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PRESERVATION OF CUT PINEAPPLES AND JUICES FROM TWO VARIETIES USING NATAMYCIN.

BY

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A THESIS SUBMITTED TO THE DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY IN PARTIAL FULFILMENT OF THE REQUIREMENTS OF MASTER OF SCIENCE IN FOOD SCIENCE AND TECHNOLOGY.

DECLARATION

I hereby declare that this work, except where duly acknowledged, is a true reflection of my research work carried out at the Department of Food Science and Technology in the Kwame Nkrumah University of Science and Technology under the able supervision of Rev.J Adubofuor a lecturer in this Department.

This work has never been presented either in whole or part for any award to the Kwame Nkrumah University of science and Technology.

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ABSTRACT

Fresh-cut fruit and juice are increasingly becoming popular with consumers because of its convenience. A major challenge faced by this industry is the appropriate method of preservation to maintain the quality of fresh -cut fruits and juices so that the shelf-life is long enough to ensure efficient marketing. This study was done to evaluate the effects of natamycin on the physiochemical, sensory properties and microbial stability of cut pineapple and juices from MD-2 and Sugar loaf during thirty six days of storage. Natamycin concentration of 10 and 20ppm were used for juices. Optimization of condition for the preservation of cut pineapple was with the help of response surface methodology. The central composite design with 24 experimental combinations was used to optimize the factors and responses. The optimum factors were 1cm thickness of cut pineapple, a time of 2 minutes, natamycin concentration of 30 ppm and a volume of 100 ml. The actual natamycin concentration of 10 ppm which diffused into the cut pineapple after soaking in 30 ppm for 2 minutes was used for the treatment of bulk storage of cut pineapple under refrigeration and ambient conditions. Sensory analysis, determination of pH, titratable acidity, Vitamin C, Brix as well as the enumeration of total moulds and yeasts were carried out on both treated and control samples. The cut pineapple and juices were analysed at 3-day interval of the 36 days storage period. There were significant difference ($p \leq 0.05$) in the pH, tittratable acidity, Brix and vitamin C of natamycin treated cut pineapples and juices and their respective controls. However, there were no significant difference in the physiochemical properties of the juices treated with 10 and 20ppm. The natamycin treated cut pineapple and juices were preferred during sensory evaluation, while yeasts and moulds were not detected throughout the storage period. On the other hand, control samples of cut pineapples

and juices from the 9th day till storage period were unacceptable by the panellist as they were also highly contaminated with yeast and moulds. Natamycin proved to be suitable and effective antifungal preservative which increases the shelf-life of cut pineapple and juices without changing the characteristics of the product. Comparative analyses showed that there were significant differences ($p \leq 0.05$) in vitamin C, total soluble sugars and pH of MD2 and sugar loaf varieties of the pineapple whiles differences in the titratable acidity, yeasts and moulds growth of MD-2 and sugar loaf varieties were not significant ($p \geq 0.05$).

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CHAPTER ONE

1.0 INTRODUCTION

1.1BACKGROUND

The world today is characterized by a rise in health consciousness and a growing interest in the role of food for maintaining and improving human well-being and consumer health. In addition to their nutritional and sensory properties, foods are presently recognized as active and protective agents and fresh-cuts, juices and products of minimal processed fruits stand out as convenient novel foods that fit the health needs of the consumer (Corbo *et al.*, 2010).

Fresh and processed fruits consumption has continued to grow rapidly in recent times, mainly because of the need for a balanced diet, the health benefits, low calories in fruits and the superior flavour of the fresh fruits as compared to canned fruits. These are an essential component of a healthy diet, able to decrease the risk of cardiovascular diseases and cancer (Mohammed, 2007, Ragaert *et al.*, 2004,).

The International Fresh-cut Produce Association (IFPA) defines fresh-cut products as fruit or vegetables that have been trimmed and/ or peeled and /or cut into 100% usable product that is bagged or pre-packaged to offer consumers high nutrition, convenience, and flavour while still maintaining its freshness (Corbo *et al.*, 2010). Fresh-cut fruits and juices attract consumers because they are fresh, nutritious, low priced, and ready-to-eat. Consequently, a wide variety of minimally processed fruits has been developed to meet consumer's needs for convenient products and to save time (Allende *et al.*, 2006). Minimally processed products are important to food service industry such as restaurants and catering companies as they offer many advantages over traditional products, with respect to convenience, expense, labour,

and hygiene. Regardless of its popularity, the production of minimal processed products is limited due to rapid deterioration and senescence (Mohammed, 2007).

Pineapples (*Ananas cosmous*) are a composite of many flowers whose individual fruitlets fuse together around a central core (Wood, 1988). The Hawain islands are now the leading producers of the fruits. Today, pineapples are marketed as fresh and canned fruits in the United States and other nations and they are mostly used as tropical foods for many recipes including fruit salads, jams, juices and other products (Cho *et al.*, 2004). Pineapples have exceptional juiciness and a vibrant tropical flavour that balances the tastes of sweet and tart. They are second only to bananas as America's favourite tropical fruit. Although the season for pineapple runs from March through June, they are available year-round in local markets (Wood, 1988).

Pineapple production is widespread in Ghana and is largely grown in the Central, Eastern and Western regions of Ghana. In Ghana, tropical fruits produced during bumper harvest are consumed fresh, sold at relatively cheap prices or allowed to go waste due to inadequate processing facilities (Yeboah and Kunze, 2004). Much of these fresh pineapple fruits go to the industry for slices and juice production, while about five million tonnes are exported (Smart and Simmonds, 1995).

Fresh cut fruits and juices such as pineapple are more perishable than the unprocessed form because of higher susceptibility to microbial spoilage, increased respiration rate and ethylene production, which leads to enzymatic browning, texture decay, rapid microbial growth, weight losses and undesirable volatile production, reducing highly the shelf life of the product (Carbo *et al.*,2009). However, the short life of the fruit has seen food scientists attempting to develop new technologies that would improve the quality and quantity of fresh-cut products and juices with the

primary aim of increasing their production without affecting quality and the environment (Mohammed, 2007).

Several methods of preservation such as chemical and natural have been carried out by researchers to increase the microbial stability and quality of freshness of minimal processed products. A research carried out by Abano (2010) in assessing the drying characteristics and physio-orgnoleptic properties of dried sliced pineapple under different pre-treatment showed that all products treated using lemon, ascorbic acid, salt and honey extended the shelf life beyond five months. While some preservatives are coming under increasing regulatory pressure, the more natural ones and antimicrobial preservatives such as lysozyme, nisin and natamycin are receiving increased attention and gaining importance and acceptability in preserving food products (European Food Safety Authority, 2009).

Natamycin is a natural antimicrobial food addictive used to protect food from moulds and yeast growth (European Food Safety Authority, 2009). This product has been used decades in the food industry as hurdle to moulds and fungi growth in dairy product, meat, and other foods. Various reports suggest that natamycin is effective in the treatment of fresh berries, tomatoes, strawberries and raspberries and has been applied to food products in several ways. It has been added in dry form to liquids, slurries, pastes and semisolids food product (Morris and Hart, 1987). According to (Morris and Hart, 1987) natamycin has an anti-yeast effect when added to wine and various fruit juices like orange, pineapple and apple juices.

1.2. PROBLEM STATEMENT

Fresh cut fruits have been a new industry with limited preservation procedures available to the average Ghanaian and also pose a threat to the health and safety of consumers because of microbial contamination, nutritional loss and limited shelf-life. It is estimated that out of the achievable yield of 32,000 to 40,000 kgacre^{-1} of pineapple produced, 25% goes to waste (Yeboah and Kunze, 2004). A baseline study with some vendors of cut pineapples and juices without preservatives revealed that the limited shelf-life of these products has caused loss of revenue to them and the situation seems to be threatening the sustainability of the sale of these products.

1.3. JUSTIFICATION

Fresh cut produce (FCP) is rapidly becoming popular with consumers because of its convenience (Gonza´lez-Aguilar *et al.*, 2005). Preservation of cut pineapples and juices using natamycin would extend the shelf-life of fresh cut pineapple fruits and juice and this would help minimize postharvest losses of pineapple as well as enhance effective marketability. It will also restrain the occurrence of fermentation which is commonly associated with cut pineapples and unpreserved juice and make fresh cut pineapple and juices much more available for consumers for them to enjoy the health benefits associated with consumption of such products.

1.4. OBJECTIVE AND SPECIFIC OBJECTIVES

The main objective is the preservation of fresh cut pineapples and juices from two varieties of pineapple using natamycin.

Specific objectives are,

1. The effects of Natamycin on microbial stability, sensory and physiochemical properties of pineapple juice from two varieties (MD-2 and Sugar loaf) under refrigerating conditions.
2. Optimizing the conditions for the preservation of cut pineapple from MD2 and Sugar loaf with Natamycin
3. The effects of Natamycin on microbial stability, sensory and physiochemical properties of cut pineapple from two varieties under refrigerating and ambient conditions.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 BACKGROUND ON PINEAPPLE PRODUCTION

Pineapples (*Ananas comosus*) are a composite of many flowers whose individual fruitlets fuse together around a central core. Each fruitlet can be identified by an "eye," the rough spiny marking on the pineapple's surface. They have a wide cylindrical shape, a scaly green, brown or yellow skin and a regal crown of spiny, blue-green leaves and fibrous yellow flesh. The area closer to the base of the fruit has more sugar content and therefore a sweeter taste and a more tender texture than the upper part. Pineapples have exceptional juiciness and a vibrant tropical flavour that balances the tastes of sweet and tart. They are second only to bananas as America's favourite tropical fruit. Although the season for pineapple runs from March through June, they are available year-round in local markets (Wood, 1988).

Thailand, Philippines, Brazil and China are the main producers of pineapple in the world supplying nearly 50 % of the total world output (Arthey, 1995). Other important producers include India, Nigeria, Kenya, Indonesia, México and Costa Rica.

Pineapple production is widespread in Ghana and is largely grown in the central and western regions of Ghana. In Ghana, tropical fruits produced during bumper harvest are consumed fresh, sold at relatively cheap prices or allowed to go waste due to inadequate processing facilities (Yeboah and Kunze, 2004). Much of the fresh pineapple fruits go to the industry for slices and juice production, while about five million tonnes are exported for the fresh fruit market (Smart and Simmonds, 1995).

In Ghana, the volume of pineapple export in 2005 was 46,694 tonnes as against 71,804 tonnes in 2004. This marks a percent change of -34.97%. Pineapple export in

2005 had a value of $12,784,300 ($12.7 million) as against $22,068,600($22 million) in 2004 representing a percent change of -42.07%. These earnings from pineapples stand out as markedly high when compared to its closest competitor, banana (Wardy *et al.*, 2009).

2.2 VARIETIES OF PINEAPPLE

There are numerous varieties of pineapple world wide, however, Smooth Cayenne' is one of the most common variety grown. Other varieties which are cultivated include 'Red Spanish', 'Singapore Spanish', 'Green Spanish', 'Sugarloaf', and 'Queen'. More recently a new variety have been developed which is called 'Del monte Gold' or 'MD-2' (Crane, 2009).

Red Spanish

This type of pineapple is not widely available as Smooth Cayenne. Its Leaves are spiny with the fruit weighing from 0.9-1.8 kg and have a pale yellow flesh with pleasant aroma (Crane, 2009).

Smooth Cayenne

This is one of the most widely grown varieties in the world. Its leaves are about 0.9 m with some spines at the base and top. The fruit weighs about 2.3-2.7 kg and have a pale yellow to yellow pulp. The fruit are cylindrical in shape with high sugar and acid content (Gilman, 2007 and Crane, 2009).

Queen.

This variety is also not widely available as Smooth Cayenne. The leaves are spiny with the fruit weighing from 0.9-1.4 kg and have a golden yellow flesh, crisp texture and delicate mild flavour. It keeps well after ripening (Crane, 2009).

Sugarloaf

This type is sometimes called 'White Sugarloaf or 'Kona Sugar Loaf'. It is the third variety in addition to MD2, and smooth cayenne which is cultivated on a large scale in Ghana. The fruit weighs from 2.3-2.7 kg with a white flesh edible core (Crane, 2009). Sugar loaf has a high juice content or volume of 205.72 ml/kg fruit, followed by MD-2 with values ranging from 134.12-191.43 ml/kg fruit and Smooth cayenne having the least volume between 91.7-108.65 ml/kg fruit. Comparatively sugar loaf has higher Brix content than MD2 and other varieties. It has a sweetness index of 15.14; followed by the MD-2 with 12.72 and the Smooth Cayenne have the lowest index of 6.98 (Wardy *et al.,* 2009). Plate 2.1 shows a picture of sugar loaf pineapple.

Plate 2.1: Sugar loaf pineapple (Pineapple Best Practice Manual, 2009).

MD-2

This new variety (picture shown in plate 2.2) has various names including Del Monte Gold, Extra Sweet and MD-2. It has a barrel-like appearance with the pulp having deep gold/yellow colour inside (Crane, 2009). The MD-2 features an extra sweet flavour, golden colour, and a higher vitamin C content. This has become very popular in the fresh cut market and the processing industry because of its low acid

content and the additional nutritional benefits it offers to the consumer. It was developed to reduce the inequality in shape, and at the same time to increase its sugar content and to make it more attractive to fresh fruit consumers (Sauls, 1998). The MD2 has been described as super-sweet, self ripening and have a longer storage life with a value twice as much as that of the smooth cayenne variety (Achuonjei *et al.,* 2003). It has Brix content between 14.4 and 18.8, acidity between 0.7 and 1.3% and Sugar/acid ratio between 1.65 and 2.14 (Ramsaroop and Saulo, 2007).

Plate 2.2. MD-2 pineapple. (Ramsaroop and Saulo, 2007)

2.3. NUTRITIONAL COMPOSITION AND HEALTH BENEFITS OF PINEAPPLE

Pineapple is mainly cultivated world wide for its fruits. It is consumed fresh, canned, frozen and in juice forms. According to Francis (1982), nearly 70% of pineapple is consumed in the fresh state.

Pineapples contain Vitamin C, the B-complex vitamins, Vitamins A, E , K, essential amino acids and minerals such as calcium, iron, magnesium, phosphorous,

potassium, zinc, copper, manganese and selenium. They are especially high in potassium and vitamin C and provide the body with powerful antioxidant activity. The proteolitic enzymes (bromelain) in the fruit aids in digestion (Walker *et al.*, 2002). In general, acidity of pineapple ranges from 0.6-1.2 % of which 87 % is citric acid and 13 % is malic acid. The pH of pineapple is 3.71 and the composition of the juice varies with geographical, cultural, seasonal harvesting and processing (Masniza *et al.*, 2010).

Pineapples are excellent sources of the trace mineral manganese, which is an essential cofactor in a number of enzymes important in energy production and antioxidant defences. In addition to manganese, pineapple is a good source of thiamine, a B vitamin that acts as a cofactor in enzymatic reactions central to energy production (Sauberlich, 1987).

Pineapple is rich in vitamin C which is the body's primary water-soluble antioxidant, defending all aqueous areas of the body against free radicals that attack and damage normal cells (Sauberlich, 1987). In addition, vitamin C is vital for the proper function of the immune system, making it a nutrient to turn to for the prevention of recurrent ear infections, colds, and flu. The body uses vitamin C to help metabolize fats and cholesterol, absorbs iron, and synthesizes amino acids and collagen. Collagen is one of the primary building blocks of skin, cartilage and bone (Walker *et al.*, 2002). Table 2.1 shows the nutrient content of three pineapple varieties with water content and total sugars of sugar loaf much higher as compared to the MD-2 and smooth cayenne.

Table 2.1: Nutrient content of three pineapple varieties.

(Value per 100grams of edible portion of raw fruit).

Nutrient	MD-2	Sugar loaf	Smooth cayenne
Water	86	92	87
Energy (KJ)	215	214	190
Protein(g)	0.53	0.52	0.55
Total lipid (fat) (g)	0.11	0.12	0.13
Carbohydrate (g)	13.50	13.10	11.82
Fibre(total dietary) (g)	13.50	13.10	-
Total sugars(g)	10.32	13.56	8.29
Sucrose (g)	6.47	8.3	4.59
Glucose (dextrose) (g)	1.70	1.82	1.76
Fructose (g)	2.15	2.31	1.94
Calcium (g)	13	13	13
Iron (mg)	0.25	0.26	0.28
Potassium (mg)	108	112	125
Sodium (mg)	1	1	1
Zinc (mg)	0.12	0.12	0.08
Manganese (mg)	0.818	0.825	1.593
Magnesium (mg)	12	12	12
Phosphorous (mg)	9	9	9
Vitamin C (mg)	56.4	42.5	16.9
Vitamin B1 (mg)	0.080	0.079	0.078
Vitamin B2 (mg)	0.033	0.031	0.029
Vitamin B3 (mg)	0.507	0.502	0.47
Vitamin B6 (mg)	0.114	0.114	0.106
Folate (mcg)	19	19	11
Choline (mg)	5.4	5.2	5.6
Vitamin A (IU)	57	55	52
Carotene, beta (mcg)	34	31	31
Vitamin E(mcg)	0.02	0.02	-
Vitamin K	0.7	0.7	0.7

Source: (USDA, 2009)

According to USDA National Nutrient Database for Standard Reference (2009), the vitamin C content in MD-2 and Sugar loaf varieties are 56.4 and 42.5mg/100ml, respectively which is sufficient enough to meet the nutritional requirement of ascorbic acid since the new average daily intake or the recommended dietary allowances (RDA) for adults (>19 yr) are 90 mg/day for men and 75 mg/day for

women. Based on clinical and epidemiological studies it has been suggested that a dietary intake of 100 mg/day of ascorbic acid is associated with reduced incidence of mortality from heart diseases, stroke and cancer (Naidu, 2003).

Pineapples are high in the enzyme bromelain which is a natural anti-inflammatory that has many health benefits and encourages healing. Bromelain is very effective in treating bruises, sprains and strains by reducing swelling and pain. This powerful anti-inflammatory effect can also help relieve rheumatoid arthritis symptoms and reduce postoperative swelling. Additionally, the bromelain contained in fresh pineapple can relieve indigestion. This enzyme helps break down the amino acid bonds in proteins, which promotes good digestion (Walker *et al.*, 2002).

2.4 PROCESSING OF PINEAPPLE INTO CUT FRUITS AND JUICES.

Fruits and vegetables are consumed as fresh, minimally processed, and processed forms such as canned, frozen, dried, preserves and fermented products (Mohammed, 2007). UNIFEM (1988) reported that fruits can be processed into various products, namely fruit juices, cuts-fruits, dried or dehydrated fruits and fruit salads. The most commonly manufactured product is fruit juice. The International Fresh-cut Produce Association (IFPA) defines fresh cut fruits and vegetables as trimmed or peeled or cut into 100% usable product that is bagged or pre-packaged to offer consumers high nutrition, convenience and flavour while still maintaining its freshness. Codex Alimentarius defines juice as "unfermented but fermentable juice, intended for direct consumption, obtained by a mechanical process from sound, ripe fruits, preserved exclusively by physical means. The juice may be turbid or clear. The juice may have been concentrated and later reconstituted with water suitable for the purpose of maintaining the essential composition and quality factors of the juice. The

manufacturing process of pineapple products which includes cuts and juices involves many steps and different sub-processes. Ripe and matured pineapples are washed, graded and peeled. Fresh cut products are produced at the small vendor level, supermarket level and by small and big processors in the world. Steps in the processes vary in accordance with the target market (Figure 2.1a). Small vendors, who target the mass market, generally keep produce on ice, while cutting on demand. On the other hand Supermarkets, which target consumers who are increasingly safety and quality conscious, cut and package fresh-cut fruits under hygienic conditions on a daily basis and display them on ice or under chilled conditions. Small and big processors, who also target supermarkets and the food service sector, often include an anti-browning treatment as a processing step to ensure longer shelf-life of their product (James and Rolle, 2010). Pineapple juice also involves many steps and different sub-processes as shown in the flow chart (Figure 2.1b).

Figure 2.1a: Steps in the production of fresh cut fruits for different target market (James and Rolle, 2010).

Pineapple Juice

Fruit ⟶ reduced crown size⟶weights ⟶ washing ⟶sorting ⟶

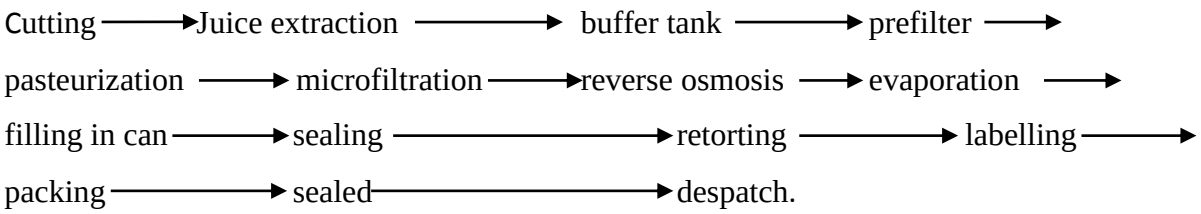

Figure 2.1b: Flow chat of commercial processing of pineapple juice (Pineapple Best Practice Manual, 2009).

