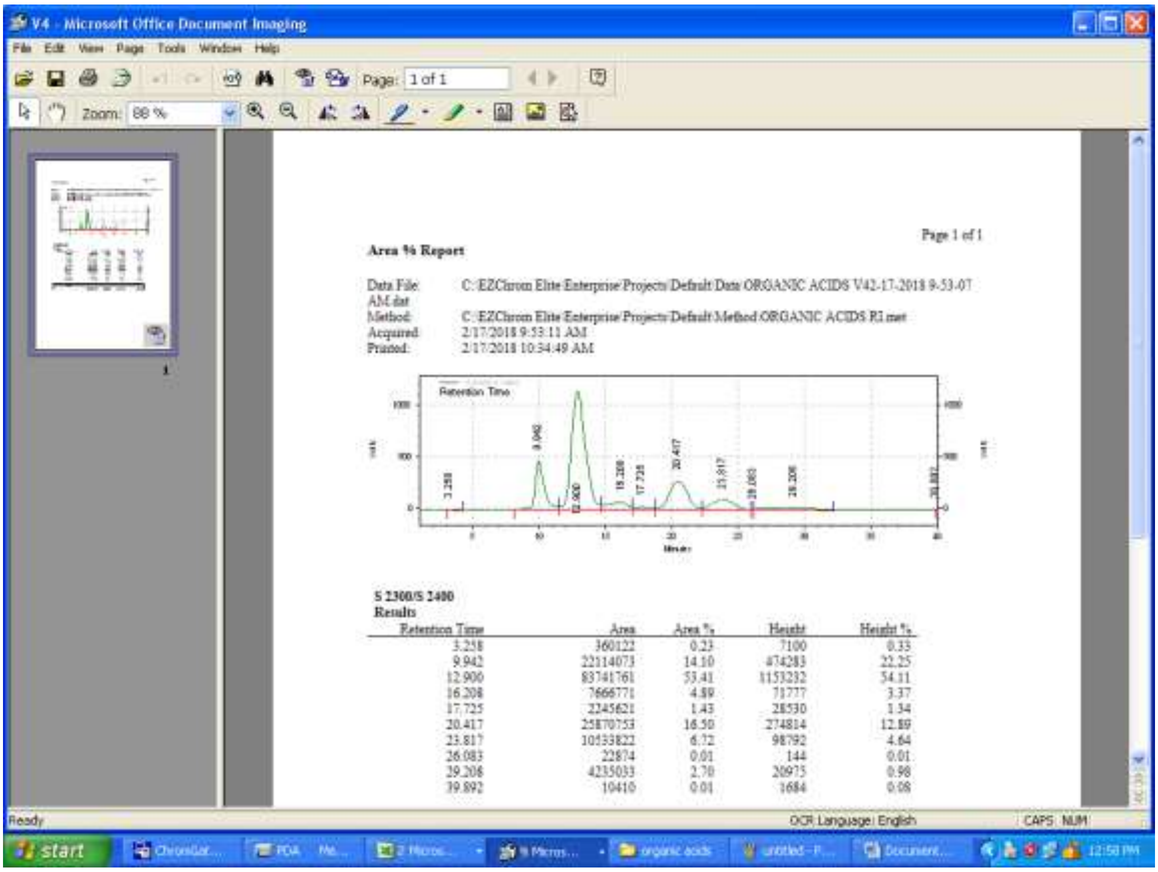
Printed: 2/16/2018 11:19:22 AM

S 2300/S 2400
Results

Retention Time	Area	Area %	Height	Height %
5.667	1543200	0.84	14771	0.40
10.025	9433612	5.14	287263	7.71
12.800	146143577	79.59	3140933	84.33
20.417	20286109	11.05	229860	6.17
23.742	2731456	1.49	33124	0.89
25.400	24783	0.01	5705	0.15
36.992	3459623	1.88	12719	0.34
Totals	183622360	100.00	3724375	100.00

Graph 6.2.3: organic acid stored under vacuum conditions 3rd week



Page 1 of 1

Area % Report

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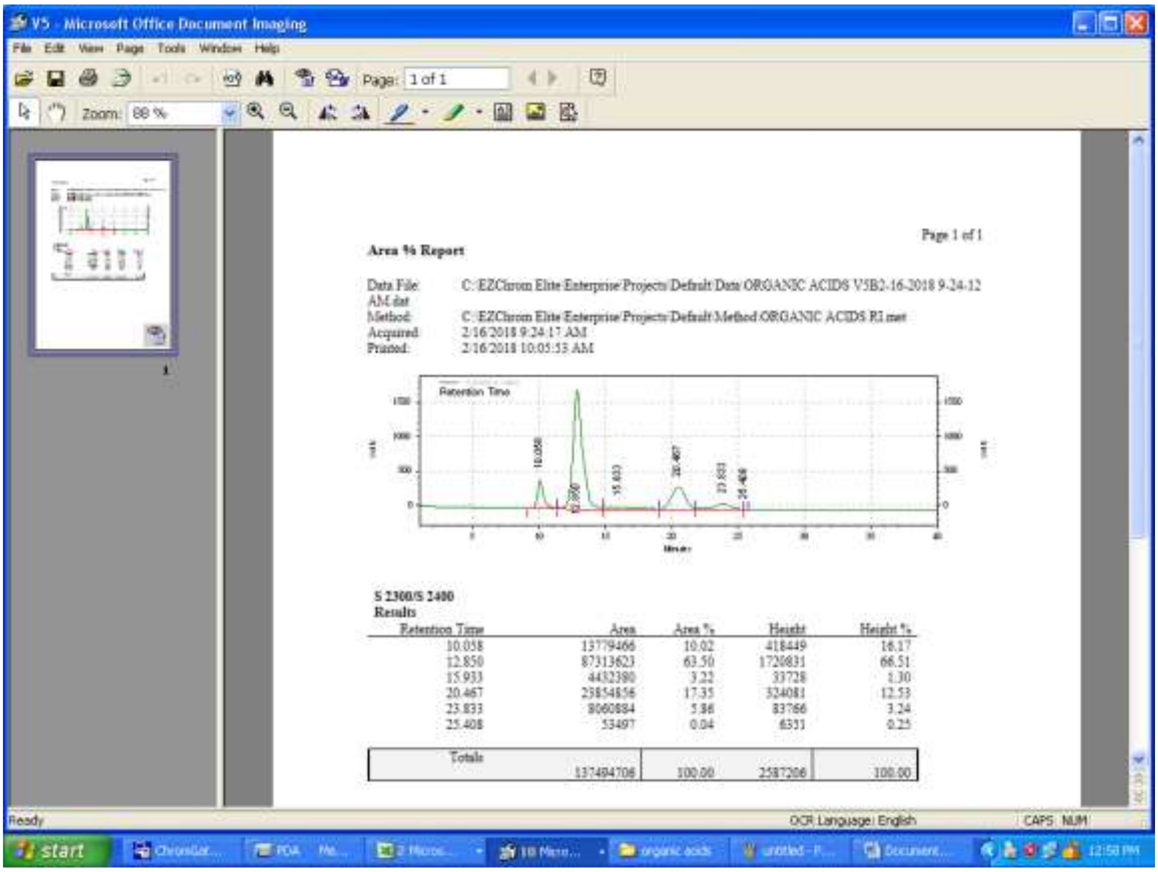
Printed: 2/17/2018 10:34:49 AM

S 2300/S 2400
Results

Retention Time	Area	Area %	Height	Height %
3.258	360122	0.23	7100	0.33
9.942	22114073	14.10	474283	22.25
12.900	83741761	53.41	1153232	54.11
16.208	7666771	4.89	71777	3.37
17.725	2245621	1.43	28530	1.34
20.417	25870753	16.50	274814	12.89
23.817	10533822	6.72	98792	4.64
26.083	22874	0.01	144	0.01
29.208	4235033	2.70	20975	0.98
39.892	10410	0.01	1684	0.08

Graph 6.2.4: organic acid stored under vacuum condition 4th week



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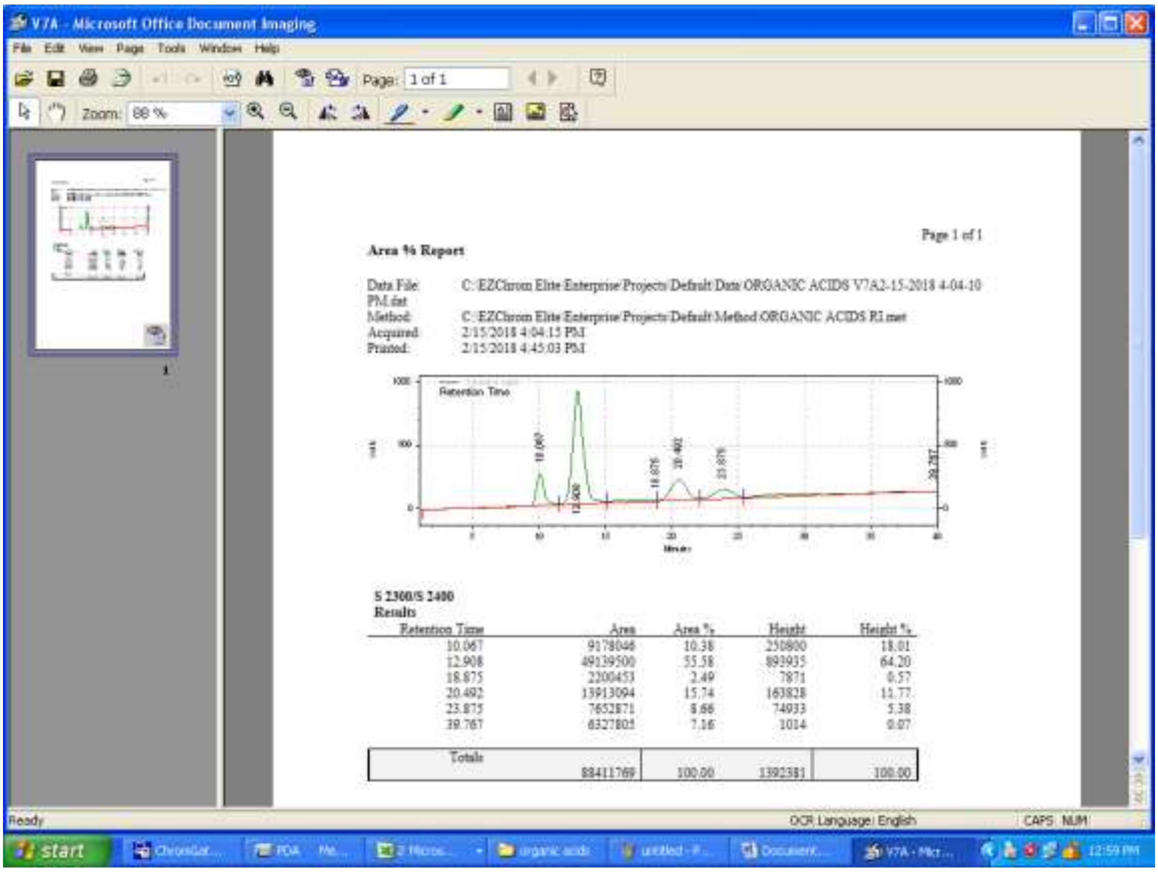
Area % Report

Data File: C:\EZChrom Elite Enterprise\Projects\Default\Data\ORGANIC ACIDS V5B2-16-2018 9-24-12 AM.dat
Method: C:\EZChrom Elite Enterprise\Projects\Default\Method\ORGANIC ACIDS RI.met
Acquired: 2/16/2018 9:24:17 AM
Printed: 2/16/2018 10:05:53 AM

S 2300/S 2400
Results

Retention Time	Area	Area %	Height	Height %
10.058	13779466	10.02	418449	16.17
12.850	87313623	63.50	1720831	66.51
15.933	4432380	3.22	33728	1.30
20.467	23854856	17.35	324081	12.53
23.833	8060884	5.86	83766	3.24
25.408	53497	0.04	6331	0.25
Totals	137494706	100.00	2587206	100.00

Graph 6.2.5: organic acid stored under vacuum cnditions 5th week



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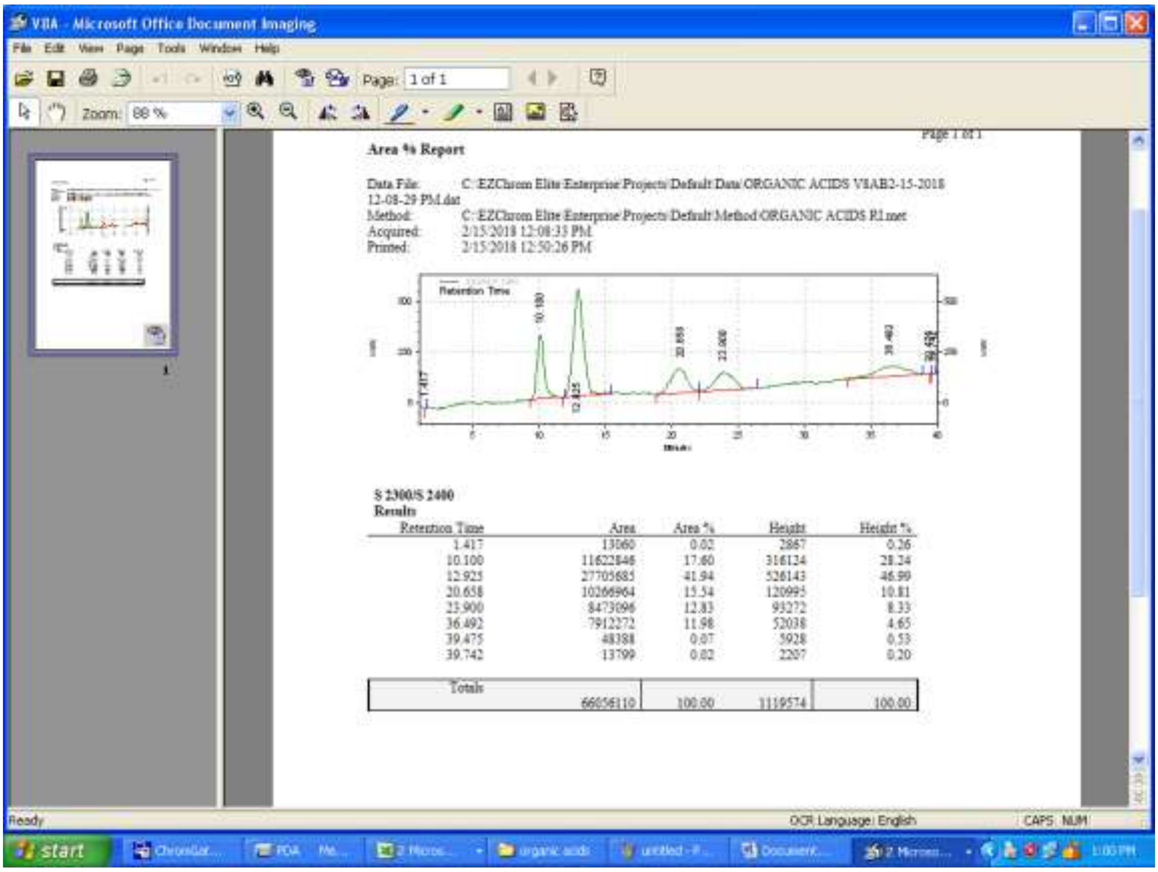
Area % Report

