USE OF MILD HEAT PRE-TREATMENT OF WHOLE PINEAPPLES FOR QUALITY RETENTION AND IMPROVED SHELF-LIFE OF ITS FRESH-CUT FRUITS

by

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DECLARATION

STUDENT

I hereby declare this thesis is the outcome of my own original research and that it is neither in part nor whole been presented for another certificate in this university or elsewhere

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DEDICATION

This work is dedicated to my family, both present and in the future. To God be the glory.

ACKNOWLEDGEMENT

If the Lord had not been on my side, let Israel now say, [Psalm 124:1], where would I have been. All glory and honour be unto HIS holy name. My sincere gratitude goes to my mother, Miss Mercy Mensah, my twin fathers, Mr. A. A. Mensah and Mr. E. A. Mensah and my entire family, for their love, care, support and encouragement through it all. God bless you abundantly. To my supervisors, Prof. Ibok Oduro, Rev. Adubofour and Mr. I. W. Ofosu who contributed immersely to this work, I am sincerely grateful to you for your direction, encouragement, dedication and valuable contribution.God bless you. I would like to express my gratitude to the head and technical staff of the Biochemistry and Biotechnology and Food Science and Technology Department Laboratory. My sincere thanks also go to Mr. William GaribaAkanwariwiak, the Head of Department and Mr. Eric Acheampong of the microbiology laboratory all of the Department of Theoretical and Applied Science, for their enormous help. Special thanks to the head and technical staff of the chemistry department, Food Research Institute, CSIR, for their help and support. To Alfred Kofi Gardemor who has been through it all with me, Akpesia! For your love, support, and encouragement and to Peter Osam Samful for his support, direction and encouragement. I am very grateful. Finally to all and sundry who in diverse ways have contributed to my research work, you are not the least. Thank you all and God bless you.

When I cast my mind back to where I began and examine where I stand now, I can only say that I have indeed come a long way by the grace of the Almighty God. To Him be Glory, Honour, Power and Praise now and forever more. Amen.

ABSTRACT

Mild heat pre-treatment has been used by a number of researchers to improve on the shelf-life and sensory qualities of fruits. The effect on sensory and nutritional attributes of fresh-cut MD2 and Smooth Cayenne pineapples subjected to mild pre-heat treatment at 60 °C, 70 °C, and 80 °C for 10, 20 and 30 min was studied. The optimum treatment condition that maintained the highest sensory and nutritional quality and variety was determined. Further studies was conducted to determine the effect of mild heat pre-treatment and storage condition (ambient and refrigeration) on the chemical (vitamin C content, pH values, TSS content and moisture content of treated and untreated pineapples), microbial (yeast, mould and aerobic bacteria) and sensory attributes of MD2 pineapple variety. A preference test to asses taste (sweet, sour balance), texture (firm, soft), and appearance/colour; and chemical (vitamin C and sugar content) analyses were carried out on the samples after a 24 h storage period during the first phase. Results showed that vitamin C content reduced with increased temperature and time of treatment in both varieties of pineapples. Total Soluble Solids (TSS) content of samples from both varieties increased with increase temperature at 10 min treatment. Sensory attributes (Taste, texture and appearance/colour) improved at some treatment conditions used in this experiment (80 °C -10min, 70 °C -10 min, 70 °C - 20 min, 60 °C -10 min). The optimum treatment condition and variety were 73.49 °C – 10 min and MD2 pineapple variety respectively where pineapples maintained the golden brown colour typical of MD2 variety and had an improved firmer texture and a good balance of sweet and sour taste highly preferred by consumers. Moisture, pH and TSS loss was reduced in treated MD2 fruits in both storage conditions during the second phase of the work. Shelf-life of the treated fruits was improved about a double fold in terms of microbial count (ambient treated and untreated = 2.3, 1.3; refrigerated treated and untreated = 6.2, 3.0) and vitamin C content (ambient treated and untreated = 12.0, 7.5; refrigerated treated and untreated = 27.0, 17.9).Thus mild heat pre-treatment technology for extending shelf-life of fresh-cut pineapples and maintaining sensory attributes will provide an alternative to the use of chemical additives.

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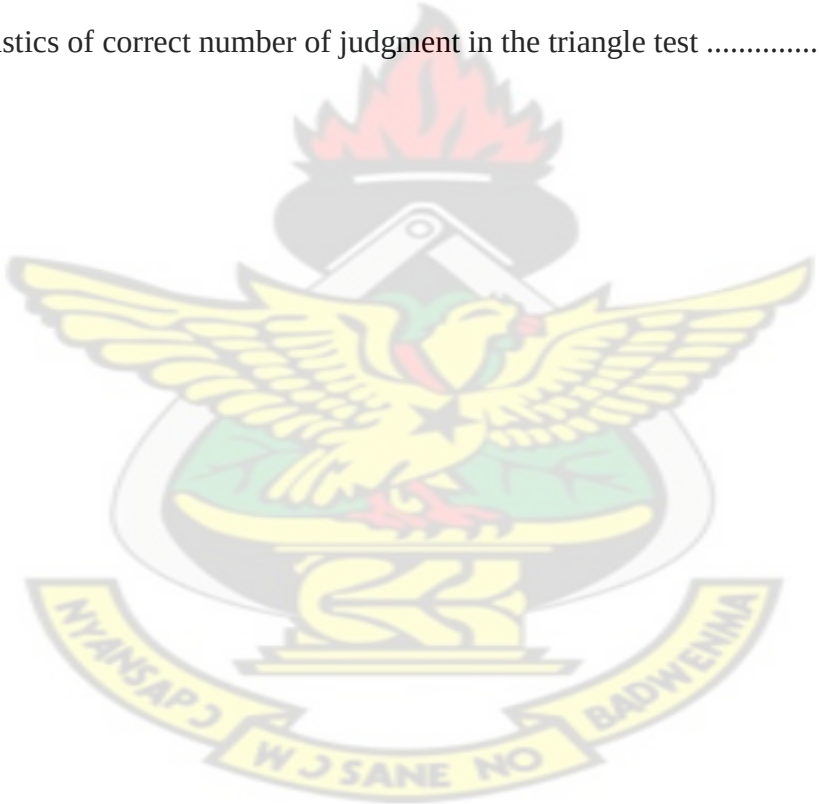

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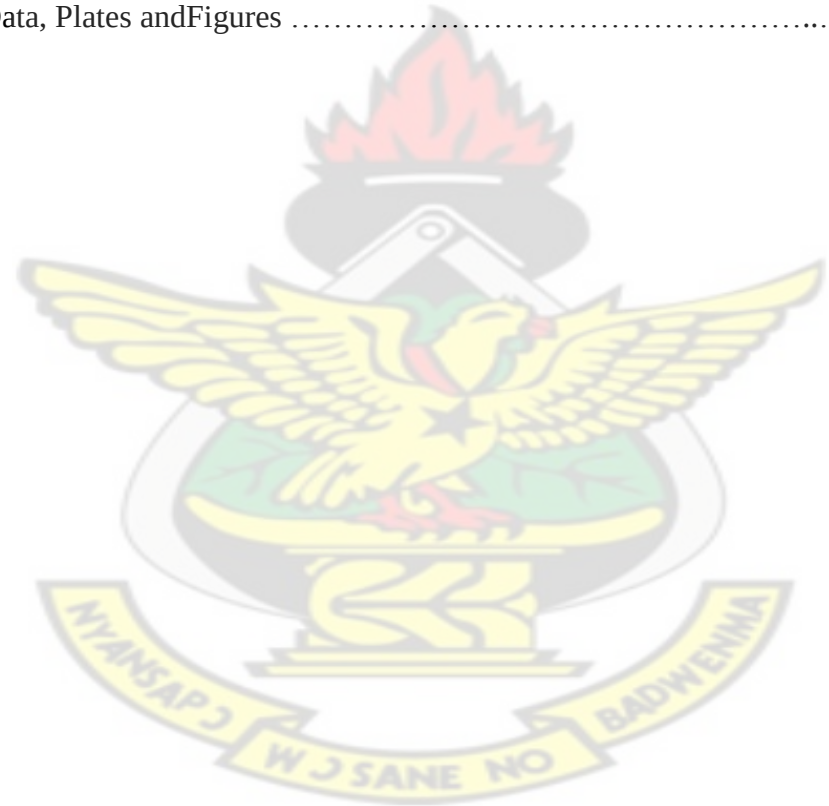


CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

Food preservation is critical for sustaining the global food supply through various production and processing technologies. These technologies improve the quality, quantity and public health standards of food products while preserving the environment and fulfilling consumer expectations (González- Anguilar *et al.*, 2008).

When fruits are harvested, microbiological and chemical changes occur which reduce the time the fruits remain acceptable and safe for consumption. Since most of the post-harvest changes in fruits lead to spoilage, various methods of food preservation are used to prolong the length of time for which the fruit retains quality and appeal (Eubanks *et al*., 2006). Postharvest quality loss in fruits is primarily as a result of respiration, onset or progression of ripening, water loss, enzymatic discoloration of cut surfaces, decay, senescence and mechanical damage suffered during preparation, shipping, handling and processing (Schlimme and Rooney, 1994; Watada *et al*., 1996).

Fresh-cut produce is one of the fastest growing convenience food industries in history (Schneider *et al*., 2009). The International Fresh-cut Produce Association defines a fresh-cut product as fruits 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 maintaining freshness (Schneider *et al*., 2009). Fresh-cut fruits and vegetables offer a number of advantages over whole produce, including cost control, waste reduction, variety selection, consistent quality and safety, and less in-store labour (Luo, 2007). Consumers are able to buy smaller portions of the fruit needed at lower prices, thus save money and prevent waste on left overs. It is more convenient for consumers to buy two or more varieties of cut fruits at a time compared to whole fruits. Processing and packaging is done under the same conditions therefore consistent quality and safety is always assured. There has been increasing demand for various fresh-cut tropical fruits over the years. However, their short shelf-life has limited their market increase. Quality changes with respect to firmness, colour, total soluble solids (TSS), titratable acidity (TA), sensory quality and microbial safety of fresh-cut mangoes, melons and mixes of

these fruits have been found to be improved upon by pre-heat treatment (Chonhenchob *et al*., 2007).

Maintaining the quality of a fresh-cut fruit or vegetable product is a major concern and a priority in the development and production of fresh-cut products of the industry. The industry has been searching for alternative methods to protect fresh-cut produce from decay and to prolong shelf life (Martínez-Ferrer and Harper, 2005). Both consumers and food producers require more research about fresh-cut produce. Producers demand inexpensive and effective technologies to safely preserve the quality of the products. In the same manner, consumers want quality low priced fresh-cut produce. Food scientists attempt to solve problems in fresh-cut processing and quality preservation to fulfill this need (González-Anguilar *et al.*, 2008).

Heat treatment has been used for many years to control fungal spores and insect infestation in fruits and vegetables (Seo *et al*., 1997 and Lurie, 1998). The beneficial effect of heat treatment has been attributed to the syntheses of Heat Shock Proteins (HSPs.) (Wang *et al.*, 2001). HSPs are a class of functionally related proteins whose expression is increased when cells are exposed to elevated temperatures or other stress. They stabilize proteins and are involved in the folding of denatured proteins (De Maio, 1999). Heat also allows demethylation of pectin by pectin methyl esterase to form anionic carboxyl group with which calcium can form salt bridge links thereby strengthening cell walls (Alonso *et al*., 1997). This makes cell walls less accessible to the enzymes that cause softening and thus increases shelf-life (Sam *et al.,* 1993). According to Wang, *et al.,* (2001), heat treatment of apples increases HSPs content and reduces ethylene production. Cell wall degrading enzymes and ethylene production are also frequently disrupted, delayed or sometimes not produced at all following heat treatment (Paull and Chen, 2000). Vicente, *et al.,* (2002), also reported that heated strawberries stored better than unheated fruits. Pre-cut heat treatment of cantaloupe melon according to Lamikanra, *et al.,* (2005) could be used to improve the shelf life of fresh-cut cantaloupe melon. They reported that heat treatment reduced respiration and moisture loss during storage of the cut melon fruit. The descriptive sensory evaluations indicated that heat treatment increased intensities of desirable flavour attributes, and reduced undesirable flavours. Heat treatment again reduces lipase (enzyme that breaks down lipids) and peroxidase (enzyme that are produced in response to wound stress)

activities in fruits (Lamikanra *et al.*, 2005). However, none of their works indicated the direct effect mild heat treatment has on the pineapple fruit.

Pineapples are good source of manganese as well Vitamin C and Vitamin B1. Due to their fibrous nature, pineapples help prevent intestinal disorders and act as natural anti-inflammatory agent with analgesic properties (Monzon, 1995). They may be used fresh, juiced, dried, made into candies, and incorporated into cooked dishes and desserts. The fruit is a good source of potassium, vitamin C and vitamin A (Crane, 2005). The ripe pineapple is a delicious diuretic which destroys intestinal worms and soothes bile. It is beneficial to the heart and effective in abdominal disorders, jaundice and anaemia. Fresh pineapple juice exercises a soothing effect on the throat and is very useful in preventing infections of the vocal organ. It also gives relief in cellulitis (Helms, 2006). It contains bromelain which has anticoagulant and anti-inflammatory properties. It has also been proved to enhance the effect of some antibiotics amoxicillin, erythromycin and penicillin (Maurer, 2001).

1.2 JUSTIFICATION

Pineapples are one of the major fruits in tropical developing countries including Ghana. Fresh-cut fruits have recently become a common commodity on the Ghanaian market. The most common fruits sold in their fresh-cut state are water melons, papaya and pineapples (Angba, 2007). The production and marketing of the fruit is a source of livelihood for most people especially those in the rural areas where these fruits are produced. Cut-fruits have recently been a viable marketing option for many retailers and provide a convenient alternative to satisfy various consumer budgets and demand (Gonzalez-Anguilar *et al*., 2005); (Angba, 2007). However, this emerging industry has limited preservation procedures available to the ordinary Ghanaian and its associated health and safety issues (nutritional loss, microbial contamination and reduced sensory attributes and shelf-life). These issues have discouraged consumers from patronizing the product and further threaten the growth and sustainability of this emerging industry in the country. Postharvest losses in cut pineapples due to short shelf-life have coursed static business growth as well as loss of income for the unsuspecting retailers involved in the business (Angba, 2007).

Maintaining the quality of a fresh-cut fruit product is a major concern and a priority in the development and production of such products in the food processing industry. These issues associated with cut-fruit, relative to that of intact fruit, are mainly due to physiological and biochemical changes typical of the senescence process such as increased respiration and ethylene production, and loss of membrane integrity. Various preservative procedures have been research with regards to pineapples, to reduce or eliminate these issues. However, these efforts have been limited to post-cut treatment which requires skill and expertise to carry out and thus unfamiliar to the ordinary retailer (Toivonen and DeEll, 2002). Known post-cut methods of treatment include: Use of pineapple juice of the same brix/acid as the cut fruit to be preserved. Here the surface-disinfected freshly cut fruit, preferably chilled to just above freezing, is immersed in the selected juice, which may be a blend of several batches, and is stored and shipped preferably close to freezing. The resulting fruit has a long shelf life and a balanced flavour but is relatively expensive and require the use of chemicals (Chenchin *et al.*, 1999). Another method, according to Martínez-Ferrer, (2005), is by the exposure of the cut-fruits to methyl jasmonate in a sealed container to increase shelf life by decreasing microbial growth. Yet another method involves the use of antioxidants like ascorbic acid and 4-hexylresorcinol in a modified atmosphere to inhibit browning and reduce microbial growth (Mohammed and Wickham, 2005).

However, mild heat treatment of a number of horticultural crops has been reported to improve product quality and shelf-life (Wang *et al.*, 2001). Heat treatment also inhibits ethylene synthesis, tissue response to ethylene, and cell wall degradation associated with hydrolytic enzymes (Lurie, 1998). Mild heat treatment has been reported to reduce microbial load and improve fruit texture and taste of a number of fruits (Valero *et al.*, 2002; Abreu *et al.*, 2003; Lamikanra *et al.*, 2005).

This research aims at determining the effect of mild heat pre-treatment shelf-life and sensory attributes of cut pineapple in storage.

1.3 MAIN OBJECTIVE

To determine the effect of mild pre-heat treatment on the sensory attributes and shelf-life of fresh-cut pineapples.

1.4 SPECIFIC OBJECTIVES

1. To determine the optimum temperature and period of treatment that maintains the highest cut pineapple sensory (taste, texture and appearance/colour of cut-pineapples) and nutritional (vitamin C and Total Soluble Sugar) quality.
2. To determine the effects of mild treatment and storage conditions on the shelf-life, and sensory quality of fresh cut pineapples.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 PINEAPPLE PRODUCTION

Pineapple is the 9th largest fruit crop after pear and ahead of peach and nectarine grown worldwide. The world's pineapple production totals nearly 16 million tones with just 12 countries accounting for 80 % of production (World Trade Atlas, 2002). These counties are found in Asia, Central and South America and Africa. Pineapple production has increased more rapidly than most other fruits generally available (World Trade Atlas, 2002).

In Ghana, pineapple export accounts for more than 50 % of the total horticultural exports and is a source of income and employment to many people (Obeng 1994). The three categories of pineapple producers for export in the country include the smallholders, nonresident commercial farmers, and large-scale producer-exporters. Smallholders are the indigenous rural inhabitants who operate their farmland in their own villages. Nonresident commercial farmers are those who reside in cities but lease land from traditional rulers in rural areas for pineapple production. Large-scale producer-exporters are those who have integrated production and export (Takane, 2004). Export of fresh pineapples in Ghana has increased constantly since the mid- 1980s (Table 2.2). The estimated value of pineapple export from Ghana to European Union countries in 2001 was about 30 million Euros (World Trade Atlas, 2002).

Table 2.1 Export of Fresh Pineapples from Ghana, 1978-2000 (tons)

Year	**1978**	**1979**	**1980**	**1981**	**1982**	**1983**	**1984**	**1985**
Pineapple exported	**48**	**54**	**8**	**10**	**44**	**57**	**650**	**1,807**
Year	1986	1987	1988	1989	1990	1991	1992	1993
Pineapple exported	2,668	4.130	4,191	7,947	9,440	10,674	9,753	13,157
Year	1994	1995	1996	1997	1998	1999	2000	
Pineapple exported	14,954	15,766	27,603	25,124	21,940	33,440	29,322	

Source: Obeng (1994) and Ghana Export Promotion Council.

2.2 VARIETIES OF PINEAPPLES

There are hundreds of varieties of pineapple, ranging from very large to miniature size. There are dwarf varieties with edible core mainly from Thailand and South Africa, an intermediate variety (Abacaxi) which can weigh up to eleven pounds and the Giant Kew which is known to reach twenty pounds (Crane, 2005). Some of the common varieties include: Smooth Cayenne, one of the most common variety grown, Red Spanish, Singapore Spanish, Green Spanish, Sugarloaf, and Queen (Crane, 2005). More recently, several new varieties have been developed and released of which Del Monte Gold (MD2) is one of them (Morton, 1987). The main pineapple varieties found in Ghana include Smooth Cayenne, Sugarloaf, Queen Victoria Pine and MD2.

2.2.1 Smooth Cayenne

Smooth cayenne is one of the most widely grown varieties in the world and in Ghana. It is known by its common name "Spineless pineapple" in some parts of the world. Smooth cayenne was for a long time the only variety exported fresh and canned (Gillman, 2007). Its leaves are about 3 ft long with some spines at the base and top. The fruit weights about 5 to 6 lbs (2.3-2.7 kg) with a pale yellow to yellow pulp. The fruits are also cylindrical in shape, as indicated by Plate 2.1 and a medium texture with high sugar and acid content. It is also well adapted to canning and processing (Gilman, 2007). It is often planted for their tropical appearance and as a horticultural novelty.