2.5 REFRIGERATION OF CUT FRUITS AND JUICES.

Fruits and vegetables have tender texture, contain high moisture content (60%–95%) and water activity and undergo various biochemical reactions. The rate of biochemical reactions in fruits and vegetables are related to temperature, such that lower storage temperatures lead to slower degradation of foods (Gorny, 2001). Hence storage of food is necessary at all points of the food chain from raw materials, through processing, distribution, retailers and final purchasers. Today's consumers expect a much greater variety and quality of products to be available throughout the year and effective storage systems for fruits and vegetables are essential to meet this need (Brennan, 2006).

Refrigeration is the process by which the temperature of fluids or foods in general is decreased. In particular, it is used for the temporary storage of perishable and processed products by the forced lowering of the temperature. Refrigeration slows down the proliferation of microorganism in food and is the gentlest method of food preservation. Refrigeration has few adverse effects on the taste, texture, nutritive value and other attributes of foods (Practical Action Technical Brief, 2011).

Fresh cut and juices undergoes changes during refrigeration. These changes are influenced by the varieties, growing and processing conditions of the product (Gorny *et al.,* 1998). The storage time of most food items is increased by storing them at low

temperatures. The main foods that require conservation at low temperature among others are fruit, juices, drinks and vegetables. Low refrigerating temperatures has been reported to cause damage called “chill injury” to fruits and vegetables. Chilling injury is a term when fruits and vegetables from tropical and subtropical origin exhibit a physiological dysfunction when exposed to non-freezing temperatures below 12°C (Martinez-Javega *et al.,* 1992). Hence the need to have optimum storage conditions for fresh cut and juice (Gorny, 2001). Optimum storage conditions of 7 to 12 °C are recommended for whole pineapples for 14 to 20 days whiles 5^{o}C ±3 are for juices and fresh cut product (Paull, 1993).

Quality changes and nutrient retention during storage of juices and fresh-cut fruits were examined in a very comprehensive report by (Gil *et al.,* 2009). These authors measured changes in visual quality, colour, the pH, the titratable acidity, the soluble solids, phenolics, carotenoids, and vitamin C in pineapple, mango and other fruits stored for up to 9 days at 5°C. In general, they determined that fresh-cut fruits visually spoil before any significant nutrient loss occurs. Based on visual appearance, the postcutting life was less than 9 days for fresh-cut pineapple, cantaloupe and strawberry.

2.6. PHYSIOCHEMICAL CHANGES IN CUT FRUITS AND JUICES DURING STORAGE

Generally processing of fruit promotes a faster physiological deterioration, biochemical changes and microbial degradation of the products which may result in degradation of its colour, texture and flavour, even when slight processing operations are used (O’Beine and Francis, 2003).

Physical characteristics of pineapple such as moisture content, reduced crown size, fruit weight, texture, delayed ripening and physical damage to the fruit greatly affect the chemical compositions of the fruit such as soluble sugars, pH, Vitamin C, phenols content and titratable acidity (Mohammad *et al.,*2005). The rates of these changes depend on the types and degree of processing (Iqbal *et al*. 2008). Hence biochemical changes, such as the concentrations changes in vitamin C, sugars, soluble solids and phenols during storage of fresh cut pineapple and juice are very important since they are used as primary quantitative parameters of quality (Gorny, 2001).

2.6.1 Changes in vitamin C content during storage.

Vitamin C content of pineapple is dependent on factors such as the cultivar, stage of maturity, conditions of storage and the part of fruit. Its content in fresh pineapples ranges from about 20 to 34.44 mg/100 ml of juice (Ngoddy and Ihekoronye, 1985). It is well known that vitamin C is easily oxidized to dehydroascobic acid in alkaline solution whiles it is relatively stable in acidic solutions. The catalyzed oxidation pathway of vitamin C degradation is the most important reaction pathway for the loss of vitamin C in fruits. Therefore, vitamin C of fruits are readily oxidized and lost during staying of the juice or the cut fruit (Ball, 2006). According to Izuagie and Izuagie (2007), degradation of vitamin C undergoes both anaerobic and aerobic pathways. Oxidation of vitamin C in aerobic pathway occurs mainly during the processing of fruit whereas anaerobic degradation of vitamin C mainly during storage.

On the other hand, degradation rate of ascorbic acid is affected by several factors such as temperature, water activity, pH, storage time and metal ions (Fennema, 1993;

Pardio Sedas *et al.,* 1994). According to Ball (1997), a meta-oxygen-ascorbate complex is formed in the presence of molecular oxygen and trace amounts of transition metals which particularly are copper (II) and iron (III). This complex rapidly decomposes to give the ascorbate radical anion. This radical anion then reacts with the oxygen to give dehydroascorbic acid (DHAA). In the anaerobic pathway, vitamin C degradation occurs in the absence of free oxygen, the degradation is caused by the formation of diketogulconic acid. The rate of degradation is maximum at pH 3 to pH 4 and this pathway is mostly responsible for anaerobic loss of vitamin C in packaged cut-fruits and juices (Ball, 2006**).**

Several studies have been reported on the ascorbic acid loss in fruits during storage under fixed conditions of temperature and relative humidity (Nunes *et al.,* 1998; Uddin *et al.,* 2002; Tudela *et al.,* 2002). The decrease of vitamin C content with time has been recently studied in a number of papers. Murtaza *et al.,* (2004) studied the content of ascorbic acid in a strawberry drink which was stored at room temperature (25°C), refrigeration temperature (4–6°C) and high temperature (40–45°C). Minimal changes were observed in the samples stored in a refrigerator. Decrease was from 65.0 to 44 mg/100 ml after three months.

This is because the dehydoascorbic acid, the oxidized form of ascorbic acid was more stable at lower temperatures. Thus, the vitamin C, in the form of dehydroascorbic acid for refrigerated juice was well retained than non-refrigerated juice.

2.6.2 Changes in total soluble sugars (Brix) during storage.

Total soluble sugars (Brix) in fruits and vegetables has been ascribed by some authors as having an increasing trend whiles others also report of them of having a decreasing trend during storage. According to Echeverria and Ismail (1990), the increase in soluble solids during storage may not necessary reflect in sucrose, glucose and fructose but rather may result in release of soluble components from insoluble material in the fruits whiles the decrease of total soluble solids in fruits is caused by a decline in the amount of carbohydrates and pectins, partial hydrolysis of protein and decomposition of glycosides into sub-units during respiration (Ball, 1997). It has also been reported by several authors that correlation between TSS reduction, decrease in sugar content and high metabolic activity occurs when fruits are stored at high temperatures (Agar *et al.*, 1999; Lamikanra *et al.*, 2000). Several studies have been reported on the decreases in total soluble solids during storage over a period of time (Echeverria and Ismail, 1990) whiles Burns and Echeverria (1990) also reported an increase observed in total soluble solids in fruits during storage.

2.6.3. Changes in pH and titratable acidity during storage

The change in pH is associated with number of reasons; it might be due to the effect of treatment on the biochemical condition of the fruit and slower rate of respiration and metabolic activity (Jitareerat *et al*., 2007). The acidity of the fruit is an important character to determine its quality and acceptability. A study conducted by Jitareerat *et al., (*2007) on coated and uncoated fruits indicate that pH increase and titratable acidity decreased significantly along with increase storage time. These results agree with those reported by El-Ghaouth *et al*., (1991) and Garcia *et al*., (1998). Others

also have reported on the decrease in pH and increase in titratable acidity (TA) along with increase storage time. An increase in TA is associated with decrease in pH. This is also in agreement with those reported by Tovar *et al.,* (2000) on the decrease in pH and increase in titratable acidity observed in slice mangoes and pineapples. It is explained by Garcia *et al.,* (1998) that the decrease of acidity during storage demonstrates fruit senescence.

2.6.4 Micro flora associated with cut fruits and juices

A major challenge faced by the fresh cut produce industry is to maintain the quality of fresh cut produce and juice so that their shelf-life is long enough to ensure efficient marketing (Gonza´lez-Aguilar *et al.,*2005). The four sources of microbial contaminants are soil, water, air, and animals (Mohammad, 2007). Each microorganism has (i) an optimum temperature at which it grows best, (ii) a minimum temperature below which growth no longer takes place, and (iii) a maximum temperature above which all development is suppressed. Microbial growth in foods results in food spoilage with the development of undesirable sensory characteristics, and in certain cases renders the food unsafe for consumption. The pathogenicity of certain microorganisms is a major safety concern in the processing and handling of foods in that they produce chemicals in foods that are toxic to humans. Their growth on foods may also result in undesirable appearances and off-flavour (Singh, 2010). Operations such as peeling or slicing increase the tissue damage of fresh cut produce causing the released of intracellular liquids and consequently increase microbial growth (Brecht, 2006).

Among the microorganisms, yeasts and moulds have a competitive advantage over bacteria as they easily cause spoilage to fresh cut fruits and juice because of their

ability to grow at a lower pH range (2.2–5.0). In their study on the microbiological quality of fresh minimally-processed fruits, Abadias *et al.,* (2008) reported that apple, peach, orange, mango and pineapple harbour small microbial populations consisting of yeasts and moulds, while no *Enterobacteriaceae* were detected. They explained this trend with the fact that the investigated fruits were more acidic than other types and the combination of low pH and low temperature during storage also tend to inhibit growth. For most fresh-cut fruits such as pineapples and other fruits there is sufficient acid to limit spoilage primarily due to fungi and aciduric bacteria (lactic acid bacteria, *Acetobacter, Gluconobacter*), *Leuconostoc* spp., and *Enterococci* spp. (Splittstoesser, 1987). Martinez-Ferrer *et al.,* (2002) identified a relationship between increased shelf- life and reduced populations of yeasts and moulds on both cut mangoes and pineapples during storage. O'Connor-Shaw *et al.,* (1994) reported that evidence of mould growth on cut pineapples stored in closed containers was the major quality defect at both 4 and 20 ^{o}C. Specification by the Ghana Standards Authority (GSA) on yeasts and moulds for preserved fruit and juices are not to exceed $5.0x10^{1}$ whiles that for unpreserved juices are supposed to be $1.0x10^{3}$ cfu/g (GSA, 2012).

2.7 PRESERVATION OF FRESH CUT FRUITS AND JUICES

The term preservation is defined by Singh (2010) as the addition of a natural or synthetic agent to food products to prevent decomposition by microbial growth or any undesirable chemical change in the finished product. Based on the mode of action, the major food preservation techniques can be categorized as (1) slowing down or inhibiting chemical deterioration and microbial growth, (2) directly

inactivating bacteria, yeasts, moulds, or enzymes, and (3) avoiding recontamination before and after processing (Gould, 1995).

Maintaining the quality of a fresh-cut fruit, processed fruit or vegetable product is a major concern and a priority in the development and the production of fresh-cut and processed produce of the industry. This has called for the use of present food technologies that utilizes an array of physical, chemical and biological processes and agents to preserve food and prevent the transmission of disease through food products (Martínez-Ferrer and Happer, 2005). O'Hare (1994) reported that cut pineapple has a shelf life of more than 5 weeks at 1ºC.

2.7.1 Types of preservatives

Preservatives can be categorized into two types based upon their source of origin**:**

Artificial preservatives:

These are a group of synthetic chemical substances that prevents spoilage and contamination of finished products by micro organisms. Some examples of these preservatives include nitrates, sulphites, sodium benzoate, propyl gallate and potassium sorbate (Singh, 2010).

Artificial preservatives have been used to reduce microbial populations on fruit and they are still the most widely used treatments, either before processing or during pre- and post-cutting operations (Gil *et al.*, 2009).

Natural preservatives

These are chemical constituents extracted from natural sources that offer intrinsic ability to protect products against microbial growth. These include essential oil constituents, flavonoids, phenolic compounds, antioxidants and antimicrobials (Singh *et al.*, 2010).

2.7.2 Preservatives from microbial sources

Microbial preservatives (extracted from microorganisms) are used to inhibit the growth of bacteria or fungi by creating an environment hostile to them (Singh *et al.*, 2010). Many chemical food preservatives have been reported as being responsible for occasional allergic reactions in sensitive individuals, thus the interest in antimicrobial compounds found in nature and the demand for natural preservative from consumers have recently increased (Gómez-López *et al.*,2009). These antimicrobials include Nisin, Natamycin, Lysozyme, Lactoferrin and fermentate (Singh *et al.*, 2010).

2.7.2.1. Structure and mode of action of natamycin in preservation

Natamycin (Pimaricin) is a polyene macrolide antibiotic produced by submerged aerobic fermentation of *Streptomyces natalensis* and related species. Fermentation is conducted for several days, and the antibiotic is isolated either by broth extraction or by extraction of the mycelium (Farid *et al.*, 2000). It has a molecular mass of 665.725 g/mol. The Registry number of natamycin is 7681-93-8 and the molecular formula is $C_{33}H_{47}NO_{13}$ (EFSA, 2009).

Structure

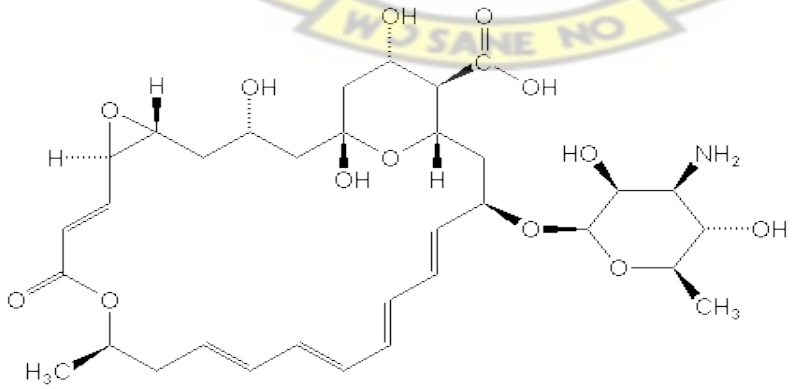

Figure 2.2: Natamycin structure. Source (Food Chemical Codex, 2011).

The solubility of natamycin is 20-50 mg/L in water. It is soluble in glacial acetic acid, methylpyrrolidone, dimethylformamide, dimethylsulfoxide, glycerol and propylene glycol. Natamycin is insoluble in higher alcohols, ethers, esters, aromatic or aliphatic hydrocarbons, chlorinated hydrocarbons, ketones, dioxane, cyclohexanol and various oils (Food Chemical Codex, 2011).

Due to the amphiphilic nature of the molecule it has low solubility in water. Natamycin is effective at low concentrations, exhibits a wide spectrum of activity and has a neutral flavour, characteristics which render it ideally suitable as a preservative (Budavari, 1989; Food Standards Australia New Zealand, 2004).

Natamycin is made up of polyenes, a large group of antibiotics with various molecular structures, which interact with fungal membranes (Franklin and Snow, 1998). The antifungal activities of natamycin and other polyenes are dependent on their binding to cell membrane sterols, primarily ergosterol, the principal sterol in fungal membranes, thereby making them leaky (Thomas and Delver- Broughton, 2003).

Natamycin has a large lactone ring with a rigid lipophilic chain containing conjugated double bonds and a flexible hydrophilic portion bearing several hydroxyl groups. It is probable that the hydrophobic region complexes with ergosterol in the membrane forming a polar pore through which small ions such as K+,H+, amino acids and other metabolites can pass freely, disrupting the cell's ionic control and killing the cell (Deacon, 1997).

2.7.2.2 Uses of natamycin in foods

Natamycin is permitted as an antimicrobial preservative in more than 70 countries, mainly for processed meat and cheese products. In South Africa it is allowed as a preservative in a wide range of products, including wine (Food Standards Australia New Zealand, 2004).

The preservative is effective at concentrations between 1 and 10 mg/kg (Thomas and Delver-Broughton, 2003). Reportedly, natamycin has been applied to food products in several ways; It has been added in dry form to liquids, slurries, pastes and semisolids when adequate mixing can be accomplished, or the pure natamycin can be mixed with one or more of the dry ingredients and then added to a given food product. Solid foods requiring surface protection can be dipped, misted, fogged or dusted with a solution or suspension of natamycin. Additionally, it has been suggested that protection from yeast and moulds may be achieved in solid food by incorporating natamycin homogeneously into the food itself (Morris and Hart, 1987). Natamycin is deemed to be a GRAS (Generally Recognized as Safe) substance in USA and is assigned the number E-235-natural preservative in European Union (Food Standard Australia New Zealand, 2004).

Natamycin has been globally used in a variety of food such as retarding spoilage of cheese, baked foods, meat, jam, jelly, marinated food, fish and chicken. Further uses include surface treatment for semi-dried, cured meat products, drinks, juice, wines, yogurts, man-made butter and others (EFSA, 2009). Natamycin has been widely used in the dip-treatment of cheeses to coat them with the fungicide which is absorbed slightly, and dries to form a solid, surface coating. Various other reports suggest that Natamycin is effective in the treatment of fresh berries, tomatoes, strawberries and raspberries. These reports indicate that Natamycin has an anti yeast

activity when added to wines, and various fruit juices, such as apple juice or orange juice (Morris and Hart, 1987).

Stark (2003) found that addition of 10 µg/g of Natamycin had an immediate reduction effect on yeast and mould counts in orange-pumpkin juice inoculated with *S. cerevisiae* as well as samples that were not inoculated. After 1 week of storage at 2.5°C to 5°C, yeast and mould counts were undetectable and the sample remained unspoiled for the 8-week duration of the test. Control samples not containing natamycin were spoiled within 1 week. A second study then compared the effectiveness of natamycin to sorbic acid. In this study juice inoculated with natural contaminants spoiled after 1 week of storage, whereas levels as low as 1.25 µg/g of natamycin prevented spoilage during the 8 weeks storage. In comparison, levels as high as 1000 µg/g of sorbic acid was needed to retard yeast growth. The sorbic acid also imparted an unpalatable favour to the fruit juice whiles natamycin did not.

Natamycin shows good stability in foods provided that the pH is in the range from 5 to 9 and the isoelectric point of natamycin is 6.5 (Stark, 1999). It is less stable in foods outside this pH range. Natamycin is sensitive to inactivation by oxidants such as peroxides, chlorine and heavy metals (Stark, 1999). Natamycin has also been found to be effective in preserving fruit juices and fresh cut fruits. However at pH below 5 natamycin degrade faster when pasteurized and stored at ambient temperature. Degradation of natamycin is minimized at extreme pH when pasteurization is done before addition of natamycin and stored at refrigeration temperature (Coryne *et al.,* 2007). According to Stark (1999), a sub–optimal concentration of 2.5 ppm of natamycin together with other preservative method such as pulse electric field is effective in preserving fresh cut and juice, however an optimal concentration of 5 ppm of natamycin alone is effective in inhibiting yeast

and mould growth in fresh cut and juice whereas a higher concentrations of 10 ppm and above would help extend the shelf life beyond ten (10) weeks when processed under hygienic conditions.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) reviewed the safety of natamycin in 1968, 1976 and 2002 and assigned an acceptable daily intake (ADI) of 0.3 mg/kg body weight (bw)/day (JECFA,2002).

Table 2.2 shows the different food product which can be preserved by natamycin, its dosages and mode of application.

Table 2.2: Different kinds of foods with Suggested Dosage Levels of natamycin and methods of application.

Food	**Dosage level(ppm)**	**Mode of application**
Hard/semi hard cheese	500-2000	Surface treatment by spray, immersion or direct addition to emulsion
Meat product, dry sausage	1250-2000	Surface treatment by spray or immersion
Yogurt	5-10	Direct addition to yogurt and mix
Bakery product	1250-2000	Surface treatment by spray
Tomato puree	7.5	Direct addition during mixing
Fruit juice	2.5-10	Direct addition
Wine	30-40	Direct addition to stop fermentation

Source: Thomas and Delves-Broughton (2003)

2.8 SENSORY EVALUATION OF FRESH CUT FRUITS AND JUICES

Sensory evaluation as defined by the Institute of Food Technology is a scientific method used to evoke, measure, analyse and interpret those responses to products as perceived through the senses of sight, smell, touch, taste and hearing (Anonymous 1975).

Sensory evaluation of food products is divided into two components analytical and affective measurements. Analytical measurements can be used to detect differences (difference tests) or to describe the product (descriptive analysis). Analytical sensory tests are usually conducted by small panels with some training of the panellists. Affective measurements determine preference (which samples are preferred over others) and usually require large numbers of panellists (Institute of Food Technologists, 1981).

Since human perception is involved in sensory testing, quality attributes are clearly defined in terms that are relevant to consumer acceptability. Affective consumer tests are the only way to determine what consumers like and what they do not like (Barrett *et al.,* 2010).

For many products, the sensory properties deteriorate ahead of microbial quality and so, in tandem with microbial tests, sensory testing can be used to determine shelf life and product variability through the supply chain (Kemp *et al.,* 2009).

Sensory attributes for cut pineapple and juice include appearance, sweetness, sourness (taste), odour, texture, firmness and its overall acceptability. Sensory analysis offers a good tool in providing quick assessment of the quality of pineapple juice (Masniza *et al.,* 2010).

2.9 PACKAGING MATERIALS FOR FRESH CUT FRUITS AND JUICES

Packaging facilitates the delivery of fresh-cut and processed products of good quality to the consumer. Packaging protects products from physical damage and prevents physical and microbiological contamination. On some occasions, as in the case of Modified Atmosphere (MA) and MAP, packaging can reduces the respiration rate of produce and slows the rate of spoilage (James and Rolle, 2010).

Packaging material for fresh-cut fruits and vegetables include plastic bags, thermoformed containers with film overwraps and rigid plastic containers. Other packaging films used include perforated thin, low density polyethylene (LDPE), high density polyethylene (HPDE), monolayer polyvinylchloride (PVC) and ethylene vinyl acetate. The main packaging material used for fresh cut and processed products are the plain low and high density polyethylene bags and bottles (James and Rolle, 2010).