Data File: C:\EZChrom Elite Enterprise\Projects\Default\Data\ORGANIC ACIDS V7A2-15-2018 4-04-10 PM.dat
Method: C:\EZChrom Elite Enterprise\Projects\Default\Method\ORGANIC ACIDS RI.met
Acquired: 2/15/2018 4:04:15 PM
Printed: 2/15/2018 4:45:03 PM

S 2300/S 2400
Results

Retention Time	Area	Area %	Height	Height %
10.067	9178046	10.38	250800	18.01
12.908	49139500	55.58	893935	64.20
18.875	2200453	2.49	7871	0.57
20.492	13913094	15.74	163828	11.77
23.875	7652871	8.66	74933	5.38
39.767	6327805	7.16	1014	0.07
Totals	88411769	100.00	1392381	100.00

Graph 6.2.6: organic acid stored under vacuum conditions 6th week



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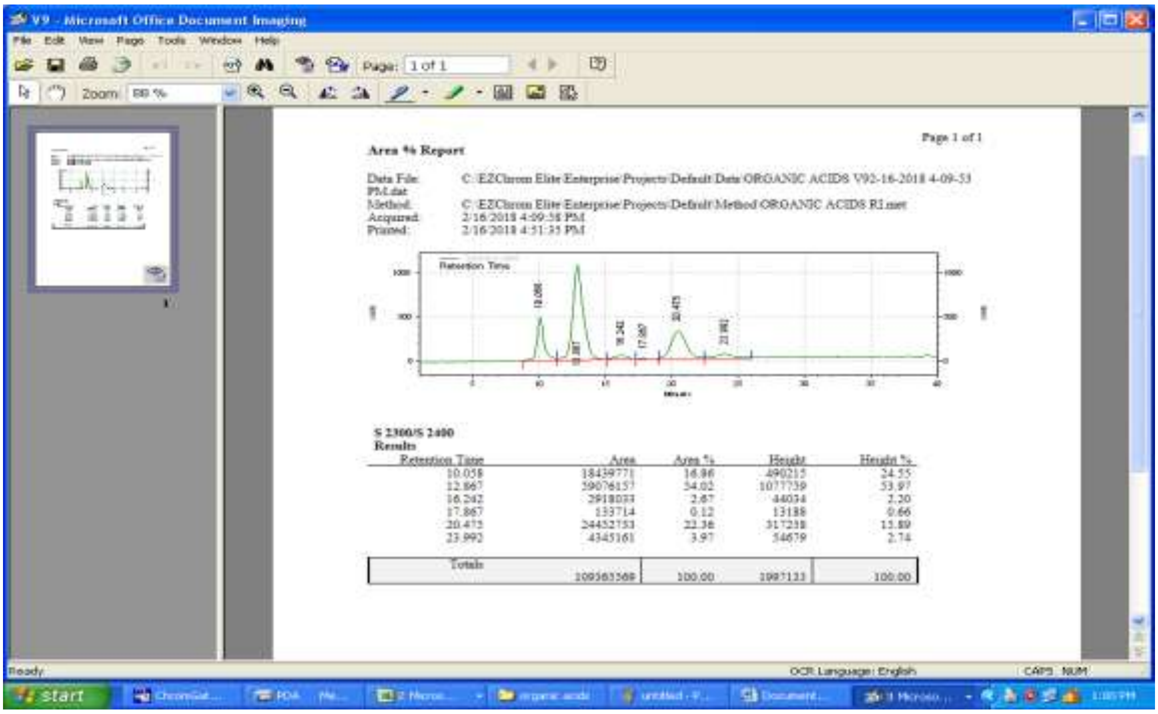
Area % Report

Data File: C:\EZChrom Elite Enterprise\Projects\Default\Data\ORGANIC ACIDS V8AB2-15-2018 12-08-29 PM.dat
Method: C:\EZChrom Elite Enterprise\Projects\Default\Method\ORGANIC ACIDS RI.met
Acquired: 2/15/2018 12:08:33 PM
Printed: 2/15/2018 12:50:26 PM

S 2300/S 2400
Results

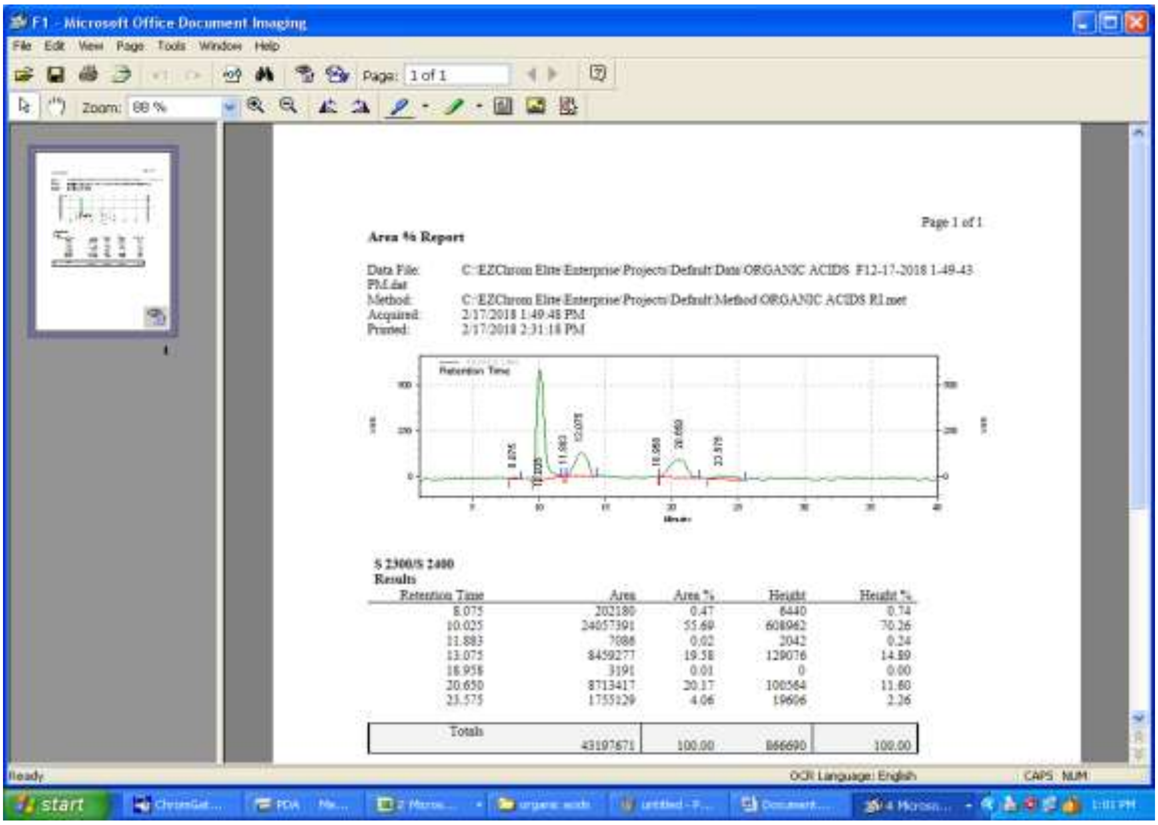
Retention Time	Area	Area %	Height	Height %
1.417	13060	0.02	2867	0.26
10.100	11622846	17.60	316124	28.24
12.925	27705685	41.94	526143	46.99
20.658	10266964	15.54	120995	10.81
23.900	8473096	12.83	93272	8.33
36.492	7912272	11.98	52038	4.65
39.475	48388	0.07	5928	0.53
39.742	13799	0.02	2207	0.20
Totals	66056110	100.00	1119574	100.00

Graph 6.2.7: organic acid stored under vacuum conditions on 7th week

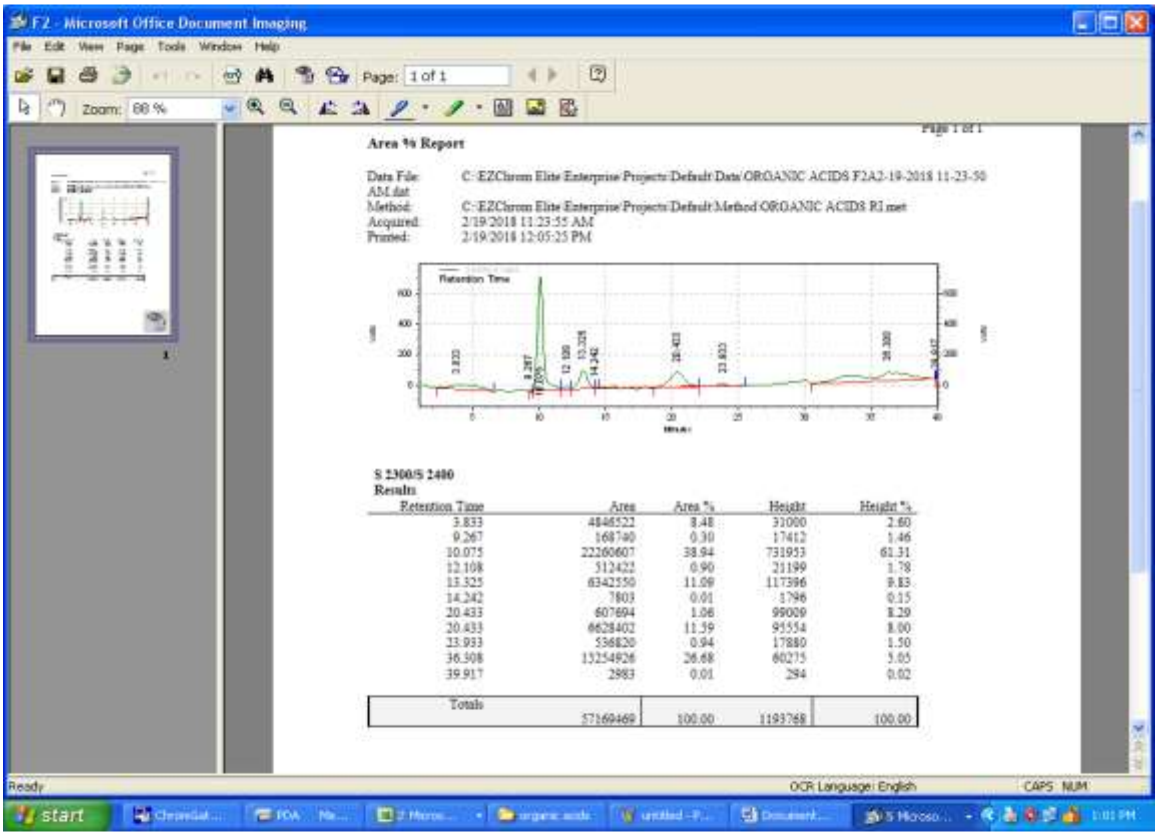


Graph 6.2.8: organic acid stored under vacuum conditions on 8th week

Appendices 6.3: cottage cheese stored under normal refrigeration temperatures (4℃) in week 1,2,3,4,5,6,7 and 8



Graph 6.3.1: organic acid under normal refrigeration conditions in 1st week



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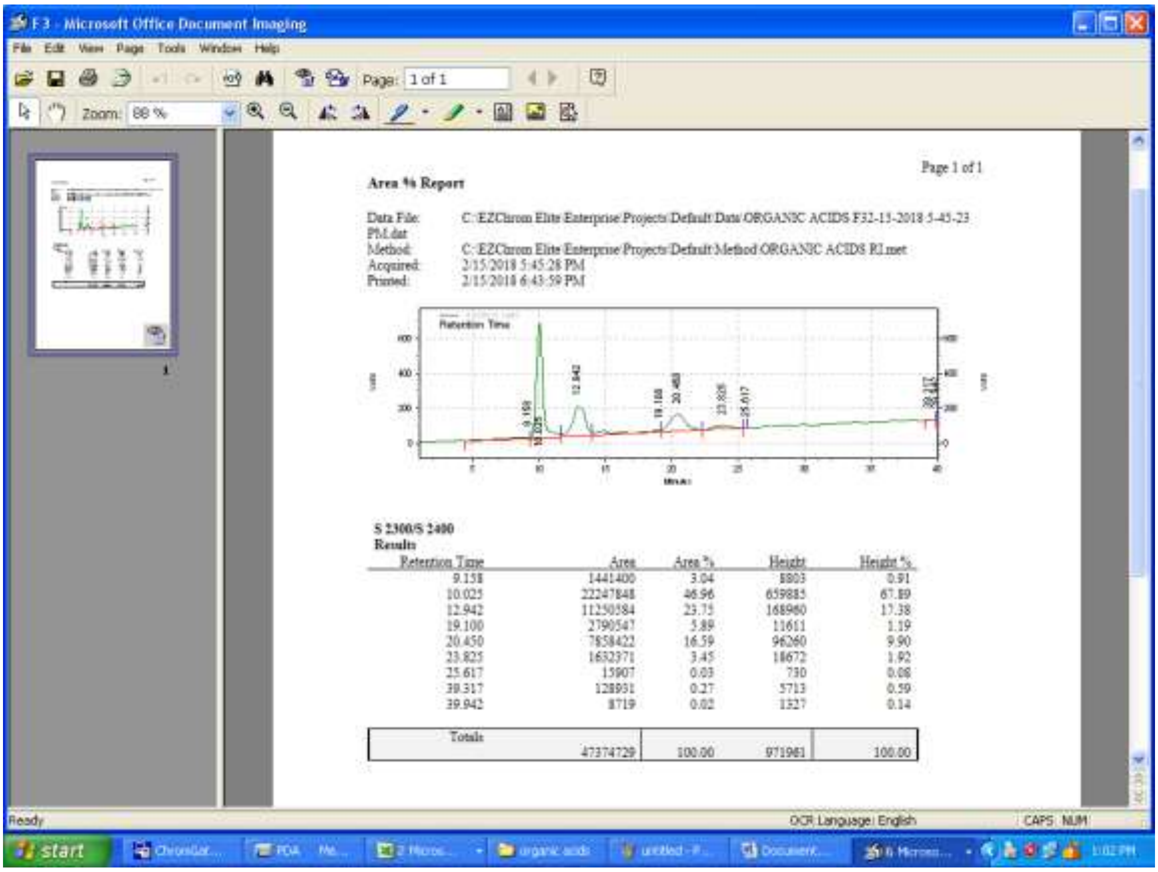
Area % Report