Fig 2.1 Smooth cayenne pineapple variety

2.2.2 MD2

The new super sweet pineapple variety is with various names including Del Monte Gold, Extra Sweet as well as MD2. The MD2 variety (Fig 2.4) is very sweet and has a barrel-like appearance. The pulp has a deep gold/yellow colour inside (Crane, 2006). MD2 is popular in the fresh fruit market because it is one of the few varieties which have low acidity content and a distinct flavour of a hint of coconut (Hrayr, 2008). This variety has a higher vitamin C content than other varieties, which gives the final consumer additional nutrition benefits. It was developed to reduce the inequality in shape, and at the same time to increase its sugar content to make it more attractive to fresh fruit consumers (Izwan, 2008). It has an extraordinary capacity for withstanding cold and transport. The MD2 has been described as super-sweet, self ripening and having a longer storage life with a value twice as much as that of the smooth cayenne variety (Achuonjei *et al.*, 2003). It has a brix between 14.4 and 18.8, acidity (meq % ml) between 6.7 and 13.3 Sugar/acid ratio between 1.65 and 2.14. MD2 has been found to have significantly higher ($P < 0.0001$) pH, °Brix/acid ratio, yellow colour, total carotenoid content and vitamin C than Smooth Cayenne, while the titratable acidity of Smooth Cayenne is significantly higher ($P < 0.0001$) (Ramsaroop and Saulo, 2007).

Fig 2.2 MD2 pineapple variety

2.2.3 Other varieties in Ghana

The Sugarloaf variety is the third variety cultivated on large scale in Ghana. It is sometimes referred to as White *Sugarloaf* or *Kona Sugarloaf*, has fruit that weigh 5 to 6 lbs (2.3-2.7 kg) with a white flesh and edible core and cylindrical in shape. It has high sugar content but no acid (Crane, 2005). The Queen Victoria pine is the final variety grown in the country.

2.3 NUTRITIONAL COMPOSITION

Pineapple is cultivated predominantly for its fruit that is consumed fresh or as canned or frozen fruit, juice, syrup or candies. Its fruit is an excellent source of vitamin C, Thiamin, potassium and manganese. It is also a good source of vitamin B1, vitamin B6, copper and dietary fiber (Table 2.3). It is very low in saturated fat, cholesterol and sodium. Pineapple bran, the residue after juicing, is high in vitamin A, and is used in livestock feed. From the juice may be extracted citric acid, or on fermentation, alcohol (Duke, 1983).

Vitamin C (also referred to as L-ascorbic acid) is the lactone 2, 3-dienol-L-gluconic acid and it belongs to the water-soluble class of vitamins. It is mainly found in fruits and vegetables and is one of the major nutrients in pineapples. In the nutritional content, it is the L-enantiomic form of ascorbic acid which also encompasses the oxidation product of dehydroascorbic acid with different oxidizing agent. It is an antioxidant that protects the body from free radical damage and boosts the immune system. It helps build and repair bodily tissue and promotes wound healing. It participates in numerous biochemical reactions, suggesting that vitamin C is important for all body processes from bone formation to scar tissue repair (Rickman *et al*., 2007). According to USDA National Nutrient Database for Standard Reference, Table 2.3, 100 g pineapple contain about 36 mg of vitamin C. In the U.S., the recommended dietary allowance (RDA) for vitamin C was recently revised upward from 60 mg daily for men and women (Food and Nutrition Board, Institute of Medicine, 2000). The RDA continues to be based primarily on the prevention of deficiency disease (scurvy), rather than the prevention of chronic disease and the promotion of optimum health (Food and Nutrition Board, Institute of Medicine, 2000). This potentially fatal disease can be prevented with as little as 10 mg vitamin C per day (Simon and Hudes, 2000). The recommended intake for smokers is 35 mg/day higher than for nonsmokers, because smokers are

under increased oxidative stress from the toxins in cigarette smoke and generally have lower blood levels of vitamin C (Food and Nutrition Board, Institute of Medicine, 2000). Pregnant and lactating women are regarded as needing more than this. At April 1999, it was being 'officially' recommended, based on new information, that the RDA ought to be changed to 120 mg/ day (Levine *et al.,* 1999). This is because the totality of the data reviewed by Anitra and Balz, **(**1999) suggests that an intake of 90 to100 mg vitamin C per day is required for optimum reduction of chronic disease risk in nonsmoking men and women. Nutritionists generally regard any serving of food that provides 10 % to 25 % of the daily vitamin C need in a relatively low calorie package as a good source (Sidibé *et al.*, 1996).

Table 2.2 Nutrient Content of 100g (3.5 Oz) of Fresh Pineapple.

Constituent	**Approximate Value**	**Constituent**	**Approximate Value**	**Constituent**	**Approximate Value**
Water	87 %	Carbohydrate	12.6 g	Phosphorus	8 mg
Calories	48 kcal	Fiber	1.4 g	Potassium	115 mg
Protein	0.56 g	Calcium	12 mg	Sodium	1 mg
Fat	0.12 g	Iron	0.26 mg	Vitamin C	36 mg
Cholesterol	0 g	Magnesium	12 mg	Vitamin A	56 IU

USDA National Nutrient Database for Standard Reference, Release 18, (2005).

2.4 IMPORTANCE OF PINEAPPLE

2.4.1 **Clinical**

Fresh pineapple juice exercises a soothing effect on the throat. It is very useful in preventing infections of the vocal organ. In diphtheria it is used for removing the dead membranes from the throat. Pineapple juice destroys intestinal worms and is beneficial to the heart. These functions are as a result of the presence of proteolytic enzyme called bromelain (Maurer, 2001). Some have claimed that pineapple has benefits for some intestinal disorders while others claim that it helps to induce childbirth when a baby is overdue (Adaikan, 2004). Pineapples are also very good source of fiber necessary for proper digestion (Montinola, 1991). According to Hartwell,

(1971), the fruit, peel, or juice is used in folk remedies for corns, tumors, and warts. The root and fruit are either eaten or applied tropically as an anti-inflammatory and as a proteolytic agent. It is traditionally used as an antihelminthic agent in the Philippines (Monzon, 1995).

2.4.2 **Industrial**

Pineapple is the only source of bromelain enzyme, a complex proteolytic enzyme used in the pharmaceutical market and as a meat-tenderizing agent (Duke, 1983). Pineapple fiber has been processed into paper with remarkable qualities, thinness, smoothness and pliability (Montinola, 1991). The stems and leaves of pineapple plant are also a source of fiber that is white, creamy and lustrous as silk (Monzon, 1995).

2.4.3 **Biological**

Ethylene (C_2H_4) is a natural plant hormone produced by plants including the pineapple plant. It affects the growth, development, ripening, and senescence (aging) of all plants. It is normally produced in small quantities by most fruits and vegetables (California Fresh Market Advisory Board, 1976). These fruits respond with uniform ripening when exposed to an external source of ethylene. Ethylene action slows at lower temperatures. That is to say that at refrigeration temperatures, ethylene activity is slowed down. This is due to the fact that at their minimum temperature levels, fruits (pineapples) are basically inactive and does not respond well to external influences including supplied ethylene (California Fresh Market Advisory Board, 1976). Due to the large amounts of natural acids (citric, malic, and tartaric) and bromelain enzyme, large amounts of fresh pineapple should not be consumed as a main dish. Despite these benefits, fresh pineapple may cause irritation of the tip of the tongue in some cases. Some may describe this sensation as a raw tingling.

2.5 SHELF LIFE OF CUT PINEAPPLES

Shelf life refers to the length of time or the period under any stated storage conditions during which a product will retain any specific qualities for which tacit or expressed claims have been made. Shelf life is affected by certain factors including exposure to light and heat, transmission of gases including humidity, mechanical stresses, and contamination by micro-organisms (Wardlaw, 2003). Major factors affecting fresh-cut fruit quality are cultivar (Kim *et al.,* 1993; Romig, 1995), pre-harvest cultural practices (Romig, 1995), harvest maturity (Gorny *et al.,* 1998), physiological status of the raw product (Brecht, 1995), postharvest handling and storage (Watada *et al.,* 1996), processing technique (Saltveit, 1997; Wright and Kader, 1997), sanitation (Hurst, 1995), packaging (Solomos, 1994; Cameron *et al.,* 1995) and temperature management during marketing (Brecht, 1999). Thus, it is particularly important to control not only the sanitation during preparation, but also the processing as these fruits often have a short storage life.

Initial quality of the fruit is also essential for optimum quality during storage. Nutritionists generally regard any serving of fruit, or natural portion (e.g. slice of melon, or a handful of berries) that gives from about 15 mg to about 30 mg of vitamin C as a very good source of vitamin C. When a fruit or natural portion (e.g. slice of melon, or a handful of berries) has more than about 30 mg per serving, it is an excellent source of Vitamin C. Obviously, when a single serving supplies a lot better than the current RDA of vitamin C, it is an exceptional source (USDA Nutrient database for standard reference, release 12, 1998; Levine *et al.,* 1999).

Whole and fresh-cut produce are unique among the food products; cut-fruits remain metabolically active and their shelf life and storage stability are shortened as consequences of these processes. Whole fruit, contrary to fresh-cut, are covered by specialized skin tissues, which serve as barrier against insect and pathogen attack, avoiding excessive water loss. Whole fruit have less water losses and gas exchange due to the natural barrier that offers the cuticle, which can be affected by storage conditions. If the epidermis or periderm is damaged or removed, which is the case with fresh-cut products, water loss can be massively increased (Ayala-Zavala *et al.,* 2008). For this reason, fresh-cut produce must be protected by a packaged system in order to increase their shelf-life (Montero-Calderon *et al.,* 2008). However, water released from fruit can accumulate inside the package and create a high relative humidity atmosphere (Ayala-Zavala *et*

al., 2008). The rate of water loss is dependent primarily on the external vapour pressure deficit. Other factors may however, affect it. Products with a large surface to volume ratio such as leafy vegetables, are prone to lose greater percentages of water than large spherical fruits. In general, fruit appearance deteriorates steadily with increasing water loss (Ayala-Zavala *et al.,* 2008). The primary products in the cut-fruits industry include watermelon and other melons, apples, grapes, pineapple, and combination fruit cups (Montero-Calderon *et al.,* 2008).

The post-cutting life of pineapple has been reported to be very dependent on temperature, from a few hours at 20 ºC to about two weeks at 1 ºC (O'Hare, 1994). In a preliminary study by Marrero- and Kader, (2001), it was found that post-cutting life of pineapple pieces ranged from 4 days at 10 ºC to over 2 weeks at 0 ºC. A sharp rise in respiration preceding an increase in ethylene production during storage at the post-cutting life of fruit pieces occurs at these conditions. Continuation of storage beyond this point led to the appearance of off-flavours and odours and microbial spoilage (Marrero and Adel, 2006).

The prolonged post-cutting life of fresh-cut pineapple products at low temperatures is contrary to the susceptibility of whole fruits to chilling. Chilling injury develops when whole fruits are stored at temperatures below 10 ºC for extended periods of time. Symptoms include dull shell colour when ripe, wilting of crown, water-soaked appearance of pulp, increased susceptibility to decay and internal browning (Paull and Rohrbach, 1985; Paull, 1997). Some of these symptoms appear after the chilled fruits have been removed to non-chilling temperatures.

Minimally processed products are one of the major growing segments in food retail establishments. However, fresh-cut fruits are still understudied because of the difficulties in preserving their fresh-like quality during prolonged periods (Soliva-Fortuny and Martin-Belloso, 2003). Mild pre-heat treatment is one of the solutions that have been adopted in solving this problem. It has been used to improve shelf-life, chemical and sensory attributes of many fruits as mentioned earlier. The use of this pre-treatment seems to be a promising methodology for extending shelf life of fresh-cut fruits, reducing the dependence on chemical additives (Atanda, 1989; Lamikanra *et al.,* 2005; Barrancos *et al.,* 2006). In these cases the temperatures used and the time of treatments were carefully chosen to minimize, if not totally eliminate, enzyme denaturation. According to Gill, *et al.,* (2006), minimal heat processing of fruit, does not significantly affect the nutritional content of pineapples even after six (6) and up to nine (9) days.

Establishing overall shelf-life limits for fresh-cut fruit, taking flavour quality into consideration, is difficult since initial product variability, potential post-cutting treatments and/or packaging affect flavour attributes differently. Washing whole products prior to processing and proper sanitation, in combination with optimum storage temperature, are critical to maintain quality and prolong product life. Little is known concerning the effect storage temperature has on volatile production, little flavour and sensory on fresh-cut fruits.

2.6 MILD PRE-HEAT TREATMENT OF FRESH FRUITS

Temperature is important in all reactions. As the temperature increases, so does the rate of reaction. This is because heat energy causes more collisions between the particles in the enzyme and particles in the substrate. However, very high temperatures damage or denature enzymes (proteins). The changes in packaged fresh-cut grape quality and microbial growth as affected by mild heat treatments and the retention of grape cap stems during 5 ºC storage was evaluated by Kou, *et al.*, (2007). The samples were subjected to mild heat treatment in a water bath (45 ºC, 8 min). The results indicated that in the package headspace for hot water treatment of stemless grapes, partial pressures of O_2 declined significantly. C_2H_4 increased significantly for the control and hot air treatment. Pre-heat treatment maintained a significantly lower decay rate than the control. Colour and texture were not significantly affected by heat treatment. Grapes that received hot water treatment had the lowest decay rate and lowest microbial growth with the absence of any negative impact on grape colour, texture, and flavour (Kou *et al*., 2007).

Also, fresh nono samples were subjected to a number of treatment conditions and pre-heat treatment at 65 °C for 30 min was most effective in leaving the least number of viable bacteria ($1{\cdot}97 \times 10^3$) without affecting the organoleptic quality of the product. Some micro-organisms isolated after the pasteurization treatments included *Lactobacillus bulgaricus* however, coliform bacteria and fungi did not survive the pasteurization treatments (Atanda, and Ikenebomeh, 1989).

Fresh-cut cantaloupes lose desirable fruity, sweet flavours, develop off-flavours and softens during storage without treatment. However, melons chilled to 4 °C, immersed in heated water at 60°C for 60 minutes were improved. Heat treatment in water reduced the rate of respiration and

moisture loss during storage. It also reduced the microbial count and controlled lactic acid bacteria growth. Heat treatment increased fruity/melon and sweet/aromatic flavours, and reduced musty, sour, bitter, chemical, and fermented flavours. The heat-treated fruit was firmer and more cohesive in texture than the untreated fruit (Lamikanra *et al.,* 2005; USDA. Agricultural Research Service Annual Report, 2008).

Mild heat treatment has been found to be a potent means of increasing the shelf-life of fruits while maintaining its sensory and nutritional attributes (Wang *et al.,* 2001; Paull and Chen, 2000; William *et al.,* 1994).

2.6.1 Effect of heat treatment on pineapples

The main factor studied which affect the quality of fresh-cut pineapple was temperature (Marrero and Kader, 2006). Within a narrow temperature range (often 0-45 °C), the rate of reaction is proportional to the temperature. It is often said that an enzyme's (in this case bromelain) rate of reaction doubles for every 10 °C rise in temperature. However, the interaction between this positive effect of increased temperature and the negative effect resulting in denaturation results in a different situation. For this reason, all enzymes are said to have an optimum temperature at which their action is maximum (Marrero and Kader, 2006). The higher the temperature to which the enzyme is subjected and the longer the heating is continued, the greater the proportion of damaged enzyme molecules. The result is that the conversion process becomes less and less efficient (Lehninger, 1982). Relatively high temperatures and long period of treatment are capable of depleting the levels of vitamin C in pineapples (Padayatty *et al.,* (2003).

2.7 STORAGE OF FRESH CUT FRUITS

The influences of processing and storage on the quality indices and nutritional content of fresh-cut fruits were evaluated in comparison to whole fruits stored for the same duration by Gill, *et al.*, (2006). Fresh-cut pineapples, mangoes, cantaloupes, watermelons, strawberries, and kiwifruits and their whole fruits were stored for up to 9 days in air at 5 ºC. The post-cutting life based on visual appearance was shorter than 6 days for fresh-cut kiwifruit and shorter than 9 days for fresh-cut pineapple, cantaloupe, and strawberry. On the other hand, fresh-cut watermelon and mango pieces were still marketable after 9 days at 5 ºC. Losses in vitamin C after 6 days at 5 ºC were less or equal to 5 % in mango, strawberry, and watermelon pieces, 10 % in pineapple pieces, 12 % in kiwifruit slices, and 25 % in cantaloupe cubes. Light exposure promoted browning in pineapple pieces and decreased vitamin C content in kiwifruit slices. In general, fresh-cut fruits visually spoil before any significant nutrient loss occurs (Gill *et al.*, 2006).

2.7.1 Storage of fresh cut pineapples under refrigeration and ambient conditions

According to Marrero and Kader, (2006), respiration rate (CO_2 production of about 0.3 Lkg^{-1} s^{-1}) of pineapple pieces at 0 ºC, 2.2 ºC and 5 ºC is very low. During storage, juice leakage from pulp pieces is an important factor of deterioration of fresh-cut pineapple. Neither refrigeration nor atmosphere modification is able to eliminate the incidence of this problem (Marrero and Kader, 2006). However, the variety of pineapple used if less prone to juice leakage, would help reduce this incidence as well as the use of mild pre-heat treatment (Lamikanra *et al.*, 2005). Thus the specific objectives of this work included the determination of the effect of mild heat pre-heat treatment on moisture retention of cut-pineapple fruits to help eliminate this problem in cut pineapples.

Some packaging and storage techniques for fresh-cut produce (e.g., MAP, refrigerated storage) may slow the rate of physical deterioration by slowing respiration of the produce. However, if packaging and storage are not properly controlled, pathogens may grow to levels that could render the product unsafe for human consumption. Water loss from fresh cut fruits occurs in both liquid (due to juice leakage from the vacuoles of the damaged cells) and in vapour form (due to vapour pressure difference between the cells and their microenvironment within the package).

Water vapour loss from the fruit tissue during storage can be minimized by proper packaging to provide a barrier and most importantly by cooling the product to 5 ºC (41 ºF) or lower (Kader, 2004). The same results can be achieved by maintaining such temperatures plus a relative humidity of 95 % or higher during handling. Since all the cells are connected, water can move from one cell to another and once the outer layer of cells loses water, water will move from the adjacent layer of cells to the outer layer to the outside of the tissue (Kader, 2004). Juice leakage can be reduced by using firm fruits, by using sharp knives to reduce cell damage upon cutting, and by minimizing mechanical damage caused by compression and vibration during packaging and subsequent handling. Immobilizing the fruit pieces within the package can help in reducing juice leakage due to vibration (Kader, 2004). It can also be achieved by the use of mild pre-heat treatment (Lamikanra *et al.*, 2005).

2.7.2 Physical and chemical changes (and characteristic) of cut pineapples on storage

Physical characteristics of pineapples, namely fruit weight, moisture content, texture, reduced crown size and delayed ripening are adversely affected by their chemical characteristics such as total soluble solid, total titratable acidity, pH, and sugars content (Mohammed *et al.*, 2006). Many chemical reactions contribute to the loss of storage life of vitamin C and hence chemical deterioration of fruits. The majority of these reactions are enzymatically driven while others are chemical reactions that occur because of the senescence (aging) processes. This involves colour, flavour, and odour changes that result from a chemical reaction between the constituents of the fruit. During storage there is also change in pH value. Changes in the pH probably affect the attraction between the substrate and enzyme, and thus the efficiency of the conversion process. A low pH prevents proliferation of food spoilage microorganisms in a given food product (Wardlaw, 2003). The optimum pH for an enzyme depends on its site of action. For example, enzymes in the stomach have an optimum pH of about 2 because the stomach is acidic, but intestinal enzymes have an optimum pH of about 7.5.

Heat treatment has been found to reduce respiration and moisture loss during storage of cut fruit (Lamikanra *et al.*, 2005). Descriptive sensory evaluations indicated that heat treatment increased intensities of desirable flavour attributes, and reduced undesirable flavours (Lamikanra *et al.*,

2005). Textural measurements showed increased hardness, chewiness, and cohesiveness, but springiness decreased in heat treated fruit (Atanda and Ikenebomeh, 1989). Sensory qualities such as texture, colour, firmness, flavour, odour, sweetness, overall acceptability as well as content, pH, total soluble solids, total titratable acidity, microbial count and nutritional quality (vitamin C) of fresh-cut fruit are significantly affected by heat treatment unlike some other means of preservation such as irradiation (Watada *et al.,* 1996; Hajare *et al.,* 2006). All these characteristics deteriorate as the fruits age. The rate at which vitamin C is lost during storage depends on the type of fruit and the storage method employed (Ajibola, 2009).