2.9.1 Polyethylene used in packaging fresh cut fruits and juices

Polyethylene (PE), commonly called polythene, is made in one of the two ways: Ethylene is polymerised at high temperature and pressure, in the presence of a little oxygen and the polymer converted into a film by extrusion. Alternatively, lower temperatures and pressures may be used to produce the polymer if certain alkyl metals are used as catalysts. The film is available in low (LDPE), medium (MDPE) and high (HDPE) density grades. Different types of polyethylene bags have different uses: The lower density grades are most widely used in food packaging because of its strength, low permeability to water vapour and ability to form a very strong heat seal. It is however not a good barrier to gases, oils or volatiles. It is used on its own in the form of pouches, bags and sacks. The MDPE on the other hand has

a good shock and drop resistance properties. It also less notch sensitive, stress cracking resistance is better than HDPE. MDPE is typically used in gas pipes and fittings, sacks, shrink film, packaging film, carrier bags and screw closures. However, HDPE has a higher tensile strength and stiffness than LDPE. Its permeability to gases is lower and it can withstand higher temperatures. It is used for foods which are heated in the package such as fruit juice and yoghurt (Brenna, 2006).

Packaging suppresses respiration and transpiration of the produce and, therefore, reduces the metabolic activity. It also prevents food contact with atmospheric oxygen, which accelerates deterioration reactions (Abbas and Ibrahim, 1996). Film bags and HPDE bottles are clear for easy inspection of its contents and readily meet high quality graphics. These are available in a wide range of thickness and grades.

Chonhenchob *et al.*, (2007) found that the atmosphere of 6% oxygen and 14% carbon dioxide that was achieve at equilibrium in the headspace of packed fresh-cut pineapple increased shelf life 7 days at 10ºC.

Film packaging reduces water loss of fruits and vegetables. However, most plastic films do not have enough permeability to achieve the in-bag high gas concentration; therefore, non aerobic respiration could be initiated (Ahvenainen 1996). Plate 2.3 shows different kinds of packaging materials that are used in the fruit cut industry.

(a)Clamshell tray with hinge interlocks

(b) Breathable bags

(c) Zip lock bag.

(d) Clear PET tray with an overwrap film

Plate 2.3: Types of packaging materials

Source: Chonhenchob et *al.*, (2007)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 SOURCE OF RAW MATERIALS AND REAGENTS

Two varieties of fresh ripe pineapple, MD2 and Sugar loaf were bought from two local markets namely "Race horse and Abgogloshie" in Kumasi and Accra respectively. Fruits were sorted to eliminate damaged ones and crowns were removed. The packaging materials (HDPE) bottles of 300 ml were obtained from Franchantony Ventures and A-1 Rubber Enterprise in Kumasi, Ghana. All chemicals and reagents with the exception of natamycin which was imported from South Africa, the rest were obtained from the Food and Drugs Authority, Department of Biochemistry and Biotechnology, as well as Theoretical and Applied Biology laboratory, KNUST. Plates 3.1 and 3.2 are pictures of samples of Sugar loaf and MD-2 varieties of the pineapple that were used for the work.

Plate 3.1: Sugar loaf variety of pineapple Plate3.2: MD-2 variety of pineapple

3.2. PREPARATION OF PINEAPPLE JUICES AND PHYSIOCHEMICAL ANALYSES

3.2.1. Preparation and packaging of pineapple juices from the two varieties.

The collected pineapples were washed thoroughly with 1.5% salt solution and hot water at a temperature of 80^{o}C. The pineapples were then peeled, sliced to remove the core and then cut into 1cm thickness. The pieces were blended and the juice extracted using a cheese cloth. The pineapple juice was poured into a stainless bucket which has a capacity of about 3,500 ml and placed in a water bath for pasteurization at 80^{o}C for 10 min. After pasteurization, the bucket of juice was immediately cooled to 30-35^{o}C by placing the bucket of juice in iced water. The cooled juice was divided into three portions. Samples of one portion of juice which served as control was analysed. Natamycin of concentrations 10 ppm and 20 ppm were prepared by weighing 10 and 20 mg of natamycin, diluted with 1000 ml distilled water into two different 1 litre volumetric flask. These concentrations were added to the remaining two portions and mixed thoroughly using a wooden spoon. The pasteurized juiced were poured into sterilized 300 ml HPDE bottles (sterilisation was with hot water at a temperature of 100^{o}C) and capped tightly. The sealed bottles were labelled as control, 10 and 20 ppm respectively and stored under refrigeration at a temperature of 5^{o}C ±2. Samples were taken for analyses every 3 days for 36 days. The plates below shows bottled juice of sugar loaf and MD-2 before refrigeration and during refrigeration.

plate 3a:Bottled juice of MD-2 before refrigeration

Plate3b: bottled juice of sugar loaf before refrigeration

plate 3c Bottled juice of pineapple stored at $5^{o}C\pm2$

Plate3.3: Bottle juices from sugar loaf and MD-2 varieties of pineapple

3.2.2 .The physiochemical analyses of refrigerated pineapple juices

3.2.2.1 Determination of total soluble sugars (Brix content)

The total soluble sugars were determined by measuring the refractive index of pineapple juice using a digital hand held refractometer (Atango manual, model Japan). Calibration of the refractometer was done by placing a drop of distilled water

on to the lens. It was then positioned at an angle of 45^{o}C to allow the entry of sunlight via the lens. A drop of MD2 juice was placed on the lens and the reading was taken in º Brix. The lens was carefully rinsed between samples and the process was repeated three times for each sample (AOAC, 1992). The same procedure was repeated for the sugar loaf variety.

3.2.2.2. Determination of pH

The pH was determined using digital pH meter equipped with an electrode. The pH meter was calibrated with buffers 4, 7 and 10 each time measurements were read. The electrode was rinsed with distilled water before dipping it into the juice. The pH reading was read from the recorder of the pH meter (AOAC, 1992).

3.2.2.3. Determination of vitamin C

Preparation of standard Ascorbic Acid Solution

Fifty milligrams (50 mg) of ascorbic acid was weighed into a 50ml volumetric flask and diluted to the mark with metaphosphoric acid–acetic acid solution and immediately used.

Preparation of Indophenols Solution

Forty two milligrams (42 mg) of sodium bicarbonate was weighed and added to 50 ml de-ionized distilled water in a 200 ml beaker and stirred to dissolve completely. Fifty milligrams (50 mg) of 2, 6-dichloroindophenol sodium salt was weighed and added to the dissolved solution in the beaker. The mixture was topped up to the 200 ml mark with deionised water. The final solution was then filtered using a filter paper (Macherey -Nagel MN615) into an amber bottle and tightly capped and stored under refrigeration at a temperature of 5±2 ^{o}C

Preparation of Metaphosphoric acid –acetic acid solution

One hundred millilitres (100 ml) of de-ionized water was added to 20 ml of acetic acid in a 250 ml beaker. Metaphosphoric acid of 7.5g was weighed and added to the solution in the beaker and stirred. The mixture was diluted to the 250 ml mark with distilled water. The mixture was filtered through a fluted filter paper into a bottle and stored under refrigeration at a temperature of 5±2 ^{o}C.

Procedure of analyses

Five millilitres (5ml) of metaphosphoric acid-acetic acid solution was pipette and added to a 2 ml ascorbic acid standard solution in Erlenmeyer flasks in triplicates. A burette filled with indophenols solution was titrated against the standard ascorbic solution in the Erlenmeyer flask until a distinct rose –pink colour forms and persists for more than 5 seconds. The initial and final readings of the burette were recorded. Blanks were prepared in the same way as above and the average titre of indophenols dye used was calculated.

Two millilitres (2 ml) of the sample was added to 5 ml of metaphosphoric acid-acetic acid solution in a 50 ml Erlenmeyer flask and performed in triplicates. The sample was titrated with the indophenols dye solution until a distinct rose-pink colour persists for more than 5 seconds. Initial and final readings of the burette were taken and used to calculate the vitamin C content (AOAC, 2000) (Calculation formula at appendix C).

3.2.2.4. Determination of titratable acidity

Ten millilitres (10 ml) of the sample was pipette into a conical flask and diluted with 50 ml of distilled water. About three drops of phenolthalein indicator was added and titrated to a faint pink end point with 0.1M NaOH solution filled in burette. The titre

value was recorded and titratable acidity based on citric acid was calculated (AOAC, 1992). (Formula shown at appendix C).

3.2.2.5. Determination of total solids

The total solids was calculated after the determination of moisture content by subtracting the percentage moisture content from 100% by using this formula.

Total Solids (%) = 100%- % moisture content.

Procedure for moisture content determination

Five (5) grams of samples from the control, 10 ppm and 20ppm of the two varieties were weighed into already weighed crucible using an analytical balance (OHAUS AS260D Model, NJ/USA). The crucible and its content were placed in a dry oven at a temperature of 105^{o}C until a constant weight was achieved approximately three hours after which the difference in weight was determined using the appropriate formula for calculation (AOAC, 1992).The samples were replicated.

3.3. MICROBIOLOGICAL ANALYSES OF PINEAPPLE JUICES.

Preparation of yeast growth count media (YGC)

Forty grams (40 g) of YGC was weighed and dissolved into 1L of distilled water. The mixture was then heated and stirred until a uniform mixture was obtained. The liquid agar was then autoclaved at a temperature of 121^{o}C for 15 minutes and cooled in a water bath to a temperature of 45 ^{o}C after which it was allowed to stand for 24 hours before use.

Determination of yeast and moulds.

The yeast and moulds were enumerated using pour plate techniques on selective media, yeast growth counts media (YGC). About 10 g each of samples from the control and treated pineapples of the two varieties MD- 2 and sugar loaf were weighed into 90 ml of Maximum Recovery Diluents (MRD) after which it was transferred into stomacher bags .The content was homogenized in a stomacher for 60 seconds to obtained 1:10 dilutions. Further dilutions (10^{-9}) were done by transferring 1ml of each of the suspension into a bottle containing 9 ml of diluents (MRD solution) to obtain 10-fold dilutions. One millilitre (1ml) was inoculated in the Petri dish and about 20 ml of YGC was poured and gently swirled to mix. The plates were then incubated at 25 ^{o}C in the Gallenkamp Incubator (Model 1h-150, UK) for 5-7 days. The colonies formed on the media were counted using the Stuart Scientific Colony Counter (Serial 7354UK). The yeast and moulds counts were expressed as colony forming units (CFU) per ml (Atlas, 1995).

3.4. SENSORY EVALUATION OF PINEAPPLE JUICES.

A preference test was the sensory tool used in this analysis. The samples were coded with random numbers. Fifty untrained panellist both male and females within the ages of 20 to 45 years participated in the evaluation of the quality of processed pineapple juice. Samples from the control, 10 and 20 ppm (juice) as well as the control and treated sample (fresh cut) of the two varieties of pineapple were served. The panellists were asked to taste one sample at a time, and record their responses and allow time between samples so that they could record their opinions. A 1 to 5 structured scale was used for sweetness, sourness, appearance, odour and overall

acceptability of pineapple juice and cut fruit. The quantification of the degree of preference and acceptability is shown on the score sheet in (Appendix I).

3.5. OPTIMIZATION OF FACTORS AND RESPONSES OF CUT PINEAPPLES.

The two pineapple varieties were received freshly from the market and were washed with hot water and 1.5% salt solution. The independent variables factors were thickness, time, volume and concentration of natamycin. The thickness of cut fruits ranged from 1.0 to 3.5 cm. Soaking time ranged from 2 to 10 minutes, concentration and volume of natamycin ranged from 10 to 30 ppm and 50 to 100 ml respectively. The responses were Vitamin C, titratable acidity, pH and total soluble sugars. The factors were then analysed to obtain optimum values. The analysis of data was done using Design Expert 7 and Minitab 16 where applicable. In all analysis using Design Expert, recommended models were based on recommendations by the software only. No theoretical justifications were made on the analysis. All hypotheses were tested at 5% significant level.

Table 3.1: Factors and responses for optimization of cut pineapples.

Run	variety	Thickness(mm)	Time(min)	Volume (ml)	Concentration.(ppm)	VitC	Brix	PH	TA	absorbance
1	0	2.25	6	75	20					
2	0	1	10	100	30					
3	0	1	2	50	30					
4	0	1	2	100	10					
5	0	3.5	2	100	30					
6	1	2.25	6	75	20					
7	1	3.5	10	50	30					
8	1	3.5	10	100	10					
9	1	1	10	50	10					
10	1	3.5	2	50	10					
11	0	1	2	50	10					
12	0	1	10	50	30					
13	0	3.5	10	100	30					
14	0	3.5	2	100	10					
15	0	3.5	10	50	10					
16	1	1	10	100	10					
17	1	2.25	6	75	20					
18	1	1	2	100	30					
19	1	2.25	6	75	20					
20	1	3.5	2	50	30					
21	0	2.25	6	75	20					
22	0	2.25	6	75	20					
23	1	2.25	6	75	20					
24	1	2.25	6	75	20					

3.5.1. Data analysis of factors and responses of cut pineapples.

ANOVA response for vitamin C, pH, titratable acidity, total soluble solids and absorbance (rate of diffusivity) were obtained using Design Expert 7.0. The response data of total soluble solids, ascorbic acids, pH and titratable acidity were loaded and analyzed. Analyses of the factors and responses data were done to select significant factors which contributed to the individual responses. In the selection of an appropriate model for the response, the model with the highest order polynomial

where the additional terms were significant and the model not aliased (aliased is when the estimate of an effect/response is influence by one more factors or there are high interactions between one or more factors) was selected. A model was said to be significant if its p-value was < 0.05. After the ANOVA studies, the responses were optimized based on the constraints imposed. In selecting the optimized response, the factor combinations which gave the most desirability were selected. The maximum desirability of the optimized formulation was found. The desirability plots of the optimised variables are at (appendix F). The fresh cut was prepared as per the optimized composition and the predicted responses were validated with the actual observed response.

Table 3.2: A constraint table of factors and responses set for optimization.

Constraints		Lower	Upper	Lower	Upper	importance
Name	Goal	limit	limit	weight	weight	
Thickness	is target=1	1	3.5	1	1	3
Time	is target=2	2	10	1	1	3
Volume	is target =100	50	100	1	1	3
Concentration	is target =30	10	30	1	1	3
VitaminC	Maximize	35.8	38.71	1	1	3
Brix	Maximize	11.9	12.5	1	1	3
pH	Maximize	4.48	4.59	1	1	3
TA	Maximize	0.56	0.61	1	1	3
Absorbance	maximize	0.448	1.2518	1	1	3

3.5.2. Determination of exact concentration of natamycin in cut pineapples

To determine the exact concentration of natamycin that diffused into the cut pineapples, the cut pineapple was prepared as per the optimized condition. The cut

pineapple with a thickness of 1mm was soaked in 30 ppm natamycin for 2 minutes after which it was removed and analyzed to determine the exact amount of natamycin that had gone into the cut pineapple. A Serial dilution of 2 to 30 ppm of natamycin concentrations was prepared and the absorbance read to obtain a standard curve. (Appendix J). Natamycin in cut pineapple was analyzed by extracting juice and dissolving in 50ml of methanol and absorbance read. The absorbance obtained was then used to calculate the exact concentration present using the equation y = 0.125x (where y= absorbance and x= concentration) which was found out to be 10 ppm

3.6. STORAGE OF CUT PINEAPPLES UNDER REFRIGERATION AND AMBIENT CONDITIONS.

Cut pineapples were prepared based on the optimized conditions established (which were of thickness 1cm, time of 2 minutes, Natamycin concentration of 30 ppm and Volume of 100 ml). Cut pineapples were soaked in 30 ppm natamycin and removed immediately after 2 minutes of soaking time (Actual natamycin concentration that diffused into the cut pineapples was 10 ppm which was used for the treatment of bulk storage of cut pineapple under refrigeration and ambient condition). They were packaged into small A1 HDPE bags each containing six pieces of cut pineapple. These were stored under 5 ^{o}C ±2 and 25 ^{o}C ±3 respectively. Three bags of pineapples samples were taken and analyzed every 3 days for 36 days.

3.6.1. Physiochemical analyses of cut pineapples.

The determinations of vitamin C, total soluble sugars, pH and titratable acidity were based on the same procedure used for the juices.

3.7. MICROBIOLOGICAL ANALYSES OF CUT PINEAPPLES

The preparations of media, determinations of yeast and mould counts were also based on the same procedure used for the juices.

3.8. SENSORY EVALUATION OF CUT PINEAPPLES

Sensory evaluation of cut pineapple which had a natamycin concentration of 10 ppm was also based on the same procedures as that of the juices. Fifty (50) untrained panellist between the ages of 20 to 45 years were used, out of which thirty–five (35) were females and fifteen (15) were males.

3.9 DETERMINATION OF NATAMYCIN IN CUT PINEAPPLE USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY.

The method was adapted as stated in the Food Chemical Codex (2011). An Agilent 2000 system (Agilent Technologies) fitted with quaternary pump, auto sampler, column oven and UVvisible diode array detector was used for the analyses. Separations were performed in reversed phase mode employing agilent zorbax eclipse C18 column (150 x 4.6 mm) with serial number USKH034381 at ambient temperature. The mobile phase was prepared by weighing and dissolving 3.0 g of ammonium acetate, 1.0 g of ammonium chloride and 5ml of tetrahydrufran in 760 ml of water (solvent A). Two hundred and forty millilitres (240 ml) of acetonitrile (solvent B) was filtered and the two solvents A and B were used in the ratio of 76:24. The flow rate was 1.0 ml/min and variable injection volumes of 20 uL up to 100 µL were used. Spectrophotometric detection was performed at 305 nm, while the run time was 15 min.

Standard preparation: A 10 ppm standard solution was prepared from a stock solution of 50ppm by weighing 5 mg of natamycin into 100ml volumetric flask. Five millilitres (5ml) of tetrahydrofuran was added and sonicated for 10 min. Sixty millilitres (60ml) of methanol and 25ml of water were added and swirled to dissolve and allowed to cool to room temperature after which it was diluted to the mark with distilled water. Twenty (20 ml) of the stock was pipette into 100ml volumetric flask and followed the same process as that of the stock solution.

Sample preparation. A 10 ml of extracted pineapple juice was pipetted into 100ml volumetric flask. Five millilitres (5.0 ml) of tetrahydrofuran was added and sonicated for 10 min. Afterwards, 60 ml of methanol and 25 ml of water were added and swirled to dissolve and allowed to cool to room temperature after which it was diluted to the mark with distilled water. The standard and sample were filled into loading vial and then loaded. Natamycin eluted at 11.5 min under the described conditions. Levels of natamycin present in pineapple were determined every three days for thirty six days during the period of analysis (calculation in appendix C).

3.10. EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS.

Design Expert 7 was used for optimization and Minitab 16 was also used for the generating of graphs and fitted equations whiles Tukey's test was used to determine the significant difference at 95% confidence level.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1. CHANGES IN THE PHYSIOCHEMICAL PROPERTIES OF PINEAPPLE JUICES PRESERVED WITH NATAMYCIN.

The juices obtained from MD2 and Sugar loaf varieties of pineapple were preserved with Natamycin concentration of 10 ppm and 20 ppm

4.1.1 Reduction in Vitamin C content of pineapple juices during Refrigeration.

Figures 4.1a and b below show a reduction in vitamin C content during refrigeration. The initial content of vitamin C in MD-2 and sugar loaf were 34.20 and 29.73 mg/100ml, respectively which falls within the stated level of vitamin C by the USDA National Nutrient Database for Standard Reference (2009), which ranges from 18 to 56.4 mg/100ml in fresh pineapple fruits depending on factors such as the cultivar, stage of maturity, conditions of storage, geographical area and the part of fruit.

The vitamin C content in untreated juice of the MD-2 decreased from 34.20 to 5.19 mg/100 ml, from 34.20 to 11.14 mg/100 ml in the 10 ppm treated sample and from 34.2 to 11.95 mg/100ml in the 20 ppm treated samples whiles that of the sugar loaf had its content decreasing from 29.73 to 6.58 mg /100 ml in the untreated samples (control), from 29.73 to 11.25 mg/100ml in the 10 ppm treated samples and from 29.73 to11.45 mg/100 ml for the 20 ppm treated samples.

Statistical analyses (in appendix B) showed that there was no significant difference ($p \geq 0.05$) between the 10 and 20 ppm treated samples of the two varieties however, significant difference ($p \leq 0.05$) was established between the untreated and the treated juices.

Contrary to other organic acids, vitamin C is quite unstable. This instability is mainly due to the activity of ascorbic acid oxidase and the reaction with oxygen in the

presence of heavy metal ions and light where ascorbic acid is oxidized to dehydroascorbic acid (Naidu, 2003). Vitamin C is also used as an indicator of fruit freshness and retention of other components. Losses of vitamin C are common in different fruits juices (Muller, 1998). Khassandra (2007) confirmed that the concentration of vitamin C in pineapple juices decrease during storage at 5^{o}C and 20^{o}C and also established that juices stored in open containers in a refrigerator for 31 days recorded vitamin C loss of 60 to70 %.

After the 36 days of storage the vitamin C retention in untreated juice of the MD2 variety of the pineapple was 15.17% whiles that of the 10 and 20 ppm treated samples were 32.57 and 34.94%, respectively. Sugar loaf had vitamin C retention of 22.13% in the control, 37.84% in the 10 ppm and 38.51% in the 20 ppm treated samples.