Data File: C:\EZChrom Elite Enterprise\Projects\Default\Data\ORGANIC ACIDS F2A2-19-2018 11-23-50 AM.dat
Method: C:\EZChrom Elite Enterprise\Projects\Default\Method\ORGANIC ACIDS RI.met
Acquired: 2/19/2018 11:23:55 AM
Printed: 2/19/2018 12:05:25 PM

S 2300/S 2400
Results

Retention Time	Area	Area %	Height	Height %
3.833	4846522	8.48	31000	2.60
9.267	168740	0.30	17412	1.46
10.075	22260607	38.94	731953	61.31
12.108	512422	0.90	21199	1.78
13.325	6342550	11.09	117396	9.83
14.242	7803	0.01	1796	0.15
20.433	607694	1.06	99009	8.29
20.433	6628402	11.59	95554	8.00
23.933	536820	0.94	17880	1.50
36.308	15254926	26.68	60275	5.05
39.917	2983	0.01	294	0.02
Totals	57169469	100.00	1193768	100.00

Graph 6.3.2: organic acids under normal refrigeration conditions in 2nd week



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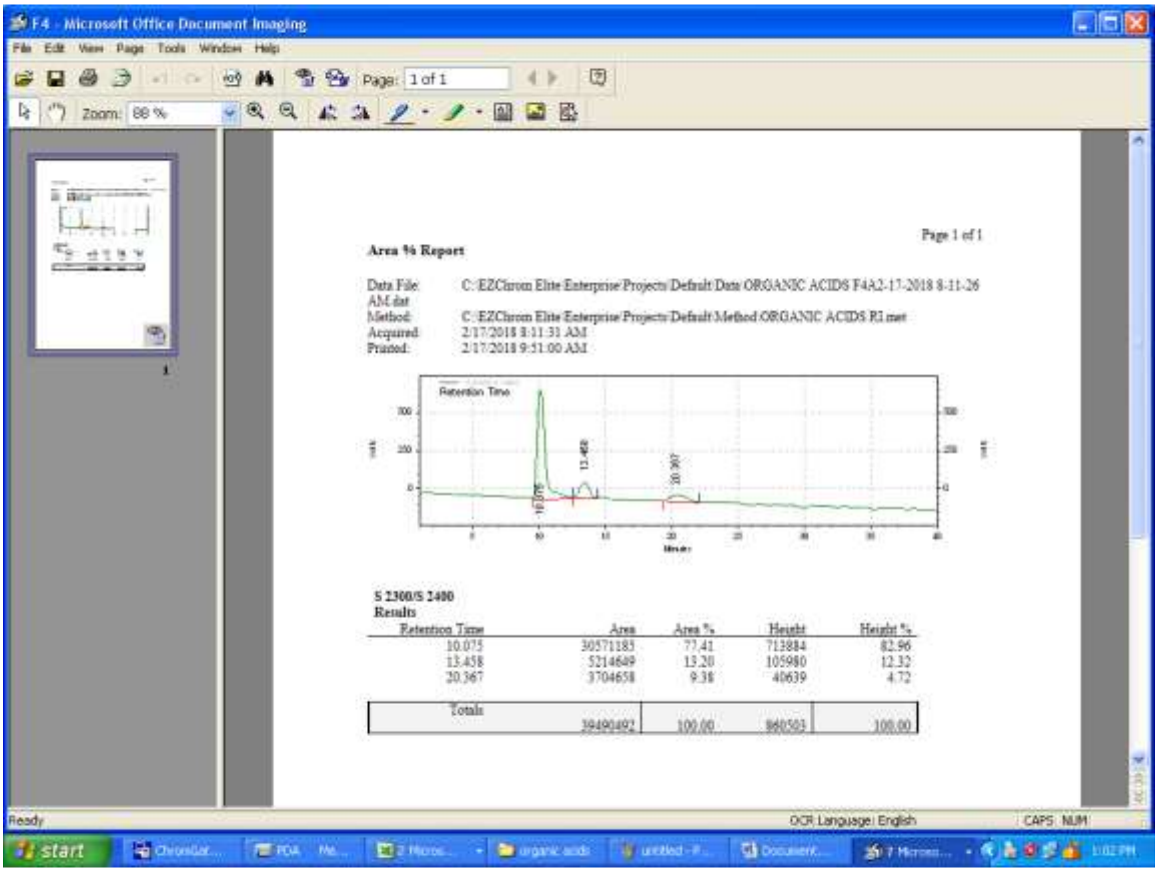
Area % Report

Data File: C:\EZChrom Elite Enterprise\Projects\Default\Data\ORGANIC ACIDS F32-15-2018 5-45-23 PM.dat
Method: C:\EZChrom Elite Enterprise\Projects\Default\Method\ORGANIC ACIDS RI.met
Acquired: 2/15/2018 5:45:28 PM
Printed: 2/15/2018 6:43:59 PM

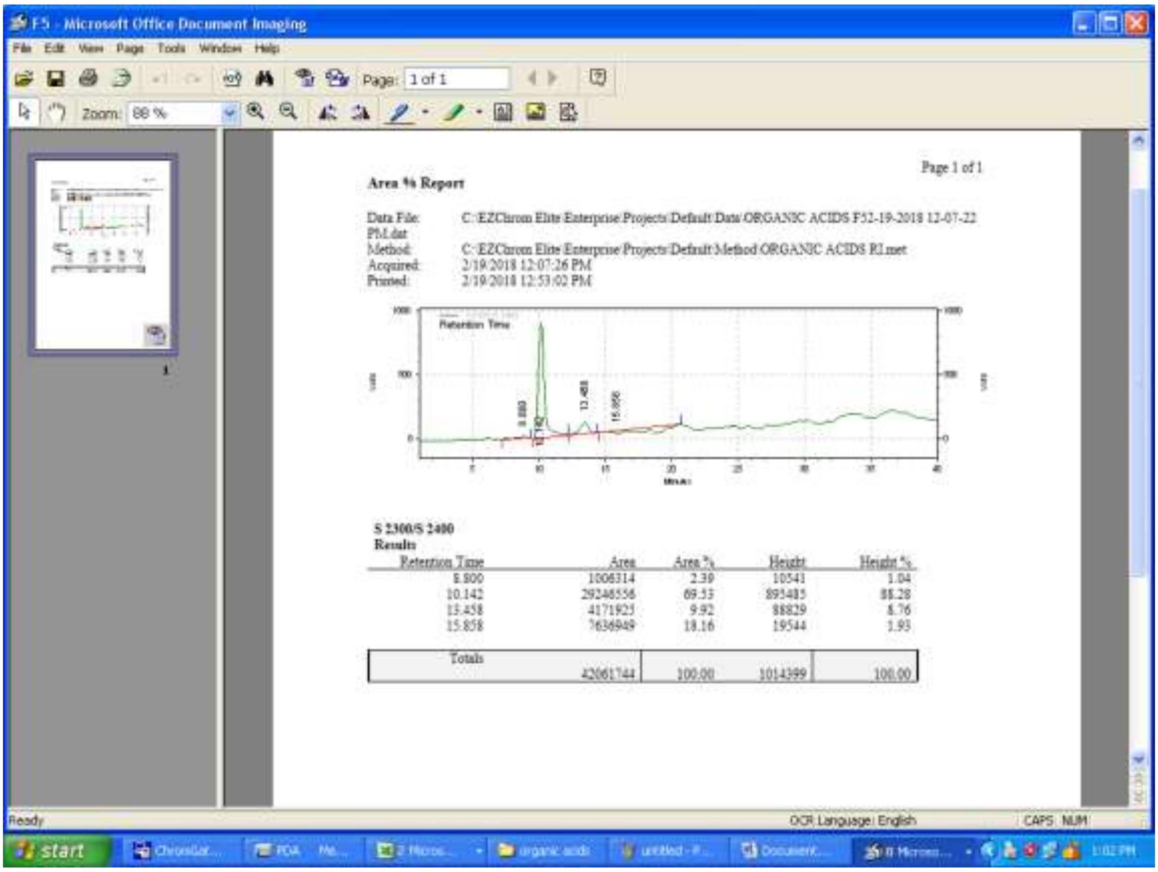
S 2300/S 2400
Results

Retention Time	Area	Area %	Height	Height %
9.158	1441400	3.04	8803	0.91
10.025	22247848	46.96	659885	67.89
12.942	11250584	23.75	168960	17.38
19.100	2790547	5.89	11611	1.19
20.450	7858422	16.59	96260	9.90
23.825	1632371	3.45	18672	1.92
25.617	15907	0.03	730	0.08
39.317	128931	0.27	5713	0.59
39.942	8719	0.02	1327	0.14
Totals	47374729	100.00	971961	100.00

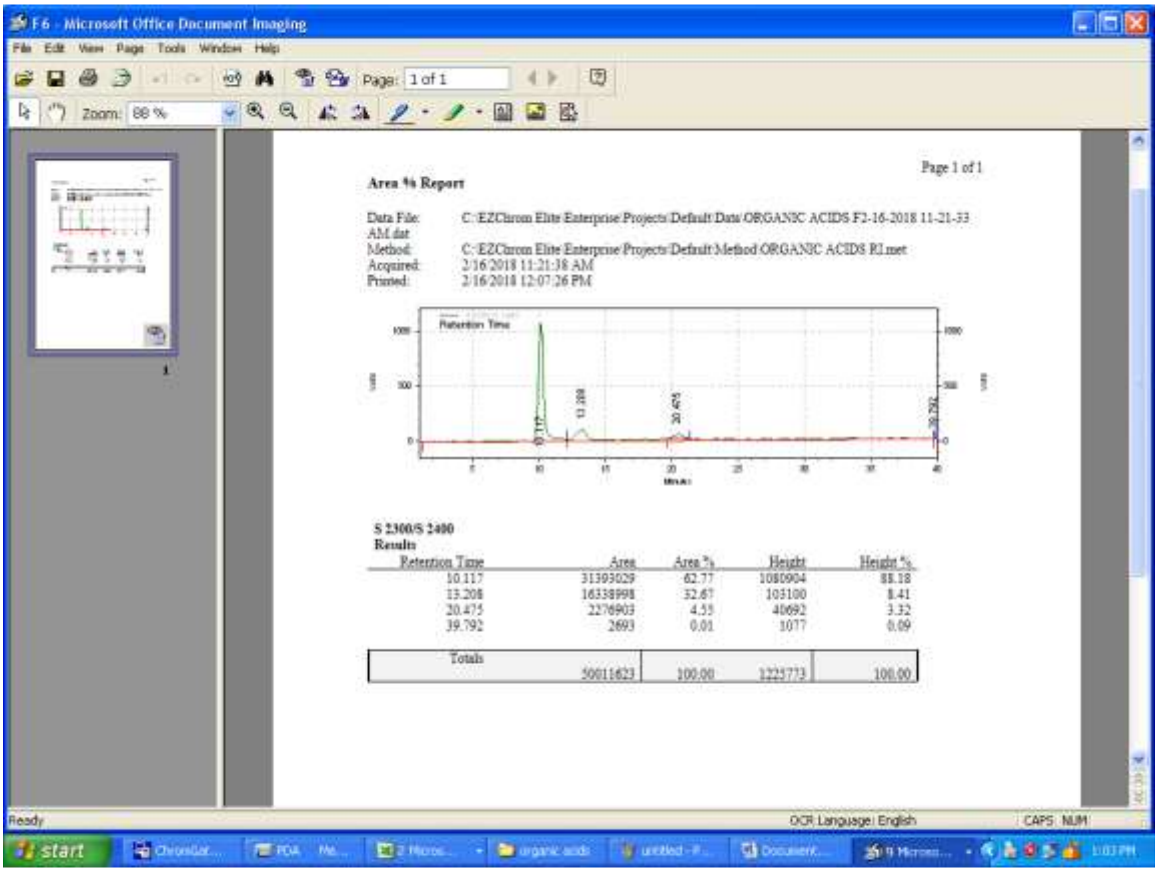
Graph 6.3.3: organic acids under refrigeration conditions in 3rd week