Processing fruits increases wound-induced C_2H_4, water activity and surface area per unit volume which may accelerate water loss and enhance microbial growth due to the availability of sugar (Watada *et al.,* 1990; Wiley, 1994; Watada and Qi, 1999). These physiological changes may be accompanied by flavour loss, cut surface discolouration, decay, increased rate of vitamin C loss, rapid softening, shrinkage and a shorter storage-life. Increased water activity and mixing of intercellular enzymes and substrates may also contribute to flavour and texture changes/loss during and after processing. Therefore, proper temperature management during product preparation and refrigeration throughout distribution and marketing is essential for maintenance of quality (Watada and Qi, 1999), as well as mild heat treatment (Lamikanra, 2005).

Reduction in flesh firmness of fresh-cut fruit products can be maintained by application or treatment with calcium compounds (Ponting *et al.,* 1972). However, calcium chloride may leave bitter off flavours on some products. Thus, the use of mild heat pre-treatment as reported by Valero, *et al.,* (2002) is a sure way of improving fruit firmness while avoiding the bitter off flavor typical of calcium chloride.

Oxidative browning in fruits also occurs. This is usually caused by the enzyme polyphenol oxidase which, in the presence of O_2, converts phenolic compounds in fruits and vegetables into dark coloured pigments. Excessive levels of O_2 in a package thus allow for cut surface discolouration to occur, while too little O_2 may cause anaerobic metabolism and production of off flavours and odours.

Oxygen is the most destructive ingredient in juice; causing degradation of vitamin C. Also, one of the major sugar found in orange juice, fructose, can also cause vitamin C breakdown. The higher the fructose content, the greater the loss of vitamin C. Conversely, higher acid level of citric acid and malic acid stabilize vitamin C (Padayatty *et al*., 2003). The retention of vitamin C is often used as an estimate for the overall nutrient retention of food products because it is by far the least stable nutrient; it is highly sensitive to oxidation and leaching into water-soluble media during storage (Davey *et al*., 2000; Franke *et al*., 2004). It begins to degrade immediately after harvest and degrades steadily during prolonged storage (Rickman *et al*., 2007). According to research, literature and results for freshly squeezed fruits, oranges had the highest vitamin C content, followed by lemons, limes, pineapple, pawpaw and carrot (Natural Food- Fruit Vitamin C Content, 2001). This is consistent with reports that, climate, especially temperature affect vitamin C level. Areas with cool nights produce citrus fruits with higher vitamin C levels. Hot tropical areas produce fruit with lower levels of vitamin C (Padayatty *et al*., 2003). This may be due to the high temperatures which are capable of depleting the levels of vitamin C. Also, environmental conditions that increase the acidity of citrus fruits also increase vitamin C levels. Light exposure was found to promote browning in pineapple juice. Ten percent losses in vitamin C have been reported after 6 days at 5 °C in pineapple pieces (Gill *et al.*, 2006)

2.7.3 Microbes associated with pineapples in storage

The microbial population of fresh-cut fruits and vegetables is determined to a large extent by the origin of fruits and vegetables, agricultural practices, conditions of harvesting, processing and storage (Fan and Song, 2008). Food biological safety is thus the top priority of the fresh-cut fruit industry. Fresh-cut produce are normally eaten raw, and for this reason they have to be considered potentially hazardous for consumtion. Most of the microorganisms affecting shelf life of fresh-cut produce need an optimal environment with a Relative Humidity higher than 80 % for their growth (Frazier and Westhoff, 1993). The main problem that makes fresh-cut fruit a highly perishable product is the microbial growth caused by the accumulation of leaked juice rich in nutrients in the bottom of containers (Brecht, 2006). During distribution and storage, high humidity in packaged fresh-cut products and cut surfaces provide favorable conditions for the growth of spoilage microorganisms (Fan and Song, 2008). Operations such as peeling, cutting, or

slicing greatly increase tissue damage of fresh-cut fruits, causing the release of intracellular liquids, and consequently increase microbial growth (Brecht, 2006).

Additionally, as a response/consequence to wounding during processing and microbial growth during storage, there is an increase in off-flavour compounds, loss of firmness and respiration, reduced fresh-cut shelf life and leads to senescence processes (Artés *et al.*, 2007). Therefore, water loss is one of the major deleterious factors that affect fresh-cut produce quality, which in turn influences firmness and enhances microbial growth.

Microbes associated with fruits and vegetables spoilage include aerobic mesophilic bacteria (aerobic plate count), psychrotrophic microorganisms, lactic acid bacteria, yeast and mould, and coliforms. Fungi are the most important microorganisms causing postharvest wastage of fresh produce (Eckert and Ogawa, 1988). This is particularly true for fruits, where the relatively acid conditions tend to suppress bacterial growth (Frazier and Westhoff, 1993). However, in vegetables, bacterial infections are more common, due to their high pH. Nevertheless, bacteria are generally the fastest growing microorganisms; and under favorable conditions bacteria usually outgrow fungi (Frazier and Westhoff, 1993). Bacteria are also important as agents of both spoilage and food borne diseases. Mold growth is suppressed by low Relative Humidity. Many pathogenic molds, such as *Fusarium* spp., *Alternaria* spp., *Aspergillus* spp., *Penicillium* spp, and *Rhizopus* spp., have been reported as the causal agents of foodborne diseases and/or food spoilage in fruits (Tripathi and Dubey, 2004). In the studies conducted by Ajibola, **(**2009), *Bacillus subtilis* and *Candida* sp. were isolated from pineapple juice both at ambient and refrigeration conditions.

The safety of foods is principally assured by control at the source, product design and process control, and the application of Good Hygienic Practices during production, processing (including labelling), handling, distribution, storage, sale, preparation and use, in conjunction with the application of the HACCP system. This preventive approach offers more control than microbiological testing because the effectiveness of microbiological examination to assess the safety of foods is limited.

2.8 SENSORY QUALITIES OF CUT PINEAPPLES

Sensory evaluation is the science of judging and evaluating the quality of a food by the use of the senses: taste, smell, sight, touch and hearing. Sensory testing has been developed into a precise, formal, structured methodology that is continually being updated to refine existing techniques. The developed methods serve economic interests and can establish the worth or acceptance of a commodity. Sensory tests offer a course to select the product that optimizes value for money. Sensory evaluation is used as a practical application in product development by aiding in product matching, improvements, and grading. Research is another area where sensory evaluation is frequently used. Evaluation of a product may be needed to determine the affects an experiment had on its subject. Finally, quality control and marketing is yet another application of sensory testing (Meilgaard *et al.,* 1999). Sensory attributes of cut-pineapples include texture, colour, firmness, flavour, odour, taste (sweetness, sourness) and its overall acceptability. The sensory evaluations of sweetness, taste, and aroma in peach and nectarine fruits of nine different cultivars were evaluated and compared. The results of the research indicated that sensory evaluation provides a good tool in the quick assessment of the quality of the fruits (Caloric *et al.,* 2006). Improving consistency in fresh-cut fruit product flavour and texture may enhance consumers desire to repeatedly purchase such products.

2.9 FRESH-CUT PACKAGING MATERIALS

Packaging fresh fruits and vegetables is one of the important steps in the long and complicated journey from grower to consumer. Bags, crates, hampers, baskets, cartons, bulk bins, and palletized containers are convenient containers for handling, transporting, and marketing fresh produce. A significant percentage of produce buyer and consumer complaint may be traced to container failure because of poor design or inappropriate selection and use. A properly designed produce container should contain, protect, and identify the produce, satisfying everyone from grower to consumer (Ashby, 1987). The choice of packaging material for food is crucial regarding shelf life. When considering a packaging material for fresh cut fruits, it is appropriate to select the material that better matches quality objectives, shelf life, storage temperature and cost of the product (Ros – Chumillas, 2007). The main packaging material used in the packaging

of fresh-cut fruits is the plain high density polyethylene bags. The limiting factor of most fresh-cut fruit and vegetables shelf life is fungi and bacteria growth, which is associated with high Relative Humidity within packages (Ben-Yehoshua *et al.*, 1998). Relative Humidity in a plastic package of fresh commodities is usually high (> 95 %); and fluctuations in the storage temperature may result in condensation, which would greatly increase the proliferation and spread of spoilage microorganisms (Grierson and Wardowski, 1978).

2.9.1 Polyethylene

Plastic bags (polyethylene film) are the predominant material used in the packaging of fresh-cut fruits. Besides the very low material costs, automated bagging machines further reduce packing costs. Film bags are clear, allowing for easy inspection of the contents, and readily accept high quality graphics. Plastic films are available in a wide range of thicknesses and grades and may be engineered to control the environmental gases inside the bag. The film material breathes at a rate necessary to maintain the correct mix of oxygen, carbon dioxide, and water vapour inside the bag. Since each produce item has its own unique requirement for environmental gases, modified atmosphere packaging material are sometimes specially engineered for each item. According to research carried out by Paine, (1987), the shelf life of fresh produce is extended considerably by this form of packaging. The different types of polyethylene bags have different uses. Film packaging reduces water loss of fruit and vegetables. Scott, *et al.*, (1982) found that fruit kept in a perforated polyethylene bags at 20 °C for 10 days lost less than 2 % of their fresh weight, while control (unpacked) fruit lost between 18 % and 30 %. According to Jessup, *et al.*, (2007), heat alone is able to reduced insect survival on fruits (mandarins, cherries, tomatoes and apples). But, when combined with sealing in 38 μm low-density polyethylene bags, even with storage at 38 °C, no insect survived treatment for more than 3 days. In a study conducted by Ben-Yehoshua, *et al.*, (1998), bell peppers packaged in perforated film lost less weight and maintained higher quality than fruit stored in open carton boxes and, at the same time, had lower decay levels than peppers kept in non-perforated packages.

The influence of 25 μm thickness polyethylene bags (polybags) with microperforations on the storage quality of Cox's Orange Pippi apples has been studied. Bags with 50 micro perforations reduced bitter pit incidence and maintained fruit quality, apparently as a result of increased CO_2 concentrations and decreased O_2 concentrations (Watkins *et al.*, 2008).

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Sources of materials

Two varieties of freshly harvested pineapples, MD2 and smooth cayenne, were received from a farm at *Nsawam* in the Eastern region of Ghana packed safely in cartons under ambient conditions. Packaging materials were obtained from a wholesale point, "A-1" Rubber Enterprise, Roman Hill, Kumasi, Ghana. All chemical reagents were obtained from Sigma- Aldrich

3.1.2 Sample preparations

PHASE ONE: The pineapple varieties were received on the day of harvest. Only fresh pineapples with no defects were selected and used for the research. The fruits were washed, weighed and immersed in a water bath set at different temperatures (60, 70, and 80 °C) for different period/time (10, 20 and 30 min) of treatment, after which they were stored immediately in a refrigerator at 5 °C for 24 h. Chemical and sensory analyses were carried out on the treated and packaged fruit as well as the untreated control fruits. Juice from both treated and untreated fruits were prepared and used for the chemical analysis. Good manufacturing practices and best possible sanitary conditions, such as use of clean potable water, clean and sanitized processing equipment etc, were strictly adhered to during processing and all subsequent handling stages. Intensity for the sensory attributes was scored on a 10 cm scale, anchored with the terms dislike very much at the low end, like moderately at the median (5) end and like very much at the high level.

PHASE TWO: Samples from MD2 variety were treated at the optimum condition obtained from phase one. These samples were packaged in polyethylene bags and stored at refrigeration condition; 6 °C (± 2) and ambient condition 27 °C (±3) throughout the second phase experimental period. During this period, chemical (Vitamin C, Brix and Moisture content) microbial (Yeast, Mould and Aerobic Bacteria count) and sensory (triangle test) analyses were

carried out every two (2) days. Based on the results obtained, the shelf-life of the cut-fruits was determined for both treated and untreated fruits.

3.2 METHODS

3.2.1 Determination of treatment factors and responses

A response surface quadratic model was projected to study the responses of three factors, temperature (60 – 80 ^{o}C), time (10 – 30 min), pineapple variety (Smooth Cayenne – MD2), on the responses. A total of 26 runs were generated in order to study six responses, including; vitamin C content, brix content, taste, texture, appearance/colour (Appendix 3). Linear and polynomial models were then projected in studying variations in microbial count, sensory attributes, vitamin C content, pH values, brix content and moisture content to study the shelf-life of the cut-pineapples in refrigeration and ambient storage conditions (Appendix 4).

3.2.2 Analysis of responses

3.2.2.1 Determination of brix content/Total Soluble Sugar

A hand held refractometer (OTANGO manual Model, Japan) was used to determine the total soluble sugar (TSS) content. The refractometer was standardized by placing a drop of distilled water on its prism. It was then positioned in a way that would allow entry of sunlight via the prism. The eyepiece was used to observe the standardization after adjusting the coarse and fine adjustments properly to a 0 % TSS. Subsequently, a drop of juice from a sample of MD2 pineapple was placed on the prism and the percentage (%) TSS determined. The process was repeated for each sample and the percentage TSS determined (AOAC, 2000).

3.2.2.2 Determination of vitamin C content

The Iodometric Titration method was used to determine the vitamin C content. A volume of 10 ml of vitamin C standard solution was measured and poured in a flat bottom flask using a 10 ml graduated cylinder. A volume of 20 ml distilled water was then added to the vitamin C solution in the flask. Two drops of 1M HCl and 15 drops of starch solution were added. The burette was filled with iodine solution and secured. The initial volume of the burette was recorded. The solution was then titrated against the standard solution. Drops of iodine solution was added to the

standard solution and swirled until a purple colour was formed and remained for more than 15 s. The procedure was repeated for two additional trials and the data recorded. Using a 10 ml graduated cylinder and a clean 125 ml flask, the procedure was repeated for three trials of the 10 ml sample of pineapple juice. The various results were recorded accordingly (AOAC, 1992).

3.2.2.3 Sensory evaluation

Preference ranking test (interval scale method) was the sensory tool used in this analysis. Fifty (50) untrained panelists participated in this analysis. Pineapple slices of approximately 3 cm x 4 cm x 4 cm were prepared in triangular wedge. Four each were packaged in plain polyethylene bags and sealed for sensory analysis (Fig 3.1). Panelists were given samples from the two varieties of pineapples, one variety at a time. They were asked to taste the samples from left to right, cleansing their palate before each sample by taking a bite of crackers and a sip of water. Panelists scored on an interval scale of 0-10 cm their degree of likeness (0= dislike very much, 10= like very much, and 5= like moderately) to assess quality attributes such as taste (sweet-sour balance), texture (firmess, softness), and appearance/colour (appendix D1). Data obtained were subjected to statistical analysis using Design Expert (2007). Both varieties of pineapples were used for sensory analysis.

Fig 3.1 Pineapple packaging.

3.2.3 Data analysis for treatment factors

3.2.3.1 Fitting the data

The response data for the vitamin C, brix and sensory evaluation data collected were loaded and fitted to models and the regression models studied. This involved studying such coefficients as the regression- (R^2), adjusted regression- (adj R^2), prediction regression-(pred R^2) and adequate precision - (adeq precision) of the models selected. Design expert, (2007) explains that adequate precision measures the signal to noise ratio and that a ratio greater than 4 is desirable. Also the adjusted and predicted R-squared values should be within 0.2 of each other to make the fitted model good.

After fitting and ANOVA studies, diagnostic plots were carried out where the normal probability plot of the studentized residuals were analyzed to check for normality of residuals. When all the model statistics and diagnostic plots were evaluated to be good, the model graphs were obtained. The vitamin C, brix, taste, texture and appearance/colour were optimized according to the constraints imposed (Table 3.1).

Table 3.1 A constraint table showing the importance of factors and responses goals set for optimization.

Constraints		Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
Name	Goal					
Temperature	is in range	60	80	1	1	3
Time	is in range	10	30	1	1	3
Pineapple	is in range	SC	MD2	1	1	3
VitC	maximize	0.095	0.423	1	1	5
Sugar	maximize	12.500	14.200	1	1	3
Taste	maximize	0.3801	0.6892	1	1	5
Texture	maximize	0.3111	0.6356	1	1	5
Appearance	maximize	0.3048	0.6692	1	1	5

At the end of the optimisation process, MD2 was selected for shelf life studies because it had the best performing characteristics of 39.00 mg/100ml vitamin C and a taste, texture and appearance

of 7.25, 6.75 and 6.69 respectively. The processing condition corresponding to these characteristics were reported to be a temperature of 73.49 ^{o}C and at a processing time of 10 min.

3.2.4 Determination of shelf-life parameters

3.2.4.1 Physicochemical Analysis

3.2.4.1.1 Determination of brix content

A hand held refractometer (OTANGO manual Model, Japan) was used to determine the total soluble sugar (TSS) content as described in 3.2.2.1 earlier (AOAC, 2000).

3.2.4.1.2 Determination of moisture content

Two (2) g of samples from both treated and untreated pineapples were weighed in a crucible using an analytical balance (OHAUS AS260D Model, NJ/USA). The weight of the crucible and each sample was determined and recorded. The crucible and its content were placed in a dry oven at a temperature of 105 ^{o}C for three hours after which the difference in weight was determined using the appropriate formula for calculation (Appendix A3) (AOAC, 1992).

The procedure was repeated for each sample and the determinations were carried out every other day for twenty (20) days on samples in refrigeration storage condition and eight (8) days for samples in ambient condition.

3.2.4.1.3 Determination of pH

The pH was measured using a digital pH meter (Beckman Model, USA) equipped with an electrode. The electrode was washed in distilled water. It was then placed into the juice. The pH reading was read from the recorder of the pH meter (AOAC, 1992). The measurements were carried out every other day for twenty (20) days on samples in refrigeration storage condition and eight (8) days for samples in ambient condition.

3.2.4.1.4 Determination of vitamin C content

The Iodometric Titration method was used to determine the vitamin C content as described in section 3.2.2.2 earlier (AOAC 2000).

3.2.4.2 Microbial Analysis

Cassava Dextrose Agar (CDA) was used as the media for enumeration of the mould load. The media was prepared by gently boiling 100 g of chopped fresh cassava pieces into 500 ml of distilled water for about 30 min. The preparation was then filtered using a sterile cheese cloth and cotton wool. The volume of the filtrate was then up to 100 ml using sterile distilled water. 20 g glucose and 15 g Agar were weighed and dispersed into the 100 ml cassava infusion and mixed thoroughly. The media was then sterilized by autoclaving and used for mould determination.

Plate Count Agar (PCA) was used as the basal medium for aerobic bacteria count and Yeast Extract Agar was used as the media for the enumeration of the yeast. An amount of nutrient agar was weighed and dissolved in 250 ml distilled water based on the manufacture's guidelines. The mixture was heated and stirred until a uniform mixture was obtained. The liquid agar was autoclaved at a temperature of 121 ^{o}C for 15 min and cooled to a temperature of 45 ^{o}C. 100 glass test tubes each filled with 10ml of distilled water and plugged with cotton wool were placed in a basket and sterilized by autoclave at 121 ^{o}C for 15 min. In preparing the serial dilution of the stored pineapples, each test tube containing 9 ml of sterilized distilled water was unplugged, flamed with a Bunsen burner and 1ml of the pineapple juice was thoroughly mixed to obtain a dilution of 10 ml.

This procedure was repeated with each dilutions as stock from which decimal dilution of 10ml were prepared. This procedure was used for the preparation of serial dilutions of the pineapples juice for the different media (and broth) in the enumeration of aerobic plate count, yeast and mould. The pour plate technique was used for the inoculation. Using sterile petri dishes, 1 ml of homogenized pineapple juice of known dilution and suitable amount (about 10 ml) of molten media (40-45 ^{o}C) were mixed by swirling. This technique was used for all the microbial determination using juice from the untreated pineapple as control (Atlas *et al*, 1995; Prescott, 1999).