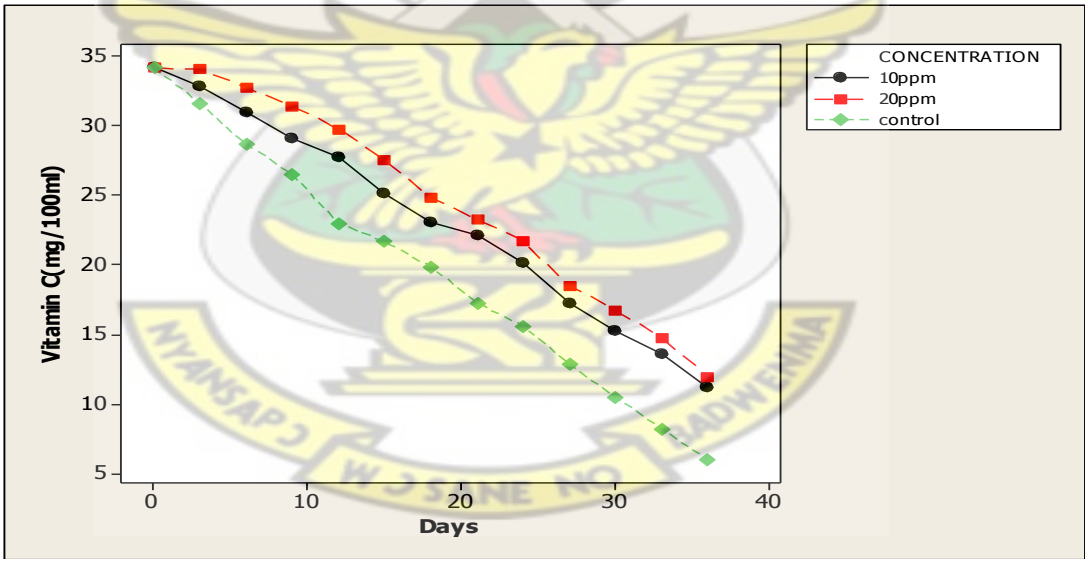

Figure 4.1a: Reduction in vitamin C content of MD-2 pineapple stored at 5^{o}C±2

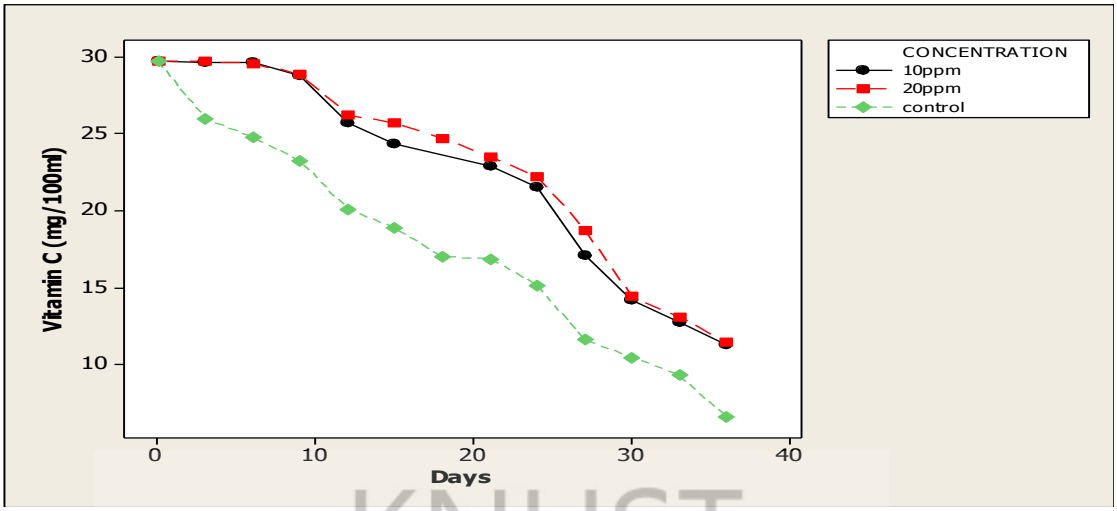

Figure 4.1b: Reduction in vitamin C content of sugar loaf pineapple stored at 5°C±2

4.1.2. Decrease in total soluble sugars of pineapple juices under refrigeration.

The soluble sugars of the two pineapple varieties MD2 and sugar loaf decreased with increasing days of storage. The results showed that untreated pineapple had a high rate of soluble sugars loss than that observed in the treated pineapple as shown in the figures 4.2a and b below. The initial soluble sugars of MD-2 and sugar loaf pineapple juices were 12.95 and 13.93 % respectively which falls within the stated level of total sugars by the USDA National Nutrient Database for Standard Reference (2009), as ranging from 8.29 to 13.56 % mg in fresh pineapple fruits. Soluble sugars of the untreated juice dropped from 12.95 to 11.34 % in the control sample, from 12.95 to 12.05% in 10 ppm and 12.95 to 12.02% in 20 ppm samples of MD-2 pineapples. Sugar loaf had its total soluble sugars reducing from13.93 to 11.70% in the control sample, from 13.93 to 12.22% in 10 ppm and from13.93 to12.25% in 20 ppm samples. There was however, no significant difference ($p \geq 0.05$) (Appendix B) between the 10 and 20 ppm treatments of the two varieties but significant difference ($p \leq 0.05$) existed between the untreated and the natamycin treated juice.

The rapid decreased in the soluble sugars in the controls may be due to increase in microbial activities as explained by (Brecht, 2006) that yeasts are known to degrade sugar and other sugar products through fermentation resulting in sugar reduction in fruit juices.

The slow reduction of sugars in treated samples may be due to the effect of the natamycin which binds to the cell membrane, the principal sterol in fungal membranes, causing destructions in the cells and making them leaky (EFSA, 2009).

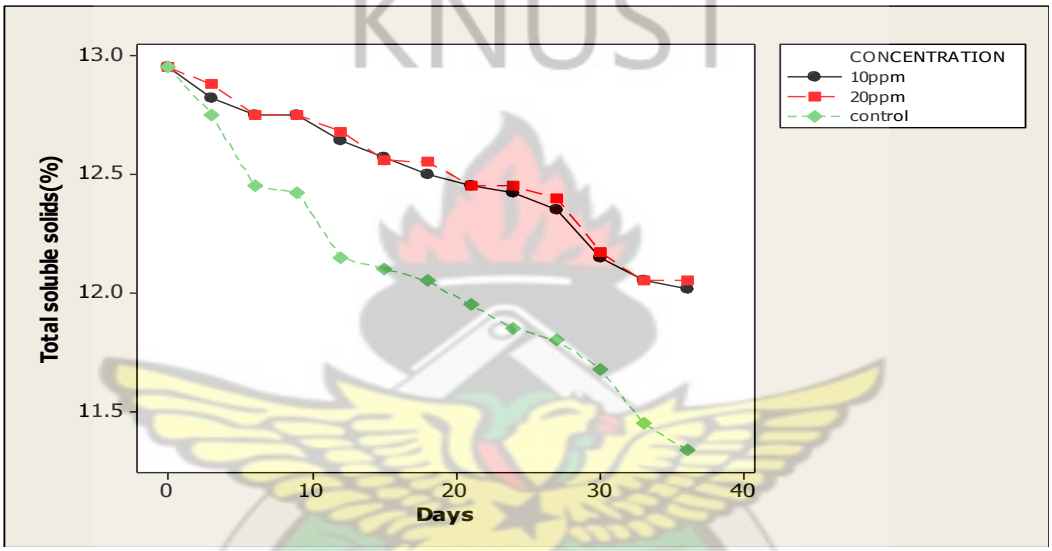

Figure 4.2a: Decrease in total soluble sugars of MD-2 pineapple juices stored at 5°C±2

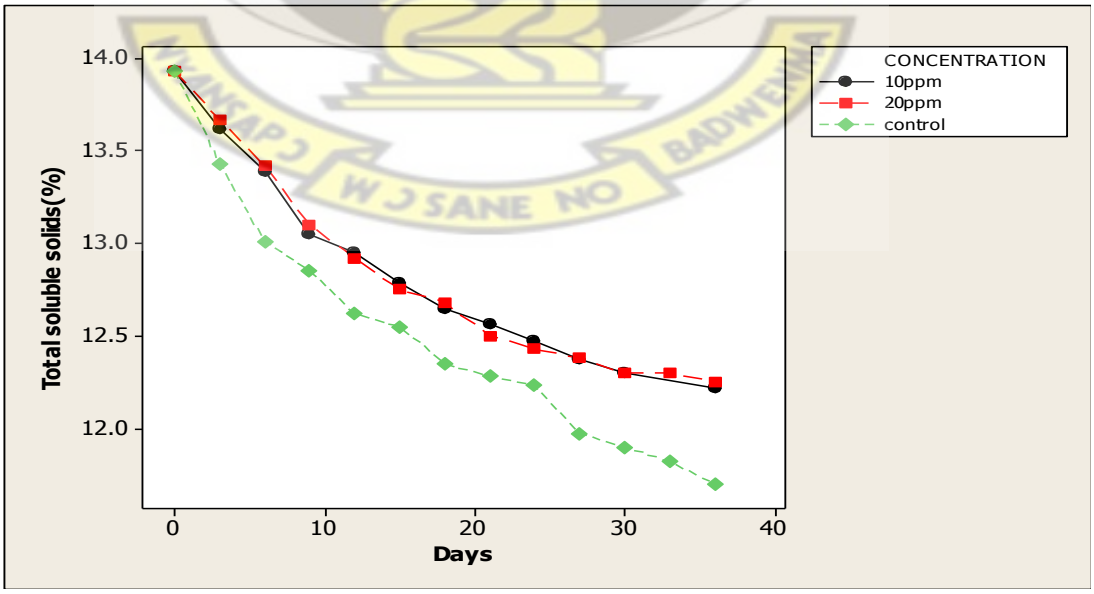

Figure 4.2b: Decrease in total soluble sugars of sugar loaf pineapple juices stored at 5°C±2

4.1.3 Changes in pH of pineapple juices during Refrigeration condition.

The pHs of the juices of the two varieties of pineapples are presented in figures 4.3. The initial pH of MD-2 and sugar loaf were 4.48 and 4.5 respectively which conforms to pH in pineapple fruits as ranging from 3.5 to 4.5 as reported by Kongsuwan *et al.,* (2009).The pH values of sugar loaf dropped quite sharply on the 3rd day from 4.50 to 4.19 in the control samples, from 4.50 to 4.26 in the 10 ppm treated samples and from 4.50 to 4.25 in the 20 ppm treated samples as compared to MD-2 which slightly dropped from 4.48 to 4.40, 4.48 to 4.44 and from 4.48 to 4.46 in the control, 10 and 20 ppm samples respectively. The pH of the two varieties increased on the 6th day and showed a decreasing trend on subsequent days until it stabilized during the end of storage. The increase in pH on the 6th day may due to the breakup of acids with respiration during storage as explained by (Pesis *et al*., 1999). In general the pH of MD-2 samples dropped from 4.48 to 3.18 in control samples, 4.48 to 3.25 in the 10 ppm treated samples and from 4.48 to 3.26 in the 20 ppm treated samples whiles that of the sugar loaf variety decreased from 4.5 to 3.17 in the control samples, 4.5 to 3.34 in the 10 ppm treated samples and from 4.5 to 3.39 in the 20 ppm treated samples at end of storage. Statistical analysis showed a significant difference ($p \leq 0.05$) (Appendix B) between the untreated and treated pineapple juices of the two varieties; however there was no significant difference ($p \geq 0.05$) between the pineapple juices treated with10 ppm and 20 ppm of natamycin. A drop in acidity is expected with increase storage time and temperature. It is therefore likely that decline in acid measured in stored pineapple juice is largely due to storage and increase in microbial activities (Burns and Echeverria, 1990). Kader (2002) confirmed that pH of fruits juices often show a noticeable reduction in acidity over a period of time during storage. Natamycin had

some effect on the pH of pineapple juices by slowly retarding its reduction. This is in agreement with work carried out by Majumder *et al.*, (2010) that Natamycin retarded the declination of pH of bottled gourd–basil leaves juice during the period of storage.

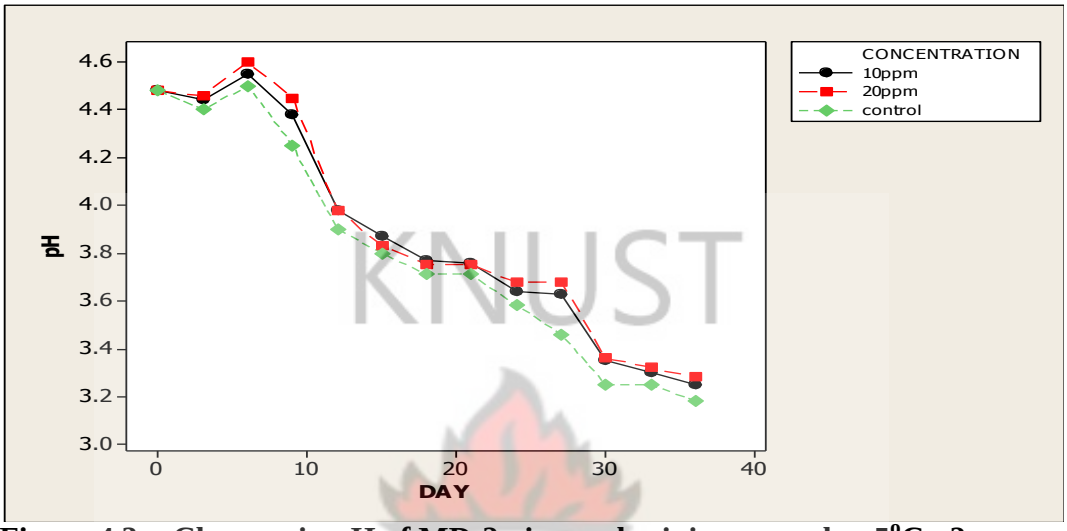

Figure 4.3a: Changes in pH of MD-2 pineapples juices stored at 5^{o}C ±2

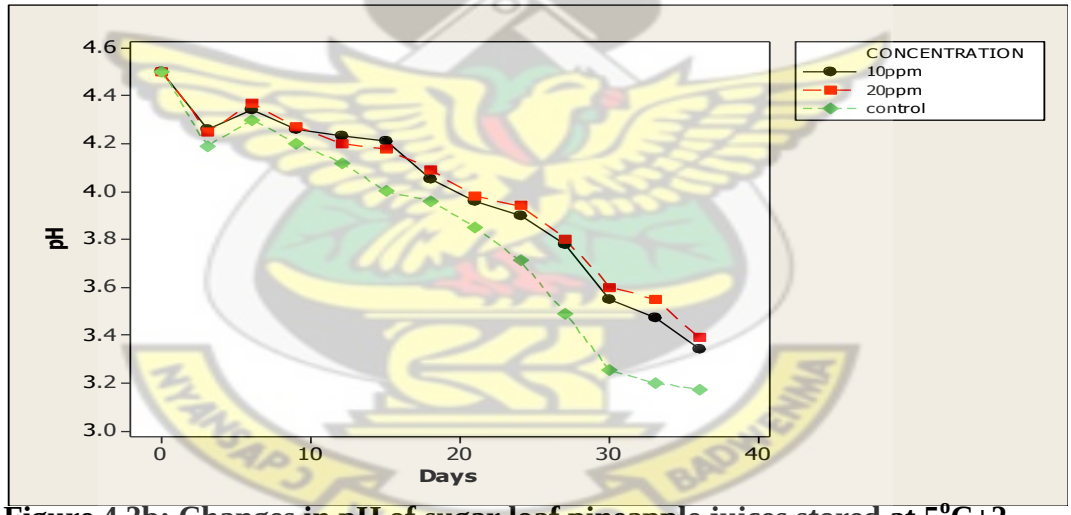

Figure 4.3b: Changes in pH of sugar loaf pineapple juices stored at 5^{o}C±2

4.1.4. Changes in titratable acidity of pineapple juices under Refrigeration.

The Titratable acidity (TA) of natamycin treated and untreated pineapple juices of the two varieties are presented in figures 4.4a and b below. The initial TA of MD-2 was 0.63 % whiles that of sugar loaf was 0.54% which agrees with work carried out by Masniza *et al.*, (2010) for pineapple fruits as ranging from 0.6 to 1.2%. The titratable acidity of MD-2 pineapples juices treated with 20 ppm of Natamycin and

the control increased from the 3rd day till the 9th day where a reduction was observed on the 12th day, increased slightly on the 15th day and continued till the end of storage. However, in the 10 ppm treated juices, there was an increase in titratable acidity on the 3rd day and decreased on the 6th day and gradually increased till the 36th day. The TA of sugar loaf showed an increase in the treated and untreated juice on the 3rd day, declined on the 6th day, increased quite steadily from the 9th day until the 15th day where a gentle increase was seen till the 24th day .TA dropped slightly on the 27th day and increased on the 30th day till the end of storage period for the untreated juices. The treated pineapple juices decline quite slowly than the control. There were however no significant difference ($p \geq 0.05$) between the 10 and the 20 ppm treated samples but a significant difference ($p \leq 0.05$) existed between the treated and the untreated pineapple juices. (Appendix B). Dharamadhikari (2007) established that higher acidity of fruit juices is often associated with lower pH values and vice versa but due to variations in buffer capacity, there are sometimes no direct relationship between pH and titratable acidity. However the results obtained from the chemical analysis showed that increase in titratable acidity corresponded with a decrease in pH.

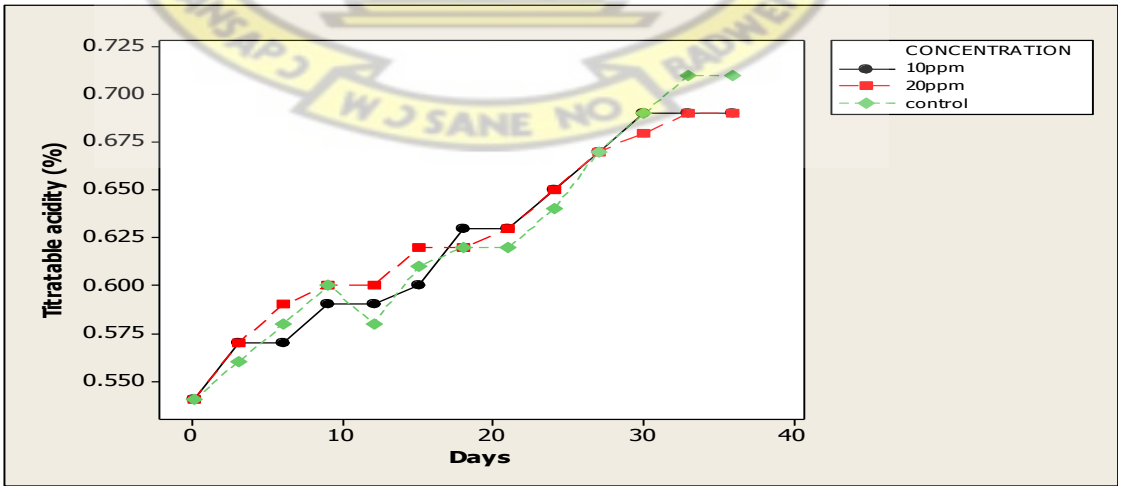

Figure 4.4a: Changes in titratable acidity of MD2 pineapple juices stored at 5°C±2

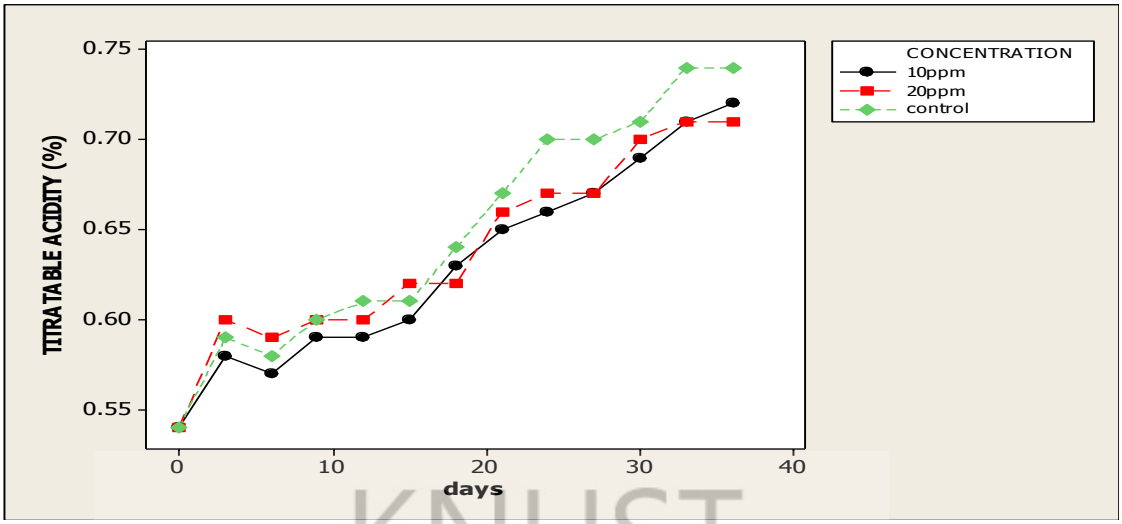

Figure 4.4b: Changes in titratable acidity of Sugar loaf pineapple juices stored at 5°C±2

4.1.5 Decrease in total solids of pineapple juices during Refrigeration.

The total solids of the juice gradually decreased with increase in storage days with the untreated samples rapidly decreasing more than the treated sample as presented in figures 4.5a and b. The initial total solids in MD-2 reduced from 15.5 to 11.97% for the control, from 15.5 to12.91% for 10 ppm samples and from 15.5 to13.05% for 20 ppm samples whereas total solids in sugar loaf reduce from15.20 to 11.95 % for the control, from 15.20 to 12.97% for 10 ppm samples and from 15.2 to 13.02% for 20 ppm samples. The initial solids of the two varieties fall within 12.10 to 16.0% as stated by Ikegwu and Ekwu (2009). According to Ikegwu and Ekwu (2009), the solid contents of food products are related to their food values and the decrease in total solids may be as a result of microbial activities. Their activities by way of utilizing the food also decrease the content of non-structural carbohydrates, protein and food energy. Statistical analysis showed no significant difference ($p \geq 0.05$) between the 10 ppm and the 20 ppm. However, significant difference ($p \leq 0.05$) was established between the treated and the untreated pineapple juices (Appendix B).