Graph 6.3.4: organic acid under normal refrigeration condition in 4th week

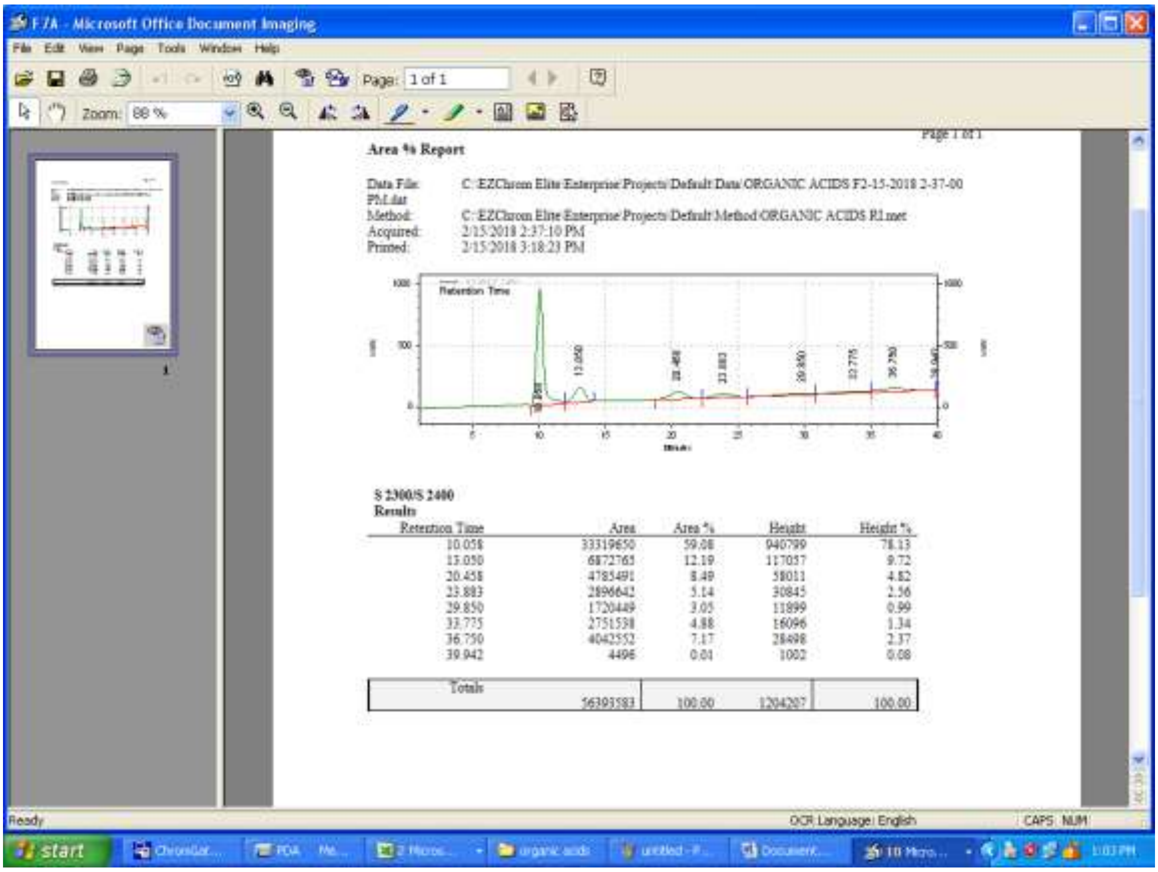


Graph 6.3.5: organic acids under normal refrigeration conditions in 5th week



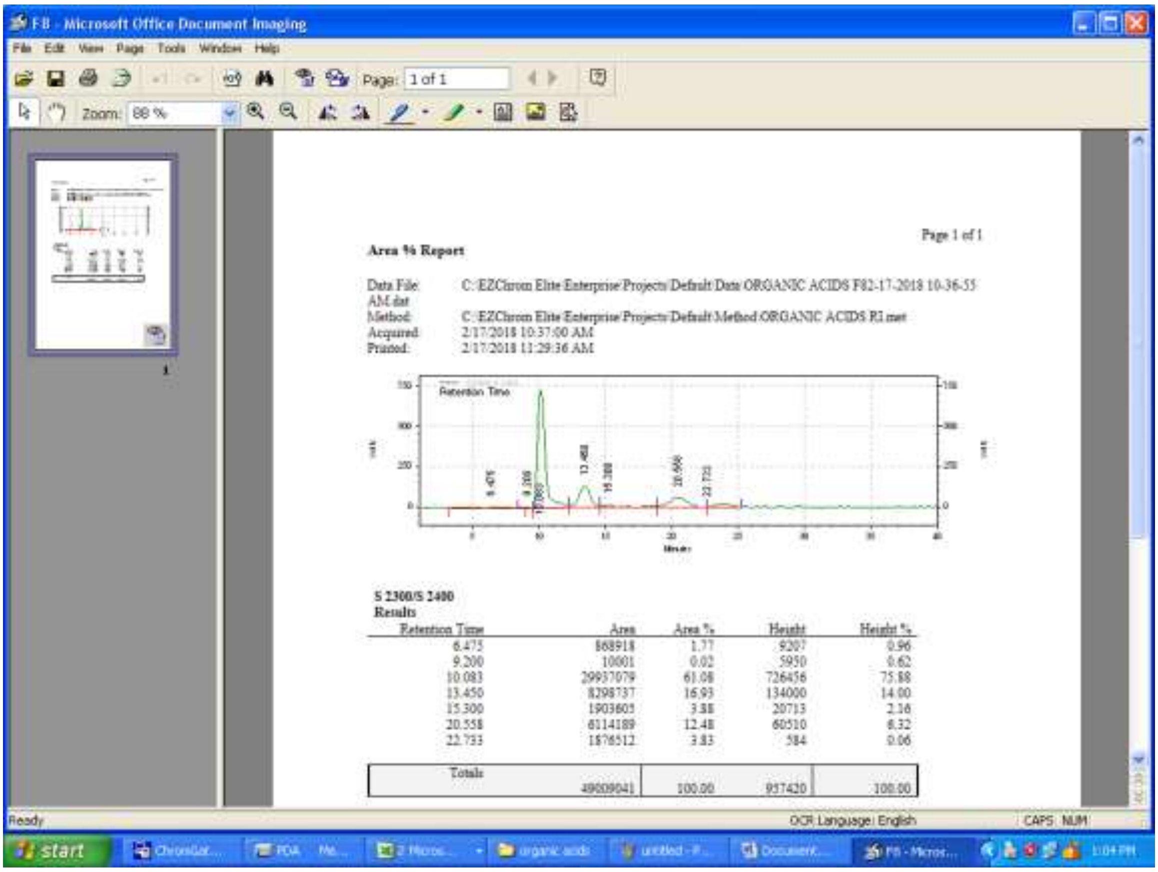
Retention Time	Area	Area %	Height	Height %
10.117	31393029	62.77	1080904	88.18
13.208	16338998	32.67	103100	8.41
20.475	2276903	4.55	40692	3.32
39.792	2693	0.01	1077	0.09
Totals	50011623	100.00	1225773	100.00

Graph 6.3.6: organic acids under normal refrigeration conditions in 6th week



Retention Time	Area	Area %	Height	Height %
10.058	33319650	59.08	940799	78.13
13.050	6872765	12.19	117057	9.72
20.458	4785491	8.49	58011	4.82
23.883	2896642	5.14	30845	2.56
29.850	1720449	3.05	11899	0.99
33.775	2751538	4.88	16096	1.34
36.750	4042552	7.17	28498	2.37
39.942	4496	0.01	1002	0.08
Totals	56393583	100.00	1204207	100.00

Graph 6.3.7: organic acids under normal refrigeration conditions in 7th week



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Area % Report

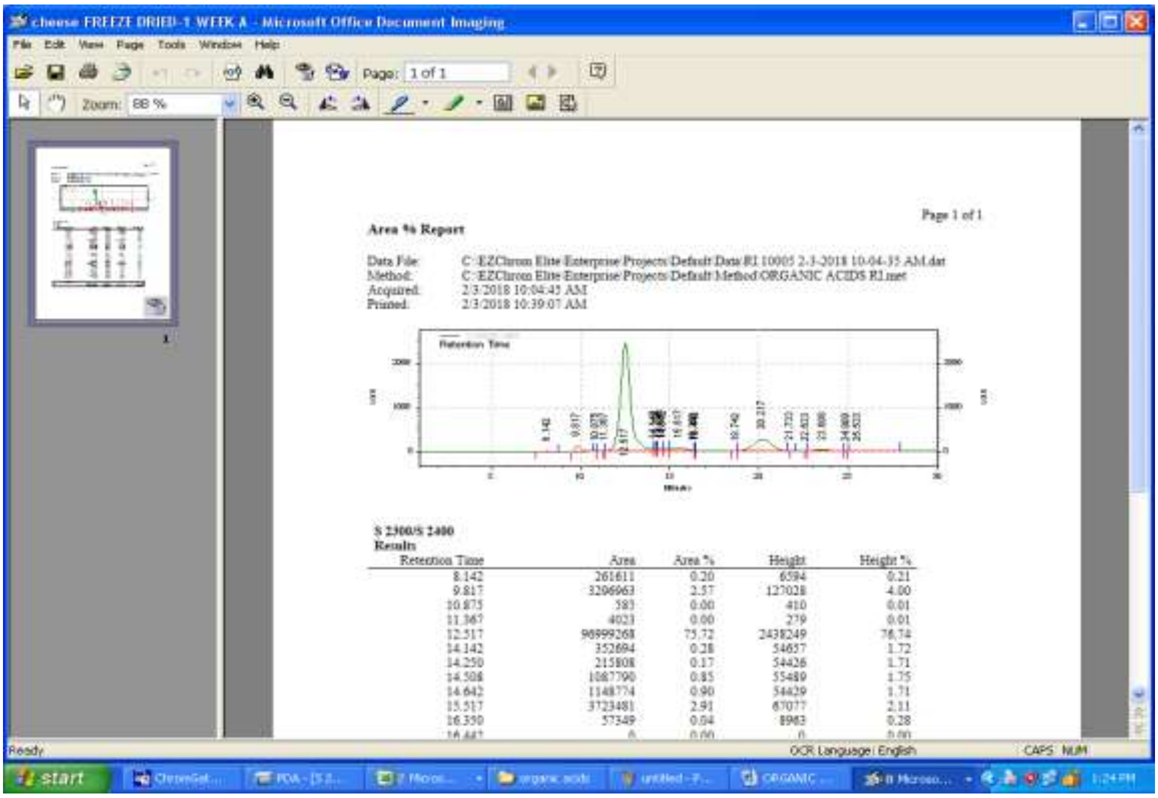
Data File: C:\EZChrom Elite Enterprise\Projects\Default\Data\ORGANIC ACIDS F82-17-2018 10-36-55 AM.dat
Method: C:\EZChrom Elite Enterprise\Projects\Default\Method\ORGANIC ACIDS RI.met
Acquired: 2/17/2018 10:37:00 AM
Printed: 2/17/2018 11:29:36 AM

S 2300/S 2400
Results

Retention Time	Area	Area %	Height	Height %
6.475	868918	1.77	9207	0.96
9.200	10001	0.02	5950	0.62
10.083	29937079	61.08	726456	75.88
13.450	8298737	16.93	134000	14.00
15.300	1903605	3.88	20713	2.16
20.558	6114189	12.48	60510	6.32
22.733	1876512	3.83	584	0.06
Totals	49009041	100.00	957420	100.00

Graph 6.3.8: organic acids under normal refrigeration conditions in 8th week

Appendices 6.4: Cottage cheese stored under deep freeze conditions in week 1, 2, 3, and6.



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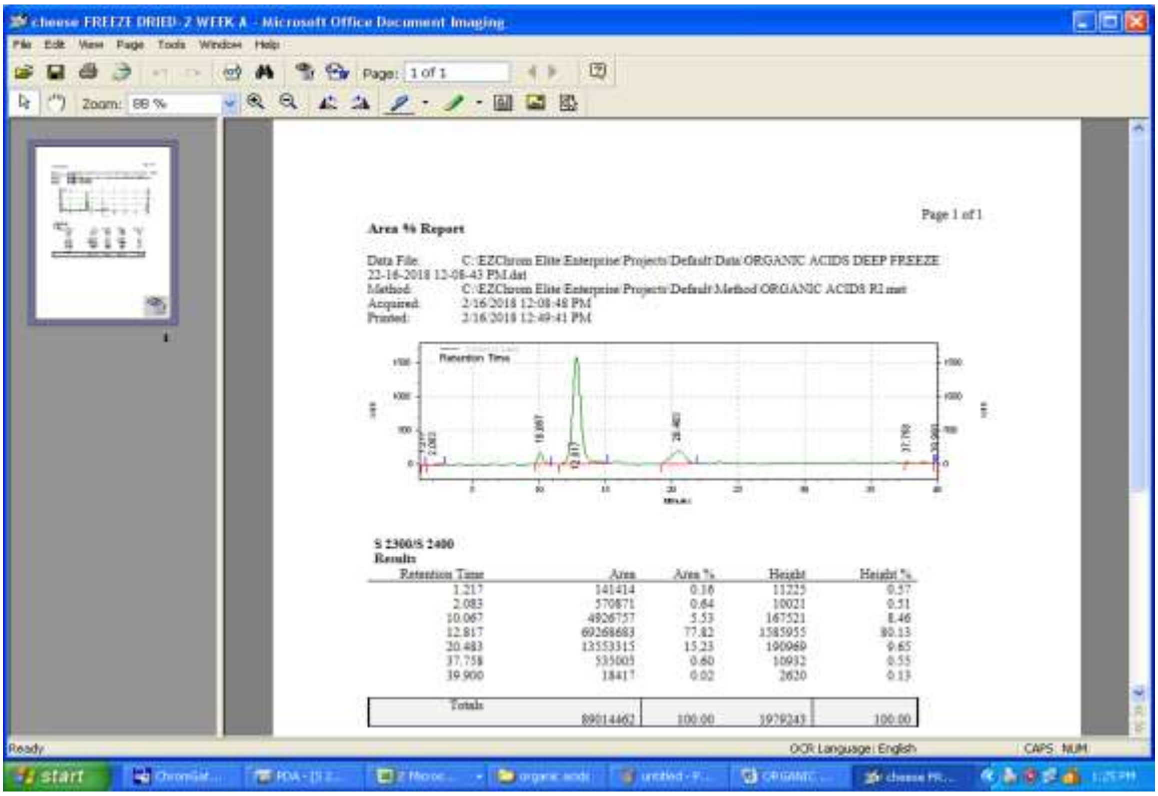
Area % Report

Data File: C:\EZChrom Elite Enterprise\Projects\Default\Data\RI 10005 2-3-2018 10-04-35 AM.dat
Method: C:\EZChrom Elite Enterprise\Projects\Default\Method\ORGANIC ACIDS RI.met
Acquired: 2/3/2018 10:04:45 AM
Printed: 2/3/2018 10:39:07 AM