The Petri dishes were incubated for 18-24 h at 37 ºC in a Gallenkamp Incubator (Model 1H-150, UK). The colonies formed on the media were counted using a Stuart Scientific Colony counter (Serial # 7354, UK). The total aerobic microbial load was expressed as the colony forming units (CFU) per 100ml (Atlas *et al*, 1995) for the aerobic, yeast and the mould counts. The microbial counts were carried out every other day for twenty (20) days on samples in refrigeration storage condition and eight (8) days for samples in ambient condition. Yeast extract agar, Cassava Dextrose Agar (CDA) and Plate Count Agar (PCA) was used as the basal medium for the determination of yeast, mould and aerobic bacteria counts respectively.

3.2.4.3 Data analysis of shelf life

The results obtained were loaded and analyzed by plotting the polynomial regression using Statsgraphics Centurion, (2008). The order was considered adequate where the largest R-squared statistics, interpreted as the percentage of the variability in the response variable was significant; i.e., $P \leq 0.05$. Such models were subsequently chosen to calculate the shelf-lives for each one of the parameters.

The shelf-life of the fruits was determined by calculating from the regression equation generated or locating on the plotted graph. The locate bar was placed such that the vertical line pointed to the exact shelf-life of the fruit when the horizontal line was positioned on the accepted limits of the parameter.

3.2.5 Sensory analysis of MD2 pineapples during storage

Triangle different test sensory tool was used in this analysis. Ten (10) panelists participated in this triangle test. Samples were packaged in plain polyethylene bags sealed by knotting as mostly seen on the Ghanaian market in urban areas, for the second phase of the research. The taste, texture and appearance/colour of the treated cut pineapples in both ambient and refrigeration conditions were compared to their respective untreated samples and conclusions drawn. Each panelist was given three samples, one of which was odd, and was asked to indicate the odd sample by circling its label on the score sheet (Appendix D2). The panelists were asked to taste the samples from left to right, cleansing their palate before each sample by taking a bite of crackers and a sip of water. They were asked to tell which sample was odd. The results obtained

was analysed using a triangle test statistical analysis table. Sensory analysis during this phase was carried out on MD2 pineapple variety.

CHAPTER 4

4.0 **RESULTS AND DISCUSSION**

4.1 **Treatment factors and responses.**

The optimum treatment condition was determined using the Design Expert software (2007). The optimum treatment condition and variety was chosen from a list of treatment condition as shown in Table 4.1. The recommended treatment condition (Number 1) and variety (MD2) was chosen and used for the second phase of the study. The optimum treatment condition and variety was 73.49 °C for 10 min for MD2 pineapple variety. This treatment condition was chosen because treated fruits had the most optimum qualities expected. Fruits had the highest vitamin C content in line with observation of Lamikanra and Watson, (2001), who reported that at temperature treatment above 60 °C for 20 min, shelf-life and sensory qualities such as texture and taste of the cantaloupe melon was improved. The sweet-sour balance and firmness of the fruits treated at this condition were improved as observed by Valero, *et al.,* (2002) in treated plums as well as Lamikanra, *et al*., (2005) in cantaloupe melons. The golden brown colour/appearance and overall preference as well as the vitamin C content of MD2 was improved (Table 4.1 number 1) as compared to the control (Table 4.2).

Table 4.1. Optimum treatment conditions and their corresponding optimum pineapple nutritional and sensory qualities.

Number	Temperature /^{o}C	Time /min	Pineapple	VitC mg/ml	Taste	Texture	Appearance
1	73.49	10.00	MD2	0.39	7.25	6.72	6.69
2	73.68	10.00	MD2	0.39	7.24	6.71	6.68
3	71.50	10.00	MD2	0.39	7.30	6.76	6.80
4	68.50	10.00	MD2	0.38	7.25	6.68	6.86
5	71.52	10.00	SC	0.16	6.77	6.51	6.26
6	71.44	10.00	SC	0.16	6.77	6.51	6.26
7	71.66	10.00	SC	0.16	6.77	6.50	6.25

Table 4.1 Runs, treatments and responses studied.

Run	FACTORS			RESPONSES				
	A: Temperature ^{o}C	B:Time minutes	C: Pineapple	VitC mg/ml	Sugar %	Taste	Texture	Appearance
1	80	20	MD2	0.384	12.8	4.20	4.70	4.60
2	60	20	SC	0.169	13.1	5.20	4.80	5.60
3	80	30	MD2	*	12.7	3.80	3.10	3.10
4	70	10	SC	0.146	12.6	6.30	5.90	5.80
5	70	20	SC	0.142	14.1	6.30	6.20	5.60
6	80	30	SC	0.095	12.8	3.80	3.10	3.10
7	60	10	SC	0.184	12.5	6.10	6.00	6.20
8	70	30	SC	0.138	12.5	4.80	5.00	5.10
9	70	20	MD2	0.384	13.2	6.80	6.30	6.70
10	60	30	SC	0.169	12.7	6.30	5.90	5.60
11	80	10	SC	0.200	13.1	6.40	5.90	6.20
12	70	20	SC	0.146	13.5	6.20	6.20	5.60
13	70	20	SC	0.145	13.8	6.20	6.20	5.60
14	70	10	MD2	0.364	13.5	6.80	6.00	6.40
15	70	20	SC	*	14.0	6.90	6.30	*
16	70	20	MD2	0.344	12.5	6.80	6.40	6.70
17	70	20	MD2	0.346	13.0	6.80	6.40	6.70
18	60	30	MD2	0.346	13.1	5.20	5.70	6.60
19	70	20	MD2	0.376	12.5	6.80	6.40	6.70
20	80	20	SC	0.143	12.7	5.00	3.90	3.90
21	70	20	SC	0.142	12.9	6.20	6.20	5.60
22	60	20	MD2	0.366	13.6	5.90	5.20	6.50
23	60	10	MD2	0.392	12.8	6.30	5.80	6.30
24	70	20	MD2	0.364	13.3	6.80	6.40	6.70
25	80	10	MD2	0.423	14.2	6.90	6.30	*
26	70	30	MD2	0.321	12.8	5.10	5.00	4.80
Control	-	-	MD2	0.424	14.8	6.60	5.60	6.80
Control	-	-	SC	0.212	14.0	6.00	5.70	4.50

***= outlier**

4.2 Analysis of the responses

4.2.1 Brix/TSS Content of Pineapple Samples

The Brix content of the pineapples after treatment is presented in Table 4.2. Brix content obtained from the treated pineapples was generally dependent on their initial brix content of the fruits before treatment. Brix content of samples from both varieties increased with increase temperature at treatment for 10min. However, a reverse of this was observed when samples were treated for 20 and 30 min at all temperature levels used (60 °C, 70 °C and 80 °C).

Valero, *et al.,* (2002), as well as Abreu, *et al.,* (2003), reported that in mild heat treated plums, heat treatment induced a greater cell wall stability and plum firmness. This explains reduced sugar loss at the temperature and time of treatment observed above. However higher temperature treatment over longer period of time disrupts the cell wall integrity making it less stable and highly porous for leaching to occur. Sugar content reduced as compared to the control but this reduction did not affect the nutritional content of the fruits as sugar content of the treated fruits remained in the accepted range of between 12 and 16 % for all treatment conditions.

4.2.2 Vitamin C Content

The ANOVA for the response surface quadratic model obtained for the behaviour of vitamin C suggested that at $p<0.05$, the model, time of processing and the vitamin C content of the two pineapples were significant (Appendix B3i). There were also significant interactions between the temperature and time of processing though processing temperature alone was not (Appendix B3i). The regression coefficient, R^2 (0.99), the adjusted regression, adj R^2 (0.98), prediction regression, pred R^2 (0.97) and adequate precision, adeq precision (35.67) of the model appeared good (Design Expert, 2007) since the adj R^2 and pred R^2 were within a difference of 0.2 and the adeq precision was greater than 4 (Appendix B3ii).

Vitamin C content of pineapple is dependent on factors such as the cultivar, stage of maturity, conditions of storage and the part of fruit (Ngoddy and Ihekoronye, 1985). Its content in fresh pineapples ranges from about 20 mg/100 ml to 34.44 mg/100 ml (Ngoddy and Ihekoronye, 1985). The optimum value obtained for vitamin C content of processed MD2 pineapples in this

research was 39.4 mg/100 ml (Table 4.2) which was even higher than that reported reports from Ngoddy and Ihekoronye, (1985). Vitamin C content decreased almost linearly with increase in period of treatment (10 to 30 min) for all treatment temperatures (60 to 80 °C) (Fig 4.1). This may be due to the relatively high temperatures and long period of treatment which are capable of depleting the levels of vitamin C as reported by Padayatty, *et al*., (2003). The tendency of vitamin C to leach into water soluble media as reported by Davey, *et al*., (2000) and Franke, *et al*., (2004), could also be a contributing factor to this loss. From the Fig 4.1 it was observed that vitamin C levels dropped to a minimum at about 70 °C for all periods of treatment and gradually increased to a maximum around 80 °C along the temperature axis. This rise observed could be explained by the work of Paull and Chen, (2000), as well as Lamikanra and Watson, (2003), who reported that increased temperature treatment above 60 °C could increase protoplasmic viscosity as well as membrane impermeability. Since loss of vitamin C is primarily through the membrane, retention observed could be attributed largely to the impermeability caused by the heat treatment.

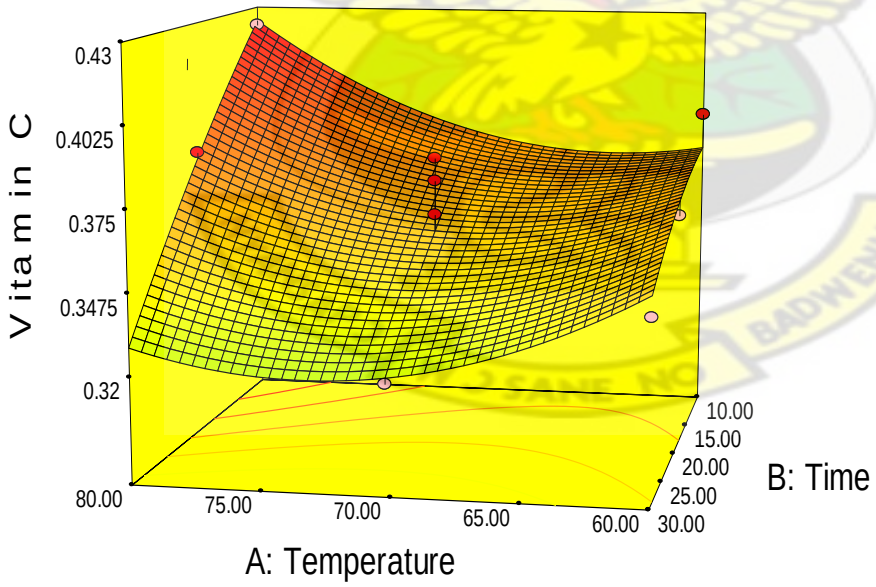


Fig 4.1. Response surface plot for the effect of treatment time/min and processing temperature (°C) on the vitamin C content (mg/100 ml) of the MD2 pineapple variety.

The retention of vitamin C is often used as an estimate for the overall nutrient retention of food products because it is by far the least stable nutrient in fruits (Davey *et al*., 2000; Franke *et al.*, 2004). It can therefore be inferred that pineapples treated at these conditions remained nutritionally wholesome after treatment since their vitamin C content was within range (Ngoddy and Ihekoronye, 1985).

4.2.3 Sensory evaluation

The ANOVA for the response surface quadratic model obtained for the behaviour of taste suggested that at $p<0.05$, the model, time and temperature of processing of the two pineapples were significant but the pineapple variety was not significant (Appendix B4i). There were also significant interactions between the temperature and time of processing though that of processing temperature and variety was not. The regression coefficient, R^2 (0.81), the adjusted regression, adj R^2 (0.72), prediction regression, pred R^2 (0.40) and adequate precision, adeq precision (12.60) of the model all appeared good (Design Expert, 2007). (Appendix B4i and 4ii).

Fig 4.2 indicates that the taste (balance between sourness and sweetness) of samples from fruits treated for 10 min was preferred to samples treated for 20 min and 30 min. There was a drop in taste with temperature to a minimum at 72 °C which then rose to a maximum from then till 80 °C. Samples of MD2 pineapples had a more preferred sweet and sour balance than smooth cayenne. Treatment at 70 °C for 10 min, 80 °C for 10 min and 70 °C for 20 min had the highest level of preference for both MD2 and Smooth Cayenne varieties. MD2 had a taste preference of 6.78, 6.89 and 6.84 at the above treatments respectively Fig 4.2 while Smooth Cayenne had a taste preference of 6.29, 6.35 and 6.24 respectively (Table 4.2). Heat treatment at these conditions resulted in fruits with more preferred sweet-sour balance.

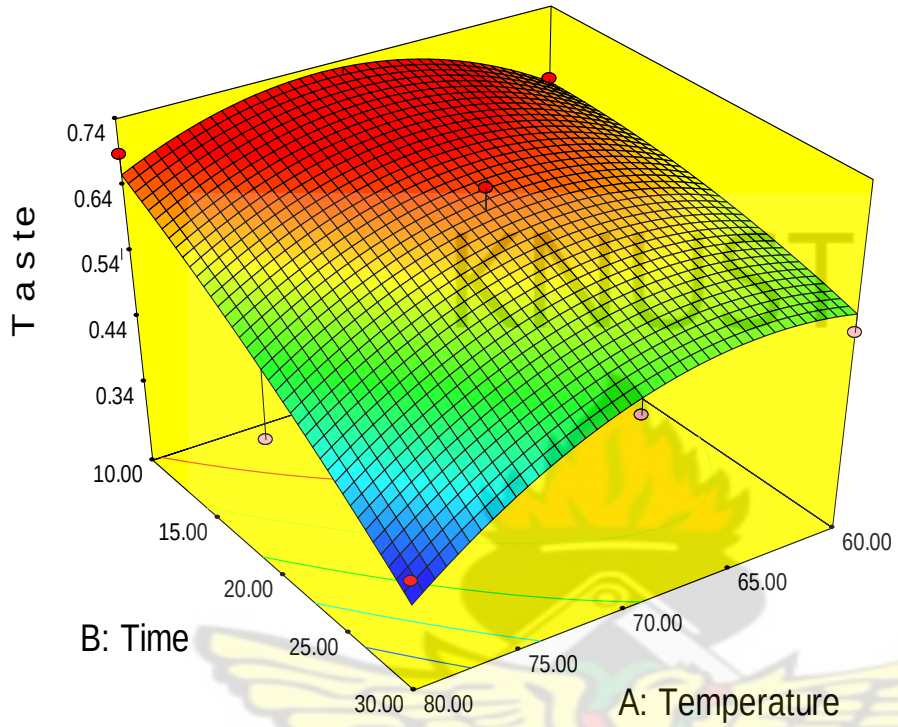


Fig 4.2 Response surface plot for the effect of treatment time (min) and processing temperature (°C) on the taste (0-1) of the MD2 pineapple variety.

The ANOVA for the response surface quadratic model obtained for the behaviour of texture suggested that at $p<0.05$, the model, time and temperature of processing and pineapple variety of the two pineapples were significant (Appendix B5). There were also significant interactions between the temperature and time of processing as well. The regression coefficient, R^2 (0.81), the adjusted regression, adj R^2 (0.98), prediction regression, pred R^2 (0.75) and adequate precision, adeq precision (19.40) of the model all appeared good (Design Expert, 2007).

The texture (firmness or softness) of the samples from both MD2 is presented in Fig 4.3 Generally the firmness of samples from the two varieties followed the same trend. The graph

indicates a general initial rise in firmness of samples with increase in temperature till a peak was reach. A drop was then observed till the end of the study. However, sample firmness reduced with increase in the period of treatment. Fruits treatment at 70 °C for 20 min had the most preference values of 6.36 and 6.18 for MD2 and smooth cayenne varieties respectively. These fruits were firmer and were very much preferred by the panelists. The increase in firmness of the samples treated at 70 °C for 20 min is due to cell wall strengthening. This was observed by Valero, *et al.*, (2002) in treated plums as well as Lamikanra, *et al.*, (2005) in cantaloupe melons. Heat treatment allows demethylation of pectin by pectin methyl esterase to form anionic carboxylic groups with which calcium can form salt bridge links thereby strengthening cell wall (Alonso *et al.*, 1997). However, at higher temperatures and period of treatment the fruit cell wall becomes disrupted. The softest fruits were treated at 80 °C for 30 min with for both MD2 and smooth cayenne. Temperature and time of treatment contributed significantly to the differences observed in the texture of the samples ($p<0.05$). Heat improved firmness however, higher temperatures and longer period of treatment reduced firmness (increased softness).

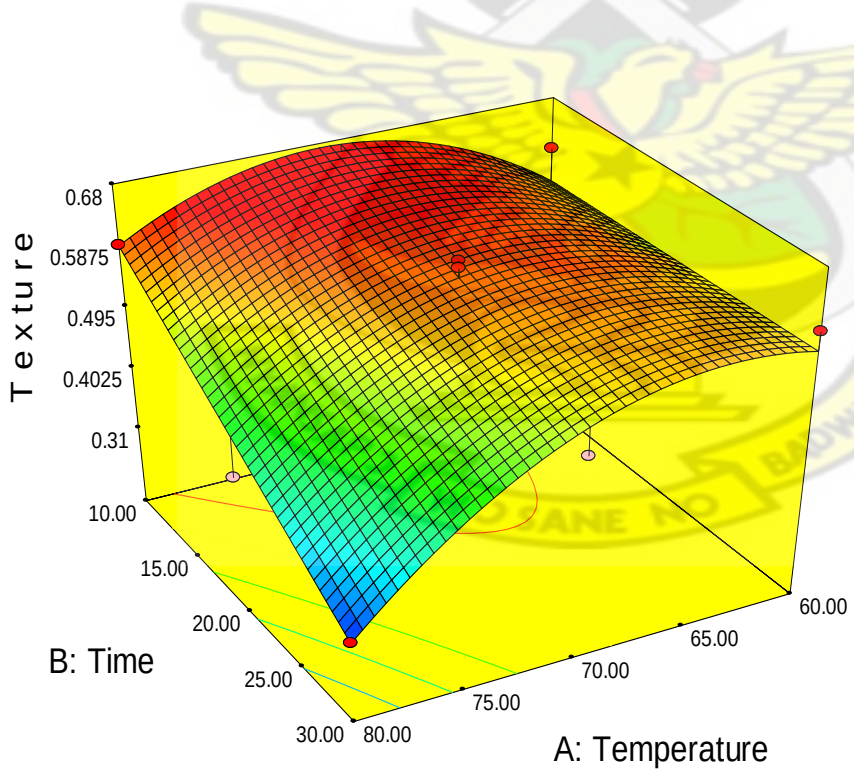


Fig 4.3 Response surface plot for the effect of treatment time and processing temperature on the texture of the MD2 pineapple variety.

The ANOVA for the response surface quadratic model obtained for the behaviour of appearance/colour suggested that at $p<0.05$, the model, time and temperature of processing and pineapple variety of the two pineapples were significant. There were also significant interactions between the temperature and time of processing as was observed in the texture above. The regression coefficient, R^2 (0.88) approached 1, making it highly significant. The adjusted regression, adj R^2 (0.86), prediction regression, pred R^2 (0.77) and adequate precision, adeq precision (21.20) of the model appeared good (Design Expert, 2007) since the adeq precision was greater than 4 (Appendix B6).

The appearance (colour) of the pineapple samples from both varieties generally decreased with increase in temperature and period of treatment (Fig 4.4). Fruits treated at 80 °C for 10 min had the highest preferred colour of 6.89* for MD2 and 6.21* for Smooth Cayenne. The least preferred fruits colour was obtained from samples treated at 80 °C for 30 min. At 70 °C for 10 min, colour preference was higher than the median point on the scale (5) which indicated that panelist preferred the appearance of the samples treated at this condition very much. The appearance of both pineapple varieties was affected significantly by all the three factors ($p<0.05$). Increase in temperature increased fruit colour. Increase in time/period of treatment reduced the colour preference of the pineapples. This was especially notice in MD2 samples treated at 80 °C for 20 min and 30 min where a dark brown colour instead of a golden brown desirable colour was observed.