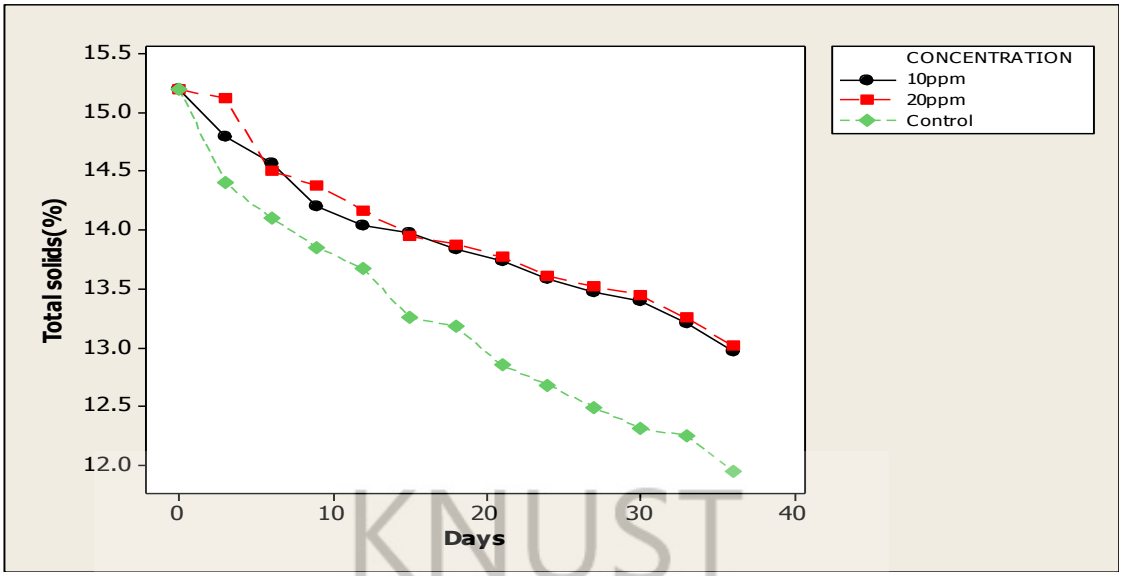

Figure 4.5a: Decrease in total solids of MD-2 pineapple juices stored at 5°C±2

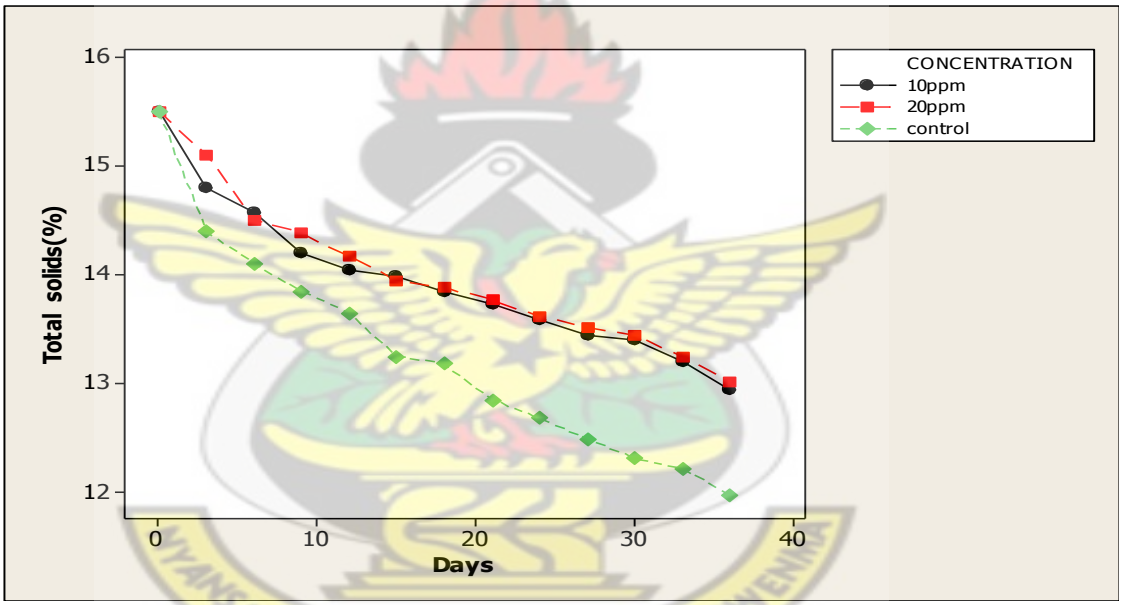

Figure 4.5b: Decrease in total solids of Sugar loaf pineapple juices stored at 5°C±2.

4.1.6. Changes in yeast count of pineapple juices under Refrigeration condition.

Figures 4.6a and b show changes in yeast count in pineapple juices. In the untreated juices the trend shows an exponential increase from the 3rd day in the yeast count up to the 24th day where the growth gradually decreased. The initial growth counts for the controls were $3.9x10^1$ and $4.0x10^1$ cfu/100 ml for MD-2 and sugar loafs

respectively whereas the final growth of yeast in juices were $1.86x10^7$ and $1.82x10^7$ cfu/100 ml for both MD-2 and sugar loaf variety. The trend was different for the treated pineapple juices since no growth was observed from the 3rd day till the end of storage period. There was an initial reduction of growth on the 1st day when treatment was applied and counts were $2.7x10^1$ and $2.8x10^1$ cfu/100ml for 10 ppm and 20 ppm for MD2 variety, whereas counts for sugar loaf was $2.6\ x10^1$ cfu/100ml for both 10 and 20 ppm treated samples (Appendix A).

Statistical analysis showed that there was no significant difference ($p \geq 0.05$) between 10 and 20 ppm treated samples, although significant ($p \leq 0.05$) difference existed in the treated juices and their controls (Appendix B). The gradual increase in the yeast count of untreated juice from the 3rd day to 27th day is due to the activities of yeast and utilization of nutrients present in the pineapple juices. The decrease in growth count from the 30th day till the 36th day is due to the depleting of nutrient. Thus when there is nutrient limitation or essential nutrients are severely depleted, growth slows down and may also cease due to the accumulation of toxic and waste products (Prescott *et al.*, 2002).

The absence of growth in the treated samples was due to the antifungal activity of natamycin that binds to the cell membrane, the principal sterol in fungal membranes, thereby making them leaky (Deacon, 1997). Stark (2003) found that the addition of 10 µg/ml of natamycin had an immediate reduction effect on yeast counts in orange-pumpkin juice inoculated with *S. cerevisia*e as well as samples that were not inoculated. After 1 week of storage at 2.5°C to 5°C, yeast counts were undetectable and the sample remained fresh for the 8-week duration of the test. This also is in agreement with a work done by Majumdar *et al.,* (2010), where after the addition of 5 ppm natamycin, yeast growth was totally absent in bottled gourd -basil leaves juice

during the 6 month storage period where as the control samples not containing natamycin were spoiled within 1 week. Comparing the results of yeast count of control with standards from Ghana Standards Authority (GSA) and Global Food Safety Standards (GFSS), count from day 0 to the 6^{th} day of the two varieties were within acceptable range. These were $9.5x10^3$ and $7.45x10^3$ cfu/100 ml for MD-2 and sugar loaf respectively for day 6, whereas from day 9 to the end of storage period yeast counts were beyond unacceptable levels. GSA specification for yeast counts for unpreserved juices is $1.6x10^4$ and $5.0x10^1$ cfu/ml for preserved juice, whereas that of the Global Food Safety Standards (GFSS, 2009) is 1.0 $x10^4$ cfu/ml. The International Commission on Microbiological Specifications for Foods on fruit juice (ICMSF, 1986) is 10^6 cfu/ml for both treated and untreated fruit juice.

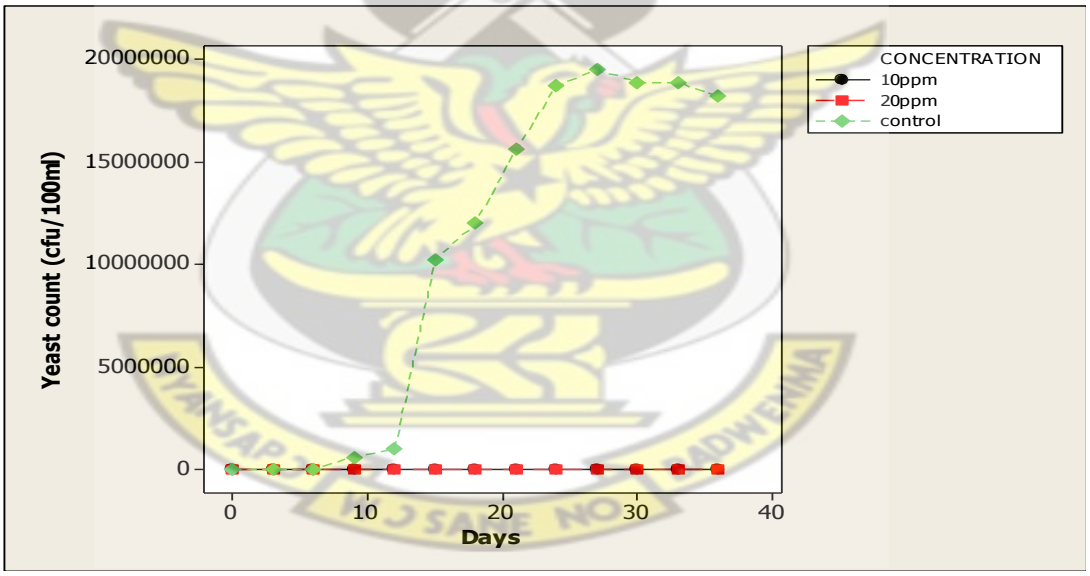

Figure 4.6a: Changes in yeast count of MD2 pineapple juices stored at 5^oC ±2

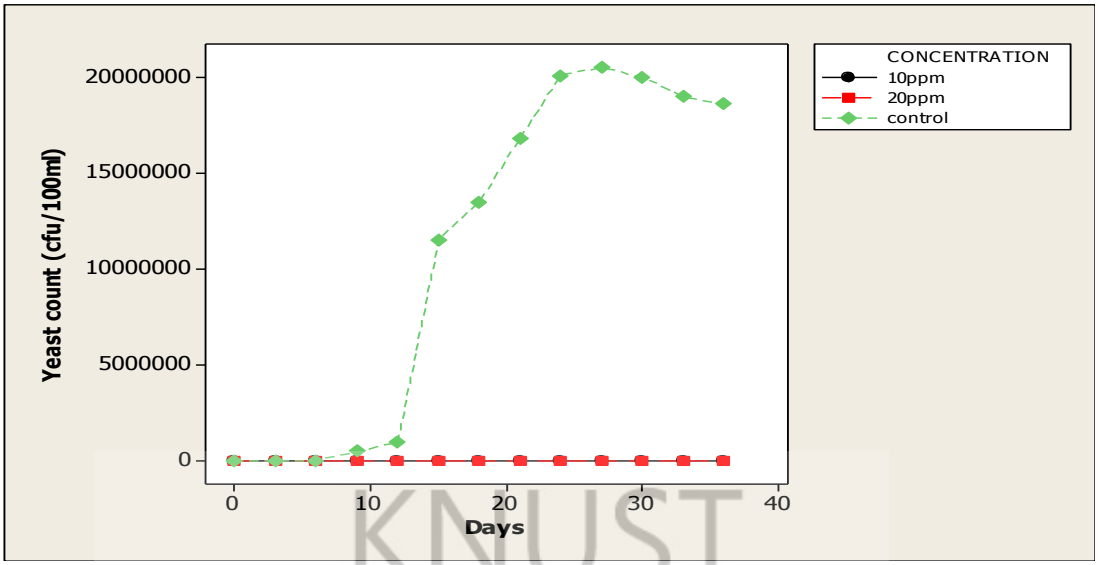

Figure 4.6b: Changes in yeast count of sugar loaf pineapple juices stored at 5^{o}C±2

4.1.7 Changes in Mould Count of pineapple juices under Refrigeration condition.

Figures 4.7a and b show changes in mould count in pineapple juices stored under refrigeration condition. The growth began with an initial count of 8.0x10^{1} and ended with 5.95x10^{3} cfu/100 ml for MD2 control samples whiles that of the sugar loaf control begun with 7x10^{1} and ended with 5.78x10^{3} cfu/100ml. The trend showed an exponential increase from the 3rdday in the mould count up to the 30th day where it gradually decreases in the untreated juices. Growth however was not observed in the 10 and 20 ppm treated samples of the two varieties. The gradual increase in the count of untreated juice from the 3rd day to 27th day is due to the activities of microbes and their utilization of nutrients present in the pineapple juices. The decrease in growth count from the 30th day till the 36th day is due to the environmental changes like nutrient deprivation and the build up of toxic wastes which slows down growth, causes death and leads to the decline in the number of microbes (Prescott *et al.*, 2002).

Statistically there was no significant difference ($p \geq 0.05$) in mould growth between the 10 and 20 ppm treated samples whereas significant difference ($p \leq 0.05$) existed between the controls and the treated juices (Appendix B). Comparing results obtained with that of GFSS and GSA, mould count for untreated juice were within acceptable limit for the first 6 days of storage period. Beyond the 6 days of storage, counts were beyond the acceptable limits thereby making the juice unwholesome for consumption. Growth was not present in the treated samples during the period of storage thereby making it wholesome for consumption. The specification of Ghana Standards Authority (GSA) for mould counts in unpreserved juice and preserve juices are $1.x10^3$ and $5.0x10^1$ cfu/ml respectively. That of the Global Food Safety Standards (GFSS, 2009) is $1.0x10^2$ cfu/ml. The International Commission on Microbiological Specifications for Foods on fruit juice (ICMSF, 1986) are 10^6 cfu/ml for both treated and untreated fruit juice. A study conducted by Stark (2003), shows that juice inoculated with natural contaminants spoil after 1 week of storage, whereas levels as low as 1.25 µg/ml of natamycin prevent mould growth during the storage period.

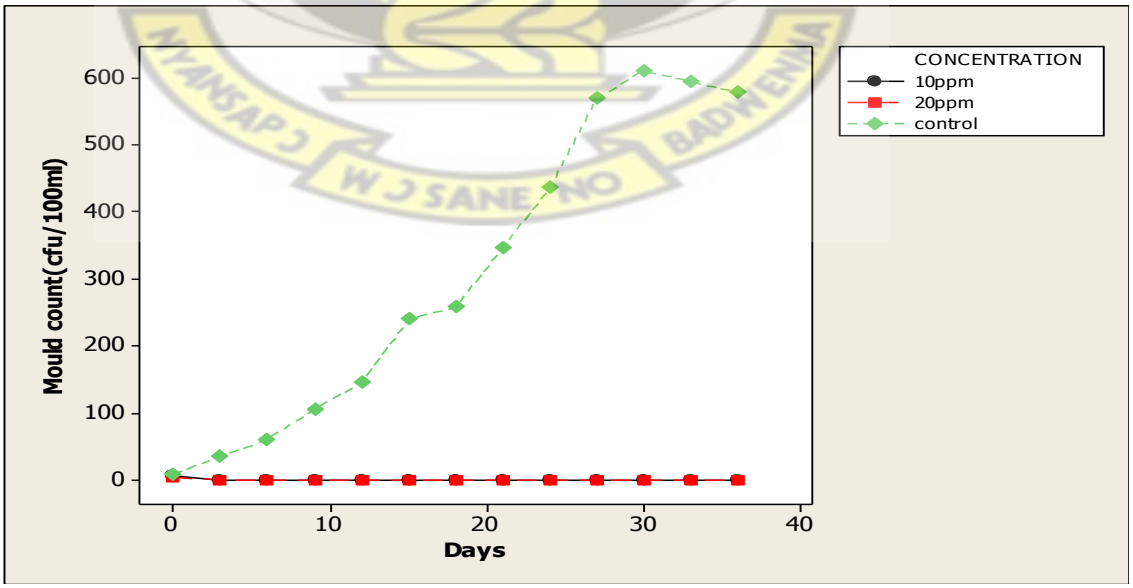

Figure 4.7a: Changes in mould count of MD-2 pineapple juices stored at 5°C±2

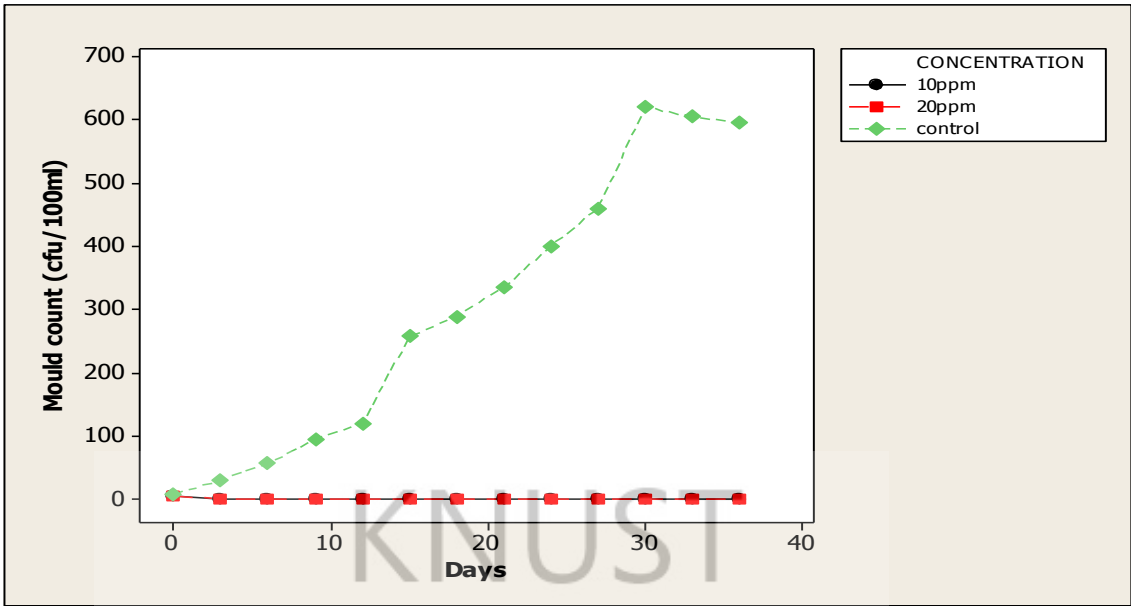

Figure 4.7 b: Changes in mould count of sugar loaf pineapple juices stored at 5°C±2

4.2. Sensory evaluation of pineapple juices during storage.

Sensory analyses of the two pineapple varieties were scored by panellist based on appearance, sweetness, odour, sourness and overall acceptability and are shown in the tables 4.1and 4.2 below. The average score by the panellists showed a high level of acceptance for all sensory parameters for the control and the treated juice samples of the two varieties within the first 6 day of storage. The results showed that there was no significant difference ($p \geq 0.05$) in the sensory parameters of both the control and treated juices during the first 6 days of storage. From the 9th day to the end of storage period sweetness and sourness were not determined as control samples had undergone fermentation; however, odour, appearance and overall acceptability were determined. After 9 days of storage period, panellist scored the treated juice higher than the control samples as they attributed it to the off- odours that was observed in the control (Appendix I).

Significant difference ($P < 0.05$) was established in the controls and treated juice in the odours, appearance and overall acceptability from the 9th day till the end of storage period (Appendix I).

About sixty eight percent (68%) of assessors preferred the natamycin treated juices because they commented that natamycin was able to preserve the strong pleasant odour, appearance of the pineapple and imparted no additional flavour to the pineapple till the end of storage while 32% commented that natamycin treated juice had a mild pleasant odour towards the end of storage period. According to Geise (1994), natamycin does not impart additional flavours to food products but rather help in maintaining its natural organoleptic properties.

Table 4.1: Sensory evaluation of MD-2 pineapple juice during refrigeration.