S 2300/S 2400
Results

Retention Time	Area	Area %	Height	Height %
8.142	261611	0.20	6594	0.21
9.817	3296963	2.57	127028	4.00
10.875	383	0.00	410	0.01
11.367	4023	0.00	279	0.01
12.517	96999268	75.72	2438249	76.74
14.142	352694	0.28	54657	1.72
14.250	215808	0.17	54426	1.71
14.508	1087790	0.85	55489	1.75
14.642	1148774	0.90	54429	1.71
15.517	3723481	2.91	67077	2.11
16.350	57349	0.04	8963	0.28
16.447	0	0.00	0	0.00

Graph 6.4.1: organic acids under freeze dried conditions in 1st week


cheese FREEZE DRIED-2 WEEK A - Microsoft Office Document Imaging

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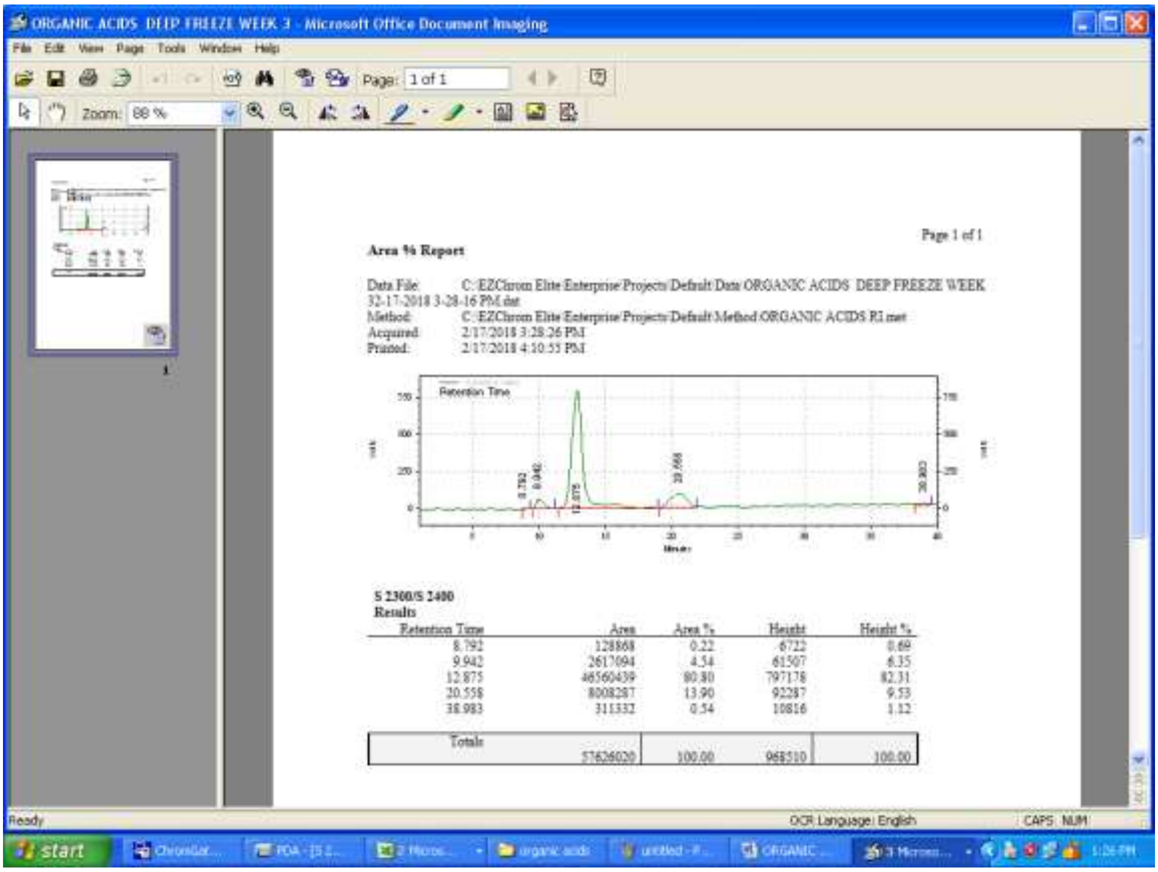
Area % Report

Data File: C:\EZChrom Elite Enterprise\Projects\Default\Data\ORGANIC ACIDS DEEP FREEZE 22-16-2018 12-08-43 PM.dat
Method: C:\EZChrom Elite Enterprise\Projects\Default\Method\ORGANIC ACIDS RI.met
Acquired: 2/16/2018 12:08:48 PM
Printed: 2/16/2018 12:49:41 PM

S 2300/S 2400
Results

Retention Time	Area	Area %	Height	Height %
1.217	141414	0.16	11225	0.57
2.083	570871	0.64	10021	0.51
10.067	4926757	5.53	167521	8.46
12.817	69268683	77.82	1585955	80.13
20.483	13553315	15.23	190969	9.65
37.758	535005	0.60	10932	0.55
39.900	18417	0.02	2620	0.13
Totals	89014462	100.00	1979243	100.00

Graph 6.4.2: organic acids under freeze dried conditions in 2nd week


ORGANIC ACIDS DEEP FREEZE WEEK 3 - Microsoft Office Document Imaging

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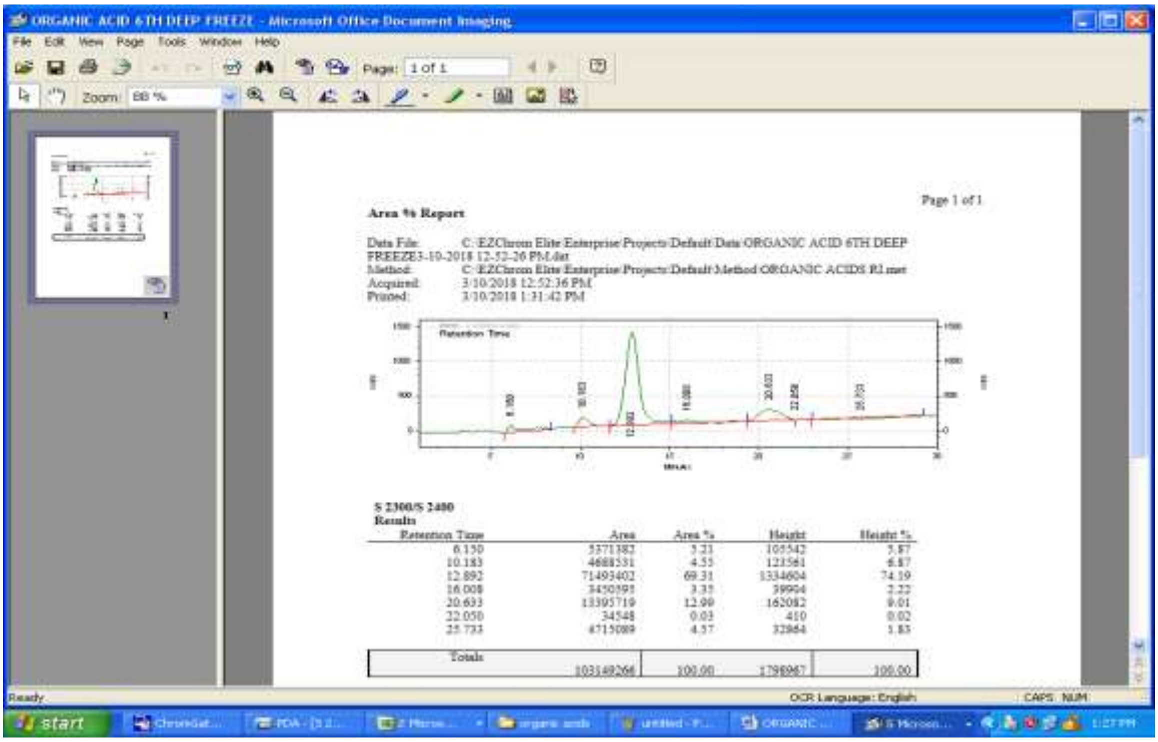
Area % Report

Data File: C:\EZChrom Elite Enterprise\Projects\Default\Data\ORGANIC ACIDS DEEP FREEZE WEEK 32-17-2018 3-28-16 PM.dat
Method: C:\EZChrom Elite Enterprise\Projects\Default\Method\ORGANIC ACIDS RI.met
Acquired: 2/17/2018 3:28:26 PM
Printed: 2/17/2018 4:10:55 PM

S 2300/S 2400
Results

Retention Time	Area	Area %	Height	Height %
8.792	128868	0.22	6722	0.69
9.942	2617094	4.54	61507	6.35
12.875	46560439	80.80	797178	82.31
20.558	8008287	13.90	92287	9.53
38.983	311332	0.54	10816	1.12
Totals	57626020	100.00	968510	100.00

Graph 6.4.3: organic acids under freeze dried conditions in 3rd week



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Area % Report

Data File: C:\EZChrom Elite\Enterprise\Projects\Default\Data\ORGANIC ACID 6TH DEEP FREEZE3-10-2018 12-52-26 PM.dat
Method: C:\EZChrom Elite\Enterprise\Projects\Default\Method\ORGANIC ACIDS RI.met
Acquired: 3/10/2018 12:52:36 PM
Printed: 3/10/2018 1:31:42 PM

S 2300/S 2400
Results

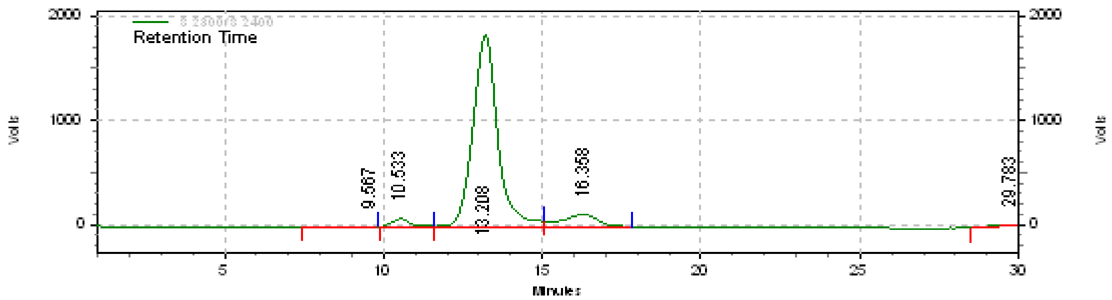
Retention Time	Area	Area %	Height	Height %
6.150	5371382	5.21	105542	5.87
10.183	4688531	4.55	123561	6.87
12.892	71493402	69.31	1334604	74.19
16.008	3450595	3.35	39994	2.22
20.633	13395719	12.99	162082	9.01
22.050	34548	0.03	410	0.02
25.733	4715089	4.57	32864	1.83
Totals	103149266	100.00	1798967	100.00

Graph 6.4.4: organic acids under freeze dried conditions in 6th week

Appendices 6.5: cottage cheese fresh samples analyses in week 1,2,3,4

Area % Report

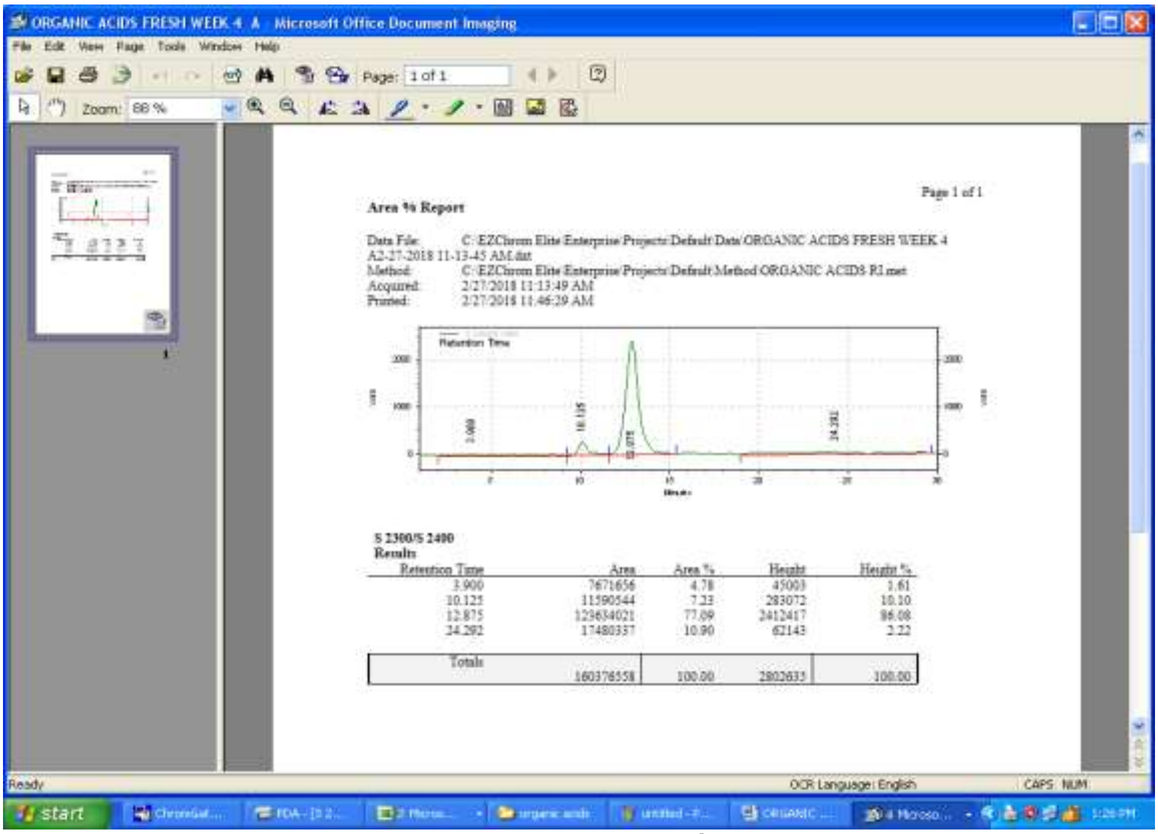
Data File: C:\EZChrom Elite\Enterprise\Projects\Default\Data\RI.10005 2-6-2018 11-49-59 AM.dat
Method: C:\EZChrom Elite\Enterprise\Projects\Default\Method\ORGANIC ACIDS RI.met
Acquired: 2/6/2018 11:50:09 AM
Printed: 2/6/2018 12:22:14 PM