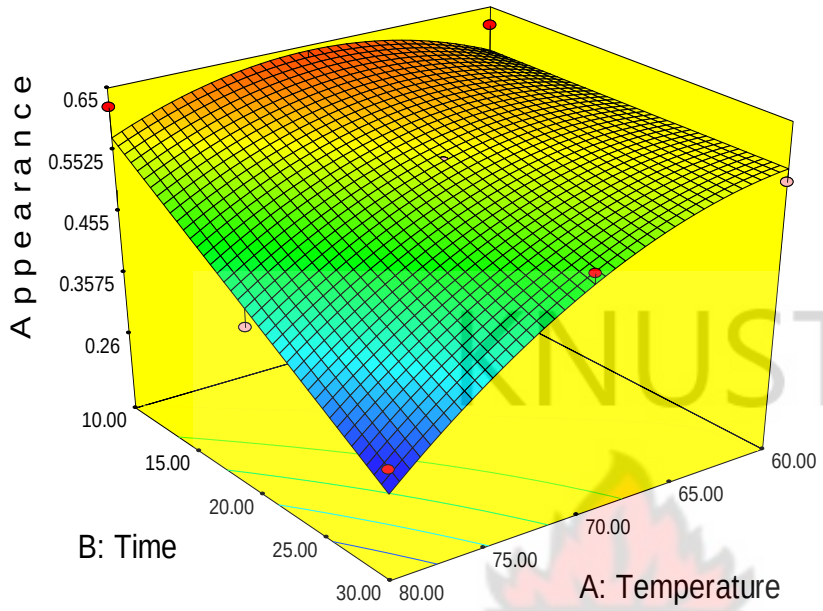


Fig 4.4 Response surface plot for the effect of treatment time and processing temperature on the appearance/colour of the MD2 pineapple variety.

Sensory descriptors such as taste, texture and appearance/colour used in the sensory analyses were improved or maintained at some treatment conditions used in this experiment. The most significant were for taste and texture. Lamikanra, *et al.,* (2005), reported a significant increase in intensities of various taste and textural descriptors in heat treated fruits. Most studies report increased firmness as the main textural changes that results from postharvest heat treatment of fruits (Kim *et al.,* 1993b; Paull and Chen 2000; Abreu *et al.,* 2003). Appearance of Smooth Cayenne was improved as compared to MD2, at high temperatures and period of treatment. Smooth cayenne had an improved yellow colour at high temperatures (70 °C for 30 min, 80 °C for 20 min and 80 °C for 30 min) while the golden brown colour of MD2 was further darken to give a dark brown undesirable colour. This resulted in the low scores obtained for MD2 varieties and the relatively high values for smooth cayenne varieties with respect to the control (Fig 4.4: Table 4.2).

4.3 Determination of Shelf-life Parameters

Evaluation of the effects of mild heat treatment (73.5°C, 10 min) on the chemical, microbial and sensory properties of MD2 pineapple variety, in ambient (Fig 4.5a) and refrigeration (Fig 4.5b) storage conditions over a period of 8 days for ambient and 20 days for refrigeration condition.

(a) (b)

Fig 4.5 Pineapples in ambient (a) and refrigeration (b) storage conditions.

4.3.1 Physicochemical analysis

4.3.1.1 Brix Content.

Brix content generally decreased with days in both treated and untreated pineapples in both ambient and refrigeration storage condition (Fig 4.6). The results showed that treated pineapples had a reduced rate of brix loss with storage than that observed in the untreated fruits. Disruption of cell wall degrading enzymes has been reported as one of the major positive effect of heat treatment (Paull and Chen, 2000). This advantage contributed to the reduced Total Soluble Solid loss observed in treated pineapples. Initial brix content of untreated fruits was higher than treated fruits. However, a rapid loss was observed throughout the storage period in both ambient and refrigeration conditions. Brix content of ambient fruits dropped rapid from 14 % to 6 % by the eighth day but a less rapid observation was made in treated fruits where brix reduced from 13.4 % to 10.5 % by the eighth day. Fruits in refrigeration condition stored better with respect to brix content than ambient fruits. This may be due to increased microbial count and moisture loss in

the ambient fruits (Brecht, 2006). Therefore low brix content of samples in ambient storage may be due to increased microbial activity and moisture loss. Soluble solids content is used as an indication of fruit maturity and quality (Paull, 1993) and for pineapples, they range between 10.8- 17.5 % (Dull, 1971) with very little variations between varieties. This indicated that refrigerated fruit samples as well as treated ambient fruit samples had brix content, characteristic of matured fruits throughout the storage period. However, untreated fruit samples had brix content, characteristic of a matured fruit only for about four (4) days of storage out of the eight (8) days storage period.

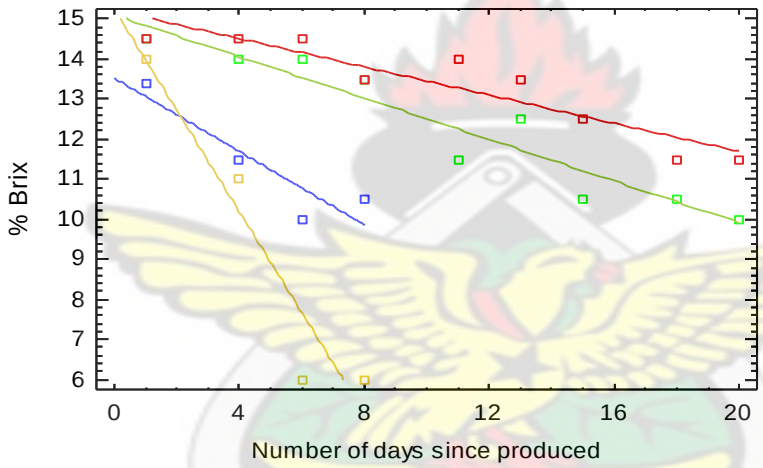


Fig 4.6 Brix content of treated and untreated pineapples in refrigeration and ambient storage condition

Legend
- Refrigerated treated samples
- Refrigerated untreated samples
- Ambient treated samples
- Ambient untreated samples

4.3.1.2 Moisture Content of Samples

Fig 4.7 shows the relationship between moisture content of treated and untreated fruits over 20 days (refrigeration condition) and 8 days (ambient) storage period. Initial moisture content of treated fruits was lower than untreated fruits. However, untreated fruits lost moisture in the form of juice much more rapidly than treated fruits. Moisture content of treated fruits in refrigeration condition reduced gradually from the first day (88.11 %) till the 20th day (84.36 %). However, it reduced rapidly in untreated fruits from 90.17 % on the first day to 80.65 % by the 15th day (Fig 4.7).

Similar moisture retention was reported for heat treated cantaloupe melons by Lamikanra, *et al.*, (2005), as well as valencia oranges by William, *et al.*, (1994), which was attributed to improved cell wall impermeability. Heat treatment increases protoplasmic viscosity and loss of membrane permeability as a result of protoplasmic streaming (Paul and Chen, 2000) thereby reducing the rate of moisture loss in the form of juice. Cell wall degrading enzymes are frequently disrupted and are sometimes not produced or delayed following heat treatment (Paull and Chen, 2000). This contributed to the moisture retention in treated fruit samples observed. In the refrigeration condition, the difference in moisture loss in both treated and untreated pineapples was significant ($p<0.05$). Moisture loss in untreated pineapples in ambient condition was relatively higher and more rapid than observed in the treated pineapples (Fig 4.7).

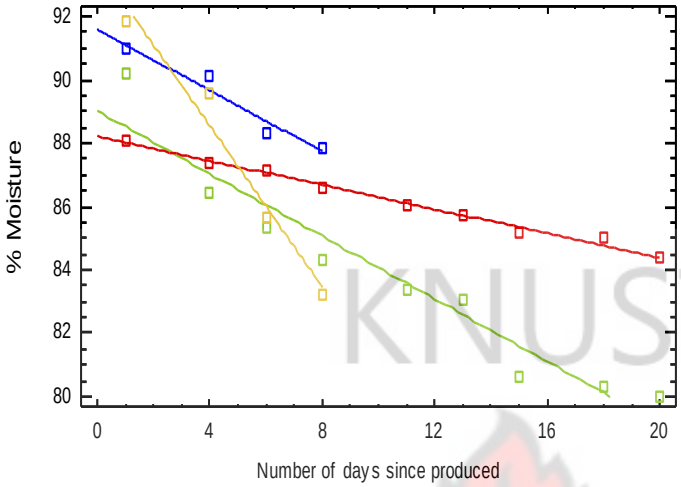


Fig 4.7 Moisture content of treated and untreated pineapples in refrigeration and ambient storage condition.

Legend

- Refrigerated treated samples
- Refrigerated untreated samples
- Ambient treated samples
- Ambient untreated samples

4.3.1.3 pH of Samples

The pH of the treated and untreated fruits in storage is presented in Fig 4.8. The pH value of pineapples in the refrigeration condition was stable in the first 4 days. However values increased in both samples from the fourth day till the twentieth day. The increase in untreated pineapples however, was rapid and sharp than that observed in the treated pineapples even though this difference was not significant ($p<0.05$). The pH of treated pineapples stored under ambient conditions was slightly lower in treated pineapples than untreated pineapples on the first day of storage. But this changed after the second day. pH of untreated pineapples increased more rapidly than treated fruits.

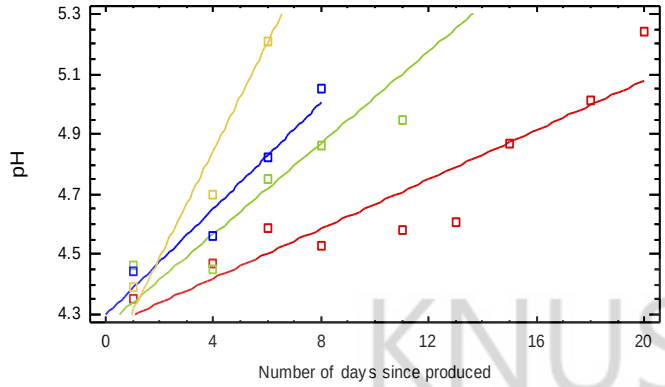


Fig 4.8 pH values of pineapples stored under refrigeration and ambient storage condition

Legend
- Refrigerated treated samples
- Refrigerated untreated samples
- Ambient treated samples
- Ambient untreated samples

Fruit acidity increases with maturity (Gortner and Singleton, 1965). Conversely high acid levels of citric and malic acid stabilizes vitamin C content (Padayatty *et al.*, 2003) in pineapple fruits. The pH values obtained indicates to a significant extent the microbial as well as vitamin C stability of the various fruit sample. Kim, *et al.*, (1993) and Lurie and Klein, (1992), reported lower total acidity in heat treated apple slices than untreated fruits as was observed here. Therefore, samples with lower pHs' as expected retained nutritional (vitamin C) and sensory (taste, texture, appearance, overall acceptability) quality better than the untreated fruits with high pH values.

4.3.1.4 Vitamin C Content

Vitamin C content of treated samples stored under ambient conditions dropped from 38.52 mg/ml on the first day to 20.40 mg/ml on the 8th day while that of the untreated fruits dropped from 34.90 mg/ml to 9.60 mg/ml over the 8 days storage period. Vitamin C content of treated

samples stored under refrigeration condition reduced from 38.28 mg/ml to 18.34 mg/ml, while untreated samples reduced from 34.42 mg/ml to 10.45 mg/ml, over 20 days storage period (Fig 4.9). Initially vitamin C content of untreated fruits were comparably higher than treated fruits but this trend change by the third day where it rapidly reduced in untreated fruits. Vitamin C was maintained in treated pineapples in both ambient and refrigeration condition over the period of storage. Shelf-life based on vitamin C content of ambient treated and untreated as well as refrigeration treated and untreated were 12.0 days, 7.5 days and 27.4 days, 17.9 days respectively. Shelf-life based on vitamin C content therefore, was expectedly higher in treated than untreated fruits. Vitamin C content was retained due to increased moisture retainability which in turn was due to cell wall stability reported by Paull and Chen, (2000).

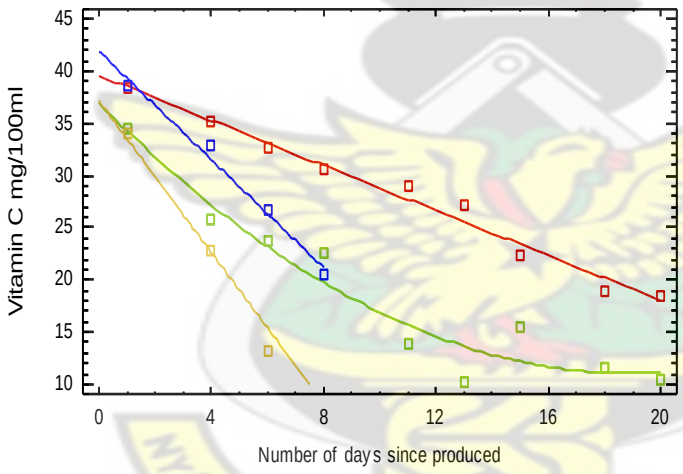


Fig 4.9 Shelf-life based on vitamin C content of treated and untreated pineapples under ambient storage conditions

Legend
- Refrigerated treated samples
- Refrigerated untreated samples
- Ambient treated samples
- Ambient untreated samples

4.3.2 Microbial Analysis

Using Statgraphics Centrurion XV.1 (2008), a shelf-life determination software, graphs and equations for the determination of shelf-life of the cut-fruits were obtained. The shelf-life of the samples were determined using the 'locate' button or calculated where applicable using regression equations of the curves. These results were based on the Global Food Safety Standards, (2009). The microbial limits used in the determination of shelf-life were: Yeast- 10000 CFU/ml; Mould- 100 CFU/ml; Aerobic Bacteria- 100000 CFU/ml.

4.3.2.1 Yeast Count

The relationship between yeast count and shelf-life of treated and untreated pineapples in ambient and refrigeration condition is presented in Fig 4.10. The growth pattern of yeast in all samples followed the microbial growth curve (Prescott, 1999). An initial yeast count of untreated fruits in ambient condition was higher as compared to treated fruits. Yeast counts were below 30 CFU/ml after the first day in storage, for ambient treated pineapples. However, there was an exponential increase from the second day till the eighth day for both treated (21000 CFU/ml) and untreated (30000 CFU/ml) pineapples. Thus, shelf-life of treated and untreated MD2 variety stored in the ambient condition, based on yeast count, was approximately 4.0 days and 2.7 days respectively (Fig 4.10).

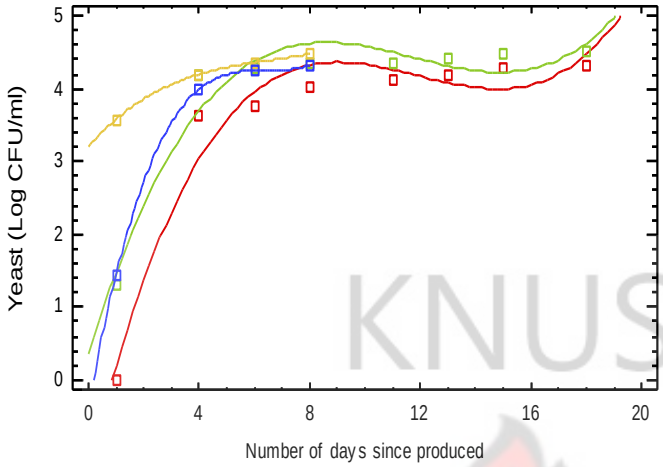


Fig 4.10 Shelf-life based on yeast count of treated and untreated pineapples in ambient and refrigeration conditions

Legend

- Refrigerated treated samples
- Refrigerated untreated samples
- Ambient treated samples
- Ambient untreated samples

Initial yeast count of untreated fruits was higher compared to the other fruits. This was due to the disadvantage of being stored in ambient condition and not being treated. Initial yeast count of treated fruits in ambient condition was slightly lower than untreated refrigerated samples as a result of pre-heat treatment. Yeast count of treated fruits in refrigeration condition was lower than untreated fruits during the first 8th day of the work but became comparable by the 20th day in storage. Treated pineapples stored in the refrigeration condition recorded yeast count of less than 10 CFU/ml after the first day in storage and increased from the third day at about 100 CFU/ml to 21500 CFU/ml on the 18th day. Shelf-life of MD2 stored in the refrigeration condition was 6.2 days. Untreated pineapples on the other hand with an initial day one count of 20 CFU/ml had 32000 CFU/ml counts. Untreated samples remained wholesome for consumption up till the 4.5 day.

4.3.2.2 Mould Count

Fig 4.11 shows the relationship between mould count and the shelf-life of treated and untreated pineapples in ambient and refrigeration condition. The acceptable limit of mould count in fresh-cut fruits is 100 CFU/ml. Mould count of treated samples in both ambient and refrigerated fruits were lower than that of untreated fruits. Mould counts were below 20 CFU/ml for both treated and untreated ambient fruits within the first 4 days of storage and increased gradually to 30 CFU/ml by the 8th day in treated fruits. However, the increase was rapid in untreated fruits from 27 CFU/ml (6th day) to 480 CFU/ml by the 8th day. Shelf-life of ambient treated pineapples based on mould count was 7.5 days. The untreated fruits had a shelf-life of 4.3 days.

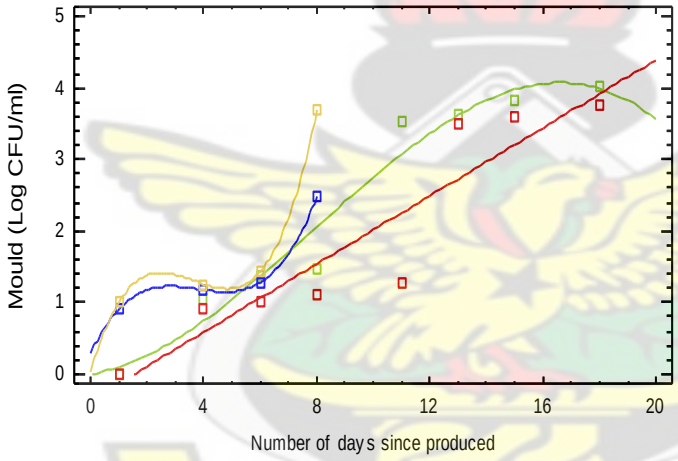


Fig 4.11 shelf-life of treated and untreated pineapples, in refrigeration and ambient storage condition, based on their mould count.

Legend
- Refrigerated treated samples
- Refrigerated untreated samples
- Ambient treated samples
- Ambient untreated samples

Mould count remained less than 10 CFU/ml for the first 6 days in the refrigerated treated fruits. This increased gradually to 19 CFU/ml by the 11th day. Growth then increased rapidly from the 13th day at 320 CFU/ml to 570 CFU/ml by the 18th day. A shelf-life of 10.5 days was located for treated pineapples in refrigeration condition with respect to mould count. Untreated fruits also had low mould counts from the first day (6 CFU/ml) till the 11th day where mould counts increased rapidly from 320CFU/ml to 570 CFU/ml. Untreated fruits were wholesome for consumption for 8.0 days

Moulds were the least counted microbes in the stored fruits. This resulted in longer shelf-life for all samples in both ambient and refrigerated, based on mould count as compared with yeast and plate count.

4.3.2.3 Aerobic Bacteria Count (ABC)

The accepted limits of ABC in fresh-cut fruits is 100000 CFU/ml. Initial ABC in treated fruits were significantly lower than that of untreated fruits in both ambient and refrigeration conditions as noted for the other microrganisms. This reduction in microbes was as a result of the pre-heat treatment which has also been reported by Lamikanra, *et al.*, 2005. ABC increased exponentially from day one till the 8th day in both treated and untreated fruits in ambient condition (Fig 4.12). However, the population and growth rate were reduced in treated fruits as compared to untreated fruits. Growth rate of Aerobic Bacteria of treated pineapples stored under ambient condition resulted in a shelf-life of 2.3 days while that of the untreated pineapple was 1.3 days, a difference of about a day.