Storage days	Concentrations	sweetness	sourness	odour	appearance	Overall acceptability
0	0ppm	4.90^{a}±0.35	3.80^{a}±0.58	4.50^{a}±0.55	4.80^{a}±0.45	4.80^{a}±0.47
	10ppm	4.85^{a}±0.45	3.75^{a}±0.45	4.60^{a}±0.54	4.80^{a}±0.45	4.88^{a}±0.40
	20ppm	4.90^{a}±0.35	3.80^{a}±0.46	4.60^{a}±0.55	4.80^{a}±0.47	4.88^{a}±0.42
3	0ppm	4.85^{a}±0.71	3.70^{a}±0.71	4.55^{a}±0.71	4.80^{a}±0.45	4.80^{a}±0.57
	10ppm	4.88^{a}±0.42	3.80^{a}±0.83	4.60^{a}±0.83	4.80^{a}±0.45	4.80^{a}±0.47
	20ppm	4.88^{a}±0.42	3.85^{a}±0.54	4.60^{a}±0.54	4.80^{a}±0.45	4.85^{a}±0.45
6	0ppm	4.80^{a}±0.47	3.80^{a}±0.55	4.65^{a}±0.55	4.75^{a}±0.54	4.80^{a}±0.45
	10ppm	4.90^{a}±0.35	3.80^{a}±0.45	4.60^{a}±0.45	4.85^{a}±0.45	4.85^{a}±0.45
	20ppm	4.90^{a}±0.35	3.75^{a}±0.55	4.65^{a}±0.84	4.85^{a}±0.47	4.84^{a}±0.47
9	0ppm	-	-	1.50^{b}±0.47	3.70^{a}±0.47	2.30^{a}±0.45
	10ppm	-	-	4.50^{a}±0.71	4.80^{b}±0.54	4.70^{b}±0.47
	20ppm	-	-	4.50^{a}±0.71	4.85^{b}±0.55	4.80^{b}±0.45

Different superscripts in the same column within every three days are significantly different ($p \leq 0.05$).

Table 4.2: Sensory evaluation of sugar loaf pineapple juice during refrigeration condition

Storage days	Concentrations	sweetness	sourness	odour	appearance	Overall acceptability
0	0ppm	$4.88^{a}\pm0.61$	$4.00^{a}\pm0.71$	$4.60^{a}\pm0.55$	$4.70^{a}\pm0.48$	$4.80^{a}\pm0.45$
	10ppm	$4.88^{a}\pm0.00$	$3.90^{a}\pm0.84$	$4.60^{a}\pm0.54$	$4.80^{a}\pm0.55$	$4.80^{a}\pm0.54$
	20ppm	$4.90^{a}\pm0.30$	$4.00^{a}\pm0.83$	$4.65^{a}\pm0.55$	$4.80^{a}\pm0.55$	$4.80^{a}\pm0.54$
3	0ppm	$4.85^{a}\pm0.55$	$3.90^{a}\pm0.54$	$4.50^{a}\pm0.71$	$4.80^{a}\pm0.55$	$4.75^{a}\pm0.55$
	10ppm	$4.88^{a}\pm0.42$	$4.00^{a}\pm0.84$	$4.60^{a}\pm0.54$	$4.80^{a}\pm0.45$	$4.80^{a}\pm0.45$
	20ppm	$4.88^{a}\pm0.42$	$3.90^{a}\pm0.71$	$4.60^{a}\pm0.55$	$4.80^{a}\pm0.45$	$4.80^{a}\pm0.45$
6	0ppm	$4.80^{a}\pm0.47$	$3.70^{a}\pm0.54$	$4.55^{a}\pm0.55$	$4.75^{a}\pm0.71$	$4.65^{a}\pm0.55$
	10ppm	$4.80^{a}\pm0.54$	$3.80^{a}\pm0.55$	$4.60^{a}\pm0.45$	$4.80^{a}\pm0.45$	$4.75^{a}\pm0.45$
	20ppm	$4.80^{a}\pm0.54$	$3.80^{a}\pm0.55$	$4.65^{a}\pm0.84$	$4.80^{a}\pm0.45$	$4.80^{a}\pm0.45$
9	0ppm	-	-	$1.70^{b}\pm0.45$	$3.40^{a}\pm0.45$	$2.50^{a}\pm0.71$
	10ppm	-	-	$4.55^{a}\pm0.45$	$4.70^{b}\pm0.55$	$4.70^{b}\pm0.71$
	20ppm	-	-	$4.55^{a}\pm0.45$	$4.70^{b}\pm0.55$	$4.70^{b}\pm0.55$

Different superscripts in the same column within every three days are significantly different ($p\leq0.05$).

4.3. DATA ANALYSES OF RESPONSES OF CUT PINEAPPLE.

4.3.1. Data analysis of Vitamin C.

Table 4.3. shows an ANOVA table for various possible models for vitamin C. None of the models with factors used in the experiment was suggested since all p-values were more than 0.05. This implies that the factors used in the experiment did not have any significant effect on the level of vitamin C. However, the 'mean vs total' model, a constant model, was selected by Design Expert7. The design also generated the coefficent estimate (Appendix H) which showed that the suggested constant

model had a constant or intercept value of 37.27 with a standard error of 0.33. With 95% confidence, the intercept value lied between 36.59 and 37.94.

Table 4.3: Fit summary Analysis for Vitamin C

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Mean vs Total	**33321.62**	**1**	**33321.62**			**Suggested**
Block vs Mean	**1.932E-003**	**2**	**9.662E-004**			
Linear vs Block	**1.61**	**4**	**0.40**	**0.16**	**0.9565**	
2FI vs Linear	**10.06**	**6**	**1.68**	**0.56**	**0.7563**	
Quadratic vs 2FI	**10.67**	**3**	**3.56**	**1.27**	**0.3492**	**Aliased**
Cubic vs Quadratic	**16.36**	**4**	**4.09**	**2.68**	**0.1813**	**Aliased**
Residual	**6.10**	**4**	**1.53**			
Total	**33366.43**	**24**	**1390.27**			

4.3.2 Data analysis of pH

Table 4.4 shows an ANOVA table for various possible models for pH. None of the models with factors used in the experiment was suggested since all p-values were more than 0.05. However, the 'mean vs total' model, a constant model, was selected by Design Expert 7. The coefficient estimate showed the suggested constant model had a constant or intercept value of 4.53 with a standard error of 8.324E-003. With 95% confidence, the intercept value lied between 4.52 and 4.55(Appendix H)

Table 4.4: Fit summary Analysis for pH.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Mean vs Total	492.95	1	92.95			Suggested
Block vs Mean	2.083E-005	2	1.042E-005			
Linear vs Block	1.959E-003	4	4.899E-004	0.31	0.8654	
2FI vs Linear	8.600E-003	6	1.433E-003	0.88	0.5428	
Quadratic vs 2FI	4.636E-003	3	1.545E-003	0.92	0.4721	Aliased
Cubic vs Quadratic	9.150E-003	4	2.287E-003	2.16	0.2366	Aliased
Residual	4.230E-003	4	1.058E-003			
Total	492.98	24	20.54			

4.3.3 Data analysis of Total Soluble Sugars (TSS)

Table 4.5 shows an ANOVA table for various possible models for total soluble sugars. None of the models with factors used in the experiment was suggested since all p-values were more than 0.05. The 'mean vs total' model, a constant model, however was selected by Design Expert 7. The suggested constant model had a constant or intercept value of 12.02 with a standard error of 0.019. At 95% confidence, the intercept value lied between 11.98 and 12.06 (Appendix H).

Table 4.5: Fit summary Analysis for Total Soluble Sugars

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Mean vs Total	3465.61	1	3465.61			Suggested
Block vs Mean	1.493E-003	2	7.467E-004			
Linear vs Block	3.444E-003	4	8.611E-004	0.098	0.9817	
2FI vs Linear	0.030	6	4.996E-003	0.46	0.8243	
Quadratic vs 2FI	0.029	3	9.517E-003	0.84	0.5110	Aliased
Cubic vs Quadratic	0.066	4	0.017	2.67	0.1820	
Residual	0.025	4	6.199E-003			
Total	3465.76	24	144.41			

4.3.4 Data analysis of titratable acidity

Table 4.6 shows an ANOVA table for various possible models for titratable acidity. None of the models with factors used in the experiment was suggested since all p-values were more than 0.05. The 'mean vs total' model, a constant model, however was selected by Design Expert 7. The suggested constant model had a constant or intercept value of 0.59 with a standard error of 3.54E-003. At 95% confidence, the intercept value lied between 0.58 and 0.59 (Appendix H).

Table 4.6 : Fit summary Analysis for Titratable acidity

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob >F	
Mean vs Total	8.31	1	8.31			Suggested
Block vs Mean	6.883E-004	2	3.442E-004			
Linear vs Block	1.711E-004	4	4.278E-005	0.14	0.9635	
2FI vs Linear	2.475E-003	6	4.125E-004	1.75	0.2002	Aliased
Quadratic vs2F	I3.739E-004	3	1.246E-004	0.45	0.7255	Aliased
Cubic vs Quadratic	1.275E-003	4	3.188E-004	1.34	0.3912	
Residual	9.500E-004	4	2.375E-004			
Total	8.31	24	0.35			

4.3.5 Data analysis of Absorbance (rate of diffusivity).

From Table H.1in appendix H for absorbance, the linear model with blocks was suggested having a p-value << 0.05, following the conditions for the selection of models as stated earlier. The models Quadratic vs 2FI and Cubic vs Quadratic were not suggested though they had p-values < 0.05 since they had aliased model terms (aliased is when the estimate of an effect is influenced by one more factors or there is high interactions between one or more factors).

The lack of fit test was further conducted to ascertain whether the suggested model fit into the data well. The p-value for the linear was 0.1744 > 0.05. Hence there is no lack of fit at the 5% significance level. Further, the model summary statistics displayed showed that the linear model is adequate since it recorded the maximum Adjusted R-Squared (Appendix H).

4.3.6 Optimization of Responses.

In selecting the optimized response, the factor combinations that gave the most desirability were selected. Desirability plot is at appendix F. A desirability of 0.750 was selected after optimization which corresponds to 1mm thickness, 2 minutes time, 100 ml volume and a concentration of 30 ppm.

Table 4.7 shows a validation response of factors, where the predicted values were validated with the actual observed response. After validation the results showed that there was no significant difference ($p \geq 0.05$) between the predicted and actual response values.

Table 4.7: Validation response of factors.

FACTORS				
Thickness	Time	volume	concentration	
1.00mm	2min	100ml	30ppm	
PREDICTED RESPONSE				
Vitamin C	TSS	pH	TA	Rate of diffusivity (Absorbance)
37.261 ± 0.05^{b}	12.183 ± 0.02^{a}	4.5321 ± 0.01^{b}	0.58614 ± 0.00^{a}	1.1733 ± 0.05^{a}
ACTUAL REPONSE				
37.30 ± 0.02^{b}	12.15 ± 0.01^{a}	4.55 ± 0.05^{b}	0.59 ± 0.05^{a}	1.1751 ± 0.02^{a}

Mean values with same letters indicate no significant difference ($p \geq 0.05$)

4.4 CHANGES IN THE PHYSIOCHEMICAL PROPERTIES OF CUT PINEAPPLES PRESERVED WITH NATAMYCIN.

4.4.1 Reduction in vitamin C content of cut pineapples under refrigeration and ambient conditions.

Tropical and subtropical fruit like pineapples are important sources of vitamin C which is one of the most important vitamins for human nutrition. In this regard, vitamin C is a quality parameter of fruits, and should be kept at an appropriate level. In general, the higher the content of vitamin C, the better the quality of the fruit (Gonzalez-Aguilar *et al.*, 2005).

The vitamin C content of the cut pineapple shown in figures 4.8a and b exhibited a decreasing trend. The untreated samples (controls) of the two pineapple varieties showed a rapid declination than the natamycin treated samples stored at 5^{o}C. The initial content of vitamin C in MD-2 and sugar loaf were 35.37 and 30.40 mg/100ml respectively, which falls within the stated level of vitamin C by the USDA National Nutrient Database for Standard Reference (2009), which ranges from 18 to 56.4 mg/100ml in fresh pineapple fruits is dependent on factors such as the cultivar, stage of maturity, conditions of storage, geographical area and the part of fruit. The vitamin C content of the control dropped from 35.37 to 5.53 mg/100ml and 35.40 to 10.50mg/100ml in the treated samples of the MD-2 variety whiles that of the sugar loaf reduced from 30.40 to 5.70 mg/100ml in the control samples and 30.40 to 10.60mg/100ml in the treated samples. Statistical analyses showed that there was a significant difference ($p \leq 0.05$) between the controls and treated samples of the two varieties. (Appendix D). However the vitamin C content of the controls and treated samples of the two pineapple varieties stored under ambient condition (25^{0}C ±2) showed no significant differences (($p \geq 0.05$). The values of vitamin C reduced from

35.30 to 16.6 mg/100 ml and from 30.40 to 14.60 mg/100ml respectively for MD-2 and sugar loaf controls. The treated samples also decreased from 35.30 to 16.70 mg/100 ml and from 30.40 to 14.50mg/100 ml for MD-2 and sugar loaf respectively. In general, vitamin C in cut fruit is sensitive to conditions such as high temperature, light exposure and ascorbate oxidase. All of these conditions have been reported to promote the transformation of L-ascorbic acid to dehydroascorbic acid. (Hernández *et al.,* 2006).

However, losses of ascorbic acid are common in different fresh-cut fruits. It has been reported that losses as high as 20 to 60 % of ascorbic acid occur in pineapple slices after 9 and 14 days of storage at 5ºC (Gonz´alez-Aguilar *et al.,* 2004).

Rapid decline in vitamin C in treated cut fruit was due to the fact that at ambient temperature natamycin degraded fast in acidic conditions. This is due to hydrolysis of the glycosidic bond to yield mycosamine and various other products causing natamycin to lose it potency (Koontz *et al.,* 2003). At the end of the storage period the vitamin C retentions were 15.58 and 29.75% for both control and treated samples of the MD2 variety whiles that of sugar loaf were 18.75 and 34.86% for the control and treated samples respectively stored under refrigeration. The cut pineapples stored under ambient condition for a period of 12 days had its vitamin C retention of 47.02 and 40.2 % for MD-2 and sugar loaf respectively. Comparative analysis of refrigerated samples showed that there was a significant difference ($p \leq 0.05$) between vitamin C of MD-2 and Sugar loaf pineapples (Appendix G).

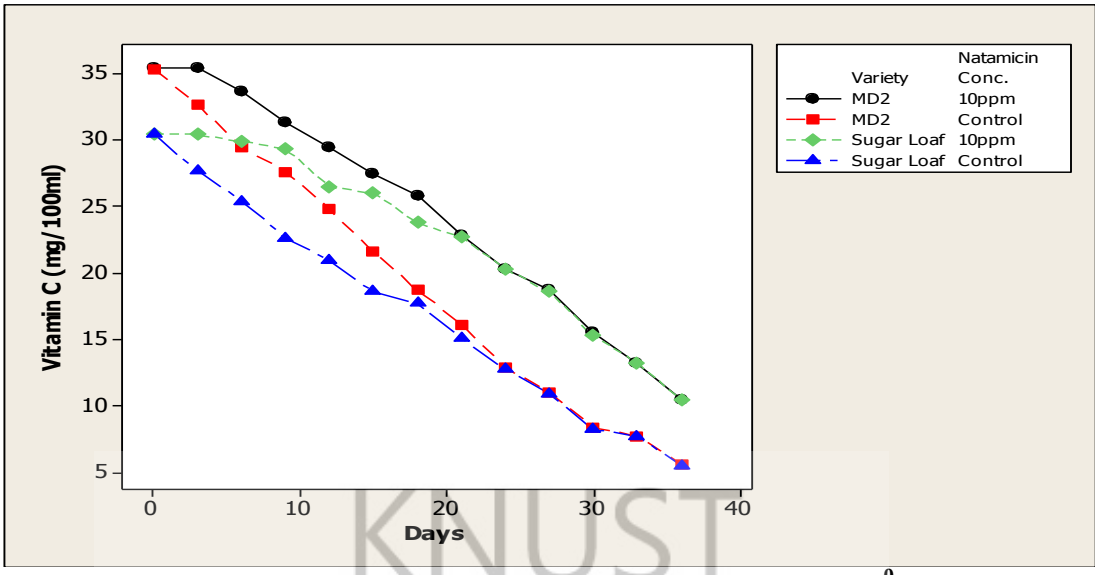

Figure 4.8a: Reduction in vitamin C of cut pineapples stored at 5^0C±2

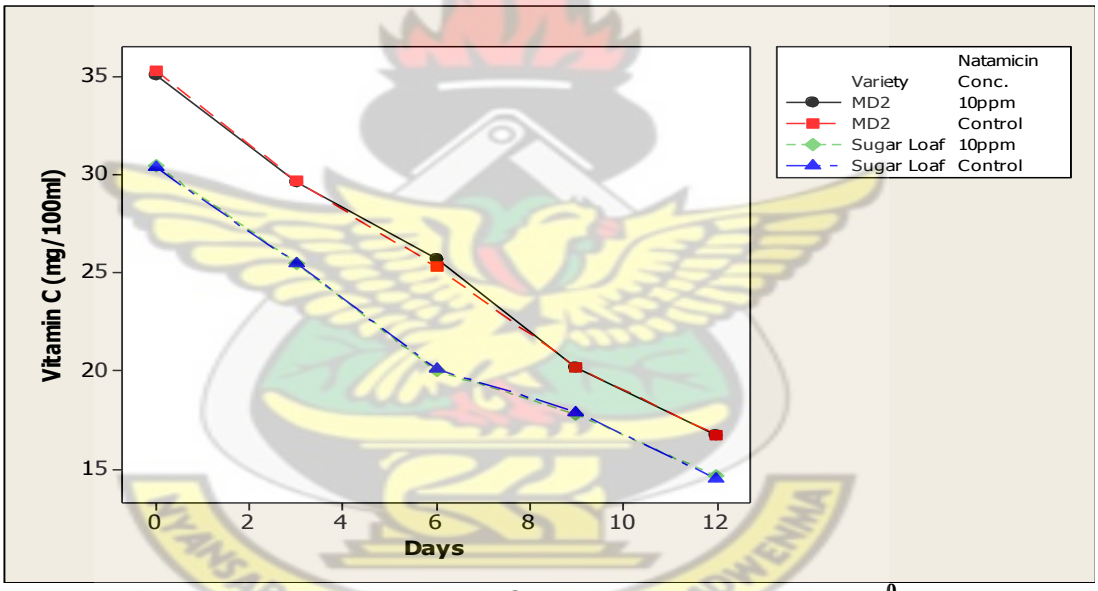

Figure 4.8b: Decrease in Vitamin C of cut pineapple, stored at 25^0C±3

Fitted Trend Equation for MD-2 pineapple (control)

Yt = 37.327 - 2.56429*t…….. (1)

(Where Yt= expected value, t = numbers of days).

Fitted Trend Equation for treated pineapple.

Yt = 39.742 - 2.16538*t…… (2)

2. Fitted Trend Equation for Sugar loaf pineapple (control)

Yt = 32.125 - 2.36128*t…… (3)

Fitted Trend Equation for treated pineapple.

Yt = 32.432 - 2.10118*t……. (4)

The trend analysis and the fitted trend equation above was established to help predict the expected outcome of vitamin C if samples were kept beyond the 36 storage days at a temperature of 5 ºC.

4.4.2 Decrease in total soluble sugars (TSS) in cut pineapples under refrigeration and ambient conditions.

The Figures 4.9 a and b below shows the reduction of total soluble sugars (TSS) in cut pineapples stored under refrigeration and ambient conditions respectively. The TSS in the MD-2 and sugar loaf cut pineapples stored at 5^{o}C±2 decreased from 14.60 to 11.50% and from 13.60 to 10.50 % respectively for the control samples whiles the treated samples dropped from 14.60 to 12.55 % for MD2 pineapple and from 13.60 to 11.75% for the sugar loaf variety. The initial soluble sugars of MD-2 of 14.60 % falls within the value of 14.4 to 18.8% as stated by Ramsaroop and Saulo (2007) whiles sugar loaf pineapple juices of 13.60 % falls within the stated level of total sugars by the USDA National Nutrient Database for Standard Reference (2009), which ranges from 8.29 to 13.56 % mg in fresh pineapple fruits.

However, samples stored at 25^{o}C±3 for 12 days decreased from 14.70 to 11.50 % for the control and 14.70 to 11.60 % for treated samples of MD2 variety, whereas sugar loaf decreased from 13.40 to11.60 % and 13.40 to 11.70 % for the control and treated samples respectively. Statistical analysis indicated that there were significant differences ($p \leq 0.05$) between the controls and the treated samples of the two varieties stored at 5^{o}C± 2 whereas no significant difference ($p \geq 0.05$) existed between samples stored at 25 ^{o}C±2 (Appendix D). The rapid decreased in the soluble

sugars in the controls may be due to increase in microbial activities as explained by (Brecht, 2006) that yeasts are known to degrade sugar and other sugar products through fermentation resulting in sugar reduction in cut fruit.

The soluble sugar is also used as an indication of fruit maturity and quality (Paull, 1993) and for pineapples; they range between 10.8 - 17.5% (Dull, 1992). The data for Sugarloaf and MD2 were within these limits. Comparative analysis showed significant differences ($p \leq 0.05$) exist between sugars of MD-2 and Sugar loaf. (Appendix G).