S 2300/S 2400
Results

Retention Time	Area	Area %	Height	Height %
9.567	545430	0.48	5068	0.25
10.533	3165300	2.80	77823	3.81
13.208	99683379	88.15	1837241	89.96
16.358	9279312	8.21	117342	5.75
29.783	406856	0.36	4919	0.24
Totals	113080277	100.00	2042393	100.00

Graph 6.5.1: organic acid of fresh sample in week 1



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Area % Report

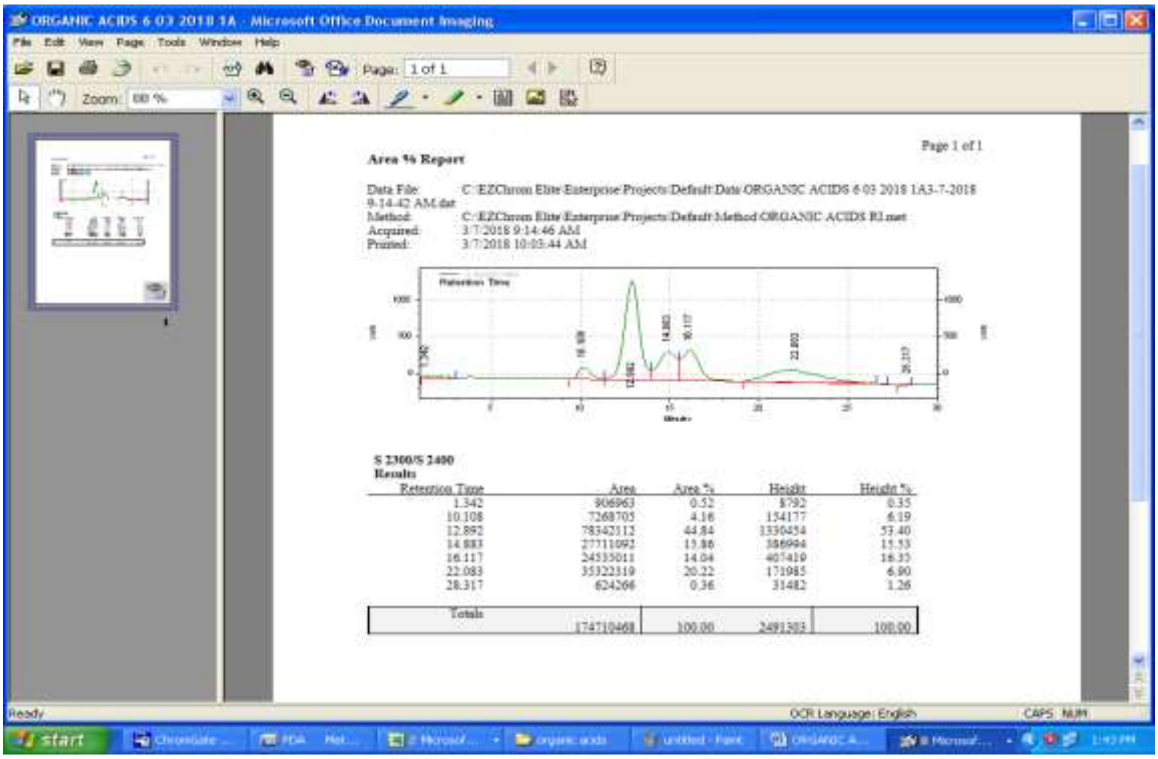
Data File: C:\EZChrom Elite Enterprise\Projects\Default\Data\ORGANIC ACIDS FRESH WEEK 4 A2-27-2018 11-13-45 AM.dat
Method: C:\EZChrom Elite Enterprise\Projects\Default\Method\ORGANIC ACIDS RI.met
Acquired: 2/27/2018 11:13:49 AM
Printed: 2/27/2018 11:46:29 AM

S 2300/S 2400
Results

Retention Time	Area	Area %	Height	Height %
3.900	7671656	4.78	45003	1.61
10.125	11590544	7.23	283072	10.10
12.875	123634021	77.09	2412417	86.08
24.292	17480337	10.90	62143	2.22
Totals	160376558	100.00	2802635	100.00

Graph 6.5.2: organic acid under fresh conditions 4th week

Appendices 6.6: Cottage cheese mixed with honey at concentration of 10%, 20% and 30% stored under normal conditions within the first week.



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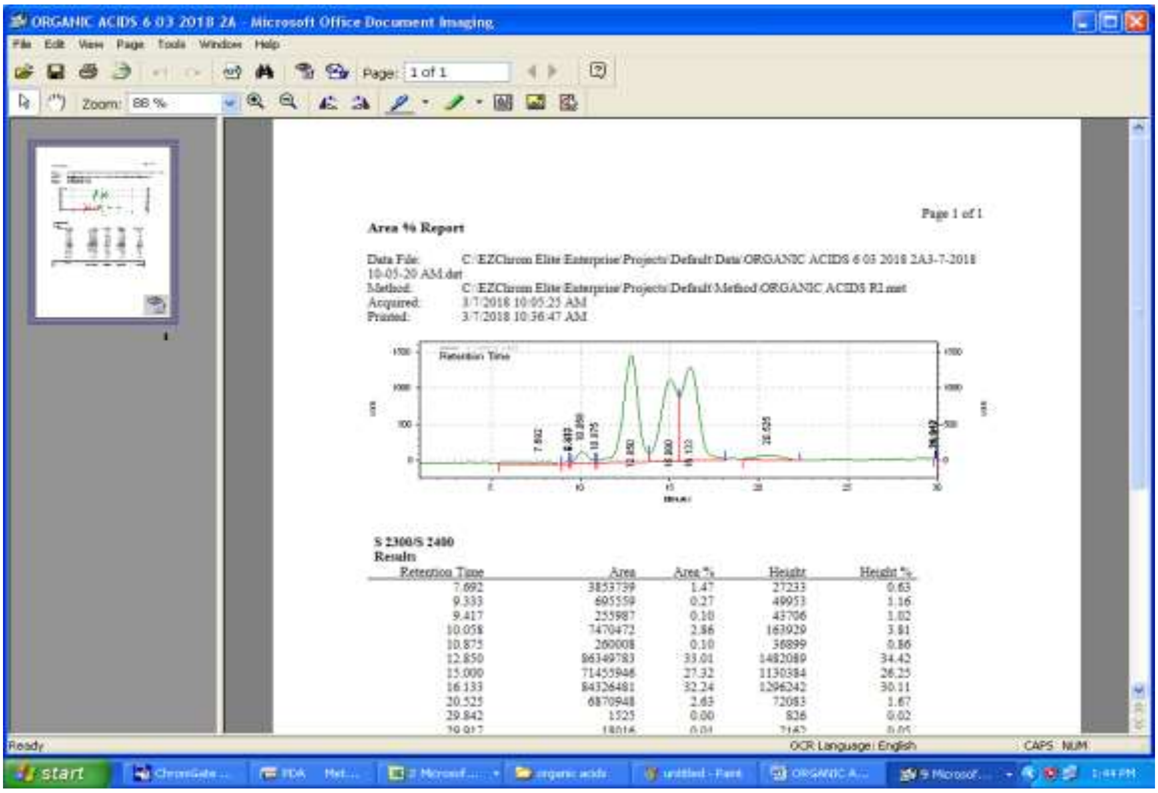
Area % Report

Data File: C:\EZChrom Elite Enterprise\Projects\Default\Data\ORGANIC ACIDS 6 03 2018 1A3-7-2018 9-14-42 AM.dat
Method: C:\EZChrom Elite Enterprise\Projects\Default\Method\ORGANIC ACIDS RI.met
Acquired: 3/7/2018 9:14:46 AM
Printed: 3/7/2018 10:03:44 AM

S 2300/S 2400
Results

Retention Time	Area	Area %	Height	Height %
1.342	906963	0.52	8792	0.35
10.108	7268705	4.16	154177	6.19
12.892	78342112	44.84	1330454	53.40
14.883	27711092	15.86	386994	15.53
16.117	24535011	14.04	407419	16.35
22.083	35322319	20.22	171985	6.90
28.317	624266	0.36	31482	1.26
Totals	174710468	100.00	2491303	100.00

Graph 6.6.1: organic acids from cheese under normal conditions at day 4 of storage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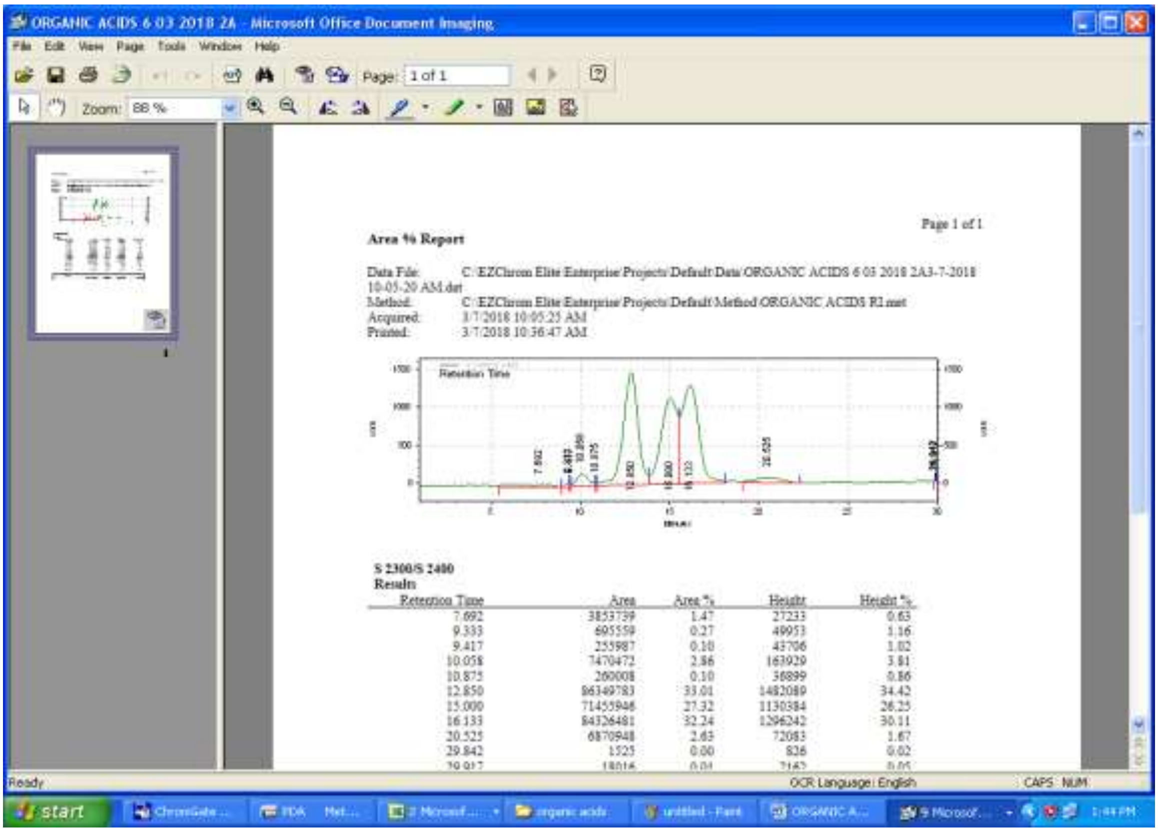
Area % Report

Data File: C:\EZChrom Elite Enterprise\Projects\Default\Data\ORGANIC ACIDS 6 03 2018 2A\3-7-2018 10-05-20 AM.dat
Method: C:\EZChrom Elite Enterprise\Projects\Default\Method\ORGANIC ACIDS RI.met
Acquired: 3/7/2018 10:05:25 AM
Printed: 3/7/2018 10:36:47 AM

S 2300/S 2400
Results

Retention Time	Area	Area %	Height	Height %
7.692	3853739	1.47	27233	0.63
9.333	695559	0.27	49953	1.16
9.417	255987	0.10	43706	1.02
10.058	7470472	2.86	163929	3.81
10.875	260008	0.10	36899	0.86
12.850	86349783	33.01	1482089	34.42
15.000	71455946	27.32	1130384	26.25
16.133	84326481	32.24	1296242	30.11
20.525	6870948	2.63	72083	1.67
29.842	1525	0.00	826	0.02

Graph 6.6.2: organic acids and sugars from cheese mixed with 10% honey under normal conditions at day 4 of storage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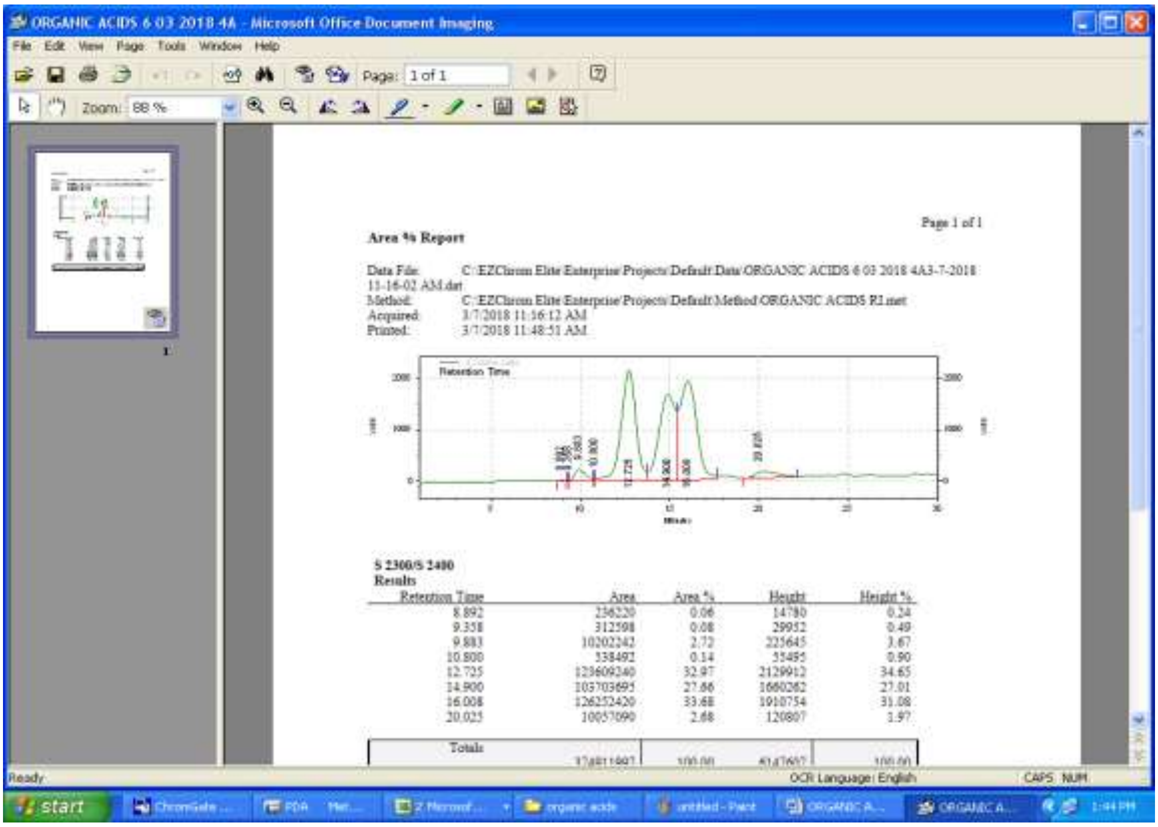
Area % Report