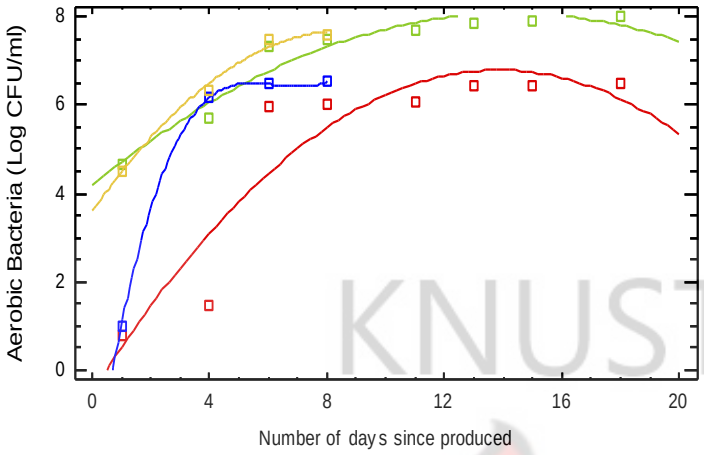


Fig 4.12 Shelf-life of treated and untreated pineapples stored under refrigeration and ambient condition based on their Aerobic Bacteria Count.

Legend
- Refrigerated treated samples
- Refrigerated untreated samples
- Ambient treated samples
- Ambient untreated samples

Shelf-life based on ABC was marked at 7.0 days for treated pineapples stored under refrigeration condition with the help of the yellow locater. Growth rate was initially slow from the first day (6 CFU/ml) till the 11th day (121×10^5 CFU/ml). Thereafter, it increased rapidly to 259×10^5 on the 13th day and reduced to 300×10^5 by the 20th day. On the other hand, growth rate in the untreated fruits even though high, was slow from the first day (46×10^4) till day 4 (90×10^4). It increased exponentially from then till the 15th day (700×10^6) where it slowed down till the 20th day (970×10^6). The untreated pineapples had a shelf-life of about 3.0 days.

Results from microbial assessment indicated that the total Aerobic Plate Count (CFU/ml) of the fruit samples in both storage conditions was higher than the mould and yeast counts (Fig 4.9-4.14). Previous studies by Lamikanra, *et al.*, (2000); (2005), indicated the prevalence of bacteria growth during storage of fresh-cut cantaloupe melons, relative to mould and yeast. The main problem that makes fresh-cut fruit a highly perishable product is the microbial growth caused by the accumulation of leaked juice rich in nutrients in the bottom of containers which provide favorable conditions for the growth of spoilage microorganisms (Brecht, 2006; Fan and Song, 2008). Microbial counts of untreated pineapples of all three microbes (yeast, mould and aerobic plate count) studied were higher than those of the treated fruits (Fig 4.10-4.12). This implies that the significant reduction in moisture loss due to heat treatment, in the form of juice as explained above, indeed reduced microbial levels in treated fruits.

4.3.3 Sensory analysis of MD2 pineapple during storage

Results were based on a null-hypothesis (H_0) that there will be no detectable difference among the three samples for the triangle test. Fruit deterioration during storage of fresh-cut pineapples is indicated by decrease in desirable sensory qualities such as taste (sweet and sour), texture (firmness), aroma (fruity) and appearance/colour. It was based on these qualities that panelists chose the odd sample out of the three samples provided in the triangle test (Table 4.3). A significant number of panelists were able to detect difference up till the 6th and 4th days in samples stored under refrigeration and ambient condition respectively. The number of assessors/panelists who made correct judgment of selecting the odd sample in refrigeration condition, reduced from day one (7 people) to day 4 (6 people). There was however a rise on the 6^{th} day (8 people) which then dropped again to 6 people by the 8th day. This rise on the 6^{th} day could be attributed to the difference between the fermented state and colour change (golden brown to dark brown) of the untreated fruits compared to the fruity golden brown treated fruits, which was absent earlier. The reduction on the 8^{th} day was due to the comparable spoilt state of both treated and untreated fruits. Heat treated fruits had higher intensities of the fruity pineapple aroma and although the intensity decreased during storage, they were consistently rated higher than the corresponding untreated fruits (Table 4.3).

On the other hand, the number of panelists who were able to correctly select the odd sample from the ambient samples reduced from day one (8 people) to the 4th day (7 people). Panelist were able to carry out sensory evaluation (identify the odd samples) till the 11th day for refrigerated samples and 6th day for ambient samples, where analysis was discontinued due to unacceptable microbial counts and deteriorated physical state of the samples. Panelists' ability to pick the odd sample was statistically significant on the first six days (refrigerated samples) till the eight day where p-value was 0.042. Panelists' were unable to determine the odd sample by comparing the aroma and appearance of the fruits without tasting them from the 11th day for refrigerated samples and 6th day for ambient samples. Their inability to identify the odd sample, apart from their inability to taste the samples, might also be due to the comparable state of the fruits samples from both treated and untreated samples by the 11th day in refrigeration condition. Sensory in the ambient condition was also discontinued from the fourth day due to the same problem. However, panelists were significantly able to pick the odd sample out of the three samples ($p<0.05$). As a result of heat treatment, treated cut-pineapples remained firmer than untreated fruits as observed by Valero, *et al.*, (2002), in plums. This was the main difference panelist used, after the first four days, in identifying the odd sample in refrigeration condition. According to Kim, (1993) and Abreu, *et al.*, (2003), post harvest mild heat pre-treatment is responsible for this firm textural improvement.

Table 4.3 Statistics of correct number of judgment in the triangle test

Number of days since produced	Refrigeration Condition		Ambient Condition	
	Number of correct judgment	p-value	Number of correct judgment	p-value
1	7	0.020	8	0.003
4	6	0.042	7	0.020
6	8	0.003	ND	ND
8	6	0.077	ND	ND
11	ND	ND	ND	ND

ND~ Not Determined

According to Luna- Guzman, *et al.,* (1999), post- cut heat treatment of cantaloupe melons have no effect on either ethylene production or respiration rate (shelf-life), during storage. A different observation was made by Lamikanra, *et al.,* (2005), where mild heat treatment improved shelf-life and sensory quality of the same fruit in storage. This study supports their observation as both shelf-life and sensory attributes such as taste (good balance between sweet and sour) and texture (firmness), of the pineapple samples were improved upon as a result of mild heat pre-treatment. This is indicative of the differences in the effect of heat on the metabolic and physiological changes that occur when whole fruits are heat treated relative to the post-cut product treatment. Previous studies on cut cantaloupe melons (Lamikanra and Watson, 2001, 2003, 2004) indicated that some enzymes such as lipase and esterase are relatively unstable when incubated at treatment conditions above 60 °C for 20 min. These enzymes are responsible for lipid and pectin breakdown in the cell and cell wall of the fruit cells which also results in cell permeability as well as increased spoilage rate. Inactivation of these enzymes as a result of pre-cut heat treatment, prior to cutting reduced the ability to synthesize these enzymes. This could also have contributed to the improved shelf-life and sensory attributes of the treated fruits compared to the untreated fruits.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

Treatment at 73.49 °C for 10 min was found to be the optimum treatment condition for MD2 pineapple variety and 71.52 °C for 10 min for smooth cayenne variety. These conditions produced golden brown fruits with improved firmness and sweet-sour balance most preferred by consumers. Vitamin C and TSS contents of the treated fruits reduced less rapidly as compared to untreated fruits. MD2 variety was better improved compared to smooth cayenne based on their nutrient and sensory qualities after treatment.

Shelf-life of refrigerated treated and refrigerated untreated fruits was 7.0 and 3.0 days respectively while that of ambient treated fruits stored for 2.3 days and untreated fruits stored for 1.3 days before they became unwholesome for consumption. This conclusion was based on the results from aerobic bacteria count for which the lowest shelf-lives were recorded.

Thus mild pre-heat treatment of whole pineapples prior to cutting could be used to extend shelf-life and improved sensory quality of fresh-cut pineapple fruit in storage.

It is recommended that further research is conducted

- Further research into the use of steam, instead of water for the treatment process, to determine its effect on the nutritive and sensory quality of pineapple fruits.
- To determine the effect of alternative packages on shelf-life and sensory quality of treated and cut pineapples.
- To determine the effect of mild pre-heat treatment on other pineapple varieties in Ghana as well as varieties elsewhere that are potentials for the Ghanaian market.

REFERENCE

Abreu, M., Beirão-Da-Costa, S., Moldão-Martins, M., Gonçalves, E.M., Beirão-Da-Costa, M.L. (2003). Use of mild heat pre-tratments for quality retaintion of fresh-cut 'Rocha' pear. *Postharvt. Biol. Technol.* **30**:153-160

Achinewhu, S. C. and Hart Kluwer, A. D. (2005). Effect of processing and storage on the ascorbic acid (vitamin C) content of some pineapple varieties grown in the Rivers State of Nigeria. Academic Publishers (Springer Netherlands) **46**(4), 335-337.

Adaikan, P., Ganesan, Adebiyi, and Adebowale, (2004). Mechanisms of the Oxytocic Activity of Papaya Proteinases. *Pharm. Biol.* **42** (8), 646–655.

Ajibola, V.O., Babatunde, O.A. and Suleiman, S. (2009). The Effect of Storage Method on the Vitamin C Content in Some Tropical Fruit Juices *Trends Appl. Sci. Res.* **4**(2), 79-84.

Alonso, J., Canet, W. and Rodriguez, T. (1997). Thermal and calcium pretreatment affects texture, pectin esterase and pectin substances of frozen sweet cherries. *J. Food Sci.* **62**:511-515.

Angba, D. (2007). Effect of storage practices on fruits and vegetables at whole sale and retail points. Term Paper (Unpublished).

Anitra, C.C. and Balz, F. (1999). Toward a new recommended dietary allowance for vitamin C based on antioxidant and health effects in humans, *Am. J. Cl. Nutr.* **69**(6), 1086-1107.

AOAC, (1990). Official Method of Analysis of the Association of Official Analytical Chemistry, 15th edition. The Association: Arlington.VA.

Artés, F., Gómez, P., Artés-Hernández, F., Aguayo, E. and Escalona, V. (2007). Improved strategies for keeping overall quality of fresh-cut produce. *Acta. Hort. (ISHS)* **746**:245–258.

Ashby, B.H. (1987). Protecting perishable foods during transport by truck, Handbook no. 669. USDA, Office of Transportation. Washington, DC.

ASTM, (1968). Manual on Sensory Testing Methods, no. 434. American Society for Testing and Materials, Philadelphia, Pa.

Atanda, O.O. and Ikenebomeh, M.J. (1989). Effect of heat treatments on the microbial load of 'nono' *Lett. Appl. Microbiol.* **9**(6), 233–235.

Atlas, R.M. (1995). Principles of Microbiology, 1st Edition, Mosby – yearbol Inc. USA, p 633 – 637.

Ayala-Zavala, F., Del-Toro-Sanchez, I., Alvarez-Parrilla, E. and Gonzelez- Aguilar, G.A. (2008). High Relative Humidity In-Package of Fresh-Cut Fruits and Vegetables: Advantage or Disadvantage Considering Microbiological Problems and Antimicrobial Delivering Systems? *J. Food Sci.* **73**(4) R41-R47

Barrancos, S., Beirão-Da-Costa, M.L., Moldão-Martins, M., Abreu, M., and Gonçalves, E.M. (2006). The Effect Of Heat Pre-Treatment On Quality And Shelf-Life Of Fresh-Cut Apples http://www.ishs.org/

Ben-Yehoshua S., Rodov, V., Fishman, S. and Peretz, J. (1998). Modified-atmosphere packaging of fruits and vegetables: reducing condensation of water in bell peppers and mangoes. *Acta. Hort. (ISHS)* **464**:387–392

Brecht, J.K. (1995). Physiology of lightly processed fruits and vegetables. *Hort. Sci.* **30**:18-22.

Brecht, J.K. (1999). Postharvest quality and safety in fresh-cut vegetables and fruits. *Coop. Reg. Res. Proj*. p S-294.

Brecht, J.K. (2006). Shelf-life limiting quality factors in fresh-cut tomatoes: anti-ethylene treatment and maturity and variety selection to ensure quality retention. Oral presentation at the "Tomato Breeders Round Table and Tomato Quality Workshop".

California Fresh Market Advisory Board, (1976). Ethylene Facts and FAQ's Informational Bulletin No. 12. http://store.yahoo.com/catalyticgenerators/whatisethylene.html (accessed 2/27/09)

Cameron, A.C., P.C. Talasila and Joles, D.W. (1995). Predicting film permeability needs for modified-atmosphere packaging of lightly processed fruits and vegetables. *Hort. Sci*. **30**:25-34.

Chenchin, E.E., Nishimoto, M.M., Abram, D.D. and Hsia S.T. (1999). Process for preserving fresh pineapple, US Patent Issued on December 28 1999.

Chonhenchob, V., Chantarasomboon, Y. and Singh S. P. (2007). Quality changes of treated fresh-cut tropical fruits in rigid modified atmosphere packaging containers Wiley, Chichester, ROYAUME-UNI (Revue), **20**:27-37

Colaric, M., Veberic, R., Stampar, F. and Hudina, M. (2006). Evaluation of peach and nectarine fruit quality and correlations between sensory and chemical attributes. John Wiley and

Son. *J. Sci. Food Agric.* http://www.cababstractplus.org/abstract/SearchResults.aspx (accessed 7/07/08)

Crane, J. H. (2005). Pineapple Growing in the Florida Home Landscape Fact Sheet HS-7, Horticultural Sciences Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, p 1-9.

Crane, J. H. (2006). USAID-RED. Oficina FHIA, La Lima, Cortes, Honduras http**://**www.usaid-red.org (accessed 2/27/09)

Davey, M.W., Van Montagu, M., Inze, D., Kanellis, A., Sanmartin, M. and Smirnoff, N. (2000). Plant L-ascorbic acid: Chemistry, function, metabolism, bioavailability and effect of processing. *J. Sci. Food Agric.* **80**:825-860.

De Maio, A. (1999). "Heat shock proteins: facts, thoughts, and dreams". *Shock (Augusta, Ga.)* **11** (1): 1–12. PMID 9921710

Design Expert, (2007). Stat Ease Inc., Hennepin Square, Suite 480, 2021E. Hennepin Ave., Minneapolis, MN55413-2726.

Duke, J. A. (1983). Handbook of Energy Crops, Unpublished

Dull, G.G. (1971). The Pineapple. In: Hulme, A.C. (ed.) The Biochemistry of Fruits and Their Products. Academic Press, New York. **2**: p 303- 324.

Eckert, J.W. and Ogawa, J.M. (1988). The chemical control of postharvest diseases: deciduous fruits, berries, vegetables and root/tuber crops. *Ann. Rev. Phytopathol.* **26**:433–469.

Eubanks, P. L., Middlecamp, C.H., Pienta, N. J., Heltzel, C. E. and Weaver, G. C. (2006). Chemistry in context: Applying chemistry to society, 5th Edition, McGraw Hill publications, New York, p 520 – 522.

Fan, L. and Song, J. (2008). Microbial quality assessment methods for fresh-cut fruits and vegetables. Stewart Postharvest Solutions, *Stewart Postharvt Rev*, **4**(3), 1-9.

FAO Trade Yearbook (2005). Food and Agriculture Organization of the United Nations, Rome. Vol.67 p15

Food and Nutrition Board, Institute of Medicine. Vitamin C. (2000). Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids. Washington D.C.: National Academy Press, p 95-185.

Franke, A.A., Custer, L.J., Araki, C. and Murphy, S.P. (2004). Vitamin C and flavonoid levels of fruits and vagetables consumed in Hawaii. *J. Food. Compos. Anal*. **17**: 1-35

Frazier, W.C. and Westhoff, D.C. (1993). Microbiología de los alimentos. 4th ed. Acribia Zaragoza , Spain, p 681.

Gill, M.I., Aguayo, E, Kader, A.A. (2006). Quality changes and nutrient retention in fresh-cut versus whole fruits during storage. *J. Agric. Food Chem*. **54**(12):4284-4296.

Gilman, E.F. (2007). *Ananas comosus* 'Smooth Cayenne' Spineless Pineapple. Publication number FPS040. http://edis.ifas.ufl.edu (accessed 2/27/09)

González-Aguilar, G. A., Ruiz-Cruz, S., Cruz-Valenzuela, R., Ayala-Zavala, J. F., De La Rosa, L. A. and Alvarez-Parrilla, E. (2008). New Technologies to Preserve Quality of Fresh-Cut Produce, Food Engineering: Integrated Approaches, Springer New York, p 105-115

Gonzalez-Aguilar, G.A., Ruiz, S., Vasquez, S., and Wang, C.Y. (2005). Biochemical changes in pineapple slices treated with antibrowning agents. *J. Agrc. Food Chem*. **40**:377-384.

Gorny, J.R. (1998). A summary of Controlled Atmosphere and Modified Atmosphere requirements and recommendations for fresh-cut (minimally processed) fruits and vegetables. 5: Fresh-cut Fruits and Vegetables and MAP. *Postharvt. Hort. Ser*. No. 19. CA '97. Proc. *7th Intl. Contr. Atmos. Res. Conf*., p. 30-66.

Gortner, W.A., and Singleton, J.L. (1965). Chemical and Physical Development of the pineapple fruit II. Carbohydrate and acid constituents. *J. Food Sci.* **30**(3): 19.

Grierson, W. and Wardowski, W.F. (1978). Relative humidity effects on the postharvest life of fruits and vegetables. *Hort. Sci.* **15**:576–4.

Hajare, S.N., Dhokane, V.S., Shashidhar, R., Saroj, S., Sharma, A. and Bandekar, J. R. (2006). Institute of Food Technologists, Chicago. *J. Food. Sci.* **71**(6), 27

Helms, S., and Miller, A. (2006). Natural treatment of chronic rhinosinusitis. *Altern Med Rev.* 11(3):196-207.

Hrayr, B. (2008). The MD2 Pineapple http://www.foodreference.com/html/artpieapple.html.

Hurst, W.C. (1995). Sanitation of lightly processed fruits and vegetables. *Hort. Sci.* **30**:22-24.

Institute of Food Technologists and FDA, (2001). Analysis and Evaluation of Preventative Control Measures for the Control and Reduction/Elimination of Microbial Hazards on Fresh and Fresh-Cut Produce.

Izwan, I. (2008). Biotech for superior pineapple produce, Tech and You. accessed on 15/14/09

Jessup, A.J., Carswell, I.F. and Dalton, S.P. (2007). Disinfestation of fresh fruits from *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) with combination mild heat and modified atmosphere packaging. *Aust. J. Ent*.. 37(2), 186 – 188.

Kader, A.A. (2004). Reducing juice leakage in fresh-cut fruit, Postharvest Technology Research Information Center, University of California.

Kim, D.M., Smith, N.L. and Lee, C.Y. (1993a). Quality of minimally processed apple slices from selected cultivars. *J. Food Sci*. **58**:1115-1117-1175.

Kim, D.M., Smith, N.L., and Lee, C.Y. (1993b). Apple cultivar variations in responds to heat treatment and minimal processing. *J. Food Sci.* **58**:111-3, 1124.

King, A.D. and H.R. Bolin. (1989). Physiological and microbiological storage stability of minimally processed fruits and vegetables. *J. Food Technol*. **43**:132-135.

Kou, L., Luo, Y., Wu, D. and Liu, X. (2007). Effects of mild heat treatment on microbial growth and product quality of packaged fresh-cut table grapes. *J. Food Sci.* **72**(8):S567-S573.

Lamikanra, O. and Watson, M.A. (2001). Effect ascorbic acid on peroxidase and polyphenols oxidase activities in minimally processed cantaloupe melons. *J. Food Sci*. **66**:1283-1286

Lamikanra, O. and Watson, M.A. (2003). Temperature and storage duration effect on esterase activityin fresh-cut cantaloupe melon. *J. Food Sci.* **68**:790-793.