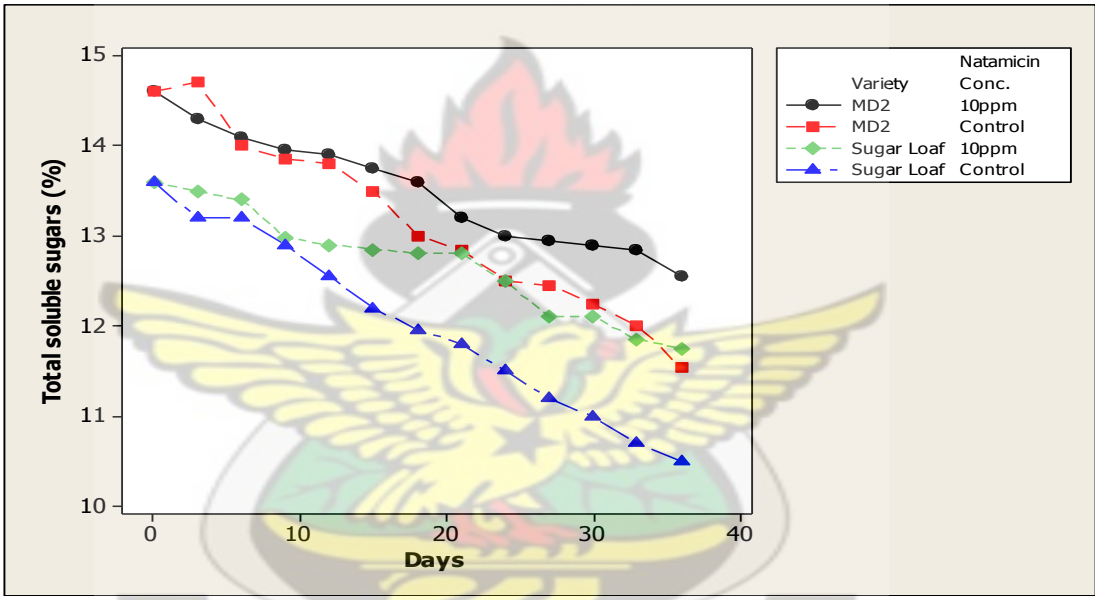

Figure 4.9a: Decrease in total soluble sugars of cut pineapples stored at 5^{o}C ±2

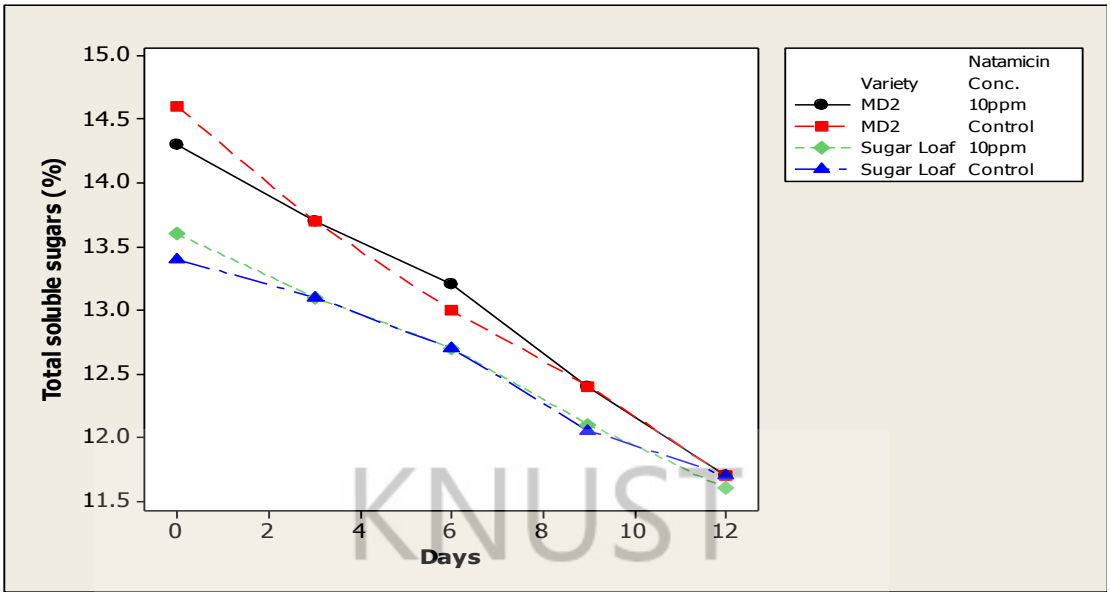

Figure 4.9b: Reduction in total soluble sugars of cut pineapples stored at 25°C ±3

Fitted Trend Equation for treated sugar loaf cut pineapple

Yt = 13.745 - 0.133187*t…….. (5)

Fitted Trend Equation for control cut pineapple.

Yt = 13.617 - 0.158736*t …… (6)

Fitted Trend Equation for treated MD-2 cut pineapple

YT=14.858- 0.141195*t…… (7)

Fitted Trend Equation for control MD-2 cut pineapple

Yt = 14.745-0.16346*t…….. (8)

The fitted trend equations above were established to help predict the expected outcome of total soluble sugars if samples were kept beyond the 36 storage days at a temperature of 5 ºC.

4.4.3 Changes in pH of cut pineapples under refrigeration and ambient conditions.

The trend analysis and the fitted trend equation below were established to help predict the expected outcome of pH if samples were kept beyond the 36 days of storage. The initial pHs of MD-2 and sugar loaf were 4.00 and 4.50 respectively which conforms to pH in pineapple fruits as ranging from 3.5 to 4.5 as reported by Kongsuwan *et al.*, (2009).

The pH of the treated and untreated cut fruits during storage are presented in Figures 4.10a and b. The figures show a clear reduction in pH of both the untreated and treated samples during the storage of the cut pineapple. Compared to the treated samples the reduction in the untreated samples of the sugar loaf variety was at a fast rate and showed a decreasing and increasing trend from the 3rd day till the 12th day and gradually decreased from the 15th day until the 27th day where the pH declined rapidly and remained steady till the 36th day. The initial pH of sugar for both the treated and untreated samples was 4.5 whereas the final pHs were 3.39 and 3.17 respectively. The reduction of pH of the MD-2 variety was not much different from that observed in the sugar loaf. The initial pH of the untreated and the treated sample was 4.0 which reduced on the 3rd day and slightly increased on the 6th day and remained quite stable on the 9th day and begun decreasing from the 12th day till the end of storage period with a value of 2.84 for treated samples and 2.76 for untreated samples. The values of the treated samples were much higher than those observed in the untreated samples. In figures 4.10 b both the treated and untreated samples of cut pineapples stored under ambient condition showed a rapid declination in pH from the 3rd day to the 12th day(end of storage for ambient). Sugar loaf had an initial pH of 4.0 and 4.20 for both the untreated and treated samples whiles its final

pH was 2.50 and 2.52 respectively. Initial pHs of treated and untreated samples of MD-2 cut pineapples were 4.18 and 4.0 whereas the final pH were 2.70 and 2.68 respectively. There was however no significant difference ($p \geq 0.05$) between the treated and untreated samples of the two varieties stored under ambient temperatures but a significant difference ($p \leq 0.05$) existed between the treated and untreated samples stored under refrigeration condition (Appendix D). Comparative analysis showed that there was a significant difference ($p \leq 0.05$) between MD-2 and sugarloaf cut pineapples (Appendix G). According to Burns and Echeverria (1990) decrease in acidity is expected with increase storage time and temperature. It is therefore likely that decline in acid measured in stored cut pineapple is largely due to storage and increase in microbial activities. Kader (2002) confirmed that pH of fruits often show a noticeable reduction in acidity over a period of time during storage.

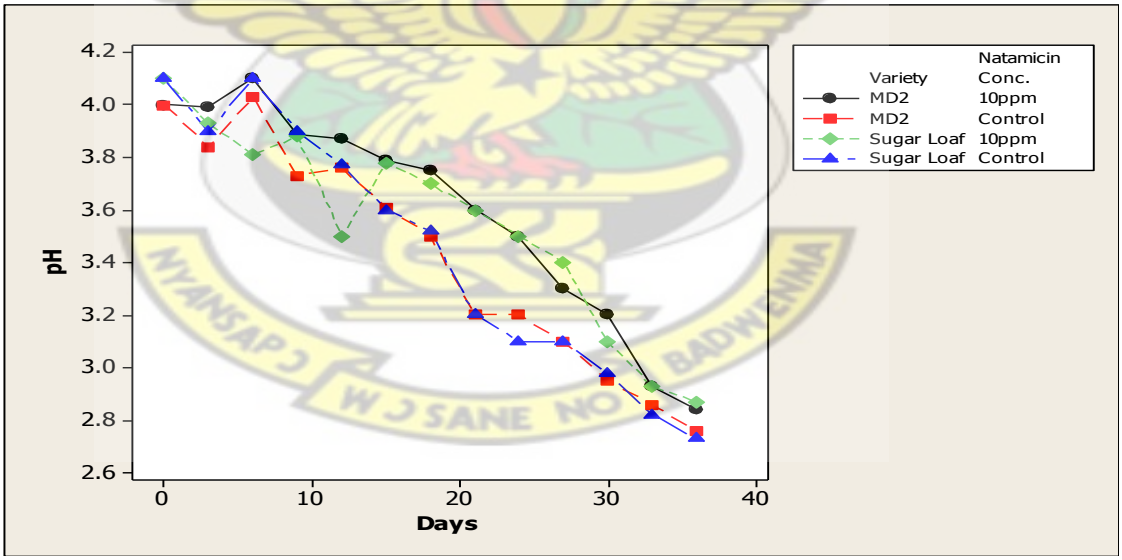

Figure 4.10a: Changes in pH of cut pineapples stored at 5°C ±2

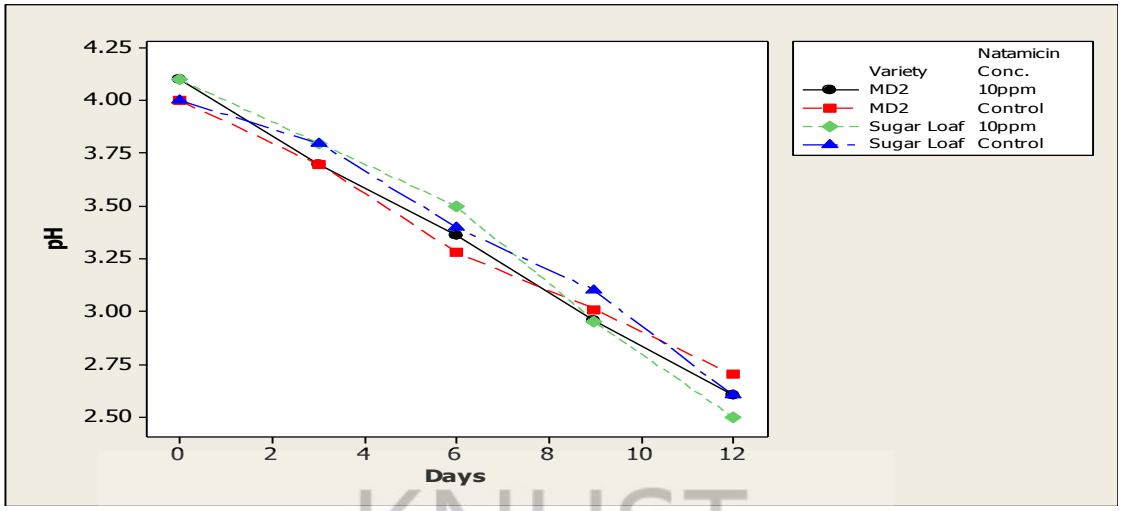

Figure 4.10b: Decrease in pH of cut pineapples stored at 25°C ±3

Fitted Trend Equation for the control pineapples

Yt = 4.4073 - 0.045989*t…… (9)

Fitted Trend Equation for the treated pineapples

Yt = 4.3965 - 0.036538*t…….. (10)

The fitted trend equations above were established to help predict the expected outcome of pH if samples were kept beyond the 36 storage days at a temperature of 5 °C.

4.4.4 Changes in titratable acidity of cut pineapple under refrigeration and ambient conditions.

The trend analysis and the fitted trend equation below were established to help predict the expected outcome of titratable acidity if samples were kept beyond the 36 storage days. The titratable acidity of sugar loaf cut pineapple shown in figure 4.11a below increased from 0.53 to 0.72% in the controls whiles that of the treated sample was from 0.53 to 0.74%. The initial TA of MD-2 of 0.58 % and sugar loaf of 0.53% falls out range with work carried out by Masniza *et al.,* (2010) for pineapple fruits

ranging from 0.6 to 1.2%. There was however some increases and decreases within the first 15 days. Titratable acidity for MD-2 increased from 0.58 to 0.72 % in the control samples whereas the treated samples were from 0.58 to 0.70%. The cut pineapple stored under ambient condition for 12 days increased rapidly in titratable acidity and showed no significant difference ($p \geq 0.05$) between the control and the treated samples (figure 4.11b). However, significant difference ($p \leq 0.05$) existed between the treated and control samples of those stored under refrigeration (Appendix B). Comparative analysis shows that significant difference ($p \geq 0.05$) does not exist between sugar loaf and MD-2 (Appendix G). Dharamadhikari (2007) established that higher acidity of cut fruit is often associated with lower pH values and vice versa but due to variations in buffer capacity, there are sometimes no direct relationship between pH and titratable acidity. However the results obtained from the chemical analysis showed that increase in titratable acidity corresponded with a decrease in pH.

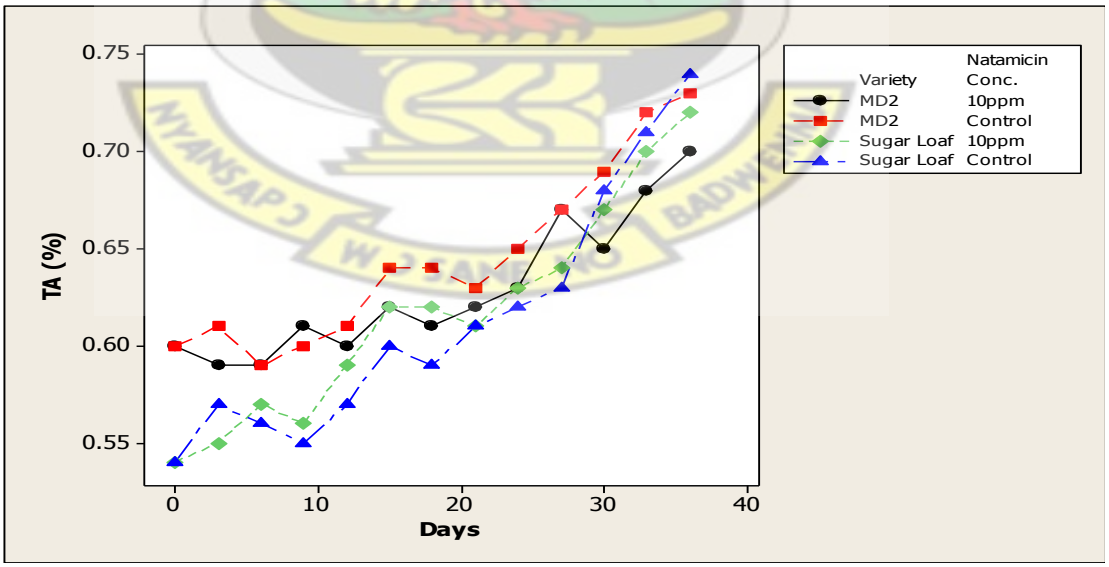

Figure 4.11 a: Changes in titratable acidity of cut pineapples stored at 5°C±2

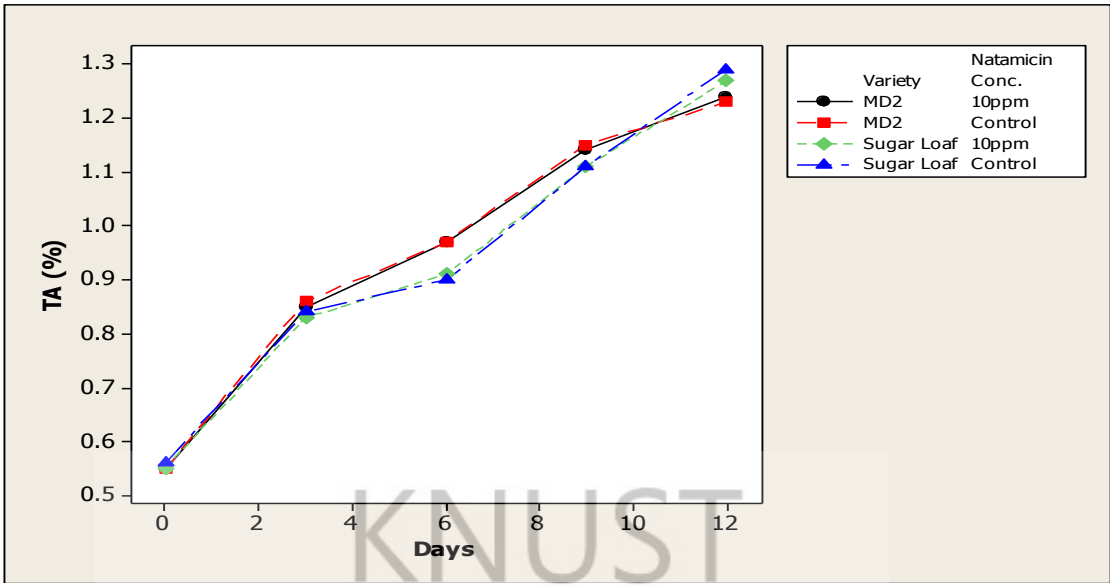

Figure 4.11 b: Increase in tiratable acidity of cut pineapples stored at 25 ^{o} C±3

Fitted Trend Equation for the treated pineapples

Yt = 0.5654 + 0.0109*t…… (11)

Fitted Trend Equation the control pineapples

Yt = 0.56923 + 0.0121*t…… (12)

The fitted trend equations above were established to help predict the expected outcome of titratable acidity if samples were kept beyond the 36 storage days at a temperature of 5 ºC.

4.4.5 Changes in yeast count of cut pineapples under refrigeration and ambient conditions.

Changes in the cut fruits stored under refrigeration were not different from those observed in the juice. The increase in growth is shown in figure 4.12a. The growth began with $1.5x10^{1}$ and ended with $6.87x10^{6}$ cfu/100 ml for the control samples of MD-2 variety whiles that of the sugar loaf began with $1.7x10^{1}$ and ended with $8.77x10^{6}$ cfu/100 ml. Growth was however not present in treated samples. The

highest growth in the control samples was on the 24th day with counts of $1.93X10^7$ and $1.91x10^7$ cfu/100 ml for MD2 and sugar loaf respectively. The ambient storage of the cut pineapples showed quite a different trend where growth began steadily and sharply increased on the 6th day till the 12th day storage period for both control and treated samples (figure4.12b). Statistical analysis shows that there was no significant difference ($p \geq 0.05$) in yeast growth in the treated and untreated cut pineapples under ambient condition, although significant difference ($p \leq 0.05$) existed in the treated and untreated samples stored at 5°C (Appendix D). The decrease in growth count from the 30th day till the 36th day is due to the environmental changes like nutrient deprivation and the build up of toxic wastes which slows down growth, causes death and leads to the decline in the number of microbes (Prescott *et al.,* 2002). However natamycin had no effect on cut pineapple under ambient condition, this is due to the fact natamycin degrade fast in acidic conditions through the hydrolysis of the glycosidic bond to yield mycosamine and methyl esters causing natamycin to further degrade rapidly (Koontz *et al.,* 2003). Comparing results obtained with that of GFSS and GSA, yeast count for untreated cut pineapples were within acceptable limit for the first 6 days of storage period. Beyond the 6 days of storage, counts were beyond the acceptable limit thereby making the cut fruit unwholesome for consumption.

Growth was not present in the treated samples during the period of storage thereby making it wholesome for consumption. Specifications by the Ghana Standards Authority (GSA) for yeast counts in unpreserved cut fruits is $1.x10^3$ and $5.0x10^1$ cfu/ml for preserved cut fruits and that of the Global Food Safety Standards (GFSS,2009) is $1.0x10^4$ cfu/ml. Comparative analysis shows that significant difference does not exist ($p \geq 0.05$) between sugar loaf and MD -2 (Appendix G).