Data File: C:\EZChrom Elite Enterprise\Projects\Default\Data\ORGANIC ACIDS 6 03 2018 2A\3-7-2018 10-05-20 AM.dat
Method: C:\EZChrom Elite Enterprise\Projects\Default\Method\ORGANIC ACIDS RI.met
Acquired: 3/7/2018 10:05:25 AM
Printed: 3/7/2018 10:36:47 AM

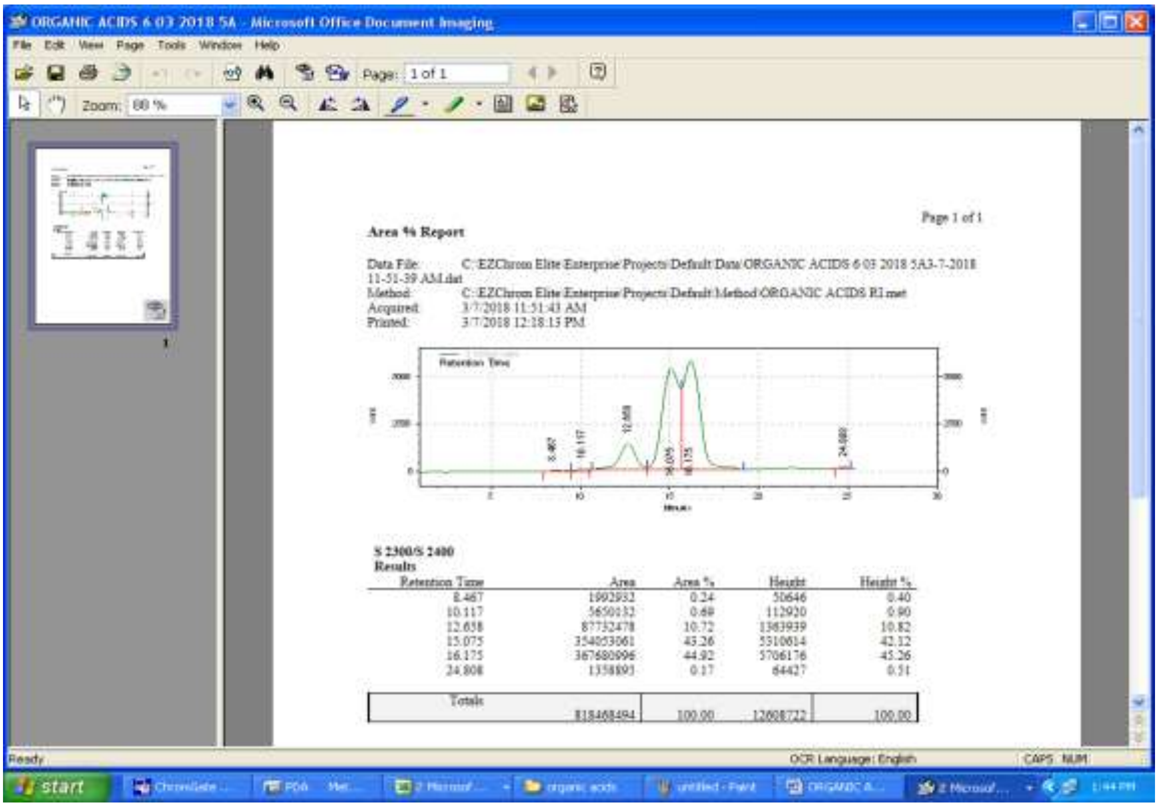
S 2300/S 2400
Results

Retention Time	Area	Area %	Height	Height %
7.692	3853739	1.47	27233	0.63
9.333	695559	0.27	49953	1.16
9.417	255987	0.10	43706	1.02
10.058	7470472	2.86	163929	3.81
10.875	260008	0.10	36899	0.86
12.850	86349783	33.01	1482089	34.42
15.000	71455946	27.32	1130384	26.25
16.133	84326481	32.24	1296242	30.11
20.525	6870948	2.63	72083	1.67
29.842	1525	0.00	826	0.02

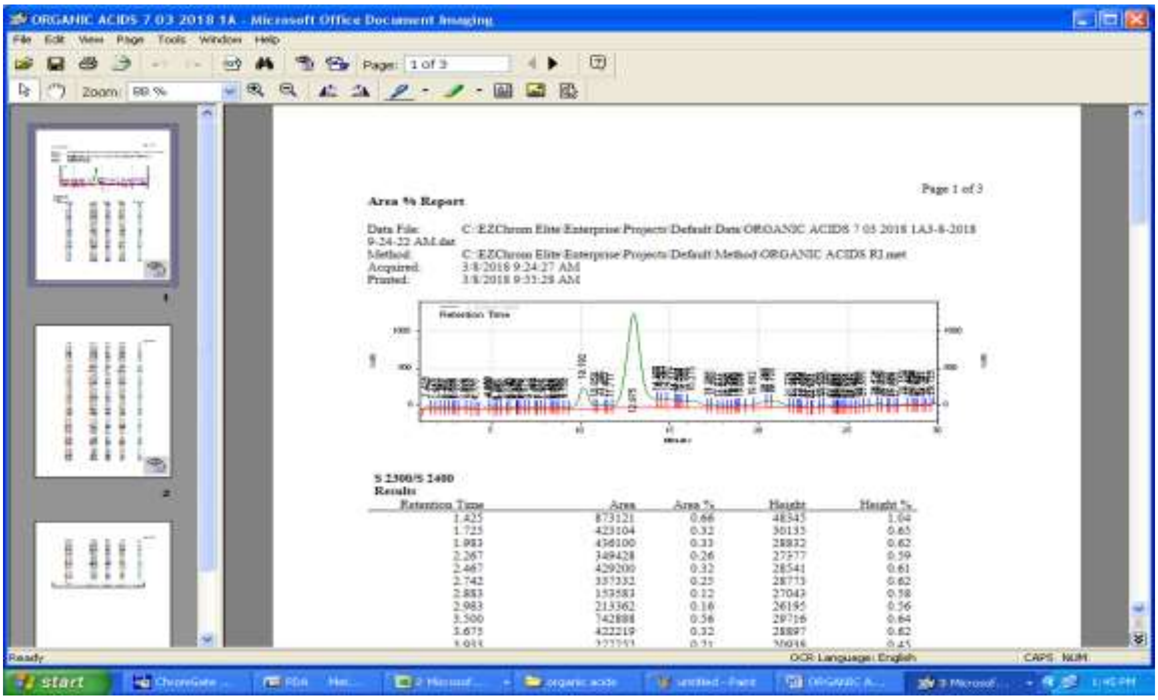
Graph 6.6.3: organic acids and sugars from cheese mixed with 20% honey under normal conditions at day 4 of storage



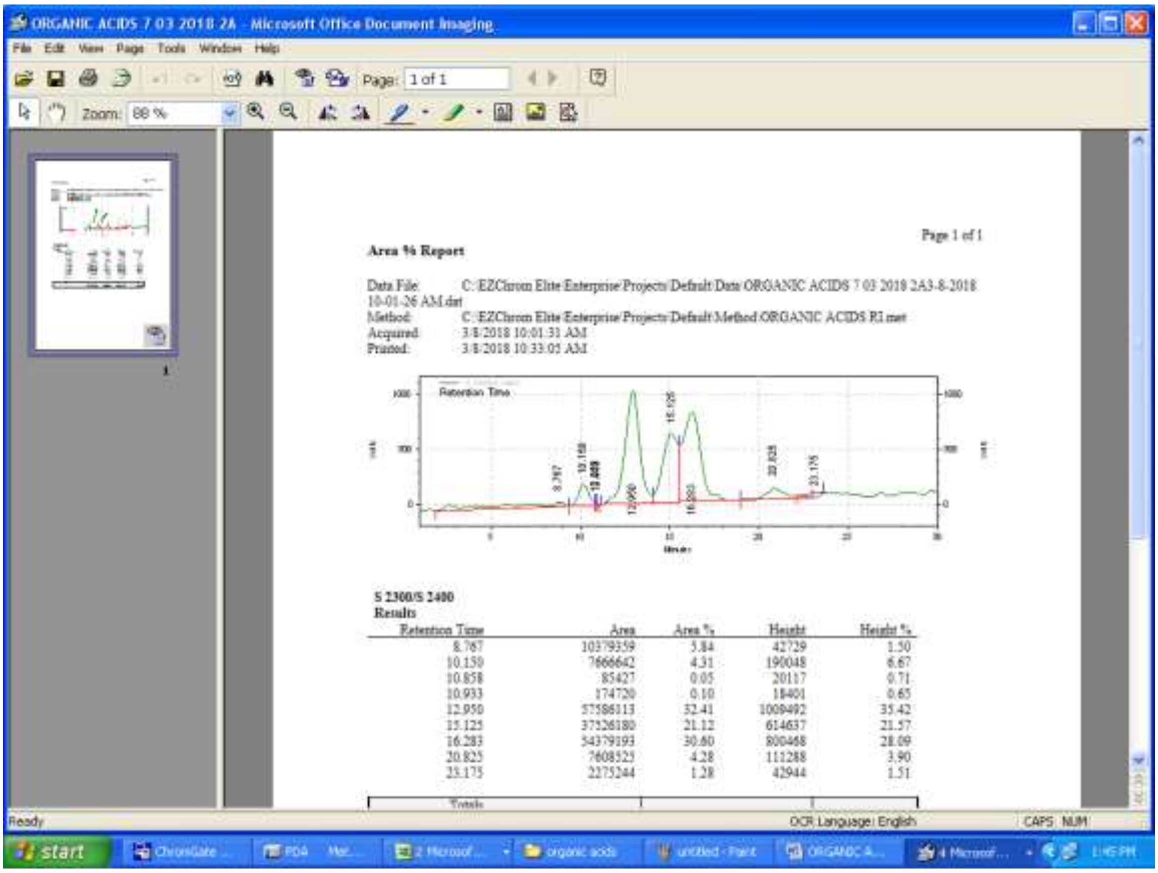
Graph 6.6.4: organic acids and sugars from cheese mixed with 30% honey under normal conditions at day 4 of storage



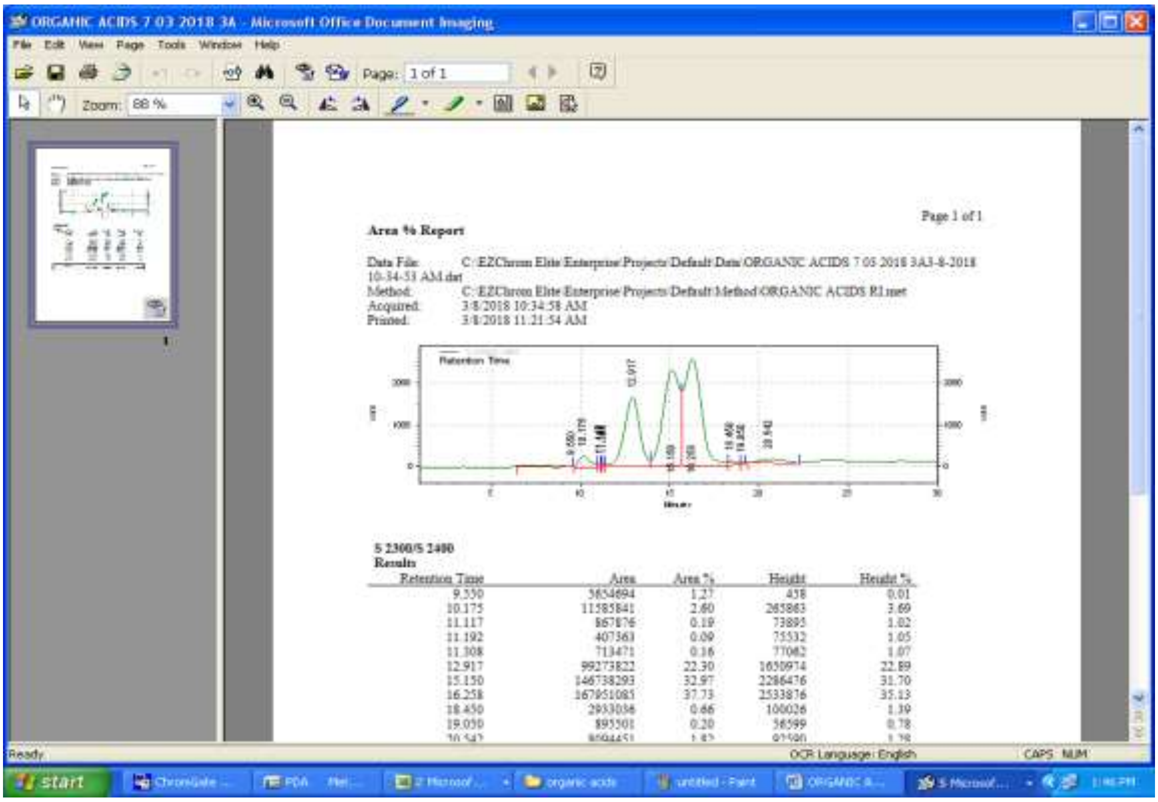
Graph 6.6.5: organic acids and sugars from pure honey at day 4