Lamikanra, O. and Watson, M.A. (2004). Storage effect on lipase activity in fresh-cut cantaloupe melon. *J.Food Sci.* **69**:126-130.

Lamikanra, O., Bett-Garber, K.L., Ingram, D. A. and Watson, M.A. (2005). Use of Mild Heat Pre-treatment for Quality Retention of Fresh-cut Cantaloupe Melon, *J. Food Sci*. **70**(1), C53-C57.

Lamikanra, O., Chen J.C., Banks, D., and Hunter P.A. (2000). Biochemical and microbiological changes during storage of minimally processed cantaloupe. *J. Agric. Food Chem.* **48**:5955-5961

Lehninger, A.L. (1982). Principles of Biochemistry, Worth Publishers, Inc., New York, p. 158. http://www.biotopics.co.uk/other/aninac.html (accessed 2/27/09)

Levine, M. (1999). Criteria and recommendations for vitamin C intake. *JAMA* **281**(15): 1415-1423.

Luna-Guzman, I., Cantwell, M. and Barrett, D.M. (1999). Fresh-cut cantaloupe: Effect of $CaCl_2$ dips and heat treatments on firmness and metabolic activity. *Postharvt. Biol. Technol.* **14**:257-269.

Luo, Y. (2007). Challenges facing the industry and scientific community in maintaining quality and safety of fresh-cut produce. *Acta. Hort. (ISHS)* **746**:213–222.

Lurie, S. (1998). Postharvest heat treatment, *Postharvt. Biol. Technol.* **14**:257-269.

Lurie, S. and Klien, J.D. (1992). Calcium and heat treatments to improve storability of "Anna" apples. *Hort. Sci.* 27: 36-39.

Marrero, A. and Kader, A.A. (2006). Optimal temperature and modified atmosphere for keeping quality of fresh-cut pineapples. *Postharvt. Biol. Technol.* **39** 163–168

Marrero, A. and Kader, A.A., (2001). Factors affecting the post-cutting life and quality of minimally processed pineapple. *Acta. Hort.* **553**:705–706.

Martínez-Ferrer, M., and Harper, C. (2005). Reduction in microbial growth and improvement of storage quality in fresh-cut pineapple after methyl jasmonate treatment.Department of Pathology, Vanderbilt University Medical Center, Nashville, TN. *J. Food Qual.* **28**(1) 3-12

Maurer, H.R., (2001). Bromelain: biochemistry, pharmacology and medical use. *Cell. Mol. Life. Sci*; 58(9):1234-1245.

Meilgaard, M., Civille, G.V. and Carr, B.T. (1999). Sensory Evaluation Techniques, Third Edition. Boca Raton,Fla.: CRC Press. P 195-208.

Mohammed, M. and Wickham, L.D. (2005). Effect of Antioxidants on Postharvest Quality Attributes Of Fresh-Cut Pineapples, www.ishs.org (accessed 03/04/08)

Montero-Calderon, M., Alejandra, R.G. M. and Martin-Belloso, O. (2008). Effect of packaging conditions on quality and shelf-life of fresh-cut pineapple (*Ananas comosus*) 2, *Postharvt. Biol. Technol.* **50**(2-3):182-189

Montinola, L.R. (1991). Pina. Amon Foundation, Manila, Philippines.

Monzon, R. B. (1995). Traditional medicine in the treatment of parasitic diseases in the Philippines. *Southeast Asian J. Trop. Med. Pub. Health* **26** (3): 421–428.

Morton, J. (1987). Pineapple. In: Fruits of warm climates, Morton, J. F. Miami, FL. p. 18–28

Naggy, S. (1980). Vitamin C contents of citrus fruit and their products. *J. Agric. Food Chem.* **28**: 8-15.

Natural Food- Fruit Vitamin C Content, (2001).

http://www.naturalhub.com/natural_food_guide_fruit_vitamin_c.htm (accessed 17/08/08)

Ngoddy, P.O. and Ihekoronye, I.A. (1985). Integrated Food Science and Technology for the Tropics. Macmillan Publishers, London. p. 73- 303.

O'Hare, T.J. (1994). Respiratory characteristics of cut pineapple tissue. Postharvest Group, DPI, Queensland, Australia, 1994 Report.

Obeng, I.S. (1994). Effects of Domestic Policies on Production and Export of Non- Traditional Agricultural Commodities: A Case Study of Fresh Pineapples in Ghana. M. Phil Thesis, Department of Agricultural Economy and Farm Management, University of Ghana.

Padayatty, S.J., Katz, A., Wang, Y., Eck, P. and Kwon, O. *(*2003). Vitamin C as an antioxidant: Evaluation of its role in disease prevention. *J. Am. Coll. Nutr*., **22**:18-35.

Paine, F.A.(1987). Modern Processing, packaging and Distribution Systems for Food. Van Nostrand Reinhold Company, New York.

Paull, R.E. (1993). Pineapple and papaya. In: Seymour, Taylor GJ, Tucker G (eds.) Biochemistry of Fruit Ripening. Chapman and Hall, London, p. 291-323.

Paull, R.E. (1997). Pineapple. In: Mitra, S. (Ed.), Postharvest physiology and storage of tropical and subtropical fruits. CAB International, Wallingford, UK, p. 123–143.

Paull, R.E. and Chen, N.J. (2000). Heat treatment and fruit ripening. *Postharvt. Biol. Technol.* **21**:21-37.

Paull, R.E. and Rohrbach, K.G. (1985). Symptom development of chilling injury in pineapple fruit. *J. Am. Soc. Hort. Sci*. **110**:100–105.

Ponting, J.D., R. Jackson and G. Watters. (1972). Refrigerated apple slices: preservative effects of ascorbic acid, calcium and sulfites. *J. Food Sci*. **37**:434-435.

Prescott, H. K. (1999). Microbiology, 4th edition, McGraw Hill Company, p 156.

Ramsaroop, R.E.S. and Saulo, A.A. (2007). Comparative consumer and physicochemical analsis of del monte Hawai'i gold and smooth cayenne pineapple cultivars, *J. Food Qual.* **30**(2), 135-159

Rickman, C., Medine, C.N., Bergmann, A. and Duncan, R.R. (2007). Functionally and spatially distinct modes of MUNC18-syntaxin 1 interaction. *J. Biol. Chem.* **282**:12097–12103

Roessler, E.B., Pangborn, R.M. and Sidel, J.L. (1978). Stone H. Expanded statistical table for estimating significance in paired-preference, paired-difference, duo-trio and triangle test. *J. Food Sci*. **43**:940-942

Romig, W.R. (1995). Selection of cultivars for lightly processed fruits and vegetables. *Hort. Sci.* **30**:38-40.

Ros – Chumillas, M., Belissario, Y., Iguaz, A. and Lopez A. (2007). Quality and shelf life of orange juice aseptically packaged in PET bottles, Technical University of Cartagena, Agricultural Equipment and Food Engineering Department, Paseo Alfonso XIII, 48, 30203 Cartagena, Spain. p. 1-12.

Saltveit, M.E. (1997). Physical and physiological changes in minimally processed fruits and vegetables. In: F.A. Tomas-Barberan and R.J. Robins (eds) Phytochemistry of Fruits and Vegetables, Claredon Press, p. 205-220.

Sam, C.E., Conway, W.S., Abbott, J.A., Lewis, R.J. and Ben-Shalom, N. (1993). Firmness and decay of apples following postharvest pressure infiltration of calcium and heat treatment. *J. Am. Soc. Hort. Sci.* **118**:623-627

Schlimme, D.V. and Rooney, M.L. (1994). Packaging of minimally processed fruits and vegetables. (In: Wiley RC (ed) Minimally Processed Refrigerated Fruits and Vegetables), Chapman and Hall, p 135-182.

Schneider, K. R., Otwell, W. S. and Garrido, V. (2009). Guidance for Processing Fresh-cut Produce in Retail Operation http://edis.ifas.ufl.edu/pdffiles/FS/FS11300.pdf [01/01/10]

Scott, M. K., G. G. Vining, D. C. Montgomery and Borror, C. M. (2006). Modifying a central composite design to model the process mean and variance when there are hard-to-change factors, *J. Royal Stat. Soc.*: Series C(Applied Statistics) **55**(5)615- 630.

Seo, Y., Oshita, S.S. and Kawagoe, Y. (1997). Mathematical and Control Applications in Horticulture, (Eds; Munack A., Tantau H.H., IFAC Hannover), Germany. p 273-276

Sidibé, M., Scheuring, J.F., Tembely, D., Sidibé M.M., Hofman, P. and Frigg, M. (1996). Baobab homegrown vitamin C for Africa. Agroforestry Today. **8**(2), 13-15. http://www.foundation.novartis.com/baobab_africa.htm (accessed 2/27/09)

Simon, J.A., and Hudes, E.S. (2000). Serum ascorbic acid and gallbladder disease prevalence among US adults: the Third National Health and Nutrition Examination Survey (NHANES III). *Arch. Intern. Med*. **160**(7):931-936.

Soliva-Fortuny, R.C. and Martin-Belloso, O. (2003). New advances in extending the shelf-life of fresh-cut fruits: a review. *Trends Food Science and Technology*, **14**(9), 341-353.

Solomos, T. (1994). Some biological and physical principles underlying modified atmosphere packaging. In: R.C. Wiley, Minimally Processed Refrigerated Fruits and Vegetables, Chapman and Hall, U.K.

Takane, T. (2004). South African study Monographs, **25**(1): 29-43.

Toivonen, P.M.A. and DeEll, R. (2002). Physiology of fresh-cut fruits and vegetables. (Ed; Lamikanra O). Fresh-cut fruit and vegetables: Science, technology and market, Boca Raton, Fla.: CRC Press p 91-132

Tripathi, P. and Dubey, N.K. (2004). Exploitation of natural products as an alternative strategy to control postharvest fungal rotting of fruit and vegetables. *Postharvt. Biol. Technol.* **32**:235–245.

Tsapatsaris, S., and Kotzekidou, P. (2004). Application of central composite design and response surface methodology to the fermentation of olive juice by *Lactobacillus plantarum* and *Debaryomyces hansenii*. *Intl. J. Food Microbiol*. **95**(2) 157-168

USDA, (1998). Nutrient database for standard reference, release 12

USDA. Agricultural Research Service Annual Report, (2008). Improving the Sensory Quality and Shelf Life of Fresh-Cut Fruit Products. http://www.ars.usda.gov/research/projects/projects.htm?ACCN.NO=409285. (accessed 18/08/2008)

Valero, D., Perez-Vicente, A., Martinez-Romero, D., Castillo, S., Guuillen, F. and Seerrano, M. (2002). Plum storability improved after calcium and heat postharvest treatments: role of polyamines. *J. Food Sci.* **67**:2571-2575

Vicente, A.R., Martinez, G.A., Civello, P.M. and Chhaves, A.R. (2002). Quality of heat-treated strawberry fruit during refrigerated storage, *Postharv. Biol. Technol.* **25**:56-71

Wang, C.Y., Bowen, J.H., Weir, I.E., Allan, A.C. and Ferguson, I.B. (2001). Heat- induced protection against death of suspension-cultured apple fruit cell exposed to low temperature. *Plant. Cell. Environ.* **24**:1199-1207.

Wardlaw, G. M. (2003). Contemporary Nutrition, fifth edition, McGraw Hill publication, New York, p 297, 546.

Watada, A.E. and Qi, L. (1999). Quality of fresh-cut produce. *Postharv. Biol. Technol*. **15**:201-205.

Watada, A.E., Abe, K. and Yamauchi, N. (1990). Physiological activities of partially processed fruits and vegetables. *Food. Technol*. **44**:116, 118, 120-122.

Watada, A.E., Kou, N.P and Minott, D.A. (1996). Factors affecting quality of fresh-cut horticultural products. *Posthavt. Biol. Technol*. **9**:115-125.

Watkins, E.W., Hewett, Thompson, C.J. (2008). Effects of Micro perforated Polyethylene Bags on the Storage Quality of 'Cox's Orange Pippin' Apples. International Symposium on Postharvest Handling of Fruit and Vegetables . ISHS *Acta. Hort.* p 258:

Wiley, R.C. (1994). Minimally Processed Refrigerated Fruits and Vegetables, Chapman and Hall, London, U.K.

Williams, M.H., Brown, M.A., Vesk, M. and Brady, C. (1994). Effect of postharvest heat treatment on fruit quality, surface structure and fungal disease in Valencia oranges. *Austr. J. Exp. Agri.* **54**(8), 1183-1190.

World Trade Atlas (2002). Trade Information System Internet Version. Global Trade Information Service Inc., Columbia.

Wright, K.P. and Kader, A.A. (1997). Effect of slicing and controlled-atmosphere storage on the ascorbate content and quality of strawberries and persimmons. *Postharvt. Biol. Technol*. **10**:39-48.

APPENDICES

Appendix A FORMULAE USED IN CALCULATIONS

A1. Vitamin C determination- Iodine method

	1	2
Initial volume		
Final volume		
Titre value		

Titre of pure Vitamin C = Titre of pineapple

10mg vitamin C Xmg vitamin C

Xmg vitamin C = Titre of pineapple × 10mg vitamin C

titre of pure Vitamin C

A2 Vitamin C content determination- Indophenol Method

	1	2
Initial volume		
Final volume		
Titre value		

Mg/1000ml=dye equivalent x titre value x dilution factor

Dye equivalent =0.188

Dilution factor=final volume of solution =

Initial volume of solution

A3 Moisture content determination

	1	2
Initial weight		
Final weight		
Moisture content (ml)		
Percentage moisture loss		

Percentage Moisture Content= $\frac{(y-m)}{(y-x)} \times 100$

y-m= Weight of Moisture

y-x= Weight of sample

x= weight of crucible

y= weight of crucible and sample

m= weight of crucible and dry sample

A4 Microbial Count in CFU/ml

CFU/standard unit volume = number of colonies x dilution factor x standard unit volume/aliquot plated

Appendix B

ANOVA TABLES FOR PRELIMINARY TESTS TO SELECT OPTIMUM TREATMENT CONDITION

B1. DESIGN SUMMARY

Study Type	Response Surface	Runs	26
Initial Design	Central Composite		
Design Model	Quadratic		

Factor	Name	Units	Type	Low Actual	High Actual
A	Temperature	oC	Numeric	60	80
B	Time	min	Numeric	10	30
C	Pineapple		Categoric	SC	MD2

Response	Name	Obs	Analysis	Minimum	Maximum	Model
Y1	VitC	24	Polynomial	0.095	0.423	Quadratic
Y2	Sugar	26	Polynomial	12.5	14.2	Mean
Y3	Taste	26	Polynomial	0.3801	0.6892	Quadratic
Y4	Texture	26	Polynomial	0.3111	0.6356	Quadratic
Y5	Appearance	24	Polynomial	0.3048	0.6692	Quadratic
Y6	Overall preference	26	Polynomial	0.4588	0.7382	Quadratic

B2.MODEL SUMMARY

Statistics Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS
Linear	0.020928	0.97035	0.965902	0.95273	0.013965
2FI	0.017048	0.983275	0.977373	0.957944	0.012425
Quadratic	0.014605	0.98917	0.983394	0.966338	0.009945
Cubic	0.012198	0.994964	0.988417	0.966054	0.010029

I+"Model Summary Statistics"0+: Focus on the model maximizing the "Adjusted R-Squared" and the "Predicted R-Squared".

B3i. ANOVA TABLE FOR RESPONSE SURFACE QUADRATIC MODEL FOR THE PERFORMANCE OF VITAMIN C IN TWO VARIETIES OF TREATED FRESH-CUT PINEAPPLES, MD2 AND SMOOTH CAYENNE.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	0.292234	8	0.036529	171.25	< 0.0001
A-Temperature	0.00018	1	0.00018	0.843647	0.3729
B-Time	0.006622	1	0.006622	31.044	< 0.0001
C-Pineapple	0.256902	1	0.256902	1204.363	< 0.0001
AB	0.001787	1	0.001787	8.376091	0.0111
AC	0.000941	1	0.000941	4.409148	0.0531
BC	0.000202	1	0.000202	0.945563	0.3463
A^2	0.00174	1	0.00174	8.159159	0.0120
B^2	0.000122	1	0.000122	0.569896	0.4620
Residual	0.0032	15	0.000213		
Lack of Fit	0.001926	8	0.000241	1.323335	0.3624
Pure Error	0.001274	7	0.000182		
Cor Total	0.295434	23			

B3ii Statistics Of The Model Regression Parameters For Response Surface Quadratic Model For The Performance Of Vitamin C In Two Varieties Of Treated Fresh-Cut Pineapples, MD2 And Smooth Cayenne.

R-Squared	Adj R-Squared	Pred R-Squared	Adeq Precision
0.99	0.98	0.97	35.67

B4i. ANOVA TABLE FOR RESPONSE SURFACE QUADRATIC MODEL FOR THE PERFORMANCE OF TASTE IN TWO VARIETIES OF TREATED FRESH-CUT PINEAPPLES, MD2 AND SMOOTH CAYENNE.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	0.1941	8	0.024262	9.015748	< 0.0001
A-Temperature	0.020411	1	0.020411	7.584389	0.0136
B-Time	0.07904	1	0.07904	29.37071	< 0.0001
C-Pineapple	0.002345	1	0.002345	0.871236	0.3637
AB	0.028393	1	0.028393	10.55079	0.0047
AC	1.52E-05	1	1.52E-05	0.005644	0.9410
BC	0.003581	1	0.003581	1.330713	0.2646
A^2	0.041887	1	0.041887	15.56482	0.0010
B^2	0.002261	1	0.002261	0.840049	0.3722
Residual	0.045749	17	0.002691		
Lack of Fit	0.042529	9	0.004725	11.74009	0.0010
Pure Error	0.00322	8	0.000403		
Cor Total	0.239849	25			

B4ii. Statistics Of The Model Regression Parameters For Response Surface Quadratic Model For The Performance Of taste In Two Varieties Of Treated Fresh-Cut Pineapples, MD2 And Smooth Cayenne.

R-Squared	Adj R-Squared	Pred R-Squared	Adeq Precision
0.809259	0.719498	0.402467	12.64331

B5i. ANOVA TABLE FOR RESPONSE SURFACE QUADRATIC MODEL FOR THE PERFORMANCE OF TEXTURE IN TWO VARIETIES OF TREATED FRESH-CUT PINEAPPLES, MD2 AND SMOOTH CAYENNE.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	0.201097	8	0.025137	12.57922	< 0.0001
A-Temperature	0.034819	1	0.034819	17.42451	0.0006
B-Time	0.055938	1	0.055938	27.99263	< 0.0001
C-Pineapple	0.001682	1	0.001682	0.841538	0.3718
AB	0.041732	1	0.041732	20.88354	0.0003
AC	0.001228	1	0.001228	0.614604	0.4438
BC	0.000116	1	0.000116	0.05802	0.8125
A^2	0.049252	1	0.049252	24.64675	0.0001
B^2	0.00113	1	0.00113	0.56533	0.4624
Residual	0.033971	17	0.001998		
Lack of Fit	0.033884	9	0.003765	347.5757	< 0.0001
Pure Error	8.67E-05	8	1.08E-05		
Cor Total	0.235068	25			

B5ii. Statistics Of The Model Regression Parameters For Response Surface Quadratic Model For The Performance Of texture In Two Varieties Of Treated Fresh-Cut Pineapples, MD2 And Smooth Cayenne.

R-Squared	Adj R-Squared	Pred R-Squared	Adeq Precision
0.837806	0.806912	0.750444	19.40099

B6i. ANOVA TABLE FOR RESPONSE SURFACE QUADRATIC MODEL FOR THE PERFORMANCE OF APPEARANCE/COLOUR IN TWO VARIETIES OF TREATED FRESH-CUT PINEAPPLES, MD2 AND SMOOTH CAYENNE.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	0.245886	8	0.030736	17.04693	< 0.0001
A-Temperature	0.067481	1	0.067481	37.42696	< 0.0001
B-Time	0.053647	1	0.053647	29.75447	< 0.0001
C-Pineapple	0.020736	1	0.020736	11.50091	0.0040
AB	0.031845	1	0.031845	17.66212	0.0008
AC	0.001365	1	0.001365	0.756983	0.3980
BC	2.78E-05	1	2.78E-05	0.015418	0.9028
A^2	0.018346	1	0.018346	10.17551	0.0061
B^2	0.002718	1	0.002718	1.507591	0.2384
Residual	0.027045	15	0.001803		
Lack of Fit	0.027045	8	0.003381		
Pure Error	0	7	0		
Cor Total	0.272931	23			

B6ii. Statistics Of The Model Regression Parameters For Response Surface Quadratic Model For The Performance Of Appearance/colour In Two Varieties Of Treated Fresh-Cut Pineapples, MD2 And Smooth Cayenne.