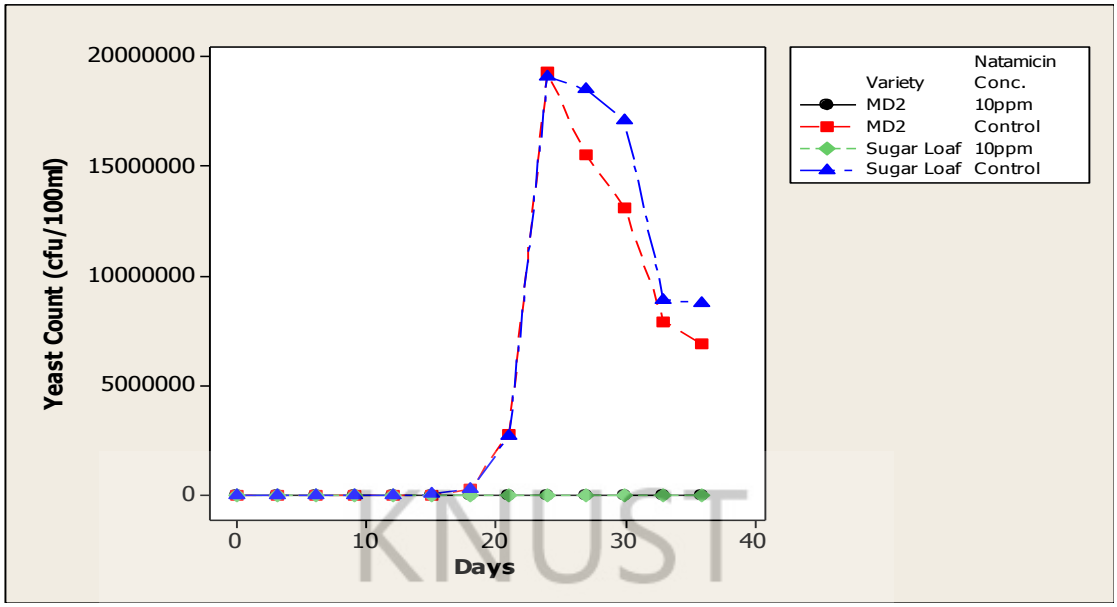

Figure 4.12a: Changes in yeast counts in cut pineapples stored at 5°C ±2

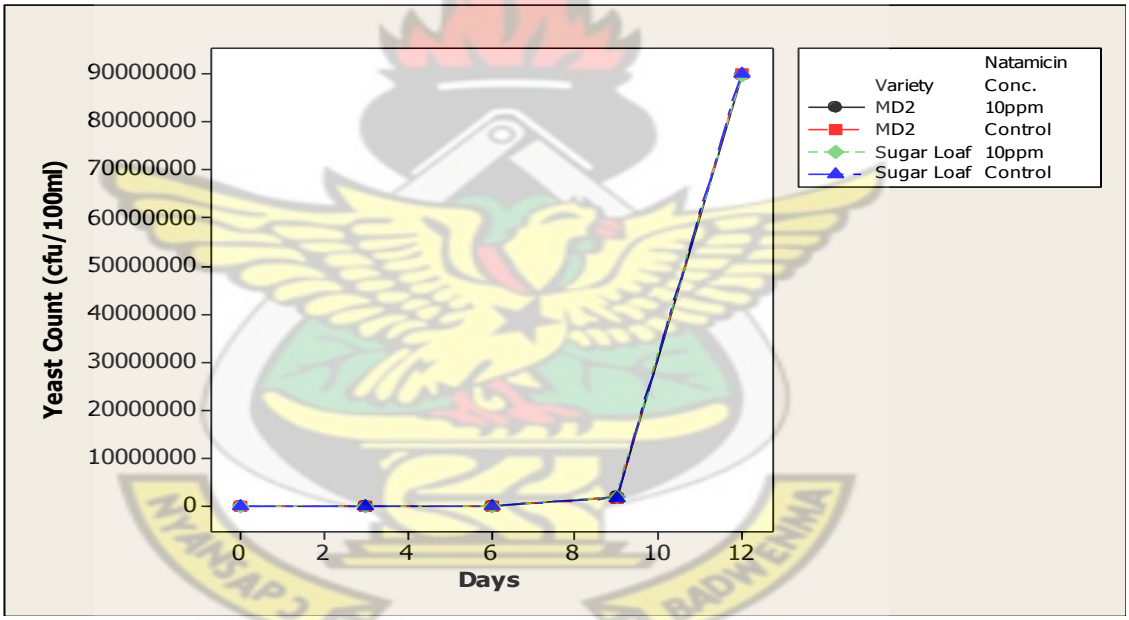

Figure 4.12b: Increase in growth of yeasts present of cut pineapples stored at 25° ±3

Fitted Trend Equation for the control pineapple

$Yt = (10^8) / (5.30115 + 128211073) * (0.0622128^{t})$..... (13)

Equations were not generated for treated samples because there were no growth.

4.4.6 Changes in Mould counts of cut pineapples under refrigeration and ambient conditions.

The figures 4.13a and b shows the growth of moulds in cut pineapples under refrigeration and ambient conditions. The mould counts in cut pineapples stored under refrigeration had an initial counts of $9x10^1$and a final counts of $5.89x10^3$ cfu/100 ml for MD2 variety whiles that of the sugar loaf had an initial counts of $8x10^1$ and a final counts of $5.96x10^3$cfu/10 0ml. Growth observed in untreated cut pineapple had a steady increase and sharply rose on the 15th day till the 30th when it decline slowly. Growth however was not observed in the treated cut pineapples. However under ambient conditions, growth sharply increased from the 3rd day till the 12th day storage for both control and natamycin treated cut pineapple. Statistically there was no significant difference ($p \geq 0.05$) in mould growth between treated and untreated samples of the two varieties stored under ambient condition whereas significant difference ($p \leq 0.05$) existed between the controls and the treated cut pineapples stored under refrigeration temperature (Appendix D). Comparative analysis (Appendix G) shows that significant difference ($p \geq 0.05$) does not exist between sugar loaf and MD-2.

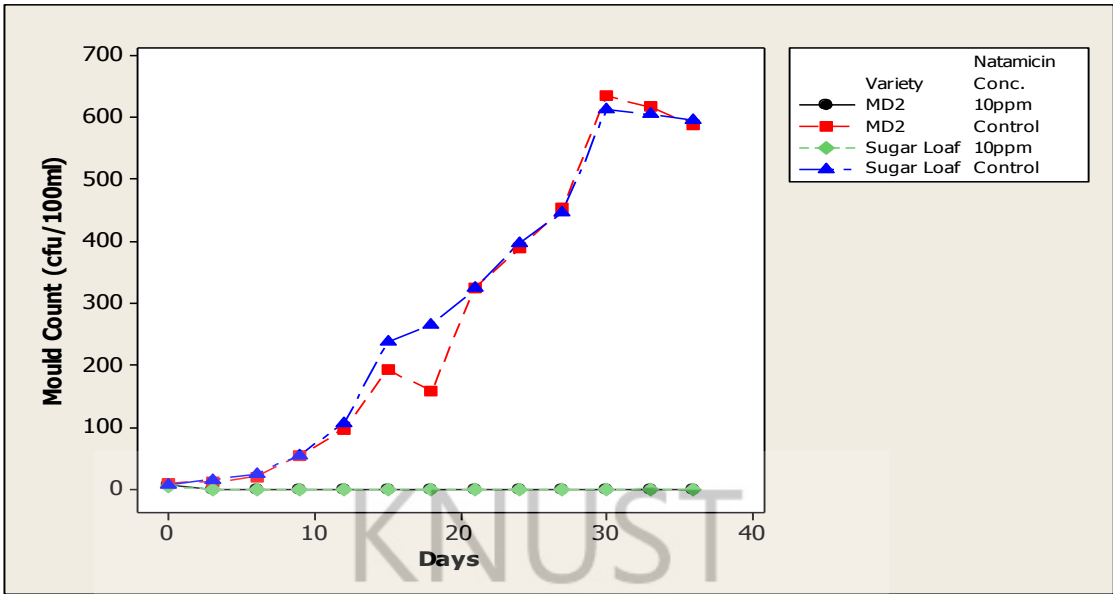

Figure 4.13a: Changes in mould count of cut pineapple stored at 5^{o}C ±2.

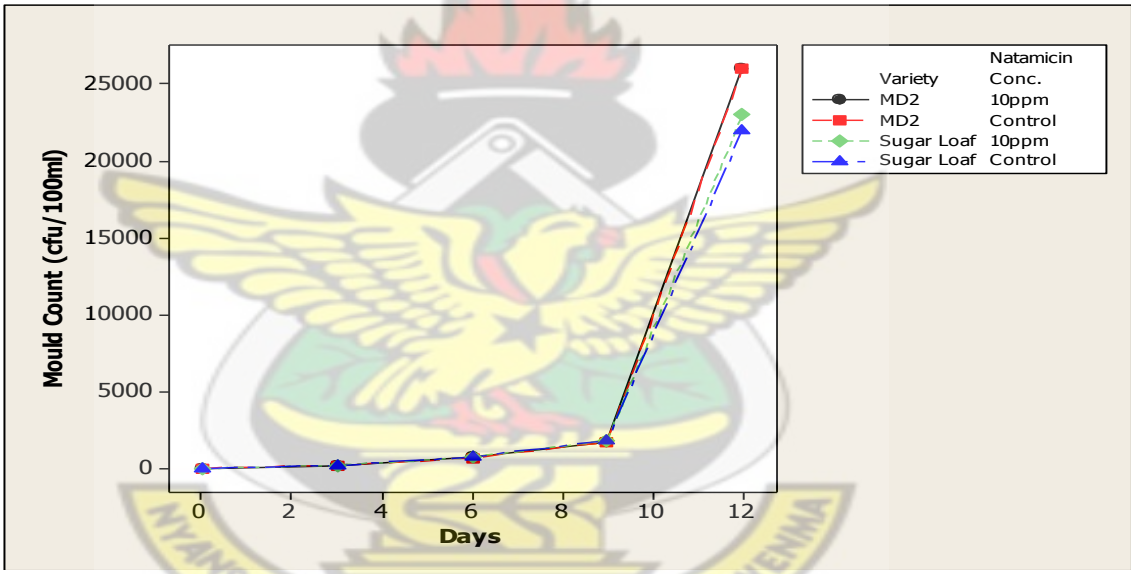

Figure 4.13b: Increase in mould counts of cut pineapples stored at 25^{o}C±3

Fitted Trend Equation for the control pineapples

$Yt = (10^{4}) / (336.891 - 18.7172)*(1.13497^{t})$…….. (14)

4.5. Degradation of natamycin in cut pineapples during refrigeration and ambient conditions.

Natamycin is unstable in solution at both low and high pH, and the instability is further influenced by temperature, light exposure and oxidation (Koontz *et al.*,).Under acidic conditions (such as encountered in pineapple), natamycin degrades rapidly via hydrolysis of the glycosidic bond to yield mycosamine and various other products (Koontz *et al*., 2003). The fact that the compound is labile under the conditions encountered in the pineapple clearly has implications for the validity of quantitative results, since its concentration is expected to decrease with time. For this reason the degradation of natamycin was studied in cut pineapple. It was observed that natamycin present in treated pineapple was 100% based on the concentration of natamycin that diffused into the pineapple which was 10 ppm and this remain stable for the 3rd until it gradually degraded to 15% during the period of storage under refrigeration condition. However under ambient condition, degradation in cut pineapples was very rapid as compared to those stored under refrigeration. This may be due to internal heat effect at lower temperature in refrigeration, thereby slowing down the rate of degradation (Paull, 1993).

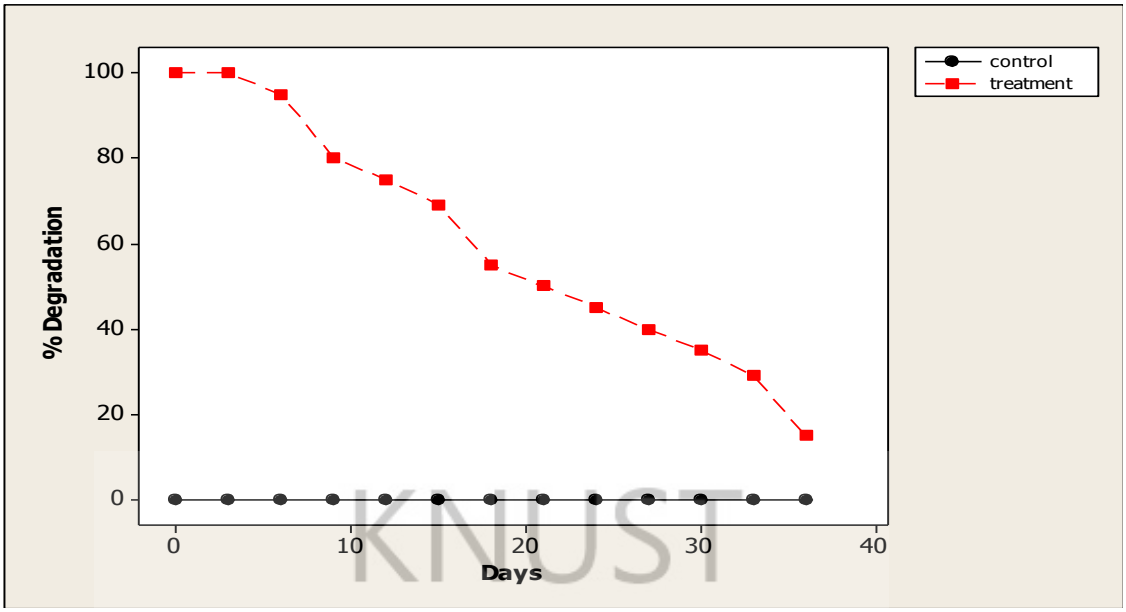

Figure4.14a: Degradation of natamycin in cut pineapples stored at 5°C±2

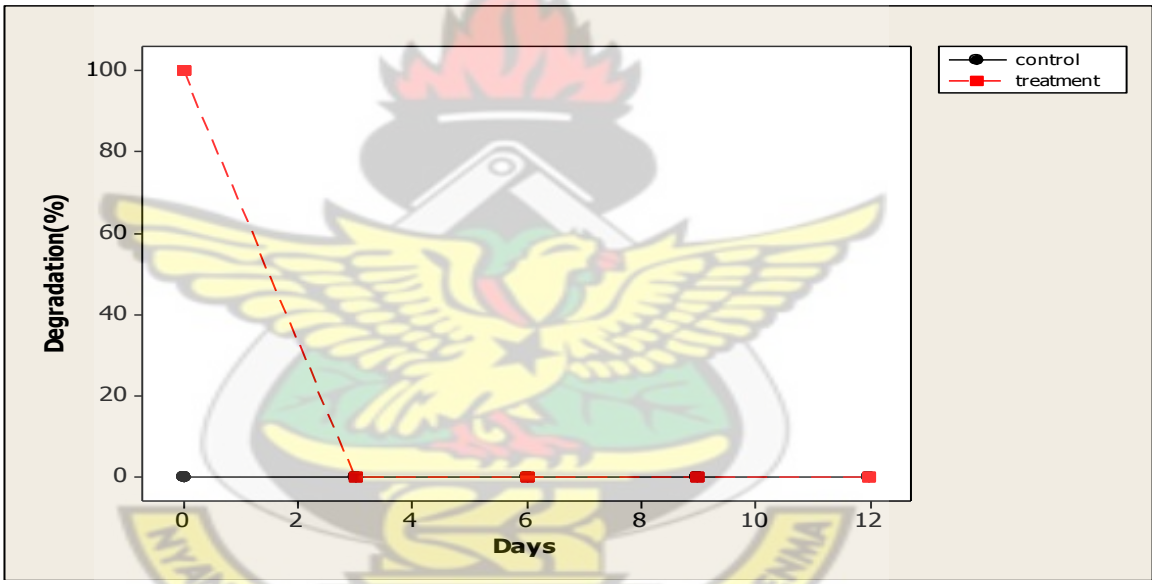

Figure 4.14b: Degradation of natamycin in cut pineapples stored at 25°C±3

4.6. Sensory evaluation of cut pineapples under refrigeration condition

Table 4.8 and 4.9 show the average scores of sensory parameters of the two varieties of refrigerated cut pineapples that were assessed. The average score by the panellists showed a high level of acceptance of all sensory parameters for the control and the treated samples of the two varieties within the first 6 days of storage. The results

showed that there was no significant difference ($p \geq 0.05$) in the sensory parameters of both the control and treated cut fruits during the first 6 days of storage.

After 9 days of storage, (based on their comment on their preferred product), about 85% of the panellists rejected the untreated cut pineapples. Furthermore, close to 100% of panellists rejected the untreated cut pineapples at the end of storage (36 days). Significant difference ($P < 0.05$) was established between the controls and treated cut fruits in terms of the odours, appearance and overall acceptability from the 9th day till the end of storage period (Appendix I). The natamycin treatment offers great advantages such as the prevention of surface browning in the treated samples (Stark, 2003).

Table 4.8: Sensory evaluation of MD2 cut pineapple under refrigeration

Storage days	concentrations	sweetness	sourness	odour	appearance	Overall acceptability
0	Control	$4.80^{a}\pm0.52$	$3.80^{a}\pm0.81$	$4.70^{a}\pm0.47$	$4.70^{a}\pm0.47$	$4.75^{a}\pm0.45$
	10ppm	$4.85^{a}\pm0.55$	$3.82^{a}\pm0.83$	$4.70^{a}\pm0.47$	$4.70^{a}\pm0.47$	$4.80^{a}\pm0.54$
3	Control	$4.80^{a}\pm0.52$	$3.80^{a}\pm0.84$	$4.55^{a}\pm0.56$	$4.70^{a}\pm0.47$	$4.70^{a}\pm0.45$
	10ppm	$4.85^{a}\pm0.43$	$3.80^{a}\pm0.82$	$4.60^{a}\pm0.45$	$4.75^{a}\pm0.45$	$4.75^{a}\pm0.45$
6	Control	$4.80^{a}\pm0.47$	$3.70^{a}\pm0.54$	$4.50^{a}\pm0.57$	$4.65^{a}\pm0.71$	$4.60^{a}\pm0.95$
	10ppm	$4.85^{a}\pm0.54$	$3.80^{a}\pm0.85$	$4.70^{a}\pm0.45$	$4.75^{a}\pm0.45$	$4.70^{a}\pm0.45$
9	Control	-	-	$1.50^{b}\pm0.45$	$3.40^{b}\pm0.85$	$2.20^{a}\pm0.41$
	10ppm	-	-	$4.40^{a}\pm0.48$	$4.70^{a}\pm0.55$	$4.65^{b}\pm0.45$

Different superscripts in the same column within every three days are significantly different ($p \leq 0.05$).

Table 4.9: Sensory evaluation of sugar loaf cut pineapple under refrigeration condition.

Storage days	concentrations	sweetness	sourness	odour	appearance	Overall acceptability
0	Control	$4.85^{a}\pm0.55$	$4.00^{a}\pm0.71$	$4.60^{a}\pm0.47$	$4.75^{a}\pm0.48$	$4.80^{a}\pm0.45$
	10ppm	$4.85^{a}\pm0.55$	$4.00^{a}\pm0.83$	$4.50^{a}\pm0.55$	$4.72^{a}\pm0.44$	$4.80^{a}\pm0.54$
3	Control	$4.80^{a}\pm0.46$	$3.90^{a}\pm0.54$	$4.50^{a}\pm0.71$	$4.70^{a}\pm0.55$	$4.70^{a}\pm0.55$
	10ppm	$4.88^{a}\pm0.43$	$3.90^{a}\pm0.71$	$4.60^{a}\pm0.45$	$4.75^{a}\pm0.45$	$4.80^{a}\pm0.55$
6	Control	$4.80^{b}\pm0.47$	$3.85^{a}\pm0.54$	$4.55^{a}\pm0.57$	$4.60^{a}\pm0.71$	$4.65^{a}\pm0.55$
	10ppm	$4.85^{b}\pm0.54$	$3.80^{b}\pm0.55$	$4.60^{a}\pm0.45$	$4.65^{a}\pm0.45$	$4.70^{a}\pm0.45$
9	Control	-	-	$1.70^{b}\pm0.45$	$3.90^{a}\pm0.45$	$2.50^{a}\pm0.71$
	10ppm	-	-	$4.50^{a}\pm0.45$	$4.70^{b}\pm0.55$	$4.60^{b}\pm0.55$

Mean values with different superscript in the same column within every three days are significantly different ($p \leq 0.05$).

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION.

Natamycin is an effective antimicrobial preservative against yeast and moulds even at low concentrations. Pineapple juice treated with natamycin concentration of 10 ppm and 20 ppm showed no significant difference ($p \geq 0.05$) in physiochemical properties analysed.

However, there were significant differences ($p \leq 0.05$) in the properties of treated and untreated juice samples. The treated samples prevented completely the growth of yeast and moulds during refrigeration. The optimization of the conditions for preservation of cut pineapples showed that none of the models with factors used in the experiment was suggested since p-values were more than 0.05. This implies the factors used in the experiment did not have any significant effect on the responses. However, the mean versus total model was suggested and further analyses gave optimum conditions of thickness 1mm, a time of 2 minutes, natamycin concentration of 30 ppm and a volume of 100 ml. The actual natamycin concentration of 10 ppm which diffused into the cut pineapple after soaking in 30 ppm for 2 minutes was used for the treatment of bulk storage of cut pineapple under refrigeration and ambient condition.

The treated samples with 10 ppm also completely prevented the growth of yeasts and moulds during refrigeration. This concentration also minimized the decrease in the content of ascorbic acid, the pH, total soluble solids and also the increase in titratable acidity of the samples of the two pineapple varieties. Sensory evaluation of preserved samples showed that 68 % of assessors preferred the natamycin treated juices. Also close to 100% of assessors' liked the treated cut pineapple as they

commented that natamycin was able to preserve the strong pleasant odour, appearance of the pineapple and added no additional flavour to the pineapple till the end of storage. Thirty two percent (32%) commented that natamycin treated cut pineapple and juice had a mild pleasant odour towards the end of the 36 days storage period. Furthermore about 100% of panellist rejected the untreated cut pineapple at end of the 36 days storage. The natamycin effect resulted to the keeping quality of cut pineapple and juice which is desired by consumers and manufacturers. However under ambient conditions natamycin was not effective in preserving pineapple due to its rapid degradation in acidic conditions.

5.2 RECOMMENDATION.

Further research should be conducted on the use of Natamycin powder on cut pineapples during storage to determine its effect on physiochemical, sensory properties and microbial stability of the fruit. The effect of Natamycin powder on other pineapple varieties and other fruits such as cut water melon and pawpaw beyond 36 days should be investigated.

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