Graph 6.6.6: organic acids under normal conditions at day 5 of storage in open condition



Graph 6.6.7: organic acids and sugars from cheese mixed with 10% honey under normal conditions at day 5 of storage



ORGANIC ACIDS 7 03 2018 3A - Microsoft Office Document Imaging

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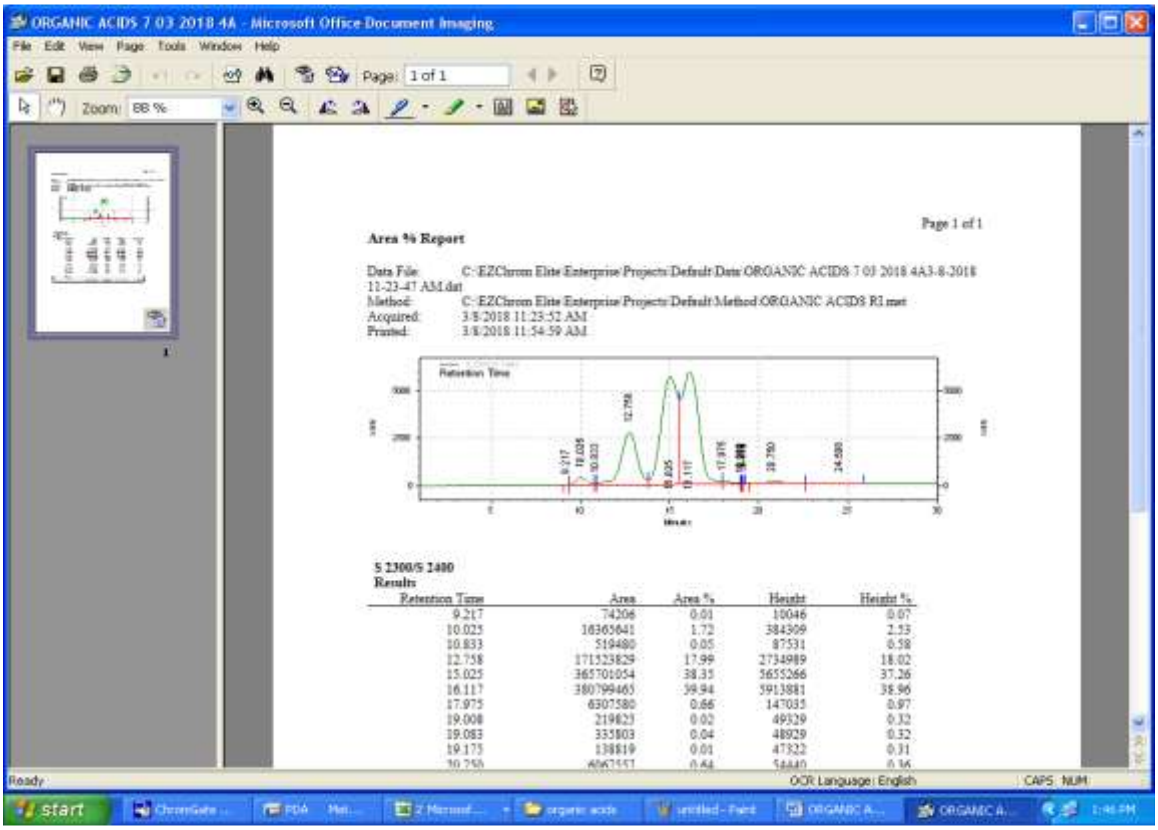
Area % Report

Data File: C:\EZChrom Elite\Enterprise\Projects\Default\Data\ORGANIC ACIDS 7 03 2018 3A3-8-2018 10-34-53 AM.dat
Method: C:\EZChrom Elite\Enterprise\Projects\Default\Method\ORGANIC ACIDS RI.met
Acquired: 3/8/2018 10:34:58 AM
Printed: 3/8/2018 11:21:54 AM

S 2300/S 2400
Results

Retention Time	Area	Area %	Height	Height %
9.550	5654694	1.27	458	0.01
10.175	11585841	2.60	265863	3.69
11.117	867876	0.19	73895	1.02
11.192	407363	0.09	75532	1.05
11.308	713471	0.16	77082	1.07
12.917	99273822	22.30	1650974	22.89
15.150	146738293	32.97	2286476	31.70
16.258	167951085	37.73	2533876	35.13
18.450	2933036	0.66	100026	1.39
19.050	895501	0.20	56599	0.78
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Graph 6.6.8: organic acids and sugars from cheese mixed with 20% honey under normal conditions at day 5 of storage



ORGANIC ACIDS 7 03 2018 4A - Microsoft Office Document Imaging

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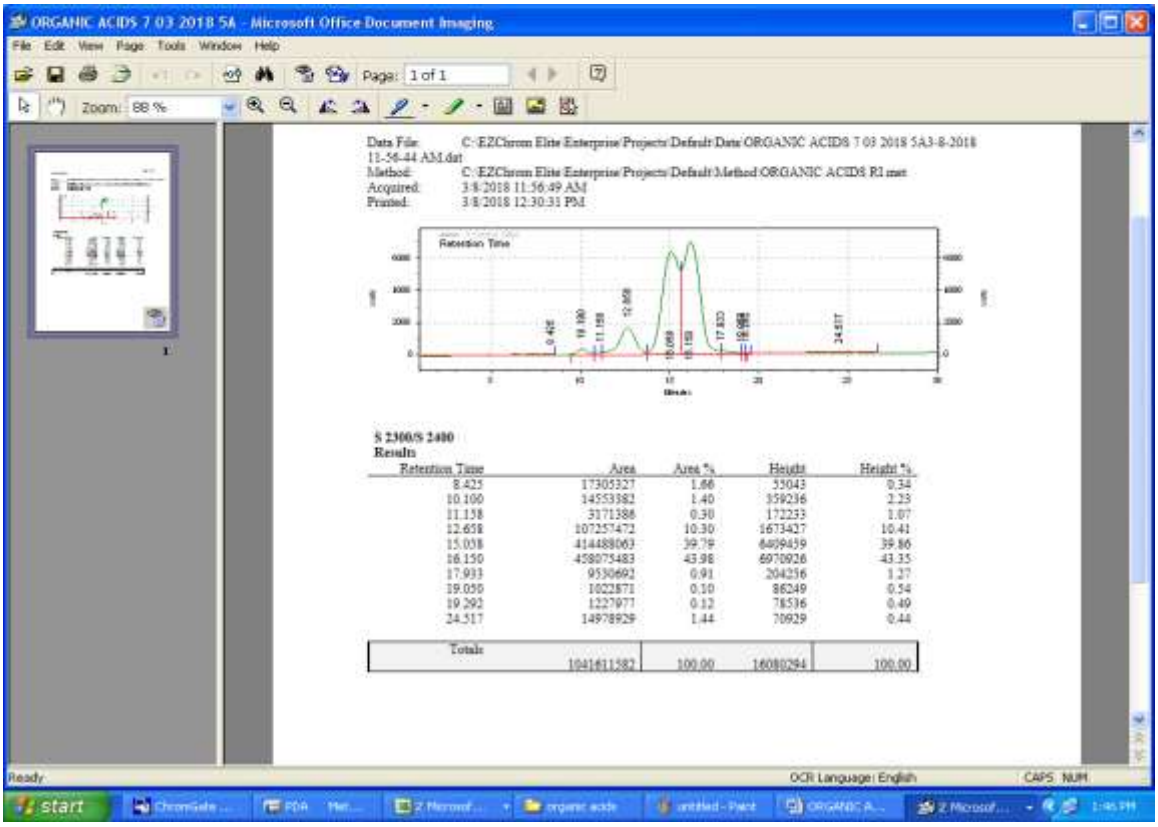
Area % Report

Data File: C:\EZChrom Elite\Enterprise\Projects\Default\Data\ORGANIC ACIDS 7 03 2018 4A3-8-2018 11-23-47 AM.dat
Method: C:\EZChrom Elite\Enterprise\Projects\Default\Method\ORGANIC ACIDS RI.met
Acquired: 3/8/2018 11:23:52 AM
Printed: 3/8/2018 11:54:59 AM

S 2300/S 2400
Results

Retention Time	Area	Area %	Height	Height %
9.217	74206	0.01	10046	0.07
10.025	16365641	1.72	384309	2.53
10.833	519480	0.05	87531	0.58
12.758	171523829	17.99	2734989	18.02
15.025	365701054	38.35	5655266	37.26
16.117	380799465	39.94	5913881	38.96
17.975	6307580	0.66	147035	0.97
19.008	219825	0.02	49329	0.32
19.083	335803	0.04	48929	0.32
19.175	138819	0.01	47322	0.31
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Graph 6.6.9: organic acids and sugars from cheese mixed with 30% honey under normal conditions at day 5 of storage


ORGANIC ACIDS 7 03 2018 5A - Microsoft Office Document Imaging

Data File: C:\EZChrom Elite Enterprise\Projects\Default\Data\ORGANIC ACIDS 7 03 2018 5A3-8-2018 11-56-44 AM.dat
Method: C:\EZChrom Elite Enterprise\Projects\Default\Method\ORGANIC ACIDS RI.met
Acquired: 3/8/2018 11:56:49 AM
Printed: 3/8/2018 12:30:31 PM

S 2300/S 2400
Results

Retention Time	Area	Area %	Height	Height %
8.425	17305327	1.66	55043	0.34
10.100	14553382	1.40	359236	2.23
11.158	3171386	0.30	172233	1.07
12.658	107257472	10.30	1673427	10.41
15.058	414488063	39.79	6409459	39.86
16.150	458075483	43.98	6970926	43.35
17.933	9530692	0.91	204256	1.27
19.050	1022871	0.10	86249	0.54
19.292	1227977	0.12	78536	0.49
24.517	14978929	1.44	70929	0.44
Totals	1041611582	100.00	16080294	100.00

Graph 6.7.0: Organic acids and sugars from pure honey at day 5 of storage.

Appendices 7: instruments used during analysis.

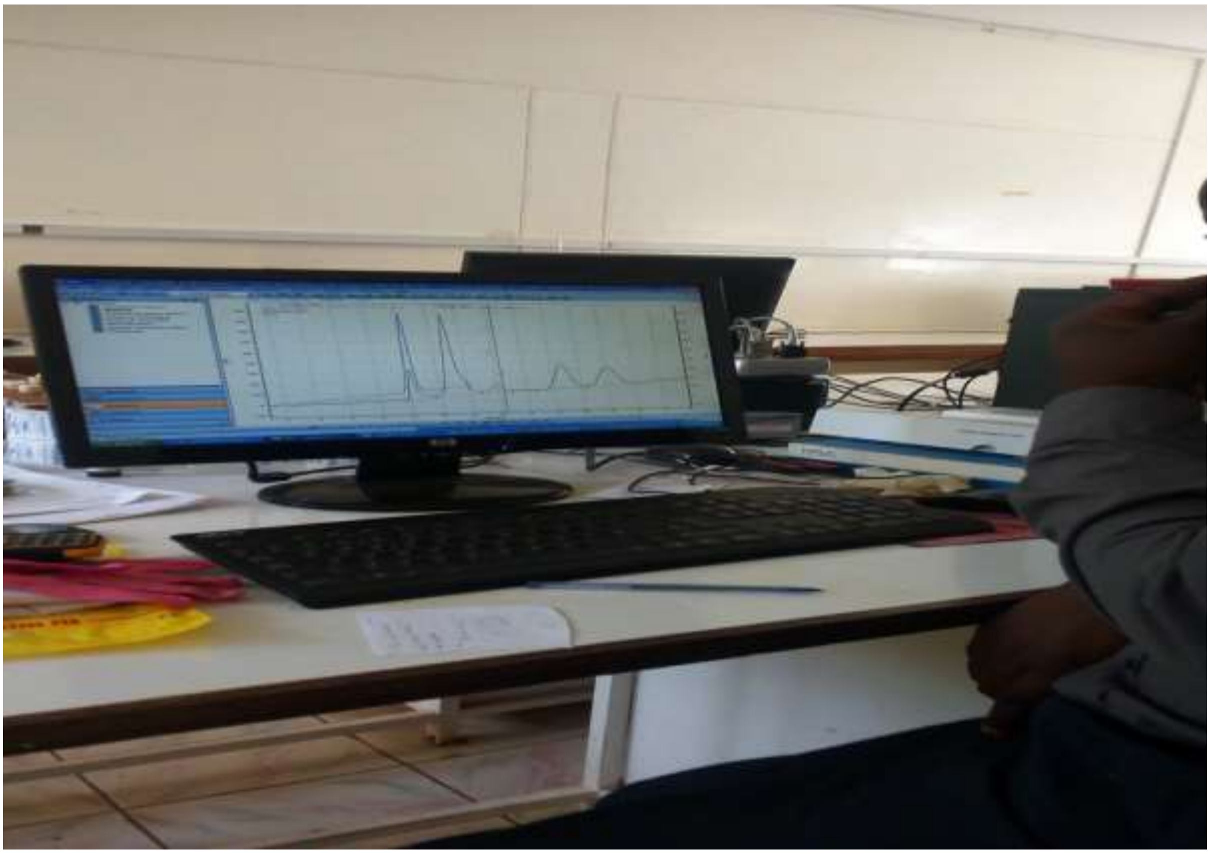


Diagram 7.1: Computer display during analysis

Diagram 7.2: HPLC machine used for analysis

Diagram 7.3: Detectors used during analysis

Diagram 7.4: ultrasonic bath for homogenization used during analysis

EFFECTS OF STORAGE CONDITIONS ON ORGANIC ACIDS CONTENT AND SENSORIAL PROPERTIES OF COTTAGE CHEESE

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