R-Squared	Adj R-Squared	Pred R-Squared	Adeq Precision
0.887077	0.855709	0.767426	21.19579

Appendix C

ANOVA TABLE FOR TESTS CURRIED OUT IN PHASE TWO

C1. ANOVA TABLE FOR YEAST COUNT (REFRIGERATED TREATED)

Source of variation	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Model	57.0852	1	57.0852	2708.29	0.0000
Residual	0.126468	6	0.0210779		
Total (Corr.)	57.2117	7			

C2. ANOVA TABLE FOR YEAST COUNT (REFRIGERATED UNTREATED)

Source of variation	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Model	43.3221	1	43.3221	459.13	0.0000
Residual	0.566141	6	0.0943569		
Total (Corr.)	43.8882	7			

C3. ANOVA TABLE FOR YEAST COUNT (AMBIENT TREATED)

Source of variation	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Model	30.1704	1	30.1704	2758.21	0.0004
Residual	0.0218768	2	0.0109384		
Total (Corr.)	30.1922	3			

C4. ANOVA TABLE FOR YEAST COUNT (AMBIENT UNTREATED)

Source of variation	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Model	3.74954E8	1	3.74954E8	1720.42	0.0006
Residual	435888.	2	217944.		
Total (Corr.)	3.7539E8	3			

C5. ANOVA TABLE FOR MOULD COUNT (REFRIGERATED TREATED)

Source of variation	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Model	3.32704E7	1	3.32704E7	49.77	0.0004
Residual	4.01072E6	6	668454.		
Total (Corr.)	3.72811E7	7			

C6. ANOVA TABLE FOR MOULD (REFRIGERATED UNTREATED)

Source of variation	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Model	1.02884E8	1	1.02884E8	194.40	0.0000
Residual	3.17538E6	6	529230.		
Total (Corr.)	1.06059E8	7			

C7. ANOVA TABLE FOR MOULD (AMBIENT TREATED)

Source of variation	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Model	0.00776763	1	0.00776763	335.81	0.0030
Residual	0.0000462617	2	0.0000231308		
Total (Corr.)	0.00781389	3			

C8. ANOVA TABLE FOR MOULD (AMBIENT UNTREATED)

Source of variation	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Model	145205.	1	145205.	40.34	0.0239
Residual	7198.4	2	3599.2		
Total (Corr.)	152403.	3			

C9. ANOVA TABLE FOR ABC (REFRIGERATED TREATED)

Source of variation	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Model	91.4273	1	91.4273	2064.38	0.0000
Residual	0.265728	6	0.044288		
Total (Corr.)	91.693	7			

C10. ANOVA TABLE FOR ABC (REFRIGERATED UNTREATED)

Source of variation	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Model	21.2287	1	21.2287	640.62	0.0000
Residual	0.198827	6	0.0331378		
Total (Corr.)	21.4275	7			

C11. ANOVA TABLE FOR ABC (AMBIENT TREATED)

Source of variation	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Model	5.90339E10	1	5.90339E10	133.98	0.0074
Residual	8.81239E8	2	4.40619E8		
Total (Corr.)	5.99151E10	3			

C12. ANOVA TABLE FOR ABC (AMBIENT UNTREATED)

Source of variation	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Model	5.06503E10	1	5.06503E10	250.94	0.0040
Residual	4.03682E8	2	2.01841E8		
Total (Corr.)	5.1054E10	3			

C13. ANOVA TABLE FOR VITAMIN C CONTENT (REFRIGERATED TREATED)

Source of variation	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Model	386.475	1	386.475	390.31	0.0000
Residual	6.93124	7	0.990177		
Total (Corr.)	393.406	8			

C14. ANOVA TABLE FOR VITAMIN C CONTENT (REFRIGERATED UNTREATED)

Source of variation	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Model	1.00257E6	1	1.00257E6	156.14	0.0000
Residual	44946.0	7	6420.85		
Total (Corr.)	1.04752E6	8			

C15. ANOVA TABLE FOR VITAMIN C CONTENT (AMBIENT TREATED)

Source of variation	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Model	180.57	1	180.57	125.63	0.0079
Residual	2.87471	2	1.43735		
Total (Corr.)	183.445	3			

C16. ANOVA TABLE FOR VITAMIN C CONTENT (AMBIENT UNTREATED)

Source of variation	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Model	0.00332118	1	0.00332118	218.20	0.0046
Residual	0.0000304421	2	0.0000152211		
Total (Corr.)	0.00335162	3			

C17 ANOVA TABLE FOR BRIX CONTENT (REFRIGERATED TREATED)

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Model	22.2771	1	22.2771	70.15	0.0001
Residual	2.22289	7	0.317556		
Total (Corr.)	24.5	8			

C17 ANOVA TABLE FOR BRIX CONTENT (REFRIGERATED UNTREATED)

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Model	10.308	1	10.308	42.64	0.0003
Residual	1.69202	7	0.241717		
Total (Corr.)	12.0	8			

C17 ANOVA TABLE FOR BRIX CONTENT (AMBIENT TREATED)

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Model	42.5818	1	42.5818	20.43	0.0456
Residual	4.16822	2	2.08411		
Total (Corr.)	46.75	3			

C17 ANOVA TABLE FOR BRIX CONTENT (AMBIENT UNTREATED)

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Model	5.60981	1	5.60981	9.67	0.0897
Residual	1.16019	2	0.580093		
Total (Corr.)	6.77	3			

C17 ANOVA TABLE FOR MOISTURE CONTENT (REFRIGERATED TREATED)

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Model	12.1543	1	12.1543	742.21	0.0000
Residual	0.11463	7	0.0163757		
Total (Corr.)	12.2689	8			

C17 ANOVA TABLE FOR MOISTURE CONTENT (REFRIGERATED UNTREATED)

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Model	81.2823	1	81.2823	92.88	0.0000
Residual	6.12578	7	0.875111		
Total (Corr.)	87.4081	8			

C17 ANOVA TABLE FOR MOISTURE CONTENT (AMBIENT TREATED)

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Model	7.7105	3	2.57017		
Residual	0.0	0	0.0		
Total (Corr.)	7.7105	3			

C17 ANOVA TABLE FOR MOISTURE CONTENT (AMBIENT UNTREATED)

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Model	43.5014	1	43.5014	59.06	0.0165
Residual	1.47307	2	0.736534		
Total (Corr.)	44.9745	3			

C 17 ANOVA TABLE FOR pH (REFRIGERATED TREATED)

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Model	0.563957	1	0.563957	43.26	0.0003
Residual	0.0912656	7	0.0130379		
Total (Corr.)	0.655222	8			

C 17 ANOVA TABLE FOR pH (REFRIGERATED UNTREATED)

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Model	1.92697	1	1.92697	166.82	0.0000
Residual	0.0808562	7	0.0115509		
Total (Corr.)	2.00782	8			

C 17 ANOVA TABLE FOR pH (AMBIENT TREATED)

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Model	0.209535	1	0.209535	31.41	0.0304
Residual	0.0133402	2	0.00667009		
Total (Corr.)	0.222875	3			

C 17 ANOVA TABLE FOR pH (AMBIENT UNTREATED)

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Model	0.857723	1	0.857723	54.85	0.0177
Residual	0.0312766	2	0.0156383		
Total (Corr.)	0.889	3			

Appendix D

QUESTIONNAIRES FOR SENSORY EVALUATION OF PINEAPPLE PRODUCTS

D1. SENSORY ANALYSIS FORM (PHASE ONE)

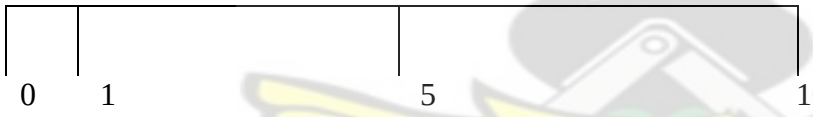
Name: ……. Occupation: ……..

Sex: ……. Phone number: …….

Age: …… Date: ……

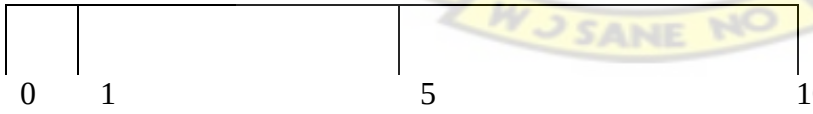
You have been provided with four (4) samples of pineapples labeled 777, 413, 936, and 200. Observe and taste in the order given you. Rinse your mouth with water after tasting each sample. Indicate on the interval scale provided, your level of acceptance (0=dislike very much, 5=like moderately and 10= like very much) in the order; colour, texture, taste and overall preference.

Colour comments:



Texture comments:

Taste comments:



Overall preference comments:

Thank you.

D2. SENSORY ANALYSIS FORM (PHASE TWO)

TEST number……

PANELIST number……..

TRIANGLE DIFFERENCE TEST

PRODUCT ……

INSTRUCTIONS: Proceed when you are ready. (Quietly so as not to disturb others.)

FOR EACH SAMPLE:

1. Take a sip of water to rinse your mouth before and after tasting.
2. Two of the samples are the same and one is different. CIRCLE the ODD sample. If you cannot, **guess.** Put the correct **(√)** sign beside it if you guessed.

117 **671** **777**

3. Describe the reason why the odd sample is different. (Please be specific)

……
……
……
……
……
……
……
……
……

Appendix E

RAW DATA, PLATES AND GRAPHS

E1. RAW DATA FROM CHEMICAL ANALYSIS IN PHASE TWO

pH

	Ambient Condition			Refrigeration Condition	
Day	treated	untreated	Day	treated	untreated
1	4.44	4.39	1	4.35	4.46
4	4.56	4.7	4	4.47	4.45
6	4.82	5.21	6	4.59	4.75
8	5.05	5.62	8	4.53	4.86
11			11	4.58	4.95
13			13	4.61	5.39
15			15	4.87	5.32
18			18	5.01	5.66
20			20	5.24	5.83

Moisture

	Ambient Condition			Refrigeration Condition	
Day	treated	untreated	Day	treated	untreated
1	90.98	91.88	1	88.11	90.17
4	90.10	89.59	4	87.34	86.45
6	88.29	85.68	6	87.17	85.33
8	87.85	83.24	8	86.62	84.33
11			11	86.07	83.33
13			13	85.71	83.07
15			15	85.16	80.65
18			18	84.98	80.31
20			20	84.36	80.01

Brix

	Ambient Condition			Refrigeration Condition	
Day	treated	untreated	Day	treated	untreated
1	13.4	14	1	14.5	14.5
4	11.5	11	4	14.5	14
6	10	6	6	14.5	14
8	10.5	6	8	13.5	13.5
11			11	14	11.5
13			13	13.5	12.5
15			15	12.5	10.5
18			18	11.5	10.5
20			20	11.5	10

Vitamin C

	Ambient Condition			Refrigeration Condition	
Day	treated	untreated	Day	treated	untreated
1	38.52	34.05	1	38.28	34.52
4	32.8	22.78	4	35.11	25.71
6	26.6	13.21	6	32.76	23.81
8	20.4	9.6	8	30.67	22.62
11			11	29.08	13.81
13			13	27.1	10.13
15			15	22.45	15.45
18			18	18.98	11.57
20			20	18.34	10.45

E2. RAW DATA FROM CHEMICAL AND MICROBIAL ANALYSIS IN PHASE TWO

DAYS	Vitamin C mg/100ml				PCR CFU/ml			
	RT	RU	AT	AU	RT	RU	AT	AU
1	38.28	34.52	38.52	34.05	6	46×10^{-4}	10	33×10^{-4}
4	35.11	28.71	28.80	22.78	30	90×10^{-4}	144×10^{-5}	213×10^{-5}
6	32.76	23.81	36.60	13.21	90×10^{-4}	210×10^{-5}	296×10^{-5}	320×10^{-6}
8	30.67	22.62	20.40	9.8	110×10^{-5}	289×10^{-5}	340×10^{-6}	387×10^{-6}
11	29.28	13.81			121×10^{-5}	480×10^{-6}		
13	27.10	10.13			259×10^{-5}	700×10^{-6}		
15	22.45	15.45			270×10^{-5}	840×10^{-6}		
18	18.98	11.57			300×10^{-5}	970×10^{-6}		
20	18.34	10.45						

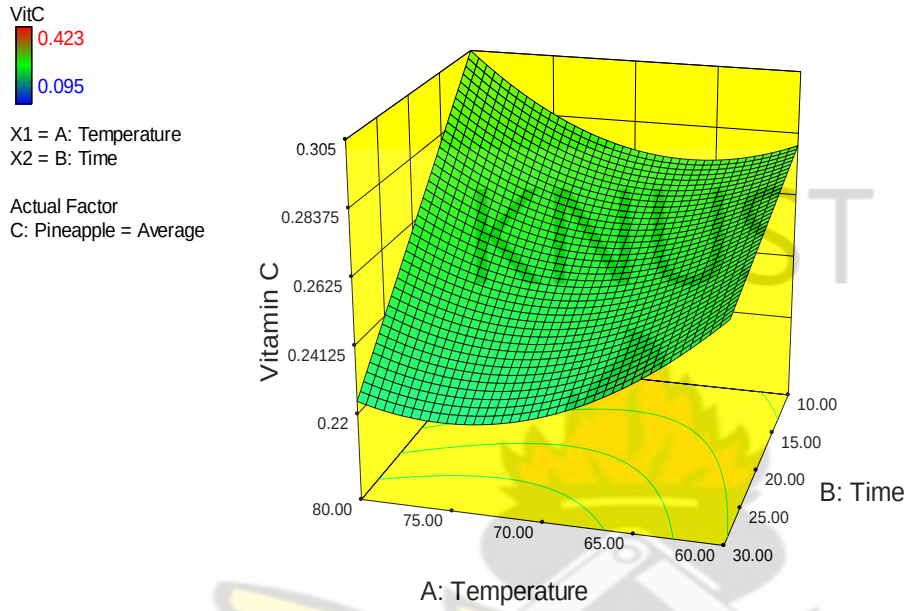
Days	Yeast ($\times10^{-2}$) CFU/ml				Mould CFU/ml			
	RT	RU	AT	AU	RT	RU	AT	AU
1	4	20	28	3800	0	0	8	10
4	4300	15000	9800	15800	8	11	15	18
6	5800	19000	17800	23000	10	24	19	27
8	10500	21800	21000	30000	13	30	30	480
11	13100	23000			19	3400		
13	15100	25100			3200	4100		
15	19000	30000			3900	6800		
18	21500	32000			5700	10400		

RT=REFRIGERATED TREATED AT= AMBIENT TREATED

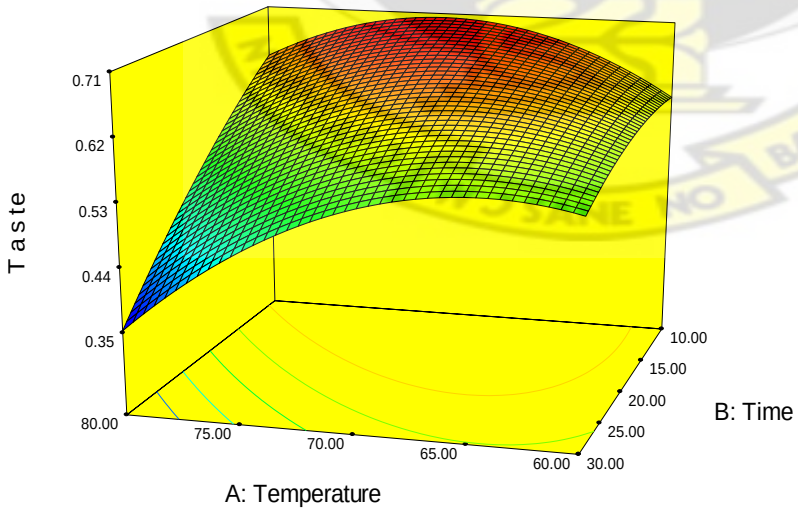
RU= REFRIGERATED UNTREATED AU= AMBIENT UNTREATED

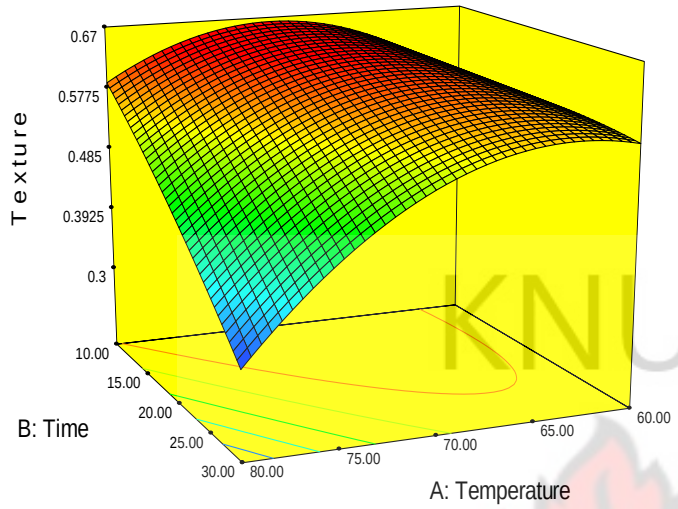
E4. GRAPHS OF THE AVERAGE BEHAVIOR OF BOTH PINEAPPLE VARIETIES

E3(a) Average Behavior Of Vitamin C Content Of Both MD2and Smooth Cayenne Pineapple Varieties

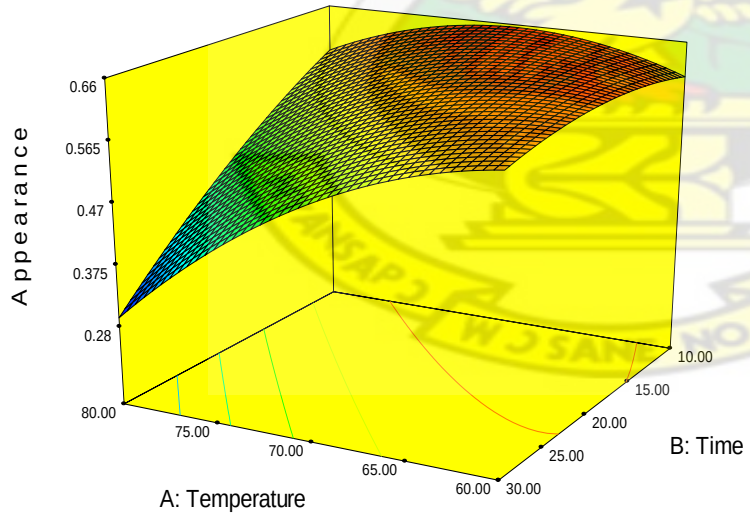


E3(b) Average Behavior Of Pineapple Taste Of The Two Pineapple Varieties





E3(c) Average Behavior Of Pineapple Texture Of Both Pineapple Varieties



E3(d) Average Behavior Of The Appearance/Colour Of Samples From Both Varieties

A (i) **A (ii)**

B (i) **B (ii)**

E (f) Treated (A) and untreated (B) MD2 pineapples (from phase two) in refrigeration before (i) and after (ii) storage for 15 days

E(g). Pineapple juice of treated (golden brown) and untreated (dark brown) samples under refrigeration storage condition of the 13 days of storage.

(a)MD2 before and after treatment at 80C FOR 10min

(b) MD2 before and after treatment at 70C for 20 min

(c) Smooth cayenne before and after treatment at 0C for 20min

E(h). Treated pineapples of the two varieties before and after treatment (PHASE ONE).